Abstracts

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

NASI KANDAR': A STUDY OF EATING HABITS AND ITS RELATIONS TO A SAFE DIET

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Introduction: 'Nasi kandar' was originated way back during the 18th Century when Indian Muslim from Southern India migrated to Penang during the British Colonial period. It has evolve over the years is enjoyed by Malaysian from all walks of life regardless of age, gender, profession, race or religion thus, becoming a multi-ethnic food.

Objectives: This study aims to identify; the pairing dishes offered at a typical 'nasi kandar' food outlet; factors that influence people to eat 'nasi kandar' and their eating habits; screen the microorganisms load in selected pairing dishes.

Methods: A total of 122 Indian-Muslim stalls and restaurants in the Klang Valley and Penang were visited in order to compare food selection availability and locality. A total of 316 randomly selected patrons were picked up to answer a set of questionnaire concerning the reason for eating at the chosen food outlet and their typical choice of pairing dishes. Data from the questionnaires and observation list were analyzed with descriptive statistics using PASW 18.0 software. Thirty three random food samples were purchased and taken for lab testing to measure the existence of microorganisms specifically *Staphylococcus aureus, Escherichia coli* and *coliforms*. Rapid method using Petrifilm™ plates from 3M were used to count the bacteria loading instead of the conventional MPN method.

Results: More than half of the total respondent (58.9%) would consume 'nasi kandar' at least once a week while 41.1% consume more than once a week. Lunch time is the most popular time to eat 'nasi kandar'. Chicken base dishes were the most diversed and widely available mainly for its cheaper price and have a more universal appeal to different race and religion thus making it the more popular choices among 'nasi kandar' partons. 'Ayam goreng' and 'ikan goreng' recorded S. aureus count of 4.4 × 102 cfu/g and 1.3 × 102 cfu/g respectively. Egg based samples showed a much higher count of *E.coli, coliforms* and *S.aureus* compared to meat based dishes, showing bacteria count ranging from 4.4 × 102 cfu/g to 1.8 × 104 cfu/g. Gravy base dishes in this study recorded of *E. coli, coliforms* and *S. aureus* count ranging from below 1.5 × 10 cfu/g to 6.5 × 102 cfu/g. 'Bendi' recorded the highest *E. coli* (1.6 × 103 cfu/g) and *S. aureus* (1.2 × 103 cfu/g) counts. 'Acar timun' recorded the highest count for coliform bacteria (5.5 × 104 cfu/g).

Conclusion: Bacteria are prone to thrive in vegetable and egg base items compare to gravies and fried items.

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BIOCHEMICAL CHARACTERIZATION OF Entamoeba histolytica CHOLINE/ETHANOLAMINE KINASES

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Introduction: *Entamoeba histolytica* is one of the most death causing protozoan parasites in the world. A study on its lipid composition revealed that its total phospholipid was pre-dominated by phosphatidylcholine and phosphatidylethanolamine. Further study showed that its phosphorylated metabolites might be *de novo* synthesized by CDP-Choline and CDP-Ethanolamine pathways, respectively. However, no further report regarding the enzymology of *E. histolytica* phospholipid metabolism was reported to date. Promising effects have been shown by anti-parasitic and anti-cancer drugs which targeted the first enzyme of CDP-Choline and CDP-Ethanolamine pathways, namely choline kinase (CK) and ethanolamine kinase (EK).

Objectives: Thus, this study aimed to characterize *E. histolytica* putative CK/EK by different approaches.

Methods: Two putative *E. histolytica* choline/ethanolamine kinases (EhCK and EhEK) were identified from *E. histolytica* genome. Sequence analysis showed that they possessed all the conserved motifs in CK family. EhEK but not EhCK was computationally predicted to be phosphorylated at the N-terminal. Notably, phylogenetic study demonstrated that both enzymes were closely related to human and *Drosophila melanogaster* EK rather than other parasites. EhCK and EhEK were cloned, expressed, purified and characterized by employing pyruvate kinase-lactate dehydrogenase coupled spectrophotometric assay.

Results: EhCK showed specific activity against choline but not ethanolamine. It was most active at pH 8.0 with temperature 40 °C. Its Michaelis-Menten kinetic study showed V_{max} of 1.9 ± 0.1 μmol/min/mg. Its Km for choline and ATP was 203 ± 26 μM and 3.1 ± 0.4 mM, respectively. EhCK showed novel preference towards Mn^{2+} rather than typical Mg^{2+} as

metal ion cofactor. Its enzyme efficiency increased 24-fold in the presence of Mn^{2+}. EhEK activity, which was originally undetectable in Mg^{2+}, showed V_{max} of 20.0 ± 0.7 µmol/min/mg with Mn^{2+}. Its K_m and $K_{0.5}$ for ethanolamine and ATP were found to be 312 ± 27 µM and 2.1 ± 0.2 mM, respectively. Interestingly, this study showed that human Δ89N-EK1 and EK2α also have similar metal ion preference, while human CKβ demonstrated metal ion-dependent substrate specificity. Current study also improved the affinity of EhCK for Mn^{2+} by Y129E mutation. This work also produced highly sensitive and specific rabbit polyclonal antibody against EhCK. The antibody did not show cross-reactivity with all human CK and EK homologues. Its ability to co-detect *Entamoeba dispar* EK provides opportunity for comparison study between these two closely-related organisms.

Conclusion: Overall, this study, for the first time, confirmed the presence of CDP-Choline and CDP-Ethanolamine pathways in *E. histolytica*.

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ANALYSIS OF CHOLINE AND ETHANOLAMINE KINASES EXPRESSION PROFILES IN MCF7, HCT116 AND HEPG2 BY REAL-TIME PCR

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Introduction: Choline kinase (CK) and ethanolamine kinase (EK) catalyse the phosphorylation of choline and ethanolamine, respectively, to yield phosphocholine (PCho) and phosphoethanolamine (PEtn) in the Kennedy pathway. The overexpression of PCho due to elevated CK activity has been reported to be associated with malignant transformation and progression. In human, ck transcript variants exist as *ckα1, ckα2,* and *ckβ* whereas ek transcript variants exist as *ek1, ek2α,* and *ek2β*. In this study, real-time PCR analysis was done to determine the gene expression profiles of ck and ek variants in MCF7, HCT116, and HepG2 cell lines.

Methods: Prior to relative quantification of ck and ek gene expressions, geNorm and NormFinder software were used to analyse the stabilities of twelve reference genes in these three cancer cell lines. Both softwares showed that UBC and YWHAZ were the two most suitable reference genes. By using absolute and relative quantification, ck and ek gene expression profiles demonstrated that transcriptional activities of each variant in these cancer cell lines were different. ckα gene was expressed most abundantly in HepG2. HCT116 exhibited the highest *ckβ* and *ek1* expressions, whereas the highest *ek2α* expression was detected in MCF7. *ckβ* showed higher mRNA expression than *ckα* in MCF7 and HCT116. Relatively low ek1 expression compared to *ek2α* was detected in MCF7 but not in HCT116 and HepG2. Both quantification approaches showed similar *ck* and *ek* variants mRNA expression patterns. To study the effect of epigenetic modifications on ck and ek gene expression, HepG2 was subjected to a DNA demethylating agent (5-Aza-2'-deoxycytidine, 5-aza-2'-dC) and a HDAC inhibitor (trichostatin A, TSA) treatments. The effects of DNA demethylation and histone deacetylase inhibition on *ck* and *ek* mRNA levels were investigated using the absolute qPCR quantification method.

Results: Result showed that 5-aza-2'-dC has no effect on ck and ek gene expression. There was no synergistic effect in the combination treatment of 5-aza-2'-dC and TSA. However, the expression of *ckα*, *ckβ,* and *ek2α* were shown to be affected by TSA alone. More than 8-fold of induction was observed in ek2α expression levels. The induction of ek2α gene by TSA was concentration- and time-dependent.

Conclusion: In conclusion, this study demonstrated that UBC and YWHAZ are sufficient for the reliable normalisation of gene expression using MCF7, HCT116, and HepG2 cells. The highest ckα and ek2α expressions were found in HepG2 and MCF7, respectively, whilst HCT116 showed the highest ckβ and ek1 expressions. In MCF7 and HCT116, *ckβ* expression was higher than *ckα*. The effect of TSA treatment on ck and ek mRNA levels showed that ck and *ek* gene expression can be regulated by epigenetic alteration. The mechanisms of TSA-induced ek2α gene expression need to be further investigated.

Supervisor:
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DEVELOPMENT OF THERMOSTABILISED ONE-STEP NESTED MULTIPLEX PCR ASSAY FOR SIMULTANEOUS DETECTION AND DIFFERENtIATION OF *Entamoeba* SPP. FROM STOOL SAMPLES

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Introduction: *Entamoeba histolytica* is the only *Entamoeba* species that causes amoebiasis in humans. Approximately 50 million people are infected with 100 000 deaths annually in endemic countries. Molecular diagnosis for *E. histolytica* is important to differentiate it from the morphologically identical *Entamoeba* dispar to avoid unnecessary treatment. Conventional molecular diagnostic tests require trained personnel, cold-chain transportation and/or storage dependent, which make them user-unfriendly. Newer molecular techniques have played an increasing role in diagnosis of amoebiasis in recent years, and polymerase chain

reaction (PCR) is one of the major techniques used. However, PCR have setbacks similar to other molecular diagnostic methods.

Objectives: Hence, the aim of this study was to develop a thermostabilised one-step nested multiplex PCR assay for the detection of *E. histolytica, E. dispar* and *Entamoeba* spp. in cold-chain free and ready-to-use form.

Methods: The PCR assay was designed based on *Entamoeba* small subunit ribosomal RNA (SSU-rRNA) gene, which detects the presence of *Entamoeba* genus and simultaneously differentiating *E. histolytica* from *E. dispar*. In addition, a pair of primers was designed to serve as an internal amplification control that helps to identify inhibitors in stool samples. All PCR reagents together with the designed primers were thermostabilised by lyophilisation. The shelf life of this novel assay was then estimated using accelerated aging technique by elevating the temperature up to 37 °C. It was estimated to be stable at 24 °C for at least six months. Analytical sensitivity and specificity of the developed assay were also determined.

Results: The limit of detection (LoD) was found to be 39 pg of DNA or 1000 *E. histolytica* cells; and 78 pg of DNA or 1000 *E. dispar* cells. Analytical specificity of the assay tested with other non-Entamoeba enteric microorganisms was 100%.

Conclusion: In conclusion, a novel cold-chain free thermostabilised one-step nested multiplex PCR assay that differentiates *E. histolytica* from other nonpathogenic *Entamoeba* spp. was successfully developed. This molecular detection kit could produce result within 2.5 hours.

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RELATIONSHIP BETWEEN QUALITY OF LIFE, NEUROCOGNITIVE STATUS AND ACADEMIC ACHIEVEMENT OF CHILDREN IN A MALAYSIAN PRIMARY SCHOOL: FROM PERSPECTIVES OF CHILD, PARENT AND TEACHER

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Introduction: Academic achievement is not the only indicator of achievement in school. The neurocognitive profile, as measured by the quality of life (QoL) and neurocognitive status are important markers that make academic achievement more relevant with regard to optimum brain utilisation.

Objectives: The main objective of this study was to determine the relationship between quality of life, neurocognitive status and academic achievement in a sample of children in a Malaysian primary school.

Methods: QoL was measured using three versions of the TNO-AZL quality of life (TACQOL) questionnaire from the children's (CV), parents' (PV) and teachers' (TV) perspectives. Neurocognitive status was assessed in two domains, executive function and visual memory using Cambridge Neuropsychology Tests Automated Battery (CANTAB®). A convenience sampling was undertaken for all 95 Standard One Zainab 2 Primary School children, 95 parents and 4 teachers. The relevant TACQOL was then administered to all respondents. The CANTAB® was then administered to all children.

Results: This study found significant discrepancies and differences between all three perspectives regarding QoL of children. Children were more pessimistic while teachers and parents often overestimated the QoL of children. Children often felt that social relationships were the most difficult, Parents felt cognitive complaints were the most relevant while teachers feel that autonomous functions were the most difficult for children. Regarding executive function, 42.1% of children experienced difficulties in extra dimensional shift which measures attention flexibility in accepting a new rule. However, they performed well in the intra extra dimensional shift, especially in Stage 4, indicating that most children responded positively to experiential learning. For visual memory, 49.5% of children experienced some difficulties in terms of their short term visual memory. The relationship between QoL, neurocognitive status and academic achievement of the children showed that only 'cognitive complaint' and 'negative mood' had a significant linear positive relationship with academic achievement. No other significant relationship was noted between neurocognitive status and academic achievement.

Conclusion: Thus, this study has shown that academic achievement does not necessarily reflect the neurocognitive status of children implying that some neurocognitive problems remain undetected. A more meaningful assessment of academic achievement, in primary schools should include an assessment of both QoL and neurocognitive profile.

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RELATIONSHIP BETWEEN IRON DEFICIENCY ANAEMIA WITH COGNITIVE FUNCTION AND ACADEMIC PERFORMANCE OF THE PRIMARY SCHOOL CHILDREN IN BACHOK, KELANTAN

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Introduction: Iron deficiency anaemia (IDA) is the

most common micronutrient deficiency and it affects millions of children worldwide. IDA may leads to serious health problems such as poor cognitive and motor development as well as poor behavior in children.

Objectives: The main objective of the study was to investigate the relationship between IDA with cognitive function of the school children.

Methods: A cross-sectional study was conducted on 249 Malay primary school children (122 males and 127 females) aged 7–9 years old by systematic sampling method in rural area of Bachok, Kelantan. Anthropometric measurement for weight and height were taken and nutritional status of the children was determined based on WHO 2007 growth reference. Birth weight was recorded from the birth certificate. Venous blood sample was drawn for hemoglobin and serum ferritin analysis. Academic performance was recorded based on marks obtained in the school final exam results in four subjects including Malay language, English, Mathematics, and science. Cognitive function was assessed using Raven's Coloured Progressive Matrices (RCPM) which has been translated to *Bahasa Malaysia* and validated accordingly.

Results: Overall, most male children experienced some form of malnutrition than female. The results showed that the prevalence of stunting and underweight were 13.8% and 16.3% respectively. Overweight (5.6%) and obesity (6.9%) was also found to be prevalent. Results revealed that the prevalence of iron deficiency without anaemia was 12.6% and 7.7% of the children was found to be iron deficient aneamia. Pearson's correlation test showed that there were no significant associations between all nutritional status indicators such as weight-for-age, height-for-age and BMI-for-age with the cognitive function of the children. However birth weight ($r = 0.159$, $P < 0.05$) and serum ferritin ($r = 0.218$, $P < 0.001$) correlated significantly with the cognitive function. Significant difference was also found between iron status of the children and the cognitive function ($F = 20.41$, $P < 0.001$). Multiple linear regression tests showed that serum ferritin contribute the most of significant factors to the cognitive performance variance ($R_2 = 0.071$, $P < 0.001$).

Conclusion: The study emphasizes the fact that iron is an important component in determining the cognitive function of the school children. Thus, it is important to overcome the problems of malnutrition especially iron deficiency anaemia among children as it affects the children's cognitive function.

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NUTRITIONAL STATUS: ITS ASSOCIATION WITH BONE RESORPTION MARKERS IN LOW SOCIO-ECONOMIC STATUS PRE- AND POST-MENOPAUSAL MALAY WOMEN IN KELANTAN

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Introduction: Good nutritional status is important in maintaining bone health. Household income is one of the factors that affect ones' nutritional status. Significant increase in bone fracture is associated with a decrease in income.

Objectives: The objective of this study is to determine the association of nutritional status and the levels of bone resorption markers in pre- and post-menopausal Kelantanese Malay women with low socio-economic status and also to determine the risk factors of bone resorption in this population.

Methods: The study method is a cross-sectional study. A total of 150 healthy pre-and post-menopausal Kelantanese Malay women were recruited for this study. The post-menopausal subjects were divided into two groups (A and B) based on the length of menopausal state. Data on socio-economic, lifestyle habit, and clinical were gained by personal interview. Calcium intake was determined by diet recall and food frequency questionnaire. Serum was collected and tested for serum levels of 25-hydroxyvitamin D (25 (OH) D) and C- and N-terminal telopeptides of type 1 collagen bone resorption markers (CTx and NTx). The mean CTx values were 0.2833 (0.1769) ng/mL for pre-menopausal and 0.423 (0.2529) ng/mL and 0.510 (0.241) ng/mL for post-menopausal groups (A and B). The NTx value for pre-menopausal group was 15.203 (15.2025) nM BCE, and for postmenopausal groups (A and B) were 17.900 (7.7959) and 19.351 (7.3775) nM BCE respectively.

Results: There was no significant different between both markers values among all groups. The mean calcium intake was 492.9 (316.51) mg/day, and 86.3% of the pre-menopausal and 91.9% of the post-menopausal groups have insufficient intake of calcium. None of the subjects have sufficient circulating level of 25(OH)D. 16.4% of the subjects were classified under Hypovitaminosis D, 82.9% insufficient vitamin D level and another 0.7% is under deficiency category. The risk factors for bone resorption in this population are duration of menopause, marital status, body mass index (BMI), physical activity, and education level. Widowed women have higher level of bone resorption, which might be due to depression. Targeting this group for intervention might be a convenient way to reduce the fracture incidence in this population. Besides that, increasing education and physical activity intervention might be effective to decrease health inequalities.

Conclusion: In conclusion, these strategies hopefully will help this populace to have higher quality of life in their late years.

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CHARACTERIZATION OF TRANSGENE INTEGRATION HOTSPOTS IN NS0 (NON–SECRETING) MYELOMA CELLS EXPRESSION SYSTEM

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Introduction: Mammalian expression system is very crucial for the production of proteins in numerous scientific and commercial areas as they are able to perform proper protein folding as compared to other hosts.

Objectives: Matrix attachment regions (MARs) are concentrated with transcription factor binding sites and have a strong effect on the level of expression of transgenes, thus highly studied to increase protein production.

Methods: In this study, an expression vector carrying green fluorescent protein was transfected into mouse myeloma NS0 cell line in order to allow random integration of the vector into the host genome. Conceptually, if the vector is integrated into a transcriptionally active site within the genome, green fluorescent protein will be highly or constitutively expressed.

Results: Transfected cells were then subjected to sorting in order to select the high producing cells using ClonePix FL (CPL). CPL is an automated high–throughput clone selection instrument. DNA sequences in the genome flanking the integrated vector in the high producing cells were subsequently retrieved using 'genome walking' method. Following this, the flanking regions were sequenced and characterized using bioinformatics tools. Functional analysis was carried out by entering the sequences into three different programs (SMARtest, MARfinder and MARscan) to determine the presence of Matrix Attachment Regions. In addition, biochemical characterization was also done using Chromatin Immunoprecipitation (ChIP) Assay.

Conclusion: As a result, putative MAR elements were identified in mammalian expression system with different lengths of MAR regions between the three algorithms. MAR binding proteins that were used in ChIP assay were also shown to interact with the mouse genome.

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A MULTIETHNIC STUDY TO DETERMINE THE ASSOCIATION OF DIETARY INTAKE AND PHYSICAL ACTIVITY WITH PLASMA ADIPONECTIN CONCENTRATIONS AMONG TYPE 2 DIABETES MELLITUS PATIENTS IN PENANG

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Introduction: Type 2 diabetes is one of the serious causes for premature illness and deaths throughout the world. Indians showed the highest prevalence of type 2 diabetes, followed by Malays and Chinese. Many studies have supported the important role of adiponectin in insulin-sensitizing which is associated with Type 2 diabetes. The influence of lifestyle factors and ethnicity on the concentration level of plasma adiponectin among Malaysian T2DM patients is unknown.

Objectives: This study is aimed to determine the levels of adiponectin concentrations among the multiethnic population with Type 2 diabetes. It also investigates the association of adiponectin concentration level with dietary intake, physical activity levels and ethnicity.

Methods: A total of 210 Type 2 diabetes patients consisting of three ethnicities were recruited in this cross-sectional study, which was conducted in Penang. Data on socio-demographic, medical history, anthropometry (weight, height, visceral fat, percentage of body fat and waist circumference), dietary intake (three days 24 hours diet recalls) and physical activity level (International Physical Activity Questionnaire) were obtained accordingly. Plasma adiponectin and routine laboratory tests (FBS, HbA1c, total cholesterol, LDL, HDL and triglyceride) were performed according to standard procedure.

Results: Adiponectin concentration level (Mean (SD): 6.01 (3.71) μg/ml) showed significant difference among Malays (Mean (SD): 6.85 (4.66) μg/ml), Chinese (Mean (SD): 6.21 (3.62) μg/ml) and Indians (Mean (SD): 4.98 (2.22) μg/ml). It was significantly associated with carbohydrate intake as well as physical activity levels. Indians showed a significantly lower level of adiponectin and HDL concentration (Mean (SD): 0.91 (0.16) mmol/L), and a significantly higher percentage of HbA1c (Mean (SD): 8.93 (1.78) %) compared to Malay and Chinese. Additionally, adiponectin concentration was significantly associated with HDL levels, but not HbA1c. In addition, no significantly difference was found between ethnic groups in term of carbohydrate intake and physical activity level. Apparently, hypoadiponectinemia in Indian respondents was independent of dietary intake and physical activity level.

Conclusion: In conclusion, hypoadiponectinemia in Indian respondents is not associated with lifestyle factors. The possibility of adiponectin gene polymorphism in Indians should be investigated.

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PREVALENCE OF METABOLIC SYDNROME RISK FACTORS AND ITS ASSOCIATION WITH DIETARY INTAKE AND PHYSICAL ACTIVITY AMONG ADULTS IN BACHOK, KELANTAN

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Introduction and Objectives: A cross-sectional study was carried out to determine the association between metabolic syndrome risk factors with dietary intake and physical activity among 298 adults aged more than 18 years old in Bachok, Kelantan.

Methods: The data collection processes included interview, anthropometric measurements, blood pressure measurements and blood sample collection. Out of total sample, 41.5% of subjects were males while 58.5% were females. The mean (SD) age of subjects was 49.01 years (SD 11.76). Majority of subjects were married (85.2%), had secondary level of education (57.2%), and working as housewives (39.7%). The median income earned by subjects was RM 500 per month. For the anthropometric measurements, 41.6% of respondents were overweight and 13.4% were obese. A total of 48.8% of subjects were at risk of abdominal obesity. Based on JIS (Joint Interim Statement), IDF (International Diabetes Federation) and NCEP ATPIII (National Cholesterol Education Program Adult Treatment Panel III) definition, the prevalence of metabolic syndrome was 37.5%, 32.4 %, and 28.5% respectively.

Results: Three most common metabolic syndrome risk factors found in subjects were high blood pressure, abdominal obesity, and low HDL-C level. The dietary pattern between non-metabolic syndrome and metabolic syndrome subjects showed subtle differences. Therefore, no significant differences in dietary intake were found between these two groups. Physical activity level was determined using International Physical Activity Questionnaire (IPAQ). A total 40.6% of subjects showed high physical activity level while 36.8% and 22.6% were in moderate and low physical activity level respectively. The median total weekly energy expenditure were 1970 MET/week for non-metabolic syndrome subjects and 1911 MET/week for metabolic syndrome subjects. There were also no significant difference of physical activity components between non-metabolic syndrome and metabolic syndrome subjects. No formal education, unemployed, housewives, BMI > 25kg/m^2, and high body fat percentage were identified as modifiable risk factors of the metabolic syndrome.

Conclusion: Weight management and preventive community based program involving housewives, unemployed and low educational adults need to be reinforced in order to effectively prevent and manage the metabolic syndrome in adults.

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IDENTIFICATION OF MARKERS FOR SERODIAGNOSIS OF LEPTOSPIROSIS IN MALAYSIA

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Introduction: Leptospirosis is one of the emerging diseases of both humans and animals worldwide, particularly in the tropics. It is a major public health problem due to the lack of diagnostic facilities for early detection which is key in preventing serious multi-organ complications. Although culture and microscopic agglutination test (MAT) are gold standard, they require time and expertise and are not useful for early diagnosis.

Objectives: This study was performed to identify antigenic components of *Leptospira* for sensitive and specific serodiagnosis of the disease.

Methods: Proteomic approach was used for extraction, separation and identification of leptospiral antigenic. Two pathogenic *leptospira* sero-groups prevalent in Malaysia namely Icterohaemorrhagiae (L44) and Javanica (L55) were used for antigen preparations. A total of 93 serum samples were divided into three categories namely leptospirosis, non-leptospirosis and healthy control sera. Several solubilizing agents such as acidic glycine, TritonX-114, urea, thiourea, Tris and CHAPS were used in five antigen preparations to maximize the number of antigenic epitopes; while SDS-PAGE, two-dimensional electrophoresis (2-DE), and Western blot were performed to analyse the immunoreactivity profiles. Six immunoreactive bands including two diffuse bands of 10-15, 15-20 kDa and four single bands of 15, 44, 63 and 72 kDa were identified. Chemical characterization of the reactive bands using periodate and proteinase K indicated that the diffuse bands were lipopolysaccharide (LPS) and the single bands were protein in nature.

Results: The immunoblot assay detected IgM antibodies against the 10–15 kDa LPS antigen in sera of 80.4% acute leptospirosis patients. The specificity of the antigen was above 95% indicating very low cross-reactivity with the serum of healthy people as well as with non-leptospiral febrile patients from endemic regions. Similar results were observed on dot enzyme immunoassay (dot EIA) developed using optimized concentration of eluted 10–15 kDa LPS marker. Among the protein antigens, 72 kDa antigenic band from sequential extraction (SEQ) method and 15kDa band from freeze thaw (FT) method showed similar high sensitivities (83.3% and 85.7% respectively) and specificities (95.2% and 93.3% respectively). Further characterizations by mass-spectrometry identified the top identities of the proteins were

as follows: 72 kDa as heat-shock DnaK protein and 15 kDa as a putative uncharacterized protein of *Leptospira interrogans*. Western blot using recombinant protein of the 72kDa antigen (r72SEQ) and a serum panel from patients and controls showed that the recombinant protein was 85% sensitive and 80% specific.

Conclusion: In summary, this study has identified one LPS and two protein antigens as potential infection markers for detection of specific anti-leptospiral IgM antibodies in the serum of acute leptospirosis patients particularly in the endemic environment of Malaysia.

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DEVELOPMENT OF DEMI-SPAN EQUATIONS FOR PREDICTING HEIGHT IN INSTITUTIONALISED MALAYSIAN ELDERLY

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Introduction: Demi-span measurement can be used as an alternative method to predict height for elderly people. However, there is little data on the accuracy of this method to be used in institutionalised Malaysian elderly.

Objectives: Therefore, the objectives of this study were (1) to determine the anthropometric differences among gender, ethnicity and age groups in institutionalised elderly; (2) to develop a demi-span equation for predicting height in institutionalised elderly; (3) to evaluate the accuracy of published demi-span equations derived from younger adults; and (4) to assess the accuracy of the body mass index (BMI) calculated using the predicted height from published demi-span equations and that from the new equation developed in this study.

Methods: A cross-sectional study was conducted on institutionalised Malaysian elderly aged 60 and older. Subjects were residents of eight shelter homes in Peninsular Malaysia; 205 men and 126 women (331 totals), from Malays, Chinese and Indians ethnic groups were recruited. Measurements of weight, height and demi-span were obtained using standard procedures.

Results: Data were analysed using SPSS, version 18.0. Results revealed that anthropometric measurements of the subjects (weight, height, demi-span and body mass index) differ by age and gender ($P < 0.001$), but not ethnicity ($P > 0.05$). The demi-span equations obtained were as follows: for men, height (cm) = 67.51 + (1.29 x demi-span) – (0.12 × age) + 4.13, and for women, height (cm) = 67.51 + (1.29 × demi-span) – (0.12 × age). Bland-Altman agreement analysis demonstrated good agreement between measured height and predicted height from new equations and was valid for BMI assessment. However, the predicted height from published demi-span equations derived from younger adults failed to yield good agreement with measured height and was less accurate for assessing BMI.

Conclusion: In conclusion, the new demi-span equations allow prediction of height and BMI with sufficient accuracy in institutionalised Malaysian elderly. However, further testing on other elderly samples is needed. Also, we recommend caution when using adult-derived equations in elderly people by considering the extent of anthropometric differences that may exist between the adults and elderly.

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EATING DISORDERS AMONG FEMALE ADOLESCENTS IN SECONDARY SCHOOL IN KUALA TERENGGANU

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Introduction and Objectives: This study was carried out to determine the prevalence and factors that affected the risk of disordered eating attitudes and eating disorders among female adolescents in secondary schools in Kuala Terengganu.

Methods: A total of 298 respondents aged 16 to 17 years were selected from three different types of school that consisted of daily school, boarding school and cluster school. Information of respondents' background and parents' background was obtained by using questionnaires. Eating Attitudes Test 40 (EAT-40) and Eating Disorder Inventory 64 (EDI-64) were used to evaluate disordered eating attitudes and eating disorders among respondents. Anthropometric measurements taken were weight, height and determination of Body Mass Index (BMI).

Results: Results from this study showed that the prevalence for risk of disordered eating attitudes was 17.8% while for risk of eating disorders, the prevalence was 55.7%. Findings showed that there was positive and significant relationship between disordered eating attitudes ($P < 0.01$) and eating disorders ($P < 0.05$) with BMI. Respondents that were classified as overweight and obese had a higher score of EAT-40 and EDI-64 compared to normal and underweight respondents. The chi-square test conducted revealed that age and socioeconomic status affected the risk for disordered eating attitudes among respondents ($P < 0.05$). Types of school also showed positive and significant correlation with

increased risk of disordered eating attitudes and eating disorders where respondents in boarding school had a highest score in EAT-40 and EDI-64. Results of the study showed that personality traits had a positive correlation with increased risk of eating disorders ($P < 0.01$ and $P < 0.05$). Positive and significant relationship was also shown in correlation between personality traits and risk of disordered eating attitudes ($P < 0.01$).

Conclusion: As a conclusion, objectives of this study had been achieved and it was found that major risk factors that lead to disordered eating attitudes and eating disorders were BMI, socioeconomic status, age, types of school, and personality traits.

Supervisor:
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DEVELOPMENT AND EVALUATION OF PARASITOLOGICAL AND NOVEL SEROLOGICAL TESTS FOR IMPROVED DIAGNOSIS OF AMOEBIASIS

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Introduction: Amoebiasis is an enteric protozoan infection caused by *Entamoeba histolytica*. It causes about 50 million infections and 100 000 death worldwide annually. In Malaysia, diagnosis of amoebiasis particularly extra-intestinal infection remains difficult due to lack of reliable and practical diagnostic tools.

Objectives: Thus, the aim of this study was to develop improved diagnostic methods for diagnosis of intestinal amoebiasis and amoebic liver abscess (ALA).

Methods: For detection of faecal *E. histolytica* trophozoites, the efficacies of eosin-Y, Wheatley trichrome and iodine in staining the characteristic features of the parasite were compared. The findings showed that the eosin-Y technique was the easiest and most rapid to perform; and could stain the characteristic features of the trophozoites as good as the Wheatley trichrome stain and better than the iodine stain. For detection of trophozoites in liver tissue of hamster with ALA, the efficacies of haematoxylin and eosin (H & E), periodic-acid Schiff (PAS) and immunohistochemical (IHC) stains were compared.

Results: The results showed that IHC stain gave more distinct and easily identifiable appearance of the trophozoites in a background of necrosis and inflammation as compared to H & E and PAS stains. Hence the IHC stain may be used as a confirmatory test for ALA if aspiration of liver abscess is available. Two indirect-ELISAs based on crude soluble antigen (CSA) and ether extract antigen (EEA) were developed. The sensitivity and specificity of CSA-ELISA was 100% and 93.33%, respectively, whereas the EEA-ELISA showed 91.67% sensitivity and 95.11% specificity. The novel EEA-ELISA is potentially important in resource-tight endemic countries as its antigen preparation is easier and inexpensive. As the CSA and EEA are not well-defined antigens, recombinant antigen was then developed and evaluated. It involved the analysis of potentially important new antigenic protein(s) from CSA of *E. histolytica*, followed by cloning and expression of a targeted gene and subsequently evaluation of the potential of the recombinant protein for serodiagnosis of ALA in an indirect ELISA format. Phosphoglucomutase (PGM) protein was identified by peptide sequencing as the novel ALA diagnostic marker, and its recombinant protein was used in rPGM-ELISA. The assay was shown to be 79.17% sensitive and 86.67% specific for diagnosis of ALA.

Conclusion: In conclusion, this study has developed improved parasitological diagnosis for invasive amoebiasis and two novel serological diagnosis for ALA.

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BIOCHEMICAL AND PROTEIN INTERACTION STUDIES OF RECOMBINANT HUMAN CHOLINE AND ETHANOLAMINE KINASES

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Introduction: Human choline kinase (hCK) and ethanolamine kinase (hEK) catalyze the phosphorylation of choline and ethanolamine to form phosphocholine and phosphoethanolamine, respectively. hCK consists of α1, α2 and β isoforms while hEK consists of 1, 2α and 2β isoforms. The activities of hCK and hEK have been associated with cell growth, tumor development, muscle development and sex determination. Inhibition of hCK has also been proposed as potential anti-tumor approach. Despite the importance of these enzymes in lipid biosynthesis, cancer pathogenesis, and tissue development, the roles, regulation and protein-protein interaction of different isoforms are still unclear.

Objectives: In this study, different isoforms of hCK and hEK were expressed in *E. coli,* purified and used to generate antibodies against hCK and hEK. PCR mutagenesis was used to identify the important residues for catalysis and BN-PAGE was used to determine the oligomeric structures of hCK and hEK.

Methods: Homo- and hetero-oligomers were purified by tandem affinity purification and their catalytic properties were determined by enzymatic assay. The formation of hCKα/β hetero-oligomers was confirmed by co-immunoprecipitation.

Yeast two hybrid method was used to screen for potential interacting proteins and the effect of RNAi knockdown of different isoforms on cell growth and morphology was determined. Full length hCK isoforms, hEK2α and Δ89NhEK1 and their antibodies have been produced. Several amino acids such as N345 in hCKα2, S120 and S150 in hCKβ, were found important for activity and substrate binding. BN-PAGE showed that hCK isoforms, Δ89NhEK1 and hEK2α were mainly present as tetramers, monomers and dimers, respectively. The specific activity of hCKα/β hetero-oligomer was between those of α/α and β/β homo-oligomers. Co-immunoprecipitation of hCKα and β from E. coli co-expressing both isoforms or mammalian cell lysate confirmed the formation of α/β hetero-oligomer. The first 98 amino acids of hCKβ were critical for hetero-oligomer formation. hsRPB7 was identified as an interacting partner of hEK2α from yeast two hybrid screening. RNAi of total hCKα resulted in cell death and knockdown of total hEK2 caused cellular morphological changes.

Results: The results suggest that hCKα is essential for cell survival while hEK2 affected the phospholipid composition of cells. This study has produced purified and active hCK and hEK together with their antibodies for detection of these enzymes.

Conclusion: Interaction between isoforms by forming hetero-oligomers and interaction of hEK2α with hsRPB7 provided new insights into the regulation of hCK and hEK by protein-protein interaction. The distinct effects of RNAi knockdown suggest that each isoform plays a different role in cells.

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CLONING AND PROMOTER ANALYSIS OF HUMAN CHOLINE KINASE ISOFORMS

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Introduction: Choline kinase (CK) is the first enzyme in the CDP-choline pathway for the synthesis of phosphatidylcholine in all animal tissues. CK phosphorylates choline into phosphocholine in the presence of ATP and Mg^{2+}. In humans, this enzyme is encoded by two separate genes, *ckα* and *ckβ* which produce at least three protein isoforms known as CKα1, CKα2, and CKβ. CK plays an important role in phospholipid synthesis, carcinogenesis and muscular dystrophy as well as bone deformities. The CK promoter region plays a significant role in the regulation of the ck gene expression.

Objectives: This study reports the cloning of *ckα* and *ckβ* promoters and the use of a reporter system for evaluating the promoter activity.

Methods: Promoter sequences of human *ckα* (2009 bp) and *ckβ* (2000 bp), located upstream of their respective genes, were cloned into a promoterless luciferase reporter vector, pGL4.10[*luc2*]. The recombinant plasmid was co-transfected with Renilla luciferase internal control vector,pGL4.73[*hRluc*/SV40] into the human breast adenocarcinoma, MCF-7, cell line. Its promoter activity was measured using the luciferase assay. Various 5'-terminal deletion mutants were constructed by PCR technique and cloned into pGL4.10[*luc2*] vector in order to identify the region of the *ckα* and *ckβ* promoters that are important for gene transcription.

Results: The results showed that the ETS transcription factor is a crucial negative regulator for the ckα promoter while ETS and GATA transcription factors are important negative regulators for the ckβ promoter activity. To confirm the importance of ETS and GATA on the regulation of *ckβ* gene transcription, several mutations were introduced to the ETS and GATA binding sites in ckβ promoter. The promoter activities of the mutant constructs were dramatically increased. Subsequently, MCF-7 cells transfected with *ckβ* promoter reporter vector were treated with phorbol 12-myristate 13-acetate (PMA) to explore the role of PMA in *ckβ* gene regulation via ETS and GATA transcription factors. PMA is the activator of PKC. The activation of PKC increases the binding of negative regulators, ETS and GATA and hence decreases the transcriptional activity of *ckβ* promoter.

Conclusion: Results showed that the activity of the *ckβ* promoter was significantly reduced after treatment with PMA. Thus, this study has identified ETS and GATA transcription factors as the important repressors in the regulation of *ckβ* gene transcription.

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