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Editorial

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Editorial

The Confluence Between Arts and Medical Science — Music and movement therapy for children with Cerebral Palsy

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The arts, specifically music and movement, are normally identified as serving an aesthetic function, principally that of entertainment. Their function as a therapeutic tool, however, is often neglected or, at the very best, misunderstood.

The arts have been used by traditional societies in healing ceremonies to address psychosomatic maladies. These societies believed that all ailments were attributable to spiritual misalignment; therefore, there was a perceived need to placate the spirits through the use of music and movement in order to redress physiological and psychosomatic conditions. Such therapeutic healing was common in early societies and is still common even in advanced societies, such as Malaysia; it is still being practised in Kelantan and Terengganu, in the form of "Main Puteri" and "Main Bageh".

In modern society, the arts have been known to relieve stress and promote a sense of well-being as well as to improve a person's physical and mental faculties (1). The act of indulging in artistic activities, such as music, dance, drama, or painting, that are both verbal and non-verbal forms of expression allows the body and mind to activate internal energy in creating an aesthetic expression. This manifestation of the inner self has a cathartic, cleansing, and calming effect: relieving stress, muscular strains, and cerebral tension.

Similarly, such therapeutic effects can be elicited through artistic involvement in special children who suffer from motor and/or cognitive deficiencies (2–5). In such cases, the goal is to activate and deploy this internal energy to extend the range of their motor functions and improve their cognitive faculties as well as to foster a sense of achievement and self-fulfilment.

To explore the efficacy and viability of therapy that brings the confluence of the arts and sciences

into focus, a pilot study was instituted by Ms Maria Soccoro of the Department of Neuroscience, School of Medical Sciences, Universiti Sains Malaysia, and myself. The study focused on the use of gamelan (a form of traditional music) to gauge and improve the gross motor functions of children with cerebral palsy (CP).

For this project, we solicited the cooperation of The Spastic Centre in Penang, a day school for children with varying severities of CP (those diagnosed with diplegic, hemiplegic, monoplegic, athetoid, and ataxic CP). It was originally proposed to have 2 groups of 15 children each for a total of 30, the objective being to have 1 control and 1 intervention group. However, the centre did not have enough children of mild spasticity with acceptable cognitive level (i.e., able to follow and understand instructions) to fill these 2 groups.

Nevertheless, Ms Soccoro, a neuro-psychologist, conducted a preliminary assessment of the sample patients using the Wechsler Abbreviated Scale of Intelligence (performance scale) test and the test for reception and grammar. Dr Abdul Rahman Ghani, a neurosurgeon, conducted the Gross Motor Function Measure (GMFM-66) analysis to select appropriate study subjects. The GMFM-66 test quantitatively evaluates 5 dimensions: lying and rolling; sitting; crawling and kneeling; standing; and walking, running, and jumping (6). Based on these tests, 9 patients with parental informed consent were selected: 5 diplagic, 1 tripelagic, and 3 others with full ambulatory function and both arms functional. The patients were all mobile but not stable. Their age ranged between 7 and 12 years, with a receptive language level of 4.00 to 4.11 years.

It was agreed that the therapy would be administered 2 days per week (Monday and Friday) for the duration of the project. Each session lasted for 1 hour from 9:00 to 10:00 am; this time coincided with the children's exercise period. For these sessions, The Spastic Centre transported the students to and from the Gamelan Studio, School of Arts, Universiti Sains Malaysia, where the therapy was administered.

The therapeutic intervention was designed around the use of music, movement, and theatre games. Gamelan music was used as part of the intervention. Almost all of the patients had not seen or experienced the hearing or playing of gamelan. This type of musical ensemble consists of large- and medium-sized circular knobbed gongs and bronze plates of varying pitches, all arranged in pentatonic scale sequence and suspended over a trough-like resonator (7). These musical instruments can be easily handled and do not require any fine technical skill, as do playing the violin, piano, or flute. The basic skill required is the ability to strike the instruments on the knob of the gong or at the centre of the metal plates. These are all percussive instruments. We were not concerned about the colour, timbre, or tonal quality of the sounds produced. Our focus was the striking actions that anyone can execute, even the special children with various degrees of spasticity. The initial objective was to get them to produce sound and by doing so getting them to exercise their arms and hands, with the purpose of relaxing and extending the range of their muscles.

Warm-up sessions included basic hand motions (raising upwards, sideways, lowering the arms from the top position to the floor, slapping the floor, vibrating the fingers, swinging from side to side, and clapping) and leg movements (simple straightening exercise in a sitting position and then retracting them, wiggling the toes, bending the knees, flexion, and pronation). Head movements were introduced, together with 4-point and 2-point crawling movements, as well as rolling from side to side. We also included vocal inflexion, shouting, and making specific animal sounds. Theatre games, such as imitating various animal movements, story-telling and magic shows, were also incorporated.

For the first few sessions, we worked towards obtaining their trust and confidence and creating a relaxed and friendly atmosphere; we did not have any specific targets at this time, intending rather to improvise as we go along to meet their interests. We worked within the general framework of playing the musical instruments and executing movements, however, and we observed the following improvements in the children after the intervention:

1. *Improvement in Posture*

Subject ADL voluntarily corrected his posture without being reminded and then sat with good posture. Similarly, subject CTN self-corrected her posture and could tolerate cross-legged sitting for almost an hour, whereas previously she could only sustain it for 15 minutes. Subject JNT improved her cross-legged sitting from 5 to 10 minutes. Subject YPG had such difficulty in sitting upright that when he first arrived, he had to be supported. After undergoing therapy, however, he sat unsupported without fear and was able to maintain upright posture for 15 minutes. Subject WCO was also able to maintain upright posture. Subject KHO normally would not tolerate sitting on the floor, but he was able to sit on the floor for

almost 10 minutes. The sitting position of subject YZL had improved, and she had been able to maintain it for almost 20 to 30 minutes.

2. Improvement in Attention Span and Concentration

Almost all of the children have shown improvement in attention span and concentration (Table 1), ranging from 5 to 15 minutes and 5 to 10 minutes, respectively.

3. Improvements in Gross Motor Function

Subject ADL's active range of motion, grasp, and release on the right side has improved. Subjects JNT and CTN can move their legs into a sitting position more easily and sustain them for a longer period of time. Subject WCO has shown improvement in the quality of movement in her upper limbs and has performed given tasks faster than before. Subject YZL improved the movement pattern of her upper limbs and her fine motor functions.

4. Confidence Level

All subjects have shown increased confidence and have been able to execute tasks more effectively. They have each shown a keen interest in attending the therapy sessions.

5. Cognitive Function

All subjects have shown memory improvement.

6. Gross Motor Function

At post-test, all subjects scored significantly higher on gross motor function as measured by standing (Table 2). They also showed significant improvement over time on the measures of walking, jumping, and running.

Mr Santosh, the therapist at The Spastic Centre, Penang, gave an outstanding testimonial on the outcome of the pilot project.

The children started the twice weekly (Mondays and Fridays) music and movement therapy sessions in March 2008. They were enthusiastic and keen in attending these sessions. During these sessions, they were instructed to play the traditional gamelan instruments and drums. While playing these instruments, they had to maintain an upright posture and forgot that they were sitting without any support. They showed keen interest in playing these instruments. In playing these musical instruments, the instructor demonstrated the method of striking

Table 1: Attention span and concentration in subjects, pre- and post-therapy

Subject	Attention span (minutes)		Concentration (minutes)	
	Pre-therapy	Post-therapy	Pre-therapy	Post-therapy
ADL	2	20	5	15
CTN	15	25	10	15
JNT	10	20	5	10
KHO	10	20	10	15
MSQ	15	25	10	15
RBT	10	20	5	15
WCO	20	25	10	15
YPG	5	10	3	8
YZL	15	20	10	15

Table 2: Gross motor function measures, pre- and post-therapy

	Standing	Walking, jumping, and running
Pre-therapy	26.56 ± 8.62	30.56 ± 21.87
Post-therapy	28.22 ± 7.63	32.11 ± 22.11

Data are expressed in mean ± SD.

the instruments, and the children copied them and followed the instructions well. During the movement sessions, which involve various types of normal movements as well as animal movements, the children imitated these movements to the best of their ability. When they were asked to do the same thing at home or in school, they would not be that co-operative. Their co-operative attitude is because they are involved in group tasks and has generated a sense of competition among them. They get so involved and enjoyed doing their tasks that they forgot their physical limitation. For example, when the instructor asked them to move towards him, they do so with their various mode of ambulation, without regard to their physical disabilities. The children attending these sessions have shown improvement in their posture and movement pattern. Even though these improvements are so small, it improves their quality of life in executing the usual daily movements. They have also become more active and self-confident. They also relate their experience in these therapy sessions to their friends who are not attending these sessions. These music and movement therapy has enhanced the children socialising and communication skills.

The use of music (gamelan) and movement as a non-invasive therapy in gauging and improving the gross motor functions of children with CP has shown positive results based on clinical and empirical findings. In fact, this therapy has a broader application than solely improvement in the gross motor functions. Besides improvement in posture, muscle strength in the upper and lower limbs, range of motion and the ability to maintain a long period of sitting cross-legged, the subjects have shown improvement in attention span, concentration and confidence. They have also improved their hand-eye coordination and their memory.

It is important to mention here that this therapy in no way replaces the subject's normal medication. The therapy does, however, aid in improving their quality of life and, through the use of music and movement, activates the dormant primordial energy of self-healing within the given configuration of their disabilities.

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References

1. Tyson F. Psychiatric Music Therapy: Origins and Development. New York: Creative Arts Rehabilitation Center; 1981.
2. Krakouer L, Houghton S, Douglas G, West J. The efficacy of music therapy in effecting behaviour change in persons with cerebral palsy. *Int J Psychosoc Rehabil.* 2001:**6;**25–32.
3. Kvam MH. The effect of vibroacoustic therapy. *Physiotherapy*. 1997:**83(6)**;290–295.
4. Rain Perry MM. Relating improvisational music therapy with severely and multiply disabled children to communication development. *J Music Ther*. 2003:**40(3)**;227–256.
5. Whipple J. Music in intervention for children and adolescents with autism: A meta-analysis. *J Music Ther*. 2004:**41(2)**;90–106.
6. Russell D, Rosenbaum P, Gowland C, Hardy S, Lane M, Plews N, et al. Gross Motor Function Measure Manual. 2nd ed. Kingston, Ontario: Gross Motor Measures Group; 1993.
7. Mohamed Ghouse N. Traditional Malaysian Music. 1st ed. Kuala Lumpur: Dewan Bahasa dan Pustaka; 2007.

Review Article

Genetic Aberrations in Childhood Acute Lymphoblastic Leukaemia: Application of High-Density Single Nucleotide Polymorphism Array

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Abstract

Screening of the entire human genome using high-density single nucleotide polymorphism array (SNPA) has become a powerful technique used in cancer genetics and population genetics studies. The GeneChip® Mapping Array, introduced by Affymetrix, is one SNPA platform utilised for genotyping studies. This GeneChip system allows researchers to gain a comprehensive view of cancer biology on a single platform for the quantification of chromosomal amplifications, deletions, and loss of heterozygosity or for allelic imbalance studies. Importantly, this array analysis has the potential to reveal novel genetic findings involved in the multistep development of cancer. Given the importance of genetic factors in leukaemogenesis and the usefulness of screening the whole genome, SNPA analysis has been utilised in many studies to characterise genetic aberrations in childhood acute lymphoblastic leukaemia.

Keywords: *B- and T-cell acute lymphoblastic leukaemia, child, cytogenetic aberrations, single nucleotide polymorphisms, oligonucleotide array sequence analysis, medical sciences*

Introduction

Leukaemia is a cancer of the haemopoietic system that affects white blood cells, which are normally responsible for combating infections. All blood cells are produced in the bone marrow (although this is not the case in utero) and develop from stem cells. Stem cells possess the potential to develop into 2 different types of white blood cell, lymphocytic or myelocytic. Mature lymphoid cells are classified as B-cells or T-cells and arise from a common lymphoid stem cell in the bone marrow (1). Normally, white blood cells grow, divide, and reproduce in an orderly and controlled manner. However, this process becomes uncontrollable in leukaemia where the cells continue to divide but not mature. Leukaemia is characterised by a diffuse replacement of the bone marrow by neoplastic cells, which leads to suppression of normal haematopoiesis. Leukaemia can be classified as acute or chronic, and lymphoid or myeloid, resulting in 4 main types of disease: acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), chronic lymphocytic leukaemia (CLL), and chronic myeloid leukaemia (CML). Childhood leukaemias are primarily acute and progress rapidly.

Genetic factors have a significant impact on the development of childhood ALL. Recent advances in cytogenetic and molecular analyses have led to a better understanding of important genes that are involved in leukaemogenesis. Conventional cytogenetic analysis has been the standard method of identifying genetic aberrations, including changes in chromosome structure and number. Cytogenetic evidence indicating that chromosomal translocations may have important prognostic implications in ALL has been used for more than a decade to assign patients to risk-directed therapy (2). It has been reported that acquired chromosomal abnormalities in the leukemic blast cells from patients with ALL strongly associate with the biology of the disease, and designate the genes involved in leukaemogenesis (3). According to Greaves, biological subsets of leukaemia that have prognostic importance can be defined by different chromosomal and gene abnormalities (4). Recurrent chromosomal translocations may provide valuable clues to the identification of fusion genes that can lead to the characterisation of known or new oncogenes or tumour suppressor genes critical for leukaemogenesis. Gene fusion events can result in altered mechanisms to activate abnormal cell growth. These mechanisms include enhanced gene expression, fusion of transcription factors, altered protein kinase patient in terms of molecular pathology, and a number of distinct genetic subtypes of ALL have been identified

according to differences in cell surface markers (at the time of presentation), clinical behaviour, and response to individual therapeutic agents (5).

Genetic alterations in childhood ALL

Numerical and structural chromosomal changes

Most of the karyotypes in ALL samples have both numerical and structural chromosomal changes. Structural abnormalities include chromosomal translocations, deletions, inversions, and other rearrangements involving genes with oncogenic potential. Numerical changes can be classified into groups according to the chromosome number such as hypodiploidy, hyperdiploidy, triploidy, and tetraploidy (3). Hyperdiploidy and hypodiploidy occur in 25% to 30%, and 1% of childhood ALL, respectively (6,7).

Molecular genetic alterations in childhood ALL

Determining the cytogenetic and molecular characteristics of childhood ALL provides a valuable tool for monitoring the disease during treatment, for the prediction of prognostic outcome, and for the management of future therapy. Molecular identification that provides detailed knowledge of important genes, such as oncogenes and tumour suppressor genes, may provide valuable clues to mechanisms of leukaemogenesis. Complete loss of an allele or an increase in copy number (CN) of one allele relative to the other can lead to the formation of allelic imbalance, commonly found in malignancies. A previous study has reported 2 forms of allelic imbalance: gene CN amplification, which reveals the presence of oncogenes; and loss of heterozygosity (LOH), which is usually associated with the presence of tumour suppressor genes (8).

Gene mutations and amplifications

Abnormalities in the coding region of genes may affect the normal function of its protein and may, in turn, impact cell division, cell growth, cell death, and the DNA damage response. Various mutations in tumour suppressor genes, proto-oncogenes/oncogenes, and DNA repair genes may contribute to tumour progression. Mutations in some tumour suppressor genes, including $p16^{INK4a}$ and *p53*, have been reported in childhood ALL (9–11). A number of oncogenes, such as *FLT3* and members of the ras family, are found to play a role in the formation of ALL. FMS-related tyrosine kinase-3 (FLT3) is a receptor tyrosine kinase expressed in early haematopoietic progenitors that plays an important role in haematopoietic development (12). Activating mutations of *FLT3* were discovered initially in AML and are found commonly in 2 subtypes of childhood ALL: infant ALL with MLL translocations and hyperdiploid ALL (13,14). Previous studies reported that the incidence of N-ras mutations is 10% in childhood ALL, but the role of this mutation is not fully understood (15,16). The Notch signalling pathway is essential in T-cell development and NOTCH1 mutations are found frequently in adult and paediatric T-cell ALL (17).

Gene amplification is a common event in many types of solid tumours. However, the occurrence of this aberration in acute leukaemia is quite rare (18). Multiple copies of *AML1 (RUNX1)*, on duplicated chromosome 21, were identified in 20 patients with ALL (childhood and adult), suggesting that chromosome 21 and *AML1* amplification were important in leukaemogenesis (19). A further study from this group found the same *AML1* amplification on 8 additional cases of childhood ALL, emphasising this as a cytogenetic subgroup in ALL and suggesting its role as an indicator of poor prognosis in ALL (20). This abnormality is known as intrachromosomal amplification of chromosome 21 with amplification of *AML1* (iAMP21) (21,22). High level amplification of *AML1* has also been reported in 2 cases of childhood ALL detected by fluorescence in situ hybridisation (FISH) and comparative genomic hybridisation (CGH) analysis (23). In ALL, *AML1* is commonly involved in translocation t(12;21)(p13;q22), which leads to the *TEL/AML1 (ETV6/RUNX1)* fusion gene. A previous study also found that *AML1* amplification was present in childhood ALL but not in adult cases, and it was not associated with *AML1* mutation (24). A few studies have reported on *ABL1* amplification in T-cell ALL cases. The first study discovered multiple copies of *ABL1* in 5 of 210 paediatric T-cell ALL cases (25). Two other studies also detected *ABL1* amplification in T-cell ALL patients and T-ALL cell lines (26,27). The study showed that *ABL1* amplification was the amplification of the *NUP214-ABL1* fusion detected in 5 of 85 T-ALL patients (27). This gene is the target of many recurrent translocations seen in different leukaemia subtypes, mostly in t(9;22)(q34;q11.2); this particular translocation results in the formation of the *BCR-ABL1* fusion gene and is one of the cytogenetic hallmarks of CML (28).

Loss of heterozygosity

Loss of heterozygosity (LOH) of human chromosomal regions is one of the most frequent genetic events found in many types of malignancies. Investigation of LOH and its effect on allelic imbalance (29) in childhood ALL may

provide important information about the genetic basis of the disease because frequent allelic deletions in tumour cells are usually indicative of the inactivation of tumour suppressor genes. Previous studies have suggested that the loss of tumour suppressor gene activity is an important event in the development of cancer. Takeuchi et al. (30) reported that inactivation of tumour suppressor genes by mutation of one allele and loss of the second allele is a crucial pathway of leukaemogenesis in childhood ALL. Informative microsatellite markers are used as an indirect method to confirm LOH and to search for inactivated tumour suppressor genes (30). Several different mechanisms at the molecular or cytogenetic level have been considered to account for LOH: deletion, gene conversion, single or double homologous and non-homologous mitotic recombination, translocation, chromosome breakage and loss, chromosomal fusion or telomeric end-to-end fusion, or whole chromosome loss with or without accompanying duplication of the retained chromosome (31).

Previously, a large number of LOH studies using microsatellite markers in childhood ALL have been performed by a Japanese group and collaborators; these studies have found that LOH of chromosomes 6q, 9p, 11q, and 12p are frequent in childhood ALL (32–36). Baccichet et al. investigated LOH using 49 highly polymorphic markers distributed over 13 chromosomal arms and found that the highest rates of allelic losses were observed in 9p and 12p regions, which were deleted in 29% and 32% of childhood ALL patients, respectively (37). They found no LOH on chromosomes 3p, 5q, 11p, 11q, 13q, or 18q (37). Cavé et al. indicated that 12p12-13 alterations at the molecular level are present in about 27% of children with B-lineage ALL, which is a higher percentage than had previously been reported by standard chromosome analysis (38). LOH on chromosome 12p12-13 was detected in 26 to 47% of childhood ALL samples analysed (34,39,40), suggesting that inactivation of a tumour suppressor gene on this region, possibly the ETV6 and CDKN1B, may play a role in leukaemogenesis (40,41). Baccichet and Sinnet investigated a number of childhood ALL samples and found that 31% (17 of 55) of samples had LOH on 12p12-13 (42). According to Takeuchi et al., in the relapse of childhood ALL, chromosome 9p was the most frequent site for LOH (occurring in 15 of 38 informative cases); they also report that the 4q and 17q regions appear to have an important role in ALL relapse (43). In 2004, Heerema et al. performed cytogenetic analyses for chromosome 7 and reported that the critical region of loss of chromosome 7 in paediatric ALL at presentation may be on the p-arm (44).

Epigenetic changes by methylation

DNA methylation is a natural modification of human DNA and is catalysed by a DNA methyltransferase enzyme that adds a methyl group to the carbon 5 position of the cytosine ring in CpG nucleotides (45). Methylation, the major epigenetic modification of human genomic DNA, takes place only at cytosine bases that are located 5′ to the guanosine residue in a CpG dinucleotide within the mammalian genome (46,47). CpG islands are GC-rich DNA regions, typically 0.5 to 4.0 kb in length, which remain unmethylated in normal tissues when found in the 5′ region of genes (46,48). These CpG islands are located frequently within the promoter regions of human genes. Methylation within CpG islands has been associated with transcriptional inactivation and functional silencing of the corresponding gene due to chromatin compaction (49,50). Robertson and Wolffe reported that CpG islands can also be found in non-coding intergenic areas rich in highly repetitive DNA elements (51).

Both DNA hypermethylation and hypomethylation might play important roles in the tumourigenic process, but increased methylation at CpG islands has been, by far, the most studied process that has a clear role in carcinogenesis (50). It has been documented that aberrant methylation may occur during the early stages of carcinogenesis, and that distinct types of cancer exhibit specific patterns of methylation changes (52). It has been proposed that methylation of the promoter region should be included in Knudson's two-hit hypothesis for inactivation of tumour suppressor genes (53,54). The first hit may be a mutation in the DNA sequence or promoter methylation, as was suggested. The second inactivating hit may be either LOH or a further mutational or methylation event in the second allele. Hypermethylation of CpG islands in a human tumour was first reported in 1986 (55). Multiple genes, such as tumour suppressor genes and DNA repair genes, have been shown to be inactivated by hypermethylation of CpG islands in many types of cancer, including haematological malignancies and solid tumours (50).

Application of high density single nucleotide polymorphism array to determine allelic imbalance in childhood ALL

Over the past few years, the application of advanced technologies, based on the large

collections of mapped single nucleotide polymorphism (SNP), to genetic studies has yielded important findings to help elucidate the molecular mechanisms responsible for the pathogenesis of human cancers. A SNP is the most common type of DNA sequence variation that is found at one specific position in the human genome and occurs in at least 1% of the human population (56). In 1998, Wang et al. illustrated large-scale identification, mapping, and genotyping of SNPs in the human genome, which led to the development of the genotyping chips that allow for the simultaneous genotyping of 500 SNPs (57).

Investigation of allelic imbalance can also be performed using a variety of techniques such as conventional cytogenetic analysis (karyotyping), FISH, Southern blotting, microsatellite analysis using highly polymorphic markers, and CGH. These techniques are either of low resolution or laborious and require the use of relatively large amount of DNA if the entire genome is to be examined. It has been suggested that the most reliable method for characterising allelic imbalances should have the ability not only to provide locus-specific genotype but also to quantify accurately the copy number of each allele (8). Recently, oligonucleotide microarrays designed for whole genome, SNP analysis have been developed, which are proving to be a powerful methodology. The SNP mapping array (SNPA), Affymetrix HuSNP GeneChip containing 1494 SNPs, was initially applied for the detection of sequence polymorphisms (58). Several studies have subsequently utilised the Affymetrix HuSNP GeneChip to identify LOH or allelic imbalance in tumour tissues from breast, bladder, prostate and small-cell lung cancer (59–62). Array-based CGH is available and provides a high resolution for CN analysis, but it cannot readily detect chromosomal LOH without CN change (63).

Development of the high-density SNPA (GeneChip Human Mapping Array by Affymetrix) started with the introduction, in 2004, of a chip that could genotype more than 10 000 SNPs. Currently, a version of a chip containing up to 900 000 SNPs is available. The GeneChip Human Mapping Array is a high resolution method for genome-wide screening and a powerful technique to detect genetic aberrations, such as LOH and CN alterations related to deletion or amplification in primary solid tumours, cancer cell lines, and leukaemias (8,63–65). Importantly, this array can characterise LOH due to acquired isodisomy (AID), which reveals the presence of LOH with no alteration of CN. This technology has been described as a fast, cost-effective, and reliable approach for whole genome AID screening (66).

A previous study by the Leukaemia Research Group at Newcastle University used SNPA analysis to detect LOH using lymphoblasts from a limited cohort of children with ALL and compared samples obtained at presentation to those obtained at relapse (64). This study was extended to observe a larger group of 78 patients at presentation and 12 at relapse, and it revealed aberrations on chromosome 9p in 26% of these patients (67). Also, they found that genomic deletion analysis showed a high degree of concordance between the 3 techniques (SNPA, array CGH, and FISH) but some deletions detected by array CGH were below the resolution of FISH (67). Genome-wide SNP analysis provides a good approach for obtaining more genetic information about the cancer cells and targeting the involvement of candidate genes in leukaemogenesis. A study of genome-wide analysis on childhood ALL by Kuiper et al. reported that loss of the 9p21.3 region was the most common lesion and revealed both CDKN2A and CDKN2B as functional candidates (68). They also found loss of the 9p13.2 region that affected only PAX5 in 20% (8 of 40) of childhood ALL cases. Furthermore, this array analysis has the potential to reveal novel genetic findings involved in the multistep development of cancer. A study by Mullighan et al. revealed new, recurrent genetic alterations in childhood ALL, which indicates the power of SNPA analysis in the study of cancer (69). Recently, a comprehensive analysis utilising 5 separate assays (mutation, methylation, SNPA, array CGH and FISH) has been reported to assess the inactivation of the target gene CDKN2A in childhood ALL (67). They discovered that CDKN2A deletion is a significant secondary abnormality in childhood ALL, which strongly correlated with the phenotype and genotype.

SNPA technology has an additional advantage over cytogenetics, array CGH, and FISH in that it can identify LOH associated with no CN alterations, which indicate the presence of copy number-neutral (CNN) LOH (66). This phenomenon has revealed the involvement of AID. A recent report using high-density SNPA analysis in a cohort of 98 patients with childhood ALL revealed CNN LOH (also known as AID and uniparental disomy, UPD) in 8% of patients (67). AID is a frequent alternative mechanism for allelic imbalance in a variety of cancers and occurs when part or all of both chromosomes of an individual pair are derived from only one parent. However, AID is a rare occurrence in the general population (70). Identification of

large regions of homozygosity, which did not correspond to deletions by SNPA analysis, were found in approximately 20% of AML cases but not in the DNA of remission cases, suggesting that it was an acquired abnormality (65). This same study also confirmed the presence of a normal CN by FISH within the homozygous regions, which then suggested that these areas consisted of AID occurring as a result of mitotic recombination. One of the mechanisms that can cause phenotypic abnormalities due to AID is homozygosity for an autosomal recessive gene. This event, as an acquired abnormality, can be correlated with homozygosity of a particular gene, suggesting that mitotic recombination acts to remove the wild type allele (71). Previous studies have shown that this mechanism of AID is associated with the presence of homozygous mutations of *JAK2* in myeloproliferative disease (72) and *CEBPA, FLT3*, and *RUNX1* in AML (73), suggesting that it is an important, novel mechanism for oncogene activation and/or tumour suppressor gene inactivation.

Comparing CN data obtained from SNPA with data obtained from conventional cytogenetics and FISH proved that SNPA is an efficient technique for CN estimation of individual chromosomes in individual patient samples. This SNPA is of great potential value in cases where cells fail to divide for the performance of standard cytogenetics and for centres where access to cytogenetic services is unavailable. It also validates DNA analysis for the majority of tumour types that do not yield suitable material for karyotyping.

Within the past year, new computer software tools have been developed by Affymetrix for data analysis of SNPA; these tools provide better and easier interpretation of results when compared with the manipulation of SNPA data in Excel spreadsheets. These application tools, available free from the Affymetrix website (http://www.affymetrix.com), include CNAT version 4.0, Bayesian Robust Linear Model with Mahalanobis distance classifier (BRLMM) Analysis Tool (BAT), and Integrated Genome Browser (IGB). CNAT 4.0 can be used to perform analysis on data from 10 K, 100 K, and 500 K arrays. It also allows combination of data from different sized arrays (virtual array). BRLMM is an improved genotype calling algorithm that allows more accurate SNP calls and can be used with 100 K and 500 K array sets. IGB is a powerful tool linked to the Ensemble database to help researchers visualise their results against the entire human genome. The IGB software can compare multiple genomic annotation sets from various data sources and, thus, is very useful for targeting areas of interest. SNPA technology has been described as a promising approach to link oncogenic pathways and to initiate the search for targets that could be exploited in the development of molecular therapeutics (56).

Conclusion

SNPA technology is a useful method to determine LOH or allelic imbalance in childhood ALL. Frequent occurrences of AID in childhood ALL may implicate this event as contributing to the presence of allelic imbalance, but the mechanisms involved in this pathogenesis remain elusive. This genome-wide screening approach is believed to develop into a powerful method for the identification of molecular targets which may be important in tumourigenesis in childhood ALL. SNPA technology has the strength to provide global analysis of CN alterations in human cancers and is capable of revealing the occurrence of LOH due to AID, which would be missed by conventional cytogenetics and CGH. SNPA technology has been shown to have accuracy, reproducibility, and improved calling algorithms. Screening the entire genome using this array requires only 250 ng of genomic DNA. This technology may provide crucial data to target any genes associated with leukaemogenesis as well as provide information to gain a better understanding for the mechanisms by which CN changes occur. These possible genes of interest may be important for future molecular therapy.

Using genome-wide SNPA, a large number of candidate genes have been found; however, it is difficult to select those genes for further investigation. Thus, priority has been given to genes which are known to be associated with tumourigenesis and located in common regions of deletion, amplification, or copy-neutral LOH (AID). These common aberrant regions revealed by SNPA must be validated by other methods, such as microsatellite analysis to confirm a LOH event, and quantitative real-time PCR to confirm the presence of gene amplification. This approach could facilitate pinpointing critical regions within the genome as well as candidate genes in those regions.

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References

1. Ross JA, Davies SM, Potter JD, Robison LL. Epidemiology of childhood leukemia, with a focus on infants. *Epidemiol Rev*. 1994;**16(2)**:243–272.

2. Rubnitz JE, Crist WM. Molecular genetics of childhood cancer: Implications for pathogenesis, diagnosis, and treatment. *Pediatrics*. 1997;**100(1)**:101–108.

3. Harrison CJ. Acute lymphoblastic leukaemia. *Best Pract Res Cl Ha*. 2001;**14(3)**: 593–607.

4. Greaves M. Childhood leukaemia. *Br Med J*. 2002;**324(7332)**:283–287.

5. Downing JR, Mullighan CG. Tumor-specific genetic lesions and their influence on therapy in pediatric acute lymphoblastic leukemia. *Hematology Am Soc Hematol Educ Program*. 2006;**508**:118–122.

6. Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. *N Engl J Med*. 2004;**350(15)**:1535–1548.

7. Armstrong SA, Look AT. Molecular genetics of acute lymphoblastic leukemia. *J Clin Oncol*. 2005; **23(26)**:6306–6315.

8. Wong KK, Tsang YTM, Shen J, Cheng RS, Chang YM, Man TK, et al. Allelic imbalance analysis by high-density single nucleotide polymorphic allele (SNP) array with whole genome amplified DNA. *Nucl Acids Res*. 2004;**32(9)**:e69

9. Lemos JAR, Defavery R, Scrideli CA, Tone LG. Analysis of p16 gene mutations and deletions in childhood acute lymphoblastic leukemias. *Sao Paulo Med J* 2003;**121(2)**:58–62.

10. Ohnishi H, Kawamura M, Ida K, Sheng XM, Hanada R, Nobori T, et al. Homozygous deletions of p16/MTS1 gene are frequent but mutations are infrequent in childhood T-cell acute lymphoblastic leukemia. *Blood*. 1995;**86(4)**: 1269–1275.

11. Kawamura M, Ohnishi H, Guo SX, Sheng XM, Minegishi M, Hanada R, et al. Alterations of the p53, p21, p16, p15 and RAS genes in childhood T-cell acute lymphoblastic leukemia. *Leuk Res*. 1999;**23(2)**:115–126.

12. Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. *Blood*. 2002;**100(5)**:1532–1542.

13. Taketani T, Taki T, Sugita K, Furuichi Y, Ishii E, Hanada R, et al. FLT3 mutations in the activation loop of tyrosine kinase domain are frequently found in infant ALL with MLL rearrangements and pediatric ALL with hyperdiploidy. *Blood*. 2004;**103(3)**:1085–1088.

14. Armstrong SA, Mabon ME, Silverman LB, Li A, Gribben JG, Fox EA, et al. FLT3 mutations in childhood acute lymphoblastic leukaemia. *Blood*. 2004;**103(9)**:3544–3546.

15. Yokota S, Nakao M, Horiike S, Seriu T, Iwai T, Kaneko H, et al. Mutational analysis of the N-ras gene in acute lymphoblastic leukemia: A study of 125 Japanese pediatric cases. *Int J Hematol*. 1998;**67(4)**:379–387.

16. Nakao M, Janssen JWG, Seriu T, Bartram CR. Rapid and reliable detection of N-ras mutations in acute lymphoblastic leukemia by melting curve analysis using LightCycler technology. *Leukemia*. 2000;**14(2)**:312–315.

17. Zhu YM, Zhao WL, Fu JF, Shi JY, Pan Q, Hu J, et al. NOTCH1 mutations in T-cell acute lymphoblastic leukemia: prognostic significance and implication in multifactorial leukemogenesis. *Clin Cancer Res*. 2006;**12(10)**:3043–3049.

18. Cuthbert G, Thompson K, McCullough S, Watmore A, Dickinson H, Telford N, et al. MLL amplification in acute leukaemia: A United Kingdom Cancer Cytogenetics Group (UKCCG) study. *Leukemia*. 2000;**14(11)**:1885–1891.

19. Harewood L, Robinson H, Harris R, Al-Obaidi MJ, Jalali GR, Martineau M, et al. Amplification of AML1 on a duplicated chromosome 21 in acute lymphoblastic leukemia: A study of 20 cases. *Leukemia,* 2003;**17(3)**:547–553.

20. Robinson HM, Broadfield ZJ, Cheung KL, Harewood L, Harris RL, Jalali GR, et al. Amplification of AML1 in acute lymphoblastic leukemia is associated with a poor outcome. *Leukemia*. 2003;**17(11)**:2249–2250.

21. Strefford JC, van Delft FW, Robinson HM, Worley H, Yiannikouris O, Selzer R, et al. Complex genomic alterations and gene expression in acute lymphoblastic leukemia with intrachromosomal amplification of chromosome 21. *Proc Natl Acad Sci USA*. 2006;**103(21)**:8167–8172.

22. Robinson HM, Harrison CJ, Moorman AV, Chudoba I, Strefford JC. Intrachromosomal amplification of chromosome 21 (iAMP21) may arise from a breakage-fusion-bridge cycle. *Genes Chromosomes Cancer*. 2007;**46(4)**:318–326.

23. Garcia-Casado Z, Cervera J, Verdeguer A, Tasso M, Valencia A, Pajuelo JC, et al. High-level amplification of the RUNX1 gene in two cases of childhood acute lymphoblastic leukemia. *Cancer Genet Cytogenet*. 2006;**170(2)**:171–174.

24. Penther D, Preudhomme C, Talmant P, Roumier C, Godon A, Mechinaud F, et al. Amplification of AML1 gene is present in childhood acute lymphoblastic leukemia but not in adult, and is not associated with AML1 gene mutation. *Leukemia*. 2002;**16(6)**:1131–1134.

25. Barber KE, Martineau M, Harewood L, Stewart M, Cameron E, Strefford JC, et al. Amplification of the ABL gene in T-cell acute lymphoblastic leukemia. *Leukemia*. 2004;**18(6):**1153–1156.

26. Kim HJ, Woo HY, Koo HH, Tak EY, Kim SH. ABL oncogene amplification with p16(INK4a) gene deletion in precursor T-cell acute lymphoblastic leukemia/lymphoma: report of the first case. *Am J Hematol*. 2004;**76(4)**:360–336.

27. Graux C, Cools J, Melotte C, Quentmeier H, Ferrando A, Levine R, et al. Fusion of NUP214 to ABL1 on amplified episomes in T-cell acute lymphoblastic leukaemia. *Nat Genet*. 2004;**36(10)**:1084–1089.

28. Melo JV, Deininger MW. Biology of chronic myelogenous leukaemia- signaling pathways of initiation and transformation. *Hematol Oncol Clin N Am*. 2004;**18(3)**:545–568.

29. Mei R, Galipeau PC, Prass C, Berno A, Ghandour G, Patil N, et al. Genome-wide detection of allelic imbalance using human SNPs and high-density DNA arrays. *Genome Res*. 2000;**10(8)**:1126–1137.

30. Takeuchi S, Tsukasaki K, Bartram CR, Seriu T, Zimmermann M, Schrappe M, et al. Long-term study of the clinical significance of loss of heterozygosity in childhood acute lymphoblastic leukaemia. *Leukemia*. 2003;**17(1)**:149–154.

31. Thiagalingam S, Foy RL, Cheng KH, Lee HJ, Thiagalingam A, Ponte JF. Loss of heterozygosity as a predictor to map tumor suppressor genes in cancer: molecular basis of its occurrence. *Curr Opin Oncol*. 2002;**14(1)**:65–72.

32. Takeuchi S, Bartram CR, Seriu T, Miller CW, Tobler A, Janssen JWG, et al. Analysis of a family of cyclin-dependent kinase inhibitors: p15/MTS2/INK4B, p16/MTS1/INK4A, and p18 genes in acute lymphoblastic leukemia of childhood. *Blood*. 1995;**86(2)**:755–760.

33. Takeuchi S, Bartram CR, Miller CW, Reiter A, Seriu T, Zimmermann M, et al. Acute lymphoblastic leukemia of childhood: identification of two distinct regions of deletion on the short arm of chromosome 12 in the region of TEL and KIP1. *Blood*. 1996;**87(8)**:3368–3374.

34. Takeuchi S, Koike M, Seriu T, Bartram CR, Slater J, Park S, et al. Homozygous deletions at 9p21 in childhood acute lymphoblastic leukemia detected by microsatellite analysis. *Leukemia*. 1997;**11(10)**:1636–1640.

35. Takeuchi S, Koike M, Seriu T, Bartram CR, Schrappe M, Reiter A, et al. Frequent loss of heterozygosity on the long arm of chromosome 6: identification of two distinct regions of deletion in childhood acute lymphoblastic leukemia. *Cancer Res*. 1998;**58(12)**:2618–2623.

36. Takeuchi S, Cho SK, Seriu T, Koike M, Bartram CR, Reiter A, et al. Identification of three distinct regions of deletion on the long arm of chromosome 11 in childhood acute lymphoblastic leukemia. *Oncogene*. 1999;**18(51)**:7387–7388.

37. Baccichet A, Qualman SK, Sinnett D. Allelic loss in childhood acute lymphoblastic leukaemia. *Leukemia Res*. 1997;**21(9)**:817–823.

38. Cavé H, Gérard B, Martin E, Guidal C, Devaux I, Weissenbach J, et al. Loss of heterozygosity in the chromosomal region 12p12-13 is very common in childhood acute lymphoblastic leukaemia and permits the precise localization of a tumor suppressor gene distinct from p27KIP1. *Blood*. 1995; **86(10):**3869–3875.

39. Stegmaier K, Pendse S, Barker GF, Bray-Ward P, Ward DC, Montgomery KT, et al. Frequent loss of heterozygosity at the TEL gene locus in acute lymphoblastic leukaemia of childhood. *Blood*. 1995;**86(1)**:38–44.

40. Baccichet A, Sinnett D. Frequent deletion of chromosome 12p12.3 in children with acute lymphoblastic leukaemia. *Br J Haematol*. 1997;**99(1)**:107–114.

41. Aissani B, Bonan C, Baccichet A, Sinnett D. Childhood acute lymphoblastic leukemia: is there a tumor suppressor gene in chromosome 12p12.3? *Leuk Lymphoma*. 1999;**34(3**-4):231–239.

42. Montpetit A, Larose J, Boily G, Langlois S, Trudel N, Sinnett D. Mutational and expression analysis of the chromosome 12p candidate tumor suppressor genes in pre-B acute lymphoblastic leukemia. *Leukemia*. 2004;**18(9)**:1499–1504.

43. Takeuchi S, Seriu T, van Dongen JJM, Szczepański T, Tsukasaki K, Takeuchi N, et al. Allelotype analysis in relapsed childhood acute lymphoblastic leukemia. *Oncogene*. 2003;**22(44)**:6970–6976.

44. Heerema NA, Nachman JB, Sather HN, La MK, Hutchinson R, Lange BJ, et al. Deletion of 7p or monosomy 7 in pediatric acute lymphoblastic leukaemia is an adverse prognostic factor: a report from the Children's Cancer Group. *Leukemia*. 2004;**18(5)**:939–947.

45. Bird AP. The relationship of DNA methylation to cancer. *Cancer Surv*. 1996;**28**: 87–101.

46. Bird A. DNA methylation patterns and epigenetic memory. *Gene Dev*. 2002;**16(1):** 6–21.

47. Gutierrez MI, Siraj AK, Bhargava M, Ozbek U, Banavali S, Chaudhary MA, et al. Concurrent methylation of multiple genes in childhood ALL: Correlation with phenotype and molecular subgroup. *Leukemia*. 2003;**17(9)**:1845–1850.

48. Drexler HG. Review of alterations of the cyclin-dependent kinase inhibitor INK4 family genes p15, p16, p18 and p19 in human leukemia-lymphoma cells. *Leukemia*. 1998;**12(6)**:845–859.

49. Jones PA, Takai D. The role of DNA methylation in mammalian epigenetics. *Science*. 2001;**293(5532)**:1068–1070.

50. Strathdee G, Brown R. Aberrant DNA methylation in cancer: potential clinical interventions. *Expert Rev Mol Med*. 2002;**4(4)**:1–17.

51. Robertson KD, Wolffe AP. DNA methylation in health and disease. *Nat Rev Genet*. 2000;**1(1)**:11–19.

52. Paluszczak J, Baer-Dubowska W. Epigenetic diagnostics of cancer—the application of DNA methylation markers. *J Appl Genet*. 2006;**47(4)**:365–375.

53. Knudson AG. Mutation and Cancer: Statistical Study of Retinoblastoma. *Proc Natl Acad Sci USA*. 1971;**68(4)**:820–823.

54. Wheeler JM, Bodmer WF, Mortensen NJ. DNA mismatch repair genes and colorectal cancer. *Gut*. 2000;**47(1)**:148–153.

55. Baylin SB, Hoppener JW, de Bustros A, Steenbergh PH, Lips CJ, Nelkin BD. DNA methylation patterns of the calcitonin gene in human lung cancers and lymphomas. *Cancer Res*. 1986;**46(6)**:2917–2922.

56. Heinrichs S, Look AT. Identification of structural aberrations in cancer by SNP array analysis. *Genome Bio*. 2007;**8(7)**:219.

57. Wang DG, Fan JB, Siao CJ, Berno A, Young P, Sapolsky R, et al. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. *Science*. 1998;**280(5366)**:1077–1082.

58. Chee M, Yang R, Hubbell E, Berno A, Huang XC, Stern D, et al. Accessing genetic information with high-density DNA arrays. *Science*. 1996; **274(5287)**:610–614.

59. Lindblad-Toh K, Tanenbaum DM, Daly MJ, Winchester E, Lui WO, Villapakkam A, et al. Loss-of-heterozygosity analysis of small-cell lung carcinomas using single-nucleotide polymorphism arrays. *Nat Biotechnol*. 2000;**18(9):**1001–1005.

60. Primdahl H, Wikman FP, von der Maase H, Zhou XG, Wolf H, Orntoft TF. Allelic imbalances in human bladder cancer: genome-wide detection with high-density single-nucleotide polymorphism arrays. *J Natl Cancer Inst*. 2002;**94(3)**: 216–223.

61. Schubert EL, Hsu L, Cousens LA, Glogovac J, Self S, Reid BJ, et al. Single nucleotide polymorphism array analysis of flow-sorted epithelial cells from frozen versus fixed tissues for whole genome analysis of allelic loss in breast cancer. *Am J Pathol*. 2002;**160(1)**:73–79.

62. Dumur CI, Dechsukhum C, Ware JL, Cofield SS, Best AM, Wilkinson DS, et al. Genome-wide detection of LOH in prostate cancer using human SNP microarray technology. *Genomics*. 2003; **81(3)**:260–269.

63. Zhao X, Li C, Paez JG, Chin K, Jänne PA, Chen T-H, et al. An integrated view of copy number and allelic alterations in the cancer genome using single nucleotide polymorphism arrays. *Cancer Res*. 2004;**64(9)**:3060–3071.

64. Irving JA, Bloodwort L, Bown NP, Case MC, Hogarth LA, Hall AG. Loss of heterozygosity in childhood acute lymphoblastic leukemia detected by genome-wide microarray single nucleotide polymorphism analysis. *Cancer Res*. 2005; **65(8)**:3053–3058.

65. Raghavan M, Lillington DM, Skoulakis S, Debernardi S, Chaplin T, Foot NJ, et al. Genome-wide single nucleotide polymorphism analysis reveals frequent partial uniparental disomy due to somatic recombination in acute myeloid leukemias. *Cancer Res*. 2005;**65(2)**:375–378.

66. Altug-Teber O, Dufke A, Poths S, Mau-Holzmann UA, Bastepe M, Colleaux L, et al. A rapid microarray based whole genome analysis for detection of uniparental disomy. *Hum Mutat*. 2005;**26(2)**:153–159.

67. Sulong S, Moorman AV, Irving JA, Strefford JC, Konn ZJ, Case MC, et al. A comprehensive analysis of the CDKN2A gene in childhood acute lymphoblastic leukemia reveals genomic deletion, copy number neutral loss of heterozygosity, and association with specific cytogenetic subgroups. *Blood*. **113(1)**:100–107.

68. Kuiper RP, Schoenmakers EF, van Reijmersdal SV, Hehir-Kwa JY, van Kessel AG, van Leeuwen FN, et al. High-resolution genomic profiling of childhood ALL reveals novel recurrent genetic lesions affecting pathways involved in lymphocyte differentiation and cell cycle progression. *Leukemia*. 2007;**21(6)**:1258–1266.

69. Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature*. 2007;**446(7137)**:758–764.

70. Karanjawala ZE, Kaariainen H, Ghosh S, Tannenbaum J, Martin C, Ally D, et al. Complete maternal isodisomy of chromosome 8 in an individual with an early-onset ileal carcinoid tumor. *Am J Med Genet*. 2000;**93(3)**:207–210.

71. Young BD, Debernardi S, Lillington DM, Skoulakis S, Chaplin T, Foot NJ, et al. A role for mitotic recombination in leukemogenesis. *Adv Enzyme Regul*. 2006;**46**:90–97.

72. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med*. 2005;**352(17):**1779–1790.

73. Fitzgibbon J, Smith LL, Raghavan M, Smith ML, Debernardi S, Skoulakis S, et al. Association between acquired uniparental disomy and homozygous gene mutation in acute myeloid leukemias. *Cancer Res*. 2005;**65(20)**:9152–9154.

Original Article

Lycopersicon esculentum (Tomato) Prevents Adverse Effects of Lead on Blood Constituents

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Abstract

Background: **Lead is known for its adverse effects on various organs and systems. In this study, the ability of lead to adversely affect blood parameters was investigated, and *Lycopersicon esculentum*, or commonly known as tomato (a source of antioxidants), was administered orally in the form of tomato paste (TP) to reduce the adverse effects of lead.**

Methods: **The study involved 56 Wistar rats divided equally into 4 groups of 14 rats each: Control, LA_G, TP_G, and $LA+TP_G$. Control and TP_G rats were given distilled water ad libitum, while LA_G and $LA+TP_G$ rats were given 1% lead (II) acetate (LA) per day. TP_G and $LA+TP_G$ rats were additionally treated with 1.5 ml of TP per day. All treatments lasted for 10 weeks, after which the rats were weighed and sacrificed, and haematological and biochemical parameters were measured. The independent samples *t* test was used to analyse the results.**

Results: **Lead caused significant reductions in the following parameters: weight; packed cell volume; red blood cell and white blood cell counts; the percentages of lymphocytes and monocytes; total serum protein, albumin, and globulin levels; and plasma superoxide dismutase and catalase activities. In contrast, lead caused a significant increase in the percentage of neutrophils and the plasma malondialdehyde concentration. TP, however, significantly prevented the adverse effects of LA.**

Conclusion: **The oral administration of TP prevents the adverse effects of lead on blood constituents.**

Keywords: *anaemia, antioxidants, biochemical marker, lead, Lycopersicon esculentum, medical sciences*

Introduction

Lead, a heavy metal, is harmful even in small amounts. Nevertheless, humans are exposed to lead through their environments and diets (1). Lead can be inhaled in dust from lead-based paints (2,3) or from fumes of leaded gasoline. It is found in trace amounts in various foods, notably in fish, which are heavily subjected to industrial pollution. Some old houses may have lead water pipes, which can easily contaminate drinking water (3). The manifestations of lead poisoning in humans are non-specific and may be hard to detect during the initial stages. Symptoms include weight loss, anaemia (4), memory loss (5), nephropathy, and infertility (6). Previous research has established that oxidation accompanies lead toxicity (7). This finding suggests that an ample source of antioxidants could reasonably prevent (or at least suppress) the manifestations of lead toxicity.

Lycopersicon esculentum, commonly known as tomato, is a good source of antioxidants (8). It contains nutrients that prevent illness, e.g., by detoxification (8,9), promoting growth (10) and proper immune system functioning (11), as well as increasing the haematocrit, RBC, and WBC content (4). Cooking helps to liberate the beneficial components of the tomato (lycopene, glutathione, vitamin C, and vitamin A) and also facilitates their absorption in the gastrointestinal tract (12,13). There is a possibility that oral administration of cooked tomatoes could reasonably prevent (or at least suppress) the manifestations of lead toxicity. Therefore, this study focused on the potential of oral administration of cooked tomatoes in preventing or suppressing lead toxicity.

Materials and Methods

Animals

A total of 56 adult male Sprague–Dawley rats (average body weight 183.45 ± 0.903 g) were used for this study. They were inbred at the Animal House of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The rats were transferred to the research section and acclimatised over a period of 2 weeks. The research section was maintained

at a temperature of 24–26 °C, relative humidity of 70%–75%, and a light/dark cycle of 12/12 hours with adequate ventilation throughout the period of research. The rats were fed with commercially available rat pellets. The maintenance, care, and treatments of the rats complied with National Institutes of Health guideline for the humane use of laboratory rats and the guideline of Ladoke Akintola University of Technology.

Preparation of tomato paste

Since no published method is available for concentrating the tomato paste, the following in-house method was adopted. Furthermore, cooking has been shown to liberate the beneficial/active components of tomatoes (lycopene, glutathione, vitamin C, and vitamin A) and facilitate their absorption in the gastrointestinal tract (12,13).

Tomato paste (TP) was prepared by grinding tomatoes and heating them in a water bath at 80°C for 45 to 60 minutes. The obtained TP was removed from the water bath when its relative density was 1.124. It was then allowed to cool to 25 °C, and then stored at 4 °C. The quantity of TP to be administered was thawed to 25 °C 1 hour prior to administration.

Treatments

The rats were randomly divided into 4 groups: 1) control given distilled water, 2) LA_G given 1% lead (II) acetate (LA), 3) TPG given distilled water and 1.5 ml of TP per day, and 4) $LA+TP_G$ given 1% lead (II) acetate (LA) and 1.5 ml of TP per day. All groups consisted of 14 rats each. All treatments lasted 10 weeks.

Sample collection and processing

Each animal was weighed and sacrificed by cervical dislocation 24 hours after the last treatment. Blood samples were collected via cardiac puncture. The blood obtained from each rat was injected into 2 tubes; a plain (no-additive) tube and an EDTA tube. The blood samples in the EDTA tubes were mixed thoroughly (although gently) with the EDTA. All the blood samples were then stored below room temperature (10–14 °C) prior to the measurement of haematological and biochemical parameters.

Measurement of haematological and biochemical parameters and data analysis

Packed cell volume (PCV) was determined using plain capillary tubes filled with anti-coagulated blood and centrifugation at 3000 rpm for 20 minutes. Red blood cell (RBC) and white blood cell (WBC) counts were determined using an improved Neubauer counting chamber following the procedure documented by Cheesbrough and McArthur (14). Field's stain A and stain B were used for the differential WBC counts. Plasma and serum were obtained by centrifugation of whole blood at 3000 rpm for 20 minutes. To serve as a guide, total serum protein (TSP), total serum albumin (TSA), and total serum globulin (TSG) levels were first estimated using the Levibond comparator (i.e., the Biuret method) following the method documented by Cheesbrough and McArthur (14), and accurate (reported) values for TSP, TSA, and TSG were then determined using photo-electric colourimeter. The total plasma protein (TPP) was on the other hand determined using the following formula derived by Kagan (15):

$$P = 340(G-1.0099)$$

where P is the total grams of protein per 100 cm^3 of plasma and G is the specific gravity of plasma at 25 °C.

Plasma superoxide dismutase (SOD) activity was determined using the method described by Fridovich (16). Plasma catalase (CAT) activity was determined using the method described by Sinha (17). Plasma malondialdehyde (MDA) concentrations were determined using the procedure described by Varshney and Kale (18). SOD, CAT, and MDA were chosen because many previous studies have reported their reliability in the measurement of antioxidant activities and level of lipid peroxidation (19–22).

The data obtained are presented as mean ± SEM. The control and the treatment groups were compared using the independent samples *t* test using SPSS version 14 (SPSS Inc, Chicago, IL). The level of significance was set at a $P < 0.05$.

Results

Weight gain

Comparison of the initial and final weights of rats showed that there was significant weight gain ($P < 0.05$) in all the groups at the end of 10 weeks of treatment (Table 1). There was no significant difference in weight gain in the TP_G, $LA+TP_G$, and control groups. However, the increase of weight in the LA_G group was significantly lower than that of the control group ($P < 0.05$).

Haematological parameters

Haematological analyses indicated that LA_G rats (unlike TP_G and $LA+TP_G$ rats) have significantly lower PCV, RBC count, and WBC count ($P < 0.01$ each) (Table 2), as well as lower

Table 1: Weight of rats before and after treatments

	Control	LA_G	TP_G	$LA+TP_G$
Initial weight (g)	183.2 ± 1.02	182.1 ± 0.34	184.0 ± 0.54	184.5 ± 0.44
Weight after treatment (g)	219.3 ± 0.99 §	198.9 ± 0.54 §	221.2 ± 0.21 §	218.5 ± 0.32 §
Weight increase (g)	36.1 ± 0.33	16.8 ± 0.99*	37.2 ± 0.76	34.0 ± 0.94

Control and TP_G rats were given distilled water ad libitum, while LA_G and $LA+TP_G$ rats were given 1% lead (II) acetate (LA) ad libitum daily. TP_G and $LA+TP_G$ rats were also given 1.5 ml of TP per day. All treatments lasted 10 weeks.
Data are expressed in mean ± SEM.
§ $P < 0.05$ indicating significant difference when compared with respective initial weight by paired samples *t* test.
* $P < 0.05$ indicating significant difference when compared with the control group by independent samples *t* test.

Table 2: Packed cell volume (PCV), red blood cell (RBC) count, and white blood cell (WBC) count in control and treated groups

	Control	LA_G	TP_G	$LA+TP_G$
PCV (%)	33.6 ± 0.31	30.2 ± 0.24*	34.1 ± 0.46	32.6 ± 0.25
RBC (thousand cells/mm^3)	6334.0 ± 3.75	5358.0 ± 26.31**	7431.3 ± 4.47	5867.3 ± 46.84
WBC (cells/mm^3)	2522.0 ± 3.02	2140.0 ± 2.14**	2437.0 ± 2.46	2340.0 ± 2.46

Control and TP_G rats were given distilled water ad libitum, while LA_G and $LA+TP_G$ rats were given 1% lead (II) acetate (LA) ad libitum daily. TP_G and $LA+TP_G$ rats were also given 1.5 ml of TP per day. All treatments lasted 10 weeks.
Data are expressed in mean ± SEM.
* $P < 0.05$ and ** $P < 0.01$ indicated significant difference when compared with the control group by independent samples *t* test.

percentages of lymphocytes and monocytes ($P < 0.05$ each), but significantly higher percentages of neutrophils ($P < 0.05$) when compared with the control group (Table 3). The percentages of eosinophils and basophils for each of the groups were, however, not significantly different from those of the control group.

Protein content

TSP, TSA, and TSG levels for LA_G rats were significantly lower ($P < 0.05$) than those of control group, while the levels in TP_G and $LA+TP_G$ rats were not significantly different from the control group (Table 4).

Antioxidant activities and lipid peroxidation

LA_G rats had significant decreases in plasma SOD and CAT activities ($P < 0.01$); whereas TP_G animals had significantly higher SOD ($P < 0.01$) and CAT ($P < 0.05$) activities when compared with the control group (Table 5). LA_G rats had significant increases in plasma MDA concentration ($P < 0.05$), while TP_G had significant decreases in MDA concentration ($P < 0.05$) compared with the control group. On the other hand, $LA+TP_G$ rats had no significant differences in SOD activity, CAT activity, and MDA concentration compared with the control group.

Discussion

The results showed that chronic exposure to lead significantly reduced weight gain, which support the findings of Wadi and Ahmad (23) and can be linked to less-efficient metabolic processes due to lead toxicity (24). Administration of 1.5 ml TP/day, however, eliminates the adverse effect of lead on weight gain. This result would be chiefly due to presence of protective antioxidants in TP, such as lycopene, vitamin C, and vitamin A (7), even though it has low protein content and low caloric value (18 kcal/100 g). Similar reasons could be attributed to the significant decrease in PCV observed in rats exposed to lead (LAG) and the non-significant difference in PCV observed in rats treated with TP and lead ($LA+TP_G$) when compared with the control group. The finding that lead decreased PCV agrees with the findings of Anetor et al. (25) that some indices of erythropoietic activity, such as haemoglobin (Hb) concentration, packed cell volume (PCV), and mean corpuscular haemoglobin concentration (MCHC), were significantly decreased in workers exposed to lead compared to the control group. However, Franson et al. (26) reported that PCV is not altered in birds after supplementation of 50 ppm of lead in their feed, and Arvind and Chopra

Table 3: White blood cell (WBC) differential count in control and treated groups

	Control	LA_G	TP_G	$LA+TP_G$
Neutrophils (%)	58.4 ± 0.29	62.4 ± 0.19*	56.4 ± 0.21	58.4 ± 0.31
Lymphocytes (%)	31.2 ± 0.24	29.6 ± 0.15*	32.0 ± 0.43	31.0 ± 0.27
Monocytes (%)	6.4 ± 0.25	4.6 ± 0.15*	7.1 ± 0.12	6.0 ± 0.22
Eosinophils (%)	3.2 ± 0.13	2.8 ± 0.18	3.3 ± 0.34	3.6 ± 0.23
Basophils (%)	0.8 ± 0.13	0.6 ± 0.15	1.2 ± 0.24	1.0 ± 0.17

Control and TP_G rats were given distilled water ad libitum, while LA_G and $LA+TP_G$ rats were given 1% lead (II) acetate (LA) ad libitum daily. TP_G and $LA+TP_G$ rats were also given 1.5 ml of TP per day. All treatments lasted 10 weeks.
Data are expressed in mean ± SEM.
* $P < 0.05$ indicated significant difference when compared with the control group by independent samples *t* test.

Table 4: Total serum protein (TSP), total serum albumin (TSA), and total serum globulin (TSG) levels in the control and treated groups

	Control	LA_G	TP_G	$LA+TP_G$
TSP (g/L)	74.8 ± 0.36	63.2 ± 0.51*	76.7 ± 0.36	70.8 ± 0.26
TSA (g/L)	45.6 ± 0.49	39.4 ± 0.38*	47.1 ± 0.23	42.4 ± 0.27
TSG (g/L)	29.2 ± 0.41	23.8 ± 0.423*	29.6 ± 0.09	28.4 ± 0.25

Control and TP_G rats were given distilled water ad libitum, while LA_G and $LA+TP_G$ rats were given 1% lead (II) acetate (LA) ad libitum daily. TP_G and $LA+TP_G$ rats were also given 1.5 ml of TP per day. All treatments lasted 10 weeks.
Data are expressed in mean ± SEM.
* $P < 0.05$ indicated significant difference when compared with the control group by independent samples *t* test.

Table 5: Plasma superoxide dismutase (SOD) activity, catalase (CAT) activity, and malondialdehyde (MDA) concentration in control and treated groups

	Control	LA_G	TP_G	$LA+TP_G$
SOD activity (/g of protein)	1.77 ± 0.05	1.12 ± 0.06 **	2.00 ± 0.19 **	1.70 ± 0.09
CAT activity (/g of protein)	0.41 ± 0.08	0.22 ± 0.03 **	0.44 ± 0.04 *	0.40 ± 0.09
MDA concentration (µg/g protein)	1400.3 ± 23.01	1813.6 ± 11.18 *	1152.5 ± 31.43 *	1419.5 ± 34.07

Control and TP_G rats were given distilled water ad libitum, while LA_G and $LA+TP_G$ rats were given 1% lead (II) acetate (LA) ad libitum daily. TP_G and $LA+TP_G$ rats were also given 1.5 ml of TP per day. All treatments lasted 10 weeks.
Data are expressed in mean ± SEM.
* $P < 0.05$ and ** $P < 0.01$ indicated significant difference when compared with the control group by independent samples *t* test.

(27) also reported no changes in PCV in calves after supplementation of 100 ppm of lead in their diets. This may considerably be due to the low concentration of lead (50 ppm or 0.005%, and 100 ppm or 0.010%, respectively) administered, and/or the different species used in the experiments.

There was no significant decrease in RBC counts in animals co-treated with TP and lead. In contrast, the RBC counts in LA_G rats were significantly lower than those of control group. The reduced in RBC counts may be caused by lead interference in the energy metabolism of erythrocytes (28), which affects the ATP concentration in erythrocytes, thus, shortening their life span and lowering the blood counts. Morse et al. (29) reported that acute lead toxicity in mice produced transient erythroid hypoplasia and impaired utilisation of RBC ^{59}Fe for haem synthesis. The administered TP would therefore

be responsible for preventing these effects of lead on RBC probably by preventing the adverse effects of lead on the energy metabolism of erythrocytes and the utilisation of RBC ^{59}Fe for haem synthesis and/or by elevating RBC glutathione level (a function of vitamin C component in tomato), which protects RBC against damaged caused by hydrogen peroxide (a toxic by-product of many metabolic reactions) by reducing the peroxide to water (30).

In a similar manner, WBC counts in rats co-treated with TP and lead were not significantly different from those of control group. Conversely, rats treated with lead had significant decreases in WBC counts. This result suggests that TP combats the adverse effects of lead on WBC, resulting in no significant differences in WBC counts observed in control group and in rats co-treated with TP and lead. This finding was most likely due to the detoxification effect of some antioxidant components of TP. Similar detoxification/protective effects have previously been documented for varieties of antioxidants, such as carotene, retinol, and bioflavonoids (31, 32).

Lead caused significant decreases in lymphocyte and monocyte counts but increased neutrophil counts. These results support the finding by Sembulingam and Sembulingam (33) that lead (like some other chemicals and drugs, e.g., mercury, camphor, benzene derivatives, venoms, and some vaccines) causes neutrophilia. Di Lorenzo et al. (34) also reported that mean absolute neutrophil counts were significantly higher in workers exposed to lead compared to workers who were not exposed to lead. TP prevented lead-induced changes to WBC and differential counts. The percentages of eosinophils and basophils in rats exposed to lead alone and those of rats treated with TP after lead exposure were not significantly different from those of control group.

The significantly lower TSP, TSA, and TSG levels in rats exposed to lead could result from the fact that lead causes disturbances in metabolism, reduces the efficiency of gastrointestinal tract digestion and absorption processes, and negatively affects protein synthesis (4,25). TP, in contrast, was able to annul the adverse effects of lead on TSP, TSA, and TSG levels of rats co-treated with TP and lead. This effect was perhaps due to some protein (sources of amino acids) components of TP (which might be used as raw materials for, or facilitate, aminogenesis/proteogenesis) and could, better still, be traced to its vitamins and lycopene components and TP ability to modulate gastrointestinal tract digestive and absorptive functions (9).

There were significant decreases in plasma SOD and CAT activities in lead-treated rats, while in rats co-treated with TP and lead, no significant differences were observed in comparison with the control group. These findings are in agreement with those of Hsu and Guo (35). Although plasma MDA concentration was significantly increased in lead-treated rats, no significant difference was observed in rats co-treated with TP and lead in comparison with the control group. This confirms that TP, as a source of antioxidants (7, 8), reduced the oxidative stress caused by lead exposure in this animal model.

Conclusion

Exposure to lead significantly reduced weight gain; PCV, RBC count, and WBC count; percentages of lymphocytes and monocytes; TSP, TSA, and TSG levels; and plasma SOD and CAT activities. In contrast, it caused significant increases in the percentages of neutrophils and eosinophils, and plasma MDA concentration. TP, however, significantly reduced these adverse effects of lead. It would be good if subsequent studies could examine if human consumption of considerable (high) amount of TP could have protective effect against lead toxicity, and if prolonged consumption of TP has any potential adverse effects in humans.

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References

1. Gidlow DA. Lead toxicity. *Occup Med (Lond).* 2004;**54(2)**:76–81.

2. Gärtner C, Stahl W, Sies H. Lycopene is more bioavailable from tomato paste than from fresh tomatoes. *Am J Clin Nutr.* 1997;**66(1)**:116–122.

3. Thompson KA, Marshall MR, Sims CA, Wei CI, Sargent SA, Scott JW. Cultivar, maturity, and heat treatment on lycopene content in tomatoes. *J Food Sci.* 2006;**65(5)**:791–795.

4. Waldron HA. The anaemia of lead poisoning: A review. *Br J Ind Med*. 1966;**23(2)**:83–100.

5. Hopkins A. Experimental lead poisoning in the baboon. *Br J Ind Med*. 1970;**27(2)**:130–40.

6. Patocka J, Cerný K. Inorganic lead toxicology. *Acta Medica (Hradec Kralove)*. 2003;**46(2)**:65–72.

7. Gurer-Orhan H, Sabir HU, Ozgüneş H. Correlation between clinical indicators of lead poisoning and oxidative stress parameters in controls and lead-exposed workers. *Toxicology*. 2004;**195(2–3)**:147–154.

8. Wang H, Cao G, Prior RL. Total antioxidant capacity of fruits. *J Agric Food Chem*. 1996;**44(3):**701–705.

9. Nguyen ML, Schwartz SJ. Lycopene: Chemical and biological properties. *Food Technol*. 1999;**53(2)**:38–45.

10. Shi J, Le Maguer M. Lycopene in tomatoes: Chemical and physical properties affected by food processing. *Crit Rev Food Sci Nutr*. 2000;**40(1)**:1–42.

11. Sandhu JS, Krasnyanski SF, Domier LL, Korban SS, Osadjan MD, Buetow DE. Oral immunization of mice with transgenic tomato fruit expressing respiratory syncytial virus-F protein induces a systemic immune response. *Transgenic Res*. 2000;**9(2)**:127–135.

12. Sayre JW, Charney E, Vostal J, Pless IB. House and hand dust as a potential source of childhood lead exposure. *Am J Dis Child*. 1974;**127(2)**:167–170.

13. Lanphear BP, Hornung R, Ho M, Howard CR, Eberle S, Knauf K. Environmental lead exposure during early childhood. *J Pediatr*. 2002;**140(1)**:40–47.

14. Cheesbrough M, McArthur J. Laboratory manual for rural tropical hospitals: A basis for training courses. Edinburgh: Churchill Livingstone, 1976.

15. Kagan BM. A simple method for the estimation of total protein content of plasma and serum. II. The estimation of total protein content of human plasma and serum by the use of the falling drop method. *J Clin Invest*. 1938;**17(4)**:373–376.

16. Fridovich I. Superoxide dismutases. *Adv Enzymol Relat Areas Mol Biol*. 1986;**58**:61–97.

17. Sinha AK. Colorimetric assay of catalase. *Anal Biochem*. 1972;**47(2)**:389–394.

18. Varshney R, Kale RK. Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. *Int J Radiat Biol*. 1990;**58(5)**:733–743.

19. Tezcan E, Atmaca M, Kuloglu M, Ustundag B. Free radicals in patients with post-traumatic stress disorder. *Eur Arch Psychiatry Clin Neurosci*. 2003;**253(2)**:89–91.

20. Sasmaz S, Arican O, Kurutas EB. Oxidative stress in patients with nongenital warts. *Mediators Inflamm*. 2005;**2005(4)**:233–236.

21. Ersoy MA, Selek S, Celik H, Erel O, Kaya MC, Savas HA, et al. Role of oxidative and antioxidative parameters in etiopathogenesis and prognosis of panic disorder. *Int J Neurosci*. 2008;**118(7)**:1025–1037.

22. Ponce-Canchihuamán JC, Pérez-Méndez O, Hernández-Muñoz R, Torres-Durán PV, Juárez-Oropeza MA. Protective effects of Spirulina maxima on hyperlipidemia and oxidative-stress induced by lead acetate in the liver and kidney. *Lipids Health Dis*. 2010;**9**:35.

23. Wadi SA, Ahmad G. Effects of Lead on the Male Reproductive System in Mice. *J Toxicol Environ Health A*. 1999;**56(7)**:513–521.

24. Struzyńska L, Dabrowska-Bouta B, Rafałowska U. Acute lead toxicity and energy metabolism in rat brain synaptosomes. *Acta Neurobiol Exp (Wars)*. 1997;**57(4)**:275–281.

25. Anetor JI, Babalola OO, Adeniyi FAA, Akingbola TS. Observations on the haematopoietic systems in tropical lead poisoning. *Nigerian J Physiol Sci*. 2002;**17(1–2)**:9–15.

26. Franson JC, Sileo L, Pattee OH, Moore JF. Effects of chronic dietary lead in American kestrels *(Falco sparverius)*. *J Wildl Dis*. 1983;**19(2)**:110–113.

27. Arvind K, Chopra RC. Lead concentration in feeds and fodders and its effect on growth, nutrient utilization and certain blood parameters in crossbred calves. *Indian J Anim Nutr*. 2003;**20(4)**:412–420.

28. Baranowska-Bosiacka I, Hlynczak AJ. The effect of lead ions on the energy metabolism of human erythrocytes in vitro. *Comp Biochem Physiol C Toxicol Pharmacol*. 2003;**134(3)**:403–416.

29. Morse BS, Germano GJ, Giuliani DG. Abnormal erythroid maturation following acute lead toxicity in mice. *Blood*. 1972;**39(5)**:713–720.

30. Johnston CS, Meyer CG, Srilakshmi JC. Vitamin C elevates red blood cell glutathione in healthy adults. *Am J Clin Nutr*. 1993;**58(1)**:103–105.

31. Obermeier MT, White RE, Yang CS. Effects of bioflavonoids on hepatic P450 activities. *Xenobiotica*. 1995;**25(6)**:575–584.

32. Neuhouser ML, Patterson RE, Thornquist MD, Omenn GS, King IB, Goodman GE. Fruits and vegetables are associated with lower lung cancer risk only in the placebo arm of the beta-carotene and retinol efficacy trial (CARET). *Cancer Epidemiol Biomarkers Prev*. 2003;**12(4)**:350–358.

33. Sembulingam K, Sembulingam P. Essentials of medical physiology. 4th ed. New Delhi: Jaypee Brothers Medical Publisher; 2006.

34. Di Lorenzo L, Silvestroni A, Martino MG, Gagliardi T, Corfiati M, Soleo L. Evaluation of peripheral blood neutrophil leucocytes in lead-exposed workers. *Int Arch Occup Environ Health*. 2006;**79(6)**:491–498.

35. Hsu PC, Guo YL. Antioxidant nutrients and lead toxicity. *Toxicology*. 2002;**180(1)**:33–44.

Original Article

Correlation of Nuclear Morphometry and AgNOR Score with Radiation Response in Squamous Cell Cancers of the Head and Neck: A Preliminary Study

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Abstract

Background: **Prediction of radiation response before the completion of the radiotherapy schedule is challenging. Information about radiation response could help oncologist to choose the appropriate combination and sequence of therapies in the multidisciplinary management of cancer.**

Methods: **The study involved 26 patients with squamous cell cancers of the head and neck region who received radiotherapy to a dose of 30 Gy in 10 fractions over a 2-week period as part of a split-course technique. Fine-needle aspiration cytology was performed on day 1 and day 5 of the schedule. The silver staining of the nuclear organiser region (AgNOR) and nuclear morphometric study were done on both days.**

Results: **The median age of the patients was 44 years old. The primary tumours were distributed in the nasopharynx (*n* = 11), larynx and hypopharynx (*n* = 5), metastatic node (*n* = 4), and miscellaneous tumours were found in the head and neck sub sites (*n* = 6). The mean initial AgNOR score was 3.0, range 1.2–7.0. The median of nuclear and nucleolar diameters were 11.07 µm, range 7.70–16.6 µm, and 2.92 µm, range 1.09–11.66 µm, respectively. Patients with a pre-radiotherapy AgNOR score of greater than 2.5 were associated with disease progression and metastasis. However, the increased of nuclear diameter on day 5 compared with baseline predicted a good radiation response in patients (*P* = 0.016).**

Conclusion: **Intra-radiotherapy nuclear morphometry combined with baseline AgNOR score could be a simple and useful tool for the prediction of radiation response in head and neck cancers.**

Keywords: *AgNORs, fine-needle aspiration, squamous cell cancer, radio-sensitivity, radiotherapy, radiology, medical sciences*

Introduction

Head and neck cancers account for 5%–10% of all cancers (1), and the malignancies have diverse biological behaviours and predictions for treatment response. Surgery, radiotherapy, and occasionally chemotherapy are the core modalities for management of head and neck cancers. Radiotherapy is used in more than 60% of head and neck cancers with the purpose being radical, adjuvant, or palliative care. Not all patients who receive radiotherapy achieve a desirable radiation response. Radio-sensitivity is not uniform, even in the similar histological subgroups in head and neck cancers (2). The response can vary, even when stage, site, tumour volume, and histology are kept constant. Additionally, histopathological subcategories do not show a consistent prediction of radiation response (3). The in vivo cytological test and the cell surviving fraction for a 2 Gy dose of radiation (SF2) are 2 known methods for predicting radiation response (4). Alternate prediction methods use radiation-induced histomorphological changes, especially changes seen in the nucleus, as a marker of radio-sensitivity. However, these methods of predicting radiation response are not practical in clinical oncology due to complicated nature of the tests.

In 1947, Graham introduced the concept of predicting radio-sensitivity using the radiation response test (5). Previously, serial cytology slides were studied to assess the radio-sensitivity of various cancers; following radiotherapy, multinucleation, and nuclear enlargement of the malignant cells were common observed changes. Past radiobiological studies have shown that induction of cell multinucleation is dose-dependent and correlated with cell survival assay, which suggests that they are non-clonogenic (6). Radiation can induce the fragmentation of

the chromosome or the formation of abnormal chromosomes, which do not take part in mitosis. These chromosomal fragments are called micronuclei, and their induction is dose-related and correlated with survival of cancer patients (7,8).

Under high magnification, it is possible to observe the nuclear and nucleolar morphometry using a computer-assisted image analyser (9). These nucleolar events can also be demonstrated with silver staining of the nuclear organiser region (AgNOR). Nuclear organiser regions (NOR) are loops of ribosomal DNA located in the short arm of chromosomes 13, 14, 15, 21, and 22, and transcribe to ribosomal RNA. NOR vary in size and shape according to the nucleolar transcription. Interestingly, they are related to the cell cycle and may also be related to cell proliferation. Binding of silver and protein occur in carboxyl and sulfhydryl groups by colloidal precipitating ionic silver. The carboxyl group on the protein reduces the silver solution that forms the micronuclei of silver. The large aggregate of silver is deposited on the disulfide and sulfhydryl group sites; they are easily observed using light microscopy. An increase in the AgNOR score suggests an increase in ribosomal activity. Studies of the predictive index based on AgNOR score are effective as early as after the first fraction of radiotherapy; the AgNOR score is correlated with local control of the disease by a full radiotherapy protocol. Knowledge of the probability of radiation response before the completion of radiotherapy would allow re-evaluation of therapeutic options (10). The nuclear roundness factor (NRF), detectable by an image analyzer, is another parameter that has been demonstrated to predict radiation response in Wilms' tumour and prostate cancer (11,12). In this study, we examined the AgNOR score as well as nuclear and nucleolar morphometry before and during radiotherapy as a predictor of radio-sensitivity.

Materials and Methods

Patient selection

A total of 26 patients with documented cases of squamous cell cancers involving the head and neck region were recruited for this study. The general physical examination, clinical tumour volume, biochemical investigation, and radiological evaluation were recorded on a case report form for analysis. The clinical tumour volume was measured as the maximum diameter in centimetres in 3 dimensions. The tumour volume was measured before (day 0), during (on 5th fraction), and after radiotherapy (on 10th fraction), as well as 6 weeks after initiation of radiotherapy.

Radiotherapy schedule

Radiotherapy was delivered with a 6 MV linear accelerator, using a 2- or 3-field technique. The radiotherapy schedule consists of 30 Gy in 10 fractions over a 2-week period: treating 5-day-a-week with 2-day break during the weekends. For cases of parallel-opposed portal, the dose was calculated at the mid-plane, but in the lower neck field, dose was calculated at the maximum depth dose (d-max). Individualised thermoplastic moulds were prepared for daily reproducibility of set-up for upper neck region tumours. The radiation dose was kept uniform in all cases, and we expected a similar outcome with this schedule. Patients with good radiation response were subjected to further radiotherapy doses (30 Gy in 10 fractions over a 2-week period) as part of a split-course technique following the exclusion of sensitive structures. At the end of radiotherapy, the response to radiotherapy was evaluated as complete response (CR), partial response (PR), or progressive disease (PD). The AgNOR scoring and nuclear morphometry was performed regardless of an additional Phase II radiotherapy course, and the last cytology sample was collected on the 6th week, counted from day 1 of the radiotherapy course.

Cytological evaluation

Fine-needle aspiration cytology (FNAC) was performed to obtain tissue materials. The tissue fluids were obtained from the measurable nodes, which were in the radiotherapy portal. The FNAC was performed before starting radiotherapy (day 1), at day 5 (after 5th fraction) of the treatment, at end of radiotherapy (after 10th fraction) and 6 weeks post-radiotherapy, counted from day 1 of radiotherapy. The AgNOR staining was performed according to the modified Crocker method (13). The cytology samples were smeared on conventional glass slides with frosted ends and then immersed in 95% alcohol as the fixative. The slides were rinsed with xylene 3 times for 5–10 minutes and then treated with 100% ethanol for 2–5 minutes. Next, the slides were washed with tap water for 5 minutes and rinsed with deionised water 2–3 times for 3 minutes. The slides were incubated in the dark for 60 minutes with 1 volume of solution-A and 2 volumes of solution-B, and then rinsed with deionised water. Solution-A (colloid developing solution) consisted of 100 ml

pure water, 2 g gelatine, and 1 ml formic acid. The gelatin was dissolved by stirring at 40–60 °C for 10–20 minutes. The solution was left at a room temperature of 40 °C. Solution-B contained 100 ml pure water and 50 g silver nitrate and was protected from light by wrapping aluminium foil around the container. The slides were washed with 5% sodium thiosulfate solution for 5 minutes followed by rinsing with 100% ethanol and, subsequently, xylene. The stained slides were then mounted with a cover slip.

The AgNOR stained as black dots within the nucleus. The number of AgNOR dots were counted in 100 cells, and the mean AgNOR count (AgNOR score) was calculated for each case. Each cytology slides were subjected to nuclear morphometric analysis using a computer assisted image analyser (Leica Qwin, Germany) at 400x magnification. The nucleus and nucleolus diameters were determined by this method.

Statistical analysis

The data obtained from the study were evaluated using SPSS version 11 (SPSS Inc, Chicago, IL). The demographic data were tabulated, and the median value of the AgNOR score, nuclear diameter, and nucleolar diameter were measured. The outcomes, in terms of radiation response, were analysed against the baseline AgNOR score and nuclear morphometry data. The difference in nuclear and nucleolar diameters on day 1 and day 5 were compared to the radiation response using the Mann–Whitney test.

Results

As per the protocol, the 26 patients who completed the radiotherapy schedule consisted of 6 females and 20 males. The median age of the patient population was 44 years. Although we attempted aspiration cytology in all 26 patients, cellular materials were evaluable for only 9 patients for nuclear morphometry and 12 patients for AgNOR score. The failure to determine the nuclear morphometric and AgNOR score in other cases were due to sampling error, failure to obtain cellular material, and quick tumour regression after brief radiotherapy. The primary tumours were distributed in the nasopharynx (n = 11), larynx and hypopharynx (n = 5), metastatic neck nodes (n = 4) and miscellaneous tumours (n = 6) of the head and neck sub-sites.

Radiotherapy

Out of 26 patients evaluated, 14 patients (54%) achieved CR, 6 patients achieved PR (23%), and the remaining 6 had PD (23%) following radiotherapy.

Cytology evaluation

Sampling was done in 54 attempts; however, only 33 aspirate yield cellular materials. After a few fractions of radiotherapy, patients showed good response to radiation, which made it difficult to obtain good tissue samples on the 2nd and 3rd FNAC procedure. Out of 26 patients, 20 patients (76%) underwent initial cytology (cytology-1), which yielded good cellular material; 10 patients (38%) yielded good cellular aspirate after day 5 of radiotherapy (cytology-2); and only 3 patients (12%) had successful 3rd round cytology. The cytology from 6th week aspirates was not successful in some cases due to sampling error and regression of the tumour following radiotherapy.

AgNOR score

Manual AgNOR score was done for 33 slides by counting the number of AgNOR dots per the given number of nuclei counted (Figure 1). Baseline AgNOR scores were available in 12 patients. The baseline mean AgNOR score was 3.0, range 1.2–7.0, and the mean AgNOR score on day 5 of radiotherapy was 2.4, range 1.2–7.1. Patients with a high baseline AgNOR score showed higher treatment failures than those with low baseline AgNOR scores. All patients, 6 out of 6, with AgNOR scores of less than 2.5 achieved a good response to radiotherapy compared with 6 out of 6 patients with AgNOR scores of more than 2.5, who achieved a poor response to radiotherapy (Table 1).

Nuclear and nucleolar morphometry

The largest nucleus and nucleolus diameters were measured. The average nucleolus diameter was 2.92 µm, range 1.09–11.66 µm, and nucleus diameter was 11.073 µm, range 7.70–16.6 µm. When the nuclear diameter increased from the baseline diameter on day 5 of radiotherapy, there was improved radiation response, local control, and longer survival (Table 2). Following radiotherapy the morphology of the nucleoli in nucleus on day 5 becomes more clumped and bizarre compared to baseline features on day 1 as shown in Figure 2. There was no correlation between nuclear/nucleolar diameters with 3-dimensional clinical tumour volume of the target lesion. When comparing the changes in nuclear diameter on day 5 to the baseline diameter,

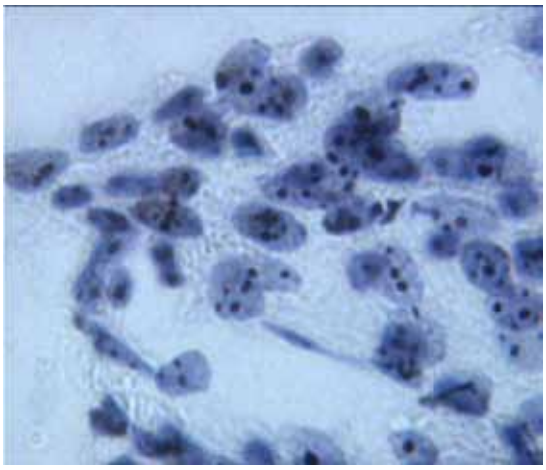

Figure 1: Photomicrograph showing silver staining of the nuclear organiser region (AgNOR) dots in the nucleus for scoring. (400x magnification)

Table 1: Silver staining of the nuclear organiser region (AgNOR) score

Response	AgNOR score
Good response	
Case-1	2.46 / nuscleus
Case-2	2.17 / nucleus
Case-3	2.36 / nucleus
Case-4	1.36 / nucleus
Case-5	1.40 / nucleus
Case-6	1.70 / nucleus
Poor response	
Case-1	4.46 / nucleus
Case-2	2.60 / nucleus
Case-3	7.00 / nucleus
Case-4	3.00 / nucleus
Case-5	2.60 / nucleus
Case-6	2.70 / nucleus

All patients (6 out of 6) with AgNOR score of less than 2.5 dots/nucleus have good response to radiotherapy, while all patients (6 out of 6) with AgNOR score of more than 2.5 dots/ nucleus had poor response.

there was statistically significant improvement in radiation response that correlated with a positive increase in diameter (P = 0.016). A similar comparison was done with nucleolar changes; however, the result did not yield any correlation (P = 0.111).

Follow-up

The patients were advised to attend a regular follow-up at 2-month intervals for the first 2 years and then at 3-month intervals thereafter. The follow-up consisted of a clinical examination; however, radiological evaluation was performed at every 6-month interval. The median follow-up interval was 7 months, range 4–20 months.

Table 2: Nuclear and nucleolar diameters and the corresponding outcome of radiotherapy

Serial No.	Nuclear diameter (µm)			Nucleolar diameter (µm)			Outcome (last visit)
	Before RT	During RT	Difference	Before RT	During RT	Difference	
1	8.77 (2.30)	16.62 (3.70)	+7.85	5.59 (3.07)	11.66 (1.06)	+6.07	14 M NED
2	14.91 (4.80)	16.2 (1.90)	+1.29	4.13 (1.09)	4.69 (1.06)	+0.56	15 M NED
3	16.21 (1.02)	7.70 (1.00)	-8.50	3.78 (0.80)	1.77 (0.34)	-2.01	6 M PD
4	8.32 (1.60)	11.56 (2.50)	-1.40	2.91 (0.60)	2.65 (0.80)	-0.26	17 M PD
5	10.2 (2.90)	16.11 (3.10)	+3.24	1.09 (0.20)	3.45 (1.36)	+2.36	12 M NED
6	9.98 (2.98)	9.75 (2.37)	+7.20	2.64 (1.09)	2.40 (0.70)	-0.24	12 M NED
7	10.51 (3.19)	8.88 (2.19)	-0.23	3.38 (1.23)	2.89 (1.09)	-0.49	6 M PD
8	9.53 (1.30)	8.48 (2.79)	-1.63	1.43 (1.05)	1.41 (0.32)	-0.02	7 M PD
9	10.91 (2.93)	9.51 (0.77)	-1.04	2.62 (0.69)	4.16 (0.69)	+1.54	7 M PD

Nuclear and nucleolar diameters are expressed in mean (SD)
RT: radiotherapy, NED: no evidence of disease, PD: progressive disease, M: months of follow-up

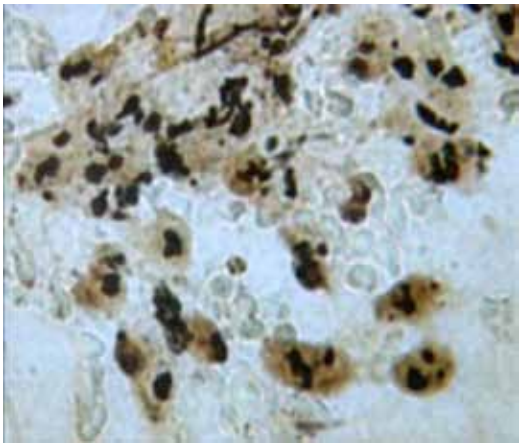

Figure 2a: Photomicrograph showing numerous nucleoli-laden nuclei before radiotherapy. (400x magnification)

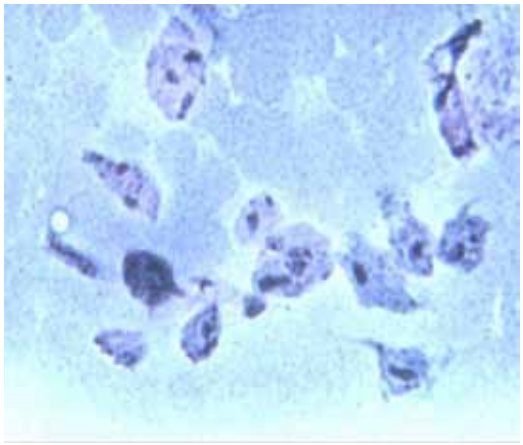

Figure 2b: Photomicrograph showing clumped and bizarre nucleoli-containing nuclei on day 5 of radiotherapy (400x) /Magnification)

Discussion

This is a prospective study to evaluate the value of AgNOR score as well as nuclear morphometry before and during a course of fractionated radiotherapy to predict radiation response. This study, even though it had a small sample size, showed that an increased trend in the nuclear diameter on day 5 compared to baseline was associated with a favourable response to radiotherapy. Furthermore, an increase in baseline AgNOR score of more than 2.5 was associated with radiation failures at a median follow-up duration of 7 months, range 4–20 months.

AgNOR count per nucleus is being used in many cancers to predict response to radiation therapy and/or outcome of treatment. In a 10-patient study, Kossard et al. investigated AgNOR dots per nucleus in small cell melanoma. They found a variation of AgNOR count: 5.83 in small cell melanoma, 8.49 in superficial spreading melanoma, and 2.71 among dermal nevi (14). This suggests that a higher AgNOR score predicts an aggressive tumour. A similar pattern was observed in our study; those cancers with a high AgNOR score per nucleus showed high failure rates. A similar study, performed by Yue et al., also showed hyperactivity of malignant cells in head and neck cancers with a high AgNOR score (15). In contrast, a study from Japan demonstrated that a higher AgNOR score correlated with a good radiation response to pre-operative radiotherapy for oral cavity cancers (16).

The nucleus and nucleolus are the main targets for radiation injury, whether the tumour is malignant or benign. Under a light microscope, the nucleolus appears as a dot-like structure positioned at the centre of the nucleus or slightly displaced towards the inner side of the nuclear membrane. A nucleolus is present in either a reticular array or as compact structures, and it has a fibrillar centre, a vacuolar portion, and a nucleolus-associated chromatin. Thus, a nucleolus consists of dense fibrils and granules, which appear as a dark staining area of varying intensity (17). A nucleolus is responsible for ribosome production and transcription of rRNA and is very sensitive to a change in ribosomal DNA synthesis. Cytochemical studies have shown a marked increase in the amount of AgNOR scores with large nucleoli that implies a higher level of ribosomal production. In our study, the nucleolus mean diameter was 2.92 μm, range 1.09–11.66 μm. However, there was no statistical correlation with treatment outcome.

Radiation therapy treatment is based on tumour factors such as location, size, and histological grade. Patients with stage III and IV head and neck cancers are treated with a fixed dose of radiation, but an increasing body of evidence shows that the response to radiation is not constant, even if the tumour-related variables are held constant. This wide variation of the radio-responsiveness to fractionated radiotherapy is probably indicated by an inherent cytological factor influencing the behaviour of the cancer after radiation exposure. Fibroblasts from patients suffering from ataxia telangiectasia are 2–3 times more sensitive than the normal cells (2). Thus, the radiation response is a product of a wide range of cellular parameters (e.g., nuclear, nucleolar, chromosomal, and genetic factors, and apoptosis).

Cancers are commonly classified according to histology and graded according to the degree of their differentiation: well-differentiated, moderately differentiated, and poorly differentiated. The poorly differentiated cancers seem to be more sensitive to radiation than well-differentiated malignancies. These histology-based variations are demonstrated in cervical cancers and some head and neck cancers. Sometimes, histopathology does not correlate with clinical curability (3). In our study, we did not find any correlation between the histopathology grade and response to radiotherapy.

Colony assay of the tumour cells has been proposed for predicting radiation response based on the fraction of cells surviving a particular radiation dose, which is defined as the ability to undergo at least 6 doublings. Intrinsic radio-sensitivity measurements with SF2 analysis have been demonstrated by Fertil and Malaise, who analysed the published studies of in vitro radio-sensitivity of tumour cell lines from different histological types and found a general correlation with clinical curability (18).

West et al. studied the SF2 assay of radiotherapy-treated squamous cells of cervical cancers. In vitro tumour SF2 values from fresh biopsy material using colony formation in agar were correlated with the treatment outcome. Patients with an SF2 value higher than the median value, 0.40, had a significantly lower survival rate than those with an SF2 value below the median (4).

An ideal radiation sensitivity test should be specific, sensitive, cost effective, and able to be practiced routinely. The chromosomal damage assay and radio-sensitive gene assay are two

new tools for the prediction of radio-sensitivity (19). The first study of a radio-sensitivity test was demonstrated using the serial cytology tests from cervical cancer, called Grahms grading (5). Subsequently, the studies have been duplicated by Gupta et al. (20). Following a course of radiation, there is alteration in the cellular and nuclear morphology. There may be an increase in nucleus size, whereby the nuclear material becomes more condensed with the appearance of prominent nuclei. Bhattathiri et al. studied serial cytological features for the analysis of micronuclei formation during fractionated radiation on squamous cell cancers of the oral cavity. They found a positive correlation between micronuclei formation and treatment outcome (21).

Nuclear morphometric analysis is a quantitation method that has been successfully employed in predicting treatment outcomes for a number of malignancies. Nuclear and nucleolar size estimation is a new concept for the assessment of tumour radio-sensitivity. McLean et al. (9) were some of the early researchers in this area; they found a correlation between large nucleoli and patient treatment outcome. In the study on induction of micronucleation, nuclear budding, and multinucleation produced by fractionated radiotherapy, Bhattathiri et al. showed that multinucleation had the greatest relation with radiation sensitivity. This study suggested that the injury to the cytokinetic apparatus was important in determining tumour radio-sensitivity (21). Another study by Memon et al. also demonstrated nuclear changes as a predictor of radio-responsiveness in oral cancer patients following radiotherapy (22).

In our study, we measured the diameters of the tumour cell nuclei before and during a fractionated course of radiotherapy. Those patients who showed an increase in the nucleus diameter following radiotherapy achieved good local control of disease compared with those who showed a decreased size. Following an initial course of radiotherapy, the nucleus of the cell increases and gradually becomes fragmented, which causes reproductive cell death.

Another dimension of radio-responsiveness is the nuclear roundness factor (NRF). In a study on prostate cancers, Hurwitz et al. noticed a positive correlation of NRF with radio-sensitivity (12). The authors used an automated imaging device to determine NRF. Sampling from aspiration cytology is an optimal method to evaluate nuclear morphometric features (23), but studies using conventional haematoxylin–eosin histology slides to determine nuclear morphometry have also been successful (24). In our experience, the failure to obtain cellular samples during radiotherapy is high, and it is more marked during subsequent aspiration cytology when the tumour is regressing.

Conclusion

The inherent radio-sensitivity to the tumour cells, the proportion of the hypoxic cell component and repopulation by the resistant clones of cells govern cancer response to radiation. The first component of radio-sensitivity can be predicted using nuclear morphometry before and during a course of radiotherapy. In borderline clinical situations, where the decision to use either radiotherapy or surgery is uncertain, nuclear morphometry test might help to decide the treatment arm before completion of radiotherapy. However, a study on a large number of patients needs to be done before it can be recommended for clinical practice.

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Authors' Contributions

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References

1. Parkin DM, Pisani P, Ferlay J. Global cancer statistics. *CA Cancer J Clin*. 1999;**49(1)**:33–64.
2. Begg AC. Prediction of radiation response. In: Liebel SA, Phillip TL, editors. Textbook of Radiation Oncology. 10th ed. Philadelphia: WB Saunders Co; 1998. p 55–68.
3. Meyer JE, Wang CC. Carcinoma of the nasopharynx. Factors influencing the results of therapy. *Radiology*. 1971;**100(2)**:385–388.
4. West CM, Davidson SE, Roberts SA, Hunter RD. Intrinsic radio-sensitivity and prediction of patient response to radiotherapy for carcinoma of the cervix. *Br J Cancer*. 1993;**68(4)**:819–823.
5. Graham RM. Effect of radiation on vaginal carcinoma; Description of cellular changes. *Surg Gynecol Obstret*. 1947;**84(2)**:153–165.
6. Bettega D, Bombana M, Pelucchi T, Poli A, Limbardi LT, Contri AM. Multinucleate cells and micronucleus formation in cultured human cells exposed to 12 MeV protons and gamma-rays. *Int J Radiat Biol Relat Stud Phys Chem Med*. 1980;**37(1)**:1–9.
7. Grote SJ, Joshi GP, Revell SH, Shaw CA. Observations of radiation-induced chromosome fragment loss in live mammalian cells in culture, and its effect on colony forming ability. *Int J Radiat Biol Relat Stud Phys Chem Med*. 1981;**39(4)**:395–408.
8. Midander J, Revesz L. The micronucleus (MN) in the irradiated cells as a measure of survival. *Br J Cancer*. 1980;**41(Suppl 4)**:S204.
9. Martano M, Damiano S, Restucci B, Paciello O, Rusco V, Maiolino P. Nuclear morphometry in canine acanthomatous ameloblastoma and squamous cell carcinoma. *Eur J Histochem*. 2006;**50(2)**:125–130.
10. Heber E, Schwint AE, Sartor B, Nishihama S, Sánchez O, Brosto M, et al. AgNOR as an early marker of sensitivity to radiotherapy in gynecologic cancer. *Acta Cytol*. 2002;**46(2)**:311–316.
11. Gearhart JP, Willmann JR, Beckwith JB, Epstein J, Partin AW. Nuclear morphometric analysis of metastasis in Wilms' tumor after multimodal therapy: Comparison with primary tumor. *Urology*. 1995;**45(1)**:119–123.
12. Hurwitz MD, DeWeese TL, Zinreich ES, Epstein JI, Partin AW. Nuclear morphometry predicts disease-free interval for clinically localized adenocarcinoma of the prostate treated with definite radiation therapy. *Int J Cancer*. 1999;**84(6)**:594–597.
13. Crocker J. Nuclear organizer region. In: Underwood JCE, editor. Current topics in pathology of the nucleus. Berlin (NY): Springer-Verlag; 1990. p 91–149.
14. Kossard S, Wilkinson B. Nucleolar organizer region and image analysis nuclear morphometry of small cell (nevoid) melanoma. *J Cutan Pathol*. 1995;**22(2)**:132–136.
15. Yue L, Iwai M, Futura I. Evaluation of argyrophilic nuclear organizer region in tongue squamous cell carcinomas. *Oral Oncol*. 1999;**35(1)**:70–76.
16. Kinoshita Y, Dohi M, Mizutani N, Ikeda A. Effects of preoperative radiotherapy and chemotherapy on AgNOR counts in oral squamous cell carcinoma. *J Oral Maxillofac Surg*. 1996;**54(3)**:304–308.
17. Nixdorf-Bergweiler BE. Nucleolar size and shape is sexually dimorphic in neurons of the lateral magnocellular nucleus of the anterior neostriatum (LAMN) in birds. *Cytogenet Cell Genet*. 1997;**76(1–2)**:77-86.
18. Fertil B, Malaise EP. Intrinsic radio-sensitivity of human tumor cell lines is correlated with radioresponsiveness in human tumors: Analysis of 101 published survival curves. *Int J Radiat Oncol Biol Phys*. 1985;**11(9)**:1699–1707.
19. Brown JM, Evans J, Kovacs MS. The prediction of human tumor radio-sensitivity in situ: An approach using chromosome aberrations detected by fluorescent in situ hybridization. I*nt J Radiat Oncol Biol Phys*. 1992;**24(2)**:279–286.
20. Gupta S, Mukherjee K, Gupta YN, Kumar M. Sequential radiation changes in cytology of vaginal smears in carcinoma of the cervix uteri during radiotherapy. *Int J Gynecol Obstret*. 1987;**25(4)**:303–308.
21. Bhattathiri NV, Bharathykkutty C, Prathapan R, Chirayathmanjiyil DA, Nair KM. Prediction of radio-sensitivity of oral cancers by serial cytological assay of nuclear changes. *Radiother Oncol*. 1998;**49(1)**:61–65.
22. Memon MH, Jafarey NA. Cytologic study of radiation changes in carcinoma of the oral cavity: Prognostic value of various observations. *Acta Cytol*. 1970;**14(1)**:22–24.
23. Liu YJ, Lee YT, Hsieh SW, Kuo SH. Presumption of primary site of neck lymph node metastases on fine-needle aspiration cytology. *Acta Cytol*. 1997;**41(5)**:1477–1482.
24. Hamilton PW, Allen DC. Morphometry in histopathology. *J Pathol*. 1995;**175(4)**:369–379.

Original Article

The Effect of Delayed Transportation of Blood Samples on Serum Bilirubin Values in Neonates

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Abstract

Background: **Delays in transporting blood samples may cause inaccurate results. Samples may be exposed to light or heat during delays, resulting in the degradation of analytes, for example, bilirubin. This study was done to determine the effect of delays in the transportation of blood samples on serum bilirubin test results.**

Methods: **Samples taken from neonates admitted to a tertiary hospital with jaundice were included in the study. The samples were collected through venipuncture in 3 labelled containers. The first container was sent immediately to the laboratory, while the second and third containers were sent after being kept in the ward for 1 and 3 hours, respectively. Bilirubin values were measured colourimetrically at a wavelength of 578 nm using a Roche Hitachi 912 Chemistry Analyser upon arrival in the laboratory.**

Results: **A total of 36 serum samples were studied. The mean of the indirect bilirubin measurements for 0-, 1-, and 3-hour samples were 174 (SD 68.65), 186.97 (SD 60.47), and 184.56 (SD 66.93), respectively. There was a significant difference in the mean indirect bilirubin measurement of 1-hour samples (*P* = 0.047, 95% CI -24.66 to -1.18) and 3-hour samples (*P* = 0.045, 95% CI -19.77 to -0.23) compared with 0-hour samples. There were no significant differences observed in either the mean total bilirubin or the mean direct bilirubin measurements of different time intervals.**

Conclusion: **This study confirms that delays in the transportation of blood samples influence the bilirubin test results.**

Keywords: *bilirubin, neonatal jaundice, transportation, time factors, specimen handling, medical sciences*

Introduction

Delay in the transportation of blood samples to the laboratory may cause alteration in test results (1,2). Of particular concern is the testing of serum bilirubin level. Unconjugated bilirubin is known to be sensitive to light, and although it is hypothesized that serum bilirubin levels decrease with delays in sample transportation to the laboratory (3), the effects of such delays on actual serum bilirubin level have not been studied.

Accurate testing of serum bilirubin level is important, especially in the treatment of neonatal jaundice since bilirubin level often dictates decision to initiate or discontinue treatment (3). Inaccurate testing of bilirubin level may cause under-treatment, putting the neonates at a risk of developing bilirubin encephalopathy; or over-treatment, leading to unnecessary separation of the baby and the mother (4).

If delays in the transportation of the blood sample systematically cause a decrease in serum bilirubin level, it may be worthwhile to undertake a large trial to generate a formula that would allow for the correction of bilirubin level if there was an inadvertent delay in transportation of sample to the laboratory. This study was performed with the aim of determining the effects of a delay in the transportation of blood samples on serum bilirubin values in neonates.

Materials and Methods

The study was conducted at the Neonatal Intensive Care Unit (NICU) and Special Care Nursery (SCN) at Hospital Universiti Sains

Malaysia (HUSM) from May 2007 until July 2007. Neonates requiring serum bilirubin values were included in this study. Written informed consents were obtained from the parents or guardians.

Blood sample of each neonate was collected through venipuncture into 3 separate containers, labelled A, B, and C. Container A was sent to the laboratory immediately after blood collection. The containers labelled B and C were sent after 1- and 3-hour delays, respectively. A dedicated person ensured timely delivery of the samples to the laboratory, and care was taken in the laboratory to perform the test upon arrival of the samples. Containers B and C were kept on a table in the ward while waiting to be sent to the laboratory. Each neonate was subjected to only 1 blood taking in this study.

Total bilirubin was determined by the colourimetric method using Roche Hitachi 912 Chemistry Analyser (Japan) bought by HUSM in 1999. Bilirubin level was determined based on the method described by Jendrassik and Groft (5), which involved the reaction of bilirubin with diazotised sulphanilic acid (for direct bilirubin measurement) in the presence of caffeine benzoate(for total bilirubin measurement). Caffeine benzoate was used to split the bilirubin–protein complex, thereby enabling bilirubin reaction with diazotised sulphanilic acid. The reaction was measured at a wavelength of 578 nm using the Roche Hitachi 912 Chemistry Analyzer. Indirect bilirubin level was calculated from the difference between total and direct bilirubin measurements (6).

The required sample size was calculated using the sample size for the paired t test equation in the Power and Sample Size Calculation PS Software (Version 2, 2003). Sample size calculation was done by using the mean and standard deviation of total bilirubin from a study by Wilson et al. (7). A sample size of 36 neonates was needed to achieve a power of 80% with a significance level of 0.05.

Data entry and analysis were performed using SPSS version 11.0 (SPSS Inc, Chicago, IL). The mean and standard deviation of serum bilirubin values for each delay interval were calculated. Differences in total bilirubin, direct bilirubin, and indirect bilirubin levels between the 0- and 1-hour, as well as 0- and 3-hour delay intervals were determined by a paired t test. The study was approved by the Ethical Committee and Research Universiti Sains Malaysia, Kubang Kerian, where the research was done.

Results

A total of 36 serum samples were studied. All samples reached the laboratory within 5 minutes of the planned timing and contained sufficient blood for testing. The mean and standard deviation for total bilirubin, direct bilirubin, and indirect bilirubin levels at different hours are presented in Tables 1, 2, and 3, respectively.

There was a significant difference in the indirect bilirubin measurements of 1-hour samples (P = 0.047) and 3-hour samples (P = 0.045) in comparison with 0-hour samples (Table 3). There were no statistically significant differences observed in the total bilirubin (Table 1) and direct bilirubin (Table 2) measurements taken at different delay intervals. The tests of assumption for the paired t test were not violated.

It was observed that 17% of the results after the 1-hour delay and 14% of the results after the 3-hour delay in transportation were very similar to the results of the samples sent immediately after sampling (Figures 1 and 2). On the other hand, 38% of the results after the 1-hour delay and 32% of the results after the 3-hour delay showed decrease in values compared with non-delayed samples, and 45% of the results after the 1-hour delay and 54% of the results after the 3-hour delay showed increase in values. The highest increases in the results after the 1-hour and 3-hour delays were 142 mmol/L and 164 mmol/L, respectively, while the greatest decreases were -28 mmol/L and -35 mmol/L, respectively.

Discussion

This is the first study undertaken to determine whether a delay in the transportation of neonatal blood samples for the measurement of bilirubin level would systematically affect the results. It was found that for some patients, delays in the transportation of bilirubin samples to the laboratory had no major effect on the treatment plan, especially on deciding the need for phototherapy. However, for a substantial minority of patients, the results were markedly different after a delay in the transportation of the samples, resulting in significant differences in the mean indirect bilirubin values between the immediate samples and the delayed samples.

It is impossible to predict which patient will still have reliable bilirubin measurement after a delay in sample transportation. Increase in bilirubin level after a delay could be due to

Table 1: Total bilirubin levels in different delay intervals (*n = 36*)

	Mean (SD)	Mean difference (95% CI)#	*t* value (P value)#
0-hour	179.03 (69.73)	-	-
1-hour	189.72 (60.03)	-10.69 (-23.22 to 1.83)	-1.733 (0.092)
3-hour	186.75 (67.98)	-7.72 (-18.32 to 2.87)	-1.480 (0.148)

Comparison with 0-hour

Table 2: Direct bilirubin levels in different delay intervals (*n = 36*)

	Mean (SD)	Mean difference (95% CI)	*t* value (P value)#
0-hour	4.75 (4.82)	-	-
1-hour	3.61 (4.66)	1.14 (-2.64 to -0.37)	1.536 (0.133)
3-hour	3.86 (4.21)	0.89 (-2.77 to -0.99)	0.966 (0.343)

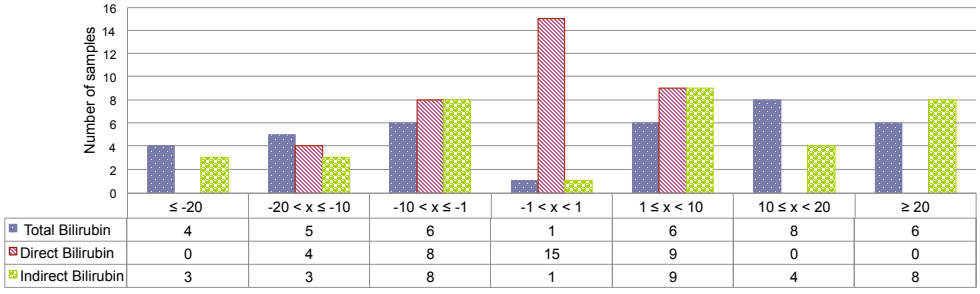
Comparison with 0-hour

Table 3: Indirect bilirubin levels in different delay intervals (*n = 36*)

	Mean (SD)	Mean difference (95% CI)#	*t* value (P value)#
0-hour	174.56 (68.65)	-	-
1-hour	186.97 (60.47)	-12.42 (-24.66 to -1.18)	-2.059 (0.047)*
3-hour	184.56 (66.93)	-10.00 (-19.77 to -0.23)	-2.078 (0.045)*

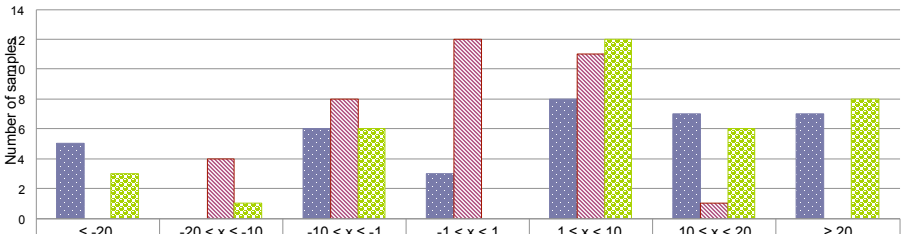
Comparison with 0-hour

* Significant difference observed ($P < 0.05$)



	≤ -20	-20 < x ≤ -10	-10 < x ≤ -1	-1 < x < 1	1 ≤ x < 10	10 ≤ x < 20	≥ 20
Total Bilirubin	4	5	6	1	6	8	6
Direct Bilirubin	0	4	8	15	9	0	0
Indirect Bilirubin	3	3	8	1	9	4	8

Figure 1: Differences in total, direct, and indirect bilirubin levels (mmol/L) between 0- and 1-hour (n=36)



	≤ -20	-20 < x ≤ -10	-10 < x ≤ -1	-1 < x < 1	1 ≤ x < 10	10 ≤ x < 20	≥ 20
Total Bilirubin	5	0	6	3	8	7	7
Direct Bilirubin	0	4	8	12	11	1	0
Indirect Bilirubin	3	1	6	0	12	6	8

Figure 2: Differences in total, direct, and indirect bilirubin levels (mmol/L) between 0- and 3-hour (n=36)

haemolysis before the sample was processed (1). Decrease in serum bilirubin level could be explained by the breakdown of bilirubin due to light exposure (3). Developing a formula to adjust for delays in transportation does not seem to be feasible considering the erratic differences in bilirubin level. Inevitable delays in analysing the sample can also occur in the laboratory despite timely arrival of the samples. As such, strict measures were taken in this study to analyse the samples immediately upon arrival in the laboratory.

Conclusion

There was no consistent trend towards the increase or decrease in serum bilirubin level in delayed transportation of the sample to the laboratory. However, in a substantial number of cases, the decision to start or discontinue phototherapy could be altered due to erroneous test results that may be caused by such delays.

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Authors' contributions

Conception and design: PS, KV, NR, JO, HVR
Provision of study materials or patients: PS, MRM
Analysis and interpretation of data: PS, KV, JO, HVR
Statistical expertise: KV
Drafting of the article: PS, NR
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References

1. Cvitkovic L, Mesic R. Various preanalytical variables and their effects on the quality of laboratory results. *Diabetologia Croatica* [Internet]. 1999 [cited 2010 Jan 3];**28(2)**. Available from http://www.idb.hr/diabetologia/99no2-2.html.

2. Murphy JM, Browne RW, Hill L, Bolelli GF, Abagnato C, Berrino F, et al. Effects of transportation and delay in processing on the stability of nutritional and metabolic biomarkers. *Nutr Cancer*. 2000;**37(2)**:155–160.

3. Dale JC, Hamrickk HJ. Neonatal bilirubin testing practices: Reports from 312 laboratories enrolled in the College of American Pathologists Excel Proficiency Testing Program. *Arch Pathol Lab Med*. 2000;**124(10)**:1425–1428.

4. Behman RE, Kliegman RM, editors. Nelson essentials of paediatrics. Pennsylvania: Saunders; 2002.

5. Jendrassik L, Groff P. Simplified photometric method for determination of blood bilirubin. *Biochem J*. 1938;**297(81)**:81–89

6. World Health Organization. Blood safety and clinical technology. In: Guidelines on standard operating procedures for clinical chemistry [Internet]. New Delhi (IN): World Health Organization Regional Office for South-East Asia; 2006 [cited 2010 Jun 14th]. Available from http://www.searo.who.int/EN/Section10/Section17/Section53/Section481_1757.htm.

7. Wilson SS, Guillan RA, Hocker EV. Studies of the stability of 18 chemical constitutes of human serum. *Clin Chem*. 1972;**18(12)**:1498–1503.

Original Article

Reliability and validity of a Malay-version questionnaire assessing knowledge of breastfeeding

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Abstract

Background: **There is a need to identify the knowledge that mothers have about breastfeeding to help promote it. Therefore, it is important to develop a valid and reliable questionnaire to provide useful and comparable data about breastfeeding knowledge. The objectives of this study were to determine the reliability and validity of a Malay version of a questionnaire assessing breastfeeding knowledge and describe the breastfeeding knowledge level among respondents.**

Methods: **A cross-sectional study was conducted from 1 January to 31 May 2008 among female staff working at the Universiti Sains Malaysia Health Campus. A self-administered questionnaire containing 53 items assessing breastfeeding knowledge was given to respondents. The questions were adapted and modified from a breastfeeding questionnaire developed by a team of Hospital Universiti Sains Malaysia paediatric nurses. Exploratory factor analysis, internal consistency reliability, and descriptive analysis of respondents' knowledge were conducted.**

Results: **A total of 252 female staff participated in the study. Factor analysis constructed 10 domains of knowledge and excluded 6 items, leaving 47 items in the final questionnaire. Cronbach's alpha of the final questionnaire was 0.77. Respondents' lowest knowledge was on the practical aspects of breastfeeding.**

Conclusion: **The questionnaire is reliable and valid to assess the breastfeeding knowledge of Malaysian women.**

Keywords: *breastfeeding, knowledge, reliability and validity, questionnaires, medical sciences*

Introduction

Breastfeeding is the best method for feeding an infant. The World Health Organisation (WHO), the United Nations Children's Fund (UNICEF), and other agencies are promoting exclusive breastfeeding up to 6 months of age. The recommendation refers to giving breast milk only, without any other food or drink (1). Malaysia also adopted the same recommendation as stated in the National Breastfeeding Policy. Despite many studies showing the benefits of breastfeeding for infants and mothers, the practice of breastfeeding, especially exclusively, is still far below the standard recommendation. In Malaysia, the prevalence of exclusive breastfeeding up to infant age of 6 months in 2006 was only 14.5%, and only 37.4% of mothers continued breastfeeding for 2 years (2). There is a need to conduct more research that may help to identify the problems and obstacles hindering mothers from breastfeeding their babies. Using a questionnaire is a common tool to get information on the knowledge related to breastfeeding.

It is important to conduct reliability and validity studies so that we may trust the data derived from data collection instruments and procedures. Validity refers to the appropriateness, meaningfulness, and usefulness of the specific inferences made from test scores. An instrument is valid if it measures what it is supposed to measure (3). Among other types of validity, construct validity refers to the extent to which a particular measure relates to other measures consistent with theoretically derived hypotheses concerning the concepts that are being measured (4). One commonly used statistical approach for assessing construct validity is factor analysis. Factor analysis is a statistical tool used for analysing scores on large numbers of variables to determine whether there are any identifiable dimensions that can be used to describe many of the variables under study. One of the types of factor analysis is exploratory factor analysis, which summarises

data by grouping together variables that are inter-correlated.

Usually, factor analysis is followed by computation of Cronbach's alpha coefficient, which is a measure of internal consistency reliability (5). The term reliability refers to the degree to which a measurement procedure can be replicated (6). Internal consistency reliability measures the degree to which the items "hang together", that is, the degree to which items relate to one another (7). Items that form a strong factor in factor analysis yield acceptable alpha coefficients when grouped in a scale, thus providing evidence of internal consistency reliability, as well as supporting initial evidence of construct validity for a developing scale (5).

There is a need to develop a validated questionnaire that assesses knowledge about all aspects of breastfeeding. A questionnaire that is best suited to local culture, beliefs and practices may provide good information about breastfeeding knowledge. These beliefs and practices may include certain issues specific to women in Malaysia, especially among the Malay ethnic group. Examples are beliefs about giving water to infants and ways to identify whether the infant is obtaining sufficient milk, because insufficient milk production is a common reason for introducing formula. In addition, knowledge about storage of expressed breast milk is also important; many women do not know much regarding breast milk expression and storage.

Therefore, it will be valuable for other studies within the same research area to use this newly developed questionnaire by helping to develop comparable data and information from various studies concerning breastfeeding. The objectives of this study were to determine the reliability and validity of a Malay-version questionnaire assessing breastfeeding knowledge and to describe the knowledge of breastfeeding among the respondents.

Materials and Methods

A cross-sectional study was conducted from 1 January to 31 May 2008, with the target population of all female staff working at Universiti Sains Malaysia (USM) Health Campus. They consisted of lecturers, administrators, nurses, technicians, and other employees at 3 different schools: the School of Medical Sciences, the School of Health Sciences, and the School of Dental Sciences. One hundred and eleven (44.0%) of them had received formal education on breastfeeding during their training and service. The Research Ethics Committee (Human) at Universiti Sains Malaysia approved the study protocol on 9 April 2008 (USMKK/PPP/JEPeM [200.4.(1.1)]). The study applied universal sampling, in which the research team recruited all female staff with at least one child. Those who did not understand the Malay language were excluded from the study; thus, all 290 staff eligible for this study were contacted and informed about the study. For the sample size, the study needed a minimum of 159 respondents to validate the questionnaire because there were 53 items for the whole questionnaire with an average of 3 options of answer for each item.

The questionnaire assessing knowledge of breastfeeding was adapted and modified from a breastfeeding questionnaire developed by a team of paediatric nurses at the Hospital Universiti Sains Malaysia (HUSM). Written consent for using the questionnaire was obtained from them before the study. The study began as a pilot study conducted among 60 nurses working at 7 specialist clinics in HUSM. The nurses all completed the same questionnaire. There was then a small discussion between the researchers and 5 of the nurses to hear their opinion about the questionnaire used. Their suggestions for the improvement of the questions on respondent's background were noted. Reliability analysis was conducted and the Cronbach's alpha was 0.70.

All 53 items in the modified questionnaire covered the following scopes of knowledge on breastfeeding: general knowledge, colostrum, advantages to mothers and babies, effective feeding method, duration of feeding, expressed breast milk (EBM), storage of EBM, complementary feeding, and problems with breastfeeding. The respondents were given self-administered questionnaires, and the complete forms were collected back on the same day to reduce information bias. Each item had categorical responses of true, false, or not sure. A correct response scored as '1', whereas a wrong and a not sure response scored as '0'. Total knowledge score ranged from 0 to 53, with higher scores indicating more knowledge. Exclusive breastfeeding is defined in this study as the practice of giving the infant breast milk without any additional food or drink, including water (1).

Statistical Analysis

A breastfeeding consultant, a family health specialist, a nutritionist, and 3 nurses involved with breastfeeding promotion evaluated and discussed the questionnaire to verify its content validity. For construct validity, exploratory factor

analysis using principal components and Varimax rotation method were performed. Items with factor loading of 0.2 and above were grouped into 1 factor and domains were constructed. Internal consistency reliability was carried out using Cronbach's alpha statistic. The item analysis was considered satisfactory if the Cronbach's alpha value was 0.7 or above (8).

Later, we calculated the scores for each domain of breastfeeding knowledge and the total knowledge score for each respondent. They were then converted to percentage scores by dividing by the possible maximum score and multiplying by 100. The percentage scores were presented in median because they were not normally distributed. Interquartile range (IQR), which was a measure of statistical dispersion, was calculated as the difference between the third and the first quartiles of each score. A higher percentage of knowledge indicated higher knowledge of the item tested. All statistical analyses were done using SPSS Version 12.0.

Results

From 290 eligible staff who were invited to take part in the study, 252 respondents completed the questionnaire, resulting in a response rate of 87%. The mean age of respondents was 38.2 (SD 7.6) years old. Most of them (243 respondents, 96.4%) were Malay, while others were Chinese (6 respondents, 2.4%), Indian (1 respondent, 0.4%) and Siamese (2 respondents, 0.8%). The respondents were mainly from the School of Medical Sciences (177 respondents, 70.2%), and the rest were from the School of Health Sciences (38 respondents, 15.1%), and the School of Dental Sciences (37 respondents, 14.7%). As for educational level, 64 (25.4%) of them completed education up to secondary school, 109 (43.3%) have a diploma certificate and 79 (31.3%) of them graduated with a bachelor degree or higher qualification. A majority of them (241 respondents, 95.6%) were not involved with shift work. Among the respondents, the median number of children they had was 3 (IQR 2).

From factor analysis, the study constructed 10 domains of knowledge on breastfeeding. Table 1 shows factor loading for each domain of knowledge. From the initial 53 items, 6 items with factor loading below 0.2 were excluded because there were other additional items testing on similar topics and this exclusion would not affect the content validity of the questionnaire; therefore, the final questionnaire consisted of 47 items with an overall factor loading between 0.20 and 0.88. Table 2 shows the items in each domain of breastfeeding knowledge. As for the internal consistency reliability, Cronbach's alpha for knowledge was 0.77, suggesting a good internal consistency.

Using the finalised questionnaire, the median percentage score for total breastfeeding knowledge among the respondents was 76.6% (IQR 14.9). Table 3 shows the median percentage score for each domain of breastfeeding knowledge. The lowest percentage score was for the practical aspects of breastfeeding. Respondents also had low knowledge about breast engorgement and complementary feeding. However, they had high knowledge of the advantages of breastfeeding, colostrum, and effective feeding.

Table 1: Factor loading for domains of breastfeeding knowledge

Domains	No. of items (Total = 47)	Median scale score	IQR	Factor loading
Advantages to baby	6	6.0	1	0.42–0.71
Advantages to mother	6	6.0	1	0.33–0.75
Colostrum	4	4.0	1	0.56–0.88
Effective feeding	3	3.0	0	0.42–0.77
Breast milk expression	8	6.0	2	0.20–0.75
Duration of feeding	4	3.0	2	0.30–0.64
Complementary feeding	2	1.0	1	0.20–0.30
Problem with breastfeeding	5	4.0	2	0.3–0.69
Breast engorgement	2	1.0	1	0.59–0.61
Practical aspects of breastfeeding	7	3.0	2	0.26–0.60

IQR: interquartile range

Table 2: Items in each domain of breastfeeding knowledge

Domains	Items
Advantages to baby	Breastfeeding reduces the risk of lung infection among babies
	Breastfeeding increases the baby's intelligence
	Breastfeeding helps to reduce the incidence of child abuse and neglect
	Baby who received breastfeeding is less prone to get diarrhoea
	Breast milk provides baby with more protection from allergy compared to formula milk
	Breastfeeding causes good development of baby's teeth and gum
Advantages to mother	Exclusive breastfeeding is beneficial in spacing birth
	Breastfeeding helps to stimulate uterine contraction
	Mothers who practised breastfeeding may achieve pre-pregnancy weight faster
	Frequent breastfeeding may prevent breast engorgement
	Mother who practised breastfeeding has a low risk of getting breast cancer
	Breastfeeding may protect against osteoporosis
Colostrum	Colostrum is the mother's early milk, which is thick, sticky, and yellowish in colour
	Colostrum is difficult to digest and needs to be discarded
	Colostrum causes constipation among babies
	Colostrum is not able to protect babies from jaundice
Effective feeding	Babies will gain weight if they receive effective feeding
	Correct positioning helps to achieve effective breastfeeding
	Babies sleep well after they receive adequate breastfeeding
Breast milk expression	Breast milk expression may be done every 3 hours
	Expressed breast milk may be stored for 3 months in a freezer of a 2-door refrigerator
	Expressed breast milk may be stored for 24–48 hours in a lower part of a refrigerator
	It is necessary to express breast milk from one side of the breast only
	Expressed breast milk may be mixed with the previous expressed milk
	Expressed breast milk may be warmed on a fire
	Expressed breast milk may be warmed in a microwave
	The leftover expressed breast milk that has been used may be stored again
Duration of feeding	Breastfeeding should be initiated within 30 minutes after delivery
	Breastfeeding should be given on demand
	Baby should be allowed to breastfeed for at least 10–20 minutes for each feeding
	Breastfeeding should be continued up to 2 years even though the baby has received complementary food

Complementary feeding	Complementary feeding should be introduced at 6 months of age
	Mothers may mix breastfeeding and formula feeding once baby starts taking complementary food
Problem with breastfeeding	Breast milk production is influenced by breast size
	Mothers with inverted nipples cannot breastfeed their babies
	Breastfeeding must be discontinued if mother has cracked nipple
	Breastfeeding must be discontinued if baby has jaundice
	Breastfeeding must be discontinued if mother has breast engorgement
Breast engorgement	Breast engorgement may be reduced with cold packs
	The use of cabbage may help to reduce breast engorgement
Practical aspect of breastfeeding	Exclusive breastfeeding must be practiced until the infant is 6 months old
	Massage may reduce breast engorgement
	Giving water to baby is encouraged after every breastfeeding
	Belching after feeding shows that the baby is full
	Babies who get enough feeding will pass urine more frequently
	Babies may also be given formula milk in the first 6 months of life
	Oral thrush frequently happens to babies who breastfeed

Table 3: Respondents' score on each domain of knowledge on breastfeeding (n = 252)

Domains of knowledge on breastfeeding	Median percentage score (IQR)
Advantages to baby	100.0 (16.7)
Advantages to mother	100.0 (16.7)
Colostrum	100.0 (25.0)
Effective feeding	100.0 (0.0)
Breast milk expression	75.0 (25.0)
Duration of feeding	75.0 (50.0)
Complementary feeding	50.0 (50.0)
Problem with breastfeeding	80.0 (40.0)
Breast engorgement	50.0 (50.0)
Practical aspects of breastfeeding	42.9 (28.6)

IQR: interquartile range

Discussion

There is a need for an objective, reliable, valid, and sensitive questionnaire to assess knowledge of breastfeeding as part of breastfeeding interventions. There are some existing validated tools assessing knowledge, attitude, confidence, self-efficacy, or satisfaction towards breastfeeding. These tools include the Modified Breastfeeding Evaluation Scale, the Breastfeeding Attrition Prediction Tool, and the Breastfeeding Self-Efficacy Scale (9). However, we developed a new questionnaire in this study to ensure its appropriateness with the local culture. Malaysian women have cultural and traditional beliefs and practices related to breastfeeding that are passed down from generation to generation. For example, many believe that they need to give an infant water to avoid thirst or constipation. As a result, they were not exclusively breastfeeding, even though they were not introducing formula to the infants. They also believe that mothers who have just given birth need to perform breast massage to stimulate milk production. Therefore, the questionnaire tested details of breastfeeding knowledge in all these areas, including the assessment of the respondents' mistaken perceptions of the need for discontinuation of breastfeeding when a baby developed jaundice or a mother has breast engorgement, and the need for discarding colostrum.

Reliability refers to the stability or consistency of information; a reliable measurement tool is one that produces similar information when a measurement is performed more than once. It is an important concept for health education evaluators because an evaluator must ensure the data collection instruments are free from measurement error. Just as there can be measurement error when assessing the physical characteristics of an individual, for example, measurement of height or weight, there is also possible measurement error present when assessing a person's knowledge, attitude, and behaviours (3). In addition to reliability, validity is also important to ensure quality of data derived from the use of the instrument. Therefore, we conducted this study to ensure that the questionnaire yields reliable and valid data accurately and reflects respondents' breastfeeding knowledge.

This study used factor analysis to test the validity of ideas about questionnaire items to determine how items should be grouped into subscales as well as to choose the relevant items and remove some of them from the instrument. Exploratory factor analysis in this study had constructed 10 domains of breastfeeding knowledge. It may provide justification for assessing summated scales for each domain, thus providing information on which aspects of knowledge the respondents are lacking. For example, by using the questionnaire and calculating scores for each domain, researchers may be able to assess in which aspects of knowledge the respondents were lacking, and further intervention can be specifically targeted towards that aspect. Based on factor analysis, we excluded 6 items with factor loading below 0.2. It is common to drop some items from a scale based on factor analysis results (5). Furthermore, most of the areas covered in those items were already included in other domains of the questionnaire.

Because this study used items to form a scale, it required internal consistency. This study found that Cronbach's alpha for the knowledge component of the questionnaire was above 0.7; therefore, the items in that component were measuring the same parameters and correlated with one another. Even though this study used 0.7 as a cut-off level for Cronbach's alpha, there is no specific cut-off value for what level of reliability is acceptable. However, a minimum value of 0.60 is desirable for basic research or evaluation studies (3); therefore, this questionnaire is reliable to assess the knowledge on breastfeeding.

Using the questionnaire, we assessed breastfeeding knowledge among USM female staff. Respondents had low knowledge of the practical aspects of breastfeeding. These practical aspects include the instructions to exclusively breastfeed for 6 months, not to give water after every feeding, and how to detect whether the baby has received sufficient breast milk. It is important to educate mothers that they do not need to provide babies with water, especially in the first 6 months of life. The practice of giving water is widespread throughout the world despite the recommendation and definition of exclusive breastfeeding. Studies conducted in several communities in the Gambia, the Philippines, Egypt, and Guatemala reported that more than 60.0% of newborns were given sugared water and tea (10).

They also lacked adequate knowledge of dealing with the problems of breastfeeding such as breast engorgement or the storage and use of expressed breast milk. Other studies also showed a low level of breastfeeding knowledge, even among health workers. A study conducted among doctors and community health workers in rural South Africa found significant discrepancies in breastfeeding knowledge compared with

the WHO recommendations (11). In North Carolina, many staff nurses who were involved in breastfeeding support had incorrect information and negative attitudes towards breastfeeding (12). It is important to overcome this problem because nurses' support of breastfeeding was best predicted by their breastfeeding knowledge and attitude (13). As for the mothers, receiving accurate information from health workers on every aspect of breastfeeding is important because their decision on infant feeding method is strongly dependent on their knowledge of and attitude towards breastfeeding. Because a majority of the respondents in this study were nurses, their knowledge must be accurate and complete to promote breastfeeding success.

Conclusion

The questionnaire used in this study is reliable and valid for assessing knowledge of breastfeeding; therefore, it can be used for subsequent studies on breastfeeding among women in Malaysia. Low knowledge levels in certain aspects found in this study, such as the false need to provide water to the baby, dealing with problems encountered during breastfeeding, as well as storage and use of expressed breast milk, need to be addressed in a more strategic intervention.

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Authors' Contributions

Conception and design, obtaining of funding, provision of study materials or patients, collection and assembly of data, drafting of the article: TATI
Analysis and interpretation of the data, statistical expertise, final approval of the article: TATI,ZS
Critical revision of the article, administrative, technical, or logistic support: ZS

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References

1. Kramer MS, Kakuma R. The optimal duration of exclusive breastfeeding: A systematic review [Internet]. Geneva (CH): Department of Nutrition for Health and Development; Department of Child and Adolescent Health and Development, World Health Organization; 2002 [cited 2008 Jan 1]. Available from: http://whqlibdoc.who.int/hq/2001/WHO_NHD_01.08.pdf.

2. Institute for Public Health (MY). Infant feeding. In: Institute for Public Health (MY). The third national health and morbidity survey (NHMS-III) 2006. Kuala Lumpur (MY): Ministry of Health (MY); 2008.

3. McDermott RJ, Sarvela PD. Health education evaluation and measurement: A practitioner's perspective. 2nd ed. Boston: McGraw–Hill; 1999.

4. Abramson J, Abramson ZH. Research methods in community medicine: Surveys, epidemiological research, programme evaluation, clinical trials. 6th ed. England: Wiley; 2008.

5. Munro BH. Statistical methods for health care research. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2005.

6. Last JM, editor. A dictionary of epidemiology. 3rd ed. New York: Oxford University Press; 1995.

7. Nunally JC, Berstein IH. Psychometry theory. Chicago: McGraw–Hill; 1994.

8. Jackson C, Furnham A. Designing and analyzing questionnaires and surveys: A manual for health professionals and administrators. London: Whurr Publishers; 2000.

9. Chambers JA, McInnes RJ, Hoddinot P, Alder EM. A systematic review of measures assessing mothers' knowledge, attitudes, confidence and satisfaction towards breastfeeding. *Breastfeed Rev.* 2007;**15(3)**:17–25.

10. LINKAGES. Academy for Educational Development. Exclusive breastfeeding: The only water source young infants need-frequently asked questions [Internet]. Washington: Academy for Educational Development (US); 2004 [cited 2009 July 17]. Available from http://www.linkagesproject.org/media/publications/frequently%20asked%20questions/FrequentlyAskedQuestions_Water_eng.PDF.

11. Shah S, Rollins NC, Bland R. Breastfeeding knowledge among health workers in rural South Africa. *J Trop Pediatr*. 2005;**51(1)**:33–38.

12. Register N, Eren M, Lowdermilk D, Hammond R, Tully MR. Knowledge and attitude of paediatric office nursing staff about breastfeeding. *J Hum Lact*. 2000;**16(3)**:210–215.

13. Bernaix LW. Nurses' attitude, subjective norms, and behavioural intentions towards support of breastfeeding mothers. *J Hum Lact*. 2000;**66(3)**:201–209.

Original Article

Big Sib Students' Perceptions of the Educational Environment at the School of Medical Sciences, Universiti Sains Malaysia, using Dundee Ready Educational Environment Measure (DREEM) Inventory

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Abstract

Background: **A cross-sectional descriptive study was conducted among Big Sib students to explore their perceptions of the educational environment at the School of Medical Sciences, Universiti Sains Malaysia (USM) and its weak areas using the Dundee Ready Educational Environment Measure (DREEM) inventory. The DREEM inventory is a validated global instrument for measuring educational environments in undergraduate medical and health professional education.**

Method: **The English version of the DREEM inventory was administered to all Year 2 Big Sib students (n = 67) at a regular Big Sib session. The purpose of the study as well as confidentiality and ethical issues were explained to the students before the questionnaire was administered.**

Results: **The response rate was 62.7% (42 out of 67 students). The overall DREEM score was 117.9/200 (SD 14.6). The DREEM indicated that the Big Sib students' perception of educational environment of the medical school was more positive than negative. Nevertheless, the study also revealed some problem areas within the educational environment.**

Conclusion: **This pilot study revealed that Big Sib students perceived a positive learning environment at the School of Medical Sciences, USM. It also identified some low-scored areas that require further exploration to pinpoint the exact problems. The relatively small study population selected from a particular group of students was the major limitation of the study. This small sample size also means that the study findings cannot be generalised.**

Keywords: *curriculum, environment, perception, questionnaires, medical student, medical sciences*

Introduction

Increasing attention is being paid to the contribution of the educational environment to learning. A conducive environment has a positive and significant impact on students' learning, academic progress, and well-being (1–4). Learning itself depends on many factors, but perhaps the most crucial factor is the engagement of the learner (5). The professional development of medical practitioners depends, to a large extent, on the attributes of the environment where they study or work (6). The educational environment includes social, cultural, and psychological elements, as well as the physical surroundings (7,8). The new-student orientation also influences students' perception of their learning environment. There is a positive association between good orientation programmes and a positive attitude toward learning (9). A warm, supportive, and challenging educational environment is generally considered an essential pre-requisite for optimal learning (10). Studies of educational environments have been conducted by researchers in medicine, nursing, and other disciplines in various countries using the Dundee Ready Educational Environment Measure (DREEM) instrument. DREEM has been validated and exhibits consistently high reliability in variety of settings (4,11–14).

The participants in this pilot study were Big Sib students. The Big Sib programme is a part of the Personal & Professional Development Programme (PPDP) at the School of Medical Sciences (SMS), Universiti Sains Malaysia. A group of Year 2 medical students are selected as Big Sibs based on their academic achievement,

attitude, and behaviour in Year 1. The programme allows the Year 2 Big Sib students to interact with Year 1 students with the aim of reducing Year 1 students' stress and help them to adapt to their new environment and gain confidence during their first year of study. It also improves medical students' soft skills and professionalism (15).

The objectives of the study were to explore Big Sib students' perceptions of the educational environment at the SMS using the DREEM questionnaire and identify weak areas in the educational environment. Presumably, the Year 2 Big Sibs' perceptions of the educational environment influence their role as mentors to the Year 1 students and thereby influence the Year 1 students' perceptions as well. We hope to use the study findings as a basis for future studies of medical students' perceptions about the educational environment of the entire SMS.

Materials and Methods

This cross-sectional descriptive study was conducted among the Big Sib students at the SMS. The data were collected using internationally validated English version of the DREEM questionnaire. The questionnaires were distributed to all SMS Big Sib students (n = 67) at a regular face-to-face Big Sib session. Before the questionnaire was administered, the students were thoroughly briefed about the purpose of the study and the data collection process. They were also assured of their anonymity and the confidentiality of their responses. We also emphasized that the students' participation was voluntary and the return of the completed questionnaire would be considered implied consent. The students were asked to provide detailed information about their perceptions of the educational environment, and the completed questionnaires were collected by the researchers at the same session. Some educational terms and phrases, such as "factual learning", "ridicule", and "authoritarian", were explained before the respondents began the questionnaire. The entire data collection process took about 20 minutes. The collected data were analysed using SPSS version 12. A descriptive analysis of the collected data was completed.

The universal DREEM inventory

The DREEM inventory gives a total global score of 200 for 50 items. Each item is scored 0–4 (4 = strongly agree, 3 = agree, 2 = unsure, 1 = disagree, and 0 = strongly disagree) on a 5-point scale. There are 9 negative items (Items 4, 8, 9, 17, 25, 35, 39, 48, and 50) scored in a reverse manner; high scores on these items indicate disagreement. The guidelines for interpreting the overall DREEM score are 0–50, very poor; 51–100, many problems; 101–150, more positive than negative; and 151–200, excellent. To indicate the different areas of the educational environment, the DREEM items are grouped into 5 subscales:

1. Students' Perception of Learning (SPoL) has 12 items, with a maximum score of 48 (satisfactory score = 24).
2. Students' Perception of Teaching (SPoT) has 11 items, with a maximum score of 44 (satisfactory score = 22).
3. Students' Academic Self-Perception (SASP) has 8 items, with a maximum score of 32 (satisfactory score = 16).
4. Students' Perception of Atmosphere (SPoA) has 12 items, with a maximum score of 48 (satisfactory score = 24).
5. Students' Social Self-Perception (SSSP) has 7 items, with a maximum score of 28 (satisfactory score = 14).

Items with a mean score of 3.5 or more are true positive points. Items with a mean of 2.0 or less should be examined more closely, as they indicate problem areas. Items with a mean between 2.0 and 3.0 are aspects of the educational environment that could be enhanced (16).

Results

Out of 67 students, 42 responded to the questionnaire, resulting in a response rate of 62.7%. Table 1 shows the DREEM global and subscale mean scores for the school. The global score was 117.9/200 (SD 14.6). The global score indicates that the Big Sib students' perceptions of the educational environment of the school were more positive than negative. The total mean score for SPoL was 28.3/48 (SD 4.1); SPoT was 26.0/44 (SD 3.7); SASP was 19.7/32 (SD 3.6); SPoA was 28.1/48 (SD 5.1); and SSSP was 15.8/28 (SD 2.6). The students perceived the educational environment of SMS positively for all 5 DREEM subscales.

Table 2 shows the individual item analysis of DREEM according to the 5 different subscales. For the SPoL subscale items, 9 out of 12 items scored between 2.00 and 3.00, and 4 items scored 2.00 or less. The mean score for Item 22 (*The teaching helps to develop my confidence*) was 1.88 (SD 0.89), which indicates that the teaching is not providing enough experiences that allow students

to develop their confidence. Item 25 (*The teaching over emphasizes factual learning**) was scored 1.69 (SD 0.78), meaning that students agreed with the statement. Students were unsure about Item 48 (*The teaching is too teacher centered**), which scored 2.00 (SD 0.82).

In the analysis of individual item of SPoT subscale, Items 8 (*The teachers ridicule the students**) and 9 (*The teachers are authoritarian**) scored 1.98 (SD 0.57) and 1.83 (SD 0.74), respectively. The negative-item scoring scheme indicated that students agreed with these 2 items. Items 39 (*The teachers get angry in teaching**) and 50 (*The students irritate the teachers**), both negative items, scored 2.43 (SD 1.06) and 2.40 (SD 0.88), respectively, indicating the students' disagreement with the items. The other 7 items scored between 2.00 and 3.00, indicating aspects of this domain that could be enhanced.

Out of 8 items in the SSAP subscale, only Item 27 (*I am able to memorize all I need*) had a mean score of 1.45 (SD 0.83), which indicates that students are mostly memorizing in their study. The other items scored between 2.00 and 3.00, indicating areas in this domain that could be improved.

Items 17 (Cheating is a problem in this school*) and 35 (I find the experience disappointing*), both negative items, in the SPoA subscale scored 2.02 (SD 0.92) and 2.24 (SD 0.92), respectively. The scores suggest that students did not fully agree with both statements. All items scored between 2.00 and 3.00 and could be improved.

The analysis of individual SSSP subscale, item indicates a problem area is Item 14 (*I am rarely bored in this course*) , which had a mean score of 1.64 (SD 1.00). Item 15 (I have good friends in this course) had mean score of 3.07 (SD 0.77), indicates a fairly good social life for the students. The other 5 items scored between 2.00 and 3.00, indicating a need for further enhancement.

Of the 50 items on the DREEM inventory, 42 items had mean scores between 2.00 and 3.00 and could be improved to enhance the educational environment at SMS. A total of 7 items scored below 2.00. These were identified as real problem areas in our school educational environment that require further exploration to pinpoint and rectify the underlying problems. Only 1 item scored 3.07, which indicates that there are no absolute positive aspects of our school educational environment.

Discussion

The DREEM questionnaire provided an overview of Big Sib students' perceptions about the educational environment at SMS and highlighted areas of concern. The overall DREEM mean score was 117.9/200, indicating that the Big Sib students' perceptions of the educational environment were more positive than negative. In comparison, the global DREEM global scores reported for different medical and allied health sciences schools were 133/200, 134/200, and 125/200 in Malaysia (17–19); 119/200, 114/200, and 107/200 in India (12,20); 108/200 in Sri Lanka (21); 130/200 in Nepal (13); 118/200 in Nigeria (13); 109/200 in Trinidad (14); and 139/200 in the United Kingdom (22). The DREEM score of 89/200 for the College of Medicine at King Saud University, Saudi Arabia (23) is reported to be the lowest score among published studies, followed

Table 1: The Dundee Ready Educational Environment Measure (DREEM) global and subscale mean scores for School of Medical Sciences (SMS), Universiti Sains Malaysia

Subscales	Maximum score	Mean	SD
1 Students' Perceptions of Learning (SPoL)	48	28.3	4.1
2 Students' Perceptions of Teachers (SPoT)	44	26.0	3.7
3 Students' Academic Self-Perceptions (SASP)	32	19.7	3.6
4 Students' Perceptions of Atmosphere (SPoA)	48	28.1	5.1
5 Students' Social Self-Perceptions (SSSP)	28	15.8	2.6
Global DREEM score	200	117.9	14.6

Table 2: Individual item analysis of DREEM by different subscales

Items		Mean	SD
Students' Perception of Learning (SPoL)			
1	I am encouraged to participate during teaching sessions	2.57	0.80
7	The teaching is often stimulating	2.40	0.73
13	The teaching is student-centred	2.29	0.65
16	The teaching helps to develop my competence	2.79	0.78
20	The teaching is well-focused	2.57	0.73
22	*The teaching helps to develop my confidence*	1.88	0.88
24	The teaching time is put to good use	2.31	0.86
25	*The teaching over-emphasizes factual learning**	1.69	0.78
38	I'm clear about the learning objectives of the course	2.43	0.77
44	The teaching encourages me to be an active learner	2.55	0.94
47	Long-term learning is emphasized over short-term learning	2.64	0.65
48	*The teaching is too teacher-centred**	2.00	0.82
	Total mean score	28.30	4.10
	Maximum score	48	
Students' Perception of Teachers (SPoT)			
2	The teachers are knowledgeable	2.88	0.63
6	The teachers adopt a patient-centred approach to consulting	2.42	0.67
8	*The teachers ridicule the students**	1.98	0.57
9	*The teachers are authoritarian**	1.83	0.79
18	The teachers have good communication skills with patients	2.63	0.69
29	The teachers are good at providing feedback to students	2.17	0.74
32	The teachers provide constructive criticism here	2.37	0.69
37	The teachers give clear examples	2.24	0.87
39	The teachers get angry in teaching*	2.43	1.06
40	The teachers are well-prepared for their teaching sessions	2.50	0.86
50	The students irritate the teachers*	2.40	0.88
	Total mean score	26.00	3.70
	Maximum score	44	
Students' Academic Self-Perception (SASP)			
5	Learning strategies that worked for me before continue to work for me now	2.52	0.59
10	I am confident about my passing this year	2.40	0.88
21	I fell I am being well prepared for my profession	2.62	0.85
26	Last year's work has been a good preparation for this year's work	2.68	0.78

27	*I am able to memorize all I need*	1.45	0.83
31	I have learnt a lot about empathy in my profession	2.69	0.86
41	My problem-solving skills are being well developed here	2.45	0.86
45	Much of what I have to learn seems relevant to a career in healthcare	2.98	0.51
	Total mean score	19.70	3.60
	Maximum score	32	
Students' Perception of Atmosphere (SPoA)			
11	The atmosphere is relaxed during ward teaching	2.15	0.88
12	This school is well time-tabled	2.24	1.00
17	Cheating is a problem in this school*	2.02	0.92
23	The atmosphere is relaxed during lectures	2.52	0.83
30	There are opportunities for me to develop my interpersonal skills	2.74	0.76
33	I feel comfortable in class socially	2.55	0.73
34	The atmosphere is relaxed during class/ seminars/tutorials	2.45	0.88
35	I find the experience disappointing*	2.24	0.90
36	I am able to concentrate well	2.17	0.82
42	The enjoyment outweighs the stress of the course	2.05	1.08
43	The atmosphere motivates me as a learner	2.50	0.94
49	I feel able to ask the questions I want	2.20	0.50
	Total mean score	28.10	5.10
	Maximum score	48	
Students' Social Self-Perception (SSSP)			
3	There is a good support system for students who get stressed	2.12	0.68
4	I am too tired to enjoy the course*	2.02	0.88
14	*I am rarely bored in this course*	1.64	1.00
15	I have good friends in this course	3.07	0.77
19	My social life is good	2.52	0.80
28	I seldom feel lonely	2.19	1.04
46	My accommodation is pleasant	2.14	1.00
	Total mean score	15.80	2.60
	Maximum score	28	

: negative item; italic: item scored 2 or less; italic: low-scored negative item

by 97/200 in the Canadian Memorial Chiropractic College study (2).

As is observed in this study, the scores for all 5 DREEM subscales reflected positive perceptions by the students. However, these ratings also indicated that there is ample room for improvement in all 5 domains of the educational environment at SMS. These results are comparable with many other reported findings(12–14,16,17,19).

There were 7 DREEM items that scored 2 or less (items in italic). Out of the 7 items, 4 of them were negative (items in italic, with asterisk); 2 of them belonged to the SPoL subscale (*The teaching over-emphasizes factual learning*, The teaching is too teacher-centred**) and the other 2 belonged to the SPoT subscale (*The teachers ridicule the students*, The teachers are authoritarian**). To a great extent, the students felt that the course is overloaded with factual information and is teacher-centred. They also felt that the teachers were strict and, at times, sarcastic about the students' shortcomings. These findings are consistent with the findings of Mayya & Roff (12). These findings are particularly interesting because they contradict our school teaching and learning philosophies. The school is using a student-centred, problem-based, integrated, and community-oriented approach in its teaching and learning. The students' reported perceptions to the contrary may be because the study was conducted at the very beginning of the new academic session. In Year 1, the students studied basic subjects in an integrated manner and they experienced fewer problem-based or independent sessions than students in other years, which may explain why the Year 2 Big Sib students perceived the program as more teacher-centred and fact-based.

Item 27 (*I am able to memorize all I need*) was the lowest-scored item, with a score of 1.45/4 (SD 0.83). This item scored below 2.0 in many other published articles (13,14,24,25). This finding might indicate that the curriculum volume needs further review and reduction for our school. Another low-scored item was Item 22 (*The teaching helps to develop my confidence*), which scored 1.88, indicating that the current teaching is not providing enough opportunities for the students to develop confidence. Item 14 (*I am rarely bored in this course*) scored 1.64 and needs to be explored further to identify what causes such boredom and whether the courses can be made more engaging. Students' perceptions for Item 4 (I am too tired to enjoy the course*) were average, 2.02. Items 4 and 14 indicate a considerable amount of stress on the students.

There were 16 items that scored between 2.50 and 3.00. They were Items 1 (2.88), 16 (2.79), 44 (2.55), and 47 (2.64) of the SPoL domain; Items 2 (2.88), 18 (2.63), and 40 (2.50) of the SPoT domain; Items 5 (2.52), 21 (2.62), 26 (2.68), 31 (2.69), and 45 (2.98) of the SASP domain; Items 23 (2.52), 33 (2.55), and 43 (2.50) of the SPoA domain and Item 19 (2.52) of the SSSP domain. Students felt their teachers were knowledgeable, were well-prepared for their teaching and stimulated them to participate in teaching sessions. They also felt that the teachers were good at communicating with them, and that their teaching helped the students to develop professional competence. They also considered the overall atmosphere of school is comfortable and reported better-than-average social lives in Item 19 (2.52). Only 1 item, Item 15 (I have good friends on this campus) of the SSAP domain scored 3.07; this indicates that students have an overall good social life on campus. However, not a single item scored 3.50 or higher, which means there is no particularly excellent aspect of the educational environment of our medical school. Although this lack of any excellent aspect may be considered a shortcoming at the moment, it only means that we have a lot of room for improvement and improvisation in the school educational environment.

Considering all of the study's findings, our overall assumptions about the educational environment of SMS are as follows:

1. Overall, the SMS has a reasonably positive educational environment with ample room for improvement.
2. The teachers are knowledgeable and well-prepared for teaching, but they are overloading the students with factual information.
3. Teachers are strict and, to some extent, authoritarian.
4. Students are experiencing a considerable amount of stress.
5. Students' social life on campus is generally good.

Future directions

The small study population selected from a particular group with different sociocultural and educational backgrounds was identified as the main limitation of the study, meaning that the study findings cannot be generalised. We feel it is important to conduct a large scale study among all SMS students regarding their perceptions of the educational environment. We also recommended including more student-centred teaching and problem-based learning in Year 1. The Department of Medical Education should address issues such as factual teaching, teacher-centred teaching, and

the emphasis on memorizing over understanding by organizing more teacher-training programmes as a part of its regular faculty development programmes.

Conclusion

This pilot study revealed that Big Sib students perceived the educational environment of USM School of Medical Sciences positively. It also identified many problem areas (indicated by mean scores between 2.0 and 3.0 for most items) where remedial measures need to be introduced. Areas with scores of less than 2.0 need further exploration to pinpoint the underlying problems. Eventually, we need to ensure a favourable educational environment that will help our medical students achieve better academic performance and the personal and professional growth that will make them indispensable assets to our country.

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Authors' contributions

Conception and design, drafting, critical revision and final approval of the article: HA
Provision of study materials or patients, collection, analysis and interpretation of data, statistical expertise, administrative support: MSBY, SPC

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References

1. Whittle SR, Whelan B, Murdoch-Eaton DG. DREEM and beyond; Studies of the educational environment as a means for its enhancement. Educ Health [Internet]. 2007 [cited 2008 Sep 10];20(1):7. Available from: http://www.educationforhealth.net.
2. Audinet K, Davy J, Barkham M. University Quality of Life and Learning (UNIQoLL): An approach to student wellbeing, satisfaction and institutional change. *J Further High Educ.* 2003;**27(4)**:365–382.
3. Genn JM. AMEE Medical Education Guide No. 23 (Part 2): Curriculum, environment, climate quality and change in medical education –– A unifying perspective. *Med Teach.* 2001;**23(5)**:445–454.
4. Pimparyon P, Caleer SM, Pemba S, Roff S. Educational environment, student approaches to learning and academic achievement in a Thai nursing school. *Med Teach.* 2000;**22(4)**:359–364.
5. Hutchinson L. The ABC of learning and teaching: Educational environment. *BMJ.* 2003;**326(7393):**810–812.
6. Rotem A, Godwin P, Du J. Learning in hospital settings. *Teach Learn Med.* 1995;**7(4)**:211–217.
7. Harden RM. The learning environment and the curriculum. *Med Teach.* 2001;**23(4)**:335–336.
8. Hiemstra R. Aspects of effective learning environments. *New directions for adult and continuing education.* 2010;**1991(50)**:5–12.
9. Ramsden P, Entwistle, NJ. Effects of academic departments on students' approaches to studying, *Brit J Educ Psychol.* 1981;**51(3)**:368–383.
10. Harth SC, Bavanandan S, Thomas KE, Lai MY, Thong YH. The quality of student–tutor interactions in the clinical learning environment. *Med Educ.* 1992;**26(4)**:321–326.
11. Roff S, McAleer S, Harden RM, Al-Qahtani M, Ahmed AU, Deza H, et al. Development and validation of the Dundee Ready Education Environment Measure (DREEM). *Med Teach.* 1997;**19(4)**:295–299.
12. Mayya S, Roff S. Students' perceptions of educational environment: A comparison of academic achievers and under-achievers at Kasturba Medical College, India. *Educ Health.* 2004;**17(3)**:280–291.
13. Roff S, McAleer S, Ifere OS, Bhattacharya S. A global diagnostic tool for measuring educational environment: comparing Nigeria and Nepal. *Med Teach.* 2001;**23(4)**:378–382.
14. Bassaw B, Roff S, McAleer S, Roopnarinesingh S, De Lisle J, Teelucksingh S, et al. Students' perspectives on the educational environment, Faculty of Medical Sciences, Trinidad. *Med Teach.* 2003;**25(5)**:522–526.
15. Yusoff MSB, Rahim AFB. Big Sib Programme guidebook 1st ed. Malaysia: KKMed publications; 2009 [cited 2009 Sep 8] Available from: http://saifulbahri.com/bigsib/BigSib_Programme_Guidebook.pdf

16. McAleer S and Roff S. A practical guide to using the Dundee Ready Education Environment Measure (DREEM). In: Genn JM, editor. AMEE Medical Education Guide No.23 Curriculum, environment, climate, quality and change in medical education; A unifying perspective. Dundee (UK): Association of Medical Education in Europe; 2001. p. 29–33.

17. Lai N, Nalliah S, Jutti RC, Hla Y, Lim VK. The educational environment and self-perceived clinical competence of senior medical students in a Malaysian medical school. *Educ Health* [Internet]. 2009 [cited 2009 Aug 7];**22(**2):148. Available from http://www.educationforhealth.net.

18. Hassan II. A study of stressors and coping strategies among first year nursing students in the college of Polytech Mara, Kota Bharu, Kelantan [master's thesis]. [Kota Bharu (Kelantan)]: Universiti Sains Malaysia; 2007.

19. Zamzuri AT, Ali AN, Roff S, McAleer S. Students perceptions of the educational environment at dental training college. *Malaysian Dent J*. 2004;**25**:15–26.

20. Abraham R, Ramnarayan K, Vinod P, Torke S. Students' perceptions of learning environment in an Indian medical school. *BMC Med Educ*. 2008;**8**:20.

21. Jiffry MT, McAleer S, Fernando S, Marasinghe RB. Using the DREEM questionnaire to gather baseline information on an evolving medical school in *Sri Lanka*. *Med Teach*. 2005;**27(4)**:348–352.

22. Verma R, Tiyagi E, Gupta JK. Determining the quality of educational climate across multiple undergraduate teaching sites using the DREEM inventory. *BMC Med Educ*. 2005;**5(1)**:8.

23. Al-Ayed IH, Sheikh SA. Assessment of educational environment at the college of Medicine of King Saud University, Riyadh. *East Mediterr Health J*. 2008;**14(4)**:953–959.

24. Till H. Identifying the perceived weaknesses of a new curriculum by means of the Dundee Ready Education Environment Measure (DREEM) Inventory. *Med Teach*. 2004;**26(1)**:39–45.

25. Al-Hazimi A, Zaini R, Al-Hyiani A, Hassan N, Gunaid A, Ponnamperuma G, et al. Educational environment in traditional and innovative medical schools: A study in four undergraduate medical schools. *Educ Health*. 2004;**17(2)**:192–203.

Original Article

The Risk Factors of External Ventricular Drainage-Related Infection at Hospital Kuala Lumpur: An Observational Study

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Abstract

Background: **External ventricular drainage (EVD) has been widely used for the purpose of cerebrospinal fluid (CSF) diversion at Hospital Kuala Lumpur (HKL).**

Method: **This prospective observational study was conducted in HKL from December 2006 to December 2008 among patients who were subjected for EVD, following strict inclusion and exclusion criteria.**

Results: **The frequency of EVD-related infection was as high as 32.2% (95% CI 23.3% to 42.57%) among 87 patients studied. This study clearly demonstrates that tunnelling the catheter for more than 5 cm under the scalp, from the burr hole to the exit site of the skin, carried a significantly lower risk of infection compared with tunnelling the catheter for 5 cm or less (OR = 0.184, 95% CI 0.083 to 0.406, *P* < 0.001). The majority of cases (19 out of 28) with EVD-related infection occurred among patients catheterised for more than 10 days (OR = 0.334, 95% CI 0.171 to 0.652, *P* < 0.001).**

Conclusion: **The technique of subgaleal tunnelling of more than 5cm and the duration of the ventricular catheterisation of 10 days and less should be implemented as standardised protocol at health institutions to reduce the risk of EVD-related infections.**

Keywords: *cerebrospinal fluid shunts, hydrocephalus, in-dwelling catheters, infection, neurosciences*

Introduction

External ventricular drainage (EVD) has become an excellent adjunct to measure the intracranial pressure (ICP) in severe head trauma. The technique also represents an important tool to manage patients with diseases associated with hydrocephalus. However, the main disadvantage of EVD is the risk of contracting a ventriculostomy-related infection (VRI), which has been reported to affect 0.0%–21.9% of patients (1–11).

One of the major indications for EVD insertion is hydrocephalus secondary to intraventricular or subarachnoid haemorrhage. Previous studies have shown that patients with blood in the CSF system have a higher rate of EVD infection compared with those with blood-free CSF (4,5,12). Generally, as is common in other surgical practices, factors that are crucial to the risk of infection include the surgeon's experience, the venue of the procedure, and the surgical technique. The need to remove the initial EVD and reinsert the new catheter after a certain amount of time has elapsed is currently under debate.

This is a prospective observational study of the surgical technique in relation to the extra-cranial length of the catheter and other factors among patients subjected to EVD insertion. This study is meant to determine the association of predisposing factors with EVD-related infection over a period of 2 years in the Neurosurgical Department, Hospital Kula Lumpur (HKL). The main purpose of this study was to determine whether the length of the ventricular catheter from the burr hole of the skull to the site of exit will show any significant difference in terms of central nervous system (CNS) infection rate. Other measures, such as the duration for which catheters have been in situ, the surgeon's experience, the venue at which the procedure was performed, as well as the primary pathology or aetiology that required drainage, were analysed to identify any correlation with infection rate. A total of 87 patients who fulfilled the criteria were included in this study. Statistical analyses (Pearson chi-square test, Fisher's exact test, and multiple regression test) were performed to determine the associations between EVD-related infections and surgical methods as well as other related factors.

Materials and Methods

This was an observational prospective study of patients who were admitted to the Neurosurgical Department and underwent the initial EVD insertion at HKL over a period of 2 years (2006–2008). Patients were selected according to the inclusion and exclusion criteria.

Inclusion criteria

1. All patients requiring an EVD system due to
   a. hydrocephalus secondary to subarachnoid haemorrhage (SAH), spontaneous or traumatic intraventricular haemorrhage (IVH), tumour associated with hydrocephalus, and intraparenchymal bleed
   b. cerebral oedema
2. The EVD system was initiated between December 2006 and December 2008
3. The EVD system was in situ for at least 48 hours
4. The material used for the EVD system was standardised for all samples

Exclusion criteria

1. Evidence of CSF leakage during the period when the system was in situ, either from the system itself or other routes, including nostril, ear, or meningocele
2. Evidence of cerebrospinal fluid infection prior to the procedure, including meningitis, infected implant (shunt system), or ventriculitis
3. Any clinical suspicion of CNS infection prior to the procedure, including subdural empyema, cerebral abscess or infected pathological disease such as toxoplasmosis or cryptococcal infection
4. Reinsertion or revision of the EVD system

Data were collected by a single investigator to minimise the risk of error in the evaluation of specific data needed for the study. After patient selection and data collection, the length of tunnelling between the burr hole and the exit skin was measured. The measurement was performed post-operatively using the digital palpation method.

The patients were classified as having EVD-related infection if the following criteria were fulfilled (13):

1. Positive CSF culture and Gram stain
2. Presence of other supportive CSF laboratory findings
   a. pleocytosis with microscopic examination showing presence of white blood cells of more than 11/mm3
   b. a decrease in the CSF glucose level (normal level = 2.5 mmol/L)
   c. an increase in the CSF protein level (normal level = 0.4g/L)

The time of infection was recorded as the time when the sample was obtained. Patients were not classified as having EVD infections if only an isolated CSF culture was positive but other parameters were within normal values.

Analyses of the data were done using SPSS version 12.0 (SPSS Inc, Chicago, IL). Descriptive analysis was performed for all variables. The univariate analysis was performed using the chi-square test or Fisher's exact test, and multiple regression analysis, with $P < 0.05$ considered to be significant.

Results

From December 2006 to December 2008, there were more than 400 cases of EVD insertion at HKL. The strict inclusion and exclusion criteria previously outlined yielded a database of 87 patients for analysis.

The youngest patient was 14 years old and the oldest was 73 years old. The median age of the patients was 50 years old, with an interquartile range of 27.0. The age group of 50–59 years old comprised the most patients (n = 20). The ventriculostomy insertion procedure was noted to be slightly more common in female patients. Out of 87 cases, 47 patients (54.0%) were female and 40 patients (46.0%) were male. The female to male ratio was 1.17:1.

Hypertensive bleeding with intraventricular extension associated with hydrocephalus is the common cause of EVD insertion (28 patients, 32.2%), followed by subarachnoid haemorrhage (25 patients, 28.7%), and ICP monitoring in trauma cases (20 patients, 23.0%). Brain tumour and other factors (including posterior fossa and supratentorial ischaemia or infarct) were the least common indication for EVD insertion.

Among the population surveyed, 28 patients (32.2%, 95% CI 23.3% to 42.6%) were diagnosed with EVD-related infection, whereas 59 patients (67.8%) were not infected. The ratio of infected to non-infected patients was 1:2.1. Most patients with EVD-related infections were female. However, for non-EVD-related infections, the numbers of cases were similar among males (31 patients) and females (28 patients).

There were less EVD-related infections in the group subjected to tunnelling of more than 5 cm (6 out of 52 patients, 11.5%) compared with the group that required tunnelling of 5 cm or less (22 out of 35 patients, 62.9%). The group of patients subjected to tunnelling of more than 5 cm had a significantly lower risk of EVD-related infection (P < 0.001) compared with the other group, which was subjected to a shorter tunnelling length (Table 1).

The bacterial cultures of CSF samples from patients with EVD-related infections showed the presence of various microorganisms: *Acinetobacter* sp. in 12 patients, methicillin-resistant *Staphylococcus aureus* (MRSA) in 10 patients, as well as *Pseudomanas* sp., *Enterobacter* sp. and *Klebsiella* sp., which accounted for 3 patients each.

In this study, most of the patients who had the EVD in situ for less than 10 days (42 out of 51 patients, 82.3%) showed no evidence of EVD-related infection. EVD-related infection was significantly more common (P < 0.001) among the group subjected to EVD for more than 10 days (19 out of 36 patients, 52.8%). Gram-negative bacteria (12 species) were primarily responsible for EVD-related infections that lasted for more than 10 days.

In this investigation, most of the procedures were performed by the resident physicians (73 out of 87 procedures); only 14 procedures were performed by a specialist or consultant. Majority of the EVD-related infection occurred in the group of cases performed by the resident group (26 out of 28 infected cases, 92.6%). There was no significant association (P = 0.21) between surgeon status and EVD-related infection.

In this study, the venue for the procedure varied, although it was typically performed in the operation theatre (85 out of 87 procedures). Only 2 procedures were performed in the Intensive Care Unit (ICU) setting for ICP monitoring. We found that there was no significant correlation (P = 1.00) between the venue for the surgery and the risk of EVD-related infection.

The majority of patients with EVD-related infections (26 out of 28 patients) presented with non-traumatic aetiologies. Cases of non-EVD-related infections were also more frequent among these patients with non-traumatic aetiologies (69.5%). It was found that there was no significant association (P = 0.16) between the

Table 1: Analysis of variables in association with external ventricular drainage (EVD)-related infection

	EVD-related infection *n* (%)		x2 stat(df)	OR (95% CI)	*P* value
	Yes	No			
Length of tunnelling *More than 5cm* *5cm or less*	6 (21.4) 22 (78.6)	46 (78.0) 13 (22.0)	25.24 (1)	0.18 (0.08, 0.46)	<0.001 [a] ***
Duration of in situ EVD *More than 10 days* *10 days or less*	19 (67.9) 9 (32.1)	17 (28.8) 42 (71.2)	11.93 (1)	0.33 (0.17, 0.65)	<0.001 a ***
Venue of surgery *Operation theatre* *Bedside/intensive care unit*	28 (100.0) 0 (0.0)	57 (96.7) 2 (3.3)		0.67 (0.58, 0.78)	1.00 [b]
Indication for EVD *Non-trauma* *Trauma*	26 (92.8) 2 (7.2)	41 (69.5) 18 (30.5)	5.87 (1)	3.88 (1.01, 12.95)	0.16 [a]
Indication for EVD *Non-trauma* *Trauma*	26 (92.8) 2 (7.2)	41 (69.5) 18 (30.5)	5.87 (1)	3.88 (1.01, 12.95)	0.16 [a]
Surgeon status *Resident* *Specialist/consultant*	26 (92.6) 2 (7.4)	47 (79.7) 12 (20.3)		2.49 (0.67, 9.33)	0.21 [b]

[a] Pearson chi-square test applied.

[b] Fisher's exact test applied.

*** P < 0.001 indicates extreme significance between the 2 groups.

traumatic and non-traumatic groups subjected to EVD with regard to the outcome of EVD-related infection. Therefore, EVD-related infection was independent of aetiology in this study.

There were differences in terms of the duration of the EVD in situ among cases with different aetiologies. Patients with hypertensive-related aetiology subjected to EVD represented the largest subpopulation (18 patients, 20.7%), with duration of in situ EVD of more than 10 days.

Multiple logistic regression analysis (Table 2) of controlled variables including sex, race, placement of the EVD, status of the surgeon, tunnelling length, and the duration of in situ EVD showed that EVD-related infection was significantly associated with both the length of tunnelling (P = 0.001) and the duration of in situ EVD (P = 0.024). EVD-related infection was noted to be 10 times less likely to develop in the group subjected to more than 5 cm of tunnelling. The EVD in situ of less than 10 days was noted to be 4 times less likely to develop EVD- related infection.

Discussion

The limited sample size in this study in comparison to the total number of patients subjected to EVD insertion and referred to the computed operation theatre database system, COTDS, (415 cases) results primarily from a high drop-off rate following the strict exclusion criteria. This prospective study included only patients with catheters constructed of similar material who had received only one EVD system. Traumatic patients who underwent parenchymal catheter insertion were also labelled as subjected to EVD insertion in the COTDS system, even though most of these patients did not undergo ventricular assessment for CSF sampling. Most patients with EVD had infected shunt systems and meningitis; these patients were excluded from the study.

The overall incidence of EVD-related infection in patients with this EVD system has been reported to range from 0.0–21.9% of patients (1–11). However, various authors used different definitions of EVD-related infection, which may have either over- or under-estimated the true infection rate. Therefore, strict criteria based on literature review (13) were used to plan this study. The following categories were used to classify patients: infection, contamination, and colonisation. These strict definitions were created before the initiation of the study and the collection of data.

The incidence of EVD-related infection at Hospital Kula Lumpur was found to be 32.2% (95% CI 23.3% to 42.6%), which is a relatively large fraction. This infection rate, as stated above, was obtained using strict inclusion and exclusion criteria; in particular, the system had to have been in situ for at least 48 hours before the patient could be included in this investigation. This minimum required duration of in situ catheterisation was meant to prevent misinterpretation of the results, as published studies have reported the infection to go undetected before day 3 of catheterisation (11,14). Most patients with traumatic head injury who required the EVD for ICP monitoring usually needed the system for only 48 hours. Furthermore, the measurement of tunnelling was performed at only day 3 of catheterisation to coincide with wound inspection and thereby avoid wound contamination during the study. Demographic data showed there was no significant difference for sex, race, or age in association with EVD-related infection.

The majority of patients subjected to EVD insertion at HKL underwent surgery with catheter tunnelling of more than 5 cm. Tunnelling length has been found to be a significant risk factor

Table 2: Multiple logistic regression analysis on the risk of external ventricular drainage (EVD)-related infection

	Adjusted OR (95% CI)	b	Wald Statistic	*P* value
Length of tunnelling				
More than 5cm	1	2.35	16.59	0.001 **
5cm or less	10.46 (3.38, 32.32)			
Duration of in situ EVD				
More than 10 days	1	1.28	5.13	0.024 *
10 days or less	3.61 (1.19, 10.94)			

* P < 0.05 indicates significance between the 2 groups.

** P < 0.01 indicates high significance between the 2 groups.

for EVD-related infection; tunnelling of more than 5 cm length is associated with a lower rate of EVD-related infection. The use of 5 cm as the cut-off point is based on prior studies (15–17). Our findings are supported by another study (18), which noted no infection during the first 16 days of catheterisation when the extended tunnelling technique was used.

Among 87 total samples, 60.8% of those with haemorrhagic-related disease, such as SAH or intracerebral haemorrhage, required an EVD. Previous research has documented a strong association between EVD-related infection and aetiological disease requiring an EVD. This is particularly prominent in cases of IVH and SAH (4,5,12). Previous studies have also shown a significant relationship between haemorrhagic CSF and EVD-related infection. This finding was consistent with our study, although the relationship was not significant. In cases of haemorrhagic-related diseases, the duration of in situ EVD is usually longer, which leads to an increased risk of drainage system blockage. The incidence of catheter blockage ranges from 19%–41% (6,19), which is why the catheter needs to be flushed and irrigated. A study by Aucoin et al. (10) noted a 24% infection rate in patients with flushed EVD systems, whereas Mayhall et al. (4) reported a higher incidence of EVD-related infection among those with catheter irrigation. In addition, the blood can act as a good medium for bacterial colonisation and subsequently promote infection (20).

Patients were divided into 2 groups: those with duration of catheterisation of 10 days or less, and more than 10 days. The cut-off point was set at 10 days because published studies found a significant risk of EVD-related infection when catheterisation lasted for more than 10 days (1,7,21). Our findings are comparable with data from the literature (13), which reported an association between the duration of catheterisation and EVD-related infection.

This study, as well as most others, demonstrated the lack of a significant association between the venue of EVD placement and EVD-related infection. However, the finding may be misleading because 85 of the patients in our sample (97.8%) underwent EVD insertion in the operation theatre. The same skewed distribution may colour conclusions related to the association between surgeon status and EVD-related infection because almost 83.9% of the EVD procedures were performed by a medical officer at our institution.

Among the cases that have been classified as EVD-related infections in this study, *Staphylococcus* sp. and *Acinetobacter* sp. were most frequently isolated pathogen. Most of the bacteria isolated were Gram negative. Notably, a longer duration of EVD may facilitate microbial infection or colonisation. On the other hand, all patients subjected to EVD insertion were given a prophylactic dose of antibiotic to protect against contamination of the wound site by Gram-positive skin flora. Both the duration of EVD as well as the administration of antibiotic against Gram-positive bacteria may lead to subsequent infection by Gram-negative bacteria as opposed to the traditional Gram-positive flora. These findings are basically comparable to those observed by other authors, although some have noted a high rate of infection by *Klebsiella* sp. (22).

This study has revealed that patients with haemorrhagic-related hydrocephalus with intraventricular extension will be managed with the EVD system in situ for more than 10 days. Such patients usually remain on the drainage system until the CSF system is conducive for a conversion to a permanent shunt. Researches have shown that patients who suffer from IVH will be dependent on the shunt system in about 55% of the cases (23,24). On the other hand, up to 63.4% of SAH patients will be shunt-dependent mainly due to communicating hydrocephalus (25–27). The CSF needs to be quite clean prior to shunt insertion, as post-haemorrhagic debris typically causes catheter or valve occlusion, especially in patients with IVH. However, not all patients need a permanent shunting system; a study revealed that up to 30% of IVH cases are resolved without evidence of hydrocephalus (28). This is probably due to the presence of blood products and associated inflammation in the cerebrospinal fluid. These factors dissipate with time, and once the cerebrospinal fluid is clear, hydrocephalus due to intracranial haemorrhage may resolve.

Multivariate analysis comparing all the variables that can predispose patients to the risk of EVD-related infection showed that tunnelling length of more than 5 cm and catheterisation of 10 days and less can significantly reduce the incidence of infection.

Conclusion

Based on the findings reported here, subgaleal tunnelling of a catheter of more than 5 cm in length under the scalp is the recommended surgical technique, as this approach involves a significantly lower rate of EVD-related infection.

A prolonged duration of catheterisation also plays an important role in relation to EVD-related infection, as it has been demonstrated that catheterisation of more than 10 days in duration is associated with a significant risk of developing EVD-related infection. Therefore, both subgaleal tunnelling of more than 5cm and 10 days and less of ventricular catheterisation should be implemented as standardised protocol at health institutions to reduce the risk of EVD-related infections.

Authors' Contributions

Conception and design, final approval of the article: MAO, MSMH
Obtaining of fund, provision of the study materials or patients, collection and assembly of data, statistical expertise, analysis and interpretation of the data, drafting of the article, critical revision of the article: MAO
Administrative, technical, or logistic support: MSMH

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References

1. Schultz M, Moore K, Foote AW. Bacterial ventriculitis and duration of ventriculostomy catheter insertion. *J Neurosci Nurs*. 1993;**25(3)**:158–164.

2. Bader MK, Littlejohns L, Palmer S. Ventriculostomy and intracranial pressure monitoring: In search of a 0% infection rate. *Heart Lung*. 1995;**24(2)**:166–172.

3. Smith RW, Alksne JF. Infections complicating the use of external ventriculostomy. *J Neurosurg*. 1976;**44(5)**:567–570.

4. Mayhall CG, Archer NH, Lamb VA, Spadora AC, Baggett JW, Ward JD, et al. Ventriculostomy-related infections. A prospective epidemiologic study. *N Engl J Med*. 1984;**310(9):**553–559.

5. Stenager E, Gerner-Smidt P, Kock-Jensen C. Ventriculostomy-related infections—An epidemiological study. *Acta Neurochir (Wien)*. 1986;**83(1–2)**:20–23.

6. Bogdahn U, Lau W, Hassel W, Gunreben G, Mertens HG, Brawanski A. Continuous-pressure controlled, external ventricular drainage for treatment of acute hydrocephalus—Evaluation of risk factors. *Neurosurgery*. 1992;**31(5)**:898–903; discussion 903–904.

7. Holloway KL, Barnes T, Choi S, Bullock R, Marshall LF, Eisenberg HM, et al. Ventriculostomy infections: The effect of monitoring duration and catheter exchange in 584 patients. *J Neurosurg*. 1996;**85(3)**:419–424.

8. Winfield JA, Rosenthal P, Kanter RK, Casella G. Duration of intracranial pressure monitoring does not predict daily risk of infectious complications. *Neurosurgery*. 1993;**33(3)**:424–430; discussion 430–431.

9. Jones CC, Cayard CH. Care of ICP monitoring devices: A nursing responsibility. *J Neurosurg Nurs*. 1982;**14(5)**:255–261.

10. Aucoin PJ, Kotilainen HR, Gantz NM, Davidson R, Kellogg P, Stone B. Intracranial pressure monitors. Epidemiologic study of risk factors and infections. *Am J Med*. 1986;**80(3)**:369–376.

11. Narayan RK, Kishore PR, Becker DP, Ward JD, Enas GG, Greenberg RP, et al. Intracranial pressure: To monitor or not to monitor? A review of our experience with severe head injury. *J Neurosurg*. 1982;**56(5)**:650–659.

12. Sundbarg G, Nordstrom CH, Soderstrom S. Complications due to prolonged ventricular fluid pressure recording. *Br J Neurosurg*. 1988;**2(4)**:485–495.

13. Lozier AP, Sciacca RR, Romagnoli MF, Connolly ES Jr. Ventriculostomy-related infections: A critical review of the literature. *Neurosurgery*. 2008;**62 Suppl 2**:S688–S700.

14. Alleyne CH Jr, Hassan M, Zabramski JM. The efficacy and cost of prophylactic and perioprocedural antibiotics in patients with external ventricular drains. *Neurosurgery*. 2000;**47(5)**:1124–1127; discussion 1127–1129.

15. Hong J, Sang DK, Jang BL, Dong JL, Jung YP. Clinical analysis of external ventricular drainage related ventriculitis. *J Korean Neurosurg Soc*. 2007;**41(4)**:236–240.

16. Park P, Garton HJ, Kocan MJ, Thompson BG. Risk of infection with prolonged ventricular catheterization. *Neurosurgery*. 2004;**55(3)**:594–599; discussion 599–601.

17. Wong GK, Poon WS, Wai S, Yu LM, Lyon D, Lam JM. Failure of regular external ventricular drain exchange to reduce cerebrospinal fluid infection: Result of a randomised controlled trial. *J Neurol Neurosurg Psychiatry*. 2002;**73(6)**:759–761.

18. Khanna RK, Rosenblum ML, Rock JP, Malik GM. Prolonged external ventricular drainage with percutaneous long-tunnel ventriculostomies. *J Neurosurg*. 1995;**83(5)**:791–794.

19. Rhodes TT, Edwards WH, Saunders RL, Harbaugh RE, Little CL, Morgan LJ, et al. External ventricular drainage for initial treatment of neonatal posthaemorrhagic hydrocephalus: Surgical and neurodevelopmental outcome. *Pediatr Neurosci.* 1987;**13(5)**:255–262.

20. Harker J, Moore K. Tissue management and wound pathophysiology. *Br J Community Nurs*. 2004;**9(9 Time Sppl)**:S5–S11.

21. Luerssen TG. Intracranial pressure: Current status in monitoring and management. *Semin Pediatr Neurol*. 1997;**4(3)**:146–155.

22. Lyke KE, Obasanjo OO, Williams MA, O'Brien M, Chotani R, Perl TM. Ventriculitis complicating use of intraventricular catheters in adult neurosurgical patients. *Clin Infect Dis*. 2001;**33(12)**:2028–2033.

23. Bhattathiri PS, Gregson B, Prasad KS, Mendelow AD. Intraventricular hemorrhage and hydrocephalus after spontaneous intracerebral hemorrhage: Results from the STICH trial. *Acta Neurochir Suppl*. 2006;**96**:65–68.

24. Yilmazlar S, Abas F, Korfali E. Comparison of ventricular drainage in poor grade patients after intracranial haemorrhage. *Neurol Res*. 2005;**27(6)**:653–656.

25. Vale FL, Bradley EL, Fisher WS 3rd. The relationship of subarachnoid haemorrhage and the need for postoperative shunting. *J Neurosurg*. 1997;**86(3)**:462–466.

26. Klopfenstein JD, Kim LJ, Feiz-Erfan I, Hott JS, Goslar P, Zabramski JM, et al. Comparison of rapid and gradual weaning from external ventricular drainage in patients with aneurysmal subarachnoid haemorrhage: A prospective randomized trial. *J Neurosurg*. 2004;**100(2)**:225–229.

27. Komotar RJ, Olivi A, Rigamonti D, Tamargo RJ. Microsurgical fenestration of the lamina terminalis reduces the incidence of shunt-dependent hydrocephalus after aneurysmal subarachnoid haemorrhage. *Neurosurgery*. 2002;**51(6)**:1403–1412; discussion 1412–1413.

28. Hoh BL, Lang SS, Ortiz MV, Chi YY, Lewis SB, Pincus DW. Lower incidence of reoperation with longer shunt survival with adult ventriculoperitoneal shunts placed for haemorrhage-related hydrocephalus. *Neurosurgery*. 2008;**63(1)**:70–74; discussion 74–75.

Case Report

Embolisation of a Bronchial Artery of Anomalous Origin in Massive Haemoptysis

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Abstract

Massive haemoptysis is the most dreaded of all respiratory emergencies. Bronchial artery embolisation is known to be a safe and effective procedure in massive haemoptysis. Bronchial artery of anomalous origin presents a diagnostic challenge to interventional radiologists searching for the source of haemorrhage. Here, we report a case of massive haemoptysis secondary to a lung carcinoma with the bronchial artery originating directly from the right subclavian artery. This artery was not evident during the initial flush thoracic aortogram. The anomalous-origin bronchial artery was then embolised using 15% diluted glue with good results. An anomalous-origin bronchial artery should be suspected if the source of haemorrhage is not visualised in the normally expected bronchial artery location.

Keywords: *bronchial arteries, glues, haemoptysis, therapeutic embolisation, medical sciences*

Introduction

Massive haemoptysis, which is defined as a pulmonary haemorrhage of 500–600 ml in 24 hours, is the most dreaded of all respiratory emergencies (1,2). Bronchial artery embolisation (BAE) has become an established procedure in the management of massive and recurrent haemoptysis (2,3). BAE is effective in the immediate control of haemorrhage, with a success rate ranging 88%–100% (2–4).

Bronchial artery of anomalous origin (BAAO) is known as a source of non-evident haemorrhage and persistent haemoptysis post-embolisation (1,5). The normal bronchial arteries usually arise from the descending thoracic aorta at the level of the T5 to T6 vertebrae, with an intrapulmonary course along the major bronchi (1,6). BAAO is defined as bronchial arteries that arise outside of these areas (1,5). Here, we report a case of a BAAO that originated directly from the right subclavian artery in a patient with right lower lobe lung carcinoma and the role of BAE in its management.

Case Report

A 58-year-old female Malay non-smoker presented in October 2008 with intermittent mild haemoptysis associated with loss of weight and appetite for duration of 2 months. A chest radiograph showed a mass in the right lower zone. A computed tomography (CT) scan of the thorax revealed a soft tissue mass in the mediobasal segment of the right lower lobe with collapse of the posterobasal segment (Figure 1). Bronchoscopy revealed an endobronchial mass obstructing the right intermediate bronchus. An endobronchial ultrasound did not show any enlarged mediastinal lymph nodes. Percutaneous fine needle aspiration of the mass was done and confirmed a diagnosis of large cell carcinoma. Chemotherapy was initiated with cisplatin and gemcitabine. Unfortunately, the patient developed thrombosis of the left superficial femoral vein after 2 cycles of chemotherapy, upon which she was anticoagulated with warfarin. Three weeks later, she presented with massive haemoptysis that was complicated by Type 2 respiratory failure and haemodynamic instability. She was resuscitated, ventilated, and then managed in the Intensive Care Unit (ICU). Urgent rigid bronchoscopy revealed huge blood

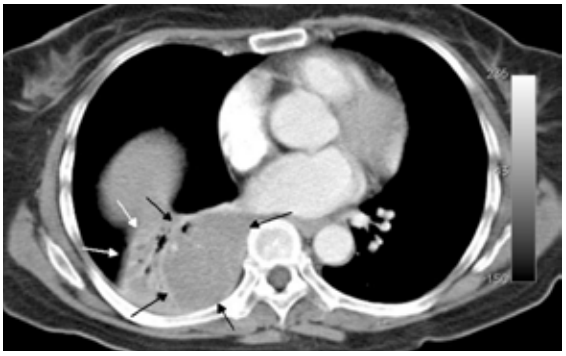

Figure 1: Axial-enhanced CT scan of the thorax showing the large mass in the mediobasal segment of the right lower lobe (black arrows). There is also an associated collapse of the posterobasal segment of the right lower lobe (white arrows).

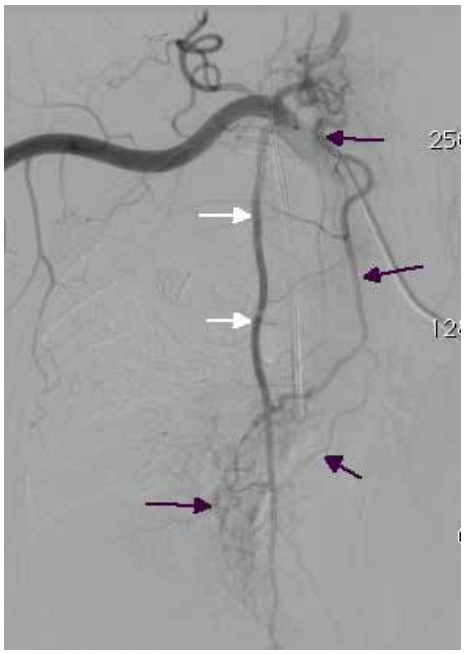

Figure 2: Selective right subclavian digital subtraction arteriogram. The right bronchial artery (purple arrows) originates directly from the right subclavian artery and is coursing inferiorly to supply the right lower lobe lung tumour. The right internal mammary artery (white arrows) is separate from the right bronchial artery.

clots occluding both the main bronchus and lower trachea. The blood clots were removed, and a double lumen endotracheal tube (ETT) was inserted to protect the left lung. The patient remained stable after the procedure. The double lumen ETT was later replaced with a single lumen ETT. The following day, fresh blood was noted from the ETT, causing intermittent desaturation. The patient was then referred to the interventional radiologist, where a thoracic angiogram was performed under general anaesthesia with the intention of transcatheter embolisation. A flush thoracic aortogram did not show any dilated or tortuous bronchial arteries supplying the right

lower lobe lung tumour. No evidence of contrast extravasation or pulmonary shunting was seen. Selective angiogram of the intercostal arteries was also performed with a 4F Cobra catheter, revealing similar results. A right subclavian arteriogram showed a dilated right bronchial artery supplying the tumour that originated directly from the right subclavian artery (Figure 2). The right bronchial artery was well-separated from the right internal mammary artery. A selective arteriogram of the right bronchial artery confirmed the initial finding (Figure 3a). A microcatheter (Miraflex, Cook) was introduced co-axially through the 4F Cobra catheter, with the tip of the microcatheter parked distally about 9.5 cm from the origin of the right bronchial artery. A superselective arteriogram was then performed pre-embolisation (Figure 3b). The decision to use 15% diluted glue (1.5 ml of Histoacryl Glue, B Braun diluted with 8.5 ml of Lipiodol, Guerbet) was made in view of the non-availability of other preferred embolic materials, such as polyvinyl alcohol (PVA). A total of 3 ml of 15% diluted glue was injected through the microcatheter until reflux was observed beyond the tip of the microcatheter under fluoroscopic guidance. The microcatheter was then pulled swiftly in one rapid motion and removed from the 4F Cobra catheter. An angiographic run post-embolisation revealed the complete obliteration of the right bronchial artery supplying the right lower lobe lung tumour (Figure 4).

The bleeding stopped after the embolisation. The patient was extubated 7 days later and remained stable. However, she developed persistent left hemiparesis, and CT scan of the brain showed a large left parietal lobe metastasis. She was then transferred to another tertiary centre for whole brain radiotherapy. Unfortunately, after completion of the radiotherapy, which took place about 2 weeks after the embolisation, she

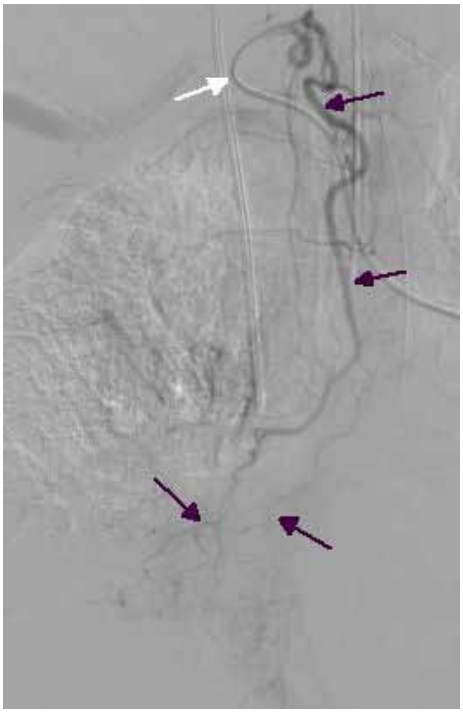

Figure 3a: Selective right bronchial digital subtraction arteriogram. The right bronchial artery was selectively cannulated with a 4F Cobra catheter (white arrow). The right bronchial artery (purple arrows) was seen dilated and tortuous while supplying the right lower lobe lung tumour.

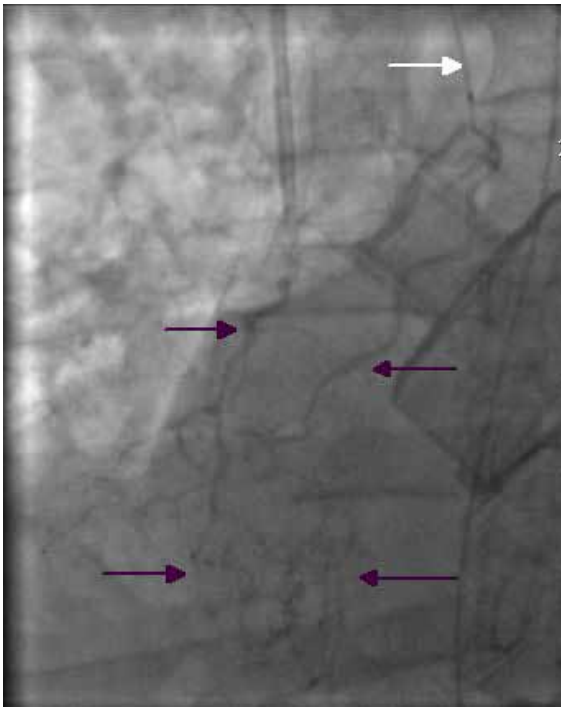

Figure 3b: Superselective arteriogram of the right bronchial artery with a microcatheter (white arrow) showing the terminal arteries supplying the right lower lobe lung tumour (purple arrows).

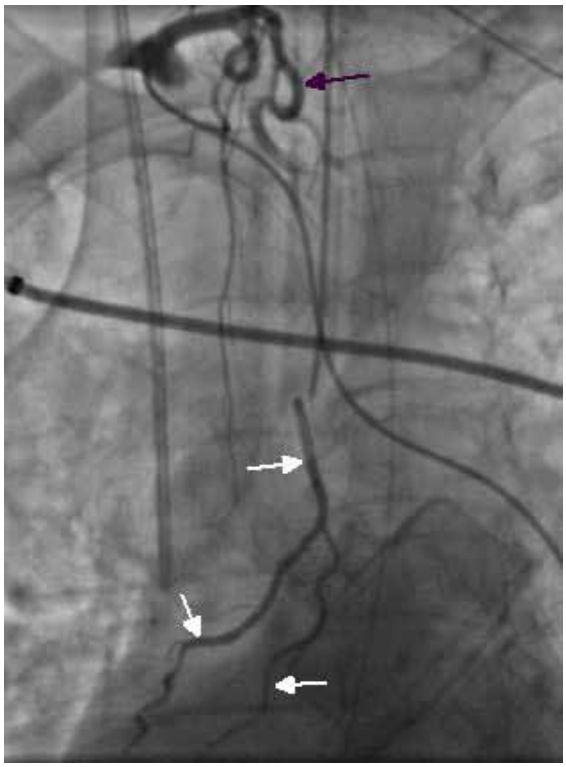

Figure 4: Selective subclavian arteriogram post-embolisation. The glue cast (white arrows) was within the distal branches of the right bronchial artery. Only the proximal right bronchial artery (purple arrow) became opaque post-embolisation.

developed another bout of massive haemoptysis and succumbed to it.

Discussion

A good and thorough knowledge of bronchial artery anatomy is essential to the interventional radiologist prior to a BAE, because 90% of the source of massive haemoptysis is from the bronchial circulation (1). The normal bronchial artery usually arises from the descending thoracic aorta between the T5 and T6 vertebral levels (1, 5). BAAO is defined as a bronchial artery that arises outside the region between the T5 and T6 vertebral levels (1,5). Sancho et al. reported that 8.3% of their patients had anomalous bronchial arteries (5). Out of the 27 anomalous bronchial arteries in their series, only 1 originated from the right subclavian artery. The rest originated from the aortic arch ($n = 24$), thyrocervical trunk ($n = 1$), and lower descending thoracic aorta ($n = 1$). Other sites of anomalous origin have been reported such as the costocervical trunk, brachiocephalic artery, pericardiophrenic artery, inferior phrenic artery, abdominal aorta, and coronary artery (1,7). These anomalous-origin bronchial arteries enter the pulmonary parenchyma through the pulmonary ligament or the adherent pleura, and their course is not parallel to the major bronchi (5).

BAE is currently an established procedure in the management of massive and recurrent haemoptysis, with success rates ranging 88%–100% (2–4). It has the advantage over surgery, especially in patients with poor pulmonary reserve, poor co-morbid conditions or massive haemoptysis (2). In surgical candidates, BAE has a role in controlling the haemorrhage while preparing the patient for an elective surgery (2).

Several types of embolic materials have been used for BAE, such as coils, gel foam, PVA, and glue (2–5,7). Among all of these, PVA with a diameter of 350–500 μm is the recommended embolic material because, in the process of BAE, the embolic materials should not be allowed to pass through the largest bronchopulmonary anastomosis, which measures 325 μm (1). However, the usage of a large-sized PVA has its

own problems. The delivery of large-sized PVA through a microcatheter is difficult since it can form a plug within the lumen (4). Because PVA is also mixed with water-soluble contrast materials that have higher viscosities than the iodised oil contrast material (Lipiodol, Guerbet) used to mix the glue, there will be a high risk of reflux during the later phase of embolisation as pressure within the distal vascular bed increased (4). Considering all of these potential problems, diluted glue was then used in this situation.

Since the 1990s, glue was exclusively used in the treatment of cerebral and spinal arteriovenous malformations, which led to the increased experience of interventional radiologists (IRs) in the control of its behaviour (8). The usage of glue is not without its own risk. The most important factor in controlling the risk in glue embolisation lies with a well-trained IR. The IR needs to mix an optimal glue concentration to enable the control of the time needed for glue polymerisation, and it also must perform a slow but continuous pace of injection to allow cast formation within the arterial lumen and tumour bed.

The technique of bronchial artery embolisation with glue in massive haemoptysis has been described by Razavi and Murphy (9). The usage of a microcatheter through a 5 or 6F guiding catheter is recommended for more secure catheter positioning. An optimal glue concentration is essential for preventing early polymerisation. The field of injection, microcatheter hub and gloves must be rinsed with a 5% dextrose solution to prevent glue from sticking to it. The microcatheter is then flushed with the 5% dextrose solution to prevent early polymerisation of glue within the microcatheter. The injection of the glue mixture can be done by either a single embolisation technique or a sandwich technique (9). During the glue injection, the IR needs to closely observe the occurrence of reflux to prevent the microcatheter from being embedded within the glue cast (4). Upon completion of embolisation, the syringe is aspirated and the microcatheter is rapidly removed. Baltacıoğlu et al. obtained an immediate success rate of 100% in controlling haemoptysis using 12.5% diluted glue in their series (4). Using glue, Razavi and Murphy had lower rebleeding rates (16.6%) as compared with the use of PVA (33%) in patients with massive haemoptysis (9).

In our patient, the flush thoracic aortogram failed to identify the source of pulmonary haemorrhage. Selective arteriograms of all of the visualised intercostal arteries also failed to identify the source of haemorrhage. Only a selective arteriogram of the right subclavian artery managed to identify the right bronchial artery supplying the right lower lobe tumour originating from the right subclavian artery. Embolisation of the right bronchial artery with a total of 3 ml of 15% diluted glue (1.5 ml of Histoacryl Glue, B Braun diluted with 8.5 ml of Lipiodol, Guerbet) produced an immediate control of the haemorrhage.

However, upon completion of radiotherapy, the patient developed a second bout of massive haemoptysis in another centre exactly 2 weeks after the embolisation. This recurrent massive haemoptysis commonly occurs in 20% to 30% of patients due to several factors (1,9), which include incomplete initial embolisation, recanalisation of embolised vessels, progression of disease, non-bronchial systemic arterial supply, or revascularisation by collateral circulation. In view of these problems, many centres have used multidetector CT (MDCT) angiography of the thorax to depict the bronchial or non-bronchial systemic arteries in patients with massive haemoptysis prior to BAE (6,10). Hartman et al. was able to depict ectopic (anomalous origin) bronchial arteries in 36% of their studied population using MDCT angiography (10). MDCT angiography of the thorax gives important anatomical information to the IR prior to a BAE.

In conclusion, a bronchial artery of anomalous origin must be suspected in patients in whom the source of haemorrhage is not evident and who have persistent haemoptysis post-embolisation.

Authors' Contributions

Conception and design: ARMR
Provision of study materials or patients, collection and assembly of data: ARMR, NTH, HSH
Drafting of article, final approval of article: ARMR, NTH, HSH, ASM
Critical revision of article: ARMR, ASM

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References

1. Yoon W, Kim JK, Kim YH, Chung TW, Kang HK. Bronchial and nonbronchial systemic artery embolization for life-threatening hemoptysis: A comprehensive review. *Radiographics*. 2002;**22(60)**:1395–1409.

2. Shigemura N, Wan IY, Yu SC, Wong RH, Hsin MK, Thung HK, et al. Multidisciplinary management of life-threatening massive hemoptysis: A 10-year experience. *Ann Thorac Surg*. 2009;**87(3)**:849–853.

3. Swanson KL, Johnson CM, Prakash UB, McKusick MA, Andrews JC, Stanson AW. Bronchial artery embolization : Experience with 54 patients. *Chest*. 2002;**121(3)**:789–795.

4. Baltacıoğlu F, Cimşit NC, Bostanci K, Yüksel M, Kodalli N. Transarterial microcatheter glue embolization of the bronchial artery for life-threatening hemoptysis: Technical and clinical results. *Eur J Radiology*. 2008;**73(2)**:380–384.

5. Sancho C, Escalante E, Domínguez J, Vidal J, Lopez E, Valldeperas J, et al. Embolization of bronchial arteries of anomalous origin. *Cardiovasc Interventl Radiol*. 1998;**21(4)**:300–304.

6. Chung MJ, Lee JH, Lee KS, Yoon YC, Kwon OJ, Kim TS. Bronchial and nonbronchial systemic arteries in patients with hemoptysis: Depiction on MDCT angiography. *AJR Am J Roentgenol*. 2006;**186(3)**:649–655.

7. Cho J, Shin T, Jun K, Ryoo J, Choi H, Choi B, et al. Transcatheter embolization of bronchial artery arising from left circumflex coronary artery in a patient with massive hemoptysis. *Cardiovasc Intervent Radiol*. 2010;**33(1)**:169–172.

8. DeMeritt JS, Pile-Spellman J, Mast H, Moohan N, Lu DC, Young WL, et al. Outcome analysis of preoperative embolization with N-butyl cyanoacrylate in cerebral arteriovenous malformations. *AJNR Am J Neuroradiol*. 1995;**16(9)**:1801–1807.

9. Razavi MK, Murphy K. Embolization of bronchial arteries with N-butyl cyanoacrylate for management of massive hemoptysis: A technical review. *Tech Vasc Interv Radiol*. 2007;**10(4):**276–282.

10. Hartmann IJ, Remy-Jardin M, Menchini L, Teisseire A, Khalil C, Remy J. Ectopic origin of bronchial arteries: Assessment with multidetector helical CT angiography. *Eur Radiol*. 2007;**17(8)**:1943–1953.

Case Report

Atretic Cephalocele — An Uncommon Cause of Cystic Scalp Mass

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Abstract

A 6 year-old girl presented with a midline parietal scalp swelling that had been gradually enlarging since birth. Magnetic resonance imaging revealed communication of the cyst with the subarachnoid space through a calvarial defect, with concomitant findings of vertically positioned straight venous sinus and subependymal grey matter heterotopia. A diagnosis of atretic cephalocele was thus made based on these classical imaging findings.

Keywords: *: cysts, encephalocele, scalp, magnetic resonance imaging, medical sciences*

Introduction

Lumps and bumps in the scalp of a child are common presentations and may be difficult to diagnose because the differential diagnoses are very broad. Clinical examination alone is often inadequate for a final diagnosis. A good imaging strategy is required for proper diagnosis, particularly in cases with intracranial association. The possible causes of scalp lumps and bumps in a child include encephalocele, lipoma, sebaceous cyst, inclusion cyst, sinus pericranii, vascular lesion (e.g., haemangioma), tumours (e.g., Langerhans cell histiocytosis, fibrosarcoma, rhabdomyosarcoma, and metastatic disease), bone and soft tissue infection, and traumatic lesions (1). An accurate diagnosis is imperative in every case as it bears significant therapeutic implications.

Case Report

A 6 year-old girl with uneventful antenatal history presented with a painless midline scalp swelling over the parietal region starting in early infancy, with slow progressive enlargement of the swelling over the years. The parents expressed concern as the child had recently developed the habit of scratching the swelling, resulting in localised eczema. The child was otherwise asymptomatic with normal developmental milestones. There was no report of seizures.

Physical examination showed a midline scalp mass that was cystic and non-mobile, measuring 3 x 4 cm in diameter (Figure 1). The scalp skin showed mild eczema and no discolouration. The hair overlying the swelling appeared sparse. The swelling was non-pulsatile and non-reducible. There was no bruit heard.

Figure 1: A midline scalp mass.

Magnetic resonance imaging (MRI) revealed a parietal scalp cyst measuring 2.5 x 2.5 x 2 cm. There was a narrow cerebrospinal fluid (CSF) tract that connected the base of the cyst to the prominent posterior interhemispheric fissure through a small midline calvarial defect (Figure 2). No cerebral tissue was noted within the cyst. The straight venous sinus was nearly vertical in position and was observed just anterior to the CSF tract connecting the intracranial subarachnoid space to the scalp cyst. There was prominence of the superior cerebellar cistern and the cisterna magna. At the base of the scalp cyst, there was varicosity that drained into the superior sagittal

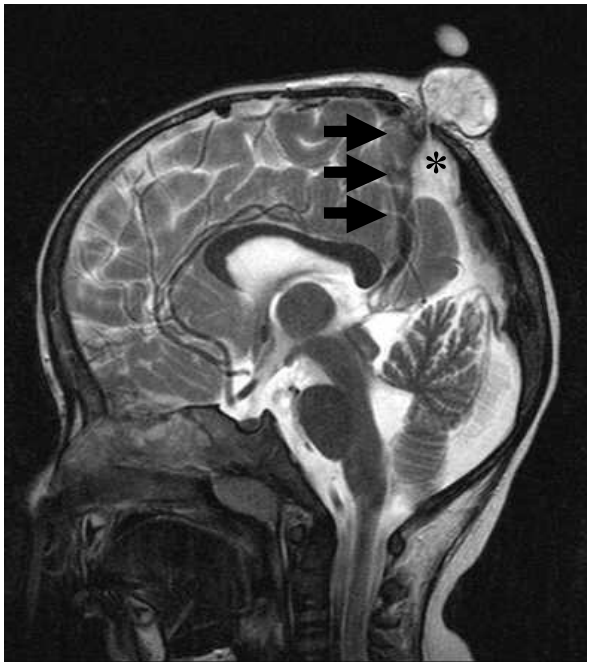

Figure 2: Midsagittal T2WI section showing the parietal scalp cephalocele with communication with the prominent posterior interhemispheric fissure (*) through a small midline calvarial defect. Vertical orientation of the straight venous sinus (arrows), prominent superior cerebellar cistern and cisterna magna were also observed.

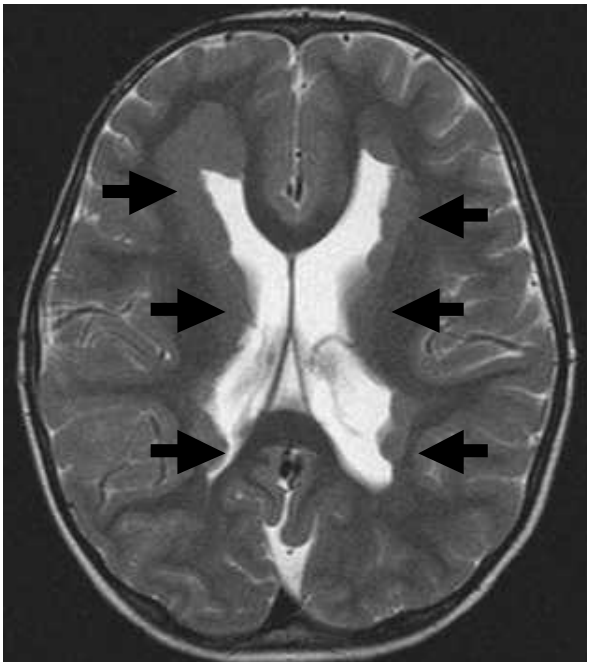

Figure 3: Axial T2WI section showing nodular subependymal grey matter heterotopia (arrows) along the lateral margin of the lateral ventricles.

venous sinus. Nodular subependymal grey matter heterotopia was noted along the lateral margin of the lateral ventricles (Figure 3). Midline structures, particularly the corpus callosum, were normal. Patient had the scalp cyst removed by the neurosurgeon and had an uneventful recovery thereafter.

Discussion

Atretic cephaloceles are small, skin-covered, subscalp lesions that contain meninges, neural rests, and glial rests. They are also known as atypical or rudimentary meningoceles, meningeal heterotopias, and meningoceles manqué. The development of atretic cephalocele has not been clearly elucidated. Atretic cephaloceles probably represent a manifestation of defects in closure of part of the neural tube (2). All previous reports of atretic cephalocele have been due to sporadic causes, with the exception of one series that reported three siblings having the same clinical diagnosis without any syndromic association (3).

There is a wide range of clinical presentations of patients with atretic cephaloceles. A child may be normal with regard to neurodevelopmental milestones, or may have severe mental retardation if the atretic cephalocele is associated with severe intracranial anomalies, such as malformations of cortical development, Walker-Warburg syndrome, or ventriculomegaly (4). On examination, a small scalp nodule is palpable and generally covered with hairless scalp. In some cases, the cranial defect may be palpable.

MRI is the best imaging modality to depict this entity. Upon imaging, an atretic cephalocele presents as a subscalp cyst with an associated calvarial defect at the vertex. Persistent vertical embryonic positioning of the straight sinus is seen in a majority of cases (4). There may be associated intracranial abnormalities, such as grey matter heterotopia, Walker–Warburg syndrome (with characteristic lissencephaly, hypoplasia of the metencephalon, and moderate to marked ventricular enlargement), lobar holoprosencephaly, Dandy–Walker syndrome, hypogenesis of the corpus callosum, extra-axial cysts, microphthalmia, and retro-ocular cysts (4,5).

The prognosis of atretic cephalocele is generally good. Of the reported cases, only one

case, which was associated with severe intracranial abnormalities (i.e., moderate hydrocephalus, lissencephaly, and metencephalic hypoplasia), showed profound psychomotor delay; the patient died at 3 years of age (4). Other reports of atretic cephalocele, even with associated with grey matter heterotopia, showed normal development as per the case presented herein. The treatment is surgical excision of the cyst and oversewing of the tract formed by the dura (5).

Acknowledgements

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Authors' contributions

Conception and design, collection and assembly of data, analysis and interpretation of data, drafting of article, final approval of the article: SLW
Provision of study materials or patients: HLL
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References

1. Morón FE, Morriss MC, Jones JJ, Hunter JV. Lumps and bumps on the head in children: Use of CT and MR Imaging in solving the clinical diagnostic dilemma. *RadioGraphics*. 2004;**24(6)**:1655–1674.

2. Atlas SW, editor. MRI of the Brain and Spine [CD-ROM]. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2003.

3. Martinez-Lage JF, Martinez Robledo A, Poza M, Sola J. Familial occurrence of atretic cephaloceles. *Pediatr Neurosurg*. 1996;**25(5)**:260–264.

4. Patterson RJ, Egelhoff JC, Crone KR, Ball WS Jr. Atretic parietal cephaloceles revisited: An enlarging clinical and imaging spectrum? *AJNR Am J Neuroradiol*. 1998;**19(4)**:791–795.

5. Saatci I, Yelgec S, Aydin K, Akalan N. An atretic parietal cephalocele associated with multiple intracranial and eye anomalies. *Neuroradiology*. 1998;**40(12)**:812–815.

Case Report

Migration of the Abdominal Catheter of a Ventriculoperitoneal Shunt into the Mouth: A Rare Presentation

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Abstract

A 1-year-old boy with a history of exomphalos and hydrocephalus had surgeries to correct these pathologies. The ventriculopleural (VP_L) shunt inserted for hydrocephalus was complicated by pleural effusion, for which a ventriculoperitoneal (VP) shunt was inserted on the contralateral side. He subsequently presented with protrusion of the distal VP shunt tip from the mouth due to perforation through the gastrointestinal tract. The child also had a history of peritonitis post-exomphalos repair, which may have predisposed him to this relatively uncommon shunt complication. Probable causes and risk factors of the perforation are discussed.

Keywords: *foreign-body migration, in-dwelling catheters, intestinal perforation, post-operative complications, ventriculoperitoneal shunt, medical sciences*

Introduction

Ventriculoperitoneal (VP) shunting is a common neurosurgical procedure for hydrocephalus. Rates of complications of VP shunts are widely reported, ranging 24%–47% (1), of which abdominal complications account for about 25% (2). Bowel perforation is reported to occur between 0.1% and 0.7% of the time (3–5) and may lead to significant morbidity and mortality as a result of peritonitis or meningoventriculitis. After bowel perforation, the catheter may present at one of the natural orifices, of which perianal protrusion is more common than perioral protrusion.

We report a case of an infant who presented with perioral protrusion of the distal end of the VP shunt catheter as a delayed complication of the procedure. Probable causes, risk factors, and management principles are discussed.

Case Report

A 1-year-old boy was admitted to the neurosurgical unit after his mother noticed a tube coming out of his mouth after an episode of vomiting (Figure 1). He was a preterm baby born at 35 weeks of gestation via normal vaginal delivery with birth weight of 2936 grams. He had exomphalos major and was repaired at 20 hours of age by the paediatric surgical team. The repair was complicated by post-operative peritonitis, which was managed conservatively with parenteral antibiotics. He also had *Haemophilus influenzae B* meningitis complicated by hydrocephalus, for which a ventriculopleural (VP_L) shunt was placed on the right side at one month of age. The decision to shunt the cerebrospinal fluid (CSF) to the pleural space was made due to the recent post-operative peritonitis.

The VP_L shunt was complicated by pleural effusion, and a VP shunt was inserted on the left side at the age of six months. The VP_L shunt was ligated but not removed as we were uncertain if the VP shunt would function well with the possibility of adhesions from the previous peritonitis obstructing the peritoneal end of the VP shunt. The post-operative period was uneventful and the pleural effusion resolved spontaneously.

The boy remained well until six months later, at 1 year of age, when he presented with protrusion of the distal end of the VP shunt through the mouth after an episode of vomiting. The child was afebrile and was clinically well and alert. There was no clinical evidence of meningitis or increased intracranial pressure (ICP). Examination of the abdomen did not reveal any signs of peritonitis. Chest and abdominal X-rays revealed that the peritoneal catheter had passed through the stomach and ascended up the oesophagus. There was no free gas under the diaphragm on the X-ray images (Figure 2 and 3). Intravenous vancomycin and ceftriaxone were started prior to the removal of the shunt and subsequent insertion of an external ventricular drain (EVD). We continued treatment with parenteral antibiotics and checked the CSF for leukocytosis and growth of organisms

Figure 1: Ventriculoperitoneal shunt tip protruding periorally

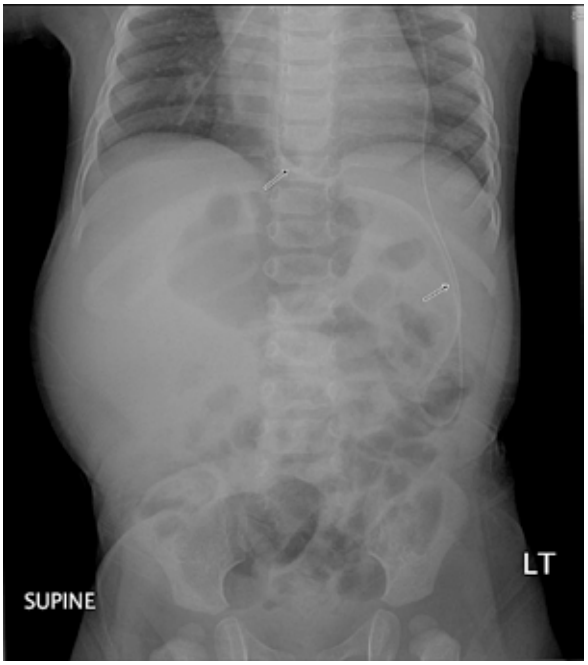

Figure 2: Abdominal X-ray showing ventriculoperitoneal shunt tip perforating the stomach and migrating up the oesophagus (black arrow)

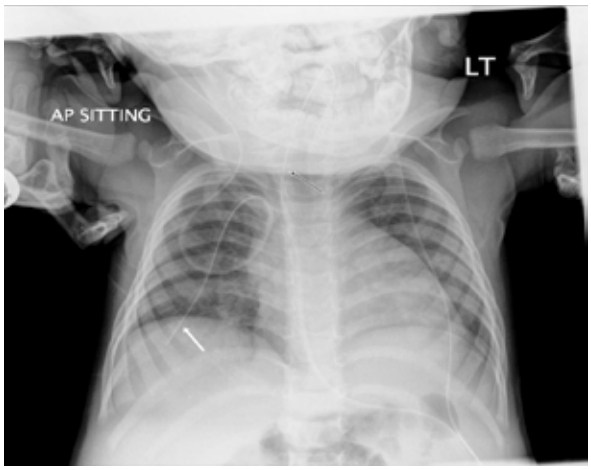

Figure 3: Chest X-ray showing ventriculoperitoneal shunt tip ascending the oesophagus (black arrow). Right-sided ventriculopleural shunt could be seen (white arrow)

on the 14th post-operative day. There was no evidence of infection and the decision was made to reinsert a VP shunt on the left side.

The operation and post-operative recovery was uneventful. The child was discharged well from our unit on the 7th post-operative day. The patient has been followed in the outpatient clinic since discharge. He has had regular follow-up for one year and has remained well with no complications related to the surgery. There were also no signs or symptoms of obstruction of the VP shunt.

Discussion

The VP shunt is the most widely used procedure in the treatment of hydrocephalus. As with any surgery, complications can occur. Although infrequent, abdominal complications have been reported. These include pseudocyst formation, intestinal obstruction, and bowel perforation and penetration (3). Most of these abdominal complications are delayed in occurrence. The incidence of bowel perforation by a shunt catheter is known to be low, occurring in 0.1%–0.7% of cases of complications (3–5). These complications can result in potentially fatal ventriculitis, meningitis, intraperitoneal abscess, faecal fistulae, peritonitis, or sepsis (6,7).

There are at least 119 cases of catheter-induced gut perforations reported in the literature (5), with the colon being the most common site of

perforation (70%), followed by the stomach (16%), and the small bowel (14%). It is postulated that the incidence of perforation is related to the mobility of the gut. The colon, being the most immobile, is the most frequently perforated viscus. The literature reviews (4,5) also revealed that, at all levels of perforation, the catheter was more likely to extrude through the anus (61.9%) or not at all (31.4%). Oral route protrusion was relatively uncommon as compared to anal extrusion, with a total of 10 cases reported in the literature (4,5,8–15).

In this case report, the transoral presentation of the VP shunt catheter occurring six months after its insertion indicates delayed gut perforation. This complication was likely a result of a chronic process as opposed to an early bowel perforation that is usually due to direct injury or perforation during the shunting procedure itself. Delayed gut perforation has been found to be associated with young age, male gender, malnutrition, silicon allergy, length of catheter, previous abdominal surgery, and infection (9,13). Age seemed to be the main risk factor for bowel perforation. It was postulated that, in children, their weaker intestinal musculature and stronger intestinal peristaltic activity result in higher incidence of bowel perforation (4). The catheter tip causes a chronic irritative process to the serosal surface of the bowel wall and subsequently leading to repeated pressure and inflammation, and eventual ulceration and perforation. Our patient is a male and presented at one year of age with a history of peritonitis after abdominal surgery for exomphalos major. Furthermore, the distal length of the peritoneal catheter that was placed in the peritoneal cavity was about 20 cm, and it was inserted into the left hypochondrium. The previous peritoneal infection and adhesions together with the long distal length of the VP shunt contributed to the pressure necrosis of his stomach wall. We postulated that this eventually resulted in the penetration and subsequent fibrotic wall formation along the perforated site of the peritoneal cavity. As such, the child did not present with signs of peritonitis or gas under the diaphragm in the abdominal X-ray. The tube seemed to have penetrated the stomach wall and then ascended along the oesophagus and protruded out of the mouth during the child's forceful emesis.

Diagnosis of shunt perforation of the bowel may not be obvious if the shunt catheter does not protrude through the natural orifices (13), and patients do not always present with significant case report. This fact is because the fibrous tract formed at the perforated site usually seals the perforation, preventing spillage of faecal matter into the peritoneum, which would otherwise lead to peritonitis. Hence, the correct diagnosis may be delayed until a later stage at which ventriculitis or central nervous system (CNS) infection has been fully established, leading to significant morbidity and/or mortality. Radiologic investigations, such as plain X-ray and contrast CT, are useful in cases when the diagnosis of bowel perforation is suspected.

The management principles of such VP shunt complications include removal of extruding shunt tubing, attention to the perforated viscus, and treatment of possible peritonitis and/or meningoventriculitis. For this patient, we removed the extruding shunt though the oral orifice after the removal of the shunt valve and ventricular catheter through the cephalic incision. There was no need for exploratory laparotomy as the child did not show any signs or symptoms of peritonitis. Intravenous antibiotics were given for 14 days before reinsertion of the VP shunt as per our hospital protocol, although the duration may not need to be this lengthy as no clinical signs of meningitis or ventriculitis were noted.

In conclusion, the appearance of the tube in the mouth represented bowel penetration. Spontaneous bowel penetration or perforation is a rare complication of VP shunt surgery. A high index of suspicion is essential, particularly in paediatric patients, to diagnose perforation or penetration as the abdominal signs and symptoms may be vague (16). Abdominal radiology, including plain X-ray and contrast CT, may be required in some cases (5). CSF culture is also mandatory for diagnosis of retrograde CSF infection to ensure early and appropriate antibiotic treatment. Early removal of the suspected shunt and conversion to external ventricular drain for CSF diversion are important measures for control of CNS infection. The selection of a clean site for CSF diversion is imperative for successful management of complicated paediatric cases.

Authors' Contributions

Conception and design, provision of study materials or patients, collection and assembly of data, analysis and interpretation of data, drafting of article: SWL, LS
Critical revision of the article: SWL, TTY, NC
Final approval of article: TTY, NC

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References

1. Christoph CL, Poole CA, Kochan PS. Operative gastric perforation: A rare complication of ventriculoperitoneal shunt. *Pediatr Radiol*. 1995;**25 Suppl 1**:S173–S174.

2. Agha FP, Amendola MA, Shirazi KK, Amendola BE, Chandler WF. Unusual abdominal complications of ventriculo-peritoneal shunts. *Radiology*. 1983;**146(2)**:323–326.

3. Sathyanarayana S, Wylen EL, Baskaya MK, Nanda A. Spontaneous bowel perforation after ventriculoperitoneal shunt surgery: Case report and a review of 45 cases. *Surg Neurol*. 2000;**54(5):**388–396.

4. Park CK, Wang KC, Seo JK, Cho BK. Transoral protrusion of a peritoneal catheter: A case report and literature review. *Child Nerv Syst*. 2000;**16(3**):184–189.

5. Odebode TO. Jejunal perforation and peroral extrusion of a peritoneal shunt catheter. *Brit J Neurosurg*. 2007;**21(2)**:235–236.

6. Azimi F, Dinn WM, Naumann RA. Intestinal perforation. An infrequent complication of ventriculoperitoneal shunt. *Radiology*. 1976;**121**:701–702.

7. Ansari S, Nejat F, Dadmehr M. Extrusion of ventriculoperitoneal shunt catheter through the rectum and retrograde meningitis. *Pediatr Infect Dis J*. 2005;**24(11)**:1027.

8. Griffith JA, DeFeo D. Peroral extrusion of a ventriculoperitoneal shunt catheter. *Neurosurgery*. 1987;**21(2)**:259–261.

9. Fermin S, Fernandez-Guerra RA, Sureda PJ. Extrusion of peritoneal catheter through the mouth. *Child Nerv Syst*. 1996;**12(9)**:553–555.

10. Jiménez Moya A, Penela Vélez De Guevara T, Gracia Remiro R, Romero Escós D, Santana Rodríguez C, Reig Del Moral C, et al. Extrusion of a ventriculoperitoneal shunt catheter through the mouth. *An Esp Pediatr*. 2001;**54(6)**:609–610.

11. Kothari PR, Shankar G, Kulkarni B. Extruded ventriculo-peritoneal shunt: An unusual complication. *J Indian Assoc Pediatr Surg*. 2006;**11(4)**:255–256.

12. Murali R, Ravikumar V. Transoral migration of peritoneal end of ventrriculoperitoneral shunt: A case report of a rare complication and review of literature. *J Pediatr Neurosci*. 2008;**3(2)**:166–168.

13. Berhouma M, Messerer M, Houissa S, Khaldi M. Transoral protrusion of a peritoneal catheter: A rare complication of ventriculoperitoneal shunt. *Pediatr Neurosurg*. 2008;**44(2)**:169–171.

14. Sridhar K, Karmarkar V. Peroral extrusion of ventriculoperitoneal shunt: Case report and review of literature. *Neurol India*. 2009;**57(3)**:334–336.

15. Sinnadurai M, Winder MJ. Silicone spaghetti. *J Clin Neurosci*. 2009;**16(10):**1348–1350.

16. Silvav G, Tun K, Dolgun H, Unlu A, Selcuki M. The spontaneous umbilical perforation of the distal end of ventriculoperitoneal shunt. *Neuchirurgie*. 2002;**48(2–3 Pt 1)**:128–130.

Case Report

Two Cases of Congenital Vallecular Cyst: A Reminder of the Potentially Fatal Cause of Upper Airway Obstruction in Infants

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Abstract

Vallecular cyst, a benign yet rare laryngeal lesion, may cause stridor and even life-threatening upper airway obstruction in infants. It can cause apnoea and poor feeding habits, thus reducing the chance of survival. Although laryngomalacia remains the most common cause of stridor in this age group, awareness and a high level of suspicion for this condition can help lead to early management and intervention. Direct laryngoscopy is accepted as the gold standard for diagnostic purposes, and marsupialisation of the cyst is the preferred treatment. We describe 2 cases of vallecular cysts in infants admitted to our hospital where timely diagnoses led to appropriate treatment.

Keywords: *cyst, infant, laryngoscopy, magnetic resonance imaging, stridor,medical sciences*

Introduction

A vallecular cyst is a rare type of laryngeal cyst seen in infants. Stridor with or without respiratory distress is the most common presentation secondary to narrowing of the airway. Even though there have been many cases reported in the literature, we strongly believe that medical officers in district hospitals or even smaller clinics, including paediatricians and general practitioners, need to be repeatedly reminded and made aware that this condition exists so that it can be quickly identified and affected children can be managed promptly and appropriately. Here, we describe 2 cases of vallecular cysts seen within a 1-year period in our hospital. In both cases, patients were initially seen by a general practitioner and then a medical officer in a district hospital—the typical scenario for vallecular cyst patients, which highlights the importance for every medical professionals and clinicians to recognize and be reminded of this lesion.

Case 1

A 2-month-old baby girl was referred to us for noisy breathing and progressive respiratory distress. She was born at term by vaginal delivery with an uneventful neonatal period. Noisy breathing was noted by her parents a few days after birth and continued to worsen; 2 days prior to the referral, she had increasingly noisy breathing, respiratory distress, and refused to feed. The baby was brought to a general practitioner, who then referred her to the hospital.

Upon examination, her vital signs were stable and there were no dysmorphic features. She was in respiratory distress with marked inspiratory stridor, and she was also tachypnoeic with suprasternal and subcostal recession that improved slightly with oxygen supplementation. The lung air entry was decreased bilaterally. Other systems were unremarkable. Laboratory results were normal.

An initial diagnosis of severe laryngomalacia was made. A flexible upper airway scope showed a cystic mass at the base of the tongue. Urgent direct laryngoscopy under general anaesthesia (GA) confirmed the cystic mass at the base of the tongue, which was pushing the epiglottis against

the posterior pharyngeal wall and obstructing the laryngeal inlet. The aryepiglottic folds were short with the presence of redundant mucosa over the arytenoids. An emergency tracheostomy was performed to view the upper airway obstruction and allow further radiological characterisation of the mass prior to a definitive surgery.

Magnetic resonance imaging (MRI) of the neck, performed once the baby's condition had been stabilised, showed a well-defined, non-enhancing, fluid-filled mass located at the base of the tongue, measuring approximately 1.5 x 1.3 cm in size (Figures 1 and 2). The airway was narrowed while the epiglottis was obliterated. Based on the MRI findings, differential diagnosis of a thyroglossal duct cyst or a vallecular cyst was made.

Marsupialisation of the cystic mass (Figure 3) and bilateral laser aryepiglottoplasty were subsequently performed. The cyst contained a clear mucoid secretion. The tracheostomy tube was decannulated a week later. A final diagnosis of a vallecular cyst was made based on the operative and MRI findings.

The stridor markedly improved and the baby had an uneventful recovery. At the 3-month follow-up examination, the child was thriving well with no evidence of stridor. A flexible pharyngoscopy showed no evidence of cyst recurrence, and the laryngeal inlet was well-visualized.

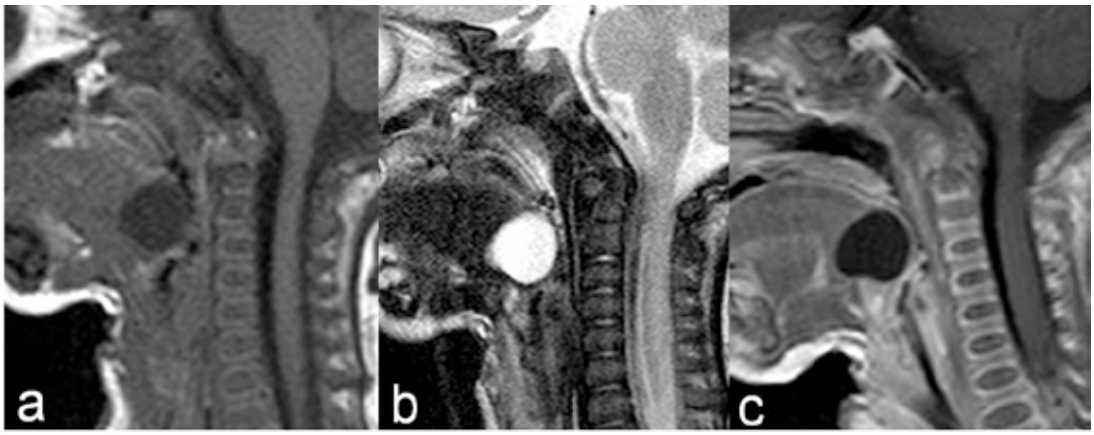

Figure 1: Case 1. Sagittal magnetic resonance imaging, MRI (a) T1-weighted, (b) T2-weighted, and (c) T1 post-intravenous Gadolinium, demonstrating a well-defined, rounded, non-enhancing cystic mass located at the base of the tongue pushing the epiglottis posteriorly and causing narrowing of the supraglottic region.

Case 2

A previously well, term 3-month-old baby boy was referred to our hospital for progressive difficulty in breathing. He was asymptomatic until he developed stridor and progressive respiratory distress from 2 months old. He was initially brought to a district hospital and was noted to be cyanosed upon examination and required intubation. He was then treated as having bronchopneumonia and was extubated a few days later. A flexible upper airway scope revealed a cystic lesion in the supraglottic area. He was then referred to our hospital for further management.

Upon examination in our hospital, he was not dysmorphic and his vital signs were stable. We also observed inspiratory stridor as well as suprasternal and subcostal recession. The air entry was decreased in both lungs. Other systems were normal. Laboratory results were also normal.

A diagnostic direct laryngoscopy under GA demonstrated a cystic mass adherent to the base of the tongue and vallecula. The epiglottis was compressed posteriorly and the laryngeal inlet was obscured. The aryepiglottic folds were short. The vocal cord and subglottic region were normal. An emergency tracheostomy was done in view of the impending airway obstruction.

A computed tomography (CT) scan showed a rounded, hypodense lesion at the base of the tongue measuring approximately 1.2 x 1.1 cm in size, which was causing obliteration of the airway (Figure 4). Minimal enhancement of this lesion was noted post-intravenous contrast.

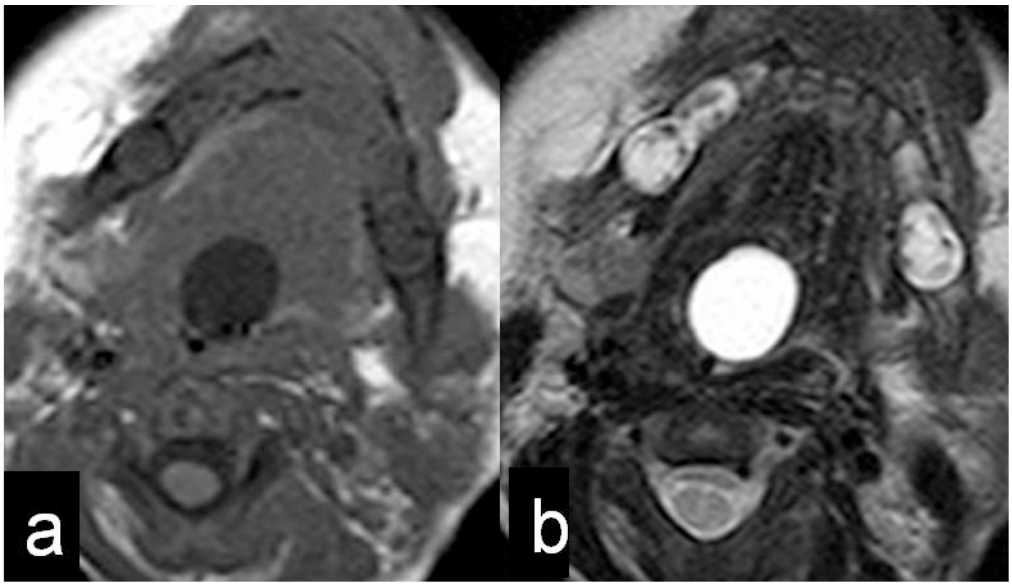

Figure 2: Case 1. Axial magnetic resonance imaging, MRI (a) T1-weighted and (b) T2-weighted showing the same cystic mass in Figure 1 obliterating the airway.

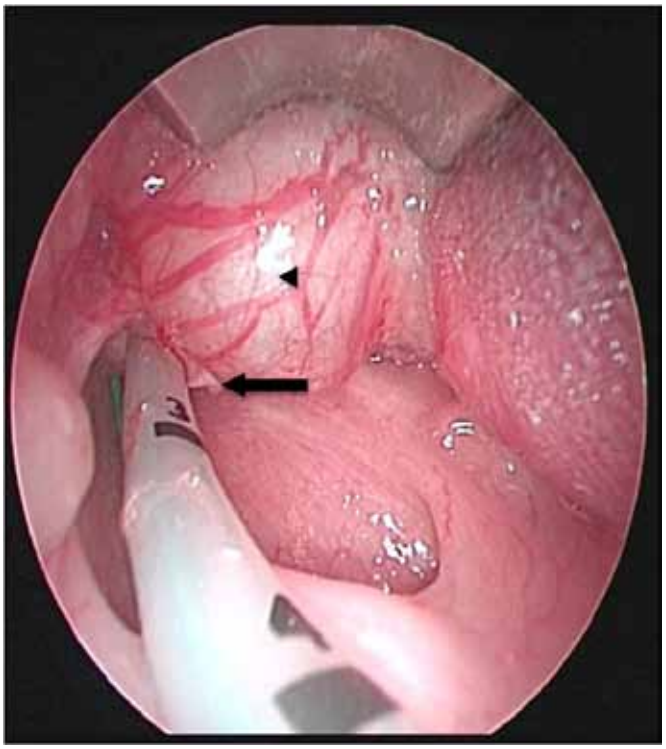

Figure 3: Case 1. The cystic mass (arrow head), shown here just prior to marsupialisation, obscuring the laryngeal inlet by pushing the epiglottis (arrow) downward.

Figure 4: Case 2. Axial contrast-enhanced computed tomography (CT) image showing a rounded minimally enhancing low attenuation mass (arrows) at the base of the tongue protruding into and causing some obliteration of the airway.

Marsupialisation of the vallecular cyst was performed, and the cyst contained a clear fluid. A repeat flexible upper airway scope 1 week later revealed reaccumulation of the cyst with collapsed epiglottis. The previous incision was reopened under GA, and this time, the aryepiglottic folds were released with a laser. The laryngeal inlet was observed, and no recurrence of the cyst was seen in the subsequent repetition of upper airway scope.

Discussion

The 2 cases described in this article illustrate that even a mass that is small in size, located within the larynx of infants and younger children, has the potential to cause significant upper airway obstruction because of the relatively small airway. Such a condition may be fatal if not clinically identified.

Inspiratory stridor, which was observed in both our patients, is an important clinical finding or symptom and usually indicates a lesion arising from the supra- glottis and glottis region. Therefore, the underlying cause must be determined. The stridor occurs due to a "ball–valve" effect caused by the obstructing lesion or mass. The most usual cause of inspiratory stridor and supralaryngeal airway obstruction in infancy is laryngomalacia (1–3). Another clinical manifestation that may develop in the presence of a laryngeal lesion is repeated apnoea. Unless there are alarming symptoms or signs of respiratory distress from upper airway obstruction, diagnosis of a laryngeal lesion may be delayed, and appropriate treatment may not be achieved in a timely manner.

Differential diagnosis of a lesion that may cause airway obstruction in this region would include haemangioma, vascular malformation, thyroglossal duct cyst, dermoid cyst, brachial cleft cyst, congenital ranula, and even alimentary tract duplication (4,5).

Laryngeal cysts represent a collection of benign laryngeal abnormalities and classifications that have been reported based on their histologic appearances, size, contents, or localisation within the larynx (6). Aryepiglottic cysts represent the most common laryngeal cysts in children (4,7). Laryngeal cysts located in the vallecular space, as seen in our patients, are thus called vallecular cysts. This entity is a rare but known cause of congenital stridor, respiratory distress, and

airway obstruction in infants (3,5,8). It can also cause interrupted feeding due to distress that may lead to failure to thrive (1,7,8). The vallecular cyst is a unilocular cystic mass of variable size that arises from the lingual surface of the epiglottis and usually contains clear, non-infected fluid (7,9). It is a ductal type of laryngeal cyst, which arises from fluid accumulation secondary to obstructed submucosal glands. The other type is a saccular cyst, which is submucosal cyst caused by mucous accumulation within the laryngeal saccule (2).

When a laryngeal lesion is suspected, visualisation by direct laryngoscopy is the main investigative tool in the diagnosis of a vallecular cyst, as the presence of the lesion can be confirmed and the degree of airway compromise can be assessed. Imaging modalities such as CT or MRI are useful, as they can provide information concerning the nature and extent of the lesion, particularly in the pre-operative setting. Although the lesions were already seen via endoscopy in both of our cases, further radiological imaging was required preoperatively to confirm their cystic nature, assess the vascularity of the lesions, and determine their actual sizes. CT or MRI can explicitly demonstrate the relationship of the lesion with surrounding structures, particularly the neurovascular structure, which is helpful during surgical exploration. Large lesions may obliterate or displace adjacent structures.

CT of most cystic masses will show similar low-density attenuation. Using MRI analysis, a vallecular cyst will appear hypointense on T1, hyperintense on T2, and does not enhance following intravenous Gadolinium. Although ultrasound can provide information about the structure, location, and vascularity of the lesion, further evaluation with CT or MRI is useful for the aforementioned reasons.

A vallecular cyst suspected during antenatal ultrasound and diagnosed with fetal MRI has been previously reported (4). Prenatal detection of this condition allows appropriate counselling and planned preparation for intervention. For example, an emergency tracheostomy may be warranted upon delivery when oral intubation is impossible or, in some centres, where the ex-utero intrapartum treatment (EXIT) procedure is performed.

Awareness and a high level of suspicion are required for a quick diagnosis of a laryngeal lesion or, as in our cases, vallecular cysts. Laryngeal lesion can be suspected from a thorough history and physical examination, especially when an infant presents with a congenital stridor. Imaging with MRI or CT would be helpful pre-operatively to further characterize the lesion. Direct laryngoscopy remains the major diagnostic tool for a definitive diagnosis. Surgical excision of the cyst is the treatment of choice and, when performed in a timely manner, gives a good prognosis.

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Authors' Contributions

Conception and design, drafting of the article, final approval of the article, administrative, technical, or logistic support: AAA
Provision of study materials or patients, collection and assembly of data, analysis and interpretation of the data, critical revision of the article: AAA, AFA, RARA

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References

1. Sands NB, Anand SM, Manoukian JJ. Series of congenital vallecular cysts: A rare yet potentially fatal cause of upper airway obstruction and failure to thrive in the newborn. *J Otolaryngol Head Neck Surg*. 2009;**38(1)**:6–10.

2. Brown DJ. Pediatric stridor. In: Mitchell RB, Pereira KD, editors. Fundamentals of pediatric otolaryngology for the clinician. New York: Humana Press; 2009. p. 137–147.

3. Breysem L, Goosens V, Vander Poorten V, Vanhole C, Proesmans M, Smet M. Vallecular cyst as a cause of congenital stridor: Report of five patients. *Pediatr Radiol*. 2009;**39(8)**:828–831.

4. Cuillier F, Samperiz S, Testud R, Fossati P. Antenatal diagnosis and management of a vallecular cyst. *Ultrasound Obstet Gynecol.* 2002;**20(6)**:623–626.

5. Mahajan V, Mathew JL, Singh M, Gupta R, Das A. Vallecular Cyst- Revisited. *Indian J Pediatr.* 2008;**75(10)**:1081–1082.

6. Ahrens B, Lammert I, Schmitt M, Wahn U, Paul K, Niggemann B. Life-threatening vallecular cyst in a 3-month-old infant: Case report and literature review. *Clin Pediatr (Phila).* 2004;**43(3)**:287–290.

7. Yang MA, Kang MJ, Hong J, Shin SH, Kim SD, Kim EK, et al. A case of congenital vallecular cyst associated with gastroesophageal reflux presenting with stridor, feeding cyanosis, and failure to thrive. *Korean J Pediatr.* 2008;**51(7)**:775–779.

8. Shah M, Mehta A. Letter to the Editor. Neonatal vallecular cyst. *Indian Pediatr.* 2006;**43(7)**:652.

9. Gutierrez JP, Berkowitz RG, Robertson CF. Vallecular cysts in newborns and young infants. *Pediatr Pulmonol.* 1999;**27(4)**:282–285

Abstracts of Theses Approved for the MMed at the School of Medical Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan, Malaysia

PAIN SYMPTOMS IN DEPRESSED OUTPATIENTS

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Introduction: Major depressive disorder is an important health problem and a major cause of disability worldwide. There is a strong association between depression and pain, which is influenced by various biological and psychosocial mechanisms. The combination of chronic pain and depression is associated with high rate of disability, socio-economic disadvantage, greater utilization of health care resources, as well as a considerable mortality rate.

Objectives: The study was conducted with the objectives of determining the proportion of adult depressed patients who attended the psychiatric clinic with pain symptoms and assessed the characteristics and personality traits of the patients. In addition, the study aimed to determine the association between specific personality traits, depression, and pain symptoms in the respondents.

Patients and Methods: A survey was carried out on patients aged 18 years and above with a diagnosis of major depressive disorder according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), who attended the psychiatric clinic in Hospital Universiti Sains Malaysia. Convenience sampling was carried out between February 2008 and June 2009. A questionnaire containing the socio-demographic data form, Hamilton Rating Scale for Depression (HAM-D), the Malay-translation of the Brief Pain Inventory (BPI), and Malay-translation of the Crown Crisp Experiential Index (CCEI) was filled after each patient had given to inform consent to participate in the study. Patients who did not consent, those with a co-morbid psychiatric diagnosis, and those with medical or surgical conditions associated with pain symptoms were excluded from the study.

Results: The study consisted of 51 respondents with mild depression; about half had neurotic traits. Although 80.4% of respondents experienced pain, the overall severity of the pain was mild. When compared with the presence of pain, there was no difference in the socio-demographic characteristic of respondents. However, Fisher's exact test revealed the statistically differences in the status of depression (depressed versus remitted) and anxious depressionî characteristic, whereby those who were still depressed and those with anxious depression were more likely to experience pain ($P < 0.05$ in both cases). Logistic regression analysis of socio-demographic and clinical variables did not show any significant finding with regard to their pain status (presence or absence). There were positive correlations observed between the pain severity and the free-floating anxiety, FFA ($r = 0.363$, $P = 0.009$), somatic concomitants of anxiety, SOM ($r = 0.394$, $P = 0.004$), depression subscale of CCEI ($r = 0.478$, $P < 0.001$), as well as the CCEI total score ($r = 0.415$, $P = 0.002$). The CCEI total score accounted for 17.2% of the variance of BPI total score. Positive correlations were also observed between the pain severity and 12 items from the HAM-D pertained to depressed mood and various types of anxiety (including depressed mood, work and interests, psychic anxiety, somatic anxiety, general somatic, and genital symptoms), as well as HAM-D totalscore ($r = 0.608$, $P < 0.001$) which was accounted for 33.2% of the variance of BPI total score.

Conclusion: The proportion of adults with major depressive disorder having pain was 80.4%. The study showed that there are correlation between anxiety, personality traits, and the severity of depression with the severity of pain experienced by depressed patients.

Supervisor :
Professor Dr Haji Mohd Razali Bin Salleh

THE ROLE OF SOCIO-CULTURAL AND DIETARY FACTORS IN EXPLAINING LOW PREVALENCE OF HELICOBACTER PYLORI AMONG KELANTANESE MALAYS

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MMed (Internal Medicine)

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Introduction: The prevalence of *Helicobacter pylori* is low in North–Eastern Peninsular Malaysia. A sero-epidemiological survey showed a prevalence of only 4.2% among 496 blood donors and 4.8% among 921 subjects who attended health-screening clinic in Hospital Universiti Sains Malaysia. The infection rate among the Malays is lower than among the non-Malays. Few studies have shown that socio-cultural and dietary factors play an important role in explaining the unexpectedly low prevalence of *H. pylori*. However, no study has been conducted locally to investigate the association between socio-cultural, dietary factors, and *H. pylori* infection in the Malay population.

Objectives: The study aimed at comparing the socio-demographic, socio-cultural, and dietary differences between

H. pylori infected and non-infected individuals, as well as identifying the differences in clinical presentation of both groups.

Patients and Methods: A total of 161 subjects were recruited in this case control study. The index cases were identified from the patients who underwent oesophago-gastric-duodenoscopy (OGDS) at Hospital Universiti Sains Malaysia and tested positive for *H. pylori* through histology. The control group comprised patients who underwent OGDS but were negative for *H. pylori*. Stratified random sampling was applied and consented respondents were interviewed using a validated questionnaire. The questionnaire consisted of data on demography, socio-cultural practices, diet, and clinical presentation of disease..

Results: *H. pylori* infection was associated with 8 variables in multiple regression analysis: body mass index, BMI ($P = 0.018$, adjusted odds ratio of 1.17, 95% CI 1.08–1.34), type of toilet used at home ($P = 0.01$, adjusted odds ratio of 4.63, 95% CI 1.89–11.27), symptom of regurgitation ($P = 0.001$, adjusted odds ratio of 0.19, 95% CI 0.07–0.52), frequent use of traditional complementary medicine, TCM ($P = 0.009$, adjusted odds ratio of 0.29, 95% CI 0.11–0.74), frequent intake of "pegaga" or pennywort ($P = 0.024$, adjusted odds ratio of 0.32, 95% CI 0.12–0.86), frequent intake of "budu", a type of fermented seafood product ($P = 0.010$, adjusted odds ratio of 0.26, 95% CI 0.09–0.73), frequent consumption of tea ($P < 0.001$, adjusted odds ratio of 0.03, 95% CI 0.11–1.00), and frequent consumption of coffee ($P = 0.021$, adjusted odds ratio of 3.45, 95% CI 1.20–9.86).

Conclusion: This study has shown that patients with higher BMI and use pit latrine at home had higher risk of *H. pylori* infection. Patients who had regurgitation were less likely to be infected with *H. pylori*. The intake of "pegaga", "budu", and tea also lowered the risk of infection, whereas frequent coffee intake increased the risk. There was no significant association observed between socio-cultural practices and *H. pylori* infection.

Supervisor :
Dr Lee Yong Yeh
Co-supervisor:
Dr Nazri Bin Mustaffa

AN EXPERIMENTAL STUDY ON THE USE OF FIBRIN GLUE IN CORNEAL WOUND REPAIR IN RABBITS

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Objectives: This study was conducted to evaluate the clinical outcomes and histopathological changes after the use of fibrin glue in repairing different types of corneal wound in rabbits.

Patients and Methods: 10 rabbits were divided into 2 groups, A and B, with each group consisting of five rabbits. Full thickness central corneal wound of 6 mm in length was created in a group A, and peripheral wound of 6 mm was created in a group B. The wounds then were closed using the fibrin glue. Serial clinical examination was conducted using portable slit-lamp at day 1, 3, 7, 14, 21 post-procedure to evaluate wound apposition, corneal clarity, and anterior chamber depth. Enucleation was done on day 21 of study. Histopathology evaluation was performed to assess stromal inflammation, avascularization, granuloma formation, and microscopic wound gapping.

Results: Good wound apposition was achieved in 80% of a group ***A*** by day 1 and 100% by a day 3 post-procedure. No wound leakage was reported in group B throughout the study. Clear cornea with a deep anterior chamber was obtained from all rabbits at the end of study. Histopathology examination revealed scanty stromal avascularization in a group B. Majority of the rabbits had a mild inflammatory reaction; however, no granuloma formation or microscopic wound gapping was observed in the two groups of study.

Conclusion: Fibrin glue has shown satisfactory clinical outcomes with a good wound apposition, clear cornea, and deep anterior chamber. These clinical findings correspond with the histopathological results, which revealed acceptable inflammatory reaction without any granuloma formation or microscopic gapping.

Supervisor:
Dr Raja Azmi Mohd Noor
Co-supervisors:
Associate Professor Dr Hasnan Jaafar
Associate Professor Dr Wan Hazabbah Wan Hitam

PPREVALENCE OF IRRITABLE BOWEL SYNDROME (IBS) AMONG HEALTHY SUBJECTS USING VALIDATED BAHASA MALAYSIA VERSION OF ROME III IBS QUESTIONNAIRE

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MMed (Internal Medicine)

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Introduction: Irritable bowel syndrome (IBS) is a very common chronic gastrointestinal disorder characterized by recurrent abdominal pain and altered bowel habit without major organic disease. ROME diagnostic criteria, which are based on subjective gastrointestinal (GI) complaints, are the most widely used criteria for diagnosing IBS. We have developed the Malay-translation of ROME III IBS

questionnaire for the study of IBS in Malaysia. This validated questionnaire was used to study the prevalence of IBS among healthy subjects.

Objectives: The aims of this study were to validate the Malay-translation of ROME III IBS and ROME III psychosocial alarm questionnaires, and investigate the prevalence of IBS among normal healthy subjects in Universiti Sains Malaysia (USM) Health Campus, Kelantan, using the validated Malay-translation of ROME III IBS and ROME III psychosocial questionnaires.

Patients and Methods: This study was divided into 2 phases: 1) the validation process of the proposed translated questionnaire, and 2) a cross-sectional prospective study to examine the prevalence of IBS among healthy subjects in a university campus.

Results: The validated Malay-translation of ROME III IBS questionnaire was shown to have good colonometric properties. The prevalence of IBS among healthy subjects in USM Health Campus was 11.8%. Prevalence of IBS was significantly associated with age, ethnicity, and level of formal education. Red flag and psychosocial alarm symptoms were higher in subjects with IBS compared to non-IBS subjects.

Conclusion: This validated Malay-translation of ROME III IBS questionnaire have a good colonometric properties suitable as a tool for research. Prevalence and characteristics of IBS among healthy subjects in USM Health Campus were almost similar to those reported in another Asian population.

Supervisor:
DrLee Yeong Yeh
Co-supervisors:
Dr Amry Abdul Rahim
Dr Sarimah Abdullah

A COMPARATIVE STUDY BETWEEN A TARGET CONTROLLED INFUSION (TCI) AND MANUAL CONTROLLED INFUSION (MCI) OF PROPOFOL FOR SEDATION DURING CEREBRAL PROTECTION IN SEVERE TRAUMATIC BRAIN INJURED PATIENTS

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Introduction: Propofol is the most common sedative agent administered to traumatic brain injured (TBI) patient using manually controlled infusion (MCI) technique. The development of target controlled infusion (TCI) technique has given rise to a new alternative method in propofol administration, and this technique has never been tested in TBI patients.

Objective: The aim of this study was to compare the effectiveness of different methods of propofol infusion (TCI versus MCI) for sedation during cerebral protection in TBI patient in Neurosurgical Intensive Care Unit (NICU).

Patients and Methods: In this prospective, double-blinded, randomized controlled trial, 50 patients (age 16-50 years old) who had TBI with a Glasgow Coma Scale (GCS) between 3 and 8 with no severe medical illness and undergoing craniotomy were randomized into two groups (MCI and TCI) of 25 patients in each using block randomization. However, 1 patient in MCI group had to be excluded due to very agitated state to the extent of requiring muscle relaxant in spite of adequate sedation. During surgery, both groups received anaesthesia according to the standard protocol, and observation began once patients were admitted to NICU for cerebral protection. Sedation level was monitored using Bispectral Index (BIS) monitor and Sedation Agitation Scale (SAS). BIS index of 60-70 and SAS score of 2-3 was considered as adequate sedation. The same infusion pumps that can operate either TCI or MCI was used during drug administration, which was done via a dedicated central venous line lumen. *Hemodynamic* parameters (blood pressure, mean arterial pressure, MAP, and heart rate) and neurological parameters (intracranial pressure, ICP, and cerebral perfusion pressure, CPP) were recorded. Time and volume of propofol used to achieve BIS 70, total volume of propofol used for 24 hours, and time taken to achieve BIS 90 after stopping infusion at the end of cerebral protection were measured. Fentanyl infusion at 1 mcg/kg was given for pain relief and was stopped four hours before the end of the 24-hour study.

Results: There were some significant differences between 2 modes of infusion. TCI mode achieved BIS 60-70 significantly sfaster than MCI, with mean of 6.32 minutes (SD 2.88) and 19.71 minutes (SD 7.00), respectively. Time taken to recover from sedation to achieving BIS 90 was also significantlyfaster in TCI, 22.44 minutes (SD 11.50), compared with MCI, 57.29 minutes (SD 19.89). In view of ICP, there was a differentiation between 2 modes; however, no significant difference was observed in MAP and CPP.

Conclusion: TCI mode of propofol is shown to be more effective in sedating neurotrauma patients as it gave adequate sedation faster with lesser volume, as well as faster recovery. It also lowered the ICP to 20 mmHg lower than MCI.

Supervisor:
Professor Dr Nik Abdullah Nik Mohamad
Co-supervisor:
Dr Wan Mohd Nazaruddin Wan Hassan

THE EFFECTIVENESS OF PRETREATMENT WITH LOW DOSE ETOMIDATE OR PROPOFOL IN REDUCING ETOMIDATE-INDUCED MYOCLONUS IN PAEDIATRIC PATIENTS

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MMed (Anaesthesiology)

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Introduction: A good choice of intravenous anaestheticagent is very important, especially in an extreme age group either pediatric or elderly patient. Etomidate is one of the anaesthetic agent of choice for general anaesthesia and as alternative to propofol and barbiturates for rapid intravenous induction of anesthesia because of etomidate properties in maintaining hemodynamic stability. Nevertheless, there is a high incidence of myoclonicmovement following induction of anesthesia with etomidate. The myoclonic movement will affect patients' intraoperative monitoring such as blood pressure monitoring, pulse oxymetry reading, and electrocardiogram monitoring.

Objectives: The goal of this study was to compare the effectiveness of pre-treatment with low-dose etomidate or low-dose propofol in reducing etomidate induced myoclonus in pediatric age group. In addition, the study was conducted to observe any significant changes in hemodynamic parameters and incidence of pain following intravenous etomidate injection.

Patients and Methods: Pediatric patients of ASA physical status I or II (n = 88) were randomized to two groups. The patients received pre-treatment of either intravenous etomidate (0.03 mg/kg) or intravenous propofol (0.2 mg/kg), followed by induction dose of intravenous etomidate (0.3 mg/kg). Presence of myoclonus and/or pain following etomidate induction was observed and charted in observational data collection.

Results: Pre-treatment with etomidate caused fewer myoclonus compared with propofol (occurring in 31 or 45.6% of patients and 37 or 54.4% of patients, respectively); however, this observation was not statistically significant (P = 0.132). Among the patients who had experience myoclonus, it was found that the condition was significantly correlated with age (P = 0.002) and body weight (P = 0.001). Mean age group for the patients experiencing myoclonus was 10.7 years old (SD 3.21), whereas the mean body weight was 33.4 kg (SD 12.48). Pre-treatment with etomidate caused less pain during subsequent etomidate injection compared with propofol (occurring in 10 or 22.7% of patients and 13 or 29.5% of patients, respectively); however, results were not relevant. There were also no significant changes in the baseline, pre-treatment, and post-induction blood pressure and heart rate in both groups.

Conclusion: Incidence and severity of myoclonus after etomidate injection were related to the patients' age and body weight. There was nosignificant difference in myoclonus and pain reduction between pre-treatment with low-dose etomidate and low-dose propofol in the pediatric patients, and nosignificant changes were observed in the hemodynamic parameters following etomidate induction.

Supervisor:
Dr Mohd Nikman Ahmad

EFFECTIVENESS OF PAP SMEAR SCREENING PROGRAMME IN DETECTING CERVICAL SQUAMOUS CELL CARCINOMA AND HIGH GRADE SQUAMOUS INTRAEPITHELIAL LESIONS IN KELANTAN: 2002-2006

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Introduction: Cervical cancer is a preventable cause of death in women. Cervical cytology had been proven to be effective as a screening test. This is demonstrated by decreased incidence and mortality rates in many countries worldwide. The specificity of the Papanicolaou smear frequently has been reported to be higher than 90%. However, the sensitivity as well as false negative and false positive rates have been inconsistent and dependent on the study design.

Objective: This study was conducted to determine the effectiveness of cervical cytology screening in detecting high grade squamous intraepithelial lesion and squamous cell carcinoma using histopathology as the gold standard.

Patients and Methods: This is a retrospective study of all patients diagnosed with carcinoma intraepithelial neoplasia (CIN 2 and CIN 3) and squamous cell carcinoma by histopathology in Hospital Universiti Sains Malaysia and Hospital Raja Perempuan Zainab II from 2002 to 2006. The cervical cytology results were reviewed and divided into 3 categories: concordant, minor, and major discordant. The concordance rate between cytological diagnoses and histopathological diagnoses were determined. The smears from the minor and major discordant categories were traced and subjected to re-screening. The 2001 Bethesda system was used to classify the revised diagnoses of re-screening.

Results: There were 182 cases of CIN 2, CIN 3, and invasive squamous cell carcinoma diagnosed by histopathological examination. We found that only 54.5% of the cases had a previous Pap smear. Of the patients who received Pap smears, 65.7% were histopathologically diagnosed as concordant, 15.1% as major discordant category, and 19.2% as minor discordant. The concordance rate between the histopathology and Pap smears was statistically significant (P < 0.05). 23 (23.2%) smears from the major and minor discordant categories were subjected to re-screening. The revised diagnoses of the re-screening were similar to the initial screening diagnoses in only 5 (21.7%) smears. In 18 (78.3%) smears, the revised diagnoses were changed. The false negative rate was 15.2% and the sensitivity of Pap smear was 84.8%. This study showed that Pap smear was found to be underutilized in 24.2% of cases, where it was used for diagnosis rather than for screening. It was also noted that there is a higher diagnostic error in the laboratory with the higher workload.

Conclusion: Pap smear is effective in detecting high grade squamous intraepithelial lesions and squamous cell carcinoma with a high sensitivity. The false negative rate is within an acceptable range.

Supervisor:
Dr Mukarramah Che Ayub
Co-supervisor:
Professor Dr Nor Hayati Othman

A LOCAL STUDY ON THE INCIDENCE AND RISK FACTORS OF POST-TRAUMATIC SEIZURES AMONG PATIENTS WITH TRAUMATIC BRAIN INJURY

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Introduction: Post-traumatic seizure is a well-known and serious complication of traumatic brain injury (TBI). The incidence and risk factors vary among study populations. Very little data has been published concerning post-traumatic seizures in the Malaysian population.

Objective: The aim of this study was to investigate the incidence and risk factors for the development of post-traumatic seizures among patients with TBI in Hospital Universiti Sains Malaysia, Kelantan, Malaysia.

Patients and Methods: A total of 157 patients who were diagnosed with TBI from June to December 2007 were included in this prospective observational study. Patients were divided into high-risk and low-risk groups and randomized to receive phenytoin for either 1 week or 1 year (for the high-risk group) and either phenytoin for 1 year or no phenytoin (for the low-risk group). The patients were then followed up for 12 months, until their death or first post-traumatic seizure. Survival analyses were conducted using Kaplan-Meier curves and Cox proportional hazard regression.

Results: Out of 157 patients, 26 patients (16.6%) developed post-traumatic seizures. From these 26 patients, 11 patients (42.3%) developed seizures early, within 7-day post-trauma of the trauma, whereas the remaining 15 patients (57.7%) developed seizures later, between 8-days and 12 months after trauma. The mean duration for development of late post-traumatic seizures was 9.4 months (SD 3.2). The risk factors for developing early and late post-traumatic seizures were different. For early post-traumatic seizures, the risk factors were young age (P = 0.021, 95% CI 0.806-0.982) and intubated patients (P = 0.029, 95% CI 1.194-25.913). For late post-traumatic seizures, the significant risk factor was a severe head injury with a Glasgow Coma Scale of 3-8 (P = 0.036, 95% CI 1.065-6.464). Log-rank tests for phenytoin treatment in both high-risk and low-risk groups were insignificant (log-rank statistic of 0.31 with P = 0.5784 for the high-risk group; a log-rank statistics of 0.23 with P = 0.6283 for the low-risk group).

Conclusion: Incidence of post-seizures in the local population was 16.6%. Risk factors for early post-seizures were young age and intubated patients, whereas only severity of head injury was found to be significantly correlated with late postpost-seizures. Phenytoin was not beneficial as a prophylaxis against post- seizures.

Supervisor:
Associate Professor Hillol Kanti Pal

DETERMINATION OF THE OVER-PENETRATION LENGTH DURING DRILLING PROCEDURE WITH K-WIRE AND DRILL BIT AMONG ORTHOPAEDICS MASTER STUDENT IN UNIVERSITI SAINS MALAYSIA

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MMed (Orthopaedics)

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Introduction: Drilling procedure is the most fundamental surgical skill required by orthopaedic surgeons as many fractures, nowadays, are treated operatively. Neurovascular injuries are known to be one of the complications of over-penetration during drilling procedures.

Objective: The purpose of this study was to determine the length of over-penetration in drilling technique among orthopaedics master students in Universiti Sains Malaysia.

Patients and Methods: A total of 14 junior postgraduate students (Year 1 and 2) and 14 senior postgraduate students (Year 3 and 4) were recruited in this study. Each student performed drilling procedure on a bovine bone block using the battery-powered drill with 3.2 mm drill bit and 2.0 mm K-wire. Drilling was stopped immediately when penetration of the far cortex was felt. The distance from the tip of the instrument to the far cortex was measured. The procedure was repeated three times for each instrument. The mean values were calculated and compared using parametric paired t test. The length of over-penetration in Experience Level group and Exposure to an AO course group for each drilling instrument were compared using non-parametric Mann-Whitney U test.

Results: The mean length of over-penetration for the drill bit and K-wire were 21.50 mm (range 10.33-35.66 mm) and 10.18 mm (range 3.00-18.66 mm), respectively. Using the drill bit, the median lengths of over-penetration for junior students and senior students were 25.55 mm and 16.66 mm, respectively, while the use of K-wire resulted in shorter over-penetration, 16.50 mm and 5.50 mm for junior students

and senior students, respectively. For the group of students exposed to AO course, themedian lengths of over-penetration were 13.33 mm with drill bit and 5.00 mm with K-wire, while for those students without AO course exposure, the lengths were 21.66 mm with drill bit and 10.66 mm with K-wire. The differences were statistically significant with regard to the type of instrument and experience level ($P < 0.001$ in all comparison) and exposure to AO course ($P = 0.02$ for drill bit and $P = 0.05$ for K-wire). There was a significant correlation between postgraduate training years in with personal experience in Orthopaedics ($r = 0.55$, $P = 0.003$).

Conclusion: The study provides the overall results regarding the depth of over-penetration in drilling procedure among orthopaedicss master students in Universiti Sains Malaysia. It may benefit as the reference for related studies in the future.

Supervisor:
Dr Amran Ahmed Shokri
Co-supervisor:
Dr Ahmad Tajuddin Abdullah

A CROSS SECTIONAL STUDY OF THE PROXIMAL FEMUR MORPHOLOGY OF THE NORMAL MALAY POPULATION USING COMPUTED TOMOGRAPHY IMAGES (SCANOGRAM) AT HOSPITAL UNIVERSITI SAINS MALAYSIA

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MMed (Orthopaedics)

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Introduction: Previous studies have shown significantly smaller proximal femur parameters in the Asian population in comparison with the Caucasian population. Currently used proximal femur implant size was based on Caucasian studies. However, due to the smaller size of the proximal femur in the Asian population, improvement in the design of implant is required to optimize component fixation and restore the normal biomechanics of the hip joint.

Objectives: This study was conducted to quantify the proximal femur dimensions in Malay patients, compare the parameters of the proximal femoral bones between male and female patients, and determine the correlation between these parameters.

Patients and Methods: This cross-sectional study involved 140 Malay patients (70 males and 70 females), aged between 18 and 80 years, who were treated in Hospital Universiti Sains Malaysia. Each patient was confirmed as having normal proximal femur morphology by analysing the computerized tomographic scan, which was conducted for various pelvic pathologies. The following parameters of the proximal femoral bones were studied: 1) femoral neck shaft angle, 2) femoral head diameter, 3) femoral neck isthmus diameter, 4) vertical offset of femur, and 5) horizontal offset of the femur. The data obtained were statistically analysed using independent *t* test to determine the difference between male and female patients. The mean of all the parameters was compared with published Western and Asian studies using one sample t test. Significant level was set at $P < 0.05$.

Results: There were significant differences between male and female patients in the femoral head diameter, femoral neck isthmus diameter, vertical offset, and horizontal offset of the femur. However, there was no significant difference in the neck shaft angle. Female patients have significantly smaller dimensions in all parameters measured except for the neck shaft angle, which was larger than the male patients'. When observation in the current study was compared to radiographic study by Sugano et al. and Noble et al., significant differences were found in the head diameter, neck isthmus diameter, vertical offset of femur, and neck shaft angle. The head diameter, neck isthmus diameter, and vertical offset of the femur observed in this current study were significantly smaller compared with their reports; however, the neck shaft angle in this study was significantly larger.

Conclusion: The current study has shown that the proximal femur morphometry of the Malay population was smaller compared to the Caucasian and Thailand population.

Supervisor:
Dr Amran Bin Ahmed Shokeri

A STUDY ON GLYCEMIC CONTROL AMONG INSULIN-TREATED TYPE 2 DIABETES PATIENTS ATTENDING DIABETES CENTRE, HOSPITAL UNIVERSITI SAINS MALAYSIA, KELANTAN

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MMed (Family Medicine)

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Introduction: The goal in management of type 2 diabetes mellitus is to achieve a good control of HbA1c. Unfortunately, many diabetic patients were unable to this target. Combination of insulin and oral anti-diabetic (OAD) agent, or full dose insulin, is an alternative treatment to improve the glycaemic control. Recent studies indicate that insulin isable to improve HbA1c level and reduce the risk of microvascular and macrovascular complications, thus reducing the economic burden of diabetes mellitus.

Objectives: The study was conducted to examine the percentage of insulin-treated diabetes patients with good glycaemic control and determine the factors that contribute to glycaemic control.

Patients and Methods: This cross-sectional study was conducted by collecting case report form (CRF) or proforma from 236 insulin-treated type 2 diabetes mellitus patients who attended the Klinik Diabetik and Klinik Rawatan Keluarga, Hospital Universiti Sains Malaysia (HUSM), from January until November 2008. The CRF was divided into 2 sections: Section 1 consisted of socio-demographic data while Section 2 consisted of background history of diabetes and clinical characteristics that was filled up by the researcher by reviewing the patients case notes.

Results: The result showed that the percentage of insulin-treated diabetes patients with goodglycaemic control was 21.2%. The factors that contribute to good glycaemic control were female, high education level, combination of insulin and OAD regime, basal bolus insulin, and fasting blood sugar. Factors that were found to be protective of glycemic control include financial status, high low-density lipoprotein (LDL), high total cholesterol, and normal weight (non-obese).

Conclusion: Glycaemic control among insulin-treated type 2 diabetes mellitus attending the Diabetes Centre, HUSM, was still unsatisfactory. Assessment of glycaemic control should be a routine during follow-up. By recognizing the factors that contribute to good glycemic control, healthcare providers can plan an intervention program to improve glycaemic control and subsequently reduce the cost of treating the diabetes mellitus and its complications.

Supervisor:
Dr Adibah Hanim Ismail
Co-supervisor:
Dr Nor Azwany Yaacob

PREVALENCE OF FALLS AND ITS ASSOCIATED FACTORS AMONG ELDERLY DIABETES ATTENDING KLINIK PAKAR PERUBATAN AND KLINIK RAWATAN KELUARGA HOSPITAL UNIVERSITI SAINS MALAYSIA

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MMed (Family Medicine)

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Objectives: The study aimed to assess the prevalence of fall and its associated factors among elderly type 2 diabetes mellitus patients attending outpatient clinics (Klinik Pakar Perubatan & Klinik Rawatan Keluarga), Hospital Universiti Sains Malaysia (HUSM).

Patients and Methods: This cross-sectional study was conducted among 288 elderly type two diabetes mellitus in HUSM, from April 2007 until March 2008. The patients were selected using systematic random sampling method. Baseline data on socio-demographic, diabetes history, co-morbid diseases, drug use, and activity of daily living (Barthelís index) were collected. The patients were examined physically, and gait assessment was carried out. Information on laboratory investigation results, medication, and concomitant illness were obtained from reviewing the patients medical records. Prevalence of fall was determined, where fall was defined having at least one history of fall in the past one year from the interview date.

Results: Prevalence of fall among elderly diabetes was 18.8%; 72.2% out of these patients had experienced more than one falls. Female gender (OR 2.54, $P < 0.05$), age of more than 75 (OR 2.97, $P < 0.05$), retinopathy (OR 2.19, $P < 0.05$), and orthostatic hypotension (OR 2.87, $P < 0.05$) were associated with higher risk for falls in elderlydiabetes; in contrast, high balance and gait scores was associated with reduced risk of fall (OR 0.89, $P < 0.05$).

Conclusion: In this cross-sectional study among elderly diabetes type 2, the prevalence of falls was found to be lower compared with previous studies in the Western countries. The factors associated with higher risk for fall where gender (female), age of more than 75, retinopathy, and orthostatic hypotension. Those who had higher balance and gait scores were found to be less likely to experience fall compared with those with lower scores.

Supervisor:
Dr Azidah Abdul Kadir
Co-supervisor:
Dr Zunaina Embong

SOFT TISSUE CHARACTERISTICS OF REPAIRED CLEFT LIP AND PALATE OF MALAY CHILDREN IN KELANTAN

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Introduction: Facial growth and soft tissue are essential parameters in the assessment of a cleft surgery outcome. Many studies have been focusing on the effects of the cleft surgery on the facial development, mainly on the skeletal development. Only a few studies have investigated the influence of surgical intervention on the soft tissue morphology.

Objectives: This study aimed to examine the facial morphology of Malay cleft lip and palate (CLP) patients who had been treated surgically without any osteoplasty and received a complete lip repair at 3-6 months, and palate closure at 9-12 months.

Patients and Methods: A total of 26 CLP patients of Malay ethnicorigin with age range of 4-10 years old were recruited in this cross-sectional comparative study. Serial

analyses of the soft tissue morphology using 2-D photographs pre-operatively and 3-D computer generated images post-operatively were conducted, and the data were compared with non-CLP subjects of same ethnicorigin, which acted as the control group. The observations were then compared with other studies on CLP patients.

Results: The results revealed statistically significant changes in intercanthal distance, mouth width, and nose width between pre-operative and post-operative measurements. Compared with the control group, CLP patients had wider intercanthal distance and nose width. However, CLP patients showed restriction of facial growth at the prolabial and anteroposterior dimension of the midface. Results of this study suggested that all recorded changes occurred in the horizontal plane, and that the dento-facial growth disturbance can extend up to the orbital region.

Conclusion: The facial profile changes in CLP patients following cleft surgeries were statistically significant when compared to the non-CLP subjects. Most of these changes that occurred in the orbital and nasal regions were dueto intrinsic factors. The effect of cleft surgeries played a major role in the changes at the prolabial region. Highest degree of dento-facial differences between CLP patients and control subjects was in the horizontal dimensions of the nose. There were no significant differences in vertical dimensions between these groups. These soft tissue characteristics of repaired CLP would be useful in the development of a more ideal treatment protocol, which can enhance the quality of life in CLP patients.

Supervisor:
Dr Wan Azman Wan Sulaiman
Co-supervisor:
Professor Dr Ahmad Sukari Halim

CORRELATION OF SIGNIFICANT LEFT MAIN CORONARY ARTERY STENOSIS WITH 12-LEAD ECG

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MMed (Internal Medicine)

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Objectives: The purpose of this study is to determine the correlation of significant leftmain coronary artery stenosis with 12-lead ECG. We also determine the sensitivity and specificity of ECG in predicting left main coronary artery disease.

Patients and Methods: A total of 220 patients who had left main coronary involvement were included in this study. Their ECGs were reviewed blindly by the investigator under 11 different ECG criteria which previous studies have formulated. The patients were then subdivided into 2 groups depending on whether there was significant LMCA stenosis or not.

Results: After establishing the frequency of ST elevation in leads aVR < V1 (criteria 5) occurred in 79.5% cases. The ST elevation in aVR and V1 (criteria 7) occurred in 0.5% of cases. This showed that a combination of ECG criteria only occurred in very few cases. After using the McNemar test as a non-parametric test for the data, it was found that ECG criteria 5 had the highest sensitivity (75%) but with a low specificity (12.5%). Other ECG criteria having high specificity however did not have high sensitivity. Therefore, new ECG criteria or a new combination of criteria needed to be devised that have both high sensitivity and good specificity. After several attempts at defining new ECG criteria, we came across new criteria (aVR = V1) which had a sensitivity of 92.9% but still having low specificity of 13.3%. We suggest using ST depression, I, II, V4-V6 (criteria 10) which has high specificity of 97.3% to exclude cases of significant LMCA stenosis.

Conclusion: We can conclude that aVR = V1 (new criteria) can be used to identify patients with significantLMCA disease and ST depression, I, II, V4-V6 (criteria 10) to exclude significant LMCA disease.

Supervisor:
Dr Mohd Sapawi Mohamed
Co-supervisor:
Assoc. Associate Professor Dr Zurkurnai Yusof

ACCURACY AND RELIABILITY ANALYSIS OF LOWER LIMB LENGTH DISCREPANCY MEASUREMENT USING TAPE MEASURING METHOD AND CT SCANOGRAPHY

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Introduction: Clinical measurement using the tape measurement method (TMM) is easily performed and required no special instrument. However, it was reported to be less accurate compared with radiological measurement. Currently, there is no available data on the accuracy of TMM in the clinical measurement of the lower limb length.

Objectives: The study was design to assess the reliability and accuracy of TMM in comparison with computed tomography (CT) scan in measuring the length of lower limb.

Patients and Methods: This was a cross-sectional study involving 50 patients who had been treated in Hospital Universiti Sains Malaysia: 37 patients with limb-length discrepancy (LLD) and 13 patients without LLD. All of them had limb-length measurement done using TMM by 2 blinded observers. From 37 patients with LLD, only 25 of them had undergone CT scan, and their CT scanogram were used for radiological measurement done by 2 blinded radiologists. Intra class correlation coefficient (ICC) and mean difference were

calculated to assess interobserver reliability for TMM and CT scanogram measurement. Accuracy of the TMM was assessed by comparison with CT scanogram as the gold standard.

Results: The interobserver reliability for TMM was high,with Intraclass correlation coefficients (ICC) of 0.912 (0.851 to 0.949) and mean difference of -0.7 (-3.3 to 1.9) mm. The interobserver reliability for CT scanogram is also high,with ICC of 0.964 (0.921 to 0.984) and mean difference of -1.14 (-3.4 to 1.1) mm. When compared to CT scanogram, the accuracy of one measurement by TMM was fair, with ICC of 0.672 and mean difference of 3.1 (-3.6 to 9.8) mm. A mean of two measurements by TMM was good, with ICC of 0.781 and mean difference of -3.4 (-9.0 to 2.1) mm.

Conclusion: Both measurements of LLD using TMM and CT scan to have been high interobserver reliability. In comparison with CT scanogram, the accuracy of the TMM was improved from fair to good if the mean of two measurements by TMM was taken instead of a single measurement. The TMM can either over- or underestimate the LLD up to 10 mm.

Supervisor:
Associate Professor Dr Abdul Razak Sulaiman
Co-supervisor:
Associate Prof Dr Mohd Ezane Aziz
Dr Juhara Haron

OUTCOME OF IMMEDIATE PARTIAL WEIGHT BEARING FEMORAL SHAFT FRACTURES, TREATED BY SINGLE PROXIMAL LOCKED INTRAMEDULARY NAILING, IN HOSPITAL IPOH

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MMed (Orthopaedics)

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Introduction: Femoral shaft fracture is a common fracture encountered in daily orthopaedic practice. Literature review showed that this type of fracture had been treated with different modalities since many years ago, and the treatment method has advanced rapidly over the past few decades. Single proximal locked femoral nail fixation has not been widely practiced. This method of fixation was only used for proximal third and mid-shaft fractures of the femur. Femur fractures with simple transverse configuration and up to 50% contact of the cortices were suitable for this method of fixation. Dynamic intramedullary femoral stabilization had been previously studied by Brumback et al. using interlocking screws at only one end of the nail, and the authors concluded that dynamic intramedullary femoral stabilization should be performed for transverse or short oblique fractures of the femoral isthmus with Winquist–Hansen type-I and type-II comminution.

Objectives: The objective of this study was to review the results and clinical outcomes of immediate partial weight bearing in the treatment of femoral shaft fractures using single proximal locked intramedullary nailing for type-0, type-I, and type-II comminution according to Winquist-Hansen classification.

Patients and Methods: This is a case-review, retrospective study using secondary data. Study subjects were 31 patients who were admitted to the ward after sustaining a single femoral fracture, with no other associated injuries, from June 2005 until May 2007. Patients with femur fractures that were beyond the mid-shaft were not included in this study due to rotational instability with single proximal locking screw fixation. Patients in this study were categorised according to the types of fracture using the Winquist–Hansen classification: type-0, type I, and type II. All patients underwent single proximal locked intramedullary nail fixation. Patients were advised for partial weight bearing on the fractured limb using crutches starting on the second day post-operation in order to achieve primary dynamization to enhance fracture healing process. They were followed up every 6 weeks for a period of 18 weeks. Throughout the follow-up period, fracture union and clinical outcome were assessed by a senior orthopaedic surgeon. The union of the fracture was considered delayed if the fracture had failed to unite by 18 weeks.

Results: Out of the 31 patients, 25 patients were males (81%) and the remaining 6 patients were females (19%). Patients' age ranged 20–40 years with a mean of 27 years. There were 5 patients with type-0 fractures (16%), 20 patients with type-I fractures (65%), and 6 patients with type-II fractures (19%). The cause of fractures was motor vehicle mishaps; 71% involved motorcycle accidents. The common site of fracture was midshaft (54.8%) compared with proximal third (45.2%). All patients were operated within a week post-injury. The operation time ranged 30–86 minutes with average of 46 minutes. Independent *t* test analysis showed statistically significant reduction in the operation time for the fixation of femur using single proximal locking method compared with proximal and distal locking method. In this study, all fractures achieved union. The average time to union for Winquist–Hansen type-0 fractures (14 weeks) was significantly shorter compared to Winquist–Hansen type-I (18 weeks) and type-II (25 weeks). There was 3.2% incidence of post-operative superficial wound infection; all were superficial and successfully treated with intravenous antibiotic given for 2 weeks. In 2 Winquist–Hansen type-II fracture cases, there was shortening of 1 cm each. None of them had post-operative complication such as nerve injury, mal-union, non-union, and implant failure.

Conclusion: There was a significant reduction in the operation time for the fixation of femur using single proximal locking method compared with proximal and distal locking method. The average time to union for Winquist–Hansen type-0 fractures were significantly shorter compared with Winquist–Hansen type-I and type-II. Based on the result, we conclude that single locked intramedullary nail is safe and beneficial to patients who sustained Winquist–Hansen type-0

and type-I femur fracture.

Supervisor:
Dr Bal Kishan
Co-supervisor:
Dr Liau Kai Ming
Dr K Manoharan

RESPIRATORY SYMPTOMS AND LUNG FUNCTIONS AMONG DOMESTIC WASTE COLLECTORS IN KOTA BHARU, KELANTAN

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MMed (Occupational Health)

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Introduction: A variety of common injuries and diseases involving eye, skin, musculoskeletal systems, respiratory, and systemic diseases exist as a result of exposure, via inhalation or skin absorption, to a large variety of chemical compounds, bioaerosols, dusts, gases, and pathogens encountered by domestic waste collectors during the handling of solid waste.

Objectives: This study was aimed to determine the respiratory status, level of knowledge, attitude, and practice (KAP), and associated factors that could potentially impair the respiratory function among male domestic waste collectors in Kota Bharu.

Patients and Methods: A comparative cross-sectional study was conducted in September–October 2008 among 95 male domestic waste collectors (exposed group) and 96 male office workers (non-exposed group) in Kota Bharu. Subjects were individually interviewed using 2 sets of questionnaire to obtain information pertaining socio-demography, personal, occupation, respiratory symptoms as well as knowledge, attitude, and practice (KAP) towards health and respiratory risks from workplace exposure to biohazards. Physical examination and lung function test were conducted to each study subjects at their workplace.

Results: All 191 participants were Malay males with mean (SD) age of 39.9 (9.14) years among the domestic waste collectors compared with 41.4 (9.06) years among office workers. The mean (SD) working duration among domestic waste collectors was 11.2 (8.76) years compared with 10.3 (9.36) years among office workers. The most frequently reported respiratory symptom was shortness of breath (42% waste collectors, 31% office workers), followed by chest tightness (37% waste collectors, 27% office workers), morning phlegm (33% waste collectors, 17% office workers), and morning cough (20% waste collectors, 3% office workers). Physical examination revealed normal findings and there were no significant differences in all respiratory function parameters among exposed and non-exposed groups. Exposed group had significantly lower mean percent score for knowledge and attitude, but higher mean percent score for practice; there was no significant difference of mean percent score for total KAP between exposed and non-exposed group. Height, duration of smoking, duration of working, and age were factors significantly related to FEV1 and FVC, and age was a factor significantly related to FEV1/FVC.

Conclusion: The study showed that chronic exposure to domestic waste could lead to higher prevalence of respiratory symptoms and mild effect on respiratory health among domestic waste collectors compared with office workers. Height, duration of smoking, duration of work, and age were factors associated with at least 1 of the lung function parameters among the exposed group. The level of knowledge, and attitude towards health and respiratory risks from biohazard exposure at workplace in the exposed group was unsatisfactory compared with non-exposed group.

Supervisor:
Dr Mohd Nazri Shafie
Co-supervisor:
Associate Professor Dr Mohamed Rusli Abdullah

A STUDY OF FEMAL SEXUAL DYSFUNCTION BY USING FEMALE SEXUAL FUNCTIONAL INDEX (FSFI) AMONG WOMEN AT THE AGE OF 40–55 YEARS OLD IN UNIVERSITI SAINS MALAYSIA, KUBANG KERIAN, KELANTAN

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MMed (O & G)

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Introduction: Sexual life is considered as one of the important aspect in a woman's quality of life; it reflects on physical, psychological, and mental well-being of the individual. Middle age women are additionally influenced by the hormonal changes that occur during menopause that affects their sexual functions, especially libido and orgasm.

Objectives: The study aimed to determine the prevalence of sexual dysfunction among 40–55 years old women, socio-demographic and sexual factors that influence libido (sexual desire) and orgasm domain, and the impact on HRT on sexual domain.

Methods: This cross-sectional descriptive study involved 434 local women, age of 40-55 years old, attending the Hospital Universiti Sains Malaysia. Sexual function was measured by using a self-administered, validated, Malay-version of the Female Sexual Function Index questionnaire. The questionnaire consists of socio-demographic data and a 19-item, multidimensional, self-report measure of female sexual functioning, which covers 6 basic domains of female

sexual functions: desire, arousal, lubrication, orgasm, satisfaction, and pain. Statistical analyses were performed using chi-square test and multivariate regression analysis.

Results: The mean age of the women was 47.5 (SD 4.3) years old. The overall prevalence of sexual dysfunction was 8.0%. The prevalence of sexual dysfunction for each domain was 18.4% for desire, 5.1% for arousal, 4.4% for lubrication, 2.1% for orgasm, 7.4% for satisfaction, and 6.9% for pain. The most significant predictors for desire were age of 40–45 years old (P value = 0.047) and more than 50 years old (P value = 0.017), Malay ethnicity (P value = 0.024), Chinese ethnicity (P value = 0.013), sexual arousal ($P < 0.001$), lubrication ($P < 0.001$), and satisfaction ($P < 0.001$). Lubrication and illness (renal disease) had significant association with the lack of orgasm ($P < 0.05$). Ageing, parity (2–5 and more than 5 children), and lack of lubrication were significantly associated with sexual pain disorders ($P < 0.05$). Hormone replacement therapy (HRT) did not significantly improve any sexual domain. Other socio-demographic variables, such as level of education, type of occupation, duration of marriage, and medical conditions, were less likely to result in significant disturbances on sexual function.

Conclusion: The prevalence of sexual dysfunction among the middle age, local women was not high. There is a negative association between age and sexual response in these women; the most affected sexual function domain was sexual desire. From this study, it was concluded that HRT did not have any positive impact on the sexual domain.

Supervisor:
Associate Professor Dr Shah Reza Johan Noor

REVIEW OF COMPLICATIONS OF ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY (ERCP) IN HOSPITAL UNIVERSITI SAINS MALAYSIA FROM 2000 TO 2006

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MMed (General Surgery)

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Introduction: Endoscopic retrograde cholangiopancreatography (ERCP) is an important procedure for the investigation and management of pancreaticobiliary diseases; endoscopists perform ERCP on a regular basis. However, the procedure has many risks. There is a recognized potential for significant morbidity, and a number of studies have identified patient and operator risk factors for the development of complications, such as small case volume. ERCP practice may vary between different institutions, endoscopic settings and health care environments, as well as physician's expertise and skill; all these variables may affect the outcome of the ERCP. The ERCP service in Hospital Universiti Sains Malaysia (HUSM) has been in existence since the last two decades. However, until now, no formal study has been done to audit the ERCP service, and no information on the outcomes and complications of this procedure is available.

Objectives: This study was performed to review ERCP complications in HUSM. The information on complications of ERCP was obtained from a tertiary referral centre and compared with published figures.

Patients and Methods: Data were collected retrospectively from medical records of patient who had ERCP from January 2000 to December 2006. Complications were reviewed, and several factors were analyzed as risk factors for post-ERCP complications using univariate and multivariate analyses.

Results: The study involved 195 patients, for which 246 ERCPs were performed during the study period. There were 54 major complications with 7 cases of ERCP-specific mortality. The incidence of ERCP complications and mortality in HUSM were within the reported global range. Post-ERCP pancreatitis was the most frequent complication and occurred in 25 patients: mild in 15 patients, moderate in 5 patients, and severe in 5 patients. Cholangitis occurred in 19 patients: mild in 3 patients, moderate in 6 patients, and severe in 10 patients. Bleeding occurred in 4 patients, while only 2 patients had perforation during the procedure. ERCP was performed for 2 pregnant patients; 1 had abortion 10 days after the procedure. However, this patient also had post-ERCP pancreatitis preceding her abortion. The other patient continued her pregnancy and delivered with no complications. Other major complications were acute pulmonary embolism in 1 patient, cardiorespiratory arrest after sedation in 1 patient, and atrial fibrillation in 1 patient. Based on univariate analysis, several factors were identified as significant risk factors for post-ERCP overall complications: history of pancreatitis, performance of pancreatogram, use of pre-cut cannulation, normal cholangiogram, absence of stones, and failure of complete stone clearance. Only 2 factors were independently associated with high risk of overall complications by multivariate analysis, i.e., pancreatic duct injection and absence of stones. For post-ERCP pancreatitis, several conditions have been identified to be significant risk factors using univariate analysis: young age, history of pancreatitis, pancreatic duct injection, normal serum bilirubin, normal cholangiogram, absence of biliary stones, and pre-cut cannulation. Young age, history of pancreatitis, and pancreatic duct injection were also identified as independent risk factors using multivariate analysis. Several conditions were also identified as significant risk factors for cholangitis. Based on univariate analysis, significant factors were malignant jaundice, normal serum bilirubin, co-morbidity with high American Society of Anaesthesiologists (ASA) score, and presence of stricture or stenosis. Successful removal of bile ducts stones was found to contribute to lower risk of cholangitis compared with other therapeutic procedures. Multivariate analysis showed that, out of the tested factors, only the presence of stricture or stenosis was independently increasing the risk of post-ERCP

cholangitis.

Conclusion: The study verified the ERCP complication rate in HUSM, the nature of these complications, and their associated factors. Furthermore, the study highlighted the need for actions to limit or reduce the incidence and severity of the ERCP complications. Hopefully, this will result in better ERCP practice, providing a clearer picture of the risk–benefit ratios in different clinical scenarios and a greater ability in advising patients about their options.

Supervisor:
Dr Mohd Nor Gohar Rahman
Co-supervisor:
Dr Syed Hassan Syed Abd Aziz

NEOANGIOGENESIS AND CYCLIN D1 EXPRESSION IN COLORECTAL CARCINOMA

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MPath (Anatomic Pathology)

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Introduction: In Malaysia, colorectal carcinoma is the most common type of cancer among men. Clinical behaviour of colorectal cancer is currently determined by prognostic parameters, which include tumour size, site, stage, and histological grade. Many patients are having recurrences despite the tumour being diagnosed as a local disease.

Objectives: The aim of this study was to determine Cyclin D1 expression and its relationship with clinicopathological variables, especially the Modified Dukes stage.

Patients and Methods: In this cross-sectional study, 61 colorectal carcinoma patients were selected after applying inclusion and exclusion criteria. The patients' archived, paraffin-embedded tissue blocks were obtained from the Department of Pathology, Hospital Universiti Sains Malaysia. Cyclin D1 expression was evaluated based on the intensity and percentage of positive nuclei. In addition, microvessel density, especially at the peritumoural and intratumoural area, was assessed using CD34 endothelial marker. The relationships between Cyclin D1 expression, microvessel density, and clinicopathological variables (particularly with the Modified Dukes stage, which was assessed using immunohistochemical EnVision technique) were analysed.

Results: For the expression of Cyclin D1, samples were divided into low expresser and high expresser groups. 93.5% of cases expressed Cyclin D1 as nuclear brownish precipitate with 55.7% of cases showing low staining intensity. Univariate analysis showed no significant correlation between staining intensity and clinicopathological variables, i.e., sex, race, size of tumour, site of tumour, histological grade, lymph node metastasis, and tumour stage. 54.1% of colorectal carcinoma cases were in the distribution of less than 10% positive nuclei. Univariate analysis resulted in significant correlations between percentage of positive nuclei and sex, race, lymph node metastasis, and stage of tumour. Other parameters, i.e., size, site of tumour, and histological grade, were not significantly correlated with the percentage of positive nuclei. Multivariate analysis showed that the percentage of positive nuclei was significantly correlated with only lymph node metastasis and tumour stage with ($P < 0.05$). The mean (SD) of peritumoural and intratumoural microvessel densities were 66.8 (17.9) vessel per mm2 and 76.7 (28.7) vessel per mm^2, respectively, indicating the presence of more vessels in the intratumoural areas. The microvessel densities were later grouped into low vascularity and high vascularity; both peritumoural and intratumoural areas had low vascularity. Peritumoural area did show some correlations with clinicopathological parameters, i.e., size, histological grade, stage, and lymph node metastasis; however, the results were not statistically significant. In contrast, intratumoural area did not show any correlation with the clinicopathological parameters.

Conclusion: Percentage of positive nuclei is the most important and useful way to evaluate Cyclin D1 expression. It was significantly correlated with sex, race, tumour stage, and lymph node metastasis. In addition to this, the percentage of positive nuclei was found to be an independent parameter in predicting lymph node metastasis and tumour stage. Microvessel density does not play any role as a prognostic factor in colorectal carcinoma.

Supervisor:
Dr Venkatesh R Nair

EFFECT OF THE SEA CUCUMBER EXTRACT AND TRICHLOROACETIC ACID (TCA) IN THE HEALING PROCESS OF THE TYMPANIC MEMBRANE PERFORATION—PILOT STUDY

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MMed (ORL–Head and Neck Surgery)

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Introduction: Gamat is a marine invertebrate that has high therapeutic value, such as its capability to rejuvenate tissues and hasten wound healing. Its effectiveness is species-specific and dose-dependant. Trichloroacetic acid (TCA) has been utilized to promote tissue generation; the acid cauterization breaks up the fibrosis on the perforation's rim and promotes the formation of granulation and proliferation of new tissues. It has been proposed to be used in treating tympanic membrane perforation. Tympanic membrane perforation is a common problem in otorhinolaryngology.

Many materials and procedures have been used to promote the healing process of the tympanic membrane perforation; these include the use of amniotic membrane, cigarettes paper, and fat plug.

Objective: The objective of this study was to evaluate the healing effect of co-treatment of gamat with TCA versus TCA alone in tympanic membrane perforation.

Patients and Methods: This was a prospective single-blind randomized control trial involving patients with perforated tympanic membrane at the ORL–HNS department, Hospital Universiti Sains Malaysia. The patients were divided into 2 treatment groups: gamat with TCA, and TCA alone. Treatments were performed following the Derlacki method, with some modifications. Both groups were serially examined every 2 weeks. Their tympanic membrane were visualized using rigid otoscope (otoscope size = 2.7 mm), and a photo was taken in every procedure and during follow-up. The perforation area was measured using Material Word Station–Image Analyzer.

Results: There were significant healing improvements of perforated tympanic membrane by using TCA cauterization alone. There were also significant healing improvements of perforated tympanic membrane by using gamat extract with TCA cauterization in perforated tympanic membrane. There was no significant difference of mean healing pattern between the co-treatment of gamat with TCA and single treatment of TCA.

Supervisor:
Professor Dr Dinsuhaimi Sidek
Co-supervisor:
Professor Dr Syed Mohsin Syed Sahil Jamalullail

AN EVALUATION OF QUALITY OF LIFE AMONG PATIENTS UNDERGOING ANGIOGRAM / PERCUTANEOUS CORONARY INTERVENTION IN HOSPITAL UNIVERSITI SAINS MALAYSIA

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MMed (Internal Medicine)

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Introduction: Percutaneous coronary intervention (PCI) has emerged as an important mode of treatment in coronary artery disease (CAD). Despite extensive and complexity of PCI, there is no local data to support the benefits of PCI, including the improvement of the quality of life (QOL).

Objective: The goal of this study was to evaluate the impact of PCI on self-perceived QOL among patients after PCI by using Medical Outcome Survey Short Form-36 (MOS SF-36) questionnaires.

Patients and Methods: This is a single centre cross-sectional study in Hospital Universiti Sains Malaysia (HUSM) conducted among patients electively planned for PCI. We administered the MOS SF-36 questionnaire which has eight domains of QOL such as a physical function, social function, physical role, vitality, general health, mental health, bodily pain, and emotional role. Total scores of improvement were calculated at Day 1 (pre-PCI) and Day 30 (post-PCI).

Results: 75 patients were enrolled in this study. The results showed significant improvement of QOL, with increments in all domains: total score (from 426.1 to 671.1), physical role (from 32.3 to 86.7), emotional role (from 40.0 to 92.9), general health (from 52.4 to 84.8), bodily pain from (54.6 to 83.9), physical function from (56.7 to 84.3), vitality from (56.2 to 77.0), social functioning from (69.5 to 84.0), and mental health from (64.4 to 77.6). All improvements in domains were statistically significant with $P < 0.001$.

Conclusion: Post PCI / angiogram showed significant improvement of QOL assessed by MOS SF-36 questionnaires at Day 30 post-procedure. SF-36 questionnaires provide a simple, reliable, and better predictor in overall QOL assessment by summarizing all eight domains of QOL. Early time return to normal activity with minimum hospital stay along with significant improvement of QOL perceived at Day 30 post-intervention are favourable factors to be considered in choosing PCI as a mode of treatment in CAD.

Supervisor:
Associate Professor Dr Zurkurnai Yusof
Co-supervisor:
Dr Suhairi Ibrahim

RELATIONSHIP BETWEEN INTERVERTEBRAL DISC HEIGHT, LATERAL FORAMEN SIZE, AND NERVE ROOT IMPINGEMENT OF LUMBAR VERTEBRA

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MMed (Orthopaedic)

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Introduction: Degenerative disc disease is a common condition in elderly patients, and its incidence increases with ageing. Patients usually present with back pain and, sometimes, with symptoms of nerve root compression. The degenerative disc disease usually worsens with time. It can be recognized radiographically as a decrease in the intervertebral disc height. Reduction of disc height results in the reduction of lateral foramen size, where the nerve roots exit from the spinal canal.

Objectives: The main objective of this study was to establish the relationship between the lateral foramen size and nerve root impingement in degenerated disc disease. In addition, the study aimed to determine the correlation

between intervertebral disc height and lateral foramen size in degenerative disc disease and radiographically normal disc.

Patients and Methods: This cross-sectional study analysed data taken from the magnetic resonance imaging (MRI) of 62 subjects randomly selected from the radiology archives. The data consisted of measurements of the intervertebral disc height, lateral foramen size, and nerve root size, bilaterally. Reports on symptoms of nerve root compression (such as numbness and weakness) were attained from the patient medical record dated around the day MRI was performed. The findings were analysed using SPSS bivariate correlation test and independent sample t test.

Results: The mean intervertebral disc height was 6.7 ± 1.4 mm at L1L2 level and showed increasing trend at subsequent levels: L2L3 (7.8 ± 1.4 mm), L3L4 (8.7 ± 1.6 mm), and L4L5 (9.0 ± 1.8 mm). At L5S1 level, the mean intervertebral disc height was 8.7 ± 2.0 mm. The lateral foramen height measurements (L1L2–L4L5) were between 17.75 ± 2.30 mm and 19.65 ± 2.25 mm. The mean L5S1 foramen height was 16.40 ± 2.71 mm. The nerve root compression symptoms most commonly occur at L5S1. Statistic analysis showed strong correlation between the intervertebral disc height and lateral foramen height at all levels of the lumbar spine in both degenerated disc and normal disc; however, no correlation was observed between intervertebral disc height and lateral foramen width. There was also no significant correlation between lateral foramen size and nerve root compression at lumbar vertebral.

Supervisor:
Associate Professor Dr Mohd Imran Yusoff
Co-supervisor:
Dr Mohd Shafie Abdullah

NON-ATTENDANCE TO THE PAEDIATRIC CLINIC IN HOSPITAL UNIVERSITI SAINS MALAYSIA (HUSM)

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MMed (Paediatrics)

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Introduction: Non-attendance is suspected to be a major problem in the paediatric clinic. Non-attendance could lead to severe consequences to the patients, and this could contribute a major burden to the health care system. Several studies have reported more than 30% of non-attendance cases. In Malaysia, no such report was available.

Objectives: The study aimed to determine the rate of non-attendance in the Paediatric Clinic, Hospital Universiti Sains Malaysia (HUSM), and to verify the efficacy of a phone call, given to fix a new appointment for every contactable non-attending patient, in improving clinic attendance.

Patients and Methods: This cross-sectional study involved all non-attending patients who had an appointment in the Paediatric Clinic during the study period, from 1 January 2009 to 28 February 2009. Non-attendees who were contactable by phone were included for the interventional segment of the study. A prepared proforma was completed for every non-attendee. Primary outcome measures included attendance rate and efficacy of a phone call to improve attendance.

Results: During the 1-month study period, 1563 patients had appointment in Paediatric Clinic. A total of 497 patients (31.8%) failed to attend their appointment. There were significant differences of attendance depending on clinic sessions, weather condition, and types of clinic. From 160 patients who were successfully contacted, 95 patients were interested in having a new appointment. There were 55 patients who already had a new appointment at the time they were contacted, 3 non-attendees had died at home, 3 had been admitted, and 2 had moved to another state and were followed-up there. The other 2 patients were not interested in getting a new appointment. 23.3% from the intervention group did not attend the clinic after the given new appointment.

Conclusion: Non-attendance rate is high in the Paediatric Clinic, HUSM. A phone call to fix a new appointment is effective in reducing the non-attendance rate.

Supervisor:
Professor Dr Hans Amin Rostenberghe
Co-supervisor:
Associate Professor Dr Nik Zainal Abidin Nik Ismail

ASSESSMENT OF KNOWLEDGE, ATTITUDE, AND PRACTICES AMONG OPERATING ROOM STAFF NURSES TOWARDS THE STANDARD AND TRANSMISSION-BASED PRECAUTION IN UNIVERSITY HOSPITAL

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Introduction: The standard and transmission-based precautions are strongly recommended as the guidelines for preventing infections and ensuring occupational safety. This survey discussed the level of knowledge, attitude, and practices in relation to the standard precautions and transmission-based precautions among the staff nurses.

Objective: The objective of this survey was to identify relationship between demographic profiles of participants and the knowledge, attitude, and practices towards standard precautions and transmission-based precautions.

Patients and Methods: This self-reported survey was conducted in April 2009 among operating room staff nurses. The questionnaire comprised 3 major components:

knowledge, attitudes, and practices towards the standard and transmission-based precautions.

Results: Out of 100 staff nurses, 75 of them had returned the completed questionnaires on the same day of distribution. There was a significant difference between male and female staff nurses in terms of the practices of the standard and transmission-based precautions. There was a weak correlation between the level of knowledge and the age of the female staff. However, in overall, the results did not show any clear correlation, and there was no statistical significance observed in the different demographic profiles concerning the knowledge, attitudes, and practices towards the standard and transmission-based precautions.

Conclusion: This study on knowledge, attitudes, and practices of operating room staff may benefit healthcare educators in planning and developing appropriate educational programmes, assist organizations to provide a safe workplace climate, and aid healthcare workers to learn the importance of personal responsibility in preventing the transmission infectious disease.

Supervisor:
Associate Professor Dr Saedah Ali
Co-supervisor:
Associate Professor Dr Wan Aasim Wan Adnan

ASSOCIATED FACTORS AND SALIVARY BIOMARKER OF STRESS AMONG ASSISTANT MEDICAL OFFICERS IN MINISTRY OF HEALTH (MOH) HOSPITALS IN KELANTAN AND TERENGGANU

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Objectives: The objectives of this study were to determine the prevalence of stress among assistant medical officers and the factors contributing to stress. In addition, the presence of biomarker of stress (cortisol) in the saliva was assessed.

Patients and Methods: Study subjects were assistant medical officers from the government hospitals in Kelantan and Terengganu. A questionnaire consisting of demographic, work-related, and stress-related information were completed and returned by the subjects. Salivary cortisol level was also measured.

Results: The prevalence of stress among assistant medical officers was 13.7% (95% CI 8.61 to 18.79). Simple linear regression showed that age (P = 0.012), duration of employment (P = 0.023), skill discretion (P = 0.039), decision authority (P = 0.039), decision latitude (P = 0.012), psychological job demand (P = 0.015), job insecurity (P = 0.000), co-worker support (P = 0.001), social support (P = 0.003), hazardous conditions (P = 0.001), toxic exposures (P = 0.008), and total physical hazards (P = 0.001) were significant factors associated with stress. However, only decision latitude (P = 0.025), psychological job demand (P = 0.021), job insecurity (P = 0.009), and total physical hazards (P = 0.008) were significant by general linear regression. There was no significant (P = 0.393) correlation between salivary cortisol and stress score. The observed Spearman correlation was 0.066, which suggested no correlation between these 2 parameters. However, salivary cortisol was found to be significantly higher (P = 0.033) among stressed compared to non-stressed assistant medical officers (0.78 μg/dL versus 0.67 μg/dL, respectively).

Conclusion: The prevalence of stress was 13.7%. Decision latitude, psychological job demand, job insecurity, and total physical hazards were the significant associated factors of stress. There was no correlation between salivary cortisol and stress score. However, salivary cortisol was significantly higher among stressed assistant medical officers. Hence, improving the working environment, joint workplace initiatives, team-oriented approaches practice, empowering, and giving more autonomy would improve the work climate and alleviate stress among assistant medical officers.

Supervisor:
Dr Aziah Daud
Co-supervisor:
Dr Wan Mohd Zahiruddin Wan Mohammad

THE EFFECT OF SINGLE DOSE GABAPENTIN ON HAEMODYNAMIC CHANGES FOLLOWING LARYNGOSCOPY AND TRACHEAL INTUBATION

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Introduction: Adrenergic responses manifested by tachycardia and hypertension are known to occur following laryngoscopy and intubation. Various drugs have been used in attempts to attenuate this response; however, all have their limitations. A more satisfactory approach for this situation might be to use 800 mg gabapentin, which can provide good control for a short period of laryngeal tracheal intubation for a planned elective case that require general anaesthesia. Gabapentin also have other complimentary actions. Many studies using gabapentin as a single agent peri-operatively showed promising outcomes. In this study, the capability of this drug to attenuate the haemodynamic response to laryngoscopy and tracheal intubation was evaluated.

Objectives: The objectives of this double-blinded,

randomized study were to compare the effect of haemodynamic changes after laryngoscopy and tracheal intubation in patients pre-treated with placebo or a dose of gabapentin (600 mg or 800 mg) and to determine the optimal dose of gabapentin to attenuate the haemodynamic changes.

Patients and Methods: A total of 111 patients of ASA physical status I or II undergoing elective surgery were selected and divided into 3 pre-treatment groups: placebo (control group), 600 mg gabapentin, and 800 mg gabapentin, all as pre-treatments prior to intubation. All patients were then induced using intravenous fentanyl (1.5 μg/kg), propofol (2 mg/kg), and esmeron (1.5 mg/kg). Intubation was performed 3 minutes following esmeron injection. Anaesthesia was maintained with nitrous oxide in oxygen with a ratio of 2:4 and 2.0% sevoflurane. Selected parameters, i.e., heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) were recorded prior to injection of study drugs as baseline, at 1 minute after laryngeal tracheal intubation, and every minute after laryngoscopy and tracheal intubation for 5 minutes. Patients were also monitored for complications, such as bucking, moving limbs, bronchospams, hypotension, and arrhythmia.

Results: The results showed that mean HR, SBP, DBP, and MAP were increased in all groups after laryngoscopy and tracheal intubation compared with baseline. However, percentages of increase in the HR, SBP, DBP, and MAP after laryngoscopy and tracheal intubation in the 800 mg gabapentin group were the least compared with the other groups.

Conclusion: This study has shown that the dose of gabapentin at 800 mg was able to give the minimum percentage changes of the mean HR, SBP, DBP, and MAP after laryngoscopy and tracheal intubation within a certain period (5 minutes). It can be concluded that pre-emptive treatment with gabapentin (800 mg) can be used to attenuate the haemodynamic response during laryngeal tracheal intubation.

Supervisor:
Dr Nizar Abd Jalil

EFFECTIVENESS OF OCCUPATIONAL STRESS MANAGEMENT PROGRAM AMONG PETROCHEMICAL INDUSTRY WORKERS IN KERTEH, TERENGGANU

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Introduction: Occupational stress is an increasingly important occupational health problem that can lead to a significant economic lost. Petrochemical industry workers are one of several occupational groups potentially experiencing high level of stress. They work in high-pressure environment, handling hazardous material, and managing large amount of chemical energy.

Objectives: The aim of this study was to determine the prevalence and associated factors of depression, anxiety, and stress among workers in the petrochemical industry. In addition, an intervention program on occupational stress was introduced and evaluated.

Patients and Methods: This was an intervention study to assess the effect of stress management intervention program based on DASS42 score. The stress management program adopted consisted of lectures on stress and stress management, video sessions, question and answer sessions, pamphlet distribution, and aerobic exercises. Validated Malay-versions of the Depression, Anxiety, and Stress score (DASS42) and Job Content Questionnaire (JCQ) were used. The respondents were asked to complete DASS42 and JCQ at the beginning of the program. This gave the prevalence and associated factors of depression, anxiety, and stress. The respondents were again asked to complete the DASS42 immediately after the intervention program and 1 month post-intervention. Multiple linear regression analysis was done to evaluate the stress-associated factors, and repeated measures of analysis of variance (ANOVA) were done to determine the effect of intervention.

Results: Out of 51 participants, there were 50 Malays and 1 Indian. The mean (SD) age was 33.57 (7.39) years old and duration of work was 7.27 (3.47) years. The prevalence (95% CI) of depression, anxiety, and stress of moderate severity or more were 23.53 (95% CI 11.48 to 35.58), 31.37 (95% CI 18.19 to 44.55), and 21.57 (95% CI 9.89 to 33.25), respectively. Type of work, decision latitude, and psychological job demand were found to be associated with depression. Level of education and exposure to toxic material were associated factors for anxiety. Marital status and psychological job demand were associated factor for stress. In the assessment of effectiveness of stress management program, it was found that there were significant reduction in occupational stress post-intervention (immediate and 1 month after) with estimated marginal mean of depression score, anxiety score, and stress score ($P < 0.001$).

Conclusion: There is an evidence of occupational stress in the petrochemical workplace where prevalence of stress, anxiety, and depression were considerably high. It indicates the needs to improve the work environment, in terms of decision latitude, psychological job demand, and toxic exposure. A regular, short stress management program is effective in reducing some aspect of depression, anxiety, and stress in the petrochemical workers.

Supervisor:
Associate Professor Dr Mohamed Rusli Bin Abdullah
Co-supervisor:
Dr Aziah Binti Daud
Dr Nor Azwany Yaacob

THE PROPORTION OF METABOLIC SYNDROME PATIENTS AND ASSOCIATED FACTORS AMONG SUBJECTS UNDERGOING CORONARY ANGIOGRAM IN HOSPITAL UNIVERSITI SAINS MALAYSIA (HUSM), KOTA BHARU, KELANTAN, USING IDF AND ASIAN MODIFIED NCEP ATP III CRITERIA

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Introduction: There is an increasing prevalence of metabolic syndrome worldwide, which is closely related to the presence of coronary artery disease. Metabolic syndrome worsens the severity of coronary artery disease. However, information on the percentage of metabolic syndrome among heart disease patients in Malaysia is lacking.

Objectives: The objectives of this study were to determine the proportion of metabolic syndrome using both the Internal Diabetes Federation (IDF) and Asian-modified National Cholesterol Education Program—Third Adult Treatment Panel III (NCEP ATP III) criteria, and to determine the factors associated to metabolic syndrome among patients undergoing coronary angiogram in Hospital Universiti Sains Malaysia.

Patients and Methods: A cross-sectional study was conducted among 236 patients who fulfilled the inclusion criteria and were suspected of having coronary artery disease. These patients came for elective coronary angiograms from September 2006 to April 2008; 228 patients underwent angiogram procedure while 8 patients were unable to do so due to several reasons. Metabolic syndrome was defined using both the IDF and Asian-modified NCEP ATP III criteria. The required information on demographic, physical, and biochemical parameters related to the metabolic co-morbidities was recorded in a data collection form. The patients' medical records were then reviewed to assess the degree of coronary artery disease severity.

Results: The proportion of metabolic syndrome based on IDF criteria among patients undergoing coronary angiogram in HUSM was 50.0%, and based on Asian-modified NCEP ATP III criteria, 76.8%. The majority of patients who underwent angiograms were diagnosed with coronary artery disease (73%). There were 20% patients with one-vessel disease while 53% patients had multi-vessel disease; 22% patients with two-vessel disease and 31% patients with three-vessel disease. There was a significant association between metabolic syndrome patients (based on IDF criteria) and smoking (OR = 2.08, 95% CI 1.20 to 3.63). Based on the Asian-modified NCEP ATP III criteria, there were associations between metabolic syndrome and age (OR = 2.20, 95% CI 1.04 to 4.68), female sex (OR = 3.99, 95% CI 1.28 to 12.30), and smoking (OR = 7.00, 95% CI 3.05 to 16.00).

Conclusion: The proportion of metabolic syndrome among patients undergoing coronary angiogram was similar to other studies. The proportion of metabolic syndrome was higher if the Asian-modified NCEP ATP III criteria were used compared with IDF criteria. Age of more than 60 years old, female sex, and smoking were significantly associated with presence of metabolic syndrome.

Supervisor:
Dr Azidah Abdul Kadir

MULTIMODALITY ASSESSMENT OF MILD AND MODERATE HEAD INJURY PATIENTS

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Objectives: The aim of this research was to compare the disability, which may be proven using the analysis of the Barrow Neurological Institute (BNI) Screen for Higher Cerebral Functions, as well as the disturbance in balance control, sensory perception, and the presence of nystagmus in patients with mild and moderate head injury.

Patients and Methods: This was a prospective study conducted from August 2006 until November 2007. The selection criteria were patients who sustained mild and moderate head injury and admitted or referred to the Hospital Universiti Sains Malaysia. Qualified patients were called within 4 to 6 weeks after the occurrence of head injury. Their complaints of headache, memory loss, sensory disturbance, as well as gait and visual abnormalities would be noted. The patients were given a series of tests, starting with the BNI Screen for Higher Cerebral Functions, conducted in the local, Malay language. The Sensory Organization Test (SOT) was conducted using the SMART Balance Master (NeuroCom International, Inc, USA) to test for balance, i.e., how visual, somatosensory, and vestibular inputs affect a patientsí ability to maintain functional balance. The presence of nystagmus was tested using the Visual Eyes Nystagmography (Micromedical Technologies, USA) where a stimulus was delivered via a tower, and the movement of the eye was followed, recorded, and analyzed for abnormalities. The cold detection threshold (CDT) was measured with Computerised Assisted Sensory Evaluator (WR Medical Electronics, USA) using the 4, 2, and 1 stepping algorithm with null stimuli. The test was performed on the dorsal aspect of the palm of the left hand; a subject indicated that a stimuli was felt by pressing the ìyesî button (and no button, if otherwise), and the just noticeable difference (JND) was calculated from the subjects responses. Data's analyses were done using the SPSS version 12.0.

Results: The study involved 11 male patients (92%) and one female patient (8%). The age of the patients ranged over 18-63 years, with a mean of 33.3 years and a median of 29.0 years. A majority of the patients cases were categorized under moderatehead injury (75%), and all (100%) injuries resulted from motor vehicle accidents. Patients with mild head injury have higher BNI score compared with those with moderatehead injury, with mean values of 45.00 and 43.11, respectively. A similar pattern was observed in BNI sub-test scores, particularly in speech and language, attention/ concentration, visuo-spatial and visual problem solving, and memory. However, none of the results were significant by Mann-Whitney test. There was no evidence to support the hypothesis that moderate head injury causes significant disturbance in balance. Both groups of patients, with mild and moderatehead injury, has normal balancing reflex when tested using the computerized post urography. In addition, no significant sensory disturbance or post-traumatic nystagmus was observed.

Conclusion: The disability reported by the patients, such as complaints of forgetfulness and lack of concentration, could not be demonstrated by using the BNI Screen for Higher Cerebral Functions. Other tests carried out failed to prove any significant disturbances. Therefore, the cause and effect of a post-concussive syndrome can only be diagnosed clinically.

Supervisor:
Professor Dr Jafri Malin Abdullah

ANAEMIA AMONG HIV-INFECTED PATIENTS: PREVALENCE, ASSOCIATED RISK FACTORS, AND QUALITY OF LIFE

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MMed (Family Medicine)

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Introduction: Anaemia is a common manifestation of HIV infection, with reported prevalence ranging from 30% in asymptomatic HIV patients to 80% in clinical AIDS patients. It is associated with disease progression, poor quality of life, and increased mortality in HIV-infected individuals.

Objectives: The objectives of the study were to determine the prevalence of anaemia, its associated factors, and impact on quality of life in HIV-infected individuals. Correlation between haemoglobin level and CD4 count was also assessed.

Patients and Methods: A cross-sectional study was conducted among 248 HIV-infected adults attending the Infectious Disease Clinic, Hospital Raja Perempuan Zainab II, Kota Bharu, from 1 January 2008 until 31 December 2008. Patients who were pregnant or on combination antiretroviral therapy were excluded from the study. The socio-demographic and medical data, including the latest haemoglobin and CD4 results, were taken for analysis. Assessment on the quality of life was done using Medical Outcome Study–HIV (MOS–HIV) questionnaire.

Results: Overall prevalence of anaemia was 46.0% (95% CI 39.77 to 52.10), with a mean (SD) haemoglobin value of 12.3 (2.4) g/dL. In multiple logistic regression analysis, several factors were observed to be significantly associated with anaemia: CD4 count of less than 200 cells/µL (OR 5.28, 95% CI 2.53 to 11.01), oral candidiasis (OR 5.02, 95% CI 1.82 to 13.87), history of blood transfusions (OR 4.87, 95% CI 1.53 to 15.49), and income of RM501–RM1000 (OR 0.40, 95% CI 0.17 to 0.94 and more than RM1000 (OR 0.24, 95% CI 0.12 to 0.52). There was a significant correlation between the haemoglobin value and CD4 count ($P < 0.001$, Spearmen correlation = 0.443). Anaemic patients had significantly lower MOS-HIV subscale scores in general health perception, pain, physical function, role function, energy, and quality of life ($P < 0.05$).

Conclusion: The high prevalence of anaemia among HIV-infected individuals was comparable with other countries. Anaemia significantly impaired the quality of life of these individuals. Knowing the associated factors for anaemia should prompt a more careful evaluation into the presence of anaemia in any HIV-infected individuals.

Supervisor:
Dr Juwita Shaaban:
Co-supervisors:
Dr Amaluddin Ahmad
Dr Mahiran Mustafa

FAMILY PRESENCE DURING CARDIOPULMONARY RESUSCITATION: ATTITUDES AND EXPERIENCES OF MEDICAL PERSONNEL AND PARENTS

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Introduction: Presence of family during cardiopulmonary resuscitation (CPR) is not a widespread practice; however, it is becoming more common nowadays. Normally, family members are not allowed to be present during CPR, and this is a true medical practice in most hospitals in Malaysia.

Objectives: The study aimed to determine the attitudes and experiences of medical personnel and parents in Hospital Universiti Sains Malaysia and Hospital Sultanah Nur Zahirah regarding family presence during CPR in children and the associated demographic factors that contribute toward such attitudes.

Patients and Methods: This was a prospective cross-

sectional study. Respondents include medical personnel caring for sick children and convenience sample of parents whose children were admitted in these hospitals. A questionnaire was developed based on previous literature review; the questionnaire incorporated demographic data, questions on CPR experiences, and a series of attitude statement that were rated using 5-point Likert scale. A self-administered questionnaire was given to the medical personnel while the parents were interviewed using a standard questionnaire after giving their verbal consent. A brief video was showing to the parents before the interview to increase the understanding of CPR. The attitude, experiences, and association of demographic factors with the acceptance of family presence during CPR in children were studied.

Results: Only 86 (19.9%) of medical personnel had previously participated in the CPR in children in the presence of family members. Out of 100 parents, 19 (19%) had experienced being presentduring CPR of their children. A majority of 279 medicalpersonnel (64.5%) did not approve allowing family members to be with their children during CPR; However, a majority of 253 medicalpersonnel (58.6%) would like to be presentduring CPR of their own children. On the other hand, 74 parents (74%) were of the opinion that family members should be allowed to be present during CPR. Logistic regression analysis demonstrated no significant association between the agreement of family presence during CPR with the demographic factors in both medical personnel and parents.

Conclusion: Medical personnel and parents showed different attitudes toward family presence during CPR in children.

Supervisor:
Professor Dr Quah Ban Seng
Co-supervisor:
Dr Noraida Ramli

THE PREVALENCE OF URINARY INCONTINENCE, ITS ASSOCIATED RISK FACTORS, AND THE IMPACT ON QUALITY OF LIFE AMONG MARRIED WOMEN IN HOSPITAL SULTANAH NUR ZAHIRAH, KUALA TERENGGANU

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MMed (O & G)

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Introduction: Urinary incontinence is defined by the International Continence Society as the complaint of any involuntary leakage of urine. Urinary incontinence is a common condition with significant medical, social, and psychological problems in women. It is very common in women that it is often mistakenly viewed as a natural process of ageing.

Objectives: The aim of this study was to determine the prevalence of urinary incontinence as well as its risk factors and impact among married women.

Patients and Methods: A cross-sectional descriptive analysis was conducted among 300 married women attending Hospital Sultanah Nur Zahirah. A structured questionnaire, prepared in Malay language, was used to collect the data. The questionnaire consisted of 3 parts. Part 1 was on the demographic data and medical history of the respondent, e.g., age, education, occupation, weight, height, parity, menopause status, surgical, obstetrical, gynaecological history. Part 2 and 3 were the validated Bristol Female Lower Urinary Tract Symptom (BFLUTS) questionnaire consisting of 34 questions that provided the assessment of incontinence, other urinary symptoms, sexual function, and aspect of quality of life. Statistical analyses were performed using chi-square test and multiple logistic regression analysis.

Results: The mean age of the women was 41.0 (SD 9.86) years old. The overall prevalence of urinary incontinence was 37.7%. The prevalence of stress incontinence was 16.7%, urgency incontinence, 9.3%, and mixed incontinence, 11.7%. Factors that influenced overall urinary incontinence were parity, menopause, duration of menopause, and mode of delivery. Among the different modes of delivery, spontaneous vagina deliveries and instrumental deliveries had significant influence on urinary incontinence. Mode of delivery and the increases in parity and duration of menopause were significantly influencing stress incontinence. Mixed incontinence was significantly influenced by menopausal status of the women and the increases in age and duration of menopause. No risk factor that could be significantly associated with urgency incontinence was observed in this study. All types of urinary incontinence (stress, urgency, and mixed) significantly affected the quality of life of the women, especially in physical activities ($P < 0.05$), functional activities ($P < 0.05$), social life ($P < 0.05$), and sexual life ($P < 0.05$). Only 14.3% of the women experiencing incontinence sought treatment.

Conclusion: The prevalence of urinary incontinence was high among the studied population. Stress incontinence had the highest prevalence, followed by mixed incontinence and urgency incontinence. All types of urinary incontinence were significantly affecting the quality of life of the women. The percentage of incontinence women who sought treatment was very low. It was recommended for public education to focus on cognitive and affective learning in order to increase the public knowledge about the causes and treatments of incontinence, and to remove the stigma surrounding incontinence.

Supervisor :
Dr Mohd Pazudin Ismail
Co-supervisors:
Professor Dr Nik Mohamad Zaki Nik Mahmood
Dr Nasir Tak Abdullah

EVALUATION OF 8-HYDROXYDEOXYGUANOSINE ENZYME AND MICROSCOPIC FEATURES IN PRIMARY AND RECURRENT PTERYGIA

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MMed (Ophthalmology)

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Introduction: Pterygium is a common external ocular disease that normally occurred in the tropical regions because of the exposure to sunlight, which is considered as a major factor in the development of pterygium. Sunlight contains ultraviolet B radiation that causes oxidative damage to the DNA of the cells. This leads to the production of 8-OHdG enzyme, which is highly mutagenic, that increase cellular proliferation and development of pterygium.

Objectives: The study objectives were to demonstrate the association between the presence of 8-OHdG enzyme and the type of pterygium, as well as to determine the microscopic features (cell maturation, epithelial cell layers, and stromal vascularity) of primary and recurrent pterygia, and comparing these features between those with positive and negative 8-OHdG enzyme in pterygia.

Patients and Methods: A total of 92 samples were collected from the pterygium patients. The samples were processed for histopathology examination and immunohistochemistry test to detect the presence of 8-OHdG enzyme.

Results: The presence of 8-OHdG enzyme was 63.6% in primary pterygium, 16.6% in recurrent pterygium, and 16.0% in normal conjunctiva (control) group. There was a significant association between the presence of 8-OHdG enzyme and primary pterygium ($P < 0.001$). The highest cell maturation (Grade 3 of 10 or more nucleated cells) was observed in normal conjunctiva (in 5 samples), followed by primary pterygium (in 2 samples) and recurrent pterygium (none). The mean epithelial cell layer in primary pteryngium was 6, and in recurrent pterygium, 5; both were higher than the mean of control group, which was 3. The means of stromal vascularity were 9 in primary pterygium and 12 in recurrent pterygium, while in normal conjunctiva it was 3. There was no significant difference in the mean cellular maturation, epithelial cell layers, and stromal vascularity between those with positive and negative 8-OHdG enzyme in all types of pterygium.

Conclusion: There was significant association between the presence of 8-OHdG enzyme and primary pterygium. Histopathology examination revealed increased cellularity and vascularity in recurrent pterygium, which is higher than primary pterygium; both showed higher figures than in normal conjunctiva. There was no significant difference in the mean cellular maturation, epithelial cell layers, and stromal vascularity between those with positive and negative 8-OHdG enzyme in all types of pterygia. There was no significant difference in the mean cellular maturation and epithelial cell layers associated with 8-OHdG enzyme between the different pterygium groups; however, there was significant difference in the mean stromal vascularity associated with 8-OHdG enzyme between primary pterygium and normal conjunctiva.

Supervisor:
Associate Professor Dr Mohtar Ibrahim
Co-supervisor:
Associate Professor Dr Hasnan Jaafar

A STUDY OF BLOOD GLUCOSE LEVEL DURING ADMISSION AND 24 HOURS POST-OPERATION WITH THE OUTCOME OF TRAUMATIC BRAIN INJURY IN HOSPITAL KUALA LUMPUR: AN OBSERVATIONAL STUDY

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Introduction: Traumatic brain injury (TBI) has been associated with acute stress response via sympathoadrenomedullary axis of which, clinically, can be reflected in the increase of blood sugar level.

Objectives: This study aimed to determine whether isolated TBI would cause any increase in blood sugar level on admission and 24 hours after having surgical intervention for the injury.

Patients and Methods: This prospective observational study was conducted among patients treated for TBI in Hospital Kuala Lumpur from January 2007 until December 2007. A total of 294 patients were selected following the inclusion and exclusion criteria; the patients include those with mild, moderate, and severe TBI. A standard performa was used to collect the data.

Results: The patients' age ranged 18–65 years old with mean (SD) of 34.2 (13.0) years old. The median age was 31 years old with a mode of 22 years old (50th centiles at 31 years old and 75th centiles at 43 years old). The majority of the cases were young adult patient. Out of 294 cases, 83.0% (n = 244) patients were male and 50 patients 17.0% (n = 50) were female, with male to female ratio of 5:1. The subjects comprised mostly Malay ethnicity, 53.4% (n = 157), followed by Chinese, 20.8% (n = 61), Indians, 14.6% (n = 43), and others, 11.2% (n = 33). The majority of cases were direct admission from Emergency Department, 57.8% (n = 170), followed by referrals from district hospitals, 39.8% (n = 117), and others, 2.4% (n = 7). Road traffic accident presented as the most common type of injury, 91.5% (n = 269), whereas fall presented 6.8% (n = 20) and assault cases, 1.7% (n = 5). From this study, computed tomography scan of the brain revealed mass lesions in 93.2% of patients (n = 274). These lesions include subdural hematoma,

45.6% (*n* = 134), extradural hematoma, 27.9% (*n* = 82), and intraparenchymal/contusion hematoma, 26.5% (*n* = 78). The mean (SD) of Glasgow Coma Scale upon admission was 9.3 (2.5). Most patients suffered severe head injury, 47.6% (*n* = 140), followed by moderately severe head injury, 35.4% (n = 104), and mild head injury 17.0% (*n* = 50). Patients subjected for major operation was 82.3% (*n* = 242), minor operation, 6.6% (*n* = 19), and conservative treatment, 11.2% (*n* = 33). The mean (SD) admission blood glucose level was 6.26 (1.30) mmol/L, while for 24 hours post operative, it was 6.64 (1.44) mmol/L. There is only slight increment of mean glucose level to suggest that isolated TBI was the main cause for raise blood sugar level ($P < 0.001$ in analysis of variance). Mild TBI group has a mean (SD) glucose level of 5.04 (0.57) mmol/L, moderate TBI group, 5.78 (0.89) mmol/L, and severe TBI, 7.04 (1.24) mmol/L. There is significant difference of admission glucose level for severe TBI compared with mild and moderate TBI ($P < 0.01$ in independent *t* test). Mean (SD) admission glucose in isolated TBI patient associated with poor outcome was 6.98 (0.10) mmol/L; however, it was not significant ($P > 0.05$ in independent *t* test). However, patients with mean (SD) admission glucose of 5.56 (0.88) mmol/L were more likely to have favourable outcome ($P < 0.001$ in independent *t* test).

Conclusion: This study showed significant differences of blood glucose level in isolated TBI. Mild, moderate, and severe TBI would cause a raise in blood sugar level during admission, and the mean increased according to the severity of isolated TBI. Surgical intervention did not cause any significant changes in the blood glucose level. Isolated TBI with minimal increase of blood sugar level would have favourable outcome.

Supervisor:
Dr Mohammed Saffari Mohammed Haspani

PROSPECTIVE RANDOMIZED CONTROL TRIAL COMPARING METFORMIN AND CLOMIPHINE CITRATE AS OVULATION INDUCTION AGENT IN WOMEN WITH POLYCYSTIC OVARIAN SYNDROME AT ALOR STAR HOSPITAL, MALAYSIA

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Objectives: The study was design to evaluate the effects of metformin on weight reduction, waist–hip ratio (WHR), menstrual cycle, and ovulation rates among women with polycystic ovarian syndrome and infertility.

Patients and Methods: A total of 115 patients diagnosed with polycystic ovarian syndrome (according to the revised Rotterdam ESHRE/ASRM criteria, 2003) attending Infertility Clinic of Alor Star Hospital were recruited and later randomized into 3 group: Group A, metformin alone (*n* = 38), Group B, clomiphene citrate alone (*n* = 39), and Group C, combination of metformin and clomiphene citrate (*n* = 38). During the first visit, the WHR of each patient was measured. Baseline levels of serum follicular stimulating hormone, luteinising hormone, testosterone, liver function test, renal function test, and serum prolactin, were evaluated. In Group A, metformin (500 mg tds) was given for a period of 6 months. In Group B, clomiphene citrate (100 mg) was given daily, from day 2 to day 6 of menstruation. The dosage was increased by 50 mg for each cycle should anovulation was noted, to a maximum of 200 mg. In Group C, a combination of metformin and clomiphene citrate were given according to the dosage received by Groups A and B. Ovulation was confirmed by performing transvaginal scan. The evaluation of the WHR and levels of serum follicular stimulating hormone, luteinising hormone, and testosterone was repeated every 3 months.

Results: Metformin has no effects on weight reduction or WHR. Only 9 out of 38 patients (23.7%) who took metformin had ovulation compared with 28 out of 39 patients (71.8%) who took clomiphene citrate. This difference was statistically significant ($P < 0.001$). There was no significant difference in the number of patients who had ovulation between those taking clomiphene citrate alone (28 out of 39 patients, 71.8%) and combination of clomiphene citrate and metformin (26 out of 38 patients, 68.4 %).

Conclusion: Metformin has no effect on weight and WHR reduction. It should not be used to regulate menstrual cycle in patients with polycystic ovarian syndrome. Its usage was not superior to the traditionally used clomiphene citrate in inducing ovulation among patients of polycystic ovarian syndrome with fertility problem, and no additional advantage could be gained if it was combined with clomiphene citrate. Therefore, the current protocol of using clomiphene citrate as the first-line drug in the induction of ovulation among patients with polycystic ovarian syndrome and infertility should remain.

Supervisor:
Associate Prof Adibah Ibrahim
Co-supervisors :
Dr Murizah Md Zain
Dr Mohd Rushdan Md Nor

A STUDY OF DEPRESSION AND/OR ANXIETY AMONG ADULT TYPE 2 DIABETES MELLITUS PATIENTS ATTENDING DIABETIC CENTER, HOSPITAL UNIVERSITI SAINS MALAYSIA

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Objectives: The study objectives were to determine the prevalence of depression and/or anxiety and their associated factors among patients with diabetes mellitus in USM.

Patients and Methods: This was a cross-sectional study involving 260 Type 2 Diabetes Mellitus adult patients attending the Diabetic Centre, Hospital Universiti Sains Malaysia from November 2007 until March 2008. The patients were interviewed using a set of questionnaires on socio-demographic, family dynamic, and medical history. Screening for depression and anxiety was done using self-administered Hospital Anxiety and Depression Scale. Patients who obtained a score of 9 and above were considered positive for anxiety and depression. They were referred to psychiatrist for further assessment to confirm the diagnosis according to the Diagnostic and Statistical Manual of Mental Disorder (DSM-IV) criteria.

Results: The prevalence of depression was 20.8%, and anxiety, 10.8%. The factors that significantly increase the risk for depression in these patients were working in private sector, receiving income from other family members, cared by children during acute illness, and the presence of diabetic retinopathy as well as diabetic foot ($P < 0.05$ in each). Those who had received secondary and tertiary level of education, satisfied with their income, and had retired had a significantly lower risk for depression ($P < 0.05$ in each). Patients with diabetic foot were significantly more prone to anxiety ($P < 0.05$); in contrast, result showed that males and those with occasionally dominance in family decision-making process were associated with less anxiety ($P < 0.05$ in each).

Conclusion: Prevalence of depression among Type 2 Diabetes Mellitus patients was similar with most studies; however, the prevalence of anxiety was lower in this group of patients. Depression and anxiety risks increased with presence of one or more problems related to occupation, family matters, and the disease complications.

Supervisor:
Dr Azidah Abdul Kadir
Co-supervisors:
Dr Asrenee Abdul Razak
Dr Azriani Abd Rahman

THE EFFECT OF INTRATHECAL BUPIVACAINE VS ROPIVACAINE ON SEDATION REQUIREMENT OF PROPOFOL

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Introduction: Regional anaesthesia with supplementary sedation is often performed for patient's comfort during surgery. Continuous infusion of propofol is a useful sedation method because of the easy management by titration and rapid emergence. Spinal anaesthesia is known to have the sedative effect and decreases the sedative or anaesthetic dose requirement of general anaesthetics. Therefore, it is possible that after a spinal anaesthesia, even routine doses of sedatives can lead to some unwarranted effects.

Objectives: The study was designed to evaluate whether 2 different types of local anaesthetic (plain bupivacaine and plain ropivacaine) can affect the quality of sedation of propofol during bispectral index (BIS)-guided sedation in lower limb orthopaedic surgery.

Patients and Methods: A double-blind randomized clinical trial was conducted among 70 ASA Grade I and II patients who underwent orthopaedic surgery of the lower limb, with the duration of operation of less than 3 hours. These patients received an intrathecal injection of either 15 mg ropivacaine (3 ml of 0.5% solution) or 15 mg bupivacaine (3 ml of 0.5% solution). 15 minutes after obtaining the appropriate level of spinal anaesthesia, propofol infusion was started at a rate of 6 mg/kg/hr to achieve a BIS level of less than 75 (onset time), then reduced to 3 mg/kg/hr and titrated to maintain the BIS level between 65 and 75. Propofol infusion was stopped at 50th minutes after spinal anaesthesia to measure the time taken to reach a BIS level of 90 (recovery time).

Results: Median anaesthetic level was T4 in the bupivacaine group and T10 in the ropivacaine group. In both the bupivacaine and the ropivacaine groups, the onset times were 243.1 (SD 48.1) seconds versus 269.1 (SD 50.7) seconds ($P < 0.05$), the recovery times were 278.2 (SD 46.8) seconds versus 251.0 (SD 47.5) seconds ($P < 0.05$), the loading doses of propofol were 0.50 (SD 0.09) mg/kg versus 0.54 (SD 0.10) mg/kg ($P < 0.05$), and the maintenance doses were 2.13 (SD 0.38) mg/kg/hr versus 2.40 (SD 0.33) mg/kg/hr ($P < 0.01$), respectively. Haemodynamic variables during sedation period did not show any significant difference between the 2 groups.

Conclusion: Bupivacaine at 15 mg, 0.5% solution was associated with a faster onset time, delayed recovery time, and lower dose of propofol sedation requirement compared with ropivacaine at the same dosage. The height of spinal anaesthesia and potency of local anaesthetic were factors that influenced the requirement of propofol for sedation. Haemodynamic variables in bupivacaine group and ropivacaine group were comparable and did not show significant difference.

Supervisor:
Professor Dr Nik Abdullah Nik Mohamad

FEATURES AND PATHOGENS IN COMMUNITY-ACQUIRED PNEUMONIA

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Introduction: Community-acquired pneumonia (CAP) is associated with significant mortality rate. Despite that, the initial treatment is mainly empirical because the precise aetiology is usually unknown at the time of initiating the antibiotic therapy.

Objectives: The objectives of this study were to determine the prevalence and the local pattern of aetiological pathogens causing CAP among hospitalized patients, and to look at the associated risk factors (old age, co-morbidities, and smoking), the severity of CAP at presentation (based on CURB-65 score), as well as the clinical and radiological features with any specific group of pathogens in order to guide for empirical antibiotic therapy and its subsequent management.

Patients and Methods: The cross-sectional study was performed from June 2008 to March 2009 in Hospital Universiti Sains Malaysia. All adults aged 18 years old and above who were admitted to general medical wards for CAP, willing to participate with informed consent, and fulfilled the inclusion criteria were selected. The largest sample size required was 152 samples. Diagnostic microbial specimens that were collected were tested in blood and sputum cultures, serology, viral studies, and sputum acid-fast bacilli (AFB) or tuberculosis (TB) culture. Demographic data, risk factors, clinical and radiological features, CAP severity at presentation, and laboratory results were recorded. Variables obtained were analysed for the prevalence as well as significant associations using chi-square test and logistic regression analysis.

Results: A total of 143 subjects were enrolled in this study. The prevalence of identified pathogens in CAP was 38 ± 5% (95% CI 30% to 47%). The most commonly isolated pathogens were the Gram-negative bacteria, namely, *Haemophilus influenza* (6.3%) and *Klebsiella* spp. (6.3%), including *Klebsiella pneumoniae*. *Mycobacterium tuberculosis* was found in 6.3% of samples. Multivariate analysis revealed that antibiotic therapy given prior to the test reduced the ability to correctly identify the pathogens (P = 0.040, OR = 0.42). Underlying bronchiectases (P = 0.026, OR = 4.19) or presence of alveolar infiltrates (P = 0.045, OR = 3.54) were significantly associated with identified pathogens. Presence of purulent sputum (P = 0.001, OR = 9.43) or underlying bronchiectases (P = 0.005, OR = 6.73) were associated with Gram-negative bacteria. Diabetes mellitus (P = 0.013, OR 8.53) and cavitation on chest radiograph (P = 0.003, OR = 19.32) were associated with *Mycobacterium tuberculosis*. Univariate analysis on Gram-positive pathogens showed its association with only fever of more than 38.5 °C (P = 0.014). Blood culture were more likely to be positive in the presence of fever of more than 38.5 °C (P < 0.001), haemodynamic instability with systolic blood pressure of less than 90 mmHg and diastolic blood pressure of 60 mmHg and less (P = 0.002, OR = 15.50), or underlying diabetes mellitus (P = 0.047, OR 5.81). Presence of purulent sputum (P = 0.024, OR 3.47) or underlying bronchiectases (P = 0.030, OR = 4.46) were associated with positive sputum culture. Subjective feeling of breathlessness were less likely to be associated with identified pathogens (P = 0.012, OR = 0.35) or Gram-negative pathogens (P = 0.030, OR = 0.321). There was no significant association between old age (more than 60 years old), current cigarette smoking status, or severity of CAP on admission with any specific group of pathogens (P > 0.05). Atypical pathogens were not associated with any factors or features.

Conclusion: The prevalence of identified pathogens in our study was within the expected range. Gram-negative bacteria were the most common pathogens identified; this was similar to a previous local study. *Mycobacterium tuberculosis* was not an uncommon cause of CAP. Certain factors and clinical presentation of CAP were associated with specific group of pathogens. Prescription of empirical antibiotic therapy that covers Gram-negative pathogens as well as further investigation to look for tuberculosis in high risk patients are recommended.

Supervisor:
Associate Prof Che Wan Aminud-din Hashim
Co-supervisors:
Dr Shaharudin Abdullah
Dr Siti Suraiya

THE ASSOCIATION STUDY BETWEEN MAGNETIC RESONANCE IMAGING (MRI) AND PERCUTANEOUS TRANSPEDICULAR BIOPSY (HPE) FINDINGS IN VERTEBRAL LESIONS

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Introduction: Magnetic resonance imaging (MRI) has become the instrument of choice for disease detection in spine pathology. More recent reports have questioned the specificity of MRI detection and concluded that signal intensity change alone is not diagnostic of a metastatic lesions. The introduction of percutaneous biopsy has substantially benefited the diagnosis of skeletal diseases. It avoids the need for open surgical biopsy in most patients. Percutaneous transpedicular biopsy for vertebral lesions can be performed with minimal morbidity and good diagnostic yield with accuracy of up to 92% in bone metastases.

Objectives: The main objective of this study was to determine the sensitivity and specificity of MRI in the diagnosis of a vertebral spinal lesions, specifically in vertebral tuberculosis and metastases. The association between MRI and transpedicularbiopsy (histopathological) findings was to be studied. The secondary objective was to determine the correlation of transpedicularbiopsy done under C-arm fluoroscopy with respect to major influencing variables such as spinal level, tissue type (lytic and sclerotic), and ultimate histopathological examination (HPE).

Patients and Methods: In this retrospective case-series study, 72 patients admitted to the Department of Orthopaedic in Hospital Kuala Lumpur and Hospital Universiti Sains Malaysia between January 2002 and December 2007 were reviewed. All patients with suspected case of metastases, tuberculosis, and pyogenic disease (based on their clinical sign, symptoms, and X-ray findings involving spinal vertebra) who underwent MRI and percutaneous transpedicular biopsy were included in the study. The data collected were reviewed, including all data pertaining to laboratory investigations to support clinical diagnosis, i.e., full blood count (FBC), erythrocyte sedimentation rate (ESR), tumour marker, sputum acid-fast bacilli (AFB), Mantoux test, and polymerase chain reaction (PCR) analysis. In addition, the MRI findings of each case reported by the radiologist and the HPE results from tissues taken through percutaneous transpedicular biopsies (which were performed later to MRI) were also examined. The data were analyzed for association between the different diagnostic assessments.

Results: Theoverall HPE diagnoses obtained through a percutaneous transpedicular biopsy were 65.3% positive (47 out of 72 cases). And 34.7% negative (25 out of 72 cases) for both vertebral tuberculosis and metastases, while theoverall disease detection by MRI was 97.2% (70 out of 72 cases). There was no significant association between MRI findings and percutaneoustranspedicular biopsy (HPE) for disease detection ($P > 0.05$). The disease prevalence was 0.653 for diagnosis via percutaneoustranspedicular biopsy (95% CI 0.543-0.763), and 0.972 for MRI (95% CI 0.934-1.011). The sensitivity and specificity of MRI compared with percutaneous transpedicular biopsy, which was taken as the gold standard in this study, which were 0.979 (95% CI 0.937-1.020) and 0.040 (95% CI 0.018-0.062), respectively. There was a significant association observed between the level of vertebral lesion and HPE by percutaneous transpedicular biopsy ($P = 0.021$); however, HPE is not significantly associated with the types of tissue in the vertebral body lesion ($P > 0.05$).

Conclusion: MRI is highly sensitive for disease detection in vertebral lesion. However, MRI has low specificity and is not reliable in detecting true negative cases. There is no significant association between MRI and percutaneoustranspedicular biopsy with regards to disease detection. For inconclusive MRI findings or MRI findings with vertebral metastases and tuberculosis, percutaneoustranspedicular biopsy clinically useful and reliable for vertebral disease confirmation.

Supervisor :
Associate Prof Mohd Iskandar Mohd Amin

24-HOUR AMBULATORY BLOOD PRESSURE MONITORING AMONG HYPERTENSIVE PATIENTS ATTENDING FAMILY MEDICINE CLINIC, HOSPITAL UNIVERSITI SAINS MALAYSIA, KELANTAN

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Introduction: Hypertension is a common and an important, modifiable risk factor for cardiovascular, cerebrovascular, and renal diseases. Although the percentage of patients who are being treated for hypertension has increased, the percentage of those who demonstrate control of blood pressure (BP) has declined. Clinicians increasingly rely on ambulatory BP monitoring (ABPM) to improve the diagnosis and treatment of hypertension, as there is a firm evidence of its prognostic value in predicting cardiovascular outcome.

Objectives: The objectives of this study were to illustrate the circadian BP profile of hypertensive patients attending Family Medicine Clinic, Hospital Universiti Sains Malaysia (HUSM), to describe the prevalence of uncontrolled hypertension based on 24-hour ambulatory BP monitoring (ABPM) and office BP, and to identify the associated cardiovascular risk factors among dippers and non-dippers.

Patients and Methods: This cross-sectional study was conducted from 1 January 2008 to 30 June 2008 among hypertensive patients attending Family Medicine Clinic, HUSM. All patients who fulfilled the inclusion criteria were selected via systematic random sampling. Schiller BR-102 plus was put on patients to get 24-hour BP reading. Mean of 2 office BPs were also taken. Non-dippers we defined as systolic or diastolic nocturnal drop of less than 10%. Statistical analyses were done using SPSS version 12.

Results: A total of 105 patients were recruited: 59 (56.2 %) males and 46 (43.8%) females with a mean age of 51.8 (SD 9.34) years old. The patients' mean 24-hour SBP and DBP were 128.4 (SD 12.7) mmHg and 79.7 (SD 8.74) mmHg, respectively. The m daytime SBP and DBP were 132.1 (SD 11.72) mmHg and 82.4 (SD 9.41) mmHg, while the mean nighttime SBP and DBP were 123.3 (SD 12.78) mmHg and 76.2 (SD 9.01) mmHg, respectively. Percentages of non-dippers were 68.6% for systolic and 61.9% for diastolic BP. Percentages of uncontrolled systolic and diastolic 24-hour ambulatory BP were 26.7% and 23.8%, respectively, and the percentage of uncontrolled diastolic nighttime BP was 56.2%. Percentages of uncontrolled office BP was high, 57.1% systolic and 61.0% diastolic, and the difference between office BP and 24-hour ambulatory BP were statistically significant. However, the simple logistic regression analysis performed to determine the relationship between cardiovascular risk factor and non-dippers were not significant.

Conclusion: The means for 24-hour and daytime ambulatory BP were normal; however, the mean diastolic nighttime was above normal value. The majority of patients were categorised as non-dippers. The percentage of uncontrolled office BP was high compared with 24-hour ambulatory BP. Therefore, rather than using simple clinical measurements, ambulatory BP was clinically and practically important to get a better understanding of BP fluctuations over 24-hour periods.

Supervisor:
Dr Juwita Shaaban
Co-supervisors:
Dr Harmy Mohamad Yusoff
Dr Tengku Alina Tengku Ismail

CLINICAL CHARACTERISTICS AND HOSPITAL COST OF HOSPITALIZED CHILDREN WITH RESPIRATORY SYNCYTIAL VIRUS INFECTION IN HOSPITAL UNIVERSITI SAINS MALAYSIA, KUBANG KERIAN

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Introduction: Insertion RSV is a major pathogen for respiratory illness in children less than 2 years of age, causing significant morbidity and mortality, especially in those with underlying disease. RSV infection shows a seasonal variation in its yearly distribution, and it imposes a substantial burden to the health care provider. In the East Coast of Malaysia, there has been no study reporting the medical cost incurred by the hospital in managing patients with RSV infection.

Objectives: This study aimed to describe the epidemiological characteristics, clinical profile, and seasonal trends of infection, and to determine the direct medical cost in managing hospitalized children with RSV infection in Hospital Universiti Sains Malaysia (HUSM) from 2006 to 2008.

Patients and Methods: This was a cross-sectional retrospective study conducted at HUSM, Kubang Kerian, Kelantan. Data of children admitted to wards 6S, Neonatal Intensive Care and High Dependency Unit with positive RSV respiratory specimens from the Laboratory of Virology in Department of Microbiology and Parasitology from 1 January 2006 until 31 December 2008 were collected. Medical charts were reviewed retrospectively. The epidemiological and clinical profiles were obtained from the medical records. Seasonal variations were determined by analyzing the monthly RSV-positive isolation rate and comparing with local meteorological parameters. The direct medical cost was calculated by using raw data of various costing derived from the various departments in HUSM.

Results: There were 155 positive cases from 2006 to 2008. However, only 131 cases were included as the medical records for the remaining 24 cases could not be traced. Bronchopneumonia was the predominant diagnosis (60.3%), followed by acute bronchiolitis (26.0%). From 131 patients, 23 patients (17.6%) had underlyingdiseases, with congenital heart disease being the most common condition, occurring in 13 patients (9.9%). The patients were older (11 vs five months, $P < 0.05$), had a longer length of stay (17 vs 8 days, $P < 0.01$), and 10 times higher risk for ventilation ($P < 0.001$). The mortality rate was 3.8%, all involving those with underlyingdiseases. Patients who were younger than six months of age required more intensive care admission (19% vs 1.5%, $P < 0.001$) and oxygen therapy (48.9% vs 31.3%, $P < 0.05$). On the contrary, they required less bronchodilator therapy (64.9% vs 86.0%, $P < 0.01$) and less of them presented with fever (74.3% vs 96.5%, $P < 0.001$). Apnoea was a common atypical presentation in neonates. The significant risk factors for ventilation were previous underlying diseases ($P < 0.001$) and age younger than 6 months ($P < 0.01$). RSV infection distribution patterns had changed from previous trends, but still maintaining its peak during the North–East monsoon from October to December. Themean direct medical cost for all patients was estimated at RM 74 301.66 per annum. Themean cost per patients for those with underlying diseases was higher than those without diseases (RM 2624.30 vs RM 1505.00).

Conclusion: RSV infection is associated with significant morbidity and mortality in children withunderlying diseases. The seasonality of RSV infection in Kelantan showed an association with the rainfall distribution, with its peak during the rainy season (October-December). The economic burden of RSV infection to the health care provider was significant, and this was even more so for those withunderlying diseases, especially congenital heart disease.

Co-supervisor :
Dr Noraida Binti Ramli
Co-supervisor :
Dr Mohd Ismail Bin Ibrahim

PERCEIVED STIGMA AMONG PATIENTS WITH SCHIZOPHRENIA IN KELANTAN

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Introduction: Stigma has been identified and recognized as a major concern in healthcare services. Stigma is manifested by bias, distrust, stereotyping, fear, embarrassment, anger, and avoidance; it leads the society to avoid living, socializing, or working with, renting to, or employing people with mental disorders. Although perceived stigma in schizophrenia is a known fact, it has not been widely studied in Malaysia.

Objectives: The aims of the study were to determine the distribution of perceived stigma toward patients with schizophrenia and its association with self-esteem, clinical factors, and socio-demographic variables.

Patients and Methods: This was a cross sectional study conducted in Hospital Raja Perempuan Zainab II and Hospital Universiti Sains Malaysia from September 2008 to March 2009. The subjects consisted of 227 stable patients with schizophrenia according to the definition of the Diagnostic and

Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), selected through convenience sampling. The perceived stigma and self-esteem were assessed with the Devaluation and Discrimination Scale (DDS) and the Shamsunnisah Self-Esteem Scale (SSES), respectively. The psychopathology was assessed with the Brief Psychiatric Rating Scale (BPRS). The main measured outcome in this study was the mean stigma score of the DDS, which is considered as a dependent variable. Descriptive analysis, simple linear regression, and analysis of variance (ANOVA) were applied in data analyses.

Results: The majority of the patients were Malays, 223 (98.2%); male, 151 (66.5%); single, 142 (62.6%); unemployed, 129 (56.8%); and had mild or doubtful symptoms on BPRS psychotic sub scale, 136 (59.9%). The mean duration of illness was 11.6 (SD 8.1) years. The mean stigma score was 2.38 (SD 0.67), with a majority of 132 patients (58.1%) scoring lower than the mid-score, which indicated low level of perceived stigma. A majority of 131 patients (57.7%) had a moderate levels of self-esteem, with a mean score of 91.4 (SD 14.5). There was a significant difference in the mean stigma score between the three levels of self-esteem ($P < 0.001$). Other socio-demographic and clinical factors were not significantly associated with a perceived stigma.

Conclusion: This study found that perceived stigma was low among patients with stable schizophrenia. The perceivedstigma was significantly associated with the level of self-esteem, where subjects with a high level of perceivedstigma tended to have a low level of self-esteem.

Supervisor:
Professor Dr Haji Mohd Razali Bin Salleh
Co-supervisor:
Dr Mohd Nawan Hamzah

RETROSPECTIVE CROSS-SECTIONAL STUDY FOR COMPLETENESS OF PRE-OPERATIVE SECTION OF ANAESTHETIC RECORD IN HOSPITAL UNIVERSITI SAINS MALAYSIA

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Introduction: The pre-operative section of the anaesthetic form is the main concern in this study. Proper pre-operative assessment is very important, and this is also true for the documentation of pre-operative assessment in the anaesthetic form for patients who will undergo any type of anaesthesia.

Objective: This study was done to assess the difference in the quality of completing the pre-operative section of the anaesthetic form between emergency and elective operations, as well as between anaesthetic records in January 2008 and May 2007.

Patients and Methods: This retrospective cross-sectional study was conducted from May 2007 to January 2008 in Hospital Universiti Sains Malaysia (HUSM). Patients' names and identification numbers were traced from the operation theatre records, and patients were selected according to the inclusion criteria. Selected patients' medical records were obtained from the record office. Anaesthetic records were assessed. The completeness of 22 variables was evaluated based on their respective criteria, and the global quality index (percentage of completeness) was scored. SPSS version 12 was used for data tabulation and analyses.

Results: There were 325 anaesthetic records traced and evaluated. The mean percentage of completeness of all the anaesthetic records in the pre-operative section was 67.98%. For anaesthetic records in January 2008, the mean percentage of completeness was 73.1%; whereas, in May 2007, it was 62.3%. For anaesthetic records from elective operations, the mean percentage of completeness was 74.55%; in contrast, mean for anaesthetic records from emergency operations was 64.38%.

Conclusion: Although the form-completeness is improving (as seen in the higher percentage of completeness in January 2008 compared with May 2007), important measures are needed to further improve the quality of completing the pre-operative segment of the anaesthetic form in HUSM.

Supervisor :
Associate Professor Dr Wan Hashim Wan Adnan

A DIAGNOSTIC VALUE OF MANTOUX TEST IN TUBERCULOSIS: A CASE CONTROL STUDY IN HOSPITAL UNIVERSITI SAINS MALAYSIA IN 2009

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Introduction: The prevalence of tuberculosis (TB) is increasing despite aggressive strategies and control programmers exercised. Although new methods had been introduced to diagnose TB, these tests are expensive and not widely available. Mantoux test remains important in developing countries, especially in extra-pulmonary, pediatric, and smear-negative cases. However, its accuracy in the diagnosis of active TB remains uncertain.

Objectives: This study aimed to determine Mantoux test diagnostic accuracy and the best cut-off points of positive Mantoux, as well as to identify the associated factors that influence Mantoux induration among TB patients.

Patients and Methods: Case-control design was used for diagnostic study, and cross-sectional study using

confirmed activeTB was applied to identify the associated factors of Mantoux reading. A total of 140 subjects were involved, including 50 cases of activeTB and 90 subjects in the control group. Control was defined as those who had never been exposed to or diagnosed with TB in the past. TB cases were retrieved from Chest Clinic, Hospital Universiti Sains Malaysia (HUSM) from 1 January 2008 until 31 March 2009, and the control subjects were chosen from around Kota Bharu, the capital of Kelantan. The patientsí folder and TB wallet were reviewed by the researcher. The required information on demography, Mantoux result, and other related parameters were recorded into the data collection form.

Results: Based on the diagnostic study, three cut-off points showed comparable results. The sensitivity of 8, 10, and 12 mm cut-off points were 72%, 66%, and 50%, respectively. Among these cut off points, 12 mm induration showed greater specificity and positive predictive value rate, 96% and 86%, respectively. In contrast, 10 mm induration was observed to be the best cut-off point based on a receiver operating characteristic (ROC) curve. Multivariable logistic regression analysis showed no association between Mantoux size and factors such as gender, race, Bacille Calmette-Guerin (BCG) vaccination, co-morbidities, degree of TB involvement as reflected by sputum smear positivity, and radiological involvement.

Conclusion: It is concluded that, in population of high TB burden, Mantoux test is fairly sensitive and specific in the diagnosis of TB. The analysis showed that 10 mm induration was thebest cut-off points of positive Mantoux test. However, 12 mm induration should be reconsidered as thebest cut-off points due to its greater specificity and positive predictive values. There is no association between Mantoux size and confounding factors such as age, BCG vaccination, gender, co-morbidities, and degree of TB involvement.

Supervisor :
Associate Professor Dr Che Wan Aminuddin
Co-supervisor:
Dr Shaharuddin Bin Abdullah

RISK PERCEPTION ON FOOD POISONING AMONG COMMUNITY IN SALOR, KOTA BHARU, KELANTAN

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Introduction: Food poisoning is the most common infectious disease that has come to the public health attention in Malaysia. In a 2003 survey, typhoid, cholera, and food poisoning were considered as endemic diseases in Kelantan.

Objectives: This study aimed to explore the knowledge, attitude, and practice (KAP), as well as to determine the risk perception and socio-demographic factors associated with the risk perception on food poisoning in Salor, Kota Bharu, Kelantan.

Patients and Methods: A cross-sectional study was conducted in Salor from June to September 2008. A total of 447 members of the community were selected for the study using enumeration block sampling. Data were collected using a self-administered, validated questionnaire, which consisted of information on socio-demography, KAP, and risk perception on food poisoning.

Results: The majority of the respondents were Malays (99.3%), married (81.4%), and females (57.5%), with the mean (SD) age of 38.1 (12.58) years old. Most of the respondents had good knowledge (71.1%), positive attitude (88.4%), and positive practices (87.2%) towards food poisoning. Majority of the respondents had perceived risk of food poisoning. However, respondents' perceived risk of food poisoning (64.7%) was better than perceived severity of food poisoning (37.1%). The significant factors associated with the risk perception on food poisoning were female (OR = 1.91, 95% CI 1.26 to 2.91, P = 0.003) and higher household income (OR = 2.48, 95% CI 1.13 to 5.47, P = 0.024). Age, marital status, and education level were not associated with the risk perception on food poisoning.

Conclusion: Data from the study showed that the community in Salor had high percentage of good KAP and risk perception on food poisoning. However, in a detailed assessment of KAP, it was demonstrated that there were deficit areas that should be addressed in the health education program. Better risk perception among females and high-income group indicated showed that these people were more aware and interested in the food safety issues.

Supervisor :
Dr Nor Azwany Yaacob
Co-supervisor:
Dr Zaliha Ismail
Dr Zaharah Sulaiman

FACTORS INFLUENCING QUALITY OF LIFE IN PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION

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Introduction: The availability and efficacy of medical treatment have delayed disease progression and prolonged the survival in patients with human immunodeficiency virus infection and acquired immunodeficiency syndrome (HIV/AIDS). Consequently, the quality of life (QOL) has emerged a significant medical outcome measure for people living with

HIV/AIDS.

Objectives: The aim of this study was to determine the socio-demographic, clinical, psychological, and spiritual factors influencing quality of life (QOL) in patients with HIV/AIDS.

Patients and Methods: This cross-sectional study was conducted using convenience-sampling method. The study population consisted of 271 patients with HIV/AIDS from April 2008 to March 2009. These patients completed the Malay-version of the Functional Assessment of HIV Infection (FAHI) and the Spiritual Well-being (SpWB) Scale. Statistical analyses were performed using SPSS version 12.0. Multivariable linear regression analyses were performed using socio-demographic, clinical, spiritual, and psychological factors as the independent variables. The total FAHI score and 5 domains or subscales from FAHI, such as the physical well-being, social well-being, and cognitive functioning, were analysed as the dependent variables.

Results: The mean (SD) of FAHI total score was 113.03 (29.08) and it ranged 0–176, with higher score indicating better QOL. The most affected domain was social well-being. Patients who were non-Malays, employed, had $CD4^+$ count of more than 200, and not having anxiety or depression had better overall QOL. Lower educational level, longer duration of illness, low $CD4^+$ count, anaemia, and possible anxiety and depression seemed to compromise the patients' physical well-being. Those of older age, Muslim, and having possible anxiety and depression had a poorer emotional well-being. Those who were employed, had no anxiety or depression, and had higher spirituality level significantly showed better functional and global well-being. Older patients, those of heterosexual source of infection, possible anxiety and depression, as well as poor spirituality were significantly associated with poorer social well-being. In addition, 65% of male patients did not disclose their HIV status, and non-disclosure was also significantly associated with poorer social well-being. Being unemployed, with possible anxiety and depression, and weaker spirituality showed significant association with lower cognitive functioning.

Conclusion: This study showed differential effects on the different domains of QOL of patients with HIV and AIDS. The worst impaired domain was the patients' social well-being. The high percentage of male patients failing to disclose their HIV status to their significant others and the fact that women were being infected via heterosexual relationship (and seemed to suffer more impairment) should be addressed with a better primary prevention and management approaches. It appears that the HIV/AIDS requires the sufferers to stop being silent, and the caregivers as well as the policy makers to be more vocal in order to curb this epidemic.

Supervisor :
Associate Professor Dr Hasanah Che Ismail
Co-supervisor :
Dr Mahiran Mustaffa

ANTI-TUBERCULOSIS DRUG-INDUCED HEPATITIS: OUTCOME OF ANTI-TUBERCULOSIS DRUG RE-CHALLENGE IN HOSPITAL UNIVERSITI SAINS MALAYSIA

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Objective: The study was conducted to determine the outcome of a re-challenge of anti-tuberculosis drugs in patients with anti-tuberculosis drug-induced hepatitis.

Patients and Methods: This was a single-centre, retrospective study conducted in Hospital Universiti Sains Malaysia from January 2006 until December 2008. The medical records of patients with tuberculosis were examined to determine the durations of developing anti-tuberculosis drug-induced hepatitis and its resolution.

Results: Only 22 out of 595 tuberculosis patients were included in the study. The review showed that 31.8% of the patients developed anti-tuberculosis drug-hepatitis in week 2, and another 22.7% in week 3. Resolution of the drugdrug-hepatitis occurred during week 1 after the cessation of anti-tuberculosis drugs in 22.7% of patients, and another 31.8% recovered during week 2. All the patients could tolerate the first-line anti-tuberculosis drug regime during the re-challenge period.

Conclusion: Anti-tuberculosis drug-induced hepatitis usually occurred in week 2 or 3 after the initiation of anti-tuberculosis drug treatment and usually resolved within two weeks after cessation of the drugs.

Supervisor :
Associate Professor CheWan Aminud-din Hashim
Co-supervisor:
Associate Professor Dr Zainal Darus

Abstracts

Abstracts of Theses Approved for the MSc and PhD at the School of Health Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan, Malaysia.

MODULATION OF CELL GROWTH AND PPAR-γ EXPRESSION IN HT-29 AND COLO 205 CELLS BY CIGLITAZONE

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Introduction: Colon cancer is one of the most common malignancies in both men and women in Malaysia. Most therapies for the disease are still unsatisfactory since they cause adverse effects to patients. Current research are now focusing on a better understanding of the tumour response and resistance to treatment, apart from finding drugs that will not cause such adverse effects. The nuclear receptor, peroxisome proliferator-activated receptor (PPAR)-γ, is reported to be expressed in various cancer cells, including breast, prostate, and colorectal cancer. PPAR-γ is a ligand-dependent transcription factor that regulates expression of genes involved in cellular proliferation, differentiation, and apoptosis. Although this receptor has been shown to be highly expressed in colorectal cancer, its exact role in colorectal carcinogenesis is still unclear.

Objectives: This study was carried out to study the possible involvement of PPAR-γ in modulating the growth of 2 human colorectal carcinoma cell lines, HT-29 and COLO 205. A synthetic PPAR-γ ligand, ciglitazone, which is also a member of the antidiabetic drug thiazolidinediones (TZDs), was used in this study to treat the colorectal cancer cell lines.

Materials and Methods: To evaluate whether ciglitazone induced inhibition of cell growth, both cell lines were treated with increasing doses of ciglitazone (2.5–100 μM) for 6 to 72 hours. Cytotoxicity was determined by measuring the lactate dehydrogenase (LDH) leakage from the cell membrane. In addition, a fluorescein-conjugated monoclonal antibody against cytokeratin 18 (CK18) that recognizes the caspase-cleaved epitope within the CK18 was used to measure apoptosis by flow cytometry. Additionally, the mRNA expression levels of PPAR-γ1 and PPAR-γ2 from the cell lines were quantified by real-time quantitative PCR technique using specifically developed homologous internal standards for each of the genes. The level of PPAR-γ protein was determined by Western blotting.

Results: Ciglitazone significantly inhibited the growth of colorectal cancer cell lines in a dose- and time-dependent manner ($P < 0.01$). The EC50 values of ciglitazone obtained after 48 hours of incubation was about 20 μM for HT-29 cells and about 30 μM for COLO 205 cells, and these concentrations were then used in subsequent experiments to treat the corresponding cell lines in a time-dependent manner. Flow cytometry results demonstrated that ciglitazone significantly induced apoptosis in the cell lines ($P < 0.01$). However, real-time PCR results revealed that the levels of PPAR-γ1 and PPAR-γ2 mRNA expression were significantly reduced following treatment with ciglitazone compared to controls ($P < 0.05$). Furthermore, ciglitazone also decreased the level of PPAR-γ protein expression as shown by Western blotting.

Conclusion: Treatment with ciglitazone suppressed colon cancer cell growth and cell death. It is postulated that the antitumour effects of this synthetic PPAR-γ ligand may not depend solely on PPAR-γ activation. Since previous reports shown that PPAR-γ was upregulated in cancer cells, including colon cancer cells, compared with their normal counterparts, the downregulation of PPAR-γ expression following ciglitazone treatment suggested the potential of ciglitazone to be used as an adjuvant for the treatment of colon cancer. However, further research is needed to evaluate the anticancer activity of TZDs, which appear to be both dependent and independent PPAR-γ pathway.

Supervisor:
Professor Dr Norazmi Mohd. Nor

HLA POLYMORPHISM IN MALAY SUB-ETHNIC GROUPS IN PENINSULAR MALAYSIA

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Materials and Methods: In this study, the Human Leukocyte Antigen (HLA) class I and II were examined through Sequence Specific Primer (SSP) typing in 176 unrelated individuals from 6 Malay sub-ethnic groups of Peninsular Malaysia: Kelantan (n = 25), Minangkabau (n = 34), Jawa (n = 30), Bugis (n = 31), Banjar (n = 33), and Rawa (n = 23). The common HLA alleles in all the sub-ethnic groups were HLA-A*24 (26%–48%), HLA-B*15 (22%–41%), -Cw*07 (21%–32%), DQB1*03 (25%–55%), and DRB1*12 (15%–40%).

Results: The Malay sub-ethnic groups studied showed close relationship to each other and to Asian populations despite specific differences between them. Banjar, Jawa, and Bugis Malays showed no significant differences to each other, which could be a result of their related origin from the islands around the Java Sea. Besides sharing in the most common haplotype found, phylogenetic and principal coordinate (PCO) analysis showed a genetic similarity between Minangkabau

and Rawa Malays. This could be a consequence of their common origin from Sumatera. The Kelantan Malays, show statistical significant difference with the other groups and revealed differences for the most frequent haplotypes, which could be related to their different origin, and the different populations influence along time. Statistical analysis on the Malay sub-ethnic groups HLA data also revealed credible forensic parameters for forensic applications.

Conclusion: The HLA data obtained from this study can be applied for vaccine development, searching for suitable donor for transplantation, disease association studies, and as a guideline for infectious disease prevention programs

Supervisor :
Dr Zafarina Zainuddin

THE DEVELOPMENT OF A CANDIDATE TUBERCULOSIS DNA VACCINE EXPRESSING Mtb8.4 AND Ag85B OF *MYCOBACTERIUM TUBERCULOSIS*

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Introduction: Tuberculosis (TB) is still one of the major health problems worldwide. The only TB vaccine currently available is an attenuated strain of *Mycobacterium bovis*, Bacille Calmette–Guerin (BCG). However, the efficacy of BCG vaccine continues to be debated. Therefore, a more effective vaccine against TB is urgently needed. DNA vaccination is a new approach to the control of infectious agents.

Materials and Methods: In this study, a DNA vaccine encoding the candidate TB antigens Mtb8.4 and Ag85B was developed using assembly PCR. Balb/c mice were immunized intramuscularly with 50 μg of the DNA vaccine, pNMN023, containing the 2 antigens in each hindleg.

Results: Reactivity against the Ag85B peptides, P1, and P3 as well as MtbB.4 showed a consistent Th1 type of immune response by virtue of the increased expression of IL-2, IFN-γ and lgG2a. Splenocytes from immunized mice were also found to proliferate more aggressively when stimulated with the antigens compared to the vector alone. In order to improve the vaccine efficacy, a preliminary prime-boost approach was used. Priming with pNMN023 and boosting with recombinant BCG (rBCG) in Balb/c mice was carried out. Flow cytometric intracellular cytokine analyses of splenocytes from mice immunized with the DNA-rBCG prime-boost regime showed that both $CD4^+$and $CD8^+$T cells showed an increase in IL-2 and IFN-γ production following stimulation with either antigens at significantly higher levels than those immunized with rBCG-DNA prime-boost.

Conclusion: The data obtained from this study suggested that DNA vaccination in combination with the prime-boost approach provide a potential strategy for developing a candidate vaccine against TB.

Supervisor:
Professor Dr Norazmi Mohd. Nor

MAGIC POLYMER GEL DOSIMETRY USING X-RAY COMPUTED TOMOGRAPHY: A FEASIBILITY STUDY

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Introduction: The aim of this project was to carry out feasibility study of developing Methacrylic and Ascorbic Acid in Gelatin Initiated by Copper (MAGIC) polymer gel dosimetry system by utilizing helical multislice X-ray computed tomography (CT) available in Hospital Universiti Sains Malaysia to determine dose.

Materials and Methods: The MAGIC gel was prepared based on the formulation proposed in the literature by Fong et al. (2001), with some modifications. The characteristics of the gel were studied for its water-equivalent properties. The mass density (ρ) was determined based on Archimedes' principle. The weight fraction of elemental composition and the effective atomic number (Zeff) were calculated. The electron density was also measured with 90-degree scattering angle at room temperature. The linear attenuation coefficient (μ) of unirradiated gel, irradiated gel, and water were determined using Am-241 based on narrow beam geometry.

Results: The measured linear attenuation coefficient of unirradiated MAGIC gel and water was found to be 0.84 (SD 0.04) cm^{-1} and 0.85 (SD 0.02) cm^{-1} respectively. The results showed that the MAGIC gel is water-equivalent. The data obtained using irradiated gel showed a linear relationship between linear attenuation coefficient and absorbed dose in the range 2-40 Gy. The protocol for CT imaging to obtain the best quality image of irradiated MAGIC gel was determined for evaluating dose information. The irradiated gel placed inside the cylindrical water phantom was scanned using various available scan parameters (kV, mA, and reconstruction algorithm) with the field of view 25 x 25 cm and 5 mm slice thickness. The signal to noise ratio (SNR) and standard deviation (SD) were the parameters chosen to determine the image quality after image averaging process was carried out. The image that has the highest SNR and lowest SD was the best quality image, and the corresponding scan parameters were used as the protocol for scanning the irradiated gel. The scan parameters of 140 kV and 400 mA with 5 mm slice thickness, 1000 ms exposure time, standard reconstruction algorithm and 25 x 25 cm field of view were chosen as scanning protocol. Using this scanning protocol, the irradiated MAGIC gels of different doses were imaged to establish relation between average CT numbers and doses. A linear relation was found

between average CT numbers and doses in the range 2–40 Gy with CT number (HU)-dose sensitivity of 0.30 (SD 0.02) HU Gy-1. In order to verify the usefulness of the CT based gel dosimetry to measure dose, the percentage depth dose (PPD) and isodose curve (beam profile) of 8 x 8 cm field size photon beam from 6 MV linear accelerator were measured. The measured PDD and isodose curves were compared with that calculated in water using radiotherapy treatment planning computer system (TPS).The disagreement of irradiated gel PDD compare to TPS at 5 cm and 10 cm depth were found to be + 1.8% and + 2.1%, respectively. The maximum disagreement of gel PDD compare to TPS calculation in the water was + 3%. The maximum disagreement of gel isodose curves compare to TPS calculation in the water at 5 cm and 10 cm measurements were + l0% and + 11.6%, respectively.

Conclusion: The results show that the CT based MAGIC gel dosimetry system using HUSM CT scanner could determine the dose of high energy photon in the range 2–40 Gy.

Supervisor:
Professor Dr Ahmad Bin Zakaria

THE ALLELE AND GENOTYPE VARIATIONS OF FIFTEEN SHORT TANDEM REPEAT (STR) LOCI IN MALAY, CHINESE, INDIAN, AND JAVANESE GROUPS OF THE MALAYSIAN POPULATION

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Materials and Methods: Allele and genotype frequencies for 15 short tandem repeats (STR) loci (D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX, and FGA) among 185 Malays, 216 Chinese, 195 Indians, and 109 Javanese were determined.

Results: For these 4 distinct groups investigated, the observed heterozygosity (HO) at these STR loci ranged from 56.0% to 82.1%. The values of the combined power of discrimination of these loci for the Malays, Chinese, Indians, and Javanese were 1 in 4.362 x 1018, 1 in 6.268 x 1018, 1 in 2.370 x 1018, and 1 in 2.543 x 1017, respectively. The values of the polymorphism information content (PIC) ranged 0.52–0.91. The probability of paternity for the Malays, Chinese, Indians, and Javanese were 0.99999932, 0.99999937, 0.99999971, and 0.99999888 respectively. The distribution patterns of the STR alleles were different in each group, especially the Indian group. The genetic data indicated the Penta E locus to be the most highly polymorphic and the TPOX locus the least polymorphic. Several loci demonstrated deviations from the Hardy–Weinberg Equilibrium (HWE) when tested with chi-square goodness-of-fit and exact test. Comparison of the population databases showed close genetic relationships between the Malay and Javanese groups and with greater variations when compared with the Chinese and Indian groups. The overall co-ancestry coefficients, θ, were estimated and the value ranged 0.005–0.015, which is consistent with the National Research Council II recommended value of 0.03. The pair-wise comparisons between the various groups of population based on co-ancestry identity were performed and were demonstrated by using phylogenetic tree.

Conclusion: The results showed that the genetic relationship between the Malays and Javanese are very close, and supported the possibility that there were genetic ties between the Malays and Chinese. The result also clearly showed that genetically the Indians were different from the Malays, Chinese, and Javanese.

Supervisor:
Dr S Panneerchelvam

IMMUNOGENICITY STUDY OF DNA VACCINE AND DNA VACCINE CARRIER EXPRESSING VP1 OF ENTEROVIRUS 71 IN THE PRIME BOOST VACCINATION STRATEGY

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Introduction: Enterovirus 71 (EV71) is a highly infectious causative agent of hand, foot, and mouth disease (HFMD) in children and could lead to severe neurological complications. In Malaysia, the first epidemic occurred in 1997 in Sarawak and caused 34 deaths due to severe neurological syndrome. There is currently no vaccine available against EV71. Vaccination is considered the most effective means to control EV71 infection.

Materials and Methods: A candidate vaccine was developed, involving the construction a synthetic VP1 gene of EV71 fused to a ubiquitin (UbGR) gene and cloning into a DNA vaccine vector with a strong eukaryotic promoter known as pVAX1, to create the candidate DNA vaccine pVaxUbVP1. The immunogenicity of the constructed DNA vaccine was evaluated in BALC/c mice involving 2 methods of delivery, as a naked DNA vaccine delivered intramuscularly or delivered orally via the live attenuated bacteria *Salmonella typhi* Ty21a, of which the recombinant strain carrying pVaxUbVP1 was designated as StUbVP1. Both candidate vaccines were used in homologous and heterologous prime boost approaches: Formats A (pVaxUbVP1 alone), B (StubVP1 alone), C (StUbVP1 as primer vaccine and pVaxUbVP1 as booster), and D (pVaxUbVP1 as primer vaccine and StUbVP1 as booster).

Results: The results indicated that total lgG levels in serum was significant in Formats A and D whereas lgG subclasses assay showed that IgG2a levels were higher than

IgG1 levels in both immunization formats. Production of in vitro IFN-γ was significant in mice vaccinated using Formats A, B, and D, whereas IL-4 production was relatively low in all groups of immunization but shows a significant increase in Format D. The percentage of intracellular cytokine (IFNγ, IL-2, and IL-4) production by $CD4^+$ and $CD8^+$ population of T cells showed a moderate to high response in Formats A and D. The analyses also showed that the use of pVaxUbVP1 in a homologous prime boost format (Format A) resulted in a Th1 type of immune response whereas using Format D (pVaxUbVP1 as primer vaccine and StUbVP1 as booster) gave a mixed Th1–Th2 types of immune response.

Conclusion: The pVaxUbVP1, used alone in a homologous prime boost approach or as the primer vaccine in a heterologous prime boost immunization format together with StUbVP1, showed potential for further development as a vaccine against EV71.

Supervisor:
Professor Dr Zainul Faziruddin Zainuddin

CONSTRUCTION OF RECOMBINANT BCG EXPRESSING THE VP1 ANTIGEN OF ENTEROVIRUS 71 FOR THE DEVELOPMENT OF A CANDIDATE VACCINE

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Introduction: Enterovirus 71 (EV71), the causative agent of outbreaks of hand, foot, and mouth disease (HFMD) in children require urgent control due to the high number of cases. Vaccination is one of the most effective methods to control disease outbreaks.

Materials and Methods: In this study, a recombinant BCG vaccine candidate was constructed against EV71. The recombinant BCG (rBCGV1) expresses a synthetic gene encoding the VP1 protein of EV71 fused to ubiquitin complex (UbGR), which was constructed using the technique of assembly PCR. The synthetic gene was codon optimized for expression mycobacterium. The AgB5A promoter and signal peptide sequence from M. tuberculosis was used to drive the expression and secretion of the synthetic gene.

Results: The expression of the UbGR-VP1 fusion protein was confirmed by Western blotting using rabbit polyclonal antibody specific to the VPI protein and was found in the cell pellet of the recombinant BCG. rBCGV1 showed the ability to induce moderate antibody production BALB/c (H-2d) mice when sera from immunized mice were tested against purified UbGR-VP1 fusion protein. IgG2a subclass antibody was shown to be induced at a significantly higher level than lgG1. Splenocytes obtained from rBCGV1 immunized mice showed significant higher level of lymphocyte proliferation when stimulated with UbGR-VP1 compared to control. Analyses of intracellular cytokines show that $CD4^+$ T cells and $CD8^+$. T cells from rBCGV1-immunized mice were stimulated by UbGR-VP1 protein to express significant levels of IL-2, IFN-γ, and IL-4 when compared to the control. Extracellular cytokine analyses also showed significantly higher levels of IFN-γ compared to control.

Conclusion: Overall, the immunogenicity studies results suggested that the rBCGV1 enhanced the stimulation of immune system towards the Th1 pathway. Data from this study also suggested the potential of rBCGV1 to be developed as a vaccine, and further studies must be carried out to evaluate the efficacy of this candidate vaccine.

Supervisor:
Professor Dr Zainul Faziruddin Zainuddin

THE EXPRESSION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR- (PPAR-y1 AND PPAR-y2) IN NAIVE AND MEMORY $CD4^+$ T LYMPHOCYTES

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Introduction: Peripheral $CD4^+$ T cells can be divided into 2 functional groups based on the expression of distinct isoforms of the surface molecule that contains an intracellular 2-domain phosphatase portion, known as CD45. Memory T cells express the lowest molecular weight CD45RB isoform, whereas naive T cells express CD45RA (human) or CD45RB (mouse) isoforms. CD45 is a protein tyrosine phosphatase which plays an important role in TCR-mediated signaling through its activation of Lck by dephosphorylating the regulatory Tyr505. Human naive and memory $CD4^+$ T cells differ in the requirements for activation and magnitude of the cellular responses. The nuclear receptor, peroxisome proliferator-activated receptor (PPAR)-γ has been reported to be involved in regulating the activities of immune cells such as macrophages or monocytes and T lymphocytes.

Materials and Methods: Given their roles in immune regulation, the current study was carried out to determine the expression of PPAR-γ in human naive and memory $CD4^+$ T cells, since it is possible that PPAP-γ may be differentially expressed in the different isoforms of CD45. In addition, the differential signaling patterns and cytokine secretion of these subsets of T cells may require engagement with PPAR-γ isoforms—a possibility that has not been explored thus far. To further dissect the role of PPAR-γ in the regulation of naive and memory $CD4^+$ T cell activation, the PPAR-γ agonist ciglitazone, was used to modulate the activation status of naive and memory $CD4^+$ T cells, as well as the expression of PPAR-γ and selected cytokines.

Results: From real-time PCR, it was observed that unstimulated naive and memory $CD4^+$ T cells did not express PPAR-γ1 and PPAR-γ2, whereas stimulated naive and memory $CD4^+$ T cells express high levels of these receptors, with PPAR-γ2 expression being higher than PPAR-γ1 in both cell types ($P < 0.01$). In addition, the PPAR-γ1 expression was higher in stimulated memory compared with stimulated naive $CD4^+$ T cells ($P < 0.05$), whereas there was no significant difference between PPAR-γ2 expression in both types of stimulated cells. The addition of the PPAR-γ agonist, ciglitazone significantly increased the expression of PPAR-γ1 by about 61-fold and 175-fold in stimulated naive and memory $CD4^+$ T cells, respectively ($P < 0.01$ in each). In contrast to PPAR-γ1, the addition of ciglitazone significantly decreased the expression of PPAR-γ2 by about 650-fold and 140-fold in stimulated naive and memory $CD4^+$ T cells, respectively ($P < 0.01$ in each). In addition, the expression levels of TGF-β and IL-1β gene were higher in unstimulated naive and memory $CD4^+$ T cells, but were decreased in their stimulated state ($P < 0.01$). IL-8 gene was expressed at low levels in unstimulated but elevated in stimulated naive and memory $CD4^+$ T cells ($P < 0.01$). However, there were no significant differences in the levels of these cytokines between naive and memory CD4+ T cells of both states. IL-2, IFN-γ, IL-5, IL-13, TNF-α, GM-CSF, and IL-6 were only expressed in stimulated naive and memory CD4+ T cells, but not in their unstimulated state. The expression levels of IL-2 and IL-13 were significantly higher in stimulated naive compared with stimulated memory $CD4^+$ T cells ($P < 0.01$). In contrast, the expression levels of IFN-γ were significantly higher in stimulated memory as compared to stimulated naïve $CD4^+$ T ceils ($P < 0.05$). However, there were no significant differences in the expression of IL-5, IL-6, TNF-α, and GM-CSF between both stimulated cell types. The addition of ciglitazone decreased the expression levels of TGF-β, IL-1β, IL-8, IL-2, IFN-γ, IL-5, TNF-α, and GM-CSF in stimulated memory and naive $CD4^+$ T cells. The induction of PPAR-γ1 and suppression of PPAR-γ2 expression in naive and memory $CD4^+$ T cells in the presence of ciglitazone suggested that the PPAR-γ isoforms may have different functions in T cell regulation.

Conclusion: The expression of selected cytokine genes in activated naive and memory $CD4^+$ T cells is consistent with previous studies. The exact mechanism of how PPAR-γ inhibit cytokine expression in stimulated naive and memory $CD4^+$ T cells and which PPAR-γ isoforms is responsible for this effect remain uncertain. It is possible that PPAR-γ inhibit the expression of cytokine genes in these stimulated cell subsets via interacting with NF-κB, AP-1, and STATs, which are important transcription factors for these cytokines, as shown by previous studies in other cells.

Supervisor:
Professor Dr Norazmi Mohd. Nor

CLONING, EXPRESSION AND IMMUNOGENICITY OF RECOMBINANT BACILLE CALMETTE-GUERIN (BCG) CONTAINING T AND B CELL EPITOPES OF *MYCOBACTERIUM TUBERCULOSIS*

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Introduction: Tuberculosis (TB) remains as one of the leading causes of morbidity and mortality in humans. The only TB vaccine currently available is an attenuated strain of *Mycobacterium bovis*, Bacille Calmette–Guerin (BCG). However, the efficacy of BCG continues to be debated. The BCG protection against adult pulmonary TB ranged 0%–80 % in randomized control trials. In addition, the rising rates of multi-drug resistant *M. tuberculosis* have worsened the situation. Thus, an improved TB vaccine is urgently needed. Recombinant BCG (rBCG) is one of the most potential approaches in evoking the immune response against TB.

Materials and Methods: In this study, 2 different types of rBCG were constructed: rBCG expressing T cell epitopes from *M. tuberculosis* Ag85B antigens and Mtb8.4 protein (rBCG018) or a combination of the antigens fused to B cell epitopes from ESAT-6, CFP10, and MTP40 proteins (rBCG032). Polyclonal anti-Mtb8.4 was successfully raised in rabbit and subsequently used for rBCG expression. Immunogenicity study of the vaccine constructs were used for immunization of Balb/c mice. Specific lgG response was obtained against the ESAT-6 and CFP10 in the sera of rBCG032-immunized mice.

Results: Splenocytes from these mice showed a high response against the Ag85B antigens and the Mtb8.4 protein, whereas splenocytes from rBCG018-immunized mice elicited a lower response against Ag85B epitopes and a high response against Mtb8.4 protein. Mice immunized with the rBCG strains produced a Th1 pattern of response against the T cell epitopes. Six weeks after the final immunization, the rBCG constructs were recovered from spleen, lung, liver, and peritoneal washout. The presences of both constructs in the colonies grown from the organ were detected by PCR.

Conclusion: The data obtained from this study demonstrated that T and B epitopes expressed in a single rBCG construct induced appropriate humoral and cellular immune responses against immunogenic epitopes from *M. tuberculosis.*

Supervisor:
Professor Dr Norazmi Mohd. Nor

DEVELOPMENT OF RECOMBINANT *MYCOBACTERIUM BOVIS* BACILLE CALMETTE–GUERIN (rBCG) EXPRESSING THE 22 kDa SERINE REPEAT ANTIGEN (SE22) OF *PLASMODIUM FALCIPARUM*

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MSc Biomedicine (Vaccinology)

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Introduction: The *Plasmodium falciparum* serine repeat antigen (SERA) is one of the promising blood stage malarial vaccine candidates.

Materials and Methods: In this study, we have developed a recombinant Mycobacterium bovis Bacille Calmette Guerin (rBCG) expressing a synthetic 22 kDa protein (SE22) from the 47 kDa N-terminal domain of SERA using assembly PCR. This synthetic gene was driven by the 65 kDa heat shock protein (hsp65) of *M. tuberculosis* and the signal peptide from MPT63 M. tuberculosis. Immunoblotting analysis using a monoclonal antibody against SE47' revealed that the SE22 protein was detected in the cytoplasm. The rBCG carrying SE22 protein (rBCG/SE22) was administered intra-peritoneally into Balb/c mice to determine the humoral and cellular immune responses against the SE22 antigen.

Results: Significant SE22-specific lgG and lgG subclasses were observed after immunization with the rBCG/SE22. In addition, the lymphocytes proliferative response to SE22 antigen was significantly higher in the rBCG/SE22 vaccinated group compared to the control groups. The expression of cytokines (IL-2, IL-4, and IFN-γ) in $CD4^+$ and $CD8^+$ splenocytes were also detectable following stimulation with SE22. The rBCG expressing SE22 antigen induced a mixed Th1/Th2 response. The IFA results showed that rBCG could induce SE22 specific antibodies in mice and react against SE22 protein expressed on the merozoites.

Conclusion: These results indicate that the rBCG/SE22 could enhance and regulate both humoral and cellular immune responses, therefore it is proposes as a potential vaccine.

Supervisor:
Professor Dr Norazmi Mohd. Nor

THE ANTICANCER MECHANISM OF IBUPROFEN AND INDOMETHACIN IN COLORECTAL CANCER CELLS

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Introduction: Ibuprofen and indomethacin are among the frequently studied non-steroidal anti-inflammatory drugs (NSAIDs) for their anticancer activities. Besides being non-selective cyclooxygenase (COX)-2 inhibitors, both NSAIDs are also direct ligands for peroxisome proliferators-activated receptor (PPAR)-γ. However, the precise mechanism(s) of action whereby both NSAIDs exert their anticancer effect remain unclear.

Objectives: In this study, the effects of both NSAIDs in constitutively COX-2-expressing (HCA-7 and HT29) and non-constitutively COX-2-expressing (HCT116) cell lines were investigated with the initial aim of determining the NSAID growth inhibitory effect, as well as the effective concentration to inhibit 50% of cell growth (EC50) of each NSAID in each cell line, assessed by using lactate dehydrogenase (LDH) release assay.

Materials and Methods: The apoptosis mechanism was then investigated using M30 CytoDEATH assay prior to flow cytometry analysis. The apoptotic-related proteins, such as caspase-8, -9, -3, and -7, were also investigated using Western blot analysis, whereas the modulation of mRNA expression of relevant molecular targets such as COX-2, c-myc, β-catenin, TCF-4, and PPAR subtypes (α, δ, γ1, and γ2) mRNA was quantified using real-time PCR analysis.

Results: The results demonstrated that both NSAIDs produced remarkable inhibition on the growth of all 3 cell lines tested. The inhibitory effect occurred in a concentration- and time-dependent manner, with indomethacin (EC50 value > 100 μM) being more potent compared to ibuprofen (EC50 value > 1000 μM). Furthermore, the ability of both NSAIDs in inhibiting the growth of cells is likely not to be associated with COX-2 expression. The evidence from M30 CytoDEATH assay suggested that the major mode of cell death caused by both NSAIDs was caspase-dependent apoptosis. This evidence was further supported by Western blot analysis, which indicated that the induction occurred via caspase-9-dependent pathway, whereas the real-time PCR analysis showed that both NSAIDs appear to modulate gene expression via a variety of different molecular targets in COX-2-dependent and/or independent pathway(s) depending on the colorectal cancer (CRC) cell type. However, alteration of TCF-4 and PPAR-γ1 mRNA expression are likely essential for both NSAIDs to induce apoptosis. Thus, Wnt and PPAR-γ signaling pathways may be involved in mediating the apoptosis induced by both NSAIDs in CRC cells. In addition, PPAR-δ was found to be another essential molecular target for indomethacin-induced CRC cell apoptosis.

Conclusion: This study may provide additional information and evidence of the various mechanisms and actions of NSAIDS in human CRC cells, which may be useful in selecting effective apoptotic drugs against specific CRC types. As shown in this current study, as well as others, both NSAIDs have anti-CRC activities and are potential anti-CRC agents. Further studies on the effect of both NSAIDs on CRC cells are important, as drugs may be developed as chemotherapeutic agents for human CRC.

Supervisor:
Associate Professor Dr Nik Soriani Yaakob
Co-supervisor:
Professor Dr Norazmi Mohd. Nor

THE DEVELOPMENT AND EVALUATION OF A NASBA SYSTEM FOR THE DIAGNOSIS OF CHOLERA USING ELISA AND BIOSENSOR METHODS

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Introduction: Cholera is a diarrheal disease caused by *Vibrio cholerae*. Cholera is potentially lethal if not diagnosed on time. Hence, early detection is crucial for patient treatment and containment of outbreak. Conventional culture and biochemical tests are laborious, time-consuming, and lacking of sensitivity. Although molecular-based methods are rapid, more sensitive and specific, they require expensive equipments and cold storage of reagents. Furthermore, DNA-based tests, such as PCR, do not distinguish between viable and nonviable cells. Nucleic acid sequence-based amplification (NASBA) is an isothermal RNA amplification technique that specifically detects viable cells. The objective of this study was to develop and evaluate a thermostabilized cholera-NASBA-ELISA assay with biosensor detection for *V. cholerae* based on the lolB gene.

Materials and Methods: RNA transcripts as positive control were first constructed and specific primers and probes were designed. NASBA and ELISA conditions were optimized and the analytical specificity was tested with 41 reference strains comprising of *V. cholerae, Vibrio* spp., and enteric pathogens. The analytical sensitivity was tested with serial dilutions of RNA transcripts and *V. cholerae* cells. Clinical evaluation of the assay was performed using spiked stool samples (n = 200). Subsequently, biosensor detection for the NASBA-ELISA assay was optimized and the results compared to spectrophotometry. The NASBA mix was thermostabilized by freeze-drying, and its stability at different temperatures was determined periodically. In addition, suitability of lolB mRNA as a viability indicator was investigated by subjecting cultures to lethal treatments and detecting the NASBA signal. The optimized cholera-NASBA-ELISA assay detected amplicons using fluorescein-labeled probes and TMB/HRP signal.

Results: The analytical specificity of the assay was 100%, while the analytical sensitivity was 102 molecules/µl RNA transcripts and 10 CFU/ml cells. Clinical evaluation gave 100% sensitivity, 84.52% specificity, 89.92% PPV, and 100% NPV. Biosensor detection was comparable to spectrophotometry, yielding similar analytical sensitivity level, excellent correlation (R2 < 0.964) and near perfect kappa agreement (95.1%, κ = 0.828). Thermostabilization of the NASBA mix was able to preserve its stability at 8 °C and -20 °C for 2 months. In the viability assay, lolB mRNA was detected even after 48 hours post-treatment, therefore precluding its use as a viability indicator.

Conclusion: We have, for the first time, developed a sensitive cholera-NASBA-ELISA assay with biosensor detection that can be performed using simple equipments within 4 hours. The dry NASBA mix reduces multiple pipetting steps and facilitates transportation and storage. The test is suitable for use as a rapid diagnostic test or screening test in the field.

Supervisor:
Dr P. Lalitha

DEVELOPMENT OF RECOMBINANT *MYCOBACTERIUM BOVIS* BACILLE CALMETTE–GUERIN (rBCG) EXPRESSING THE 19 kDa C-TERMINUS OF MEROZOITE SURFACE PROTEIN-1 (MSP-1C) AND THE 22 kDA OF SERINE REPEAT ANTIGEN (SE22) OF *PLASMODIUM FALCIPARUM* AS A POTENTIAL BLOOD-STAGE MALARIAL VACCINE

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Introduction: Recombinant *Mycobacterium bovis* Bacille Calmette-Guerin (rBCG) expressing the 19 kDa C-terminus of merozoite surface protein-1 (MSP-1C) and a 22 kDa protein (SE22) from the 47 kDa N-terminal domain of serine repeat antigen (SERA) of *Plasmodium falciparum* is a potential blood-stage malarial vaccine candidate. In the present study, the MSP-1C and SE22 were synthetically generated in favour of mycobacterial codon usage by assembly PCR. More importantly, the synthetic MSP-1C was mutated at various sites to induce the production of inhibitory but not blocking antibodies as previously reported.

Materials and Methods: The MSP-1C and SE22 fragments were cloned into a shuttle plasmid to facilitate expression by BCG. The expression of the blood-stage epitopes were driven by the heat shock protein 65 (hsp65) promoter from *M. tuberculosis* and the signal peptide from the MPT63 *M. tuberculosis* antigen. Expression of the recombinant clones were detected by specific monoclonal antibodies using Western blotting: SE47 mAb against the SE22 and 12.10 and 1E1 mAbs against the MSP-1C.

Results: The SE22 successfully reacted with SE47 mAb while the MSP-1C protein reacted with the inhibitory mAb 12.10, but not the blocking of mAb 1E1. The immunization of BALB/c mice with the rBCG elicited specific humoral responses against both blood-stage epitopes with a mixed Th1/Th2 profile. Immunized sera containing high levels of specific lgG2a against both epitopes (as determined by ELISA) were reactive with fixed *P. falciparum* merozoites as demonstrated

by the indirect immunofluorescence assay (IFA). In addition, the antibody titres against the MSP-1C and SE22 epitopes appeared to be correlated with the levels of inhibition of merozoite invasion of erythrocytes in vitro. Furthermore, the lymphocyte proliferative response to MSP-1C and SE22 from rBCG-immunized mice was significantly higher than the control groups. The expression of intracellular cytokines (IL-2, IL-4, and IFN-γ) in $CD4^+$ and$CD8^+$ cells were also detectable following in vitro stimulation with both epitopes. Preliminary and long-term in vivo stability analyses showed that the rBCG were stable in spite of being a non-integrative plasmid.

Conclusion: This study demonstrated that a single construct expressing a combination of 2 blood-stage epitopes of *P. falciparum* induced appropriate humoral and cellular responses against the parasites, paving the way for the construction of a potential blood-stage malarial vaccine.

Supervisor:
Professor Dr Norazmi Mohd. Nor

DEVELOPMENT AND EVALUATION Of hemA MUTANTS OF *VIBRIO CHOLERAE* O139 BENGAL AS A POTENTIAL VACCINE

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Objectives: The study aimed to describe the development of live attenuated oral vaccine strains VCUSM1 and VCUSM2 against *Vibrio cholerae* O139 Bengal. These strains are metabolic auxotrophs and require exogenous aminolevulinic acid (ALA) for their survival.

Materials and Methods: The auxotrophy was achieved by mutating a house-keeping gene hemA that encodes for glutamyl tRNA reductase, an important enzyme of the C5 pathway of ALA biosynthesis. Experiments carried out in infant mice and adult rabbits have shown that these vaccine strains are good colonizers of the small intestine. Subsequent experiments have revealed that these strains shed for a maximum of 4 days in the stool and elicit greater than a 4-fold rise in vibriocidal antibodies in the vaccinated rabbits. Rabbits vaccinated with VCUSM1 and VCUSM2 were fully protected against subsequent challenges with virulent wild type.

Results: Dose optimization studies have shown that as little as 1 x 106 CFU of VCUSM1 and VCUSM2, given orally 2 weeks apart, yielded 100% protection against subsequent challenge. Experiments carried out in ligated ileal loops of rabbits have shown that these strains are not absolutely non-reactogenic; however, they are 2.5-fold less toxic at a dose of 1 x 106 CFU. VCUSM1 and VCUSM2 survived no longer than 6 days in environmental waters as compared with the wild type, which was still detectable on day 20 post-inoculation.

Conclusion: These results suggested that VCUSM1 and VCUSM2 are the least toxic, highly immunogenic, promising vaccine candidates against *V. cholerae* O139 Bengal.

Supervisor:
Professor Dr Zainul Faziruddin Zainuddin

Abstracts

Abstracts of Theses Approved for the MSc and PhD at the School of Dental Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan, Malaysia

CLINICAL AND RADIOLOGICAL STUDY OF IMMEDIATE PLACEMENT OF CORAL COATED DENTAL IMPLANT

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Objectives: The study was conducted to determine clinically the efficacy of bone healing of immediate dental implantation with coral augmentation at the bone-implant interphase and to compare radiographic bone density around immediate dental implants with and without coral augmentation

Material and Methods: A comparative vertical prospective study design was employed, comparing 2 groups of patients receiving dental implants in Hospital Universiti Sains Malaysia. A group of patients received implants (Frialit-2 implant system, Germany) coated with natural coral (obtained from the National Tissue Bank, Universiti Sains Malaysia) and the other group received non-coated implants. All the implants were placed immediately into the extracted tooth socket. The inclusion criteria were healthy patients, age 18–40 years old, indicated for single tooth extraction, no endodontic and periodontic lesions at site of extraction, and extraction socket was left with intact 4 walls. The exclusion criteria were patients with systemic disease and if the extraction socket has lost 1 or more wall. Out of 13 patients selected, 8 patients had immediate implant with coral coating (test group) and 5 patients had non-coated implant (control group). However, 2 patients in the test group dropped out of the study. Clinical and densitometric assessments were done after 1, 2, 3, and 12 weeks post-operation.

Results: Clinically, all the 11 patients in both groups showed normal wound healing. Densitometric analysis showed that the bone density was significantly higher in the immediate coral-coated implant group compared with the control group on at least 1 point around the implant ($P < 001$). The values for densitometric analysis at 5 different points were higher in coral-coated implant group. However, the difference was significant only at the coronal mesial and midway distal points, with $P < 0.002$ and $P < 0.024$, respectively.

Conclusion: Locally produced coral (obtained from the National Tissue Bank) is a suitable material for coating the surface of implants since it was shown to provide primary stability to the immediate placement of the coated implants in the extraction sockets. This primary stability will ensure new bone growth to provide the more stable secondary stability. The biocompatibility of the coral graft and its role as an osteoconductor would have encouraged this very useful phenomena in dental implantology.

Supervisor:
Professor Dr Ab Rani Samsudin
Co-supervisor:
Dr Sam'an Malik Masudi

DENTAL CAST AND CEPHALOMETRIC ANALYSIS OF UNILATERAL CLEFT LIP AND PALATE USING FINITE ELEMENT ANALYSIS

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Objective: The purpose of this study was to localize differences of Malay population between non-cleft (NC) and unilateral cleft lip and palate (UCLP) in terms of the size and shape of the maxilla and mandible using Finite Element Analysis (FEA).

Material and Methods: In this cross sectional study, 31 subjects with UCLP and NC (6–12years old) were selected, and their dental casts and lateral cephalometrics were compared. The mean (SD) age was 9.4 (1) years for UCLP compared with 9.5 (1.17) years for NC. No patient had received orthodontic treatment. Dental casts were digitized into 6 elements with 12 homologous landmarks while lateral cephalometrics were digitized into 7 elements with 11 homologous landmarks.

Results: The maxilla was more affected by cleft in all dimensions than mandible. Difference in the size and shape of the mandible between UCLP and NC were limited.

Conclusion: Size and shape differences between UCLP and NC can be identified with finite element analysis.

Supervisor:
Dr Zainul Ahmad Rajion
Co-supervisor:
Dr Rozita Hassan

MICROLEAKAGE OF REPAIRED FISSURE SEALANT USING NANO-FILLED RESIN: IN VITRO STUDY

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Objectives: The aims of this study were to investigate whether differences in the technique of repair influence a seal of a repaired nano-filled, resin-based fissure sealant and to determine the effect of different curing time on microleakage of a repaired fissure sealant.

Materials and Methods: A total of 108 extracted molars were randomly allocated to 1 of 4 groups of 28 teeth each. A light cured, nano-filled, resin fissure sealant (Filtek Z350) was placed on their occlusal surface following cleaning by prophylaxis and acid etching. Following storage in artificial saliva for a week, duplication of sealant failure was carried out. The teeth were then subjected to 4 different methods of repair: Group 1, a slow-speed prophylaxis brush followed by acid etching and 10 seconds curing time (control); Group 2, prophylaxis brush, acid etching, application of bonding agent, and 10 seconds curing time; Group 3, prophylaxis brush, acid etching, and 5 seconds curing time; and Group 4, prophylaxis brush, acid etching, and 20 seconds curing time. Then, they were stored for 1 week in artificial saliva, painted with 2 layers of impermeable varnish. Their apices were sealed with wax, and the teeth were immersed in 1% methylene blue for 48 hours. The teeth were then sectioned longitudinally in a mesiodistal plane to achieve 3 cuts resulting in a maximum of 4 blocks, i.e., 6 surfaces per tooth. A total of 648 surfaces from 108 teeth were scored for microleakage using scoring system on the intact and the repaired side of the fissure sealant.

Results: Chi-square analysis indicated no significant different between the tested techniques of repair, except for Group 3 that resulted in the highest number of surfaces exhibited maximum score of microleakage ($P = 0.027$).

Conclusion: The present data did not demonstrate any single method of repair to be superior to the control method, which was the use of prophylaxis brush without any medium, followed by acid etching, applying fissure sealant, and light curing for 10 seconds. The control method seems to be the simplest and the most appropriate method of repair; therefore it is recommended.

Supervisor:
Dr Siti Noor Fazliah Mohd Noor
Co-supervisor:
Dr Dasmawati Mohamed

THE APICAL SEALING ABILITY EVALUATION OF A NEW EXPERIMENTAL NANO HYDROXYAPATITE-FILLED EPOXY RESIN BASED ENDODONTIC SEALER: IN VITRO STUDY

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MSc Dentistry

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Objectives: The study was conducted to evaluate the apical sealing ability of experimental nano hydroxyapatite (HA)-filled epoxy resin-based endodontic sealer and compare it with the commercial AH26 sealant.

Material and Methods: School of Dental Science, Universiti Sains Malaysia (USM) had prepared a new experimental nano HA-filled epoxy resin based endodontic sealer. The HA nano crystal, sizes ranging 40–60 nm, were synthesized at the School of Chemical Sciences, USM by wet chemical method using calcium hydroxide, $Ca(OH)_2$, and phosphoric acid, H_3PO_4, as the Ca and P precursors, respectively. The components of experimental nano HA-filled epoxy resin sealer were nano HA, bismuth (III) oxide, hexamethylene tetramine, and epoxy resin. A total of 76 extracted human anterior teeth were instrumented using nickel–titanium (NiTi) files and randomly divided into 2 test groups of 33 teeth each and 2 control groups of 5 teeth each. The first group was obturated using gutta-percha with AH26 sealer. The second group was obturated with the nano HA-filled epoxy resin based sealer. All teeth were coated with nail polish, except 2 mm from foramen apical, and then suspended in 2% methylene blue for 7 days. All teeth were sectioned longitudinally for the measurement of penetration of the dye using stereo-microscope (36 x magnification). The maximum length of the dye penetration in each root was examined and measured by Imaging System (LEICA, UK). All the measurements were then noted and tabulated. Every measurement was repeated twice by 2 blinded researchers, the mean of the 2 measurements was recorded for each case. Intraclass correlation coefficient (ICC) was nearly 0.983, which suggest that the measurement was almost identical or with negligible errors of measurements.

Results: Statistical analysis of the results was performed using independent sample *t* test. The mean penetration distances of methylene blue across the AH26 silver-free sealer and experimental nano HA sealer was 0.44 (SD 0.63) mm and 0.75 (SD 0.81) mm, respectively. The result showed that there was no statistically significant difference ($P > 0.05$) in apical sealing ability between AH26 silver-free sealer and experimental nano HA sealer.

Conclusions: Experimental nano HA-filled epoxy resin endodontic sealer provided an adequate apical seal against dye penetration in similar level with AH26 silver-free and could be used as an alternative to the commercial available endodontic sealer. Further study should be carried out to determine the setting time, radio-opacity, solubility, and antibacterial properties of nano HA root canal sealer.

Supervisor:
Dr Sam'an Malik Masudi
Co-supervisors:
Dr Dasmawati Mohamad
Dr Norhayati Luddin

VARIATIONS IN TOOTH SIZE, DENTAL ARCH DIMENSIONS, AND SHAPE AMONG MALAY SCHOOL CHILDREN

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Objectives: The aims of the present study were to measure the mesiodistal tooth size and dental arch dimensions in Malay schoolchildren with Class I, Class II, and Class III, and compare the tooth size and arch dimensions among different classes of malocclusion.

Materials and Method: The current study consisted of dental casts of 150 subjects: 78 males and 72 females aged 12 and 16 years. Every malocclusion group consisted of 50 subjects. An electronic digital caliper was used to measure the individual mesiodistal tooth width of all maxillary and mandibular permanent teeth (except 2nd and 3rd molars). Inter-canine and inter-molar widths were also measured. To measure maxillary and mandibular arch perimeter and length, AutoCAD software was used.

Results: The teeth width and arch dimensions were significantly larger in males than in females except for lower arch perimeter and upper arch length ($P < 0.05$). Moreover, the arch widths were significantly smaller in Class II compared with Class I normal occlusion. Significant difference was observed only in the mandibular inter-canine width ($P < 0.05$). There were no significant differences neither in arch perimeter or arch length in the maxillary and mandibular dental arches.

Conclusion: Knowledge of arch width and tooth size that is associated with malocclusion is helpful in determining orthodontic treatment goals and likely post-treatment sequence for the malocclusion.

Supervisor:
Dr Zainul Ahmad Rajion
Co-supervisor:
Dr Rozita Hassan
Dr Siti Noor Fazlia Mohd Noor

EVALUATION OF APICAL SEALING ABILITY OF A NEWLY DEVELOPED NANO HYDROXYAPATITE SEALER USING COLD LATERAL AND CONTINUOUS WAVE CONDENSATION TECHNIQUES: AN IN VITRO STUDY.

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MSc Dentistry

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Objectives: The study was designed to evaluate in vitro apical sealing ability of experimental nano hydroxyapatite (HA)-containing endodontic sealer and compare it with AH 26 sealer using cold lateral and continuous wave condensation techniques.

Materials and Methods: This was a randomized controlled single-blind experimental study involving 232 freshly extracted single rooted human teeth. Crowns of the teeth were amputated at the cemento–enamel junction using Exakt cutting system. The root canals were prepared using a crown-down technique with ProTaper nickel–titanium rotary system and randomly divided into 4 experimental groups to be obturated by the nano HA sealer and cold lateral condensation technique ($n = 53$), the AH 26 sealer and cold lateral condensation technique ($n = 53$), the nano HA sealer and continuous wave condensation technique ($n = 53$), and the AH 26 sealer and continuous wave condensation technique ($n = 53$). The remaining 20 teeth served as positive and negative control groups of 10 teeth each. All teeth were stored in an incubator, at 37 °C for 7 days to allow adequate setting of sealers. Root surfaces were covered with 2 layers of nail polish except for the apical 2 mm and then placed in an aqueous solution of 2% methylene blue dye. After 72 hours, the teeth were rinsed under running tap water and dried, and the nail polish was removed. Each specimen was then embedded in isobornyl methacrylate resin to facilitate their mounting in a hard tissue cutter. Six transverse sections of 1 mm thickness were taken starting at the apical limit of the preparation and ascending apico–coronally to a total of 6 mm of each tooth. The coronal surface of each consecutive section was assessed for dye penetration using stereomicroscope supported by image analyzer software. Overall dye penetration for each tooth was then calculated as the ratio between the total methylene blue infiltrated surface areas and the total dentinal surface areas of the 6 levels. Data was entered into SPSS software and analyzed using two-way ANOVA where $P < 0.05$ was considered as statistically significant.

Results: The positive controls demonstrated maximum dye penetration at all levels in all teeth. In contrast, the negative controls showed no evidence of leakage at any level in any of the samples. Means of overall apical dye penetration were 9.33% for nano HA sealer and 8.94% for AH 26 sealer with no significant difference ($P = 0.087$) between the 2 tested sealers. The means of overall apical dye penetration were 12.15% and 6.11% for cold lateral condensation and continuous wave condensation techniques, respectively. Cold lateral condensation leaked significantly more ($P < 0.001$) than continuous wave condensation technique.

Conclusion: Experimental nano hydroxyapatite sealer provided a comparable apical seal to that obtained by AH 26 sealer and could be used as an alternative to the commercial available endodontic sealer. In addition, continuous wave condensation technique using system B created a better apical seal than conventional cold lateral condensation technique.

Supervisor:
Dr Sam'an Malik Masudi
Co-supervisor:
Dr Wan Zaripah Wan Bakar

GENOTOXIC EVALUATION OF LOCALLY PRODUCED DENTAL PORCELAIN USING THE AMES SALMONELLA AND COMET ASSAYS

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MSc Dentistry (Prosthodontics)

School of Dental Sciences, Universiti Sains Malaysia
Health Campus, 16150 Kelantan, Malaysia

Objectives: The study was conducted to determine the genotoxicity of locally produced dental porcelain (Universiti Sains Malaysia, MY) using the Salmonella/mammalian-microsome mutagenicity assay (Ames assay) and the single cell gel electrophoresis assay (Comet assay).

Materials and Methods: In the Ames assay, 4 genotypic variants of the Salmonella strains (TA98, TA100, TA1535, and TA1537) carrying mutations in several genes were used. The dental porcelain was incubated with these 4 strains at 5 different concentrations (0.3125, 0.625, 1.25, 2.5, and 5 mg/plate) along with concurrent appropriate positive and negative controls, both in the absence and presence of metabolic activation (S9). The results were assessed based on the number of revertant colonies per plate in comparison with that of the negative control. In the Comet assay, L929 (CCL-1 ATCC, USA) mouse fibroblast cells were treated with the locally produced dental porcelain at 3 different concentrations (50, 100, and 200 mg/mL) along with concurrent negative and positive controls. Fifty cells were captured randomly from each slide and scored under a fluorescence microscope. The mean value of tail moment was used as a measurement of DNA damage.

Results: For a substance to be considered mutagenic in the Ames assay, the number of revertant colonies per plate containing the test material must be at least more than double the number of colonies per plate containing the negative control. The test material do not show more than double the number of colonies than the negative control or any dose-dependent increase in the number of revertant colonies both in the absence and presence of metabolic activation. The results of the Comet assay showed that the mean tail moment with the test material was similar to that of the negative control; there was also no dose-dependent relationship on the tail moment.

Conclusion: Locally produced dental porcelain is non-genotoxic by both Ames and Comet assays under the present test conditions.

Supervisor:
Dr Adam Husein
Co-supervisor:
Dr TP Kannan

PREVALENCE OF ORAL MUCOSAL LESIONS AND THE ASSOCIATION BETWEEN DIABETES MELLITUS AND ORAL PRECANCEROUS LESIONS AMONG MALAY PATIENTS ATTENDING HOSPITAL UNIVERSITI SAINS MALAYSIA

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Objectives: The study aimed to determine the prevalence of oral mucosal lesions among Malay diabetic patients, and the association between oral mucosal lesions and diabetes factors, as well as diabetes and oral precancerous lesions.

Materials and Methods: This cross-sectional study involved 420 diabetic patients and 420 non-diabetic subjects in Hospital Universiti Sains Malaysia, Kota Bharu, Kelantan, from January until August 2009. Demographic information, duration and type of diabetes, glycosylated hemoglobin values (HbA_{1c}), and previous and current uses of medication were obtained from medical records. Detailed oral examination of the oral cavity was done based on international criteria and World Health Organization codes. The number of remaining teeth and presence of dentures were also noted.

Results: The frequency of oral mucosa lesions was significantly higher in diabetes patients, 45%, in comparison with 38.3% in controls ($P < 0.05$). There were 12 types of oral mucosal lesions identified. Specific oral lesions that were found to be of significantly greater frequency in diabetes patients than controls include geographic tongue ($P < 0.01$), denture stomatitis ($P < 0.05$), and angular cheilitis ($P < 0.05$). The prevalence of traumatic ulcers was higher in diabetics; however, this was not significant ($P > 0.5$). About one-third of subjects in both groups used dentures and diabetic patients have a lower mean number of remaining teeth compared to non-diabetics ($P < 0.001$). There was an association between the prevalence of oral mucosal lesions and metabolic control ($P < 0.05$). However, no association between diabetes mellitus and oral precancerous lesions was found.

Conclusions: Diabetic patients showed higher prevalence of oral mucosal lesions than control subjects did. There was an association between oral mucosal lesions and metabolic control of diabetes; however, no association was observed between diabetes and oral precancerous lesions.

Supervisor:
Associate Professor Dr Hj Abdul Rashid Hj Ismail
Co-supervisors:
Dr Rajan Saini
Associate Professor Dr Hjh Noorliza Mastura Hj Ismail

DENTAL AGE IN KELANTANESE MALAY POPULATION BASED ON DEMIRJIAN'S METHOD

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School of Dental Sciences, Universiti Sains Malaysia Health Campus, 16150 Kelantan, Malaysia

Objectives: The purposes of this study were as follows: (1) Examining the applicability of Demirjian's methods for estimating the chronological age of male and female Kelantanese Malay children aged 5 to 16 years; (2) Establishing a new dental age standard, if Demirjian's methods were not applicable on the Kelantanese Malay population; (3) Comparing the dental age curves between Malay children and French–Canadian children; (4) Determining the sexual dimorphism in the dental age assessment of Kelantanese Malay children; (5) Detecting the differences in "dental ages" and "maturity scores" between the lower left permanent teeth and the lower right; and (6) Detecting the median ages of attainment of each stage of dental development according to Demirjian's stages for the lower left 7 teeth.

Materials and methods: A total number of 905 panoramic radiographs (OPG) for healthy Kelantanese Malay children aged 5 to 16 years old were collected from the Radiographic Unit, Hospital Universiti Sains Malaysia (HUSM), and the Orthodontic Clinic, Hospital Kota Bharu. The dental age was assessed cross-sectionally by using Demirjian's methods (1973 and 1976). Children who had any disease that was known to affect the dental development or have agenesis in the lower arch were excluded, as well as those poor quality OPG images.

Results: Demirjian's method (1973) overestimated the chronological age by 1.2 years for boys and 1.3 years for girls. The 4 teeth: M_2, M_1, PM_2, PM_1 method overestimated the age by 1.2 years for both sexes, while the 4 teeth: M_2, PM_2, PM_1, I_1 method overestimated the age by 0.6 year for boys and 0.7 year for girls. As the methods of Demirjian's were not applicable on Kelantanese Malay children, new modified specific standards curves and tables were produced for Demirjian's method (1973). An external sample of 47 Kelantanese Malay children (28 boys and 29 girls) randomly selected from HUSM was used to test the accuracy of the modified Demirjian's method on Kelantanese Malay population. The results showed a mean difference between the chronological age and dental age of about 2 months for both sexes. The median ages of attainment of each developmental stage according to Demirjian's stages for the lower permanent left 7 teeth for both sexes were produced. Moreover, girls showed more advancement in dental age compared with boys. In comparison between the dental development between the Kelantanese Malay and French–Canadian children, the results showed that the "dental age" for younger age groups of boys (7.00–9.99 years) and age groups of girls (7.00–8.99 years) was not significantly different from the French–Canadian children. After the age 10 years, the difference increased in boys, whereas in girls, the increase started earlier, i.e., after the age 9 years. The greatest difference between Kelantanese and French–Canadian girls was at age 12 years with mean difference of about 2 years, while for boys was 1 year earlier than the girls with a difference reach about 2 years and 3 months. In the older age groups (14.0–15.99 years) the maturation scores were similar with the French–Canadian children. No significant difference was found in the dental development of the lower left teeth when compared with the right lower teeth.

Conclusion: Demirjian's methods (1973 and 1976) were shown to be less accurate to estimate the chronological age in Kelantanese Malay children samples. Variations in the dental development should be considered especially for the genetic factor and less for non-genetic factors. The modification of the system had resulted in a new dental age system that is more precise and more applicable for the Kelantanese Malay children.

Supervisor:
Dr Siti Noor Fazliah Mohd Noor
Co-supervisor:
Dr Mohd Fadhli Khamis

THE CORONAL SEALING ABILITY OF A NOVEL NANO HYDROXYAPATITE-FILLED ENDODONTIC SEALER

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Objectives: The study aimed to evaluate the sealing ability of a novel nano hydroxyapatite (HA)-containing endodontic sealer in preventing coronal leakage before and after post preparation and to compare it with the commercial AH26 sealant.

Material and Methods: A total of 152 extracted human single-rooted teeth were instrumented using nickel–titanium (NiTi) files and the crowns were then amputated. After cleaning and shaping procedure were completed using step back technique, samples were randomly divided into 2 groups. The 2 groups were obturated with either gutta percha and AH 26 sealer, or gutta percha with the nano HA-containing sealer. All teeth were then stored at 37 °C for 7 days to allow the sealer to set. Each group was then further subdivided into 2 groups: a group was prepared for post using para-post drill and the other group was left intact. All 4 study groups consisted of 38 samples per group. The teeth were then thermal-cycled at 5°C and 55°C in water baths at dwell time of 30 seconds for a total of 500 cycles. External surfaces of the roots were coated with 2 layers of nail varnish that did not cover the coronal opening. Specimens were then submerged in 2% methylene blue dye for 24 hours. Each root was sectioned vertically into 2 halves, and microleakage was measured under microscope (36 x magnifications) by taking

the maximum linear dye penetration corono–apically. Micro leakage readings were analyzed by the independent *t* test (α = 0.05).

Results: The result showed that there was no statistically significant difference in the coronal sealing ability between the 2 sealers, before and after preparation for post. The experimental nano HA-containing and AH26 sealers with post space preparation showed significantly more leakage compared with sealers with no post space preparation.

Conclusion: Preparation for post caused a significant decrease in the coronal sealing ability of both sealers; however, it did not result in any difference between the performances of the 2 sealers. The novel nano HA-containing sealer tested had a comparable coronal sealing ability with the commercial AH26 sealer.

Supervisor:
Dr Zuryati Ab Ghani
Co-supervisor:
Dr Adam Husein
Dr Sam'an Malik Masudi

ASSESSING AND MANAGING RISK OF OCCUPATIONAL STRESS IN MALE AUTOMOTIVE ASSEMBLY WORKERS IN MALAYSIA

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Introduction: Occupational stress is a major occupational health problem in many industries. Automotive assembly industry workers are among the occupational groups reportedly experiencing disproportionately high levels of occupational stress.

Objectives: The main purpose of this study was to assess and manage the risk of occupational stress in male automotive assembly workers in Malaysia. Other objectives include validating the Malay version of the Job Content Questionnaire (JCQ) and Depression Anxiety Stress Scales (DASS) 21- and 42-item; determining the prevalence and associated factors of self-perceived depression, anxiety, stress, and quality of life (QOL); modeling the relationship between job demand, job control, and social support in relation to the self-perceived depression, anxiety and stress, and QOL; and evaluating the immediate effects of a 4-hour stress management training on the self-perceived depression, anxiety, and stress in male automotive assembly workers.

Material and Methods: As parts of the longitudinal research design, a cross-sectional study and quasi-experimental intervention were carried out between November 2004 and October 2007. A total 728 workers from 2 automotive assembly plants in Selangor and Pahang were enrolled in this study.

Results: The Malay version of the JCQ and DASS (21-item and 42-item) were reliable and valid for assessing job content, self-perceived depression, anxiety, and stress. The prevalences of self-perceived depression, anxiety, and stress were 35.4%, 47.2%, and 31.1%, respectively. Multiple linear regression (MLR) analyses revealed that psychological job demand, job insecurity, and hazardous conditions were positively associated with the DASS-Depression, DASS-Anxiety, and DASS-Stress; supervisor support was inversely associated with DASS-Depression and DASS-Stress. The prevalence of reported good or very good overall self-perceived QOL and general health status was 64.9% and 53.7%, respectively. MLR analyses indicated that created skill was positively associated with physical health and psychological domains of QOL while skill discretion was positively associated with the social relationship and environment domains of QOL. Social support was positively associated with the physical health and environment domains of QOL while co-worker support was positively associated with the psychological and social relationship domains of QOL. Job insecurity and hazardous condition were negatively associated with all domains of QOL while psychological job demand was negatively associated with the environment domain of QOL. In the structural equation modeling analysis, the final model showed that social support in the workplace was directly related to all 4 domains of QOL (physical health, psychological wellbeing, social relationships, and environmental conditions) and inversely related to self-perceived depression and stress. Job demand was directly related to self-perceived stress and inversely related to the environment domain of QOL. Job control was directly related to the social relationships domain of QOL. Surprisingly, self-perceived stress, anxiety, and depression were also found to be important mediating factors in the relationships between job demand and social support and the 4 domains of QOL. Meanwhile, in the quasy-experimental study, we found that the stress management training significantly improves self-perceived depression and anxiety in the experimental group as compared with the control group.

Conclusion: Reducing psychological job demand, job insecurity, and hazardous condition factors and promoting good support from co-workers and supervisors may improve the worker's self-perceived depression, anxiety, stress, and QOL in the automotive assembly plant. At the individual level, the findings suggest that the stress management training is effective in reducing the self-perceived depression and anxiety.

Supervisor:
Professor Dr Rusli Nordin
Co-supervisor:
Dr Lin Naing

Abstracts

Abstracts of Theses Approved for the MSc and PhD at the Institute for Research in Molecular Medicine (INFORMM) Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan, Malaysia

DEMONSTRATION OF ANTIGENIC AND SPECIFIC OUTER MEMBRANE PROTEIN(S) OF *ACINETOBACTER BAUMANNII*

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MSc Molecular Medicine

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Introduction: *Acinetobacter baumannii* has been recognized as an emerging nosocomial pathogen and is often multi-resistant to antibiotics. Current identification of *A. baumannii* is by conventional culture method and biochemical tests, which take about 2 to 7 days to produce results. Hence, there is a need for a new rapid, sensitive, and specific test that would allow better management of nosocomial infections. The aim of this study was to detect the presence of a specific and antigenic biomarker for *A. baumannii* from the outer membrane protein (OMPs) that can be used for the development of a rapid and specific diagnostic test.

Material and Methods: Protein profiles of OMP lysates from the ATCC strain and clinical isolates of *A. baumannii* were obtained by SDS-PAGE and compared. The protein profiles of the clinical isolates were 95% identical to that of the ATCC strain. Following this, the protein electrophoretograms were subjected to Western blot using sera from patients infected with *A. baumannii*, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli*, and sera from normal healthy subject using labeled anti-IgM, IgA, and IgG to check for cross reactivity.

Results: There was 1 band of molecular weight 34.4 kDa that was found only in clinical isolates of *A. baumannii* and it does not cross react with other tested sera. The observations suggested that the protein was specific for *A. baumannii* and can be used as a biomarker for development of a diagnostic test.

Conclusion: The results are encouraging in that the 34.4 kDa protein identified is specific for *A. baumannii* and can be used as a biomarker for development of a diagnostic test that would be faster and more specific than the current techniques of diagnosis. However, further studies need to be done to measure the antibody level against this specific protein, the sensitivity and specificity of the protein, and the retention time of the antibody detectable in the serum of the infected patients.

Supervisor:
Profesor Dr Asma Ismail

Co-supervisor:
Dr Kirnpal Kaur Singh Bangga Singh

CLONING, EXPRESSION, AND PURIFICATION OF TOXOCARA CANIS RECOMBINANT ANTIGENS (rTES-32, rTES-120) AND DEVELOPMENT OF SERODIAGNOSTIC TEST FOR TOXOCARIASIS

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Introduction: Routine serodiagnosis of human toxocariasis is based on indirect IgG-ELISA kits which employ native *Toxocara canis* excretory-secretory (TES) antigen. However, these assays lacked specificity especially when used in tropical countries where multi-parasitism are prevalent. In an effort to improve the diagnostic test for infection, we have developed an IgG4-ELISA assay that uses 3 recombinant antigens.

Material and Methods: Recombinant *T. canis* DNA that encodes for rTES-26, rTES-32, and rTES-120 were produced by cloning of open-reading frames (ORF) of the respective genes via reverse-transcriptase-PCR (RT-PCR) using mRNA extracted from a culture of *T. canis* second stage larvae into PCR2.1 TOPO vector. Sequence analysis revealed that TOPO/TES-32 and TOPO/TES-120 were 100% similar to the reported sequences in the GenBank; however, TOPO/TES-26 gene fragment had 4 mutations. After all mutations in TOPO/TES-26 gene fragment had been corrected, TES-26 and TES-32 were subsequently subcloned into a GST-tagged prokaryotic expression vector, while TES-120 was subcloned into a HIS-tagged vector. All constructs were expressed in *E. coli* BL21(DE3) expression host.

The recombinant proteins were subsequently purified under native condition by affinity chromatography using GST and His-Trap resins, since these recombinant proteins are abundantly expressed in soluble form. The site-specific protease, Factor Xa, was used to remove GST tag in the TES-26 and TES-32 fusion proteins. Western blot analysis revealed that these recombinant antigens were immunologically reactive and specific.

Results: Sera from patients infected with toxocariasis had IgG4 antibodies that recognized these recombinant antigens, while sera from individuals with other infections and healthy controls did not. When the 3 recombinant antigens

were tested in ELISAs specific for immunoglobulin IgM and IgE classes, as well as IgG subclasses (IgG1-IgG4), the results clearly showed that only IgG4 assay displayed good specificity. The diagnostic utility of each purified recombinant antigen and rTES-30USM (previously produced in our laboratory) was further evaluated by IgG4-ELISA assay using 242 serum samples, which included 30 sera from patients with clinical, haematological, and serological evidence of toxocariasis. Both rTES-26 and rTES-32 IgG4-ELISAs demonstrated sensitivity of 80.0%, while rTES-120 IgG4-ELISA showed sensitivity of 93.3%, which is similar to that previously reported for rTES-30USM IgG4-ELISA. The sensitivity of rTES-120/rTES-30USM IgG4-ELISA was found to be significantly higher than rTES-26/rTES-32 IgG4-ELISA ($P < 0.001$). However, the mean ODs of the 30 toxocariasis samples among the IgG4 assays using the 4 recombinant antigens were shown not to be significantly different. There was marginally no significant difference between the specificities of rTES-26 and rTES-120, rTES-26 and rTES-30USM, or rTES-30USM and rTES-120.

In the final assay, rTES-32 was excluded since it did not show better sensitivity or specificity than rTES-26. Instead, rTES-30USM was included due to its high sensitivity and the fact that a 100% detection of toxocariasis cases was achieved with the combined use of rTES-30USM and rTES-120 in IgG4-ELISA.

Conclusion: A final assay which is sensitive (80.0%–93.3%) and specific (92.0%–96.2%) for detection of toxocariasis was successfully developed using 3 adjacent wells, each separately coated with rTES-26, rTES-30USM, and rTES-120. This study is novel in several ways: it is the first report on the use of multiple recombinant antigens for serodiagnosis of toxocariasis, the use of rTES-26 (and rTES-32) in *Toxocara* serodiagnosis, the use of IgG4 assay for rTES-120 and rTES-26, and the use of GST tag in the expression and purification of *Toxocara* recombinant proteins. The test may provide a significant improvement over commercially available tests for diagnosis of toxocariasis and may be use especially in countries co-endemic with other soil-transmitted helminthes.

Supervisor:
Professor Dr Rahmah Noordin

CYTOCHROME P450 2C8: AN INVESTIGATION OF TYPES AND FREQUENCIES OF CYP2C8 POLYMORPHISM IN MALAYSIA AND IN VITRO ANALYSIS OF CATALYTIC ACTIVITY

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Introduction: CYP2C8 is a polymorphic gene. Polymorphisms and drug interactions may imply susceptibility to adverse effects with drugs of CYP2C8 substrate. The objective of this study is to investigate the types and frequencies of CYP2C8 polymorphism in Malaysia and to analyze the in vitro catalytic activity of CYP2C8.

Materials and Methods: Subjects were healthy adult Malays (n = 313), Chinese (n = 335), and Indians (n = 200), as well as male Malay patients with hypertension (n = 154). DNA extracted from the blood was subjected to PCR method developed for CYP2C8 genotyping. HPLC method was developed using Amodiaquine for the in vitro catalytic activity and inhibitory study of CYP2C8 utilizing recombinant CYP2C8 protein expressed in E. coli. The in vitro inhibitory study with natural products consisted of herbs extracts of *Eurycoma longifolia Jack* (ELJ) or locally known as Tongkat Ali, *Labisia pumila* (LP) or Kacip Fatimah, *Andrographis paniculata* (AP) or Hempedu Bumi, *Echinacea purpurea* (EP) or Purple Coneflower, and *Ginkgo biloba* (GB), as well as Tualang Honey (TH) and Policosanol, a cholesterol lowering tablet produced from sugarcane wax.

Results: The allele frequencies of CYP2C8*2 and *3 were 0.2% and 0.3% in Malays and 1.5% and 2.3% Indians. In Malay patients with hypertension, CYP2C8*2 and *4 were detected with allele frequencies of 0.3% each. The kinetic parameters, Vmax and Km for CYP2C8 were 2.41 (SD 0.014) pmol/min/pmol CYP2C8 and 1.28 (SD 0.047) µM respectively. Extracts of LP, AP, TH, and Policosanol inhibited Amodiaquine metabolism via the CYP2C8 pathway in an uncompetitive (LP and AP), competitive (TH), and noncompetitive (Policosanol) inhibition mechanism.

Conclusion: We have successfully developed a specific, sensitive, and less tedious allele-specific multiplex PCR method for genotyping CYP2C8 polymorphism in Malaysia. We also have successfully expressed recombinant CYP2C8 protein and developed a specific and sensitive HPLC method for the in vitro analysis of CYP2C8 catalytic activity and the herb and food inhibition effect on CYP2C8 activity. Future studies are required to investigate the inhibitory effects of natural products on CYP2C8 activity to improve the understanding between genotypes and drug-herb or drug-food interactions in relation to susceptible diseases.

Supervisor:
Professor Rusli Ismail

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> Theriault A, Caao JT, Gapor A. Tocotrienol is the most effective vitamin E for reducing endothelial expression of adhesion molecule and adhesion to monocytes. *Atherosclerosis.* 2002; **160**:21-30.

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> Carlson BM. Human embryology and developmental biology. 3rd ed. St Louis: Mosby; 2004.

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> Reid R, Bulusu R, Buckels J, Carroll N, Eatock M, Geh I et al. Guidelines for the management

of gastrointestinal stromal tumors (GISTs). Sept 2005. [Internet]. Available from:http://www.augis.org/news/articles/gist%20mngmnt%20gdlns%20071205%20final.pdf

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1. Council of Science Editors, Scientific Style and Format. The CSE Manual for Authors, Editors and Publishers. 7th ed. Reston (VA): The Council; 2006.
2. The Chicago Manual of Style. 15th ed. 2003.
3. ICMJE Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication [Internet] Available from: http://www.icmje.org/

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