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EDITORIAL

Influenza A / H1N1 Pandemic: The Scare of 2009

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Introduction

The world was taken by surprise with the announcement of a major influenza outbreak resulting in significant mortality in Mexico in late April 2009. The intense world media attention rapidly escalated the efforts of all countries around the world to activate their own respective influenza pandemic preparedness plans. The identification of a new 're-assorted' influenza virus as the source of this rapidly spreading infection fuelled fears that this was the next major influenza pandemic that the world had been warned about. The World Health Organisation (WHO) promptly raised the influenza alert level from 4 to 5 on April 29th, 2009 and finally to the highest level of 6 on June 10th, 2009.

On the local front, much discussion has taken place (in both print and electronic media) on the measures taken by the various agencies on the front line of this outbreak. While there are some who feel that too much is being done, so much so that everyday activities are being hampered, there is probably an equally sizeable group in our population who feel that the measures taken have been insufficient. During an outbreak, especially one of global magnitude, many forces influence our reactions to the perceived threat. The operative word here is perceived, and perception is a heterogeneous entity that is governed by myriad factors. Hence, it is imperative that our reactions be as evidence-based as possible. Our responses should therefore always be based on sound science. At the same time, they must be guided by common sense and a clear understanding of local realities, both of our strengths and our limitations.

Influenza A / H1N1 2009: The Science

Infectiousness And Severity

Within the first weeks of the outbreak, it was clear that this novel influenza A/H1N1 virus is extremely infectious. It has a secondary attack rate of 22 to 33%, which is significantly higher than that of seasonal flu, which is estimated at 5 to 15%. This is likely due to the lack of immunity in

humankind to this novel virus. The initial reports of 'high' mortality from Mexico have not been reported in other countries. The reasons for this are likely multiple, but they may include complex heterogeneity in the degree of immunity in local populations to circulating influenza strains, as well as transmission factors such as geographic conditions, social mixing and local seasonal changes, etc. The global case fatality rate as of July 15th, 2009 stands at 0.45% (with varying rates in different countries; e.g., Argentina 1.7%, Mexico 1.1%, USA 0.5% and Canada 0.31%).

Clinical Manifestations

The clinical characteristics of this novel influenza virus appear to be similar to those of seasonal strains with some minor differences. From the early patient cohorts in Mexico, the United States of America and the United Kingdom, it became apparent that 25–30% of patients with influenza A/H1N1 had diarrhoea and/or vomiting as one of their main symptoms. However, this has not been seen universally. In Malaysia, less than 5% of all patients admitted to the Sungai Buloh Hospital had these symptoms. Most patients had mild self-limiting symptoms. Common presenting symptoms include fever (94%), cough (92%), sore throat (66%), headaches (38–81%), rhinorrhoea (27%), arthralgia (56%), diarrhoea (25%) and vomiting (25%) (1,2,3). Nonetheless, patients with certain risk factors or co-morbidities were more likely to experience influenza-associated complications, the most frequent of which was pneumonia (either primary viral pneumonia or secondary bacterial pneumonia). The other noted complications include myocarditis, encephalitis, persistent diarrhoea and shock. Patients who are at risk for severe complications of influenza A H1N1 infection include but may not be limited to the following groups: children under the age of 5 years; adults 65 years of age or older; persons of any age with an underlying chronic medical condition such as chronic respiratory disease (asthma, chronic obstructive pulmonary disease, obstructive sleep apnoea, etc.), chronic renal disease, diabetes mellitus, an immunosuppressed condition (e.g.,

those with HIV infection) or malignancy; those on chemotherapy or long-term steroid treatment; and pregnant women.

In a cohort analysis of 642 influenza A/H1N1-infected patients in the US (1) from April 15th to May 5th, 2009 the Novel Swine-Origin Influenza A (H1N1) virus Investigation Team reported that only 9% of these patients required hospitalisation, and 54% of those admitted had co-morbidities that conferred an increased risk. There were eight intensive care admissions with two deaths. None of the patients, however, were 65 years of age or older.

Modes Of Transmission

The modes of transmission of this novel virus in humans are thought to be mainly through the dissemination of large droplets and possibly small-particle droplet nuclei expelled when an infected person coughs. There is also potential for transmission through contact with fomites that are contaminated with respiratory or gastrointestinal material. Since many patients with influenza A H1N1 infection have had diarrhoea, the potential for faecal viral shedding and subsequent faecal-oral transmission should also be considered.

The incubation period appears to range from two to seven days. On the basis of data regarding viral shedding from seasonal influenza, most patients with influenza A/H1N1 infections are likely to shed virus starting from one day before the onset of symptoms through five to seven days after the onset of symptoms or until symptoms resolve; in young children and in immunocompromised or severely ill patients, the infectious period might be longer. The potential for persons with asymptomatic infection to be the source of infection to others is still uncertain; nonetheless, as for other influenza viruses, viral shedding is frequently highest during the period of fever and acute respiratory symptoms.

Antiviral Therapy

During the 2008–2009 influenza season, almost all circulating human (H1N1) viruses (seasonal influenza) in the United States were resistant to oseltamivir. However, genetic and phenotypic analyses indicate that the novel influenza A H1N1 2009 virus is susceptible to oseltamivir and zanamivir but resistant to the adamantanes. However, this should be taken in the context of the announcement from WHO of oseltamivir resistance in three patients from Denmark, Japan and Hong Kong on July 8th. In light of this latest report, prudence should be applied in our use of these antivirals. As of July 8th, 2009 the Ministry of Health in Malaysia has

recommended that given the severity of illness observed among some patients with influenza A H1N1 infection, therapy with neuraminidase inhibitors should be prioritised for hospitalised patients (i.e., those with moderate to severe illness) with suspected or confirmed infections.

Mitigating The Influenza A / H1N1 2009 Pandemic

While the initial endeavours from the Malaysian Ministry of Health (the lead agency in our nation's response) was centred around containment, it is also spelt out in the National Influenza Pandemic Preparedness Plan (NIPPP) that as the number of cases increases, mitigation strategies may have to be applied. Containment as defined by many international agencies is merely an attempt to delay the initial spread of the virus in the local population. Its goal is to dampen the sharp rise in cases and prevent a sustained overwhelming demand on the healthcare system. A rapid upsurge of cases in any given community could also negatively impact essential services and the economy.

Mitigation is a collective term recommended by WHO for actions taken in countries in phases 5 and 6 of pandemic alert to reduce the impact of the pandemic (4)

The mitigation measures taken by the Malaysian Ministry of Health are centred around the following objectives:

- reducing transmission
- ensuring healthcare for those who may be infected
- maximizing care for those with disease
- protecting the most vulnerable
- ensuring the continuance of essential services and minimizing the pandemic's impact on the country's socio-economic development

Preventing the introduction of infected visitors through vigorous case-finding, as well as active contact tracing, can be a legitimate initial measure by which countries can delay the introduction of the virus into the local population. However, to be effective, this strategy needs to be comprehensively implemented, and it must target incoming travellers from all areas of sustained community transmission. To sustain these intensive efforts over any prolonged period of time would require huge amounts of resources; both in personnel and finances. Such efforts have to be made in conjunction with surveillance for local transmission, especially of those infections that cannot be linked to another case.

In Malaysia, the first case, which was a Malaysian student returning from the US, was detected on May 15th, 2009. This was followed by a constant flow of imported cases from various countries with contained local transmission until June 16th, 2009 when we reported our first case involving local transmission. This was soon followed by multiple clusters in schools, which all involved cases returning from abroad with the infection. As of July 14th, 2009 the Ministry of Health has confirmed 804 cases, with 555 (69%) imported cases and 249 (31%) involving local transmission. It is expected that the number of cases involving local transmission will continue to rise substantially. It was evident by early July that local transmission was already established in this country, and this triggered our move towards mitigation.

Moving away from a containment strategy toward a mitigation strategy mainly involves giving up public health measures actively targeting incoming travellers from affected areas and not actively pursuing case-finding outside of groups at higher risk of experiencing severe disease. Such scaling down of the delaying strategy could be phased in over a certain period (4). A mitigation strategy would focus on the following strategies:

- Providing the public, including incoming travelers, with relevant information
- Promoting self isolation of symptomatic persons and treatment according to national protocols, with special consideration given to non-nationals visiting the country (enforced quarantine and contact tracing are no longer routine procedures)
- Ensuring early treatment of all those in a country developing illness according to national policies. In Malaysia, H1N1 screening, hospitalization and antiviral therapy is recommended for those with moderate to severe illness
- Improving public hygiene and infection control in health institutions

The next phase of the pandemic will involve the much-awaited influenza A/H1N1 vaccine, which is expected in the last quarter of 2009. How the world will coordinate this enormous undertaking will be a test of its resolve to keep public health principles, ethics and humanitarian values above politics and business. We live in interesting times and there can be little doubt that we still have a lot to learn about this virus and about ourselves from this pandemic.

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REVIEW ARTICLE

Dengue: where are we today?

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Abstract

Dengue is considered the main arthropod-borne viral disease of humans. In the last few years, an increasing number of reports of mild and severe cases have been reported. The growing dengue incidence observed in recent years has been accompanied by reports of new observations, findings and global initiatives with an improvement in our understanding of this phenomenon. The epidemiology and new clinical classification of dengue, advances in the diagnostic and pathogenesis knowledge, and vaccine development as well as control methods including new global initiatives are summarised here.

Keywords: Dengue, review, management, medical sciences

Introduction

Dengue has been re-emerging in the last decades, with an estimated 50–100 million people infected annually. More than 2.5 billion people live in geographic areas where the infection is endemic, and more than 100 countries are at risk of dengue transmission (1–3). The infection is caused by any of the dengue viruses (DENV-1 to 4), an RNA virus classified as a flavivirus of the family Flaviviridae. The virus is transmitted to humans by the bite of *Aedes* mosquitoes. *Aedes aegypti* is the principal vector, although *Aedes albopictus* is also important in some settings (4,5). Clinical manifestations of the illness varies from asymptomatic infection, observed in most of infected individuals, to the mild illness named Dengue Fever (DF) and to the severe form of the disease called dengue haemorrhagic fever with or without dengue shock syndrome (DHF/DSS) (6). The increase in dengue incidence observed in recent years has been accompanied by reports of new observations, findings and global initiatives with an improvement in our understanding of this phenomenon. Here dengue situation is updated

Current dengue epidemiological situation

The vector and virus expansions throughout tropical and subtropical areas around the world have been favoured by global unplanned urbanisation, population growth, international travel, abundance of non biodegradable containers and basically poor living conditions. Today, more than 70% of dengue cases occur in Asia and the Pacific, followed by the Americas, Africa and the Middle East. While both DF and DHF/DSS were widely recognised in Asia and the Pacific in the 1960s and the 1970s, the expansion to the Americas in the 1980s and 1990s and more recently to the Middle East and Africa has been observed (7), with reports of the four viruses in circulation in endemic areas. A recent report suggests that half of the world's population is at risk of dengue infection (1–3).

The expansion of dengue has been accompanied by the report of epidemics in virgin populations such as the Galapagos Islands and the Easter Islands. An increasing number of epidemics involve more than one viral serotype, and greater dengue activity with epidemics is occurring at shorter intervals (from five to two or three years); there have also been numerous reports of dengue illness in travellers (8,9). The American region has seen a dramatic dengue increase in the last 30 years, with an increasing number of DHF cases,

from 60–80 cases before 1981 to more than 38,000 in 2008. In addition, the co-circulation of several serotypes, the report of over 1 million cases in 2008 worldwide and dengue transmission occurring in over 30 countries reflect the seriousness of the problem (6).

In this context, climate change is expected to worsen the dengue epidemiological situation. The temporal and spatial changes in temperature, precipitation and humidity will affect the biology and ecology of the vectors and consequently the risk of virus transmission. It is estimated that, with the expected temperature increase of 2°C, the mean potential of transmission will rise. If water temperatures climb, the mosquito larvae take a shorter time to mature, and consequently there is a greater capacity to produce more offspring during the transmission period. In warmer conditions, mosquitoes digest blood faster and feed more frequently, thereby increasing dengue transmission. In addition, the extrinsic period of the virus within the vector could be shorter and therefore increase the proportion of infected mosquitoes (10–12).

Two important analyses related to dengue are the estimation of the global epidemiological and economic burden of the illness. Although some studies have been performed, these topics remain priorities of research. The global burden has been recently estimated in DALY (disability adjusted life years) to be 264 DALYs per million people per year for the two billion people living worldwide in areas at risk for dengue. Another study estimated a loss of 420 DALYs per million people per year, which is comparable to the burden of meningitis, twice the burden of hepatitis and one-third of the burden of HIV/AIDS (13). However, few studies focus on the cost of dengue. Since there is no uniform methodology applied, cost variation is observed among reports. Cost per case has been estimated to be 109.16 USD (Thailand 1994), 61.00 USD (Thailand 2005), 125.00 USD (Puerto Rico) and 299.00 USD (Cuba 1981) (14,15).

Of interest is the report of possible dengue transmission associated with blood transfusion. Recently, Mohammed et al. found that 1 in 1000 blood donations in Puerto Rico contained DENV RNA. On the other hand, Linnen et al. found variable evidence of dengue viraemia among asymptomatic blood donors (from 0.30% in Honduras to 0.04% in Brazil). These new findings raise concerns regarding transfusion-transmitted DENV (16,17).

Clinical illness

According to the World Health Organization (WHO) classification, the presence of fever, bleeding, thrombocytopenia ($<100,000/\text{mm}^3$) and haemoconcentration (including pleural effusion, ascitis etc) allows the classification of a patient as DHF/DSS (18). The increasing number of patients, variation in clinical manifestation and the extension of the transmission to new areas of the world have been accompanied by difficulties in the application of this WHO classification (19,20). Significant numbers of severe dengue patients do not meet all of the criteria outlined by the WHO.

Not surprisingly, there is a wide request for a classification that is useful for the management of the acute case. Under the leadership of the TDR/WHO and as part of a multi-centre study, the WHO classification was recently reviewed. As a consequence of this study, a new clinical classification was proposed for validation in several countries of the American and Asian regions. Accordingly, cases will be classified as dengue or severe dengue. The presence of warning signs, such as persistent vomiting and intense abdominal pain, in non-severe cases will alert clinicians about a bad prognosis. Severe dengue includes not only the former DHF/DSS cases but also patients with severe plasma leakage, severe bleeding and severe organ impairment. In recent years, an increasing number of unusual manifestations of dengue such as neurological disorders and myocarditis have been reported. The new classification also considers these aspects of dengue (21). In the Bolivian dengue epidemic of 2009, the new proposed classification was applied with very good acceptance by clinicians and epidemiologists (Martinez E. and Castro O., personal communication). Interestingly, in 1981 a similar dengue classification had been successfully applied in Cuba, during the first DHF/DSS epidemic in the American region (22,23). It is expected that once this new classification is validated, a favourable impact on mortality and better case management will be observed.

Diagnosis

Dengue diagnosis is based on the isolation of the virus, the detection of the viral antigen or RNA in serum or tissues or the detection of specific antibodies in the patient's serum. Virus isolation (mainly from mosquito cell lines such as *A. albopictus*), the identification by immunofluorescence assay using specific dengue monoclonal antibodies and the genomic detection by RT-PCR or real-time RT-PCR confirm the

infection (24,25). Serum is the sample of choice for dengue diagnosis, although tissue samples (liver, spleen, lymphatic nodes, etc.) in fatal cases can be employed for virus isolation and genomic (RT-PCR) or antigen (immunohistochemistry) detection (26-28). Serum samples collected in the first five days of fever are useful for virus detection.

IgM detection by ELISA in samples collected after day 5 of illness is routinely used for dengue diagnosis. IgG seroconversion or a fourfold increase in paired serum samples is also among the criteria for dengue diagnosis (29,30). One of the current research priorities is the evaluation of diagnostic assays and commercial kits. Recently, TDR and the Pediatric Dengue Vaccine Initiative (PDVI) identified a network of laboratories in Latin America and Asia to perform diagnostic evaluations. As part of this initiative, nine IgM commercial kits based on ELISA and rapid test formats were evaluated. Good sensitivity (95–99%) and specificity (79.9–86.6%) were demonstrated in three of them (the PanBio, Standard Diagnostic and Focus ELISA tests) (31).

There is still a need for early dengue diagnosis. During viral replication, NS1 non-structural protein is secreted in the blood, appearing as early as day 1 of fever and declining after days 6–7. Considering the characteristics of the protein, several studies focus on NS1 detection as an early marker of dengue infection (32,33). Sensitivity ranges of 60.4–87.4 % and specificity ranges of 97.9–100% have been found using ELISA and rapid tests. The highest percentage of NS1-positive samples has been observed in individuals with a primary dengue infection. The presence of anti-dengue immune complexes could be an explanation of the lower sensitivity observed in samples collected from individuals with a secondary infection (33). As part of the multicentre DENCO project conducted by TDR, NS1 detection was evaluated in serum samples collected from confirmed dengue cases during the acute phase of illness. Preliminary results support the usefulness of this marker for early dengue diagnosis, but, much more importantly, these results suggest the need for a new dengue diagnostic algorithm where NS1 and IgM detection complement each other to achieve higher sensitivity.

The evaluation of available RT-PCR and real time RT-PCR protocols, the development of a single test combining antigen and antibody detection and new diagnostic tools combining high sensitivity and specificity, low cost, simplicity and, ideally, high prognostic capacity for disease severity are still priorities for dengue diagnosis (24,34).

Pathogenesis

Two exclusive hypotheses, the secondary infection by a different dengue serotype and the viral virulence, were proposed early on to explain DHF/DSS (35,36). Observations in the last 50 years support an integrated view of the problem, since the secondary infection is needed for severity (37,38). Age (a higher risk is observed in children), chronic diseases such as bronchial asthma, diabetes mellitus and sickle cell anaemia, ethnicity (a higher risk is observed in whites compared to blacks) and genetic factors (26,38–42) have been reported as the main host risk factors for DHF/DSS. In this context, the virus serotype, the sequence of infecting viruses and the virus genotype are also of importance. Genotypes of DENV-2 and 3 from Asia have been associated with DHF epidemics (43–46). The report of quasi-species and recombinant viruses adds more complexity to the problem and demonstrates the genetic diversity of dengue viruses (47–49).

The increase in vascular permeability (clinically expressed as haemoconcentration, pleural effusion, ascites, and cardiovascular hypotension after fever defervescence) characterises the severe syndrome. Molecular mechanisms involved in this syndrome are not well understood. Severe dengue has been associated with a second heterotypic dengue infection even after a long interval after primary infection (50,51). Although several sero-epidemiological studies support this observation (52), probably the “unique” epidemiological Cuban dengue situation best exemplifies the important role of the secondary infection as a risk factor for severity. In three dengue epidemics (DENV-2 in 1981 and 1997, and DENV-3 in 2001), severe cases occurred in individuals previously infected by DENV-1 in the 1977 epidemic who then had a second infection (53–56). Children suffering their primary infection during the 1997 and 2001 epidemics developed only a mild disease.

The phenomenon of antibody-dependent enhancement (ADE), whereby dengue antibodies at sub-neutralising concentrations enhance DENV infections in Fc receptor-bearing cells, was first proposed to offer a unifying basis to explain clinical, serological and epidemiological observations (36). After an initial period of cross-protection, cross-reactive antibodies waned to non-neutralising levels. These non-neutralising antibodies could mediate an increased uptake of viral particles through virus-antibody complexes, leading to increased viral replication and immune activation accompanied by cytokine release (57). Cytokines may play a direct role on the immunopathogenesis

of dengue. Their proinflammatory effects on vascular endothelial cells could lead to leaky junction and, consequently, to the increase of vascular permeability. Of interest is the association of higher viraemia to severe disease supported by several clinical studies (58–61). A complementary hypothesis explaining DHF/DSS involves the reactivation of cross-reactive memory T cells specific for the previous infecting virus resulting in a delayed viral clearance and an increase of cytokine production (62,63). A “tsunami of cytokines and chemical mediators” released from T cells, monocytes and endothelial cells has been associated with severe illness, with high levels of IL-10, TNF- α , IL-8, IL-12, IFN- γ and other cytokines found in the sera of patients (57,64–67).

Activation of complement is also involved in DHF pathogenesis since high levels of circulating C3a and C5a are observed in the plasma of severe patients. Although the mechanism of complement activation is not well known, it is assumed that complement is activated by the circulating immune complexes reported in patients. High levels of secreted NS1 and pre-existing cross reactive antibodies may mediate complement activation. Furthermore, infected monocytes and endothelial cells could activate complement via classical and alternative pathways (57,68,69).

Although the high cytokine production as a consequence of ADE and T cell activation could explain the vascular endothelial leakage and the increased capillary permeability observed during DHF/DSS, severe disease reported in infants with dengue-immune mothers cannot be explained by T cell involvement when infants suffer their primary dengue infection (70,71). Relatively recently, an autoimmune mechanism has been proposed. Some studies suggest that anti-NS1 antibodies cross react with platelets and endothelial cells, resulting in endothelial dysfunction and cytokine and complement activation (72,73).

Thrombocytopenia and bleeding also accompany the severe illness; however, the mechanisms involved are not well defined. Early bone-marrow suppression with peripheral platelet destruction has been postulated to explain the former (50). Recent studies support the key role of innate immunity in determining disease outcome. High levels of IFN- α and IFN- γ in response to DENV infection have been suggested to be associated with a protective host response. In addition, high levels of NK cells and activated NK have been related to mild illness (68,74).

A better understanding of dengue pathogenesis is needed for implications in drug and vaccine development. In particular, research on the

innate and adaptive immune response in vivo and the molecular mechanisms associated with plasma leakage and bleeding (67,75) is important.

Vaccine development

The development of a safe and effective dengue vaccine is one of the public health priorities defined by the WHO (76). The development of a dengue vaccine involves several complexities such as the need to develop a vaccine against all four viruses, to avoid the ADE phenomenon, the poor understanding of the protective dengue immunity and disease pathogenesis and the lack of an animal model for vaccine evaluation. However, significant advancements have been observed in the last ten years. Currently, several vaccine candidates are in phase I and II clinical studies, and others are in advanced preclinical phase.

The main applied strategies include the traditionally and molecularly attenuated vaccines, chimeric live virus vaccines and DNA and recombinant subunit vaccines (77). The main concerns for live vaccines include the potential risk of enhanced illness after vaccination (if an adequate immune response to the four viruses is not simultaneously achieved) and the enhanced vaccine reactogenicity in persons with pre-existing anti-flavivirus antibody. On the other hand, subunit vaccines will probably require booster immunisations to maintain high levels of immunity.

The dengue vaccine pipeline appears to be sufficiently advanced (76). In preparation are population-based efficacy trials in exposed populations both in Asia and the Americas as the vaccine should be evaluated under different patterns of dengue transmission and circulating dengue viruses. Vaccine developers and the PDVI are working together to establish field sites for vaccine evaluation. Although protective immunity against dengue viruses is not completely defined, it is accepted that neutralising antibodies play an important role in protection against the viral infection. However, the role of the cellular immune response in the protection and recovery is not well known. Limited information is available to correlate immune response with disease outcome, so the definition of the correlates of protection is still a priority of research (57,77,78). As no animal model is available, it is urgent to define correlates of protection to allow the establishment of the efficacy of a vaccine candidate.

Control

Vector control is the only available method to control epidemics and prevent transmission, but a range of control strategies is needed to face the varying situations. However, until now, sustainability is the main problem. It is recommended that the application of integrated vector control strategies, including tools for reducing larvae and adult mosquito, be complemented with strong community and intersectoral participation.

At the end of the 1990's, the WHO established the Global Strategy for Dengue Prevention and Control (79), comprising five aspects: integrated vector control based on the community and intersectoral participation, active dengue surveillance, emergency preparedness, capacity building and vector control research. New tools for vector control include integrated vector management, the eco-health approach for dengue control and prevention (to improve community health) and the integrated management strategy for dengue prevention and control, *Estrategia De Gestion Integrada*, (EGI)/Dengue, with the objective to achieve a sustainable national strategy allowing a functional integration of actions. Additional control strategies include the Communication of Behavioral Impact (COMBI), the application of geographic information systems (GIS) to epidemiological and entomological studies, and others. More research on the development and evaluation of vector control tools and strategies and surveillance and response is needed (80,81).

Conclusions

Today, dengue is considered the most rapidly expanding arboviral disease in the tropics and subtropics and is now a serious public health concern. The re-emergence of Yellow fever, West Nile Fever and Chikungunya worsen the epidemiological situation (82). The last decade are marked by major advances and the implementation of several international initiatives (PDVI, The Innovative Vector Control Consortium, Asia-Pacific Dengue Partnership, DENFRAME and Denco projects, others); however, more research is needed to improve the dengue situation (9). Recognising the severity of this situation, the global dengue research agenda, discussed by the dengue expert group convened by the TDR/WHO at the end of 2006, provides a strategic plan for reducing dengue morbidity and mortality and its negative socioeconomic impact (Scientific Working Group. World Health Organization. Report on dengue

1-5 October 2006, Geneva, Switzerland. TDR/SWG/o8) (21). It is expected that the integrated actions among countries based on the application of more advanced knowledge will positively impact dengue control and prevention.

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ORIGINAL ARTICLE

Influence of CYP2D6 polymorphisms on symptomatology and side-effects of patients with schizophrenia in Malaysia

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Abstract

Background: Our objective was to investigate the association of CYP2D6 polymorphisms with symptoms and side-effects of patients with schizophrenia.

Methods: The subjects were 156 patients with schizophrenia undergoing antipsychotic treatment at a psychiatric clinic. Patients with co-morbid diagnoses of substance abuse or mental retardation were excluded from the study. Psychopathology was evaluated using the Positive and Negative Symptoms Scale (PANSS). Extrapyramidal side-effects and akathisia were assessed with the Simpson Angus Scale (SAS) and the Barnes Akathisia Rating Scale (BARS), respectively. DNA was extracted from blood and subjected to PCR-genotyping.

Results: We found that CYP2D6 polymorphisms were significantly associated with a subtotal negative PANSS score. In addition, CYP2D6 is not related to side-effects of antipsychotic therapy, or SAS and BARS scores. The results suggest that CYP2D6 polymorphisms may have implications in treatment response.

Conclusions: Therefore, CYP2D6 may be a predictor for treatment outcomes of patients with schizophrenia. However, further investigation is required to confirm these findings in a larger sample.

Keywords: Cytochrome P450 CYP2D6, schizophrenia, treatment outcomes, neurosciences

Introduction

Schizophrenia is a highly heritable condition, as demonstrated in family, twin and adoption studies (1). Such studies have also shown that environmental factors combined with genetic predisposition contribute to the development of schizophrenia (1,2). Despite extensive research, no mutations or disease-predisposing DNA sequence variations have been identified. The mode of inheritance of schizophrenia is likely to be polygenic or multifactorial (1,2).

Pharmacological treatment of schizophrenia involves antipsychotic therapy. The variation in individual clinical responses to antipsychotic

therapy remains a critical problem in the management of patients with schizophrenia despite considerable progress in delineating different domains of this illness. These range from positive and negative symptoms to cognitive dysfunction and psychosocial vulnerabilities. Although a minority of patients experience complete symptom remission, a large proportion of patients continue to experience significant psychiatric symptoms (3,4). Moreover, there is a subset of patients who develop drug-induced side-effects such as extrapyramidal side-effects (EPS) including acute dystonic reactions, neuroleptic-induced parkinsonism and akathisia, later-onset movement disorders such as tardive dyskinesia or dystonia and life threatening

Table 1: Demographic and clinical characteristics of 156 patients with schizophrenia

Variable	n	(%)
Positive family history ^a		
No	113	(72.4)
Yes	43	(27.6)
Family support		
Good	149	(95.5)
Poor	7	(4.5)
Education level		
Primary	8	(5.1)
Secondary	123	(78.8)
Tertiary	24	(15.4)
Others	1	(0.6)
	Mean	(SD)
Age (year)	34.1	(10.01)
	Median	(IQR)
Age at first onset (year) ^b	23.2	(10.15)
Duration of illness (year)	8.8	(8.98)
Number of admission	1.0	(3.00)

^aA positive family history was defined as the presence of at least one first- or second-degree relative with schizophrenia.

^bThe age at first onset was defined as the age at which the subject presented schizophrenic symptoms noticeable to family members, or the age when the illness started as noted in medical records. The definition was chosen because the definition was equal for all patients by evaluating the medical records.

side-effects such as agranulocytosis and neuroleptic malignant syndrome, which require significant medical intervention (5). Genetic factors may be considered as one of the causes of this phenomenon. Psychopharmacogenetic studies have focused on three major phenotypes that are the clinical efficacy of antipsychotic drugs, the efficacy of antidepressant medications and the development of side-effects associated with treatment (6).

Debrisoquine 4-hydroxylase (*CYP2D6*) is a polymorphic enzyme involved in the metabolism of many centrally acting drugs. A study by Seigle et al. (7) clearly demonstrated that *CYP2D6* mRNA and protein are expressed within different regions of normal human brains. Although the total amount of *CYP2D6* in the brain is rather low, they identified specific cell types in certain areas of the brain expressing significant *CYP2D6* levels, indicating a mechanism of local drug metabolism.

The gene encoding *CYP2D6* is highly polymorphic. The *CYP2D6* allele nomenclature is available at <http://www.cypalleles.ki.se/cyp2d6.htm>. The *CYP2D6* allele subgroups are associated with absent, decreased, normal or increased

enzyme activity (8). Currently, more than 50 mutations and 90 alleles for *CYP2D6* have been discovered (9,10). Many different polymorphisms that impact *CYP2D6* activity have been reported in all parts of the world (11). These mutations include genetic alterations that lead to over expression (gene duplication), absence of an active protein product (null allele or non-functional alleles) or production of a mutant protein with diminished catalytic capacity (inactivating allele).

Patients who express dysfunctional or inactive enzyme molecules are considered PM. For example, patients with two null alleles are considered as a poor metabolisers (PM) (12,13,14,15). There are also alleles (inactivating alleles) that lead to the production of an enzyme with diminished or reduced catalytic capacity, but these changes do not lead to PM status. Patients with one non-functional allele (null allele) and those carrying two alleles that code for an enzyme with reduced activity are considered intermediate metabolisers (IMs). Patients with two functional alleles (wild-type allele, *CYP2D6**1) and those carrying one allele coding for an enzyme with reduced activity are considered extensive metabolisers (EMs) (15,16). As a general rule, the number of functional *CYP2D6* genes present in an individual dictates the drug metabolism phenotype (13,16,17). In addition, the ultrarapid metaboliser (UM) phenotype results from a gene duplication that results in multiple functional copies of a single *CYP2D6* gene. When elimination of a drug is highly dependent on *CYP2D6*, lower clearance is seen in PMs compared to EMs. At the same dosage, PMs achieve higher steady state plasma drug levels than EMs due to their reduced metabolic capacity and are therefore more prone to develop side-effects (18,19). On the other hand, UMs are susceptible to treatment-refractoriness to antipsychotics. In the present study, our objective was to investigate the association of *CYP2D6* polymorphisms with symptomatology and side-effects of patients with schizophrenia.

Materials and Methods

Patient enrolment and assessment

This was a cross-sectional study. One hundred and fifty-six patients with schizophrenia according to the DSM-IV criteria attending the Psychiatric Clinic, Hospital Universiti Sains Malaysia undergoing antipsychotic treatment or treated with antipsychotics in the past were recruited for the study. Patients with co-morbid diagnoses such as substance abuse or mental retardation were excluded from the study. Written informed consent was obtained after explaining a complete

Table 2: Association of items of PANSS scale and *CYP2D6* polymorphisms

Items of PANSS Scale	<i>CYP2D6</i> Polymorphisms		
	Allele	Genotype	Phenotype
	<i>P</i> value ^a	<i>P</i> value ^a	<i>P</i> value ^a
Positive symptom			
1. Delusions	0.330	0.815	0.868
2. Conceptual disorganization	0.966	0.854	0.796
3. Hallucinatory behaviour	0.576	0.747	0.536
4. Excitement	0.004	0.017	0.005
5. Grandiosity	0.326	0.076	0.005
6. Suspiciousness	0.547	0.299	0.906
7. Hostility	0.071	0.238	0.090
Negative symptom			
1. Blunted effect	0.021	0.035	0.014
2. Emotional withdrawal	< 0.001	0.018	0.001
3. Poor rapport	< 0.001	0.003	< 0.001
4. Passive/apathetic social withdrawal	< 0.001	0.018	0.001
5. Difficulty in abstract thinking	0.987	0.919	0.771
6. Lack of spontaneity and flow of conversation	0.446	< 0.001	0.011
7. Stereotyped thinking	ND	ND	ND
General symptom			
1. Somatic concern	0.406	0.348	0.091
2. Anxiety	0.062	0.276	0.211
3. Guilt feeling	0.210	0.489	0.498
4. Tension	0.164	0.362	0.392
5. Mannerism and posturing	ND	ND	ND
6. Depression	0.263	0.456	0.055
7. Motor retardation	0.586	0.011	0.170
8. Uncooperativeness	< 0.001	< 0.001	< 0.001
9. Unusual thought content	ND	ND	ND
10. Disorientation	ND	ND	ND
11. Poor attention	0.081	0.181	0.172
12. Lack of judgement and insight	0.614	0.033	0.006
13. Disturbance of volition	0.586	0.023	0.003
14. Poor impulse control	0.003	< 0.001	< 0.001
15. Preoccupation	< 0.001	< 0.001	< 0.001
16. Active social avoidance	0.004	0.034	0.006

^aAnalysis of variance (ANOVA). ND indicates that no statistics are computed because the values are constant.

description of the study. The patients were then called for interviews to obtain detailed histories of present illness, past treatments, side-effects during the past two years, current medications, socio-demographic variables and family histories. Psychopathology was rated using the Positive and Negative Syndrome Scale (PANSS). EPS were assessed with the Simpson Angus Scale (SAS) and akathisia with the Barnes Akathisia Rating Scale (BARS). The accuracy of the information given by patients was counter-checked against their medical records. The protocols for the study were approved by the Research and Ethical Committee at Universiti Sains Malaysia.

Genotyping Methods

Genomic DNA was obtained from peripheral leukocytes extracted from ten millilitres of blood withdrawn from the patients using previously described methods (20). Samples were screened for *CYP2D6**3, *4, *5, *6, *9, *10, *14, *17 and gene duplication. PCR was performed on a Perkin Elmer GeneAmp PCR System 9700® (Applied Biosystems, Foster City, CA, USA) according to previously described procedures with slight modifications (21).

The prediction of a patient's *CYP2D6* phenotype was based on their genotype. Patients with two non-functional alleles were classified PMs. Patients with one non-functional allele and

Table 3: Association of *CYP2D6* alleles and PANSS scores

	Subtotal Positive ^a		Subtotal Negative ^a		Subtotal General ^a		Total PANSS ^a	
<i>CYP2D6</i> *1	9.7	(3.52)	8.9	(3.86)	20.2	(4.46)	38.7	(10.11)
<i>CYP2D6</i> *4	9.8	(2.75)	7.3	(0.50)	22.3	(5.32)	39.3	(8.42)
<i>CYP2D6</i> *5	10.9	(2.78)	9.2	(3.74)	22.5	(6.26)	42.6	(11.13)
<i>CYP2D6</i> *10	9.4	(2.63)	8.8	(3.77)	20.6	(4.27)	38.9	(8.96)
Duplication	11.2	(5.01)	14.1	(7.67)	24.5	(8.76)	49.8	(19.31)
<i>F</i> statistic (<i>df</i>)	1.29	(4, 289)	4.44	(4, 289)	2.67	(4, 289)	3.22	(4, 289)
<i>P</i> value ^b	0.276		0.002		0.033		0.013	
NA	8.1	(2.19)	7.2	(0.65)	18.8	(2.90)	34.1	(4.86)
Total	9.6	(3.12)	8.9	(3.97)	20.5	(4.65)	39.1	(10.02)

^aMean (SD), ^bAnalysis of variance (ANOVA). NA represents samples that were amplifiable during first PCR, but genotypes were not determined during the second PCR. Samples were screened for *CYP2D6**3, *4, *5, *6, *9, *10, *14, *17 and duplication gene.

those carrying two alleles coding for an enzyme with reduced activity were classified as IM. Patients with two functional alleles and those carrying one allele coding for enzyme with reduced activity were classified as EMs. A patient was classified as a UM if duplication of a functional gene was detected.

Statistical analysis

Comparisons of clinical features between genotypic groups were evaluated using an Independent t-test, analysis of variance (ANOVA), or non-parametric tests (Mann-Whitney U-test and Kruskal-Wallis test), Chi-square test or Fisher's Exact test when appropriate.

PANSS total and subscales scores were used to measure the current psychopathology. We tested if the potential factors which affect drug response factors such as gender, positive family history, educational level, age, age of onset, duration of illness and number of admission influenced the treatment response using either Independent t-test, ANOVA or simple linear regression analyses. The relationship of different genotypes, predicted phenotypes and alleles with the severity of symptoms of schizophrenia was analyzed using ANOVA. The associations of the distribution of the *CYP2D6* polymorphisms between patients who experienced side-effects with antipsychotics treatment and patients without side-effects in the past two years were studied using the Chi-square test. Data analyses were done after all genotyping of the patients was completed. The statistical analysis was carried out using SPSS/Win software (Version 11.0, SPSS, Inc., Chicago, IL). The limit of significance was set to 0.05.

Results

One hundred and fifty-six patients with schizophrenia (female, $n = 76$, 48.7%) were recruited. Their age ranged from 18 to 62 years. One hundred and fifty-three were Malays (98.1%) and the other three were Chinese (1.9%). Table 1 shows the demographic and clinical characteristics of the patients.

Patients were currently on typical antipsychotics such as haloperidol (10.5%), chlorpromazine (12.5%), trifluoperazine (5.0%), perphenazine (2.5%), fluphenazine (8.0%), flupentixol (14.0%), sulpiride (4.0%) and zuclopenthixol (0.5%). Of the atypical antipsychotics, risperidone (26.5%), olanzapine (9.0%), clozapine (6.5%) and quetiapine (1.0%) were prescribed to the patients. About 47.4% of patients treated with antipsychotics were given anticholinergic drugs such as benhexol to counteract extrapyramidal side-effects (EPS). This was standard practice among some psychiatrists at the clinic to prescribe anti-parkinson drugs (anticholinergic drugs) routinely if their patients were treated with haloperidol.

The results showed that *CYP2D6* polymorphisms have no association with clinical features including positive family history, age, age at first onset, duration of illness, number of admission, educational level and current medication profiles. The associations between *CYP2D6* polymorphisms and PANSS items are shown in Table 2. PANSS total and subscales scores were used to measure the current psychopathology. Possible stratification effects, such as gender, positive family history, educational level, age, age of onset, duration of illness and number of admission were considered.

Table 4: Association of *CYP2D6* genotypes and predicted phenotypes and PANSS scores

Variable	Subtotal Positive ^a		Subtotal Negative ^a		Subtotal General ^a		Total PANSS ^a	
Genotype								
*1/*1XN	11.8	(5.85)	12.5	(6.40)	24.0	(10.17)	48.3	(22.07)
*1/*10XN	9.0	(2.83)	20.5	(13.44)	26.5	(7.78)	56.0	(18.39)
*1/*1	9.8	(3.94)	8.6	(3.57)	19.8	(4.48)	38.1	(10.32)
*1/*10	9.7	(2.97)	9.3	(4.39)	20.8	(4.56)	39.7	(10.18)
*1/*5	10.0	(2.83)	8.5	(2.12)	19.5	(3.54)	38.0	(8.49)
*10/*10	9.2	(2.49)	8.3	(2.49)	20.3	(3.89)	37.8	(7.53)
*4/*10	9.8	(2.75)	7.3	(0.50)	22.3	(5.32)	39.3	(8.42)
*5/*10	10.1	(2.30)	9.4	(4.60)	20.9	(5.33)	40.4	(11.02)
*5/*5	15.0	(0.00)	9.0	(0.00)	32.0	(0.00)	56.0	(0.00)
<i>F</i> statistic (<i>df</i>)	0.72	(8, 138)	2.97	(8, 138)	1.68	(8,	1.64	(8, 138)
<i>P</i> value ^b	0.678		0.004		0.107	138)	0.119	
Predicted phenotype								
UM	10.8	(4.92)	15.2	(8.82)	24.8	(8.70)	50.8	(19.39)
EM	9.7	(3.37)	9.0	(4.01)	20.3	(4.49)	39.0	(10.13)
IM	9.4	(2.46)	8.4	(2.80)	20.5	(4.16)	38.3	(8.05)
PM	15.0	(0.00)	9.0	(0.00)	32.0	(0.00)	56.0	(0.00)
<i>F</i> statistic (<i>df</i>)	1.40	(3, 143)	5.45	(3, 143)	3.88	(3,	3.86	(3, 143)
<i>P</i> value ^b	0.246		0.001		0.011	143)	0.011	
NA	8.4	(2.51)	7.3	(0.76)	18.9	(3.13)	34.6	(5.50)
Total	9.6	(3.12)	9.0	(4.05)	20.6	(4.70)	39.3	(10.16)

^aMean (SD), ^bAnalysis of variance (ANOVA). NA represents samples that were amplifiable during first PCR but genotypes were not determined during the second PCR. Individuals carrying two null alleles (*CYP2D6**3, *4, *5, *6 and *14) are PMs, while those carrying two alleles conferring decreased enzyme activity (*CYP2D6**9, *10 and *17), or one null allele and one decreased allele are IMs and those with one or two functional alleles (*CYP2D6**1) are EMs. Subjects with duplicated gene are classified as UMs.

However, the result did not reveal any significant effects on PANSS scores.

Patients with *CYP2D6* gene duplications had a tendency to have higher mean values of PANSS scores than patients with other *CYP2D6* alleles (Table 3).

Table 4 shows the PANSS scores of patients according to their genotypes and predicted phenotypes for *CYP2D6* polymorphisms. ANOVA revealed that mean subtotal negative scores were significantly different among *CYP2D6* genotypes. However, no significant differences were found between genotypes in relation to other PANSS scores such as subtotal positive, subtotal general scores and total PANSS scores. In terms of *CYP2D6* predicted phenotype, it was associated with subtotal negative, subtotal general scores and total PANSS scores. However, the *CYP2D6* predicted phenotype was not associated with subtotal positive scores.

We could not analyse the SAS and the BARS because the majority of patients (94.2%) had zero scores for the both scales. Only nine patients were found to have experienced side-effects during the interview. Side-effects were effectively treated since

the beginning of therapy, and thus, the assessment of the antipsychotics side-effects was invalid.

There were no significant differences in terms of side-effects of antipsychotics between the genotypic subgroups and alleles of *CYP2D6* polymorphisms. From the treatment history, we found that one third of the patients experienced side-effects during the past two years. We compared side-effects during past two years between different genotype and predicted phenotype subgroups. However, there were no significant differences between the groups (Table 5).

Among those who experience side-effects during past two years, the most common genotype was *CYP2D6**1/*10 followed by *CYP2D6**10/*10, which is the same as the overall patients (Table 5).

Discussion

Pharmacogenetics data are largely unavailable in Malaysia, especially for psychiatric patient populations. To the best of our knowledge, this is the first pharmacogenetic study conducted with patients with schizophrenia in Malaysia.

Table 5: Association of *CYP2D6* genotypes and predicted phenotypes and experience of side-effects during past two years

Variables	<i>n</i>	Experience of Side-effects				<i>X</i> ² (<i>df</i>)	<i>P</i> value
		No		Yes			
		<i>n</i>	(%)	<i>n</i>	(%)		
Genotype							
*1/*1 <i>XN</i>	4	2	(50.0)	2	(50.0)	10.42 (8)	0.237 ^a
*1/*10 <i>XN</i>	2	2	(100.0)	0	(0.0)		
*1/*1	36	27	(75.0)	9	(25.0)		
*1/*10	48	28	(58.3)	20	(41.7)		
*1/*5	2	0	(0.0)	2	(100.0)		
*10/*10	42	31	(73.8)	11	(26.2)		
*4/*10	4	2	(50.0)	2	(50.0)		
*5/*10	8	6	(75.0)	2	(25.0)		
*5/*5	1	1	(100.0)	0	(0.0)		
Predicted phenotype							
UM	6	4	(66.7)	2	(33.3)	1.52 (3)	0.678 ^a
EM	86	55	(64.0)	31	(36.0)		
IM	54	39	(72.2)	15	(27.8)		
PM	1	1	(100.0)	0	(0.0)		
NA	9	7	(77.8)	2	(22.2)		
Total	156	106	(67.9)	50	(32.1)		

^aChi-square test. NA represents samples that were amplifiable during first PCR but genotypes were not determined during the second PCR.

The *CYP2D6* allelic frequencies showed a unique distribution in Malaysian patients with schizophrenia. Compared with Malaysian healthy volunteers (21), patients reported slightly higher duplication alleles (3.2%, 95% CI 1.3–5.2% versus 0.9%, 95% CI 0.0–2.2%), but a lower frequency of *CYP2D6**4 (1.3%, 95% CI 0.0–2.5% versus 2.8%, 95% CI 0.6–5.0%) and *CYP2D6**9 (0.0% versus 3.3%, 95% CI 0.9–5.7%) polymorphisms. The most commonly occurring *CYP2D6* genotype among the patients was *CYP2D6**1/*10 (30.8%), followed by *CYP2D6**10/*10 (26.9%), which are similar to findings among healthy Malay volunteers (21). The genotypes that predicted an EM phenotype was found (55.1%, 95% CI 47.3–62.9%) to be similar to those found among healthy Malay volunteers, 62.0% (95% CI 52.5–70.9%) (21). The frequency of predicted PM phenotype was 0.6% (95% CI 0.0–1.9%) which is at the lower end of the range reported for healthy volunteers at 1.8% (95% CI 0.0–4.4%) (21).

An important finding of this study is that *CYP2D6* polymorphisms are significantly associated with a subtotal negative PANSS score. Patients who were UMs had more pronounced or severe negative symptoms of schizophrenia. This

observation is in contrast with other reports that found no association between polymorphic *CYP2D6* alleles and schizophrenia or with its symptoms (19,22,23). Hamelin et al. (22) investigated whether a relationship exists between *CYP2D6* *1, *3, *4, *5, *6 and *7 polymorphisms and schizophrenia. They found no differences among different genotypes in disease symptom severity, number and severity of adverse drug effects, or attitudes toward drug treatment at baseline and at the end of the study. They concluded that common *CYP2D6* alleles were not associated with schizophrenia or with disease symptoms, antipsychotic-related adverse effects, or attitudes toward treatment. Jaanson et al. (19) studied 52 patients with schizophrenia and schizoaffective disorder who received zuclopenthixol decanoate monotherapy for eight weeks. They found that the duration of illness and BPRS score did not differ significantly between *CYP2D6* genotypes (*CYP2D6**3/*4) (PMs), *CYP2D6**1/*4 (heterozygous EMs) and *CYP2D6**1/*1 (homozygous EMs). A recent study in Sweden found no correlation between the number of active *CYP2D6* alleles and PANSS, and Extrapyramidal Symptoms Rating Scale (ESRS) scores in 26 outpatients with schizophrenia

receiving depot haloperidol monotherapy. All patients were genotyped for *CYP2D6**3, *4, *5 and for gene duplications (23).

Unfortunately, published data originating from clinical studies regarding the impact of *CYP2D6* isoenzyme activity on the therapeutic effects of antipsychotics in schizophrenia are scarce, especially during long-term treatment (6,22). The results of our study are in agreement with a study conducted by Plesničar et al. (24). For example, we found significant differences in emotional withdrawal ($P = 0.001$), poor rapport ($P < 0.001$), passive social withdrawal ($P = 0.001$), lack of spontaneity and flow of conversation ($P = 0.011$) and uncooperativeness ($P < 0.001$) between *CYP2D6* phenotypes. These symptoms were also found to be significantly different between different *CYP2D6* genotypes.

It remains unclear whether some of the persistent negative symptoms are influenced by *CYP2D6* polymorphisms or reflect other antipsychotic-induced side-effects in patients receiving long-term antipsychotic treatment. However, we cannot speculate whether this was a phenomenological variation in the symptomatology of schizophrenia. Multiple genes, rather than just one, may play a role in complex phenotypes, including the clinical response to antipsychotics.

The present study found that *CYP2D6* alleles, genotypes and predicted phenotypes are not related to the incidence of side-effects from antipsychotic therapy and severity of SAS and BARS scores in our patients. We found that most patients were well controlled with regard to side-effects at the time of assessment. The results are in accordance with Hamelin et al. (22) who found no relationship between *CYP2D6* *1, *3, *4, *5, *6 and *7 genotypes and the number and severity of adverse drug effects. Panagiotidis et al. (23) also found no correlation between the number of *CYP2D6* alleles and side-effects. In addition, Plesničar et al. (24) also reported no statistically significant differences between PMs and patients having at least one functional *CYP2D6* allele (UMs/EMs/IMs) in relation to Abnormal Involuntary Movement Scale AIMS, SAS or BARS. In the Japanese population, Ohmori et al. (25) found no association between tardive dyskinesia TD and *CYP2D6**2. In a recent study, Tiwari et al. (26) also showed no significant association between *CYP2D6**4 with TD ($P = 0.935$) in North Indian patients with chronic schizophrenia.

There was no over-representation of patients with genetically impaired drug metabolic capacities among patients experiencing side-effects during the past two years. In particular, there was no

over-representation of PMs among patients with side-effects, which is in agreement with the results of previous studies (27,28,29) in which no over-representation of mutated *CYP2D6* alleles was reported in patients with side-effects during antipsychotic treatment.

The small number of subjects with null alleles may have influenced the apparent lack of an effect of *CYP2D6* genotypes on clinical outcomes. Individuals with these genotypes have been postulated to be more prone for side-effects during antipsychotics treatment. Prospective studies with a larger number of patients with null alleles and patients with multiple alleles are desirable, but the low frequency of these genotypes is an obstacle.

Some limitations must be pointed out in our study. First, our study has a selection bias caused by the exclusion of inpatients and the recruiting of outpatients who had given informed consent. This bias could not be eliminated because of ethical and social considerations. Second, we could not control variables such as type and dosage of antipsychotics that could affect treatment outcomes. In the future, it may be worthwhile to examine a subject group consisting entirely of first-episode patients and treated with the same type and dosage of antipsychotics. Lastly, we did not investigate the severity of symptoms of schizophrenia and side-effects prospectively, but evaluated at a single time-point only (cross-sectional assessment). A one-point assessment is not a proper assessment; it should include base line assessments to gauge the individual treatment response.

We conclude that *CYP2D6* activity may impact the treatment response and severity of schizophrenia. However, we would like to stress that our results are still preliminary because we do not understand the relationship between *CYP2D6* polymorphisms and psychopathology of the illness. Further work is required to confirm this.

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ORIGINAL ARTICLE

Molecular characterisation of Haemoglobin Constant Spring and Haemoglobin Quong Sze with a Combine-Amplification Refractory Mutation System

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Abstract

Background: The interaction of the non-deletional α^+ -thalassaemia mutations Haemoglobin Constant Spring and Haemoglobin Quong Sze with the Southeast Asian double α -globin gene deletion results in non-deletional Haemoglobin H disease. Accurate detection of non-deletional Haemoglobin H disease, which is associated with severe phenotypes, is necessary as these mutations have been confirmed in the Malaysian population.

Methods: DNA from two families with Haemoglobin H disease was extracted from EDTA-anticoagulated whole blood and subjected to molecular analysis for α -thalassaemia. A duplex polymerase chain reaction was used to detect the Southeast Asian α -globin gene deletion. Polymerase chain reaction-restriction fragment length polymorphism analysis was then carried out to determine the presence of Haemoglobin Constant Spring and Haemoglobin Quong Sze. A combine- amplification refractory mutation system protocol was optimised and implemented for the rapid and specific molecular characterisation of Haemoglobin Constant Spring and Haemoglobin Quong Sze in a single polymerase chain reaction.

Results and Conclusions: The combine- amplification refractory mutation system for Haemoglobin Constant Spring and Haemoglobin Quong Sze, together with the duplex polymerase chain reaction, provides accurate pre- and postnatal diagnosis of non-deletional Haemoglobin H disease and allows detailed genotype analyses using minimal quantities of DNA.

Keywords: Combine-ARMS, Hb Constant Spring, Hb Quong Sze, medical sciences

Introduction

Thalassaemia is a public health problem in Malaysia, with about 4.5% of Chinese and 2.5% of Malays being carriers of α^0 -thalassaemia (Southeast Asian (SEA) α -globin gene deletion, $--^{SEA}/\alpha\alpha$) (1). The loss of two α -globin genes in *cis* ($--/\alpha\alpha$) results in mild anaemia with microcytosis and hypochromic red blood cells. The loss of three α -globin genes causes deletional Haemoglobin H (HbH) disease ($--/\alpha$), whose presentation ranges from moderate anaemia to thalassaemia intermedia. Inheritance of α^0 -thalassaemia with an α -globin structural variant results in non-deletional HbH disease ($--/\alpha^T\alpha$), a disorder with a more severe phenotype than deletional HbH

disease. Patients with non-deletional HbH disease are more likely to have splenomegaly and need blood transfusions (2,3).

Non-deletional α^+ -thalassaemia mutations give rise to α -globin structural variants (e.g., Hb Constant Spring) in addition to variants with structurally normal α -globin chains but that are expressed at a decreased level. More than 30 α -globin structural variants have been listed in the human globin gene mutation database (<http://globin.cse.psu.edu>). Haemoglobin Constant Spring (HbCS) involves a TAA $\rightarrow$ CAA base pair substitution in the termination codon of the $\alpha 2$ -globin gene (HBA2 c.427T>C). The end product is an elongated α -globin chain with additional 31 amino acid residues (4). It was first observed in Constant Spring, Jamaica, in a Chinese family

with haemoglobin H disease (5). HbCS is the most common α -globin structural variant in Malaysia and in other Southeast Asian countries (6,7). In Malaysia, HbCS has been reported in Malay, Chinese and Indian populations at frequencies of 2.24%, 0.66% and 0.16% respectively (8,9). HbCS has also been observed among Aborigines ("Orang Asli" population) in East and West Malaysia (10). Haemoglobin Quong Sze (HbQS) is another non-deletional α -globin gene defect. It results from a gene mutation in the $\alpha 2$ -globin gene whereby the amino acid leucine is substituted by proline (CTG→CCG, codon 125) (11,12). HbQS (HBA2 c.377T>C) is a rare and highly unstable haemoglobin variant reported in the Chinese population and in Thailand (13,14).

Both deletional ($--/\alpha$) and non-deletional ($--/\alpha^T\alpha$) HbH disease have been observed in the different ethnic groups in Malaysia, particularly among Malays and Chinese (15). However, non-deletional HbH disease caused by HbQS has not been previously reported, as the typical α -globin structural variant encountered in Malaysia is HbCS.

The detection of HbCS and HbQS is carried out using DNA amplification techniques and restriction enzyme digestion of amplified PCR products (16–19). In Malaysia, HbCS is detected by molecular analysis, Hb electrophoresis, high-performance liquid chromatography (HPLC) and isoelectric focusing. However, this abnormal α -globin, which comprised of only 1–2% of the total haemoglobin, is unstable and the fraction of slow-moving haemoglobin can be missed when non-molecular techniques are used.

This study highlights the presence of two α -globin structural variants, HbCS and HbQS, in Malaysia, as well as their molecular characterisation using a sensitive and specific Combine-ARMS technique developed in-house.

Materials and Methods

Family study

Two families (A and B), each with a child with HbH disease, were referred for molecular characterisation for α -thalassaemia at the University Malaya Medical Centre (UMMC). Patient A was four years of age when she was admitted with jaundice, severe anaemia and a 4-cm hepatomegaly. Family B consists of a Chinese couple and their 10-month-old daughter who was referred for pallor and lethargy. Based on clinical and haematological investigations, the accompanying diagnosis from the consultants

involved in both cases suggested HbH disease with probable involvement of HbCS. Molecular characterisation of α -thalassaemia was carried out for both families. The haematological and Hb analysis data of patients A (carried out at UMMC) and B (carried out at Singapore General Hospital) are shown in Table 1.

DNA extraction

Ethical and institutional approval to carry out studies on α -thalassaemia was obtained from the Ethics Committee of the University Malaya Medical Centre (UMMC) in accordance with the Declaration of Helsinki. Informed and signed consent was also obtained from the parents of both families. Blood (5 mL) was collected from patients and family members in sodium-EDTA tubes and DNA was extracted using proteinase K and sodium dodecyl sulphate. Extracted DNA was purified using phenol-chloroform-isoamyl alcohol and precipitated with 4 M sodium acetate and ethanol.

Duplex PCR for the detection of the SEA deletion

The presence of the SEA α -globin gene deletion was detected using a duplex PCR protocol (20) in which the SEA deletion was amplified as a 730-bp fragment and the normal α -globin gene sequence between the $\psi\alpha$ - $\alpha 2$ -globin genes was amplified as a 136-bp fragment. DNA from α -thalassaemia carriers will amplify both the 730-bp SEA deletion-specific sequence and the normal 136-bp $\psi\alpha$ - $\alpha 2$ -globin gene sequence. DNA from a Hb Bart's hydrops foetalis ($--/--$) will specifically amplify only the SEA deletion-specific fragment.

PCR-RFLP analysis for HbCS and HbQS

The polymerase chain reaction-restriction fragment length polymorphism technique was used to detect HbCS in the families' genomes. A 339-bp sequence spanning the 3'-termination codon of the $\alpha 2$ -globin gene that contains the HbCS mutation was amplified and then subjected to restriction enzyme analysis using *Mse* I (17). The detection of HbQS was carried out by amplifying the same 339-bp sequence spanning the 3'-termination codon of the $\alpha 2$ -globin gene, followed by restriction enzyme digestion with *Msp* I (19).

Combine-ARMS for the detection of Hb CS and Hb QS

The C-ARMS primers for the detection of HbCS and HbQS were synthesised in two forms: the normal gene sequences and the mutant α -globin gene sequences, which contain one nucleotide substitution at the 3' end and an additional one-base pair mismatch at the third or fourth base from the

Table 1: Haematological data and haemoglobin analysis of patients A and B with non-deletional HbH disease

Parameters	Patient A HbH disease with HbCS	Patient B HbH disease with HbQS	Normal range
Sex-Age	Female - 4 years	Female -10 months	Patient A, UMMC ^a Patient B, SGH ^b
RBC (x10 ¹² /L)	2.57	5.88	4.0–5.4 3.8–5.5
Hb (g/dL)	5.86	9.2	11.5–14.5 10.0–15.0
MCV (fL)	74	53.5	77–91 76–96
MCH (pg)	22.8	15.7	24–30 27–32
MCHC (g/dL)	31	29.3	30–35 32–36
RDW (%)	19.2	21.6	12–15 10.9–15.7
Hb electrophoresis	Hb A, Hb A2 and weak, Hb H bands present	HbA, HbA2 and Hb H bands present	-
Hb A2	2.8 %	1.8 %	2.2–3.3 2.1–3.1
Hb F	-	0.2 %	0.1–1.3 0.1–5.0
HbH inclusion bodies	Positive (numerous)	Not in report	-
Blood film	Severe anisopoikilocytosis, target cells and some polychromatic cells present	Red cells are hypochromic, microcytic, moderately severe anisopoikilocytosis and numerous target cells present	-

^a University Malaya Medical Centre, UMMC; ^bSingapore General Hospital, SGH

Hb, haemoglobin; RBC, red blood cell count; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; RDW, red cell distribution width; -, not available

3' end. In each reaction, amplification of a 323-bp region between the γ - and ϵ -globin genes of the β -globin gene complex in chromosome 11 (primer E, 5'-AGTGCTGCAAGAAGAACAACCTACC-3' and primer F, 5'-CTCTGCATCATGGGCAGT-GAGCTC-3') was included as an internal control to check amplification efficiency (21).

DNA amplification was carried out in a PCR mixture that contained 10X buffer (750 mM Tris-HCl, 200 mM (NH₄)₂SO₄, 0.1% Tween 20), 0.2 mM of each dNTP, 1 mM MgCl₂, 1 U Taq DNA polymerase and 1 μ g of DNA, in a 25- μ l reaction. First, 50 pmol of forward primer CS-1 (5'-CCT-GGGCCGCACTGACCCTATT-3') was added to 20 pmol each of reverse primers CS-M (5'-AGGAG-GAACGGCTACCGAGGCTCCAGATTG-3') and

QS-M (5'-CGGTGCTCACAGAAGCCAGGAACCTT-GGCCG-3') to amplify either a 183-bp HbCS mutant band and/or a 138-bp HbQS mutant band. The mixture was denatured at 95°C for 5 min, followed by 30 cycles of 93°C for 1 min, 65°C for 1 min and 72°C for 1.5 min, and a final extension at 72°C for 3 min. A similar C-ARMS protocol was carried out for the amplification of the normal α -globin gene sequence; in this reaction, forward primer CS-1 was added to 20 pmol each of reverse primers CS-N (5'-AGGAGGAACGGCTACCGAG-GCTCCAGATTA-3') and QS-N (5'-CGGTGCT-CACAGAAGCCAGGAACCTTGGCCA-3') to amplify the 183-bp HbCS and 138-bp HbQS normal bands. Amplified DNA was visualised after electrophoresis in a 1.5% agarose gel and staining with ethidium bromide.

Results

Figure 1 shows the gel electrophoresis results of the duplex PCR (upper gel) for the detection of α^0 -thalassaemia and the C-ARMS for HbCS and HbQS (lower gel). In the duplex PCR (upper gel), the 730-bp SEA deletion-specific band was observed in DNA from patient A and her mother (lanes 3 and 4) and in DNA from patient B (lane 7) and her father (lane 6). DNA from all members of families A and B amplified the 136-bp normal $\psi\alpha$ - α 2-globin genes. In C-ARMS (lower gel), the 183-bp HbCS-specific mutant band was amplified in DNA from patient A (lane 3) and her father (lane 2). The 138-bp HbQS specific mutant band was amplified in DNA from patient B and her mother (lanes 7 and 8). The 323-bp internal control band was amplified in DNA from all the family members.

The family pedigree with complete genotypes of both families is shown above the gels in Figure 1. The two patients referred for molecular analysis were confirmed to have HbH disease; patient A is compound heterozygous for the SEA deletion and HbCS ($--^{SEA}/\alpha^{CS}\alpha$) and patient B is compound heterozygous for the SEA deletion and HbQS ($--^{SEA}/\alpha^{QS}\alpha$).

The sensitivity of the in-house-developed C-ARMS was evaluated with HbCS (50 patients) and HbQS (10 patients) control DNA. The respective 183-bp HbCS and 138-bp HbQS sequences were amplified from the control DNA. In addition, the specificity of the C-ARMS technique was evaluated using DNA from normal individuals ($n=100$); non-specific amplification was not observed.

Discussion

A rapid and simple molecular method for the detection of α -globin structural variants is especially useful in regions where non-deletional HbH disease is present. Accurate detection of non-deletional HbH disease is necessary as these disorders have been associated with more severe phenotypes, including neonatal death, compared to the deletional forms of HbH disease (2, 22–24). HbH hydrops foetalis is usually associated with non-deletion mutations that give rise to hyper-unstable α -globin chains (25). A HbH hydrops foetalis case due to the association of the $--^{SEA}$ deletion with the HbQS mutation has been reported in a Chinese woman (26). Affected fetuses have severe anaemia and symptoms consistent with the classic presentation of Hb Bart's hydrops foetalis. HbH disease is considered a public health problem in California, USA, and neonatal screening programs for HbH and HbCS have been

incorporated into the universal newborn screening program for haemoglobinopathies in that state (27,28). The haematological data show a relatively low red blood cell count for patient A. The patient is undergoing regular blood transfusion at three-weekly intervals; her pretransfusion haemoglobin ranges between 8–10 g/dL, whereas her post-transfusion haemoglobin is between 12–14 g/dL.

HbCS has been reported in the Malaysian population, with the highest frequency among Malays, followed by the Chinese and Indian populations (7,29). The presence of HbQS in Malaysia has not been previously documented and this study presents the first report of HbH disease associated with HbQS in Malaysia. In HbCS, the mRNA of the mutant gene is unstable and the small quantities of abnormal α -globins in the peripheral blood of HbCS patients make detection difficult, especially in heterozygotes (30). The non-deletional α -globin gene mutation in HbQS results in highly unstable α -globin chains (3). The detection of HbCS by gel electrophoresis requires fresh blood samples, and the highly unstable α -globin chains in HbQS make this Hb variant undetectable by routine electrophoresis (14).

Accurate detection of both HbCS and HbQS requires molecular techniques. In Malaysia, where both disorders are likely to be involved in non-deletional HbH disease, C-ARMS offers rapid and simple confirmation of diagnosis. PCR-RFLP also yields accurate diagnosis, but it is more expensive due to the higher cost of restriction enzymes in Southeast Asian countries. C-ARMS utilises five oligonucleotide primers that can be stored at -70°C . The primers E and F used for amplification of the 323-bp internal control are the same standard internal control primers commonly used in single ARMS. Using a standard PCR kit that includes Taq polymerase, 10X buffer and magnesium chloride, C-ARMS is cost-effective.

ARMS has been widely used for molecular characterisation and prenatal diagnosis of β -thalassaemia worldwide. Using ARMS, characterisation of β -thalassaemia was achieved in 98.7% of Malaysian Chinese patients (31), over 97% of patients in Pakistan (32), 84.2% of Egyptian patients (33) and 75% of patients in the Khuzestan provinces of Iran (34). A disadvantage usually associated with ARMS is false-positive and false-negative amplifications. In our laboratory, careful evaluation of primer concentrations and PCR conditions during the development of the ARMS method has resulted in sensitive and specific amplification. False-positive results due to maternal contamination can be further checked with DNA amplification of the VNTR locus DIS80

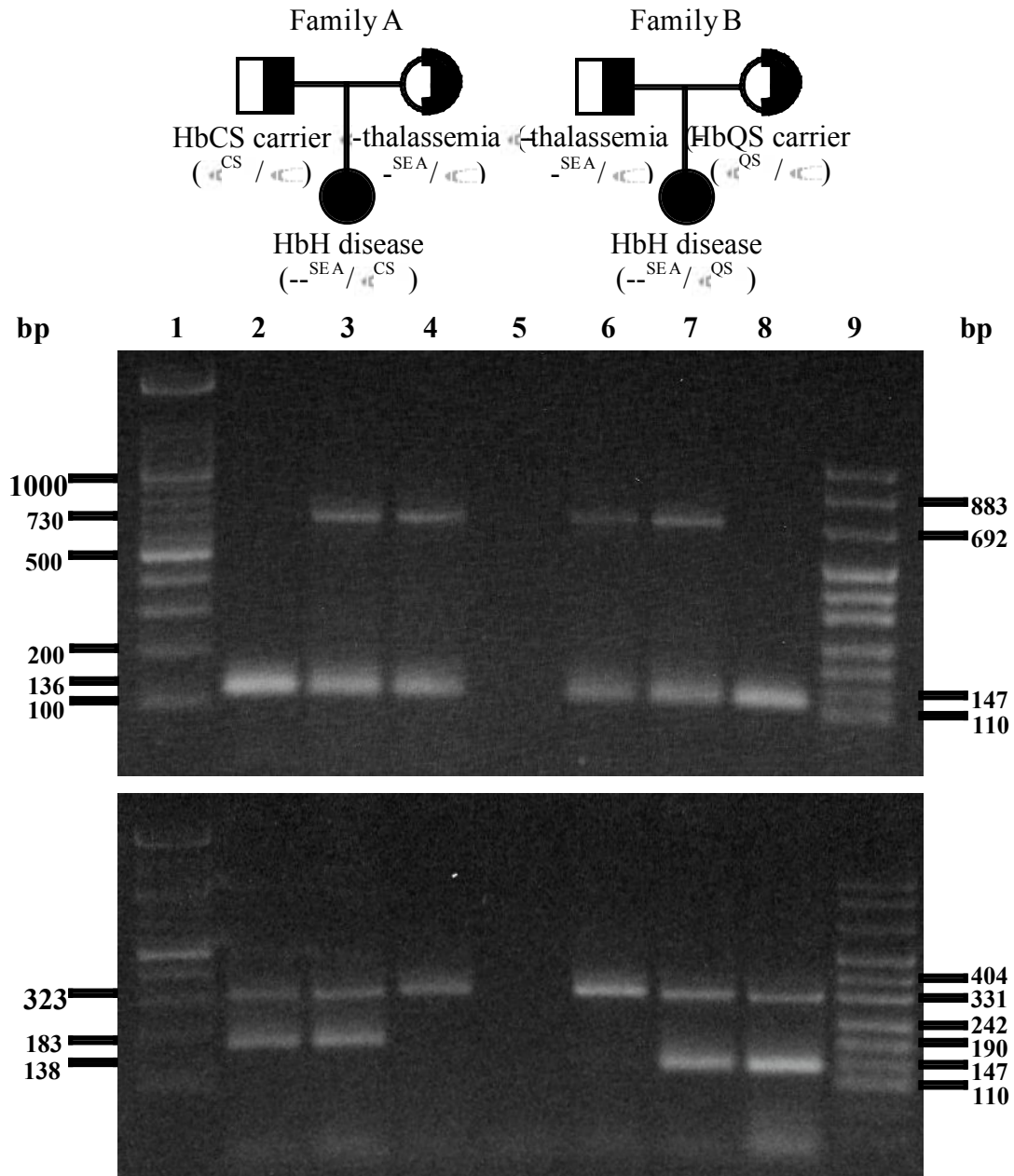


Figure 1: Gel electrophoresis of the amplified SEA deletion-specific sequence and the normal $\psi\alpha$ - $\alpha 2$ -globin genes in a duplex-PCR (upper gel) and the detection of HbCS and HbQS using C-ARMS (lower gel). The affected daughter in family A was admitted with hepatomegaly of 4cm, jaundice and severe anaemia at four years of age. The family was molecularly characterised with the SEA deletion and HbCS. Patient B was referred for pallor and lethargy, and she was characterised with the SEA deletion and HbQS

Upper gel. Duplex-PCR for the detection of SEA deletion

Lane 1: 100 bp DNA ladder; lane 2: father of patient A, 136 bp normal $\psi\alpha$ - $\alpha 2$ -globin genes region band; lane 3: patient A, 730 bp deletion-specific band and 136 bp normal band; lane 4: mother of patient A, 730 bp deletion-specific band and 136 bp normal band; lane 5: water control (no DNA added); lane 6: father of patient B, 730 bp deletion-specific band and 136 bp normal band; Lane 7: patient B, 730 bp deletion-specific band and 136 bp normal band; Lane 8: mother of patient B, 136 bp normal $\psi\alpha$ - $\alpha 2$ -globin genes region band; Lane 9: pUC Mix molecular weight marker.

Lower gel: C-ARMS for detection of HbCS and Hb QS

Lane 1: 100 bp DNA ladder; lane 2: father of patient A, 323 bp normal Gy- ϵ -globin gene region internal control band and 183 bp HbCS mutant band; lane 3: patient A, 323 bp internal control band and 183 bp HbCS mutant band; lane 4: mother of patient A, 323 bp internal control band; lane 5: water control (no DNA added); lane 6: father of patient B, 323 bp internal control band; Lane 7: patient B, 323 bp internal control band and 138 bp HbQS mutant band; Lane 8: mother of patient B, 323 bp internal control band and 138 bp HbQS mutant band; Lane 9: pUC Mix molecular weight marker.

(31) and haplotype construction at six polymorphic restriction sites along the β -globin gene cluster (35). The most common α -chain variant in Southeast Asia is HbCS, followed by the rarer HbQS in Malaysia and Singapore. The frequency of HbQS in Malaysia has not been reported, except for the fact that it is reported as a rare mutation. Hb Pakse is another rare α -chain variant and has been reported more commonly in Thailand (36). The Combine-ARMS for HbCS and HbQS was specifically established for rapid detection of the two main non-deletional α^+ -thalassaemia mutations in Malaysia. The sensitivity and specificity of this technique will allow confirmation of these two α -globin structural variants and thus yield valuable updated data on the actual frequencies of HbCS and HbQS in the Malaysian population.

Studies on the co-inheritance of β -thalassaemia with HbH disease have also shown variable clinical heterogeneity depending on whether deletional or non-deletional HbH disease is involved. Clinicians and genetic counsellors must take into account the role of HbCS and HbQS in HbH patients in whom α - and β -thalassaemias and Hb variants co-exist, particularly in Malaysia and other countries in Southeast Asia.

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Obtaining of funding: KHC

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ORIGINAL ARTICLE

Antiproliferative Properties of Clausine-B against Cancer Cell Lines

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Abstract

Background: Clausine B, a carbazole alkaloid isolated from the stem bark of *Clausena excavata*, was investigated for its antiproliferative activities against five human cancer cell lines: HepG2 (hepatic cancer), MCF-7 (hormone-dependent breast cancer), MDA-MB-231 (non-hormone-dependent breast cancer), HeLa (cervical cancer), and CAOv3 (ovarian cancer).

Methods: Chang liver (normal cells) was used as a control. The effect of clausine-B was measured using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

Results: Clausine-B was found to be active ($IC_{50} < 30 \mu\text{g/mL}$) against four of the cancer cell lines tested. The IC_{50} values for these four lines were: 21.50 $\mu\text{g/mL}$ (MDA-MB-231), 22.90 $\mu\text{g/mL}$ (HeLa), 27.00 $\mu\text{g/mL}$ (CAOV3) and 28.94 $\mu\text{g/mL}$ (HepG2). Clausine-B inhibited the MCF-7 cancer cell line at 52.90 $\mu\text{g/mL}$, and no IC_{50} value was obtained against Chang liver.

Conclusion: It is possible that the phenolic group in clausine-B responsible for the antiproliferative activities found in this study.

Keywords: Clausine-B, *Clausena excavata*, ethnopharmacology, cell survival, medical sciences

Introduction

Natural sources of foods that are believed to have a high antiproliferative activity on tumour cells have become an important element of cancer prevention and treatment strategies. For this reason, the potential of naturally-occurring dietary substances to prevent disease has become an important area of scientific interest. Several years ago, researchers began to consider whether a drug-resistant cell line could be used to screen samples for such agents and, in particular, to evaluate whether non-toxic extracts and compounds could be found that would possess this selective activity. Furthermore, with a number of compounds available, it was also regarded as an opportunity to evaluate a broad range of natural product structure types (1). Plants have many phytochemicals that possess various bioactivities, including antioxidant

and anticancer properties. For example, some studies have reported that extracts from natural products, such as fruits, vegetables and medicinal herbs, have positive effects against cancer that are comparable to those of chemotherapy or recent hormonal treatments (2–3). Many plants have therefore been examined in an attempt to identify new and effective antioxidant and anticancer compounds (4–6).

Clausena excavata is commonly known as *Chemama* among Malays. This species is also known as *Kemantu hitam*, *Pokok cherek* (diarrhoea tree), *Cherek hitam* and *Secherek* in Malaysia; *Tikusan* and *Bagal tikus* (in Java); *Bajetah* and *Ki bechetah* (in Sundanese); *Sicherek* (in Sumatra); and as *Fia fan* (in Thailand) (7). It is a strong-smelling shrub, found from the Himalayas and China to (and throughout) Malaysia, especially in the Peninsula. The leaflets have a characteristic curry-like smell

when crushed. The decoction of the roots can be used for bowel-complaints, chiefly colic. The pounded root and leaves are used as a poultice for sores, including ulceration of the nose. It has been recorded that pounded leaves have been applied to the head for headaches. Ulceration of the nose may be treated by fumigation from burning the leaves and bark (8). The flowers and leaves may be boiled and the decoction taken for colic; and a decoction of leaves has been given after childbirth. The juice of the plant was used in Java for coughs and as a vermifuge and cold treatment (9). The leaves of this plant are used as a traditional medicine to cure colds, abdominal pain, malaria and dysentery (10). The principal active constituents of this plant are carbazole alkaloids and coumarins (11–17). Some coumarins that have been isolated from the leaves have been found to inhibit tumour promotion (17).

Limited data is available on the antiproliferative properties of clausine-B, one of the carbazole alkaloids that have been isolated from the stem bark of *Clausena excavata*. Therefore, we developed this study to determine whether clausine-B has antiproliferative activity against several human cancer cell lines, including breast and liver.

Materials and Methods

Human cancer cell lines and reagents

HepG2 (hepatic cancer), MCF-7 (hormone-dependent breast cancer), MDA-MB-231 (non-hormone-dependent breast cancer), HeLa (cervical cancer), CAOV3 (ovarian cancer) and Chang (normal liver) cell lines were obtained from the American Type Culture Collection (ATCC), USA. The normal cell line, Chang, was used for comparison. The growth media: RPMI-1640, Minimum Essential Medium (MEM) and Dulbecco's Modified Eagle Medium (DMEM) with high glucose and low glucose concentrations, and the phosphate buffer solution (PBS) tablets were obtained from Sigma Chemical Co., St Louis, USA.

Foetal calf serum, penicillin-streptomycin and trypsin were from PAA Laboratories, GmbH, Austria. The MTT kit, which consists of the MTT labelling reagent and the solubilisation solution, was supplied by Roche Diagnostics GmbH, USA. Clausine-B (Figure 1), a carbazole alkaloid isolated from the stem bark of *C. excavata*, was obtained from Professor Taufiq Yap Yun Hin, from the Department of Chemistry, Universiti Putra Malaysia. It was dissolved (1 mg/mL) in dimethyl sulphoxide (DMSO) (Sigma Chemical Co).

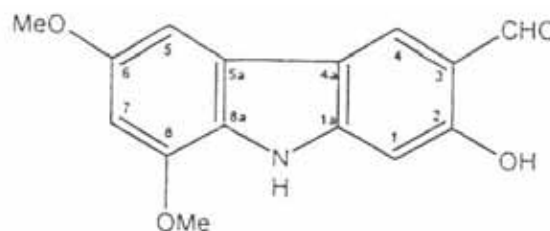


Figure 1: The chemical structure of clausine-B

Cell culture

HepG2, MCF-7 and HeLa were grown in RPMI-1640. MDA-MB-231 was grown in DMEM with low glucose, and CAOV3 was grown in DMEM with high glucose. MEM was used to grow Chang. The cells were cultured in the appropriate medium, supplemented with 5–10% foetal calf serum and 1% penicillin-streptomycin, using 25 cm² flasks in a 37°C incubator with 5% CO₂.

Cell subculture

To subculture the cells, the cells were divided and the culture medium was replaced with fresh medium as follows: First, the old medium was removed, and then the cells were rinsed briefly with PBS to wash the cells. 1–2 mL of trypsin was then added, and the flask was incubated at 37°C and 5% CO₂ for 5 minutes. After the cells had detached from the lower part of the flask, 20 mL of medium was added to the flask and the culture was divided in two parts. One part was then transferred to a new flask.

Cellular behaviour

The cells were grown until they were confluent. Then, the cells were trypsinised and the number of viable cells was counted with a hemocytometer to prepare a cell suspension. One hundred microlitres of suspension containing 1 x 10⁵ cells was seeded in each well of a 96-well microtiter plate (Nunc, Denmark). The plate was then incubated overnight at 37°C with 5% CO₂. The following day, the medium was replaced (18).

Cell proliferation assay

Each cancer cell line was grown in a 96-well microtiter plate (Nunc, Denmark) in a final volume of 100 µL culture medium per well. The cells were then treated with clausine-B at a dose of 10, 20, 30, 40, 60, 80 or 100 µg/mL and maintained at 37°C with CO₂ for 24–72 hours. After the incubation period, 10 µL of MTT labelling reagent (Roche Diagnostics, USA) was added to each well. The microtiter plate was then incubated again for 4 hours at 37°C with 5% CO₂. Then, 100 µL of the

solubilisation solution (Roche Diagnostics, USA) was added into each well. The plate was allowed to stand overnight in the incubator at 37°C and 5% CO₂. The cell viability was measured using an ELISA reader (ELx 800) at 550 nm. The experiment was carried out in six replicates.

Measurement of the growth inhibitory effect

Cells were grown in 6-well plates (Nunc, Denmark) in a final volume of 1 mL of culture medium per well. Each well contained 1×10^5 cell/mL and was incubated for 24 hours in a 5% CO₂ incubator at 37°C. The method used for cell culture was similar to that described above. The HeLa cell line was used as it has the lowest IC₅₀ value (the concentration of sample that can inhibit 50% of the cancer cells from proliferating) for clausine-B, which had been screened using the MTT assay. The cells were treated with the sample at a concentration that was similar to the IC₅₀ value. The plate was then incubated again in the 5% CO₂ incubator at 37°C for 24, 48 and 72 hours. The untreated cells (control) were also incubated for 24, 48 and 72 hours. The growth of the cells was photographed using a phase contrast microscope (Olympus, USA).

Results

Cell proliferation assay

Table 1 presents the IC₅₀ values of clausine-B. Clausine-B was found to inhibit 50% of the proliferation of HeLa cancer cells at 22.90 µg/mL, MDA-MB-231 at 21.50 µg/mL, CAOV3 at 27.00 µg/mL and HepG2 at 28.94 µg/mL. Clausine-B inhibited the MCF-7 cancer cell line at 52.90 µg/mL. There was no IC₅₀ value obtained from clausine-B against the Chang liver cell line.

Table 1: The IC₅₀ values of clausine-B

Cancer cell lines	IC ₅₀ (µg/mL) ^a
HeLa	22.90 ± 0.45
MCF-7	52.90 ± 8.49
HepG-2	28.94 ± 0.00
MDA-MB-231	21.50 ± 0.04
CAOV3	27.00 ± 0.29
Chang	None

^aValues are expressed as the mean ± standard deviation of six replicate measurements. The IC₅₀ value is defined as the concentration of sample necessary to inhibit 50% of the cancer cells from proliferating.

Growth inhibitory effect

The ability of clausine-B to inhibit proliferation of the HeLa cell line at 22.90 µg/mL was estimated by analysing its effect on the growth of the cells (Figure 2). The growth of the untreated (control) and treated HeLa cell line after incubation for 24, 48 and 72 hours was photographed using a phase contrast microscope. Figure 2 (A, C and E) shows the untreated cells after 24 to 72 hours incubation. It can be seen that the cells are growing normally and that they are attached to each other. The observation of HeLa cells showed that after 24 hours of treatment, most of the cells were attached in the culture containing 22.90 µg/mL of clausine-B (Figure 2 B). They were viable and adhered to the lower surface of the well after 48 hours of treatment, although a large proportion of the cells were poorly attached (Figure 2 D). However, all of the cells were shrunk and detached after 72 hours of incubation (Figure 2 F).

Discussion

The Micro-culture Tetrazolium Salt (MTT) assay was used in this study to measure the amount of cell viability. The effect of clausine-B on the proliferation of human cancer cells was determined. The percentage of cell viability was measured by comparing the optical density (OD) against the control. The antiproliferative activity of clausine-B is presented as a percentage of the cell viability versus concentration (Figure 3). Cells grown in a 96-well tissue culture plate were incubated with the yellow MTT solution for approximately 4 hours. After this incubation period, purple formazan salt crystals were formed. These salt crystals are insoluble in aqueous solution, but may be solubilised by adding the solubilisation solution and incubating the plates overnight in a humidified atmosphere (37°C, 5% CO₂). The solubilised formazan product was quantified spectrophotometrically using an ELISA reader. An increase in the number of living cells resulted in an increase in the total metabolic activity in the clausine-B sample. This increase directly correlated with the amount of the purple formazan crystals formed, as monitored by the absorbance.

Clausine-B, the pure compound isolated from *C. excavata*, causes 50% cell death in all human cancer cell lines tested (MCF-7, MDA-MB-231, HepG-2, HeLa and CAOV3). The best IC₅₀ values obtained were 21.50 and 22.90 µg/mL, causing 50% of the MDA-MB-231 and HeLa cancer cell lines to die, respectively. In this study, clausine-B has been proven to inhibit the proliferation of four cancer cell lines out of five that were tested

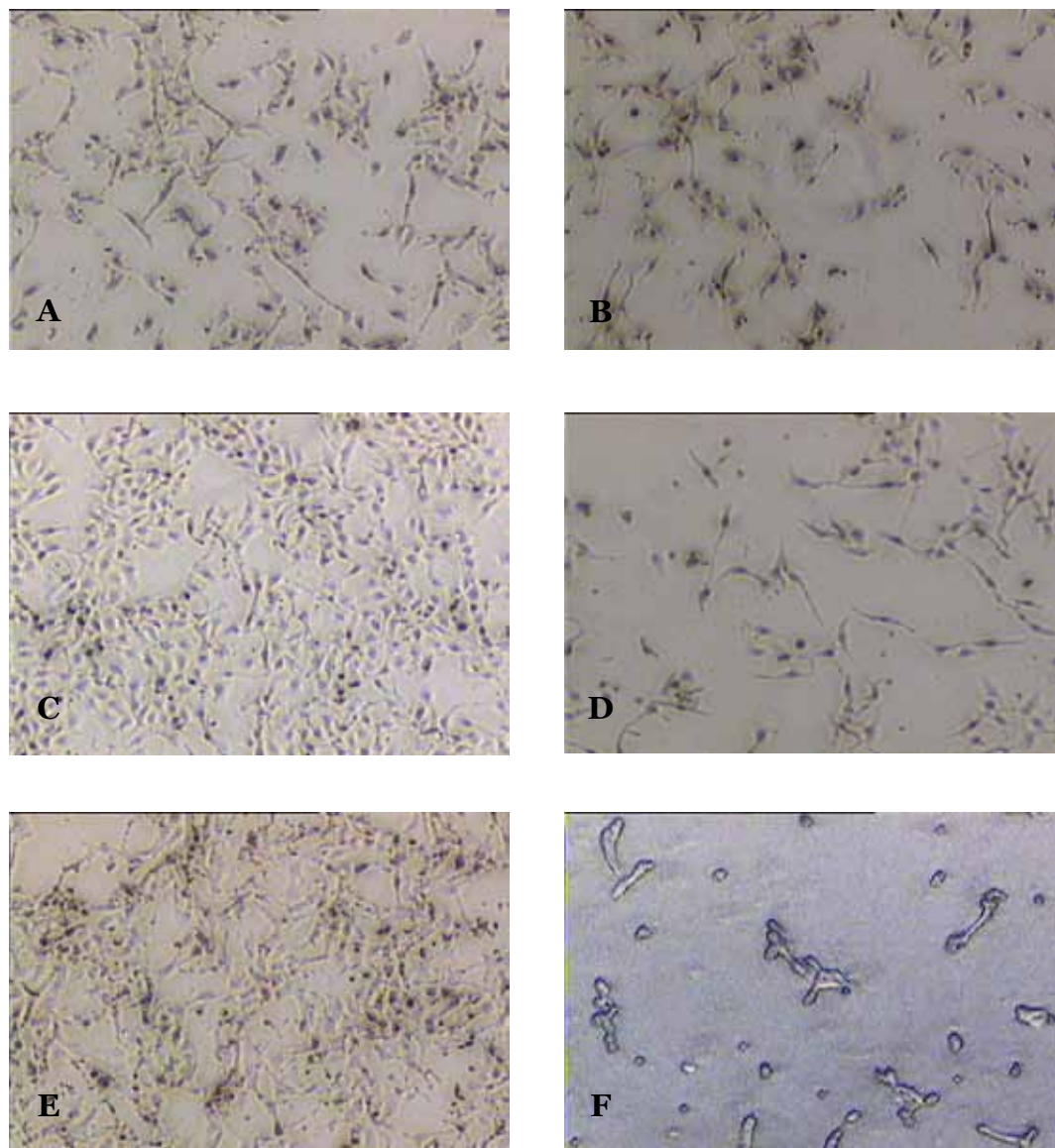


Figure 2: Phase contrast micrographs (x4 magnification) of the antiproliferative activity of clausine-B on the HeLa cell line after 24, 48 and 72 hours of incubation ($IC_{50} = 22.90 \mu\text{g/ml}$). **A.** The untreated HeLa cell line (control) after 24 hours incubation. **B.** The treated HeLa cell line after 24 hours incubation. **C.** The untreated HeLa cell line (control) after 48 hours incubation. **D.** The treated HeLa cell line after 48 hours incubation. **E.** The untreated HeLa cell line (control) after 72 hours incubation. **F.** The treated HeLa cell line after 72 hours incubation.

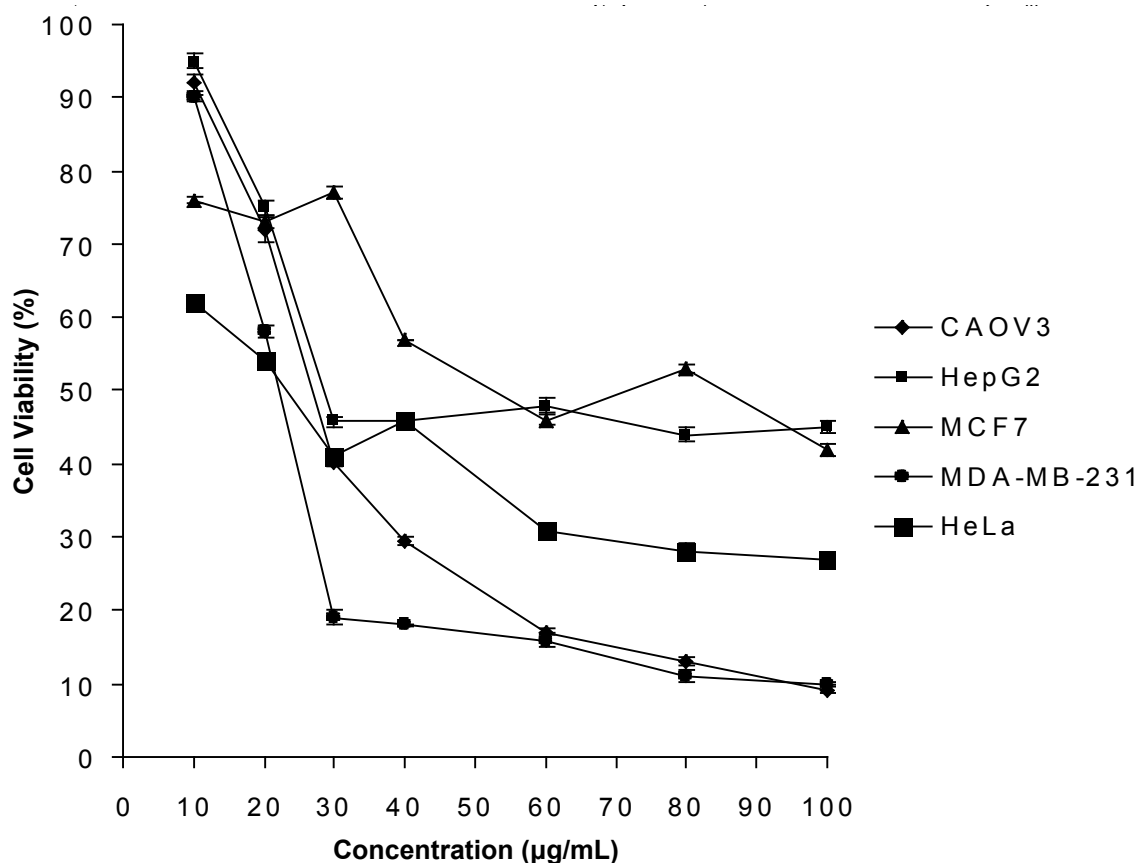


Figure 3: Antiproliferative activities of clausine-B on cancer cell lines. Values are expressed as the mean $\pm$ standard deviation of six replicate measurements.

at a strong IC_{50} value ($<30 \mu\text{g/mL}$). Clausine-B was not active ($IC_{50} > 30 \mu\text{g/mL}$) against MCF-7. It was shown that clausine-B exhibited no effect on Chang, the normal cell line that was used as a comparison.

Phenolic compounds such as furanocoumarins, flavonoids and carbazole alkaloids are the main constituents of *C. excavata* (3,17). These phenolic compounds can stimulate or suppress the immune system due to the presence of hydroxyl groups (19). Therefore, the presence of the phenolic group may be assumed to be responsible for the antiproliferative activities of clausine-B that were found in this study. Several studies demonstrating the cytotoxic effect of carbazole alkaloids isolated from *C. excavata* have been reported previously (13,14,20). Clausine-B has also been found to show significant cytotoxicity against the CEM-SS cell line (21). However, limited data was obtained on the cytotoxic effects of clausine-B. Perhaps data from this research and previous studies will yield better insights into the cytotoxic properties of clausine-B.

The morphology of HeLa cancer cells that were treated with clausine-B at $22.90 \mu\text{g/mL}$ was

studied to prove the effect of clausine-B on the cell growth. Our observations showed that the growth of the cells was inhibited. However, further study is needed to determine whether the inhibitory effect of clausine-B on the growth of the cells is due to the inhibition of the proliferation of the cells or the induction of cell death. Clausine-B proved to possess antiproliferative properties against all the cancer cell lines tested. Therefore, it may have potential as an anticancer agent. However, the mechanism behind the antiproliferative effects of clausine-B need to be studied to determine if the effect is due to an increase in apoptosis. Further investigations will yield clearer answers.

It has also been suggested that an in vivo study should be carried out in conjunction with the in vitro study, so that the results can be compared and more information regarding the antiproliferative properties of clausine-B will become clear. This is important, because there may be many factors that affect the antiproliferative activity in the in vivo study. Furthermore, in vitro studies may not produce the same results as in vivo experiments.

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ORIGINAL ARTICLE

Analysis of hair samples using microscopical and molecular techniques to ascertain claims of rare animal species

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Abstract

Background: An unidentified animal species named the Jenglot and claimed to be a rare living animal species was recently found in the deep jungle of Irian Jaya, Indonesia; brought to Kuala Lumpur, Malaysia by a businessman; and exhibited in a local museum. The owner of the Jenglot carcasses had made a request to perform DNA analysis on the Jenglot to ascertain its species.

Methods: Because the muscle appeared very dry and recovery of DNA was extremely difficult, we therefore used the animals' hair for further analysis. Hair samples were collected from three different Jenglots that were different in colour and physical appearance. The samples were labelled as A, B, C and D, respectively.

Results: Microscopic characteristics indicated that all four hair samples were of human origin, with a medullary index less than 1/3 and pigment distribution towards the periphery. The scale pattern on the hair samples was of the imbricate type, adding certainty to the hypothesis of human origin. A dried root sheath was found in samples B and C, which was contrary to expectations since the sample collection method left a few cm of hair on the body of the Jenglots. Sample D had black dye granules over the cuticular surface. Sequencing of the mitochondrial DNA (mtDNA) hypervariable segment I (HVS-I) region showed polymorphisms at positions 16140, 16182C, 16183C, 16189, 16217 and 16274 and heteroplasmy at positions 16112, 16232 and 16251, a human-specific mtDNA haplotype that was consistent across all the samples.

Conclusions: Based on these findings, it was concluded that it is unlikely that the samples of Jenglot hair originated from an animal species.

Keywords: Hair, mitochondrial DNA, microscopical analysis, health sciences

Introduction

Morphological examination of hair samples is the first step in forensic hair comparisons. The main medico-legal concerns with hair examination include identification of the species of origin, ascertainment of the hair's provenance from the body and, finally, comparison of the control hair sample from the victim to the hair sample from the crime scene. Though it is not possible to definitely identify a sample of hair originating from a particular person's head, unequivocal determination of human origin can be established based on microscopic examination of the hair's cuticle, cortex, medulla and pigment granules (1).

Human and animal hairs show similarities in having an outer cuticle, cortex and medulla. The

outer surface of the hair is covered by scales. Though there are similar morphological features, the scale pattern provides distinguishing characteristics between animal and human hairs. The scales of an animal's hair show many distinctions such as coronal (crown-like) and spinuous patterns, whereas in the case of humans the scale patterns are of the 'imbricate' type (flattened) (Figure 1) (2,3). Besides, the medullary index, which is the ratio of the medulla's width to the diameter of the hair, is 1/3 and below in humans compared to greater than 1/3 in animal hairs, due to the greater width of the medulla in animals (2,3).

With the advancement of forensic DNA typing, microscopic hair comparisons and DNA analysis can be complementary and provide information on the source of a hair. In this case analysis, hair

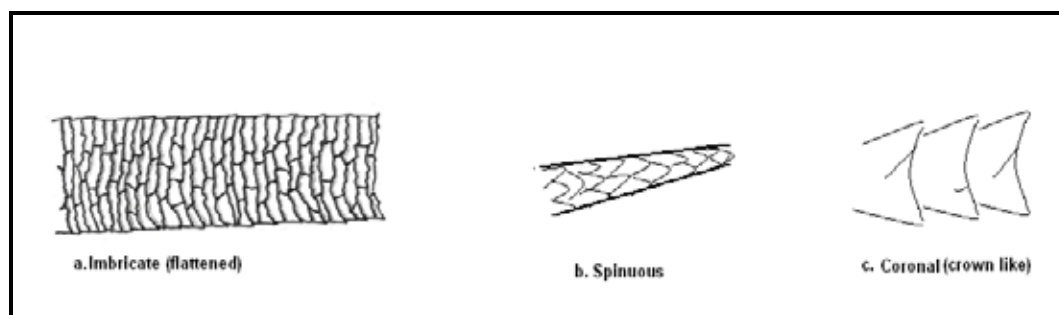


Figure 1: Representative diagram showing different scale patterns found on hairs: a, an imbricate scale pattern is unique to human; b, spinuous; c, coronal patterns are observed on animal hairs.

samples were obtained from three Jenglots, which were claimed to be a rare living animal species only found in the deep jungle of Irian Jaya, Indonesia. Three Jenglots were bought from West Java by a Malaysian businessman for his personal collection and exhibited in one of the museums in Kuala Lumpur, where they have attracted numerous visitors. A request was then made by the owner of the Jenglot carcasses to the authors to perform DNA analysis so as to ascertain the veracity of the claim that they are a rare animal species. Hair samples were collected from three different Jenglots and were labeled as A, B, C and D. Samples A and B were taken from a medium-size Jenglot carcass, which was 32 cm tall. Sample C was collected from a 16.8-cm tall Jenglot while sample D was collected from the biggest Jenglot, which was 61.3 cm tall. All hair samples were collected from the head region of the Jenglots by cutting at the distal end of the hair.

Materials and Methods

Several strands of hair were placed in parallel on a microscope slide and two drops of water were added over the hairs in order to hold them in place. A cover slip was placed over the hairs and they were scanned along their length at 100x and 400x under a compound microscope to observe the morphological characteristics of the cuticle and medulla, and the distribution of pigment in the cortex. The medullary index and the diameter of the hairs were calculated using an ocular micrometer calibrated with a stage micrometer at 100x and 400x (4).

It is often very difficult to directly observe scale patterns from hair strands on a slide. Hence, a cast was made using nail polish to obtain the impression of the scales. A thin layer of nail polish was spread on a microscope slide and a hair was placed in the middle of the slide. It was allowed to

stand for 15 minutes so that the nail polish could harden and the hair was then gently removed using forceps (4). The scale pattern was observed under a compound microscope at 100x and 400x.

DNA was extracted from each hair sample using the Promega hair and tissue extraction kit and Promega DNA IQ™ system (Promega, USA), following the manufacturer's protocols. Hair samples were thoroughly washed using soap solution and air-dried prior to the extraction to avoid contamination. The hypervariable segment I (HVS-I) region of human mtDNA was amplified using two sets of human-specific primers (L15996 and H16213; L16128 and H16432) (5) and sequenced. Polymorphisms were reported by aligning the HVS-I sequence from the hair samples with the Cambridge Reference Sequence (6).

Results and Discussion

Hair is an outgrowth of the skin produced from a structure called the hair follicle and found only in mammals. Humans develop hair follicles during foetal development and no new follicles are produced after birth. Hair is composed of the protein keratin and it is also the primary component of finger and toe nails (7).

Forensic analysis of hair centres on colour and structure, determined through microscopic magnification. The hair shaft has three forensically relevant layers: the cuticle, cortex and medulla. The cuticle has overlapping external scales, which helps in species identification. The scales of human hairs are imbricate whereas animal hairs show many distinct patterns such as coronal (crown-like) and spinuous scales (Figure 1) (1,2,4).

Within the hair cuticle is the cortex, made up of spindle-shaped cells that contain the colour pigments; the way the pigments are distributed helps to identify hairs from particular individuals. Hair colour is mostly the result of pigments and the

pigments of human hairs are distinguishable from those of other mammals. Human hairs are generally consistent in colour and pigmentation throughout the length of the hair shaft, whereas animal hairs may exhibit radical colour changes over a short distance—a phenomenon known as banding (4). The distribution and density of pigments in human and animal hairs can also be identifiable features. The pigmentation of human hairs is evenly distributed and denser toward the cuticle, whereas the pigmentation of animal hairs is more centrally distributed, i.e., denser towards the medulla (1,2,4,8,9).

At the centre of the hair shaft is the medulla, which is also valuable for species identification. Animals' medullary index (ratio of the medulla's diameter relative to the shaft's diameter) is greater than humans'. Humans have a medullary index of

less than $1/3$ while the medullary index of animals is greater than $1/3$. However, the medulla is often fragmented or interrupted, which may result in differences in the identification of hairs from the same individual (1,2,4,8,9).

The results of the examination of the four hair samples labelled A, B, C and D are shown in Table 1. All hair samples were found to have a medullary index below $1/3$, which is indicative of human origin (1,4,8,9). Moreover, moderate shaft diameter variations were observed in all four samples (Figure 2) and the scale pattern was of the imbricate type, indicating human origin (Figure 3) (1,4,8,9). The pigments were distributed more towards the cuticular margin, which is also a characteristic indicative of human origin (Figure 4) (1,4,7–10).



Figure 2: Representative hair from sample C (medullated) showing moderate shaft diameter variation (100x).

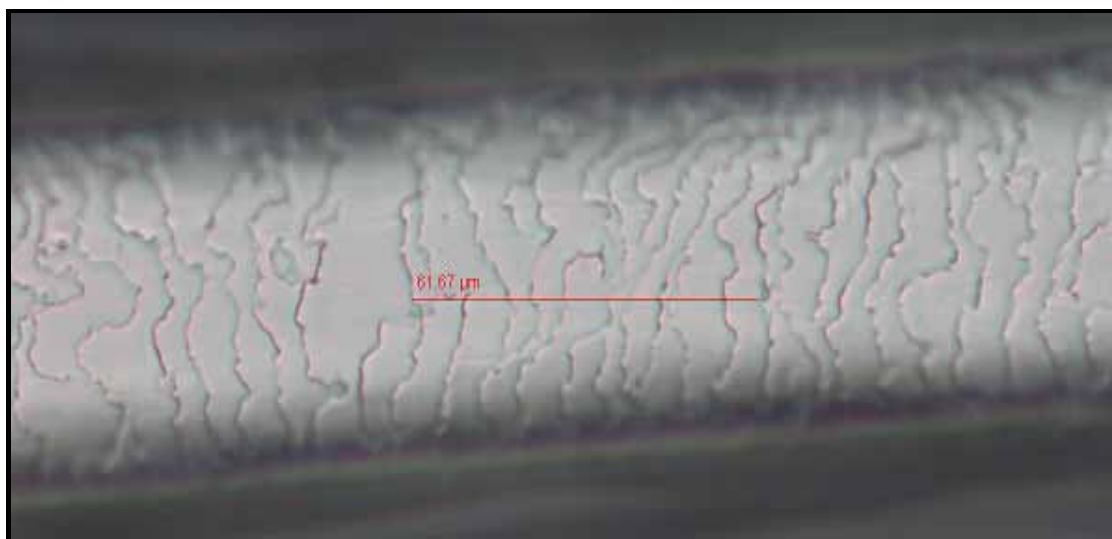


Figure 3: Scale impression cast showing the imbricate nature of the scales of the Jenglot's hair (400x).

Table 1: Results of morphological examination of hair samples

Characteristics examined	Sample A	Sample B	Sample C	Sample D
Colour	Black and grey hairs	Black and grey hairs	Brownish and grey hairs	Most hairs were dark with blackish dye granules over the cuticular surface; very few without dye granules
Medulla	Medullated and non-medullated	Medullated and non-medullated	Mostly non-medullated and a few with a medulla	Few hairs with a medulla - faintly visible
End morphology	Cut at one end and with a tapered tip	i. Mostly cut at one end and with a tapered tip ii. Few with dried hair root at one end and with cut markings at the other end	i. Mostly cut at one end and with a tapered tip ii. Few with dried hair root at one end and with cut markings at the other end	Cut at one end and with a tapered tip
Shaft diameter	Coarse and usually with little or no variation (80-110 μm)	Coarse and usually with little or no variation (80-120 μm)	Coarse and usually with little or no variation (80-110 μm)	Coarse and usually with little or no variation (80-120 μm)
Pigment distribution	Pigment was found to be more dense towards the periphery (cuticle)	Pigment was found to be more dense towards the periphery (cuticle)	Pigment was found to be more dense towards the periphery (cuticle)	Pigment was found to be more dense towards the periphery (cuticle)
Scales	Imbricate scales	Imbricate scales	Imbricate scales	Imbricate scales
Dye	-	-	-	Dye granules can be seen over the cuticular surface and were also found dissolved in acetone used to take scale impressions

Black dye granules were clearly visible under microscopic examination of the clear nail varnish imprints of the hair, indicating dyeing of the hairs in sample D (Figure 5). A few hairs from samples B and C also had an intact dried root at the other end of the cut tip, which indicated that the hairs were implanted upside down on the Jenglots' heads (Figure 6).

The mtDNA HVSI region was successfully amplified using human-specific primers,

supporting the morphological findings indicating the human origin of these hairs. Sequence analysis of the HVSI fragment showed polymorphisms at positions 16140, 16182C, 16183C, 16189, 16217 and 16274 and heteroplasmy at positions 16112, 16232 and 16251. This haplotype was consistent across all the samples, suggesting a maternal relationship between the owners of the hairs or that all the hair samples might have originated from the same individual.

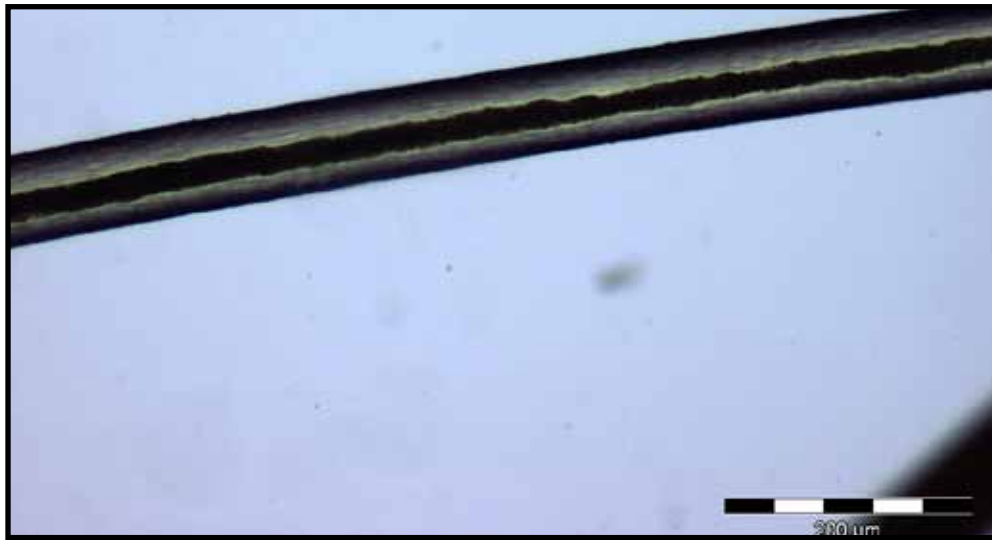


Figure 4: Medullated hair showing pigment granules more towards the cuticle (100x).

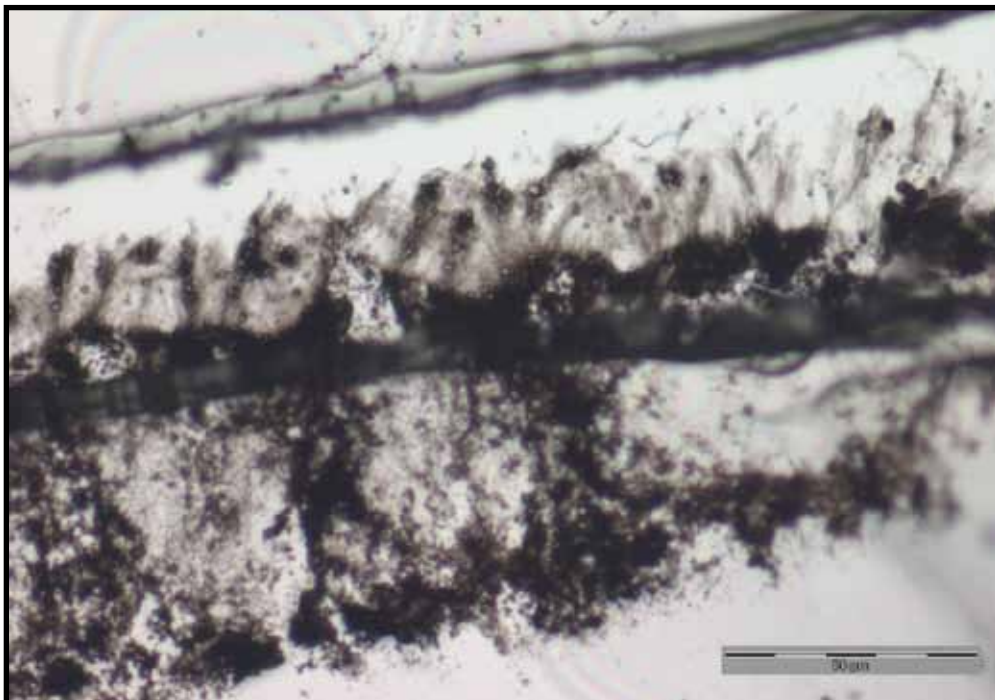


Figure 5: Black dye granules found when mounting a hair from sample D for scale casting in nail polish (100x).



Figure 6: A hair from sample B with a dried-up hair root that appeared at the other end from the cut tip, which indicated that the hair was implanted upside down on the Jenglot's head (100x).

Conclusion

Classical morphological analysis of hair samples, jointly with mtDNA sequence-based analysis, was useful in disproving the claim of a rare animal species in this case. The morphological characteristics observed and the mtDNA sequence analysis proved that the hair samples are of human origin and had been implanted on the Jenglot carcasses' heads. This certainly disproves the claim and myth that the Jenglot is a rare animal species.

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Author's contributions

Conception and design, final approval of the article: ZZ
Data collection, assembly, analysis and interpretation;
drafting of the article: SP, ZZ

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BRIEF COMMUNICATIONS

Isosporiasis in HIV/AIDS Patients in Edo State, Nigeria

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Abstract

Background: The role of opportunistic infections in accelerating disease progression in HIV-positive individuals, leading to quick death, is still receiving serious attention. The objective of this study was to determine the prevalence of *Isospora belli* infections in HIV-positive patients in Edo State, Nigeria between August 2007 and March 2008.

Methods: A total of 268 samples from HIV-positive patients and 20 samples from HIV-negative patients were processed using the modified Ziehl-Neelsen staining technique to microscopically identify the presence of *I. belli* oocysts.

Results: The overall prevalence of the coccidian was 3.1%. Gender and age had no correlation with the prevalence of the parasite ($P > 0.05$). There was a significant relationship between isosporiasis and CD4+ T cell counts in HIV-positive patients (OR=11.388, 95% CI= 2.797–46.371, $P=0.0004$).

Conclusions: Routine investigation of *I. belli* in HIV-positive subjects is advocated in tertiary health institutions.

Keywords: *Isospora belli*, HIV, CD4 + T lymphocytes, medical sciences

Introduction

Human immunodeficiency virus (HIV) infection, a worldwide phenomenon, is a serious problem in the present day (1). *Isospora belli* is a coccidian protozoan parasite endemic to many regions of the world outside the United States, including the Caribbean, Central and South America, Africa and Southeast Asia. It is common in tropical and subtropical environments. The infection is common in immunosuppressed patients, particularly those with AIDS living in tropical areas. Sporadic outbreaks have occurred in mental institutions and in day-care centres in the United States (2). About a few hundred cases of human isosporiasis were described until it became an opportunistic infection in immunocompromised, predominantly human HIV-positive patients (3).

Transmission occurs via the faecal-oral route, mainly by ingestion of infectious oocysts in contaminated food and water. In immunocompetent individuals, isosporiasis is a self-limiting gastrointestinal disease characterised by watery diarrhoea (4). Some immunodeficient individuals

are predisposed to severe and prolonged diarrhoea caused by opportunistic parasites, particularly *I. belli*, which is one of the most commonly identified causes of chronic diarrhoea in AIDS patients (5). Chronic diarrhoea in patients infected with HIV results in a significant morbidity and mortality, primarily caused by HIV wasting (6,7). The early detection of *I. belli* infection is very important in preventing complications and in prolonging a healthy life in HIV-positive patients. *I. belli* has recently been recognised as an opportunistic protozoan pathogen in patients with AIDS, though the coccidian rarely causes diarrhoea in patients with AIDS in the United States (8).

The objective of this study was to determine the prevalence of *I. belli* infections and the correlation between *I. belli* infection prevalence and CD4+ T cell counts among HIV-positive patients in Edo State, Nigeria.

Materials and Methods

The study was conducted at the University of Benin Teaching Hospital, Benin City, Edo State, Nigeria, between August 2007 and March 2008. The hospital is a tertiary care centre offering services to a large population from the mid-western part of Nigeria.

A total of 268 HIV-positive and 20 HIV-negative patients (131 males and 157 females) were analysed. The age of the study subjects ranged from 20 to 70 years (34.33 ± 9.39 years). All enrolled subjects were outpatients that were on their first visit prior to HAART management. Verbal informed consent was obtained from each subject prior to specimen collection, and samples were taken only from those who indicated their willingness to participate in the study. The study was approved by the Ethical Committee of the University of Benin Teaching Hospital.

Faecal samples were collected during two different appointments separated by an interval of four months. Patients were given a universal container with 5 mL of 10% formol saline in which to put the faecal sample. The stool samples were processed using the modified Ziehl-Neelsen staining technique to identify oocysts of *I. belli* microscopically. A concentrated smear of the stool was made on a clean grease-free slide and was fixed in methanol for 3 minutes. The slide was immersed in cold carbol fuchsin and stained for 15 minutes. It was then thoroughly rinsed in tap water and decolourised in 1% acid alcohol for few minutes. After rinsing again in tap water, the slide was counterstained with 0.4% malachite green for 30 seconds (9). The slide was then air-dried and observed under a compound light microscope to look for the presence of oocysts of *I. belli*.

About 4 mL of whole blood was also collected from each patient for CD4+ T cell counts. The blood samples were analysed for CD4+ T cell counts using flow cytometry (Partec, GmbH, Germany). Briefly, 20 μ L of CD4 PE antibody and 20 μ L of well mixed whole EDTA blood were put in a Partec test tube, mixed gently and incubated in the dark for 15 minutes at room temperature. The samples were mixed every 5 minutes during incubation. Eight hundred microlitres of CD4 buffer was added to each tube and the samples were mixed gently. The number of CD4+ cells in each tube was then measured with a flow cytometer.

Statistical analysis was done using the chi-square (χ^2) test and odds ratio analysis. A *P* value < 0.05 was considered significant.

Results

During the eight months of this study, 288 patients were examined for isosporiasis. Among these patients, 268 were HIV-positive and 20 were HIV-negative patients. An overall prevalence rate of 9 (3.1%) was observed among HIV-positive patients in this study, with 4 (3.1%) males and 5 (3.2%) females infected with *I. belli* (Table 1). There were no *Isospora* infections observed in the HIV-negative patients.

Table 1: Prevalence of *Isospora belli* infection according to sex

Sex	No. Examined	<i>I. belli</i> infection (%)
Male	122	4(3.3%)
Female	146	5 (3.4%)
Total	268	9 (3.4%)

Statistical analysis of the information displayed in Table 1 showed no significant association between infection with the parasite and gender ($P > 0.05$). Isosporiasis was only observed in HIV-positive patients, and there was a significant difference in the prevalence of *I. belli* infection between HIV-positive and HIV-negative patients ($P < 0.001$). There was a significant association between the CD4+ T cell counts and isosporiasis (OR = 11.388, 95% CI = 2.797–46.371, $P = 0.0004$) (Table 2).

Table 2: Isosporiasis in HIV-positive patients and its association with CD4+ T cell counts

CD4+ (cells/ μ L)	Number tested	Number positive (%)
<200	21	4(19.05)
200 – 500	247	5 (2.02)

The results showed that the highest prevalence for males (5.0%) was found in the 41–50 year age group and the highest prevalence for females (4.5%) was found both in the 20–30 and in the 51–60 year age groups. There was no significant association between isosporiasis and age group ($P > 0.05$) (Table 3).

Table 3: Prevalence rate of Isosporiasis according to age and sex

Age (years)	Males		Females	
	No. examined	<i>I. belli</i> (%) infection	No. examined	<i>I. belli</i> (%) infection
20-30	30	0	22	1 (4.5%)
31-40	45	2 (4.4%)	51	2 (3.9%)
41-50	40	2 (5.0%)	49	1 (2.0%)
51-60	11	0	22	1 (4.5%)
61+	5	0	13	0

Discussion

To our knowledge, this is the first study to be carried out on the prevalence of *I. belli* infection in HIV-positive patients in Edo State, Nigeria. The results obtained in this study can provide important information for future understanding of isosporiasis in HIV-positive patients.

The overall prevalence rate of 3.1% is higher than that found in several other studies. Wiest (9) observed a prevalence rate of 0.9% in Egypt, Fisseha recorded a prevalence rate of 1.4% in Addis Ababa (10). On the other hand, the prevalence rate we found is lower compared to some other studies. Cardoso reported a rate of 4.04% in the northern region of Sao Paulo state, Brazil (11), Mohammed observed a prevalence rate of 7.4% in Jimma, Ethiopia (12), Bialek recorded a prevalence rate of 23.9% in Tabingen, Germany (13), Escobedo noted a prevalence rate of 27% in India (14) and Ambriose-Thomas observed an 85% prevalence rate in Haiti (15). The observed prevalence rate (3.1%) in this study is similar to that found in Brazil (4.4%) (12).

The low prevalence rate that we found may be to the result of the low abundance of this parasite in the study area. It is possible that the observed prevalence rate would be higher with the use of a diagnostic real time PCR assay for *I. belli*, as this is regarded as a gold standard in detecting *Isospora*. It has been noted that with the commencement of HAART treatment in these patients that a low prevalence may also be observed, but with our study, the subjects were those on their first visit who are yet to commence the HAART treatment. There was no significant association between isosporiasis and gender or age group ($P > 0.05$).

There appeared to be a significant difference in *I. belli* infection rates between HIV-positive and HIV-negative subjects ($p < 0.001$). This is in agreement with work of Cardoso (11) and Mohammed (12). This could be due to a shift in

the immune status of the subjects. HIV patients with CD4+ T cells counts of <200 cells/ μ L were at increased risk of *I. belli* infection (OR= 11.388, 95% CI= 2.797–46.371, $P = 0.0004$). This higher *I. belli* infection rate may be the result of the low immune status of these patients, which exposes them to opportunistic infections. The effects of isosporiasis may lead to increases in morbidity and mortality in these patients.

This study has demonstrated that *I. belli* infection is prevalent in Edo State, Nigeria and, as a result, may increase the burden on HIV-positive patients.

In conclusion, routine investigation of *I. belli* infection is advocated in various tertiary hospitals as this will enhance better management of HIV-positive patients.

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CASE REPORT

Emergency Thyroidectomy for a Bleeding Multinodular Goitre

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Abstract

Goitre is a slow-growing thyroid mass, rarely presenting as an emergency. However, a superimposed infection or acute intralesional bleeding can cause the mass to increase rapidly in size. We report a patient with long-standing multinodular goitre who presented with bleeding from the left thyroid mass. Despite all appropriate measures, the continuous bleeding finally stopped upon thyroidectomy.

Keywords: Goitre, bleeding, thyroidectomy, medical sciences

Case Report

A 70-year-old Malay female presented with a long-standing thyroid mass of more than ten years. There was a history of left lobectomy for a similar problem in the past. However, the mass reappeared several years after the operation; the patient could not recall the exact duration of the symptom-free period. For the previous two years, she had noted that the swelling was growing rapidly. It was associated with bluish discoloration of the skin at the most prominent site of the swelling. Initially, she was examined in the clinic a few weeks before her presentation. Ultrasonography of the mass revealed that it was a thyroid mass with both solid and cystic components. There was increased vascularity at the periphery of the mass near the skin. A few paratracheal lymph nodes were also present. Fine needle aspiration showed signs of colloid goitre. She was scheduled for a complete thyroidectomy at a given date.

She presented earlier than the given date because of bleeding from the mass. There was slow but continuous oozing from the surface of the lesion (Figure 1). Neither the mass nor the bleeding was pulsatile. The bleeding surface area measured 4 x 4 cm. However, the mass had not increased in size. Her haemoglobin level on admission was 7 g/dL. She was managed conservatively with packed cell transfusion, intravenous fluid volume replacement

and compression dressing. However, the dressing was soaked with blood every 3 to 4 hours.

An emergency thyroidectomy was planned in order to arrest the bleeding. She underwent a complete thyroidectomy on the next day (Figure 2). Recurrent laryngeal nerves were preserved. As the left lobe extended infraclavicularly, a careful dissection was made in order to avoid thoracic duct injury. The wound was closed in the usual manner after securing all the bleeding. Histological reports revealed that it was nodular goitre with intralesional haemorrhage. Lymph nodes removed showed reactive changes.



Figure 1: Bleeding from the mass



Figure 2: Removed specimen

Discussion

Thyroid swelling rarely presents as an emergency situation. Surgery, if indicated, is usually carried out in an elective manner after a thorough investigation, which includes a thyroid function test, fine needle aspiration, ultrasonography and, sometimes computed tomography and thyroid scans are done. The type of surgery required is determined by the clinical, radiological and histological results of the needle aspiration study.

Most of the emergency cases involving goitre include compromised airways. Tracheal compression due to the growing goitre usually requires intubation with a smaller size endotracheal tube. Emergency thyroidectomy under regional anaesthesia (bilateral superficial cervical plexus block) has been reported for severe airway obstruction (1).

Bleeding from a benign thyroid swelling is usually managed by conservative measures such as compressive dressing and replacement of the blood loss. Usually, surgery will be undertaken when the bleeding stops. However, intrathyroidal bleeding can present as an acute problem. Extensive intralesional bleeding can result in a rapidly expanding haematoma with airway compromise (2). If the collection slowly progresses and remains confined intralesionally, the patient may develop chronic symptoms, such as dysphagia, neck pain, chronic cough and throat discomfort. Calcification of such haemorrhages may also occur (3). Potential causes of thyroid bleeding include malignant changes, systemic haematological disorders and hypervascular lesion of the thyroid. Bleeding from previous needle aspiration could be considered if a large-bore needle was used and the procedure was recently done.

Our case illustrates an intralesional thyroid haemorrhage that emerged via the thinning of the skin. As the bleeding was from the thyroid itself, performing thyroidectomy as an emergency surgery effectively arrested the haemorrhage. Superselective embolization, although it is available in our centre, may not have been beneficial to this patient, as the bleeding was very superficial, both by clinical and radiological assessments. Total thyroidectomy and central neck dissection were opted for, in consideration of the age of the patient, the long-standing nature of the swelling and the presence of multiple, small cervical lymph nodes in the paratracheal region.

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CASE REPORT

Anterior Stabilisation of Sacroiliac Joint for Complex Pelvic Injuries

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Abstract

Sacroiliac joint diasthesis from high energy trauma is always complicated with chronic pain and long term morbidity. Open anterior stabilisation with plate allow direct reduction and stabilisation with biomechanically advantages. Here we report on four cases of pelvic injury with sacroiliac joint disruption treated with anterior plate stabilisation through a surgical approach similar to that used for anterior ring fractures.

Keywords: Pelvic fracture, internal fixation, anterior, sacroiliac joint, medical sciences

Introduction

High-energy pelvic ring injuries represent a severe injury involving major disruption of the bony pelvis, ligament and surrounding soft tissues. Definitive stabilisation of the pelvic ring disruption remains a challenge for the managing team. Anterior external fixation is useful in acute situations to reduce pelvic volume and control haemorrhage; however, definitive anatomical reduction and stabilisation, particularly of the sacroiliac joint, is important to minimise chronic pain and long-term morbidity (1,2,3,4).

Case reports

Case 1

A forty-three-year-old offshore worker sustained multiple injuries after an electric pole fell and hit his right thigh and pelvis. He presented in casualty, fully conscious with tachycardia and blood pressure of 90/50 mmHg. Initial resuscitation with fluid and four pints of blood were able to stabilise his blood pressure. The trauma team review revealed that he had symphysis pubis diasthesis of 2

cm and right sacroiliac joint disruption. There was no urethral injury and minimal bruises were seen over the perineal region. He also sustained grade 1 open fracture of the right femur, closed diaphyseal fracture of the right tibia, closed segmental fracture of the fibula and avulsion fracture of the medial malleolus.

The open fracture of the right femur was debrided eight hours after the injury. The right femur, tibia and malleolus fractures were stabilised with plates and screws. Surgery was uneventful as only two pints of blood were required and the coagulation profile was normal throughout. We proceeded with symphysis pubis plating and used a retroperitoneal approach to the sacroiliac joint for plate stabilisation. Bleeding was controlled; blood loss for pelvis surgery was estimated to be 500 mL and the patient did not require any additional transfusion. He was observed in the intensive care unit for twelve hours. However, the coagulation profile was slightly abnormal and was corrected with four units of plasma and cryoprecipitate. The patient was able to sit after three days and ambulated with crutches seven days later. At present, 3 years after injury, he has full function with no disability and chronic pain (Figure 1).



Figure 1: Symphysis pubis diastasis with right sacroiliac joint vertical dislocation. Fracture reduced through anterior approach and stabilised with plate and screws.

Case 2

A nineteen-year-old motorcyclist sustained a head-on collision with a pick-up truck at the speed of approximately 100 km/h. He sustained multiple fractures of the limbs as well as pelvic injury. The hypovolaemia was transient and the patient was managed with a 3-pint blood transfusion. The pelvis was initially stabilised with external stabilisation; femoral and tibia fractures were stabilised with plates and screws within 24 hours of injury.

Further pelvic evaluation revealed a bi-columnar acetabulum fracture with central hip dislocation. The posterior sacroiliac joint was disrupted anteriorly; however, the posterior ligamentous structure remained intact (Figure 2A,B). The acetabulum was reduced and stabilised with long reconstruction plate and L-buttressing plate. The anterior sacroiliac joint was reduced and stabilised using a similar plate from the alar of the sacrum (Figure 2C). At present, 2 years after the injuries, the patient is ambulating pain-free with full hip motion and working as a petrol station attendant.

Case 3

A forty-year-old gentleman was found stuck under a fallen log. He was brought to casualty in a hypotensive state; emergency external stabilisation to the pelvic bone and eight pints of blood and fluid resuscitation were able to regain normotension. Emergency pelvic radiograph revealed complex fracture of the acetabulum, pelvic rami and iliac wing fracture. The ipsilateral sacroiliac joint was also disrupted and displaced (Figure 3A); otherwise no other injury was noted.

The fracture was stabilised through an extended ilio-inguinal approach. A long plate was placed from the alar of the sacrum at the medial iliac wall across the contra-lateral symphysis pubis. Further augmentation with another plate at the sacroiliac joint and ileum were able to achieve stable fixation (Figure 3B). At present, 1 year after the injuries, the patient has returned to his previous occupation with pain-free and full motion of the hip joint.

Case 4

A twenty-two-year-old woman presented with hypovolaemic shock following a motor-vehicle accident. Initial assessment revealed intra-abdominal bleeding and severe pelvic disruption (Figure 4A). Emergency laparotomy identified multiple serosa bleeding with 500 mL of blood collected. Twelve pints of blood and temporary external fixation were able to stabilise the patient haemodynamically.

She underwent definitive stabilisation of the pelvic ring ten days later through bilateral ilio-inguinal approach. The entire pelvic ring was stabilised with a reconstruction plate and the sacroiliac joint was stabilised with two anterior plates (Figure 4B). The incomplete trans-foramina sacral fracture was not fixed with any metal implant, as the pelvic ring was stable throughout the intra-operative spring test. At present, the patient was pain-free and able to walk unaided at three months after surgery.

Discussion

Patients with unstable pelvic fracture usually present with complex problems to the trauma team or orthopaedic surgeon. These injuries are the result of high-energy trauma, which places patients at risk of multiple life-threatening injuries. The aims of management are rapid and accurate initial assessment including x-rays and CT-scan, as well as provisional stabilisation of the pelvic ring by either external fixation or pelvic clamp to maintain haemodynamic stability. Surgical management of severe pelvic ring fracture includes life-saving surgery, "damage-control orthopaedic surgery", early total care and delayed definitive surgery (5,6). However, the displaced pelvic ring disruption has to be reduced and stabilised to prevent a long-term disability.

High-energy pelvic ring disruptions occur in predictable locations. Combination injuries, which occur in the anterior pelvic ring (particularly the pubic rami) and anterior column of the acetabulum with sacroiliac joint disruption, are common in

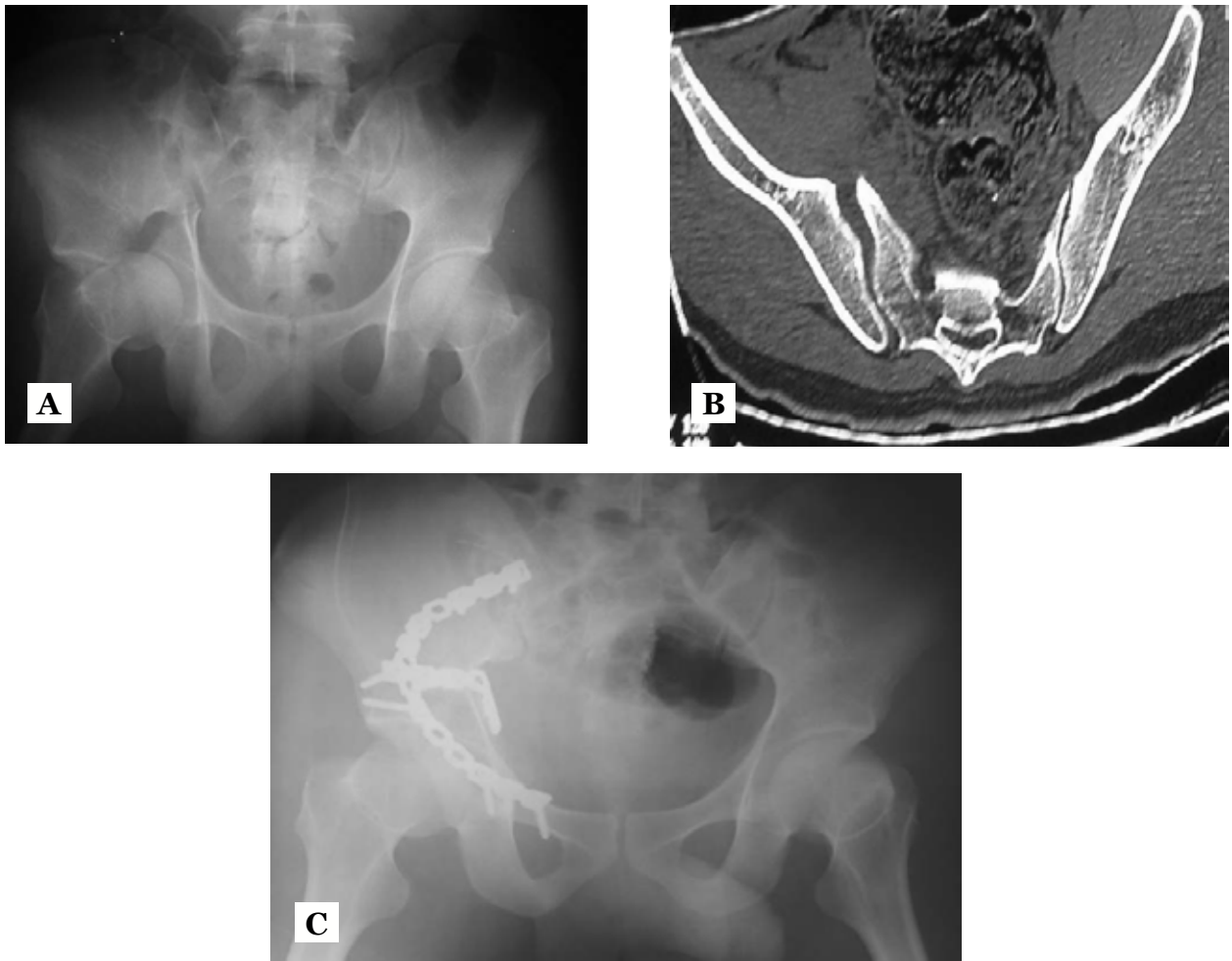


Figure 2: **2A.** Bi-columnar transverse acetabulum fracture. **2B.** Sacroiliac joint disruption confirmed on CT scan. **2C.** Acetabulum and sacroiliac joint reduced and stabilised using a single reconstruction plate through the ilio-inguinal approach.

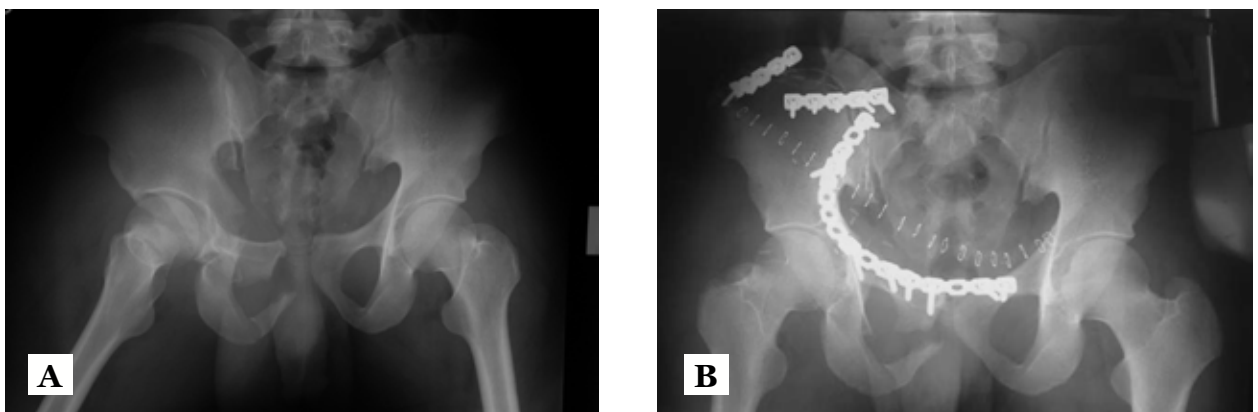


Figure 3: **A.** Radiographs showing complex bi-columnar acetabulum fracture with sacroiliac joint diasthesis. This resulted in floating segments of the ileum and grossly unstable pelvis. **B.** Acetabulum fracture was reduced and the medial segment of the pelvis was stabilised with a long plate. Sacroiliac and ileum fracture was adequately stabilised for early rehabilitation of the patient.

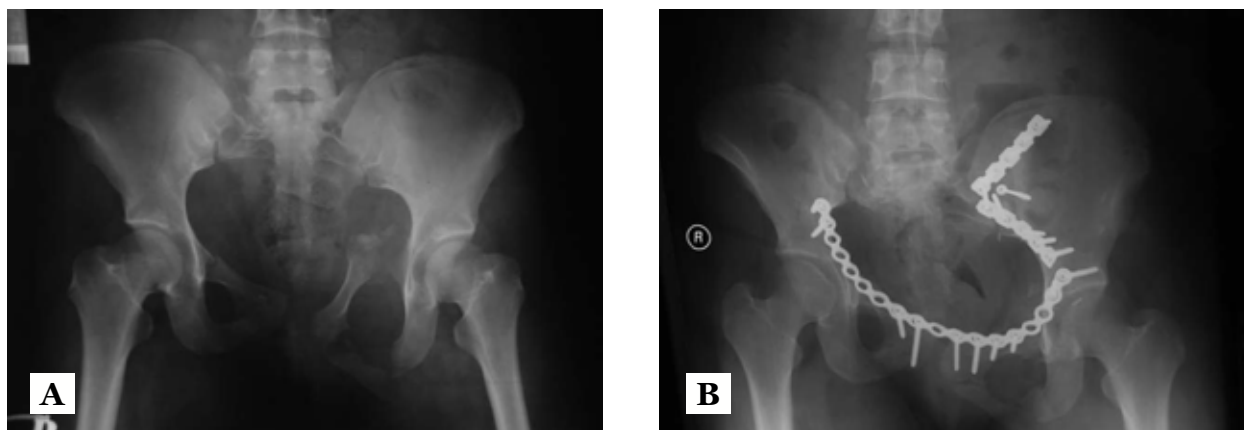


Figure 4: **A.** Severe pelvic ring disruption and unstable pelvis; symphysis pubis diasthesis, left superior and inferior pubic rami fracture, left sacroiliac joint and un-displaced trans-foramina sacral fracture. **B.** The fractures are entirely stabilised with a ring reconstruction plate and two sacroiliac joint plates.

high-energy trauma. Emergency temporary pelvic stabilisation has led to increased survival after pelvic fracture (5,6). A pelvic external fixation frame will stabilise the pelvis, as well as secure the tamponade effect and haematoma formation in the context of ongoing venous bleeding. Pelvic anti-shock clamp may be used to stabilise posterior pelvic ring injury to limit pelvic ring expansion and to control continuous bleeding. However, both constructs have limited roles, especially when the pelvic column is involved and the iliac bone is fractured, as shown in our cases.

Pelvic external fixator alone rarely provides sufficient stability to be used as definitive treatment in high-energy pelvic ring disruption. Lindahl et al. found that pelvic fixation is useful in acute resuscitation but it is of limited value in definitive treatment of unstable type C or even open-book injury (7). Pelvic fixation controls instability, decreases bleeding, diminishes risk of chronic pain and allows early patient mobilisation. Pelvic stability should be achieved immediately after injury, and it plays a vital part in the resuscitation of patients experiencing ongoing haemorrhage (6).

Early pelvic stabilisation has been shown to improve patient outcome (2,3,4,8). The suitable time for definitive stabilisation has been subjected to debate, due to a lack of clear evidence in the literature (6). Haemodynamic stability and local CT scan contrast evaluation for retroperitoneal bleeding are important factors in determining whether early surgical stabilisation is justified. The first case illustrated that early internal pelvic stabilisation did not increase additional risk, provided that the patient was haemodynamically stable throughout the initial resuscitation surgery.

Unstable posterior pelvic disruption such as dislocation of the sacroiliac joint is always complicated with significant disability (1). Adverse consequences of non-operative treatment include leg-length discrepancy, rotational mal-union; prolonged recumbency, delayed neurological compromised and chronic pain (1). Numerous techniques with variable success have been described to overcome this problem. These include anterior plate stabilisation and the posterior iliosacral screw-through percutaneous technique or open methods (2,3,4,8,9). Sacroiliac plating allows direct visualisation of the joint, removal of any intra-articular debris and anatomical reduction of the unstable disrupted sacroiliac joint (2,9). Our approach is anterior because a similar approach was used successfully to stabilise the symphysis pubis diasthesis. Furthermore, extensile Letournel surgical approach for reconstruction of acetabulum fractures provides excellent exposure of the sacroiliac joint for reduction and stabilisation. Lumbar 5 nerve root can be visualised and a nerve injury is preventable.

More than two-thirds of patients with surgically treated unstable pelvic injury returned to their original occupation without any disability, however neurological impairment may compromise the final outcome (1). Open reduction and definitive internal stabilisation provide the best long-term clinical results. Successful treatment of high-energy pelvic injuries relies on early intervention, accurate reduction, stable fixation and low rates of associated injuries and complications.

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ABSTRACTS

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A STUDY ON COGNITIVE IMPAIRMENT AND DEMENTIA AMONG OUTPATIENT CLINICS

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Objectives: To assess the prevalence of cognitive impairment and dementia and to identify its associated factors among elderly patients attending outpatient clinics (KRP & KPP), Hospital Universiti Sains Malaysia.

Patients and Methods: This was cross sectional study involving 399 elderly patients, age 65 years and above, attending outpatient clinics, (HUSM) from January till December 2006 by means of 2 phase procedure. During the phase one screening test, we used validated Malay version of MMSE or ECAQ for illiterate patients. The patients and caregivers were also interviewed using questionnaires on socio-demographic, familial factors, medical history and lifestyles factors. In phase 2, diagnostic study was done on patients with MMSE scores of ≤ 17 or ECAQ of ≤ 5 including 5% of those who scored one or two points above cut-off value in phase 1. The diagnostic study consisted of detailed history and examination of patients which included interview with patient's caregivers. Dementia was diagnosed by means of DSM-IV criteria.

Results: Amongst 399 patients screened, 47 patients were found to have cognitive impairment (43 patients scored positive on ECAQ and 4 on validated Malay version of MMSE). Thus the prevalence of cognitive impairment is 11.8%. Fifty one patients (47 patients who scored positive on screening and 4 screened negative patients) were invited for formal diagnostic testing in phase 2 however only 47 patients were successfully completed phase 2. Ten patients were found positive for dementia. Prevalence of dementia was 2.5%. Female (OR: 6.96, $p < 0.05$), no formal education (OR: 9.11, $p < 0.05$), number of living 8 and more (OR: 6.27, $p < 0.05$) and history of hypertension (OR: 0.35, $p < 0.05$) were significant associated factors for cognitive impairment. Associated factors for dementia (based on univariate analysis) were history of exposure to pesticide and history of stroke.

Conclusion: Prevalence of cognitive impairment and dementia in this study was lower compared to previously reported studies in Malaysia. Female, no education, number of living 8 and above independently predicted cognitive impairment. However, history of hypertension seems to have protective effects. Associated factors for dementia (based on univariate analysis) were history of exposure to pesticide and history of stroke.

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A STUDY ON AMBULANCE RESPONSE TIME IN KUALA LUMPUR, MALAYSIA

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Introduction: Ambulance service is a main part of pre hospital treatment. Time taken to response to emergency call is known to impact on the chain of survival. Ministry of Health (MOH), Malaysia targeted 95% of the ambulances arrive at the scene of the incident within 30 minutes of received of the emergency call.

Objectives: To study the performance of emergency ambulance service based on ambulance response time in Kuala Lumpur, by analyzing the call processing time and dispatch-to-scene time.

Patients and Methods: A retrospective study was conducted at the Medical Emergency Call Centre (MECC), Hospital Kuala Lumpur (HKL) from 1st October 2007 till 31st April 2008. The study was approved by the National Medical Research Registration, Ministry of Health (MOH) Malaysia for approval. All emergency calls for ambulance service were handled by call taker in MECC, HKL. The call taker would interact with the caller to gather critical medical information through an algorithmic set of questions as depicted in Emergency dispatch form and would manually filled up the form. The call taker would determined appropriate medical call triage according to the information collected. With all the gathered information from caller, then the call taker pass it to the call dispatcher in MECC, HKL with the exact time being recorded. Then the call dispatcher would order the responder for ambulance deployment, and the time the responder receiving order from the call dispatcher recorded. The responder would make a decision of the appropriate type (Basic or Advanced) of ambulance team to be deployed. Subsequently, the call dispatcher would obtain from the responder the time of ambulance arrival at the scene or incident site. After completion, the 'Emergency Dispatch Form' was then collected and was kept in the MECC room at HKL.

Results: A total of 525 calls were included in the study and entered for data analysis. 327 of cases involved non trauma patients and the remaining 198 numbers of cases comprised of trauma patients. In this study it was demonstrated that 87.8% ($n=461$) of the cases had a time period of being less than 5 minutes from when the calls were picked up, information obtained and phone triage done until the time responders were activated. Meanwhile 12.2% (64) of cases had registered times of more than 5 minutes. In this study, the dispatch-to-scene time has a mean of 21.9 minutes with standard deviation of 14.9. Although trauma-type calls formed only 37.7% of the calls studied, they showed higher percentages (86.4%) of achieving ART within 30 minutes as compared with non trauma-type of

calls which formed 62.3% of cases and yet only 78.3% achieved ART within 30 minutes. The difference of 8.1% was observed and it was statistically significant.

Conclusion: This study showed that the performance of ambulance service in Kuala Lumpur was still below than expected by the MOH Malaysian Guideline Standard. The achievements by components were for 'call processing time' was 87.8% and 86.4% for the 'dispatch-to-scene time' was 86.4% (trauma calls). Location of patients or incident site did play a significant role in determining the outcome of the ambulance response time. Well link roadways in Kuala Lumpur help emergency responders to attend motor vehicle accidents promptly. The use of either hospital based or NGO based emergency services did not affect the ambulance response time significantly.

Assoc. Prof. Dr. Nik Hishamuddin Nik Ab. Rahman : Supervisor
Dr. Mahathar Abdul Wahab : Co-Supervisor

A STUDY ON THE ASSOCIATION OF SERUM LIPID PROFILE WITH RETINAL HARD EXUDATES AMONG PATIENTS WITH TYPE 2 DIABETES MELLITUS IN HOSPITAL USM

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MMed Ophthalmology

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Introduction: Retinal hard exudate is a component of diabetic retinopathy which is formed due to breakdown of the inner blood retinal barrier, as a process of microangiopathy.

Objectives: This study was done to relate the association between lipid profile and retinal hard exudates in diabetic retinopathy and the association between oxidized-LDL with systemic diseases among type 2 diabetic patients.

Patients and Methods: A cross sectional study was conducted in 40 patients with diabetic retinopathy and another 40 patients without diabetic retinopathy. Demographic data was collected and comprehensive ocular examination was performed. Nine nil venous blood was taken for fasting serum cholesterol, triglycerides, MDL, LDL, ox-LDL, and for HbA_{1c}.

Results: The mean serum cholesterol level was 5.9 (1.86) mmol/L in diabetic retinopathy group compared to patients without retinopathy 5.0 (1.03) mmol/L (P=0.001). The mean serum LDL was 3.6 (1.69) mmol/L in retinopathy group compared to 3.0 (1.02) mmol/L in the control group (P=0.005). There was a higher concentration of serum cholesterol, triglyceride and LDL in patients with severe retinal hard exudates compared to those with mild and moderate, however it was not statistically significant (P= 0.082, 0.116, 0.218) respectively. The mean serum oxidized-LDL concentration was higher in diabetic retinopathy with severe retinal hard exudates compared to mild and moderate. There was no statistically significant difference in the mean oxidized LDL with other systemic diseases or duration of diabetes.

Conclusion: There was significant association between serum cholesterol and LDL with diabetic retinopathy. However there was no association between serum lipid profile with the severity of retinal hard exudates. Serum ox-LDL was also not associated with diabetic retinopathy and other systemic co-morbidities in our study.

Assoc. Prof. Dr. Mohtar Ibrahim : Supervisor
Dr. Bahiah Shaharuddin : Co-Supervisor
Dr. Shatriah Ismail
Dr. Shaharul Baryah

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A STUDY OF OUTCOME OF NEWBORN HEARING SCREENING PROGRAMME IN HUSM FROM JANUARY 2003 TO DECEMBER 2007

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Introduction: Universal newborn hearing screening has been started in HUSM since January 2003. The effectiveness and the challenges of the programme need to be evaluated.

Objectives: This retrospective study aimed to determine the outcome of the newborn hearing screening in HUSM from January 2003 to December 2007.

Patients and Methods: All infants who were delivered in HUSM were screened for hearing impairment with portable distortion product otoacoustic emission (DPOAE) before discharge. When they failed the initial screening, a second screening with DPOAE was arranged in 6 weeks. When these newborns failed the second screening, an audiologist performed a diagnostic auditory brainstem response (ABR) test to confirm the hearing loss. In this study, the data of 16,100 newborns were traced from the newborn hearing screening record. The data such as sex, race, age, results of the first, second and third screening were analyzed with SPSS 16.0.

Results: The study showed that the coverage of the UNHS was 98%. The prevalence of hearing impairment was 0.09%. The prevalence of initial screening refer rate was 25.5%. The prevalence of defaulters in second and third screening was 33.9% and 40.7% respectively. The age of detection of hearing impairment was 3.31 months (S.D. 0.86). The age of hearing aid fitting was 13.57 months (S.D.4.82)

Prof. Dr. Dinsuhaimi Sidek : Supervisor
Dr. Mohd Khari M. Daud : Co-Supervisor

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THE ROLE OF ANATOMICAL VARIATIONS IN OSTEOMEATAL UNIT THE AETIOLOGY OF CHRONIC RHINOSINUSITIS

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Introduction: The complexities of the nose and paranasal sinuses anatomy, as well as their multiple functions make the sinuses an interesting and rewarding topic of study especially at the region of osteomeatal complex (OMC). Congenital anomaly in this region, though rare, may create technical difficulties during surgery. However, the role of anatomical variations in pathogenesis of sinusitis is still unclear.

Objectives The aims of the study were to look into the anatomical variations in the osteomeatal complex in chronic rhinosinusitis patients, to determine the association between bony anatomical variations in osteomeatal complex with chronic rhinosinusitis (CRS) and to determine the main anatomical variations in the osteomeatal complex which are usually depicted by computed tomography (CT) and nasoendoscopy in patients with CRS and normal patients without CRS.

Patients and Methods : A case control study was done in

which we had reviewed the CT scan of paranasal sinuses (HRCT) images of 240 individuals, 120 cases of CRS and another 120 patients without CRS problem. Their paranasal sinuses CT scan obtained 1.25mm thicknesses in axial and coronal planes with high resolution technique were reviewed. The data were analysed using Pearson CM Square test.

Results : The anatomical variations recorded were: Concha bullosa in 49 cases (40.8%) amongst the CRS and 57 cases (47.5%) among patients without CRS, Paradoxical middle turbinate in 14 cases (12.0%) of CRS and 27 (23.0%) in patients without CRS, pneumatized uncinate process were found in 3 cases (3.3%) of CRS cases while in patients without CRS 3 cases (3.3%) as well, Hailer's cells (infraorbital ethmoid cell) in 61 (51.0%) cases of CRS while 75 cases (62.0%) cases of patients without CRS, pneumatized agger nasal cell 100 (83.0%) in CRS and 95 (79.0%) in patients without CRS, deviated nasal septum in 67 cases (56.0%) of CRS while in patients without CRS 73 (60.8%) cases and lastly pneumatized septum seen in 4 (3.3%) cases of CRS and none in patients without CRS. However 'the presence of single anatomical variation itself does not have significant association with the genesis of CRS except for paradoxical middle turbinate and infraorbital ethmoid cell.

Conclusion : The most common anatomical variation in osteomeatal complex in CRS patients are pneumatized agger nasi cell, followed by haller's cell, DNS, right concha bullosa, left cciicba buflosa, paradoxical middle turbmate, pneumatize uncinate process and lastly septum pneumatization. And the single occurrence of an anatomical Vanant itself does not establish the genesis of the CRS thsease except for paradoxical middle turbinate and infraorbital ethmoid cell.

Dr. Shamim Ahmed Khan : Supervisor
Dr. Rushdan Ismail : Co-Supervisor
Dr. Rohaizan Yunus

A PRELIMINARY STUDY TO COMPARE THE PREDICTION ERROR OF POSTOPERATIVE REFRACTION IN PAEDIATRIC CATARACT SURGERY BETWEEN 2 DIFFERENT INTRAOCULAR LENS POWER CALCULATION FORMULAS

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Introduction : The treatment of paediatric cataracts has progressed tremendously in the past 15 to 20 years. There is a growing trend towards intraocular lens implantation in infants and younger children whose eyes are still undergoing rapid growth and refractive changes.

Objective : This study assessed the predictability of desired refractive outcomes at 3 month postoperative period in paediatric patients undergoing cataract surgery with primary placement of an intraocular lens.

Patient and Methods : This was a randomized interventional study of 31 eyes (24 patients) that successfully underwent cataract surgery and intraocular lens implantations. All patients were 12 years old and below. Intraocular lens power calculations were made using either SRK II or Modified Formula For Paediatric IOL Calculation. The postoperative refractive outcome was taken as the spherical equivalent of the refraction at 3 month postoperative follow-up. The prediction error was taken as the absolute difference between the predicted and the actual refraction. The data were analysed to compare the mean prediction error between SRK II and Modified Formula

and evaluate the predictability.

Results : The mean prediction error in the SRK II group was 1.03 (0.69) D while in Modified Formula 1.14 (1.19) D. The SRK II group showed lower prediction error of 0.11 D compared to Modified Formula group, but this was not statistically significant ($p > 0.05$). 18.75% eyes in SRK II group achieved good predictability i.e. the refraction postoperatively was within ± 0.5 D from predicted refraction compared to 46.67% eyes in the Modified Formula group. However the difference of the predictability between the two formulas was also not statistically significant.

Conclusion : We concluded that the predictability of postoperative refraction in paediatric cataract surgery was comparable between Modified Formula and SRK II formula. The existence of the Modified Formula provided an alternative to the ophthalmologist for intraocular lens calculation in paediatric patients.

Dr. Zunaina Embong : Supervisor
Dr. Bakhiah Shaharuddin : Co-Supervisor
Dr. Raja Azmi Mohd Noor

A PILOT STUDY INTO THE EFFECTS OF LABISIA PUMILA (KACIP FATIMAH) VAR ALATA WATER EXTRACT ON POST MENOPAUSAL WOMEN

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MMed O & G

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Introduction: *Labisia pumila* (Kacip Fatimah) is a traditional herb commonly used by Malay women during the peripartum period to make the process of childbirth easier and also as traditional herbal preparation during the postpartum period to improve the general condition of the mother. Recent research on Kacip Fatimah has shown that it has estrogenic-like activity, behaves like a phytoestrogen and has no adverse effects in premenopausal women.

Objectives: The effects of Kacip Fatimah spray dried water extract on post menopausal Malay women by studying safety profiles such as haematological indices, liver and renal function, the cardiovascular effects from body mass index and waist hip ratio, the effects on blood pressure, were investigated ECG and chest x-ray, the effects on the breast by studying mammographic changes, the effects on the pelvis from cervical smear reports. The investigation also include a review of undesirable side effects should these occur.

Patient and Methods: This was a randomized, double blind, placebo controlled study comparing three different doses of sprayed dried water extract of Kacip Fatimah against a placebo on post menopausal Malaysian women. 70 eligible participants were divided into four groups. Group 1 received 140 mg /day Kacip Fatimah, Group 2 received 280 mg / day Kacip Fatimah, Group 3 received 560mg /day Kacip Fatimah and group 4 received placebo (maltodextrime) Pre study screening and assessment was done and if the subject was eligible informed consent was taken and baseline investigations including hematological, liver and renal function tests, chest x ray, electrocardiography (ECG), mammography, pelvic ultrasound and pap smear were performed. Those respondents with normal baseline investigations were invited to participate in this study. The study duration was six months and all participants were required to come to the clinic for clinical examination two monthly. During each visit the participants were asked

regarding any side effects of medication or occurrence of any vaginal bleeding. Participants were also required to come for a supply of medication each month and the investigator team had to ensure that all participants complied with the medication and scheduled visits. On visit 4, all blood investigations, blood pressure, chest x ray, ECG, mammography, pelvic ultrasound and pap smear were repeated again.

Results: There was no significant difference seen in demographic profile between the four groups of postmenopausal women. No significant changes were seen in the measurement of body mass index and the waist hip ratio at the end of study period. In terms of haematological indices, liver and renal function, no significant changes were seen in each of the four groups. Other investigations such as blood pressure, chest x ray, ECG, mammography, pelvic ultrasound and pap smear were all normal. There were 11 patients who developed vaginal bleeding or spotting. Ultrasound and pipelle sampling were carried out to investigate the cause of the bleeding. Secretory phase endometrium were identified in two participants, proliferative phase endometrium was diagnosed in one participant, changes compatible with postmenopausal endometrium in one participant. The results of the remaining six participants showed inadequate tissue for diagnosis. One patient had no pipelle sampling report as she refused this investigation.

Conclusion: Based on the findings of this research, consumption of spray dried water extract of *Lab Esia pumila* (Kacip Fatimah) up to 560 mg per day for six month would appear safe and has not been shown to cause any adverse effect to the body, however the incidence of vaginal bleeding in this study is of concern and require further evaluation in a larger sample size study with proper investigation of post menopausal bleeding should it occur by ultrasound scan, pipelle endometrial sampling and hysteroscopy with dilatation and curettage if indicated.

Prof. Dr. Mohd Shukri Othman : Supervisor

A STUDY OF MITOCHONDRIAL A1555G MUTATION IN AMINOGLYCOSIDE INDUCED OTOTOXICITY

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MMed ORL-HNS

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Introduction: Aminoglycoside induced ototoxicity is one of the most common causes of acquired deafness, involving the auditory and vestibular system. The Vestibular and Audiototoxicity is frequently irreversible. In developing countries, like Malaysia, aminoglycoside are more routinely used even for a minor infection, since it is relatively cheap and easily available. However, studies conducted in Asian country had proved that aminoglycoside caused 10 to 20% of hearing loss. Mitochondrial A1555G Mutation - An alteration or change, as in nature, form, or quality of a gene that maternally inherited gene during fetal life and has an association with hearing loss. People who carry the genetic mutation of mitochondrial DNA (mtDNA) A1555G, has a higher risk of having hearing loss when expose to aminoglycoside drug (eg: streptomycin, gentamycin etc) compare to them who do not carry the mutation gene. A study which was conducted in Japan, found that 40% of the patient who had hearing loss due to this drugs, also carry the mtDNA A1555G mutation. From this study, we hope that we could detect early genetic A1555G mutation, especially in newborn baby, so that prevention can be made early and precaution

should be taken before prescribing aminoglycoside drugs.

Objectives: The purpose of this study was to determine the prevalence and association of mtDNA A1555G mutation in subjects with aminoglycoside-induced ototoxicity.

Patient and Methods: A cross sectional study was carried out in Otorhinolaryngology clinic HUSM and PKB, Kota Bharu from June 2007 to May 2008. Twenty two subjects with aminoglycoside induced ototoxicity and twenty two control subjects without ototoxicity after exposed to aminoglycosides were included in this study. Ototoxicity was confirmed by pure tone audiometry and distortion product otoacoustic emission (DPOAE). All eligible and consented patient was underwent buccal mucosa swab for further genetic analysis for mtDNA A1555G mutation.

Results: There was 1 subject (4.54%) in the aminoglycoside-induced ototoxicity group identified to have the mtDNA A1555G mutation. There was no significant association between mtDNA A1555G mutation and ototoxicity in this study (P=0.500).

Conclusion: In our study, we found mtDNA A1555G mutation in one of our subject who had hearing loss secondary to ototoxic drug. Though, it was not significant statistically. There is a possibility that the prevalence of this mtDNA mutation is truly very low in our population. However, a further larger study with a bigger sample size and a wider area of coverage need to be done to confirm our finding.

Dr. Mohd Khari Md Daud : Supervisor
Prof. Dr. Dinsuhaimi Sidek : Co-Supervisor

COMPARISON BETWEEN ENDOSCOPIC DACRYOCYSTORHINOSTOMY (EDCR) WITH AND WITHOUT NASOLACRIMAL SILICONE STENT

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MMed ORL-HNS

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Introduction : Endoscopic dacryocystorhinostomy is a procedure of treating obstructed nasolacrimal duct endoscopically. All this while these cases were treated by the ophthalmologist with a procedure called external DCR. With the development of endoscopic surgery, otorhinolaryngologist gives another option to patients with obstructed nasolacrimal duct, with a scarless DCR. It was thought that stenting is crucial in maintaining the patency of post DCR cases. However, stenting is noted to cause few post operative complication and prolonged the duration of operation.

Objectives : The objectives of this study was to compare the outcome of EDCR cases with nasolacrimal silicone stenting and without stenting. Apart from that, this study was performed to compare the operative time used by these two groups and to see the complications that developed post operatively.

Patients & Methods : This was a cross sectional study of patients that underwent EDCR at Hospital Alor Setar from June 2004 till December 2007, included minimum of 6 months follow up post operatively. Forty eight patients with problem of obstructed nasolacrimal duct underwent DCR endoscopically. Twenty three patients underwent DCR with nasolacrimal stent and 25 patients underwent DCR without stenting. The demographic data, post operative patency, duration of operations and post op complications were evaluated.

Results : The EDCR without stenting showed higher patency result post operatively, compared to the group with stenting (95.9% vs 80.7 %). Apart from that, the operative time

taken for non stenting group are shorter and post operative complications, especially formation of granulation tissue and pain is statistically less than the stenting group.

Conclusion : EDCR without stenting has higher patency rate, shorter operation time and lower post operative complications.

Prof. Dr. Dinsuhaimi Sidek : Supervisor
Dr. Shamim Ahmad Khan : Co-Supervisor
Dr. Zulkifli Yusof

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THE VALUE OF ROUTINE PORTABLE CHEST RADIOGRAPH OF PATIENTS IN INTENSIVE CARE UNIT (ICU).

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MMed Radiology*

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Introduction : Since the advent of ICU set up, there has been two schools of thought regarding the usage of routine portable chest radiograph in critically ill patients. One school of thought says that routine portable CXR will give a significant number of unsuspected findings while the other claimed that it just increased radiation dose to the patient and little benefits obtained. At present, two standards of care have evolved, one using routine CXR and the other using clinically indicated CXR. In Hospital Universiti Sains Malaysia (HUSM), some of our ICU physicians practised routine CXR particularly in cardiopulmonary disease with some modification while some of them requested CXR when clinically indicated. Our study aim is to determine the diagnostic and therapeutic efficacy of routine chest radiographs in ICU HUSM.

Patient and Methods : This study was a cross sectional design conducted in HUSM starting from October 2006 till March 2007. 1016 CXRs were obtained within the study period and 501 portable CXRs were included in this study. The CXR were analysed and divided into routine and non routine groups. The CXR findings were recorded. The images were reviewed by a trainee radiologist using GE Pathspeed Diagnostic Workstation in the department of radiology. Approval from the Research Board Committee was obtained prior to study.

Results : Fifty five (17.3 %) CXRs showed new lesions or significant abnormality in routine group, compared with 132 (72.1 %). There was a significant difference of detecting new lesion between routine and non routine with p value of 0.001. The CXR with new findings that resulted in an intervention was significantly higher in non routine compared to the routine groups, 68.9 % and 9.4 % respectively with p value of 0.001.

Conclusion : The non routine protocol yielded better diagnostic and therapeutic efficacy than routine CXR. The use of routine CXR is not cost effective. It resulted in an increased radiation dose to the patient with only little benefits.

Assoc. Prof. Dr. Haji Abdul Kareem : Supervisor
Assoc. Prof. Dr. Mohd Ezane Aziz : Co-Supervisor

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OUTCOME OF FRACTURE NECK OF FEMUR TREATED BY REPLACEMENT SURGERY IN THE ELDERLY

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Introduction : In the new millennium we expected to see significant increase in life expectancy, hence an increase in elderly population. Osteoporotic related fractures such as femoral neck fractures are expected to be on the rise in incidence and carry significant morbidity and mortality. The incidence of hip fracture in Malaysia is 88 and 218 per 100,000 men and women respectively (NOF, 2005). The optimal surgical treatment of displaced femoral neck fractures in elderly with Osteoporosis remains controversial. Surgical options include prosthetic replacement (arthroplasty) and internal fixation. Arthroplasty options include hemiarthroplasty, bipolar arthroplasty, and total hip arthroplasty. The mortality rate ranged from 14% - 30% in the first year (Kenzora 1984). The decision of whether to internally fix or replace a displaced femoral neck fracture is based on life expectancy, the presence of chronic disease, bone quality, and level of function at the time of fracture, as well as on expected function.

Patients and Methods : A retrospective study to assess the outcome of traumatic fracture neck of femur in the elderly aged above 65 years, from 1st January 2001 to 31st December 2005 treated by replacement arthroplasty (Unipolar or Bipolar Hemiarthroplasty), was conducted in Hospital Universiti Sains Malaysia. Outcome was scored via follow up at Orthopaedic clinic or telephone interview from the patient or primary care giver using the Harris Hip score (Appendix 2). Prevalence of Osteoporosis in these patients was also reviewed using Singh Index (Appendix 3). There were 45 cases of traumatic neck of femur fracture treated with hemiarthroplasty or Bipolar Hemiarthroplasty that met the criteria for this study. All parameter was analysed using SPSS Version 12.0.1(2007). All data obtained from this study underwent descriptive analysis with regards to sociodemographic pattern.

Results : The mean age was 75.13 years (range, 65 to 89 years). There were 39 women (86.7%) and 6 men (13.3%). Out of these 45 patients, 15 (50 %) had returned to their pre-morbid ambulatory status. Functional Outcome, 7(15.6%) patients obtained a Harris Hip Score of excellent (90-100), 13(28.9%) obtained a score of good (80-89), and 11(24.4%) obtained a score of fair (70-79), 3(6.7%) obtained a score of poor (60-69) and 1 (2.2%) obtained scores of failed (<60), 6 represented (22.2%) patients who were not amenable for scoring. A total of 68.9 % of patients had a cumulative fair HHS score, which represented good functional outcome. Patients who had Bipolar replacement had better functional outcome. 11 out of 16 patients returned to their pre-morbid status of being community ambulators and fared well in the Harris Hip Scores. Patients who were walking independently before trauma had a better outcome than those who needed aid to walk. Only half (50%) of the patients who were initially community ambulators returned to their pre-morbid status. The degree of osteoporosis was assessed using the Singh index. 33 patients (73.3%) had significant established osteoporosis with Grade 1 (2 patients), Grade 2 (9 patients), grade 3 (22 patients) respectively. Within 1 month of surgery 2 (4.4 %) patients had died and subsequently 7 (15.6%) had died after 1 year post operatively.

Conclusion : The functional outcome of Hemiarthroplasty was 68.9% of patients obtaining good results which is acceptable and among these cases patients who underwent Bipolar Hemiarthroplasty showed better outcome with 68.75% having excellent to good outcome as compared to Unipolar Hemiarthroplasty. 11 out of 16 cases had returned to their pre-morbid status as community ambulators. Post operative functional outcome in terms of ambulation showed 50 % of patients returning back to their pre-morbid ambulatory status. There was a definite correlation between the incidence of

fracture neck of femur and Osteoporosis. In this study, 73.3 % of patients showed evidence of established Osteoporosis. This would support the decision to choose Replacement Arthroplasty rather than Internal fixation in Garden type 3 and 4. The mortality rate in this study was 15.6 % within the first year of operation which is within an acceptable range as compared to other studies which rates was as high as 30 %.

Assoc. Prof. Dr. Wan Faisham Nu'man Wan Ismail: Supervisor
Assoc. Prof. Dr. Imran Yusof : Co-Supervisor

THE PREVALENCE AND RISK FACTORS FOR IN-HOSPITAL MORTALITY AMONG COPD PATIENTS ADMITTED TO HOSPITAL UNIVERSITI SAINS MALAYSIA

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MMed Internal Medicine

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Introduction : Chronic Obstructive Pulmonary Disease (COPD) is still one of the leading causes of morbidity and mortality worldwide and it is the only disease which is showing increasing trend of mortality. However, the factors that determine the outcome of patients with COPD are still poorly understood, therefore it is very important to identify factors that influence patient's survival in order to plan for effective treatment strategy in reducing the morbidity and mortality.

Objectives : The primary objective for this study is to estimate the prevalence of in-hospital mortality of COPD patients admitted to Hospital Universiti Sains Malaysia (HUSM). The secondary objective is to determine the risks of mortality among COPD patients who had been admitted to HUSM.

Patient and Methods : A total results of 324 patients were recruited into this retrospective observational study. The prevalence of in-hospital mortality of COPD patients is 26.1%. The commonest cause of death was acute exacerbation of COPD which contribute 41% of total mortality, and the second commonest cause of death was cardiac related death (33%). The factors that had been identify to increase the risks of mortality in COPD after adjustment with multiple logistic analysis are smoking, duration of the disease, number of ICU admission, presence of pneumonia, level of serum albumin and the level of carbon dioxide arterial tension (pCO₂).

Conclusion : The mortality rate of COPD patients is high and the mortality risks are smoking, duration of disease, number of ICU admission, presence of pneumonia, level of serum albumin and the of carbon dioxide arterial tension (pCO₂).

Dr. Che Wan Aminud-din Hashim : Supervisor
Dr. Shaharudin Abdullah : Co Supervisor

A PROSPECTIVE STUDY ON THE OUTCOMES OF IN-HOSPITAL CARDIAC ARREST (IRA) CASES RECEIVING CARDIOPULMONARY RESUSCITATION (CPR) IN TWO TERTIARY HOSPITALS IN MALAYSIA.

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Introduction : Resuscitation medicine is an integral part of acute emergency care. The advancement in this field has led to evidence-based international guidelines on the practice of CPR which has improved and standardized its practice. Coupled with the usage of the Utstein style to report on outcomes of CPR, this has further enhanced increasing standardized research in this arena. However, it remains to be proven whether these results of research mainly from other countries is applicable to our local population. There is currently a severe lack of CPR research in Malaysia. This study aimed to tackle this by investigating the rates of survival outcomes for in-hospital cardiac arrest (NA) in our population. This study also served as a starting point to hopefully create a database that other centers in the nation would be able to add on to.

Objectives : The objective of this study was to determine the outcomes of CPR in In-Hospital Cardiac Arrest (11-IA) cases. The endpoints were looking at the success rate of achieving ROSC (return of spontaneous circulation), immediate survival (ROSC at least 20 minutes), 24-hour survival and survival-to-hospital discharge. Another objective is to identify any factors that could lead to the improvement in these endpoints.

Patients and Methods : This is a prospective study using convenient sampling. It was conducted from March, 2007 until December, 2007. The standard Utstein in-hospital CPR reporting form was distributed to all locations involved in this study and a briefing was given to all the staff involved in these areas at the beginning of the study. Any case of IHA requiring CPR in these areas was then included into the study and a member of the primary resuscitating team would fill in the form. The forms were then collected and the follow up of the patients that survived were then conducted until the patients were discharged from hospital or passed away in hospital.

Results : In this study, survival outcomes for IHA receiving CPR obtained were a rate of ROSC of 61.2% (n=60), immediate survival rate of 38.8% (n38), 24-hour survival of 14.3% (n14) and a survival-to-discharge rate of 8.16% (n=8). The mean age of the study population was 50 years and 59% were in the middle-age range of 40 to 70 years. The males outnumbered females by a ratio of 2.1: 1. Majority of IHA events were monitored (91%) and witnessed (99%). The main immediate causes of arrest were hypotension (41.8%), myocardial infarction (19.4%) and respiratory depression (17.3%). When divided into cardiac causes versus noncardiac, the percentages were 29.6% vs 70.4% respectively. The time from collapse to CPR was less than 1 minute in 76.5%. The initial rhythms detected at IHA were bradycardia (n=31), asystole (n=28) and PEA (n=27). The immediate survival of day time versus night time IHA was 45.6% vs 29.3%. The other survival rates were not affected by time of IRA. The only significant factor associated with 24-hour survival and survival-to-discharge was having a shockable rhythm at time of arrest.

Conclusion : In conclusion, the survival outcomes for IRA with CPR obtained were a rate of return of spontaneous circulation (ROSC) of 61.2% (w'60), immediate survival rate of 38.8% (n=38), 24-hour survival of 14.3% (n14) and a survival-to-discharge rate of 8.16% (n8). A shockable rhythm at the time of IHA was the only significant factor to affect outcomes.

Assoc. Prof. Dr. Nik Hishamuddin Nik Ab. Rahman : Supervisor
Assoc. Prof. Dr. Rashidi Ahmad : Co-Supervisor

A COMPARISON OF SUTURING TECHNIQUE FOR REPAIR OF EPISIOTOMY A RANDOMISED CONTROL TRIAL OF MALAY PRIMIGRAVIDAE IN HOSPITAL UNIVERSITI SAINS MALAYSIA KOTA BAHRU, KELANTAN, MALAYSIA.

Dr. Maizun binti ishak

MMed O & G

Department of O & G,
School of Medical Sciences, Universiti Sains Malaysia,
Health Campus, 16150 Kelantan, Malaysia.

Objective : To determine the outcome of episiotomy repair between 2 suturing techniques – continuous subcuticular technique and transcutaneous interrupted technique among Malay primigravida.

Patients And Methods: A 6 months prospective randomized study was done on 200 Malay primigravida in Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan, who had spontaneous vaginal delivery (SVD) from August 2005 till January 2006. This study was conducted to compare the outcome of two suturing techniques where continuous subcuticular technique and transcutaneous interrupted were analysed technique. The outcome measured were perineal pain at 24 hours, 10 days and 3 months and also dyspareunia after 3 months post-delivery.

Results : A total of 200 patients (100%) were able to be reviewed at 24 hours, 148 patients (74%) at day 10 and 131 patients (65.5%) were reviewed at 3 months post-delivery. There was significantly less time taken to repair episiotomy in subcuticular continuous technique compared with transcutaneous interrupted technique (17.2 mm vs 24.8 min, $p < 0.05$). However, there was no significant difference in perineal pain score at 24 hours (2.4 vs 2.3, $p = 0.674$), 10 days (2.3 vs 2.6, $p = 0.361$) and 3 months (1.3 vs 1.3, $p = 0.728$) post-delivery. There was no difference in dyspareunia at 3 months post-delivery review in both groups ($p = 0.33$). The perineal pain at 24 hours was significantly correlated with episiotomy length after repaired ($r = -0.185$, $p = 0.009$). However, there was no significant difference in pain on ambulation at 24 hours ($p = 0.487$) and 10 days ($p = 0.712$), use of analgesia at 24 hours ($p = 0.841$), 10 days ($p = 0.909$) and 3 months ($p = 0.334$) post-delivery. There was no difference in urinary or bowel problems for both groups at 24 hours and 10 days ($p > 0.05$). There was no statistically significant difference in wound morbidity of both group studied at 24 hours and 10 days post-delivery ($p > 0.05$).

Conclusion : Subcuticular continuous technique should be given as an option when repairing an episiotomy. Even though there was no reduction in the symptoms of perineal pain and dyspareunia, this method significantly required less repairing time.

Assoc. Prof. Dr. Nik Mohamad Zaki : Supervisor

A RANDOMIZED CONTROLLED TRIAL OF PARENTERAL GLUTAMINE IN NEWBORN RECEIVING TOTAL PARENTERAL NUTRITION (TPN) IN HOSPITAL UNIVERSITI SAINS MALAYSIA (HUSM)

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MMed Paediatrics

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Campus, 16150 Kelantan, Malaysia.

Introduction : Glutamine is a conditionally essential amino acid. Addition of glutamine in TPN of critically ill adults causes a reduction of complications such as infection. In neonates however, no clear benefits of addition of glutamine to TPN were shown in a limited number of studies, mainly performed in high income countries.

Objectives : The addition of glutamine to standard TPN in neonates in HUSM, Malaysia was studied to see if it would

improves selected neonatal outcomes.

Patients and Methods : This was a double blinded randomized controlled trial. Babies admitted to the NICU during the 1-year study period, requiring TPN were eligible for inclusion. Subjects were randomized either to receive glutamine added TPN (intervention) or standard TPN (control). Primary outcome measures included time taken to reach full enteral nutrition, incidence of sepsis and NEC, time taken to achieve extubation, and time to discharge from NICU.

Result : Out of 270 subjects included in the study 132 were randomized to the intervention group and 138 to the control group. There were no significant differences between the two groups in terms of baseline data. The median time taken to reach full enteral nutrition was similar for both intervention and control group (6 days in each group, $p = 0.52$). The time taken to achieve extubation was also similar in both groups (2 days in each group, $p = 0.76$). The incidence of necrotising enterocolitis in the intervention group was slightly lower than for the control group but the difference was not significant (5.8% vs. 7.1% $p = 0.68$). The incidence of clinical sepsis and culture proven sepsis was also not significantly different in the intervention and the control group (15.7% vs 10.2%, $p = 0.21$ and 16.5% vs 15.7%, $p = 0.38$ respectively). Sub-group analysis for preterm and term babies for the same outcomes also showed no statistically significant differences

Conclusion : Addition of glutamine to TPN for neonates was not shown to improve outcome.

Assoc. Prof. Dr. Hans Van Rostenberghe : Supervisor
Prof. Dr. Quah Ban Seng : Co-Supervisor
Dr. Noraida Ramli

COMPARISON FOR EASE OF INSERTION FOR LARYNGEAL MASK AIRWAY BETWEEN TRENDLENBURG AND SUPINE POSITION FOR ELECTIVE ORTHOPAEDIC, SURGICAL AND GYNECOLOGY SURGERY

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MMed Anaesthesiology

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Health Campus, 16150 Kelantan, Malaysia.

Objectives : The purpose of this study is to assess the ease of insertion of the Laryngeal Mask Airway (LMA) between supine and trendelenburg position in the elective Gynecology, Orthopedic and General surgery patient.

Patients and Methods : We measured easiness of insertion, incidence of adverse respiratory complication and hemodynamic response to LMA insertion. A randomized single blinded prospective study was conducted involving a total of 92 premedicated, ASA 1 or 11 patients, aged 18 to 65 years and were divided into 2 groups either insertion in supine or trendelenburg position. After a standardized induction of anesthesia with Fentanyl 1.5 mcg/kg and propofol 2 mg/kg, a size 3 or 4 Laryngeal mask airway was inserted and the patient breathe spontaneously through the surgery with no muscle relaxant given. Anesthesia was maintained with nitrous oxide, oxygen and sevoflurane. The LMA was removed at the end of surgery with the patient fully awake. The speed and ease of insertion and the number of attempts needed to successfully secure airway were recorded. The incidence of adverse respiratory complications like sore throat, presence of blood on LMA, laryngospasm, coughing, vomiting and desaturation was recorded. Hemodynamic changes such as systolic blood pressure, diastolic blood pressure, mean arterial pressure and

heart rate at the different time interval were recorded.

Results : We found that there was no statistically significant difference in time required for successful insertion and number of attempts for both group. We were able to insert LMA at first attempt in 73.9% within 20.20 seconds in trendelenburg position. There were no differences in incidence of adverse airway complication both in supine and trendelenburg position. Both groups had no statistical differences in hemodynamic parameters during spontaneous ventilation under anesthesia except systolic blood pressure and mean arterial pressure just after LMA insertion, which had statistically significant.

Conclusion : We concluded that, insertion of the LMA in trendelenburg position is appropriate provided with a good experience and proper patients selection and strongly indicated in the scenario of fail intubation and ventilation as an alternative to the conventional method of LMA insertion.

Dr. Mahamarowi Omar : Supervisor

Dr. Aisai Abd. Rahman : Co-Supervisor

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MULTIMODALITY ASSESMENT OF MILD AND MODERATE HEAD INJURY PATIENTS: AN ANALYSIS

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MSurg Neurosurgery

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School of Medical Sciences, Universiti Sains Malaysia,
Health Campus, 16150 Kelantan, Malaysia.**

Objective : The aim of this research was to compare the disability that may be proven using the analysis of the BNI [Barrow Neurological Institute] Screen for Higher Cerebral Functions, disturbance in balance control, sensory perception and presence of nystagmography.

Patient and Methods : This was a prospective study conducted from August 2006 till November 2007, where two groups of patients that had sustained mild and moderate head injury were selected. There were patients that would be admitted to or referred to University Sains Malaysia Hospital in Kubang Kerian, Kelantan. The qualified patients would be called within 4 to 6 weeks after the head injury. Their complaints of headache, memory loss, sensory disturbance, gait and visual abnormalities would be taken into the questionnaire. They would be given a battery of test starting with the BNI [Barrow Neurological Institute] Screen for Higher Cerebral Functions, conducted in the local language [Bahasa Malaysia] The test for balance would be conducted by using the SENSORY ORGANIZATION TEST [SOT] using the SMART Balance Master®, [NeuroCom International, Inc. 9570 SE Lawnfield Road Clackamas, OR 97015 USA]. The result would be analyzed on how visual, somatosensory, and vestibular inputs affect a patients ability to maintain functional balance. Nystagmus would be tested by using the VisualEyes Nystagmography. The stimulus would be delivered via a tower, using the VisualEyes tower Nystagmography [Micromedical Technologies, 10 Kemp Drive Chatham, Illinois 62629 United States of America]. The movement of the eye would be followed, recorded and analyzed for abnormalities. The next test would be conducted by using the Computerised Assisted Sensory Evaluator [CASE IV version 4.27.1; WR Medical Electronics Co. 123 North Second Street, 'water, MEN 55082 Minneapolis USA]. Cold detection threshold [CDT], were measured using the 4, 2, and 1 stepping algorithm with null stimuli as described by Dyck et. al, performed on the dorsal aspect of the palm on the left hand. The subjects would the "yes" or "no" buttons depending on whether they felt the stimuli and the computer will calculate the 'just noticeable

difference" [JND] from the subject's

Result : The result of the research would be calculated using the SPSS software version 12.0 [2003] 11 patients were male [91.67%] and 1 female [8.33%]. The age of the patient recruited into this study ranges from 18 years to 63 years [45 years] with a mean age of 33.25 years and median of 29.00 years. Among the number of patients admitted for the study, majority belongs in the moderate head injury category [75%]. The mode of injury sustained in our study comes from motor vehicle accident [100%]. In our study, patients in the mild head injury category have a higher BNI score and compared to those in the moderate head injury group. The mean score of the patients in the mild head injury group have a mean score of 45.00 as compared to 43.11 in the Othoderate head injury group. The BN1 sub-test score of these patients also indicated that the mild head injury patients' scores were higher than to moderate head injury although it statistically irrelevant [Mann-Whitney test]. In particular, the mean scores were noted higher in total score of BNI, and it's sub-score particularly speech and language, attention/concentration, visuospatial and visual problem solving, and memory. statistically however, none of these observed values were significant. In our study, we failed to disclose any evidence to support that moderate head injury has significant disturbance of balance. Both groups of patients had normal balancing reflex on testing the computerised posturography. There was no significance sensory disturbance noted between groups of patients. The Mann-Whitney test concluded no significance [p= 0.67]. In our study also, we failed to show any evidence of post-traumatic Nystagmography. This was in conclusion made by Mallinson, et in 1995 that ENG was rarely helpful diagnostically in head-injured group [trauma group].

Conclusion : From the test conducted, we concluded that the disability that could be demonstrated in patients with complaint of forgetfulness and lack of concentration cannot be demonstrated by using the BNI [Barrow Neurological Institute] Screen for Higher Cerebral Functions. Other tests done in this study, failed to prove any significant result. Therefore cause and effect of post-concussive syndrome can only be diagnosed by clinical diagnosis.

Prof. Dr. Jafri Malin Abdullah : Supervisor

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SLEEP DISORDERS AMONG CHILDREN ATTENDING THE PAEDIATRIC CLINIC IN USM HOSPITAL.

Dr. Nazzlin Dizana Din

MMed Pediatrics

**Department of Pediatrics,
School of Medical Sciences, Universiti Sains Malaysia,
Health Campus, 16150 Kelantan, Malaysia**

Objectives : To evaluate the frequency of sleep disorders among children attending the pediatric clinic in USM Hospital.

Patients and Methods : The Sleep Disturbance Scale for Children (SDSC) consisting of 26 items which concentrates on six sleep disorders, Disorder of Initiating and Maintaining Sleep (DIMS), Sleep Breathing Disorder (SBD), Sleep Wake Transition Disorder (SWTD), Sleep Hyperhydrosis (SHY), Disorder of Arousal (DA), Disorder of Excessive Somnolence (DOES), was translated to Bahasa Malaysia. Parents of children aged 4-16 years attending the paediatric clinic, USM Hospital from December 2005 to February 2008 were requested to complete the SDSC while awaiting consultation.

Results : Among 684 children from 8 sub-specialty clinics who completed the SDSC, there were 361 (52.8%) boys. The number

(%) of children in each age group was as follows: 6-12 years old (462, 67.5%), 4-5 years old (86, 12.6%), more than 12 years old (136, 19.9%). Sixty-three (9.2%; 95% CI 7.3% to 11.6%) children had a significant total sleep problem score. The proportion of children with specific sleep disorders were as follows: 64 (9.4%) DIMS, 55 (8.0%) SBD, 31 (4.5%) SWTD, 27 (3.9%) SHY, 31 (4.5%) DA, and 57 (8.3%) DOES. Regression analysis revealed that the frequency of SBD ($p=0.01$) and SWTD ($p=0.03$) was significantly different among subspecialty clinics, and children aged more than 12 years had a significantly higher prevalence of DOES ($p=0.03$) than other age groups. No significant gender differences were observed.

Conclusion : Sleep disorders were relatively high among children attending the paediatric clinic in USM Hospital. Given the impact of sleep disorders on children and families, there is a need for increased awareness of children's sleep problems in the clinics and community.

Prof. Dr. Quah Ban Seng : Supervisor

THE EFFICACY OF INTRAVITREAL TRIAMCINOLONE VERSUS ASER PHOTOCOAGULATION IN THE PRIMARU TREATMENT OF DIABETIC MACULAR OEDEMA

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MMed Ophthalmology

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Health Campus, Kelantan, Malaysia.

Objective: To compare the efficacy and safety of intravitreal triamcinolone injection to laser photocoagulation in the primary treatment of diabetic macular oedema.

Patients and Methods: Forty patients with newly diagnosed diabetic macular oedema were randomized into 2 groups 20 in 4mg intravitreal triamcinolone acetate (IVTA) injection of and 20 in laser photocoagulation group. Evaluation was done at three months and the macular oedema was quantified using HRT II. Intraocular pressure elevation, lenticular opacity and endophthalmitis were observed.

Results: Mean visual acuity for PITA group was 0.935(0.223) at baseline and 0.405(0.223) at three months, $p<0.01$. Mean visual acuity for laser group was 0.795(0.315) at baseline and 0.525(0.289) at three months, $p<0.01$. However, there was no statistically significant difference between the two groups, $p=0.151$. Mean macular oedema index for IVTA group was 2.539 (0.914) at baseline and 1.753 (0.577) at three months, $p<0.01$. Mean macular oedema index for laser group was 2.139 (0.577) at baseline and 1.711(0.472) at three months, $p<0.01$. However, there was no statistically significant difference between the two groups ($p=0.811$). The mean intraocular pressure was statistically significant pre and post IVTA injection ($p=0.032$). There was no significant cataract progression at three months in both groups, $p=0.688$ and no incidence of endophthalmitis post IVTA injection at three months review.

Conclusion: Both PITA and laser photocoagulation demonstrate good outcome as primary treatment in diabetic macular oedema patients. The IVTA is a relatively safe procedure.

Dr. Zunaina Embong : Supervisor
Dr. Bakiah Shaharuddin : Co-Supervisor

THE RETROSPECTIVE STUDY OF IN HOSPITAL TIME MANAGEMENT OF ACUTE STROKE PATIENT IN THE

EMERGENCY DEPARTMENT HOSPITAL KUALA LUMPUR

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MMed Emergency Medicine

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Health Campus, 16150 Kelantan, Malaysia.

Introduction: The Emergency Department Hospital Kuala Lumpur commonly receives cases of patients presented with signs and symptoms of acute stroke in which majority of them are sent for CT Brain for confirmation of diagnosis. However in a case of acute ischaemic stroke none of them had been given fibrinolytic therapy or r-tpa in which assumption made that the patient had missed the beneficial period of the therapy which is within 3 hours from the onset of symptoms.

Objectives: The objectives of the study are to analyse the in-hospital time taken for stroke patients when arrives in the Emergency Department until the diagnosis of stroke is confirmed. This will include the time interval from door to medical consultation, door to CT scan performed till door to CT scan film interpreted. Besides this the time of symptoms onset is also studied as well as the demographic profile of the stroke patients. The association between the demographic factor and stroke risk factors with the time of hospital arrival is also include in the study.

Patients and Methods: A total of 200 patients were involved in the study. All subjects were those age more than 18 years old who presented to the emergency department with the signs and symptoms of acute stroke and had CT brain done. The data analyse will be collected retrospectively through case notes in which the detail of the patients, demographic factor, history of presenting complaint, past medical history, social history, time documentation when seen by doctor till CT film interpretation will be reviewed. In this study the data analysed are stroke patients presented to the Emergency Department Hospital Kuala Lumpur in a period of 1 year from 1st April 2007 until 31st March 2008

Result: From 200 patients 42% of them are Malay followed by Chinese (36.5%), Indian (18.5%) and others (3.0%). Male accounted for 65% while female accounted for 35%. The mean age was 58.3 years old with 58.5% were more than 55 years old. The CT brain finding shows infarct in 42.5% of patients, normal CT finding accounted for 39% and haemorrhagic stroke in 18.5% of patients. The percentage of patients who arrived more than 3 hours from the onset of symptoms was 73% while less than 3 hours was 27%. Most of the patient is triaged to the yellow zone which accounted for 45.5%, followed by the green zone which is 41.5% and the red zone (13%). As for the in-hospital time analysis, it is found that the mean time interval for door to medical consultation is 24.2 minutes and the mean time interval for door to CT is 52.3 minutes. Hypertension occurs highest most among the patients (52.5%) followed by diabetes (41.5%), family history of stroke (23%), smoking (21%), ischaemic heart disease (14.5%), previous history of stroke (8%) and hyperlipidaemia (3.5%). There is a significant association between time of hospital arrival and age in which pvalue is less than 0.05. The significant independent predictors for time of hospital arrival is age (OR 1.83, 95% CI 0.958, 3.502), Hypertension (OR 1.99, 95% CI 0.989, 3.906) and ischaemic heart disease (OR 0.424, 95% CI 0.174, 1.036).

Conclusion: 73% of stroke patients arrives late to the Emergency Department Hospital Kuala Lumpur. There is also an in-hospital delay in which may jeopardise the chance of patient for fibrinolytic therapy. Critical pathway might need to be structured in the emergency department to overcome the delay, hence improving the care of stroke patient.

Assoc. Prof. Dr. Nik Hishamuddin Nik Ab. Rahman : Supervisor
Dr. Mahathar Abd. Wahab : Co-Supervisor
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STUDY OF PREVALENCE OF URINARY INCONTINENCE AMONG WOMEN AGE MORE THAN 45 YEARS ATTENDING GYNAECOLOGY CLINIC, HUSM

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MMed O & G

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Objective: The prevalence of urinary incontinence and its associated factors amongst the population of women more than 45 years attending the gynaecology clinic were studied

Methodology: The study design was prospective, which was done from May 2006 until October 2006 in the Gynaecology Clinic, Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan.

Patients and Methods: All women age 45 years and above who attended the Gynaecology Clinic for various gynaecological problems who fulfilled the inclusion and exclusion were taken. They were given a questionnaire and physical examination performed. If they were found to have the incontinence, urodynamic study was performed to confirmed the diagnosis.

Results: The overall prevalence of urinary incontinence is 32.7%. The prevalence of stress incontinence is 24% (95%CI=18.0%, 30.0%), urge incontinence 5.1% (95% CI= 2.0%, 6.2%) and mixed incontinence 3.6% (95% CI= 1.0%, 6.2%). From this study, it was found that 64 participants were clinically diagnosed having urinary incontinence from the questionnaire. Factor that is found to be associated with stress incontinence is cystocele where the p value is 0.0000, largest birth weight is significant with urge incontinence (p value <0.001) and age, perineal tear and largest birth weight is significant with mixed incontinence where p value is 0.020, 0.018 and 0.004 respectively. From 64 participants who had urinary incontinence, only 45.3% of them underwent confirmatory CMG test. Out of these 64 participants having urinary incontinence, 29(45.3%) cases perform CMG. Out of the 29 who perform CMG, 15 cases was positive(51.7%).

Conclusions: This study showed that overall prevalence of urinary incontinence is 32.7%. The prevalence of stress incontinence is 24% (95%CI=18.0%, 30.0%), urge incontinence 5.1% (95% CI= 2.0%, 6.2%) and mixed incontinence 3.6% (95% CI= 1.0%, 6.2%) It was found that the associated factor that contribute to urinary incontinence are cystocele, largest birth weight, age and perineal tear.

Dr. Mohd. Pazudin Ismail : Supervisor
Dr. Wan Abu Bakar Yusof : Co-Supervisor
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NASAL AIRWAY ANALYSIS USING 3-DIMENSIONAL SOFTWARE AMONG THE NORMAL SUBJECTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA, KELANTAN

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MMed ORL

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Introduction: In order to function well the nasal patency plays a major role to bring in inspired air and release the expired air. The patency of the nasal cavity can be assessed by variety of methods, ranging from simple subjective measurement such as visual analogue scale to more accurate objective measurement such as acoustic rhinometry. Acoustic rhinometry (AR) is the recommended technique for assessment of the nasal geometry. it quantifies subjective symptoms of nasal obstruction into an objective assessment of nasal patency. 3D software is capable in converting the data from AR (2D) into a 3-Dimensional image and this will provide a new prospect in how nasal patency can be measured and evaluated.

Objectives: The objective of this study was to convert the normal values of the Minimal Cross-sectional Area (MCA) and nasal volumes collected using Acoustic Rhinometry and analyze using the 3D software. This study also analyzed the differences between MCA and nasal volumes of male and female collected by the AR and 3D software.

Patients and Methods: This was a cross sectional study of healthy volunteer adult subjects ranging between the age of 18 years old to 70 years old, comprising of 75 males and 75 females Otorhinolaryngology Head and Neck clinic, Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian, Kelantan. A written consent was taken from the candidates after the aim and methodology as well as the procedure was explained to the candidates. A primary assessment with thorough history, systematic ear, nose and throat (ENT) examination, including rigid nasoendoscope was included and performed to each subjects. Later the subjects were examined using the AR scan. The AR scan that was be used was the SR100 (RhinoMetric, Denmark). The Acoustic rhinometry was performed following the standard procedure as described in the literature. The data was analyzed using paired T-test with p-value less than 0.05 was considered to be significant.

Results: In this study, the mean MCA1 for males were $0.49 \pm 0.14 \text{ cm}^2$ and females $0.42 \pm 0.16 \text{ cm}^2$. For the nasal volume of MCA, V1 for males were 3.46 ± 1.28 and for females were $2.9 \pm 0.98 \text{ cm}^2$. In 3D analysis the results also showed that the adult male nasal airway is significantly different from female teenagers (p=0.00), female adult (p0.00) and male teenager (p=0.004) on both the left and right nostril. There is also no significant correlation between MCA and BMI.

Conclusion: The male nasal airways differ from the female nasal airways on both the left and right nostrils. These results were produced by the Acoustic rhinometry software and also by the 3D software. The 3D software showed that the male adult nose differs from the female (adult and teenager) and even the male teenager. The male nasal airway is narrower at the anterior nasal valve and wider distal to nasal valve. Acoustic rhinometry is a valuable method of assessing geometry of nasal cavity. 3-Dimensional software can be used with AR in enhancing the data and making it more useful in diagnosis, treatment planning and ongoing post treatment or surgery.

Dr. Shamim Ahmed Khan : Supervisor
Prof. Dr. Dinsuhaimi Sidek : Co-Supervisor
Prof. G.D. Singh
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A STUDY OF CONTRAST SENSITIVITY AND QUALITY OF LIFE FOLLOWING PHACOEMULSIFICATION WITH TWO DIFFERENT TYPES OF ASPHERIC LENSES

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MMed Ophthalmology

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Health Campus, 16150 Kelantan, Malaysia.

Introduction: Aspheric lens has emerged as a new landscape of intraocular design in order to provide a better quality of vision. Measurement of contrast sensitivity and vision related quality of life would assess the optical and visual performance of these aspheric lenses.

Objectives: To compare the visual acuity, contrast sensitivity and vision related quality of life following phacoemulsification and implantation of two aspheric lenses among age related senile cataract patients attending ophthalmology clinic in Hospital Universiti Sains Malaysia, Kubang Kerian and Hospital Raja Perempuan Zainab II (HRPZ II), Kota Bharu, Kelantan.

Patient and Methods: A total of 112 eyes of 112 patients were randomized to receive either Akreos Adapt Advanced Optic (n=61) or Tecnis Z9003 (n=51). Patients were selected according to the inclusion and exclusion criteria. At 3 months postoperative period, patients were assessed for contrast sensitivity under photopic and mesopic using CSV 1000 and modified VF-14 questionnaires was used to assess the vision related quality of life. Comparison of means of each parameter was made between the intraocular lenses Data analyzed by either Chi-square or independent t test with p value less than 0.05 was considered significant The vision related quality of life using the VF-14 scores were further divided into near vision, intermediate vision and distant vision items.

Results: The mean visual acuity and contrast sensitivity in photopic and mesopic lighting at 3, 6, 12 and 18 cpd showed very similar results in Akreos Adapt AO and Tecnis Z9003. There was no significant difference between the two intraocular lenses. The VF-14 scores in Akreos Adapt AO has a mean of 98.57 (2.51) whereas the Tecnis Z9003 has a mean of 97.18 (5.46). The total quality of life scores also revealed no significant difference.

Conclusion: Postoperative visual acuity and contrast sensitivity in both intraocular lenses were almost comparable. Neither Akreos Adapt AO nor Tecnis Z9003 showed a significant difference in contrast sensitivity and vision related quality of life at 3 months postoperative period.

Assoc. Prof. Dr. Wan Hazabbah Wab Hitam : Supervisor
Dr. Shatria Ismail : Co-Supervisor
Dr. Nik Azlan Nik Zaid

MOLECULAR STUDY OF TRANSFUSION DEPENDENT THALASSEMIA PATIENTS WHO ATTENDED PEDIATRIC DAY CARE HOSPITAL UNIVERSITI SAINS MALAYSIA, KELANTAN FROM JANUARY 2006 TO APRIL 2006

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MMed Paediatrics

Department of Paediatrics,
School of Medical Sciences, Universiti Sains Malaysia,
Health Campus, 16150 Kelantan, Malaysia.

Introduction: Thalassemia has emerged as one of the most common public health problems in Malaysia, particularly among Malaysian Chinese and Malays. This study aims to determine the spectrum of Thalassemia gene mutations found in transfusion dependent Thalassemia patients who attended Pediatric Daycare Unit, Hospital Universiti Sains Malaysia, Kelantan, Malaysia. The findings are important for establishing the prenatal diagnosis in our Human Genome Centre.

Patient and Methods: This was a cross sectional study in which 38 transfusion dependent Beta Thalassemia patients were screened for six different mutations previously shown to

be prevalent in the Malaysian population. Sampel collection was started in January, 2006 till April, 2006. DNA was extracted from leucocytes collected from the peripheral vein, amplified by PCR and digested by six restriction enzymes for detection of mutations. The mutation were correlated with the clinical severity based on the following clinical parameters: age at presentation, pre-transfusion hemoglobin level, mean volume of blood transfusion per kilo body weight per year, spleen size, splenectomy and growth failure were recorded in these patients to determine the severity of each group of thalassaemia type depicted by the mutation. For the statistical analysis, Kruskal-Wallis test and univariate analysis were used.

Results: Five of the six luuown Beta-globin gene defects occurring in the Malaysian population were detected, namely, JVS-1 nt5 (G>C), IVS-1 ntl (G>T), Codon 26 (G>A), Codon 4 1-42 (4 bp del) and Codon 19 (A>G). The mutation which was not observed in this study was in Codon 15 (G>A). The two most common mutations observed were Codon 26 (G>A) (54.3%) and IVS-1 nt5 (G>C) (20%). Three patients did not show any of the six mutations. There were no significance different in age at presentation (p=0.23), pre-transfusion hemoglobin level (p=0.2), volume of blood transfusion given to the patient (p=0.42) and also spleen size (p=0.59) between groups of type of Thalassemia.

Conclusion: Our results showed that the majority of Kelantan Beta Thalassemia patients have similar beta-globin gene defects as the rest of the Malaysian population. However, mutations in the three patients were not identified. The findings complement the existing data on the Beta Thalassemia gene mutation in Malaysia.

Assoc. Prof. Dr. Zilfalil Alwi : Supervisor
Dr. Ariffin Nasir : Co-Supervisor

RELIABILITY OF PAIN ASSESSMENT BY PARENTS AND ATTENDING EMERGENCY MEDICAL OFFICERS FOR PEDIATRIC PATIENTS PRESENTING WITH ACUTE PAIN AT EMERGENCY DEPARTMENT HOSPITAL UNIVERSITI SAINS MALAYSIA, KUBANG KERIAN, KELANTAN

Dr. Sarah Shaikh Abdul Karim
MMed Emergency Medicine

Department of Emergency Medicine,
School of Medical Sciences, Universiti Sains Malaysia,
Health Campus, 16150 Kelantan, Malaysia

Introduction: Pain assessment is the first step towards effective analgesic methods. This is because pain is an individualized sensation and emotion.

Objective: This study was done to assess the agreement of pain severity assessment done by parents or guardians and attending emergency medical officers in a child in terms of inter-rater agreement between parents or guardians, attending EDMO and respective child-patients in pain.

Patients and Methods: This is a single centre cross-sectional study carried out from August 2006 till August 2007 in Emergency Department Hospital Universiti Sains Malaysia. Children between the age of 5 till 1 years old with complaint of acute pain from either trauma or medical causes were enrolled. They were administered either Faces Pain Scale - Revised or Visual Analog Scale for self assessment of pain. Their accompanying parents or guardians and attending Emergency Medical Officers (EDMO) blinded to one another were given Visual Analog Scale to assess respective child-patients pain. The scores obtained was then analyzed to obtain the Kappa value for agreement between the self assessments by the child-patients with that

of parents or guardians and attending EDMOs.

Results: A total of 118 child-patients were recruited from August 2006 to August 2007. Mean pain score by parents or guardians was 5.6 (SD $\pm$ 2.7) Mean pain score by parents or guardians was 5.3 (SD $\pm$ 2.16) and by attending EDMO 4.5 with (SD $\pm$ 2.19) Kappa value of agreement between pain assessment by parents or guardians with respective child is 0.16 and that between attending EDMO with respective child-patient is 0.11.

Conclusion: The study showed that both accompanying parents or guardians and attending EDMOs have Door level of agreement in severity of pain assessment compared to self assessment by respective child-patients. The level of agreement is good in children above 10 years of age and with trauma-related pain.

Assoc. Prof. Dr. Wan Aasim Wan Adnan : Supervisor
Prof. Dato' Dr. Abu Hassan Asaari Abdullah : Co-Supervisor

THE PREVALENCE AND RISK FACTORS FOR IN-HOSPITAL MORTALITY AMONG COPD PATIENTS ADMITTED TO HOSPITAL UNIVERSITI SAINS MALAYSIA

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MMed Orthopaedics

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Introduction: Wide resection in Limb Salvage Surgery for primary bone tumors results in segmental osseous defect. The optimum method for reconstruction distal femur and proximal tibia remained controversial. Options include the use of autografts, allografts, custom-made megaprotheses and modular endoprotheses. Endoprosthesis allows early rehabilitation with a good long term functional outcome result. The aim of this study is to evaluate the functional outcome of patient in modular endoprosthesis reconstructions surgery in the treatment of primary bone tumors of distal femur and proximal tibia of the lower limb, by using Musculoskeletal Tumor Society scoring system.

Patients and Methods: Fifty four consecutive patients with primary bone tumor of distal femur and proximal tibia were selected and reviewed to determine the functional outcome after wide resection endoprosthesis reconstruction surgery by using Musculoskeletal Tumor Society scoring system.

Results: There were 34 (63%) cases of distal femur and 20 (37%) cases of proximal tibia bone tumor. The Primary osteosarcoma are 33 (6 1.1%) and stage III GCT are 20 (37%). The mean age is 26.6 $\pm$ 1 0.61. There were 12 (22.2%) patients who had metastasis to the lung. The mean MSTS score for both DF and PT endoprosthesis was 21.13 (70.43%), MSTS score for DF was 21.94 (73.13%) and PT was 19.75 (65.83%) group into good to excellent result. The infection rate was 13% (7 cases) and high in PT endoprosthesis group. The early revision rate of endoprosthesis replacement was 11.1% (6 cases) mainly due to infection (3 cases). Infection and at site of endoprosthesis were the cause of early failure.

Conclusion: Endoprosthesis replacement for primary bone tumors had good to excellence MSTS score. There were no different in functional outcome after distal femur endoprosthesis and proximal tibia endoprosthesis. The cause of early failure in our center following endoprosthesis surgery is infection and the location of endoprosthesis replacement which is a proximal tibia.

Assoc. Prof. Dr. Wan Faisham Nu'Man Wan Ismail : Supervisor

ROLE OF TOPICAL METHICILLIN IN VENTRICULOPERITONEAL SHUNT SURGERY – A RANDOMIZED CONTROL STUDY

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MSurg Neurosurgery

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Health Campus, 16150 Kelantan, Malaysia.

Objective: To evaluate the role of methicillin as a topical installation during shunt insertion in ventriculoperitoneal shunt surgery with the aim of reducing the rate of postoperative infection.

Research procedure: A single blinded randomized control study was carried out on all patients who were admitted or referred to the Neurosurgery Department, Sultanah Aminah Hospital Johor Bahru with the diagnosis of Hydrocephalus; a ventriculoperitoneal shunt was indicated. The period of study was from November 2005 to May 2007. The follow up period for this study was of 3 months after surgery. The period of study was considered adequate to access the role of our observation. Randomization was carried out in the operation theatre prior to the procedure. The scrub nurse would pick up a sealed envelope and prepare as stated. That is Group 1 with topical methicillin, Group 2 without topical methicillin. One dose of prophylactic antibiotic, IV Cefuroxime 25mg/kg was given at induction. Stringent operative technique was followed preparing patient and draping. Ethical approval was received JTP/KKM 1- 0805. Statistical analysis was done using SPSS version 12 (2003).

Result: A total of 90 patients were recruited in the study, 13 (14.4%) patients developed infection within 3 months, of this Group 1 had a 8.9% risk of infection and Group 2 had a 20% risk, however statistically there was no significant postoperative VPS infection reduction with the use of topical methicillin in ventriculoperitoneal shunt surgery (p=0.230). Multivariate analysis showed that duration of surgery had a significant influence on the postoperative VPS infection in the non-Methicillin group.

Conclusion: Topical methicillin has no significance in reduction of postoperative VPS infection

Mr. Johari Siregar B Adnan : Supervisor
Dr. Noorazman Abd Rahman : Co-Supervisor

A COMPARISON OF SUTURING TECHNIQUE FOR REPAIR OF EPISIOTOMY A RANDOMISED CONTROL TRIAL OF MALAY PRIMIGRAVIDAE IN HOSPITAL UNIVERSITI SAINS MALAYSIA KOTA BAHRU, KELANTAN, MALAYSIA.

Dr. Stanislaus Djokomuljanto
MMed Paediatrics

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School of Medical Sciences, Universiti Sains Malaysia,
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Objective : The addition of low-cost reflecting curtain to a standard phototherapy unit could increase effectiveness of phototherapy for neonatal jaundice was determined.

Patients and Methods : This was randomized controlled clinical trial done in a level one nursery of the Hospital

Universiti Sains Malaysia, Kelantan, Malaysia. Term newborns with uncomplicated neonatal jaundice presenting in the first week of life. Phototherapy with white curtains hanging from the sides of phototherapy unit (study group, $n=50$) was compared with single phototherapy without curtains (control group, $n=47$). The primary outcome was the mean difference in total serum bilirubin measured at baseline and after 4 hours of phototherapy. The secondary outcome was the duration of phototherapy.

Results: The mean (standard deviation) decrease in total serum bilirubin levels after 4 hours of phototherapy was significantly ($p<0.001$) higher in the study group [27.62 (25.24) $\mu\text{mol/L}$] than in the control group [4.04 (24.27) $\mu\text{mol/L}$]. Cox proportional hazard regression analysis indicated that the median duration of phototherapy was significantly shorter in the study group (12 hours) than in the control group (34 hours; change 45.2; $p<0.001$; hazards ratio 0.20; 95% confidence interval 0.12 to 0.32). No difference in adverse events was noted in terms of hyperthermia or hypothermia, weight loss, rash, loose stools or feeding intolerance.

Conclusion: Hanging white curtains around phototherapy units significantly increase the efficacy of phototherapy in the treatment of neonatal jaundice without evidence of increased adverse effects.

Assoc. Prof. Dr. Hans Van Rostenberghe : Supervisor
Dr. Noraida Ramli : Co-Supervisor
Prof. Dr. Quah Ban Seng

RANDOMISED, DOUBLE BLIND CONTROLLED TRIAL ON PREOPERATIVE ANXIETY LEVEL BETWEEN PREMEDICATED AND NON-PREMEDICATED PATIENTS UNDERGOING ELECTIVE SURGERY

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MMed Anaesthesiology

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School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kelantan, Malaysia.

Introduction: Patients should not suffer needless anxiety before surgery.

Objective : This study was aimed to determine the level of anxiety that exist in patients undergoing elective surgical procedure from various departments. Comparison was also made within the various demographical data obtained with specific objective.

Patients and Methods: This was a prospective randomized, double blind controlled clinical trial on preoperative anxiety levels. Patients completed the anxiety assessment scales both before and after intervention. The scale used was Hospital Anxiety and Depression Scale(HADS) The fourteen questions were subdivided to assess anxiety and depression separately. Paired T-test within groups and independent T-test for between groups were used during statistical analysis using SPSS 11.0. A $p < 0.05$ was considered to be significant.

Results: One hundred and forty scale sets were obtained with no statistically significant difference in demographic characteristic. The intervention group had preintervention mean anxiety score of 8.3 ± 2.3 (mean $\pm$ SD) and post-intervention mean anxiety score of 3.3 ± 1.8 with $p = 0.000$ where the placebo group had preintervention score of 8.9 ± 2.3 and post-intervention score of 10.24 ± 2.9 with $p0.001$. Differences were also seen in mean anxiety score with ethnicity, gender, ASA class (American Society of Anesthesiologist) and type of operation.

Conclusion: In this study, it was found that significant level of anxiety existed preoperatively and premedication reduced it's level significantly. The placebo group had the highest level of anxiety and its level increased even more immediately before surgery. In addition, female patient, ASA 11 and patients undergoing gynaecological surgery had higher level of anxiety.

Assoc. Prof. Dr. Saedah Ali : Supervisor

A COMPARATIVE STUDY ON THE OUTCOME OF RECONSTRUCTION WITH AUTOGENOUS GRAFTS VERSUS POROUS POLYETHYLENE (MEDPOR) IN HOSPITAL UNIVERSITI SAINS MALAYSIA FROM 2004-2007

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MMed Ophthalmology

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Objective: To determine the difference in surgical outcomes of orbital floor reconstruction between the use of two different reconstructive materials.

Patients and Method: All patients who underwent orbital floor reconstruction in the study period were divided into two groups according to the materials used for the grafts. All patients underwent comprehensive ocular examinations, Goldmann perimetry, Hess chart test and exophthalmometry.

Results: Thirty-five patients underwent orbital floor reconstruction within the study period in our center. Twenty-six patients were analyzed. Autogenous grafts were used in 14 patients (53 %), and medpor in 12 patients (46.2%). Among our patients, 84.6% of them were males and 15.6 % females. The mean age was 24.5(8.2) years. Motor vehicle accidents were attributed to 96.2%. Motorcyclists were the most common victims (76.9%). The most common clinical presentations were diplopia 61.6% and enophthalmos 50 %. In our study 50% of orbital floor reconstructions were carried out within 2 weeks and 73.1 % of the cases were approached through blepharoplasty incision. Postoperatively there was no diplopia in primary gaze. In all cases however diplopia in the inferior and peripheral gaze was found 11.5 % and 26.9 % respectively in autogenous group, and 3.8 % and 26.9 % in medpor group ($P = 1.24$). Enophthalmos was 11.4 % in autogenous graft group and 15.3 % in medpor group ($P= 0.465$). Hess chart was found to be abnormal in 11.4 % for each group ($P=0.062$). Restricted extraocular movements were seen in one (3.8 %) patient of autogenous group and 7.7 % of patients in medpor group ($P=0.574$).

Conclusion: The outcome of orbital floor reconstruction by medpor was comparable and as good as autogenous graft, and there was no statistically significant difference between the two groups. Goldmann perimetry was a more objective binocular visual field test to detect diplopia and extraocular deficits.

Dr. Bakiah Shaharuddin : Supervisor

STUDY OF SHORT TERM OUTCOME AND INCIDENCE OF RECURRENCE OF INGUINAL HERNIA REPAIR IN HOSPITAL UNIVERSITI SAINS MALAYSIA.

Dr. Wan Zainira Wan Zain
MMed Surgery

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Health Campus, 16150 Kelantan, Malaysia.

Introduction: The purposes of this study are to evaluate the complications arise from the inguinal hernia repair and their correlations with the patients' factors and surgical procedure's factors. The aim is to improve our that surgical unit in Hospital Sains Malaysia offers the surgery for inguinal hernia repair.

Methods and Results: This retrospective study was done on 600 patients who undergone the hernia repair. This study started from January 2000 until January 2005. About 580 male patients and 18 female patients had undergone hernia repair within the time frame. Mean age was 52 years old with standard deviation of 18.2 years. We found patients more than 50 years old tend to develop short term complications and overall complications after the surgery ($p < 0.05$). 364 patients have right sided hernia, 200 patients have left sided hernia and 36 have bilateral hernia. 494 patients have indirect hernia, 93 patients have direct hernia, 7 patients have both direct and indirect hernia and 6 patients have sliding hernia. Indirect hernia have higher risk to develop recurrent hernia ($0 < 0.05$). Majority of patients came to hospital for the treatment after 1 to 6 years of symptoms. 460 had undergone Lichtenstein repair, 121 had undergone Darning repair, 10 had Bassini and 9 had laparoscopic hernioplasty. Type of surgery does not alter the incidence of complications and recurrence after the surgery. 537 patients had elective surgery and 63 patients had emergency surgery. Duration of operation was longer in emergency surgery in which the duration of more than 2 hours were 17.7% in elective cases comparing to 49.2% in the emergency cases ($p < 0.05$) and emergency cases stayed longer in hospital comparing to elective cases ($r < 0.05$). 87 patients had COAD (chronic obstructive airway disease), 45 had chronic constipation and 39 had BPH (benign prostatic hypertrophy). Patients who had COAD tend to come as emergency and develop short term complication after the surgery ($p < 0.05$). There were 23 wound infections. Our wound infection rate was at 3.8%. The less experienced surgeons had shown to have higher early infection rate comparing to the more experienced surgeons ($p < 0.05$). There were 19 recurrent cases. 6 patients recurred as early as less than 6 months. 6 recurred after 6 month but less than 1 year, 7 recurred after 1 year to 5 years after the surgery. The indirect hernia have higher recurrent rate. Similarly with patients who develop post operative complication, the recurrent rate was higher in this group ($p < 0.05$).

Conclusion: In summary, the commonest complications post hernia repair were wound infection, scrotal hematoma and recurrence. Patients age of more than 50 years old, patients who have COAD and less experienced surgeons had increased the risk of post operative complications. Patients who have indirect hernia and who developed post operative complications have higher risk to develop recurrence.

Dr. Mohd. Ridzuan Abd. Samad : Supervisor

WORKING MEMORY IN CHILDREN AND ADOLESCENTS WITH ATTENTION DEFICIT HYPERACTIVITY DISORDER AND THEIR SIBLINGS IN HOSPITAL UNIVERSITI SAINS MALAYIA

Dr. Wee Kok Wei
MMed Psychiatry

Department of Psychiatry,
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Health Campus, 16150 Kelantan, Malaysia

Introduction: Impairment of working memory in children with Attention Deficit Hyperactivity Disorder (ADHD) has been described with certainty. However, there have been few studies of working memory among the siblings of children with ADHD. If similar impairment in working memory of the siblings can be demonstrated, this could suggest impaired working memory as an endophenotype of ADHD.

Objective: Working memory of ADHD children, their siblings and those children with other chronic medical illnesses were assessed and compared.

Patient and Method: 57 subjects were recruited: 1) ADHD group (n 21), 2) siblings of ADHD children group (n=15), and 3) non-ADHD children with chronic medical condition as the control group (n = 21). All the subjects aged between 6 and 15 years, and ADHD was diagnosed according to DSM-IV-TR. All subjects were screened with M.I.N.I. Kid Screen. IQ was determined using the Seguin Form Board test. Those with other co-morbidity or IQ < 70 were excluded from the study. Three tests from the Working Memory Test Battery for Children (WMTB-C) were used to assess working memory. Digit Recall was used for assessment of the phonological loop component, Mazes Memory test for the visuospatial sketch pad component and Backward Digit Recall for the central executive component.

Result: IQ score and other socio-demographic factors showed no association with the working memory scores except with Backward Digit Recall Standard Score. ADHD children and their siblings showed similar impairment and both differed from the control group on the Mazes Memory test. ADHD children also showed impairment in Digit Recall test, however the siblings group did not differ from the control group. The score of Backward Digit Recall did not show any significant difference between the 3 groups.

Conclusion: Impairment of the visuospatial sketch pad component of working memory seems to cluster in ADHD children and their siblings. This suggests that impairment of visuospatial sketch pad component may point towards an endophenotype of ADHD and help facilitate the identification of genes involved in ADHD. However, the results need to be interpreted in the light of the limitations and the unique study population who were almost all from the same ethnicity.

Assoc. Prof. Dr. Mohd Jamil Yaacob : Supervisor
Dr. Norzila Zakaria: Co-Supervisor

INCIDENCE OF DEEP VEIN THROMBOSIS IN LOWER LIMB INJURY FOLLOWING CAST IMMOBILISATION A COHORT PROSPECTIVE STUDY CONDUCTED IN HOSPITAL UNIVERSITI SAINS MALAYSIA (HUSM)

Dr. Zairuddin Abdullah Zawawi
MMed Orthopaedics

Department of Orthopaedics
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Health Campus, 16150 Kelantan, Malaysia.

Introduction : Patient who had undergone lower limb immobilization have an increased risk for the development of deep vein thrombosis and subsequent pulmonary embolism, a life threatening situation. One of the known causes for the development of deep vein thrombosis is by recent cast immobilization of lower extremities. The purpose of this study is to determine the incidence of deep vein thrombosis following lower limb immobilization by cast and its predisposing factors.

Objectives: The incidence of DVT following cast immobilization of the lower limb; and which level of cast and

possible predisposing factors leading to DVT were analysed.

Patients and Methods: A prospective cohort study of a total of 40 patients participated in the study where they must be on cast for at least 6 weeks duration. The incidence of deep vein thrombosis was determined by colour Doppler ultrasound.

Results: From 40 patients assessed during the study period, deep vein thrombosis was identified in only 1 patient after 6 weeks of casting. This gave the incidence rate of only 2.5%.

Conclusion: The rate of deep vein thrombosis in our community following lower limb immobilization by cast is very low. Thus, we could conclude that cast application is still a safe mode of treatment in lower limb injury and it is not necessary to start antithrombolytic agent for prophylaxis for DVT.

Assoc. Prof. Dr. Mohd. Imran Bin Yusof : Supervisor

Dr. Tg. Muzaffar Tg. Shahabudin: Co-Supervisor

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THE MALAYSIAN JOURNAL OF MEDICAL SCIENCES

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Theriault A, Caa 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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