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ABSTRACT

Asian Conference on Biomedical Research and Laboratory Medicine was organized by three associations: Malaysian Association of Clinical Biochemists (MACB), Association of Scientific Officers Ministry of Health (ASOMH), and Malaysian Biomedical Science Association (MyBIOMED). The conference was held on 24th-25th August 2021 and a workshop on 26th August 2021 through Webex Webinar Platform with the theme of "Revolutionising laboratory medicine through research and innovation". This conference aimed to bring together leading academic, scientists, laboratory medical scientists, pathologists, researchers, and research scholars to exchange and share their experiences and research results about all aspects of laboratory medicine and health sciences. There was a total of almost 500 participants registered and over 100 abstracts submitted for this conference.

Keywords: *Biomedical research; laboratory medicine; scientific officers and clinical biochemists*

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ABSTRACTS

Proceedings of the Asian Conference on Biomedical Research and Laboratory Medicine 2021, 24 – 25 August 2021

A hospital-based study on the proportion of *Campylobacter* as a cause of diarrhoea among patients in Hospital Pakar Sultanah Fatimah, Muar Johor, Malaysia

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Abstract

Campylobacter is one of the major etiologies of bacterial gastrointestinal infection and it has been increasing in incidence reported worldwide. In Malaysia, very little information on the incidence of Campylobacteriosis is available. This study is to determine the proportion of Campylobacter gastroenteritis and to compare the proportion of Campylobacter gastroenteritis with other commonly implicated enteric pathogens among in-patients in Hospital Pakar Sultanah Fatimah Muar, Johor. A total of 854 diarrhoeic stool samples received from 1st January 2020 to 31st December 2020 were included in this study but only 179 stool samples grew enteric pathogens. *Salmonella* non-typhi species were the commonest stool bacteria pathogen isolated (10%) followed by *Campylobacter* species (4.5%), enteropathogenic *Escherichia coli* (3.8%), *Aeromonas* species (2.5%) and *Vibrio parahemolyticus* (0.35%). Out of 38 stool samples that recovered *Campylobacter*, *Campylobacter jejuni* was identified in 36 stool samples (94.7%) and the other 2 samples contained *Campylobacter coli*. 92.1% of the *Campylobacter* was isolated from children below the age of 7. All of the *Campylobacter* isolates were resistant to ciprofloxacin; 63.2% were resistant to erythromycin and 94.7% were resistant to doxycycline. In conclusion, *Campylobacter* was the second most common enteric bacterial pathogen in diarrhoeic stools. It still can be the aetiology of gastroenteritis in adults although it is more common to be recovered from paediatric age groups. This observation indicates that Campylobacter is one of the common bacterial causes of diarrhoea in Malaysia. Thus, this organism must be sought by diagnostic laboratories in their routine examination of diarrhoeic stool.

Keywords: Antibiotic susceptibility; *Campylobacter*; *diarrhoea and enteric bacterial pathogen*

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Zika virus–host interactome: a high-throughput yeast two-hybrid screen

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Abstract

Zika virus (ZIKV) became a significant health concern when outbreaks of the disease were associated with microcephaly and other neurological disorders in infants. The Zika epidemic peaked in 2016 and has affected over 80 countries worldwide. There are currently no approved antiviral drugs or vaccines for its prevention. In this study, we attempted to decipher ZIKV-human host protein-protein interactions to unravel the molecular mechanisms involved. Five ZIKV genes of a Brazilian isolate were cloned into the pGBKT7 vector and expressed in yeast cells. The human proteins targeted by ZIKV proteins were then identified through a yeast two-hybrid system (Y2H) using a universal human cDNA library. Then, an enrichment analysis was performed for the interacting human proteins using Gene Ontology (GO) database (DAVID) to characterize the molecular functions, biological processes, and cellular components. The Y2H screening identified 58 human proteins targeted by two structural and three non-structural ZIKV proteins. GO analysis revealed enrichment of human proteins involved in centrosome duplication, mitotic processes, synaptic vesicle exocytosis, regulation of endothelial barrier, ubiquitin-proteasome pathway, and protein binding. Some of the important proteins found in this study include TRAF6 (involved in inflammatory pathway and immune response), CEP192 (implicated in process related to microcephaly), and SNAPIN (involved in central nervous system development). Altogether, these findings facilitate a better understanding of cellular pathways hijacked by the virus to replicate, escape innate immunity, and induce neuropathogenesis. Importantly the identified human proteins represent attractive targets for the development of effective host-directed antiviral drugs.

Keywords: *Zika virus; protein-protein interaction; neuropathogenesis; gene ontology and host-directed antiviral drugs*

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Identification of Neuropilin 2 as a host factor in Chikungunya virus infection

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Abstract

The growing occurrence of Chikungunya virus (CHIKV) infections demands specific antiviral strategy for minimizing morbidity and mortality. One of the ways to develop an antiviral strategy is using host factors as a target. There are many important host factors that have been identified to be involved in facilitating CHIKV infection using different screening methods such as the CRISPR/Cas9 genome-wide screening, LCMS/MS tagged screening and yeast two hybrid screening. A high throughput yeast two-hybrid (Y2H) screening was used to identify the host factors via the interaction of E1 and E2 proteins (bait plasmid) with human cDNA library (prey plasmid). Bait and prey plasmid were mated together to produce interactions. Positive interactions were distinguished by the presence of blue colonies on selective agar plates. Neuropilin 2 (NRP2) was chosen out of seventy positive interactions from yeast-two-hybrid screening. An interaction map was done to see the list of proteins obtained from the screening. NRP2 was selected based on the previous finding which indicates NRP2 is one of the receptors for viral entry. Yet, downstream assays will need to be done to confirm. We have successfully obtained positive interactions for both E1 and E2 of CHIKV with human host protein through yeast two hybrid systems. It shows that yeast two hybrid systems can determine protein-protein interaction between CHIKV and human host protein. This could help in better understanding of viral pathogenesis and identification of host biological pathways involved in viral replication, maturation, and spread of infection.

Keywords: *Chikungunya virus; yeast-two-hybrid; protein-protein interaction; human proteins and Neuropilin 2*

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Investigating the relationship between differentially expressed miRNAs with PD-1, PD-L1 on the clinical variables of head and neck epithelial carcinoma

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Abstract

Head and neck cancer is the sixth most common cancer worldwide with increasing incidence year by year. With lack of clinical manifestations and hidden locations, head and neck cancers usually have poor prognosis. Tumorigenesis and disease outcome are influenced by a variety of genetic and non-genetic factors. Research has implicated that miRNAs are master regulators of cellular processes with essential role in cancer initiation, progression, and metastasis. miRNAs also possess remarkable stability in circulation due to their existential forms which, coupled with the mentioned cellular roles, make them a promising therapeutic and diagnostic molecule for cancer management. Meanwhile, advances in immuno-oncology have transformed the treatment of head and neck cancers by targeting immune checkpoints such as PD-1 and PD-L1 with immune checkpoint inhibitors like Nivolumab, Pembrolizumab and Atezolizumab. Nonetheless, there is limited information regarding the relationship between miRNA and PD-1, PD-L1. Hence, the objective of this study is to determine the relationship between circulating biomarkers with PD-1 and PD-L1, which can predict tumour staging and grading. The imaging characteristics of malignant tissues were compared to immunohistochemistry results and microRNA expression. Immunohistochemistry assay were performed on Formalin-Fixed Paraffin-Embedded tissue and qPCR were performed on patients' blood samples. New knowledge on the association between the expression of apoptotic biomarkers (PD-1, PD-L1, and miRNA) with staging and grading of head and neck malignant tumours will be obtained.

Keywords: *Head and neck cancer; biomarker; miRNA; PD-1 and PD-L1*

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H-NMR-based metabolomics approach to understanding breast lump disease in Hospital USM - preliminary study

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Abstract

Breast lump is a common symptom of breast pathology and it can be due to benign or malignant causes. The tumor markers used in breast cancer have limited capacity for early cancer detection. Metabolomics analysis has lots of potential to identify new blood biomarker and solving this issue. This study aims to determine the metabolite profile of breast lump patients using ^{1}H NMR metabolomics approach. This was a case control study conducted at BestARi unit, Hospital USM. The serum for metabolic profiling from breast lump (benign & malignant) patients were compared to a healthy group. Serums were analysed using proton nuclear magnetic resonance spectroscopy (^{1}H NMR). Partial least squares discriminant analysis (PLS-DA) was conducted in order to discriminate between the 3 groups. Variable's importance (VIP) score was used to determine the variable with the most contribution towards group differentiation. PLS-DA score plot showed discrimination between the 3 groups while PLS-DA loading plot revealed most of the metabolites were higher in malignant group. Myoinositol contributed with the highest VIP score. There were different metabolites identified between benign and malignant breast lumps. Myoinositol could be the marker to differentiate between normal and pathological breast lumps. The potential usage of metabolomics in managing breast cancer and the results of this work may enhance towards the further understanding of the underlying molecular mechanism and the quest for suitable biomarker.

Keywords: *breast lump; metabolomics; NMR; biomarker and metabolites*

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A retrospective demographic study of head and neck cancer patients at Hospital Serdang

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Abstract

Head and neck cancer is the sixth most common cancer worldwide. Tobacco consumption is known to be the major contributor to this malignancy. Nasopharyngeal carcinoma is the commonest head and neck cancer in Malaysia where Chinese is the major diagnosed patient. Histopathologically confirmed head and neck cancer patients of any gender and race aged between 21 to 80 years from January 2010 to January 2020 were selected for the analysis. A total of 318 retrospective demographic data were manually extracted from the Hospital Information System at Hospital Serdang. Data showed that male (n=228, 72%) to female (n=90, 28%) ratio is 2.5:1. The most diagnosed patients was Chinese (n=157, 49%), followed by Malay (n=124, 39%), Indian (n=28, 9%) and others (n=8, 3%). The mean age of the patients is 54 years, the median age is 55 years. Out of 318 patients, 183 patients are a smoker (57.5%), 135 patients are a non-smoker (42.5%), this gives the smoker to non-smoker ratio of 1.36:1. This analysis is limited by the inability to collect complete data due to non-standardized documentation and lost data from the information system. Our study illustrates that the incidence of head and neck cancer is more common in male than female. Chinese is the majority among all races. The common age of patients ranging from 51 years to 60 years. Patients who are smokers showed a slightly higher number than a non-smoker.

Keywords: *Demographic analysis; head and neck cancer; Hospital Serdang and retrospective study*

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Whole-genome sequencing and annotation of *Barrientosiimonas humi gen. nov., sp. nov. 39^T*, a novel rare actinobacteria from Barrientos Island, Antarctica

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Abstract

A genomic analysis study presents the whole-genome sequencing and annotation of *Barrientosiimonas humi gen. nov., sp. nov. 39^T* to unravel the genomics data of *B. humi* sequences and understand the related biological functions information expression. Whole genome of *B. humi* isolate was sequenced using PacBio Sequel and then polished by Illumina HiSeq. The data was further undergone bioinformatic analysis for genome annotation. The circular genome of *B. humi* was then visualized. Totally, 83,639 reads were predicted from its 3.6Mb genome size. It composed of 3,381 coding genes, with 95% were functionally annotated. *B. humi* was evident sharing close sequence similarity with species *Demetria terragena* and family *Dermacoccaceae*. Gene Ontology (GO) indicated cell and cell part were highly represented among cellular component, catalytic activity and binding were most enriched process within molecular function, metabolic process and cellular process were the top in biological process. Clusters of Orthologous Group (COG) revealed metabolism related genes were highly enriched, mostly mapped to amino acid transport metabolism, transcription, energy production and conversion. Kyoto Encyclopedia of Genes and Genomes (KEGG) reported that metabolism process was the most represented KEGG pathways, which biosynthesis of antibiotics and metabolic pathways associated genes were significantly functioning. There were 52 biosynthetic gene clusters involved in secondary metabolites biosynthesis. We found that *B. humi* was capable to produce antibacterial, antifungal, cytotoxic and inhibitor bioactive compounds. The natural producer of multiple ectoine biosynthesis genes were expressed in *B. humi* genome region, elucidating its potential to adapt and function in extreme environments. *B. humi* genomic sequence positions of the predicted coding regions were illustrated. Our research is the first whole-genome sequencing study in *B. humi*, which serves as a significant approach that offers the comprehensive genomic data to accelerate the future studies of novel secondary metabolites extraction.

Keywords: *Actinobacteria; annotation; Barrientosiimonas humi; biosynthetic gene clusters and genome sequencing*

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Reproductive toxicity in female rats exposed to technical xylene during preimplantation period of gestation

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Abstract

A comprehensive study on xylene toxicities to the female reproductive system and prenatal development is vital to improve the current health and safety guidelines for laboratory and industrial workers exposed to xylene on daily basis. This animal study is aimed to investigate the toxicity of technical xylene to the female reproductive system when exposed during the preimplantation stage of gestation. Mature female Sprague-Dawley (SD) rats aged six to eight weeks old at the proestrus phase were caged overnight for mating with an adult male of a similar strain. Females with the presence of sperm in their vaginal smear were considered pregnant with gestational day 0 (GD0). These pregnant females were randomly grouped (n=9) for 100, 500, and 1000ppm of technical xylene (including control) daily administration from GD1 to GD3 via intraperitoneal injection. The animals were then sacrificed on GD5 for data and sample collection. The results showed a significant decline in maternal body weight, food consumption, and total implantation sites in the pregnant rats ($p<0.05$) compared to untreated rats. Increased liver weight was observed in rats administered with 1000ppm of technical xylene compared to the control group. Histological findings of the liver, kidney, and lungs showed a systemic inflammatory response as a result of toxic insults to technical xylene in all groups. Female reproductive toxicity with tissue inflammation was evident from the exposure of technical xylene during the gestational preimplantation phase. Further investigations are warranted to explore the teratogenic effects of technical xylene.

Keywords: *Technical xylene; maternal toxicity; reproductive toxicology; female reproductive system and preimplantation*

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Managing reagent lot-to-lot variation for full blood count at core laboratory, Hospital Kuala Lumpur

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Abstract

Each new reagent lot must be validated ahead of using, according to the MS ISO 15189 accreditation standard. For Full Blood Count (FBC), we faced difficulty when it comes to verifying reagent lot-to-lot variation (LTLV). The inability to load concurrent reagent into the analyser had been a stumbling block in the deployment of lot-to-lot verification. The Clinical and Laboratory Standards Institute (CLSI) EP26-A guideline was adapted with slight modification in the assessment of new lot for FBC reagents. Due to the constraints, two analysers were involved in assessing LTLV. Prior to the evaluation, a correlation study was undertaken. In this study, Cellpack DCL, SLS, Cellpack DFL, Fluorocell RET, Fluorocell and Lysercell WNR and Fluorocell and Lysercell WDF were evaluated based on the related parameters. The number of samples required, desired concentrations and rejection limit were set for each FBC parameters excluding calculated parameters. The reagent balance in the analyser was regularly reviewed, and samples with desired concentrations were collected in advance. Based on the calculated rejection limit, acceptability of the new reagents lot was determined. Reagent in use and candidate reagent were evaluated using two well correlated analysers with acceptable bias. The difference between lot-to-lot was less than the calculated rejection limit for all parameters evaluated. Therefore, were accepted for use. Lot-to-lot testing in haematology should be continued despite the limitations to ensure accurate and reliable patient results. It is essential to plan ahead of time when assessing new reagent lots.

Keywords: *Lot-to-lot variation (LTLV); full blood count (FBC) and haematology*

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Laboratory turnaround time (LTAT) study of samples received in 2020 in Drug Detection Laboratory, Pathology Department, Queen Elizabeth Hospital Sabah

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Abstract

Laboratory turnaround time (LTAT) is considered as one of the most important indicators of work efficiency in a laboratory. In the Pathology Department HQE, LTAT is set as one of the quality objectives which a laboratory must at least achieve 80 % of LTAT agreed with clients. The objective of the study is to fulfil the requirement of the MS ISO 15189:2014 audit of timeliness quality objective. A retrospective data analysis study of 9032 samples received in 2020 was performed to investigate whether our laboratory can achieve the set up LTAT targeted for department quality objectives for screening and confirmatory tests. 1.16% rejected samples for various reasons in 2020 was excluded from the data analysis. Results showed that we achieved 99.6% of 3 working days LTAT for screening test of Amphetamine type stimulant (ATS), opiate, cannabis (THC) and ketamine group, 95.4% of 5 to 14 working days LTAT for ATS and ketamine confirmatory test, 83.6% for THC confirmatory test and 54.2% for opiate confirmatory test. The reason the opiate confirmatory test did not achieved 80% of LTAT is caused by the breakdown of fume hood used for Thin Layer Chromatography testing in September 2020. This test was held off temporarily until the fume hood was repaired Overall, we achieved more than 80% LTAT for all tests performed in the Drug Detection Laboratory except for the opiate confirmatory test and have met the quality objective set up by the Pathology Department Hospital Queen Elizabeth.

Keywords: *LTAT; ISO 15189; amphetamine; opiate and cannabis*

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Cardiac troponin request in emergency department at tertiary teaching hospital: cost-effectiveness and cost-benefit analysis

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Abstract

According to the latest guideline, cardiac troponin (cTn) is the proposed marker for myocardial infarction. Thus, our laboratory has discontinued the creatine kinase–MB (CK-MB) test in May of 2020. The study's objective is to evaluate the cost-effectiveness strategies and cost-benefit analysis of cTn-T request post-discontinuation of CKMB in the Emergency Department (ED). This cross-sectional study from January 2020 to May 2021 utilising Laboratory Information System (LIS) data. The result is analysed using SPSS and Excel software. The cost per test calculated for cost analysis. The strategies are ; (1) Consistent discussion between physicians and laboratory consultants (2) Establishment of protocols without jeopardising patient care (3) Enhancements in the LIS for appropriate request (4) Closely screening of the request. From January to April 2020, the total number of cTn-T requests is 445, with a mean (SD) of 111(67) tests per month, and the request increased to 352 in May. During six months intervention phase (Jun 2020- November 2020), the total cTn-T test was 1891 with a mean (SD) test per month of 315(40). From (December 2020- May 2021), the total cTnT test significantly reduced to 443 with the mean(SD) test per month of 74(22) with a p-value <0.001. Thus, laboratory cost reduction was significant from the intervention phase with the mean(SD) per month of MYR12606(1615) to post-intervention of MYR2953(862), respectively with the p-value <0.001. CKMB elimination raised the cTn-T request substantially. Several interventions demonstrate the cost-effectiveness of cTn-T requests following discontinuation of CKMB.

Keywords: *Cardiac troponin; cost-effectiveness; cost-analysis and CKMB*

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Monitoring of blood sample quality storage in portable car refrigerator (BIOBASE)

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Abstract

Every blood sample withdrawn from the respondent should be taken to the laboratory promptly to maintain the sample's quality thus ensuring the reliability of the test results. In this study, we investigated the association between the Hepatitis B Surface Antigen (HBsAg) test results with the delayed duration of testing. Blood samples were taken at the selected data collection site, centrifuged and stored in a portable car refrigerator with a temperature range between 2-8 ^{0}C. The presence or absence of HBsAg in the sample was determined by comparing the chemiluminescent signal in the reaction to the cut-off signal (S/CO). Out of 21 blood samples, four blood samples were randomly selected to run the HBsAg test in five storage conditions (baseline hour, 24 hours, 36 hours, 72 hours and 84 hours). The mean of the results from each group was compared with the baseline using Student's independent t-test. The 18-litre portable car refrigerator brand BIOBASE (Model CR-18) used had a temperature ranged between 0^{0}C~ 65^{0}C. All the samples (n=4) were non-reactive (S/CO values < 1.00). The mean results at baseline, 24, 36, 72 and 84 hours were 0.455±0.17, 0.360±0.04, 0.343±0.36, 0.403±0.07 and 0.348±0.04, respectively. Using baseline as a reference, there were no significant different at 24 hours [t(3)=0.996, p=0.393], 36 hours [t(3)=1.364, p=0.266], 72 hours [t(3)=0.508, p=0.646] and 84 hours [t(3)=1.487, p=0.234]. Storage of blood samples showed no deterioration in testing quality with increasing hours of up to 84 hours.

Keywords: *Blood sample; portable car refrigerator and Hepatitis B surface antigen*

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Hypertriglyceridemia-induced acute pancreatitis in pregnancy: a case report

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Abstract

Hypertriglyceridemia is a rare but well-known cause of acute pancreatitis (AP). An elevation of serum triglyceride >11.3 mmol/l is considered the threshold to trigger AP. We report a case of a 33-year-old female, gravida 2 para 1 at 28+4 weeks of gestation, who presented with a history of epigastric pain for 3 days associated with fever and vomiting. Abdominal examination revealed epigastric tenderness with guarding. The blood sample was found to be severely lipemic and laboratory analysis revealed severe hypertriglyceridemia (HTG) of 94.8 mmol/L with other biochemical evidence which supports AP. We highlight a case of hypertriglyceridemia-induced AP secondary to pregnancy. Other causes of HTG had been ruled out including a family history of dyslipidemia or premature death due to coronary artery disease. Lipemia in the patient's sample was removed by manual dilution and/or high-speed sample centrifugation. The clear supernatant (post centrifugation) was analyzed to provide reliable results to aid diagnosis and patient management. It is crucial to understand lipemia as an important interfering factor in laboratory tests and to apply a systematic approach towards lipaemia removal depending on the analytes measured as this interference will lead to the inaccurate result produced and may affect patient management.

Keywords: *Hypertriglyceridemia; pancreatitis; pregnancy; lipemia and centrifugation*

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Exploring the neuroprotective effects of *Cordyceps militaris* against Aβ42-induced neurotoxicity

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Abstract

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease, commonly caused by abnormal accumulation of amyloid beta (Aβ) and neurofibrillary tangles. The use of cholinesterase inhibitors reported side effects, leading to investigations of novel potent inhibitors that may induce minimal complications without compromising drug efficacy. Although numerous studies have indicated the vast medicinal properties of *Cordyceps militaris* (CM), minimal on neuroprotective effects. Hence, this study aims to elucidate the neuroprotective effects of CM against $A\beta_{42}$-induced neurotoxicity. Prior the $A\beta_{42}$ 24-hour incubation, SH-SY5Y human neuroblastoma cells were pre-treated with various concentrations of CM extract and/or donepezil (positive control). After incubation, the effects of single or combinatorial treatment of CM extract and/or donepezil on cell viability and proliferation were studied using MTT assay. Subsequently, molecular docking analysis was performed to study the interactions of CM ligands (adenosine, cordycepin, 3-deoxyinosine) with selected AD-related proteins (CREB1, GSK3β). Selected protein expression evaluated using Western Blot. Singular treatment of CM extract enhanced the survival and growth of $A\beta_{42}$-treated cells. Similar phenomenon was observed in singular treatment of donepezil. In combinatorial treatment, the $A\beta_{42}$-treated cell proliferation is significantly higher compared to singular treatment of CM extract and donepezil. 3-deoxyinosine displayed strongest binding energy towards CREB1, followed by adenosine and cordycepin; whereas adenosine was evidently the stronger ligand for GSK3β compared to 3-deoxyinosine and cordycepin. CM decreases cellular toxicity. The bindings are believed to inhibit the accumulation of CREB1 and GSK3β in inducing neurotoxicity. CM extract exhibit therapeutic and preventive functions against AD.

Keywords: *Alzheimer's disease; amyloid beta; natural product; Cordyceps militaris and neuroprotective effects*

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Mutation detection of a case with heritable disorder of connective tissue using next-generation sequencing and Sanger sequencing

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Abstract

Heritable disorders of connective tissue (HDCT) are a heterogeneous group of genetic conditions caused by defective fibrillin, elastin, collagen, and other related biomolecules. Specific genetic testing for HDCT is unavailable due to the genetic heterogeneity of the disorders. Hence, the use of massively parallel sequencing technology is critical to enable the dissection of a broad genome spectrum. The study was conducted with the approval of the human ethics committee. A total of 5 ml of blood was collected from an individual with connective tissue disorder history. DNA was extracted and analyzed using NGS whole-exome sequencing. Grounded on the mutation findings derived from the exome sequencing, specific primers were designed and employed in a polymerase chain reaction and Sanger sequencing for validation purposes. A likely pathogenic variant 48741078 NC_000015.9g was identified in the *FBN1* gene. This missense mutation causes the change from cysteine to tyrosine at position 1853. The upstream and downstream primer successfully flanked and amplified 818 bp of amplicon containing the variant. Double peaks at the specific site on the Sanger sequencing electropherogram validated the heterozygous of the variant. The use of NGS technology offers ultra-high throughput and scalability to capture large DNA regions at a low cost with a short running time. The potential variant derived from the analysis provided a basis for genetic counselling by clinical geneticists. The outcome from the study is imperative to understand how the disease would be inherited, therefore early identification and clinical management for at-risk relatives could be made.

Keywords: *Connective tissue disorder; mutation; Next-generation sequencing; polymerase chain reaction and Sanger sequencing*

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Cytotoxicity effects of triphenyltin (IV) dithiocarbamate compounds with different ligands on Jurkat E6.1, leukemia cell line

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Abstract

Following cisplatin, many attempts have been made to study the anticancer effects of other metal complexes. Organotin(IV) dithiocarbamate is a type of compound that is getting more attention as an anticancer agent due to its potent cytotoxic properties towards cancer cells. In this study, a series of newly synthesized organotin compounds known as triphenyltin(IV) diisopropyl dithiocarbamate (Compound 1), triphenyltin(IV) diallyl dithiocarbamate (Compound 2), and triphenyltin(IV) diethyl dithiocarbamate (Compound 3) have been assessed for their cytotoxic effects toward Jurkat E6.1 cell line. WST -1 [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium] assay was used in determining cytotoxic effects for each of the compounds for 24 hours. The mode of cell death was assessed by Annexin V-FITC/PI staining assay for 24 hours using the IC_{50} values obtained. The morphological characteristics of the treated cells after 24 hours was observed using inverted microscope. All triphenyltin(IV) dithiocarbamate compound exhibited potent cytotoxic effects toward Jurkat E6.1 and induce cell death via apoptosis. Morphological observation conducted showed the characteristic of apoptosis such as cell shrinkage and membrane blebbing after 24 hours of treatment. Different ligand on triphenyltin(IV) dithiocarbamate compounds cause different cytotoxic effects on Jurkat E6.1 cells. All compounds showed good potential to be developed into anti-leukemic agents due to their potent cytotoxicity against acute lymphoblastic leukemia cells (Jurkat E6.1) and which lead to the induction of cell death via apoptosis. However, further studies on their mechanisms of action are needed to explore the potential of these compounds before they can be developed as anti-leukemic agents.

Keywords: *Jurkat E6.1 cell line; Organotin(IV) dithiocarbamate compounds; cytotoxicity; apoptosis and anti-leukemia activity*

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Importance of reliable local geometric Mean Normal Prothrombin Time (MNPT) in procedures for validation of International Normalised Ratio (INR)

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Abstract

Verification of International Normalized Ratio (INR) is required whenever there are changes in Prothrombin Time (PT) testing system to verify the reagent's International Sensitivity Index (ISI) and local geometric Mean Normal Prothrombin Time (MNPT) prior to diagnostic use. To assess the reagent's ISI acceptability and local MNPT. Validation of INR was done for new lot number of PT reagent. We established our local MNPT using at least 20 healthy donors. The samples were analysed using HemosIL PT reagents on ACL TOP 550 CTS in the year 2018, 2019, 2020 and 2021. We used certified plasma (HemosIL INR Validate) for the INR verification study. Results obtained were within 15% of target value were reported as acceptable by the software. Performance of PT and INR tests were also monitored using internal quality control (IQC) and external quality assurance (EQA) program. Our local MNPT in 2018, 2019, 2020 and 2021 were 11.1 sec, 11.3 sec, 10.6 sec and 11.2 sec respectively. MNPT in 2020 was significantly lower than the manufacturer's mean. However, verification of INR was acceptable thus the PT reagent was used for patients' testing. Unfortunately, EQA performance of our PT and INR tests in 2020 highlighted the presence of low bias in our results. Validation of INR and comparison of local MNPT with manufacturer's mean of normal PT is required to assess acceptability of new lot number of PT reagent. The significant differences in local MNPT should trigger the possibility of reagent-related problems.

Keywords: *Geometric Mean; Prothrombin time; INR; coagulation; change lot; quality assurance and MNPT*

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Effect of *Litsea elliptica* Blume essential oil as antimalarial agent towards HSD mice infected with *Plasmodium berghei* NK 65

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Abstract

Malaria is widely known as a serious infectious disease due to the increasing of antimalarial drug resistance. Therefore the search for a new antimalarial drug is urgently needed. Pertaining to the matter, *in vivo* study was done to evaluate the effects of essential oil of *Litsea elliptica* as an antimalarial agent towards *Plasmodium berghei*. Approximately 96 HSD mice were grouped into normal control, untreated, treated with chloroquine and treated with *L. elliptica at* 100 mg/kg and 300 mg/kg dosages. Three methods of treatment employed were post-infection, prophylactic and inoculation. The parasitemia, blood profile, liver function test (AST, ALT and total protein liver), and the analysis of histology and mass of liver and spleen were evaluated. Treatment by 300 mg/kg dose in post-infection has shown a significant reduction of parasitemia and white blood cell differential counts of eosinophil, neutrophil and monocyte. There was also an effective increase in red blood cell count and haemoglobin level as well as protective effects towards the liver when 300 mg/kg was given prophylactically. The protective effects were seen in reductions of AST and ALT levels and histology for both liver and spleen. A significant reduction of white blood cells was found in the inoculated 300 mg/kg treated group. However, this essential oil did not give a significant effect on platelet and liver total protein. Essential oil of *L. elliptica* at 300 mg/kg dose demonstrated potential as an antimalaria agent.

Keywords: *L. elliptica and antimalaria*

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Effect of silencing *CTNNB1* on cells apoptosis in HAC15 human adrenocortical cells

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Abstract

Aldosterone-producing adenomas (APAs) excessively synthesizes aldosterone. Activation of *CTNNB1* (β-catenin) in transgenic mice increased aldosterone production and risk of adrenocortical tumors, suggesting that the Wnt/β-catenin pathway plays a role on APA tumorigenesis and aldosterone secretion. Herein we aim to investigate the effect of silencing *CTNNB1* on cell apoptosis in human adrenal cells. A subclone of the H295R immortalized human adrenocortical cell line, HAC15 was transfected with ONTARGET plus siRNA (Thermo Scientific) or relevant controls using the Neon™ Transfection System 100 µL Kit (MPK10096, Invitrogen) according to manufacturer's recommendations. The apoptosis assay was performed after 48 hours of transfection using the Pacific Blue™ Annexin V/SYTOX™ AADvanced™ apoptosis kit (A35136, Invitrogen) on the BD FACSVerse™ system. The supernatants and cells were harvested for aldosterone (IS-3300, IDS-iSYS) and cortisol (06687733190, Roche Elecsys e100), and RNA isolation (12183018A, Invitrogen) for real-time PCR via Applied Biosystems ABI 7000. Experiments were repeated 3 times independently. *CTNNB1* silencing significantly downregulated the *CTNNB1* and *CYP11B2* mRNA expression and decreased aldosterone secretion, with no significant change on cortisol production. Flow cytometric analysis of *CTNNB1* silencing showed no significant difference in percentage of apoptosis cells compared to control cells. Our finding demonstrated inactivating *CTNNB1* lead to reduction of aldosterone secretion with no effect on apoptosis rate in HAC15 cells. However, further experiment on cell proliferation needs to be performed to rule out if modulating *CTNNB1* can affect cell fate. Inhibiting Wnt/β-catenin pathway to decrease aldosterone secretion may be a therapeutic target for APA.

Keywords: *Aldosterone Producing Adenomas; aldosterone; CTNNB1; Wnt/β-catenin signaling pathway and apoptosis*

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In silico identification of anti-SARS-CoV-2 therapeutic peptides from scorpion venom

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Abstract

The coronavirus disease 2019 (COVID-19) outbreak posed a significant threat to human health worldwide. Although the development of anti-SARS-CoV-2 vaccines and antiviral agents have been extraordinarily rapid, only several drugs confer promising treatment outcomes in clinical recovery and a reduction in the risk of mortality in the severe COVID-19 patients. Produced by scorpion *Androctonus mauritanicus*, Mauriporin showing promising antiviral property was explored in the present study. The sequence of mauriporin was retrieved (ID: N0EAL3) and fragmentized. The physicochemical properties, lipophilicity, water solubility, pharmacokinetics, druglikeness, medicinal chemistry, toxicity hemolytic, and allergenicity of mauriporin-derived peptides (MPs), were studied for peptide safety evaluation. All MPs were then biocomputationally investigated for their inhibitor potential against SARS-CoV-2 main protease, M^{pro} (PDB ID: 6LU7). A total of 34 MPs were generated, but only two potential anti-SARS-CoV- 2 peptides were identified. These two MPs were predicted to be non-toxic and non- allergenic with low hemolytic activity. Based on the docking targeting SARS-CoV-2 M^{pro} protein, MP1 showed the strongest binding energy (-210.490) followed by MP5 (-202.441) compared to reference N3 peptide inhibitor (-215.634). The α-helical structure of MP1 could interact stably by forming four hydrogen bonds with the key binding residues (His164 and Glu166) of the viral M^{pro} target protein, thus inhibiting M^{pro} activity. Two non-toxic and non-allergenic anti-SARS-CoV-2 peptides (MP1 and MP5) were identified and could be used as a useful template in designation of potent, specific, and stable anti-SARS-CoV-2 peptides.

Keywords: *in silico; SARS-CoV-2; main protease (M^{pro}); peptides and scorpion venom*

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From serology to genotype: updates in immunohematology testing

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Abstract

Red blood cell (RBC) phenotyping test is performed daily for National Blood Centre (NBC) blood donors. However, RBC phenotyping is only done on a limited blood group system: Rh, Kidd, Duffy and Ss. Transfusion-dependent patients require more antigens testing to avoid allo-antibody production. Genotyping offers more antigens testing which include V, VS, M, N, K, k, Kp^a, Kp^b, Do^a, Do^b and etc. Pilot study was carried out from October until November 2020 involving 58 rare blood group donors from NBC. Purification of DNA was done using GeneAll kit methodologies. Red blood cell genotype was carried out via automation using the PreciseType HEA test by Immucor. Out of the 58 rare blood group donors, four donors were serologically typed as Fy(a-b-) and six donors were serologically type as Jk(a-b-). The results show that all donors with phenotype Fy(a-b-) have GATA silencing mutation present. Meanwhile, all blood donors with phenotype Jk(a-b-) were found to have presence of JK*B allele. The frequency of Jk(a-b-) and Fy(a-b-) phenotypes is quite low in most populations. Results discrepancy between serology and genotyping for Jk(a-b-) phenotype requires further investigation. Sequencing of Jk(a-b-) samples may reveal novel mutations. These findings prompt immunohematologists to initiate the Ministry of Health to consider red blood cell genotyping for all rare blood group donors. This is to avoid any clinical implication on patients if the wrong blood phenotype were transfused.

Keywords: *Red blood cell; genotyping; rare blood group and blood donor*

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Identification of unknown dried forensic larvae using cytochrome oxidase 1 gene

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Abstract

Identifying the correct fly species is the vital step for estimating the age of the oldest fly larvae to be used as an indicator for post-mortem interval (PMI). Usually, morphological comparison using taxonomy keys is used for species identification. However, it requires special expertise, often time-consuming and may be hampered by imperfect specimens as the larvae do not have perfect morphology. In this study, the cytochrome oxidase 1 gene (COI) region was used in Polymerase Chain Reaction (PCR) method to identify unknown dried larvae stored in Forensic Unit Laboratory, Universiti Kebangsaan Malaysia. All specimens, which were left unpreserved and in dried condition were subjected to DNA extraction and PCR. To analyze the species, the DNA sequences were then subjected to data analysis using the BLAST database. All the extracted DNAs were less than 10ng/µl. The sequencing analysis revealed that all specimens were *Chrysomya megacephala* (Fabricius). Fly larvae can be physically dried when exposed to heat or left unattended for a period of time. Even though the specimens were not kept in any preservative, the DNA products obtained were still intact and useful for identification. This study showed the COI gene is able to identify all dried larvae and thus, a suitable region of the larvae genomes for comparison with reference data. The DNA method is very useful for larval identification, thus can be an alternative to morphological method.

Keywords: *Forensic entomology; molecular; cytochrome oxidase 1; dried larvae and Chrysomya megacephala.*

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Case study: An investigation on the effect of different creatinine calibrator's values on quality control (QC) performance and patient results

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Abstract

Change in the calibrator lot often leads to shifting in the QC performance as observed on the Levey-Jennings plot and is a common dilemma to the laboratory. This study aims to investigate the performance of quality control and patient samples against different creatinine's calibrator values. The CFAS calibrator was run as a sample over a period of 20 days to establish assayed mean. Biorad Chemistry Control and PreciControlClinChem (PCC) were run in duplicate against the assigned calibrator value and assayed calibrator value to obtain bias and TEA value. Patient samples that cover the low-normal-high range and medical decision limit were run in duplicates against both assayed and assigned calibrator value. P values were accessed to check for significant differences between the results. The assigned calibrator value for creatinine was 370 and the mean assayed value was 341.8. All precision results were within the EFLM desirable target. Bias was lower in assigned values for both Biorad and PCC that ranged from 1.61% to 3.01% as compared to assayed calibrator value (4.24% - 6.92%). TEA for Biorad is within the ELFM desirable target. P values show less than 0.05 for all the levels of QC thus indicate statistically significant difference between assayed value and assigned value for all QC. Nevertheless, patient samples have p value more than 0.05 for all ranges of samples. Statistically significant differences are observed on the QC samples upon changes of the calibrator value. However, the creatinine values in patient samples are not affected.

Keywords: *Calibrator; change lot; trend shift; assigned value and assayed mean value*

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Observer agreement in assessing spheno-occipital synchondrosis fusion for forensic age estimation using digital photographs

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Abstract

Forensic anthropologists have long been using visual assessment of morphological characteristics to establish a biological profile to assign an identity to human remains that cannot be identified. Understanding the role of observer agreement is critical for the assessment of age-related morphology as this method can be made prone to inherent subjectivity in the qualitative phase assessment. A total of 10 observers, with varying levels of qualification and experience, scored 12 spheno-occipital synchondrosis fusion status based on digital photographs. An easily repeatable and reproducible three-stage scoring method was used in this study. Fleiss' Kappa was used in order to assess inter-observer agreement. Results showed that the overall agreement percentage for all observers was 62.78% ($\kappa = 0.44$), increasing to 83.33% ($\kappa = 0.75$) when taking into account only the score given by observers who also had experience with photographs of skeletal remains. Generally, anthropological methods are reliable and that accuracy increases with the observer's level of experience. Further, photographs are problematic to interpret without the necessary anthropological training. The present study has highlighted the importance of proper training in the recognition of key traits in skeletal morphological assessment especially in digital images.

Keywords: *Forensic science; forensic anthropology; age estimation; spheno-occipital synchondrosis and digital photograph*

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Strengthening quality assurance procedure for the correct identification of amphetamine group in urine by a semi-qualitative method using GC/MS

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Abstract

Drug laboratory Hospital Queen Elizabeth confirms Amphetamine Type Stimulant (ATS) by a semi-quantitative method using Gas Chromatography Mass Spectrometry (GC/MS) based on the retention time and ion spectrum of the targeted drugs compared to analytical standards at its cut-off concentration. Misidentification can occur as drugs in the ATS group are structurally related and produce similar ions. Thus, additional criteria need to be included to strengthen the reporting such as monitoring area under curve (AuC) of mixed standards and monitoring ion ratios of mixed standards to ensure correct reporting. Studies were performed retrospectively and data was collected from Jan to May 2019. AuC monitoring was performed by analysing data obtained from 60 batches containing 340 sets of mixed standards of Amphetamine, Methamphetamine, MDA, MDMA. Ion ratio monitoring was done by analysing each analytical standard at its cut-off. Ion ratios were tabulated and range was established. CV for long term monitoring of AuC were within acceptable limits of less than 20% for all analytes. More than 95% of analytical standards at its cut-off concentration fulfilled the ion ratios monitoring criteria outlined in CSLI C60. Standard operating procedure was updated to include AuC monitoring and ion ratios as criteria in reporting samples in addition to retention time and ion spectrum of the targeted drugs. By adding criteria on the monitoring of AuC and ion ratios as part of quality assurance procedure, the laboratory is committed to producing high quality and value added results.

Keywords: *Amphetamine Type Stimulants (ATS); GC/MS; AuC; ion monitoring and reporting criteria*

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Assessing the analytical performance using sigma metrics - a journey in achieving quality analysis for Clinical Research Center Sarawak General Hospital (CRC SGH) laboratory

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Abstract

Laboratory errors are the result of poorly designed quality systems in the laboratory. In order to achieve top-notch quality in a clinical research laboratory, it is essential to assess quality improvements of automated analytic tests which is vital in the decision-making process of clinical studies. A retrospective study was conducted on a data set consisting of internal and external quality control of 25 assays collected from January 2021 to June 2021. The sigma metrics (σCLIA, σBV) of these assays run on automated haematology analyzer (Sysmex XS1000i) and automated dry- chemistry analyzer (Fuji Dri-Chem NX500) was calculated based on imprecision (CV%), inaccuracy (bias %), total allowable error (TEa) with the formula of Sigma = (TEa-bias)/CV. Eight out of 25 assays achieved Six Sigma quality performance, which showed σ ≥ 6. Nine assays described σ>3, which met the process performance in a clinical research laboratory setting while the remaining eight assays failed to achieve the minimum six sigma quality performance with metrics less than three. Sigma metrics provides a comprehensive interpretation which helps to implement suitable quality control design for each individual assay to ensure continuous quality implementation to improve reliability of results. Sigma values provide guidance on the interpretation of individual assay performance in order to design suitable quality control measures to be implemented to improve reliability of results. Sigma metrics calculations are essential to scrutinize between the two most common sources of error; imprecision and inaccuracy. This serves as a gold standard for obtaining high quality laboratory reports.

Keywords: *Sigma metrics; allowable total error; bias; coefficient of variation and internal quality control.*

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Improvement in laboratory turnaround time performance of urgent full blood count request at Core Laboratory, Hospital Kuala Lumpur

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Abstract

Full Blood Count (FBC) test is requested as urgent for immediate patient's management and emergency cases. Malaysia Key Performance Indicator (KPI) of Clinical Services Medical Programme has set a standard that at least 90% of urgent FBC has laboratory turnaround time (LTAT) of 45 minutes or less. This study was conducted to improve our LTAT performance based on the KPI standard. The study was done in January 2019 until May 2021. Data was collected from request forms and Laboratory Information System (LIS) on monthly basis using a random sampling procedure. The data were analysed retrospectively every 6 months. Outliers were further analysed to find the root cause of the delay. As remedial measures to improve the performance, we introduced two terminals to validate our FBC and created awareness among our staff on the KPI. New LTAT Alert System was installed in August 2020 as an additional remedial measure. Our LTAT performance from January to June 2019 was only 80%. After the initial remedial measures, the LTAT performance in July to December 2019 improved to 92%. However, our performance from January to June 2020 dropped to 74%. Additional measures were implemented and our LTAT performance in July to December 2020 and January to May 2021 improved to 93% and 90% respectively. Continuous monitoring is required to maintain the quality of laboratory services. Commitment of the staff, smooth and efficient laboratory workflow and excellent LIS performance are important to achieve a good LTAT for Urgent FBC.

Keywords: *LTAT; KPI; urgent; FBC and quality*

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Verification of biomarkers of inflammation assays for Covid 19 on the Roche Cobas E602 system

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Abstract

Interleukin-6 (IL-6) and Procalcitonin (PCT) are biomarkers of inflammation, which are released as a cause or consequence of an inflammatory response. IFCC guidelines advocated the use of both IL6 and PCT as biomarkers for monitoring treatment of Covid 19 patients. As one of Covid 19 hospital, Hospital Kuala Lumpur has decided to offer these tests to manage critically ill patients. This study was carried out to evaluate and verify the performance of PCT and IL-6 assays of Roche Cobas e602 immunochemistry platform by electrochemiluminescence immunoassay (ECLIA) measurement. The performance of these analytes was assessed in terms of precision and linearity using Roche Cobas e602. A precision verification study was carried out using five replicates of QC per day for five days following which imprecision estimates in form of Within Run (Repeatability) % CV and Within Lab % CV were calculated and compared against manufacturer's claims. Linearity study using low and high concentrated calibrators were prepared in five different concentrations to plot the linearity graph. The percentage CV repeatability and CV within lab measured for PCT level 1 awere1.56% and 1.91%, PCT level 2 were 1.20% for both CV. Meanwhile CV repeatability and CV within the lab for IL6 level 1 are 0.99% and 1.13%, for level 2 are 0.77% and 1.17%. Dilution analysis for these biomarkers yield linear results across the dynamic range of the assay. As shown by their performance characteristics, the Roche Cobas e602 electrochemiluminescence immunoassay is fit for patient sample testing.

Keywords: *Covid-19; biomarker; inflammation and immunoassay*

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In silico evaluation of luteolin specific DNA aptamer

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Abstract

Luteolin is a type of flavonoid that possesses many biological and pharmacological benefits such as antioxidant, antimicrobial, anticancer and anti-inflammatory. This compound can be detected using sophisticated methods which are expensive and time-consuming. Recent studies showed that DNA aptamer can be used as a biorecognition tool that offers rapid results in detecting and characterizing luteolin in laboratory scale. However, the three-dimensional (3D) structure of luteolin specific aptamer is not available.Herein, the 3D structure of aptamer was constructed using a pipeline of bioinformatics tools including Mfold, RNAComposer, Discovery Studio Visualizer and UCSF Chimera software. The evaluation of molecular interaction between aptamer and luteolin was performed using molecular docking simulation (AutoDock Vina software). The binding energy of DNA aptamer-luteolin complex is 9.0 kcal/mol. Based on the observation, the changes in temperature and ion concentration might affect the folding structure of the aptamer. The result exhibited that the folding of aptamer varied according to the parameter in secondary structure prediction. The binding scores of the complex are reduced, which indicate that the ion concentration plays important roles in the folding of the aptamer to fold in its unique conformation. The 3D structure of luteolin specific aptamer and its interaction with the target was successfully determined. This preliminary study provides a basic understanding on binding orientation of aptamer and ligand.

Keywords: *DNA aptamers; 3D structure; luteolin and molecular docking*

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Updates on germline *RB1* gene mutations in unrelated Malaysian retinoblastoma patients

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Abstract

Retinoblastoma (RB) is the most common childhood cancer in the retina of children under 5 years old, due to mutations in the tumour suppressor *RB1* gene. RB is inherited in an autosomal dominant pattern. Globally, the incidence is approximately 1 in 15,000-20,000 live births. The purpose of this study was to identify causative *RB1* mutations in 223 clinically diagnosed RB patients referred to Institute for Medical Research in 2012-2020. Half of the patients in our cohort had unilateral RB, 44% had bilateral RB and the remainder was unknown. Mutation screening was performed in blood-derived DNA using a combinatorial approach of PCR-sequencing and multiplex ligation-dependent probe amplification (MLPA) assay which included the analysis of all 27 exons, splice sites and promoter region of *RB1* gene. A total of 54 mutations were observed in most bilateral cases and scattered throughout the *RB1* gene, of which 8 were novel. The spectrum of different mutation types among the 54 mutations were 44% nonsense mutations, followed by 20% frameshift, 16% splicing, 15% whole *RB1* gene deletion, 4% missense and 2% partial *RB1* gene deletion. Two recurrent mutations, p.(Arg579*) and p.(Cys712Arg) were detected in two and three unrelated cases, respectively. RB is a potentially curable cancer and early diagnosis is critical for survival and eye preservation. Our report expands the spectrum of *RB1* mutations. Information of the mutational signatures in RB patients would further help in accurate genetic counselling and ensure effective disease management measures for the patients and their families.

Keywords: *RB1; retinoblastoma; unilateral and bilateral*

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Correlation between procalcitonin (PCT) and uric acid (UA) among controlled and uncontrolled type 2 diabetes mellitus (T2DM) patients in Hospital USM- a preliminary study

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Abstract
PCT is a polypeptide with 116 amino acids produced by C-cells in the thyroid gland. It is a biomarker of inflammation, and its normal serum concentration is <0.05ng/mL. PCT is elevated in cases of inflammation regardless of the pathological condition. UA is an oxidative stress marker involved in inflammatory pathway. The aim of this study is to determine the correlation between PCT and UA in controlled and uncontrolled T2DM. This was a cross sectional study done at Outpatient Clinic Hospital USM from July to December 2020. Fifty diabetic patients were selected through simple random sampling and blood was taken for PCT and UA testing. PCT was analysed using immunoassay method and UA was analysed using biochemistry analyser. Patient were further categorized to controlled and uncontrolled DM based on HbA1c cut-off >7% (ADA guideline). Among the 50 DM patients, only 44 patients had their HbA1c tested. Twenty-seven patients were controlled DM and seventeen patients were uncontrolled DM. The median (IQR) for PCT and UA concentrations in controlled DM were 0.025(0.01) ng/mL and 361(105) mcg/L whereas in uncontrolled DM were 0.049(0.05) ng/mL and 367(181) mcg/L. There was a significant difference in PCT value ($p<0.05$) between controlled and uncontrolled T2DM. PCT showed positively correlated with UA ($r=0.615$, $p<0.05$). In T2DM, UA and PCT together play a role in oxidative stress and inflammatory pathway. There is significant correlation between PCT and UA in both controlled and uncontrolled T2DM. Acknowledgement: grant no 304.PPSP.6316542

Keywords: *Procalcitonin; Uric acid; Controlled; Uncontrolled and Diabetes Mellitus*

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Effects of Roselle (*Hibiscus sabdariffa* Linn.) polyphenol-rich calyx extract on the functional and structural abnormalities in diabetic cardiomyopathy rat model

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Abstract

Hibiscus sabdariffa Linn. (roselle) has been proven for its medicinal properties and recently has incited research interest for its potential in treating cardiovascular disorders. Therefore, this study aimed to determine the cardioprotective effects of polyphenol-rich extract of roselle calyx (HPE) in diabetic cardiomyopathy (DCM) rat model. Type 1 diabetes mellitus (DM) was induced by a single intraperitoneal injection of streptozotocin (55 mg/kg). The non-diabetic rats (NDM) acted as control group (n=6). All rats were left untreated for four weeks. At the end of the four weeks, the diabetic rats were randomly divided into three groups: diabetic group (DM), diabetic group treated with HPE (DM+HPE) (100mg/kg) and diabetic group treated with metformin (DM+MET) (150mg/kg). Treatment for HPE and metformin were given daily for another four consecutive weeks. The results showed that HPE treatment was able to improve cardiac function, whereby the left ventricular developed pressure (LVDP), cardiac contraction rate (+dP/dt), and coronary flow were increased significantly ($p<0.05$) while cardiac relaxation time (tau) was significantly decreased ($p<0.05$) in DM+HPE compared to DM. In addition, histological analysis showed a significant decrease in cardiomyocyte hypertrophy and cardiac fibrosis in DM+HPE compared to DM group. Roselle exhibited its anti- hypertrophy and anti-fibrotic properties in preventing cardiac remodelling, which contributes to the attenuation of cardiac functional abnormalities. Collectively, these findings suggest that HPE has a promising potential in limiting the progression of cardiac dysfunction and structural changes in diabetic cardiomyopathy rat models.

Keywords: *Diastolic dysfunction; systolic dysfunction; cardiac fibrosis and cardiomyocyte hypertrophy*

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Polyphenol-rich extract of *Hibiscus sabdariffa* Linn. calyx (HPE) limits hyperglycemia-induced oxidative stress and apoptosis progression in diabetic heart

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Abstract

Diabetes mellitus (DM) increases the risk of diabetic cardiomyopathy (DCM) development mediated by oxidative stress and apoptosis. This study was undertaken to investigate the effects of a polyphenol-rich extract of *Hibiscus sabdariffa Linn.* calyx (HPE) in limiting the progression of DCM in rats that already have developed DCM condition. Type 1 DM was induced by a single intraperitoneal injection of streptozotocin (55 mg/kg). The non-diabetic rats (NDM) acted as a control group (n=6). All rats were left untreated for four weeks prior to being randomly divided into three groups: diabetic group (DM), the diabetic group treated with HPE (DM+HPE) (100mg/kg) and the diabetic group treated with metformin (DM+MET) (150mg/kg). Metformin group acted as the positive control. Then, treatment for HPE and metformin were given daily for another four consecutive weeks. Results showed that the DM+HPE group had significantly ($p<0.05$) reduced cardiac oxidative stress status as shown by the lower levels of the advanced oxidation protein product (AOPP) and malondialdehyde (MDA) as well as improved activities of superoxide dismutase (SOD), catalase (CAT) and level of reduced glutathione (GSH) in comparison to DM group. Meanwhile, HPE improved apoptosis condition exhibited by the increment of BCL-2 expression and reduction of Bax and Bax/BCL-2 ratio significantly ($p<0.05$).Polyphenols in roselle showed their ability as antioxidants to reduce free radicals' production and hence prevent the cardiac oxidative damage and inhibit apoptosis which is among the main mechanisms mediating DCM. In conclusion, HPE showed potential in improving diabetic cardiomyopathy conditions via alleviating oxidative damage and apoptosis.

Keywords: *Diabetic cardiomyopathy; cardiac dysfunction; structural changes; antioxidant and roselle*

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Association of PIVKA-II and AFP responses with radiological response in post treatment HCC patients in a tertiary hospital in malaysia

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Abstract
Hepatocellular carcinoma (HCC) is associated with poor prognosis. Serum alpha-fetoprotein (AFP), the most widely used biomarker for HCC but has its limitation. Protein Induced by Vitamin-K Absence-II (PIVKA-II) is an emerging biomarker for HCC. This study aimed to determine the levels of PIVKA-II and AFP in healthy and HCC patients as well as their association with radiological response post-treatment in HCC patients. A one-year cohort study was conducted at Hospital Universiti Sains Malaysia, Kelantan. Blood samples were obtained and both PIVKA-II and AFP concentration were determined by chemiluminescent microparticle immunoassay using ARCHITECT Plus analyzer (Abbot, Wiesbaden, Germany). A reduction in PIVKA-II or AFP level by 50% from the baseline was defined as response to treatment. A total of 54 HCC patients and 40 healthy controls were enrolled. The median (IQR) concentration of serum PIVKA-II in HCC patients was 988.4 (23832.82) mAU/ml. It was significantly higher compared to healthy group 24.2 (10.5) mAU/ml ($p<0.001$). Similarly, the median (IQR) level of AFP in HCC group was 13.6 (647.83) ng/ml and it was significantly higher than healthy group 1.7 (1.21) ng/ml ($p<0.001$). PIVKA-II response was significantly associated with the radiological response ($p=0.016$) meanwhile, there was no significant association between AFP and radiological response ($p=0.187$) post-treatment in HCC patients. PIVKA-II and AFP levels were significantly higher in the HCC group however the association was only significant between PIVKA-II with radiological response post-treatment. PIVKA-II exhibits a promising biomarker in evaluating treatment response among HCC patients.

Keywords: *Hepatocellular carcinoma; PIVKA-II; alpha-fetoprotein; biomarker and radiological response.*

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Bisphenol F increased presence of target cells in male Sprague Dawley rats

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Abstract

Bisphenol F (BPF) is an industrial chemical utilized in the manufacturing industry such as polycarbonate plastics, epoxy resins, and thermal receipt papers as a replacement for Bisphenol A. However, the toxicity effects of BPF on red blood cells in the blood circulation is under-reported. Hence, this study aims to evaluate the effect of BPF on the oxidative stress, membrane fragility and the morphology of red blood cells (RBC) of male Sprague-Dawley (SD) rats. Adult male SD rats (n= 20) weighing between 230-250g were divided randomly into four groups: control (received 1 ml/kg of normal saline) and BPF1, BPF5 and BPF10 who received 1, 5, and 10 mg/kg/bw of BPF, respectively via force-feeding oral for 28 days. The result showed no significant differences were recorded for SOD and MDA, but the GSH level was increased significantly in the BPF1 group compared to the control group ($p<0.05$). The RBC membrane fragility did not show any significant difference among all groups. However, a significant increase of abnormal morphology of RBC with the presence of target cells found in all BPF groups as compared to the control group at $p<0.05$. BPF exposure has initiated oxidative stress but not to the chronic stage which has proven by the RBC membrane intact. The target cells found in the BPF group might show the abnormality of haemoglobin content in the RBC. BPF increased the presence of target cells in the RBC of male SD rats.

Keywords: *Antioxidant; BPF; RBC; oxidative stress and fragility.*

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Early-stage development of diabetic cardiomyopathy model in type-1 diabetic rat

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Abstract

In clinical setting, the early stage of diabetic cardiomyopathy (DCM) is characterized by diastolic dysfunction and ventricular hypertrophy. However, the time-point for early stage development of DCM in diabetic rat model is still unclear. This study aimed to determine the time-point for early stage development of DCM in diabetic rat model. A total of 10 male Sprague-Dawley rats were divided into two groups: non-diabetic (NDM) and diabetic (DM) group. Type-1 diabetes was induced with a single, intraperitoneal injection of streptozotocin (55 mg/kg). All rats were left untreated for 4 weeks. At the end of study period, all animals were sacrificed, the heart was immediately excised for cardiac function and histological evaluation. Results showed that DM rats had significantly ($p<0.05$) lower left ventricular develop pressure (LVDP), cardiac relaxation (-dp/dt) and higher level of tau relaxation as compared to NDM rats. However, the cardiac contractility (+dp/dt) showed no significant changes. The histological analysis revealed remarkable cardiomyocyte hypertrophy and early fibrosis development. Discussions: The remarkable prolonged -dp/dt and cardiomyocyte hypertrophy indicate that 4 weeks of untreated diabetic condition may lead to the development of early-stage DCM. The unaffected cardiac contractility exhibited by the insignificant change in +dp/dt further confirmed that the DCM had not yet developed into a later advanced stage. The development of early-stage of DCM can occur at 4 weeks of untreated type-1 DM rats.

Keywords: *Cardiomyocyte hypertrophy; cardiac fibrosis; diabetes mellitus; diastolic dysfunction and systolic dysfunction*

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Redundancy of thyroid function test request - A 2020 review in Hospital Universiti Sains Malaysia

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Abstract

Thyroid function test (TFT) is used to detect and monitor thyroid dysfunction. TFT consists of thyroid stimulating hormone (TSH), free thyroxine (FT4) and free tri-iodothyronine (FT3). Testing of TFT becomes redundant when multiple providers request for the same patients without checking whether the test has been requested before. This study is to determine the percentages of repeated TFT within the same day. This is a cross sectional study using retrospective data of adults TFT in Endocrinology Laboratory, Hospital USM in 2020. Repeated TFT results within the same day from the same patients were retrieved from Laboratory Information System (LIS). The data were analysed for descriptive analysis using SPSS Software and Microsoft excel. A total of 12863 TFT were requested in 2020. 148 (1.2%) tests were requested within the same day from 72 patients. Out of these patients, thyroid and non-thyroid related were 25(34.7%) and 43(59.7%) respectively. The most requests were from medical wards. The tests requested were; TSH alone 1(0.7%), FT4+FT3 1(0.7%), TSH+FT4 7(4.7%) and TSH+FT4+FT3 139(93.9%). The initial and subsequent results did not show any difference. The possible reasons for repetition within a day might be due to poor communication between health personnel and mobilization of patient from ward to another ward. However, the understanding of TSH and FT4 relationship are important in determining the interval of repeating the test to avoid redundancy. Unnecessary repetitions of TFT must be avoided to prevent burden to the patients and additional cost to the healthcare.

Keywords: *Thyroid function test; TSH; FT4; FT3 and redundancy*

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Increased protein expressions on human brain endothelial cells induced by ammonium chloride

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Abstract

Human brain endothelial cells (HBECs) are the major element of the Blood-Brain Barrier (BBB) wherein limited passage of substances is allowed from the blood into the brain. Lysosomal dysfunction plays a crucial role in BBB homeostasis as failure in waste materials degradation and clearance results in physio biological imbalances that could lead to neurodegenerative diseases. Identifying the changes in HBEC protein expressions exposed to lysosome inhibitors is crucial in understanding disease mechanisms involving brain vasculature. This can be studied via the expression of several proteins such as Claudin-5, a tight junction protein, the intracellular adhesion molecule 1 (ICAM-1), an inflammatory marker and endothelial nitric oxide synthase (eNOS). HBECs were treated with 10 mM ammonium chloride, a lysosome inhibitor at 1-hour incubation duration. The protein expressions of Claudin-5 and ICAM-1 were studied with protein load of 4 µg added with DTT and denatured at 95°C for 5 minutes before analyzed by automated capillary-based immunoassay, whereas eNOS level was detected by ELISA method. All data were analyzed by T-test. The result demonstrated that Claudin-5 and ICAM-1 expression significantly increased ($p < 0.05$) together with eNOS ($p < 0.001$). This may suggest exposure to ammonium chloride causes several cellular changes associated with blood-brain barrier integrity properties alterations and promote inflammatory responses. This research could provide an insight for a better understanding associating lysosomal dysfunction with protein expressions for future research involving brain endothelial cells.

Keywords: *Inflammation; permeability; tight junction and vasodilation*

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Cytoplasmic pYAP protein expression increased in pre-malignant stage of lung squamous cell carcinoma (LSCC) *in vivo*

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Abstract

Phosphorylated Yes-associated protein (pYAP) is a well-known tumor suppressor that negatively regulates carcinogenesis. Several studies demonstrated increased pYAP and its cytoplasm localization expression in numbers of pre-malignant (PM) stage cancer. However, the characterization of cytoplasmic pYAP expression in PM lung squamous cell carcinoma (LSCC) remains elusive. Thus, we aimed to determine cytoplasmic pYAP protein expression in N-nitroso-tris-chloroethylurea (NTCU) induced PM LSCC *in vivo.* Female BALB/c mice were assigned into two groups (n = 6); vehicle and cancer group which received 70 % acetone and 0.04 M NTCU respectively. The treatment was given twice a week for 15 weeks at the dorsal area of mouse's shaved skin. Following lung extraction, we performed hematoxylin and eosin staining to confirm lung SCC formation and immunohistochemistry staining to determine pYAP protein expression. P-value of ≤ 0.05 was assigned as statistical significance. We successfully developed PM LSCC in the cancer group, indicated by the formation of hyperplasia lesions around the bronchi. We observed a significantly higher ($p<0.05$) epithelium thickness in the cancer group which confirmed the hyperplasia lesion. Moreover, we also observed a significantly higher ($p<0.05$) cytoplasmic pYAP protein expression in the cancer as compared to the vehicle group. Cytoplasmic pYAP expression might be increased as a normal physiology mechanism to suppress LSCC carcinogenesis at an early stage. Cytoplasmic pYAP protein expression is increased at an early stage of LSCC and could be a good prognostic marker for the disease in the future.

Keywords: *Pre-malignant; lung squamous cell carcinoma (SCC); N-nitroso-tris-chloroethylurea (NTCU) and mouse model.*

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Neuroprotective potential of mixed functional food on amyloid-beta induced cognitive function impairment in rat

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disease that impairs memory and cognitive function. In Malaysia, some manufacturers have marketed mixed functional food products and claimed that their products have health benefits including improving memory. However, the mechanism on how this claim is yet to be discovered. The effect of mixed functional foods (MFF) that comprised of punicalagin, protocatechuic acid, gallic acid, ellagic acid, ferulic acid and caffeic acid on amyloid-beta 42 (Aβ-42) induced rats were analysed. Forty-five albino Wistar rats were randomly divided into five groups: NC (0.9% normal saline treatment + PBS solution injection), Abeta (0.9% normal saline treatment + 0.2 µg/µl Aβ-42 injection), MFF (4 ml/kg MFF treatment + PBS solution injection), Abeta-MFF (4 ml/kg MFF treatment + 0.2 µg/µl Aβ-42 injection) and Abeta-NAC (150 mg/kg N-acetylcysteine + 0.2 µg/µl Aβ-42 injections) as positive control. MFF and N-acetylcysteine (NAC) were given for 30 days and Aβ-42 injection via intracerebroventricular was given for 14 days. Results showed that MFF treatment decreased Aβ-42, IL-1β, IL-10 and increased Nrf2 levels ($p < 0.05$) compared to the Abeta group. Histology findings showed that MFF treatment improved cell structure and Nissl bodies compared to the Abeta group. In conclusion, our study showed that MFF has the potential as a neuroprotective agent in AD prevention via inhibition of microglial activation and inflammation. This research's future direction is to develop a healthy option of mixed functional food products for human consumption to reduce the risk of developing AD.

Keywords: *Mixed functional food; oxidative damage; inflammatory mediators and Alzheimer's disease*

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Zingiber officinale (ginger) improves oxidative status and muscle performance in aging animal model

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Abstract

Ginger (*Zingiber officinale Roscoe*) has been shown to possess antioxidant properties. This study aimed to evaluate the effect of ginger on oxidative status and muscle performance in the aging animal model. Sprague Dawley (SD) rats aged three (young), nine (adult), and twenty-one (old) months old were orally treated daily with either distilled water or ginger extract at a concentration of 200 mg/kg body weight for 3 months. Muscle performance was measured at 0, 1, 2, and 3 months of treatment by measuring muscle strength and integrity. Urine, plasma, and muscle tissue were obtained for the determination of oxidative stress markers (DNA damage, isoprostane, creatine kinase [CK-MM], lipid peroxidation [LPO], malondialdehyde [MDA]), and antioxidant enzymes (superoxide dismutase [SOD] and catalase [CAT]). Young and adult ginger-treated rats showed a significant improvement in muscle strength after 3 months of supplementation. Bone mineral density (BMD) and bone mineral content (BMC) were increased while fat-free mass (FMM) was decreased after 3 months of supplementation in young ginger-treated rats but were not changed in adult and old ginger supplemented rats. Supplementation of ginger for 3 months was able to significantly decrease the levels of damaged DNA, urinary isoprostane, CK-MM, LPO, and MDA and significantly improved SOD and CAT enzyme activity. 6-Gingerol and 6-shogoal are the main bioactive compounds in the ginger extract that contribute to antioxidant activity. Ginger has shown a protective effect and improves muscle performance in Sprague Dawley rats indicating its potential in delaying sarcopenia progression in aging.

Keywords: *Zingiber officinale; oxidative stress; muscle strength; antioxidant enzyme and sarcopenia*

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Apoptotic potency of *Ganoderma neo-japonicum* Imazeki in inhibiting pro-survival BCL-2 protein: An *in silico* evaluation

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Abstract

Colorectal cancer (CRC) is one of the deadliest non-communicable diseases worldwide. Aberrant expression of BCL-2 has been implicated in CRC progression. BCL-2 activates pro-survival signals that suppress cell death. Its activities are neutralized by BH3-only proteins such as BAD, BID, NOXA, and PUMA. On this basis, several BH3 mimics have been developed to overcome the anti-apoptotic defense mechanisms. Natural products are prolific sources of anticancer drugs against CRC. *Ganoderma neo-japonicum* Imazeki, a well-known medicinal mushroom, might comprise lead compounds that mimic the action of BH3-only proteins. Two active extracts, namely hexane fraction (Hex) and chloroform fraction (Chl), were isolated from wild fruiting bodies. They were subjected to mycochemical profiling through GC/MS and LC/MS methods, respectively. Next, the potential compounds (two from each fraction) were selected for docking assessment. Stellasterol (Hex) exhibited the highest affinity towards Bcl-2 protein, followed by 1,25-dihydroxyvitamin D3 3-glycoside (Chl), proscillaridin A (Chl), and linoleyl alcohol (Hex). All interactions occurred at the binding pocket shown by control ABT-263 (an orally bioavailable BH3 mimic). Cell deaths due to the BCL-2 inhibition have been reported with *G. applanatum* and *G. lucidum*. It was reasonable to speculate that mechanisms underlying the apoptotic effects of *G. neo-japonicum* are attributed to the presence of such inhibiting compounds against BCL-2 protein. Overall, *G. neo-japonicum* is deemed as a therapeutic opportunity to initiate cell death by suppressing BCL-2 expression in CRC.

Keywords: *Colorectal cancer; Ganoderma neo-japonicum; molecular docking; apoptosis and BCL-2 protein*

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Study on the anti-hyperglycemic properties of miang extract

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Abstract

Natural products are widely used as an alternative treatment for diabetes mellitus. Miang is an edible fermented tea prepared from Assam Tea leaves (*Camellia sinensis var. assamica*) that is produced in the northern Thailand through a unique fermentation process. The potential use of Miang extracts as antihyperglycemic agents are less explored to date. Hence, the present study aimed to investigate the antihyperglycemic and antiglycation properties of Miang leaves extracts (fresh Assam tea (FA), steam Assam tea (SA), fermented traditional Miang (MTF) and culture-starter fermented Miang (MCSF)) and the correlation with antioxidant property. The antioxidant property was assessed through the determination of total phenolic content (TPC), ferric reducing antioxidant power (FRAP), ABTS and DPPH radical scavenging activities. The antihyperglycaemic property was determined via the assessment of α-glucosidase and α-amylase inhibitory activities. The antiglycation activity was evaluated based on the inhibition of advanced glycation end-product (AGE) formation. Generally, the potency of the extracts as antioxidant, antihyperglycemic, and antiglycative agents, was in the following trend: MCSF ≥ MTF ≥ SA ≥ FA. The MCSF extract exhibited significant antihyperglycemic activities compared to the other extracts. There was a positive correlation between TPC, antioxidant, antihyperglycemic and antiglycation properties of the extracts. The bioactive compounds in Miang may have undergone biotransformation-induced by microorganisms during the fermentation process and this could have enhanced the health benefits of the fermented Miang tea. These findings revealed the potential applications of the Miang extracts as an adjuvant for the treatment of diabetes and related complications.

Keywords: *Antiglycemic; antiglycation; antioxidant; fermentation and Miang tea*

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Effects of palm tocotrienol on joint health in a rat model of osteoarthritis

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Abstract

Osteoarthritis is a degenerative joint disease lacking effective disease-modifying treatment agents. Palm tocotrienol, a natural anti-inflammatory therapy, may have potential therapeutic effects against this condition since low-grade inflammation is involved in the pathogenesis of osteoarthritis. This study aims to determine the effects of palm tocotrienol in a rat model of osteoarthritis. Male Sprague-Dawley rats (3 months old, n=6/group) were randomly assigned to 5 groups. The normal control was not induced with osteoarthritis. The rest of the groups were induced with osteoarthritis using monosodium iodoacetate and treated orally daily with olive oil plus normal saline (osteoarthritis control), palm tocotrienol (100 mg/kg) plus normal saline, olive oil plus glucosamine sulfate (250 mg/kg) or palm tocotrienol (100 mg/kg) and glucosamine sulfate (250 mg/kg) for one month. The knee joint and serum of the rats were harvested for analysis when the treatment ended. The histology scores of knee joints among different groups were similar ($p>0.05$). The cartilage oligomeric matrix protein level was significantly elevated in the osteoarthritis control compared to all other groups ($p<0.05$). The discrepancy between joint histology results and serum cartilage remodelling markers might indicate that palm tocotrienol and glucosamine sulfate could protect against cartilage damage, but the joint structure might take a longer time to heal. Palm tocotrienol may be beneficial to joint health by suppressing cartilage damage in an experimental model of osteoarthritis. Its effects are on par with glucosamine sulfate. However, the combination of both agents did not produce additive or synergistic joint protecting effects.

Keywords: *Cartilage; chondrocyte; glucosamine; joint and vitamin E*

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Minocycline improved learning and working memory impairment in lipopolysaccharide (LPS)-induced Alzheimer's disease rat model

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Abstract

Minocycline has been found to exert protected effects on learning and memory impairment in various neurodegenerative disease animal models. However, its learning and memory effects in lipopolysaccharide(LPS)-induced Alzheimer's disease (AD) rat models have not been elucidated. This study investigates the cognitive-enhancing effects of minocycline and compares it with clinically approved N-Methyl-D-Aspartate (NMDA) receptor antagonist (memantine) in LPS-induced AD rat model. Male Sprague Dawley rats were divided into 5 groups: (i) control, (ii) untreated LPS (iii) LPS treated with 25 mg/kg minocycline, (iv) LPS treated with 50 mg/kg minocycline and (v) LPS treated with 10 mg/kg memantine. Minocycline treatments were given intraperitoneally once daily for 14 days while LPS was injected once at day 5. Morris water maze (MWM) test was performed to assess learning and working memory function at days 15 until 23. The present study confirmed that LPS significantly impairs ($p<0.05$) learning and working memory. Dependent on dose, treatment with minocycline significantly improved ($p<0.05$) learning and working memory comparable to memantine. Minocycline improved learning and working memory of the LPS injected rat model comparable to memantine. Thus, minocycline has beneficial preventive-therapeutic effects for neurodegenerative diseases involving neuroinflammation such as AD.

Keywords: *Minocycline; lipopolysaccharide; Alzheimer's disease; learning and memory*

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Morphology analysis of colony forming unit-derived mouse erythroid, myeloid and lymphoid progenitors using digital image processing method

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Abstract

Hematopoietic stem cells (HSCs) play an important role for the maintenance of hematopoiesis. The colony forming unit (CFU) assay is a widely used in-vitro assay to measure the proliferation and differentiation abilities of individual hematopoietic stem and progenitor cells (HSPCs) within a sample to form colonies. CFU can be classified morphologically based on the commitment of cell lineage consisting of erythroid, lymphoid and myeloid lineages. Conventional method for CFUs analysis is done manually through an inverted microscope. However, this method carries limitations such as time-consuming, high workload and requires trained laboratory staff for analysis. Therefore, to overcome this limitation, a bio-imaging automation system is used to identify the morphology of CFUs more accurately and efficiently. This study aimed to identify morphological features for the recognition of CFUs from erythroid, lymphoid and myeloid lineages through digital image processing methods. The morphological features of erythroid CFU (CFU-E), myeloid CFU namely CFU-granulocytes (CFU-G), CFU-granulocytes-macrophages (CFU-GM) and CFU-macrophages (CFU-M) and pre-B lymphoid CFU were analyzed using the Regionprops method. Regionprops is an image processing tool that measures various features of images namely area, centroid1 (horizontal coordinate), centroid2(vertical coordinate), major axis length, minor axis length, eccentricity, orientation, equivdiameter, solidity, extend and perimeter in the form of black and white photographs. This study indicates that area, centroid2(vertical coordinate), major axis length, minor axis length, equivdiameter, solidity, extend and perimeter are able to significantly ($p<0.05$) differentiate CFUs among different lineages. Morphological features are able to differentiate CFUs among different lineages.

Keywords: *Hematopoietic stem cells; morphology; colony forming unit; image processing and regionprops*

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Whole exome sequencing analysis on childhood B-cell precursor acute lymphoblastic leukaemia: A preliminary descriptive study

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Abstract

Whole exome sequencing (WES), which is one of the next generation sequencing approaches, has become a powerful tool in the field of medical genetics. This technique is commonly applied to various diseases to reveal single nucleotide variants (SNVs) and insertion/deletion (indel) mutations within the exome or protein-coding regions of the genomic DNA. In this study, genomic DNA from sixty-one paediatric B-cell precursor acute lymphoblastic leukaemia (B-ALL) patients diagnosed between 2016 and 2017 were subjected to WES. Bioinformatics analysis was performed according to GATK best practices and the Catalogue of Somatic Mutation in Cancer (COSMIC) database was used as a reference for the most common mutated genes implicated in B-ALL. Findings from this study revealed that all patients have both SNVs and indel in *TP53* and *FLT3* genes. Majority of the patients were also found to have SNVs in the RAS-pathway genes, *NRAS* (98.3%, n=60/61) and *KRAS* (96.7%, n=59/61). Interestingly, SNVs were detected at high frequency in the ETS transcription factor, *ERG* (90.2%, n=55/61). Mutations in *TP53*, *FLT3*, *NRAS*, *KRAS*, and *ERG* genes are associated with unfavourable outcomes in newly-diagnosed childhood B-ALL. Furthermore, 8984 SNVs and 16240 indel were found to have high mutation impact in this B-ALL cohort. Further large-scale and well-designed studies are required for integration of these findings with the clinical data for risk stratification and prognostication of B-ALL patients.

Keywords: *Whole exome sequencing; acute lymphoblastic leukaemia; bioinformatics; single nucleotide variant and mutation*

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Evaluation of four rapid diagnostic test (RDT) kits for the detection of *Plasmodium species*

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Abstract

The main objective for the study is to compare the diagnostic efficacy of four commercially available RDT kits; OptiMAL-IT, BinaxNOW® Malaria, SD Bioline Malaria and Diaxis® Malaria for the detection of *P. falciparum*, *P. vivax*, *P.ovale* and *P.malariae*. This was a retrospective case-control design. Prior to product placement for diagnostic testing in Hospital Tengku Ampuan Rahimah Klang, all RDTs need internal product evaluation to assess products' quality and reliability. A total of 45 consecutive malaria patients' blood samples were selected, of which 26 were the case. The diagnostic performance of all kits was calculated against results of microscopic examination as the reference gold standard. Results shown that the sensitivity of OptiMAL-IT, BinaxNOW® Malaria, SD Bioline Malaria and Diaxis® Malaria were 88.5% (95%CI 76.2-100), 73.1% (95%CI 56.0-90.2), 84.6% (95%CI 70.7-98.5) and 80.8% (95%CI 65.6-96.0) respectively. All four RDT kits yielded 100% specificity. OptiMAL-IT produced the highest AUC value (0.94, 95%CI 0.87-1.00), followed by SD Bioline (0.92, 95%CI 0.85-1.00), Diaxis® Malaria (0.90, 95%CI 0.82-0.99) and BinaxNOW® Malaria (0.87, 95%CI 0.77-0.97). Although all kits correctly detected *Plasmodium* species for parasitaemia levels of more than 5,000/µl, they were unable to detect 2 out of 3 samples of *P. vivax* infection with less than 1,000/µl. They were also unable to detect the presence of *P. falciparum* co-infection with *P. vivax* at level of less than 500/µl. Overall, all four RDT kits showed excellent performance in detecting *Plasmodium* species. This study, however, is limited to low-density parasitaemia (≤500 parasites/*µ*l) detection. Thus, other commercial RDTs and alternative methods are recommended to overcome the limitation.

Keywords: *Evaluation; malaria and rapid diagnostic test*

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Detection and quantification of biochemical markers for primary creatine disorders by tandem mass spectrometry

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Abstract

Primary creatine disorders (PCD) is a new class of Inborn Errors of Metabolism (IEM) categorized by groups of disturbances in energy metabolism. Biochemical markers detection for PCD relies on the analysis of two main metabolites in biological fluids: guanidinoacetate (GAA) and creatine (Cr). Our aim is to develop methods with new techniques using the Flow Injection Analysis Electrospray Ionization Tandem Mass Spectrometry (FIA-ESI-MS/MS), validate and establish age-specific normal reference ranges for quantification of GAA and Cr in urine and plasma for diagnosis of PCD. Urine and plasma were obtained from 1320 hospitalized subjects with no neurological symptoms from day 1 to 50 years old. Twenty-five microliters of samples were extracted using methanol and acetonitrile containing isotopes labelled standards of GAA and Cr: d2-GAA and d3-Cr. After derivatization and formation of butyl esters, samples were analyzed using multiple reaction monitoring modes (174>101; GAA, 176>103; d2-GAA, 188>90; Cr and 191>93; d3-Cr). Forty samples were also analysed using Symmetry ® C18 column (reference technique) for method comparison. Both methods showed a good precision with inter and intra-day coefficients of variation (CV) less than 15%, recoveries ranged from 90% to 110% and bias ± 10%. The curve was linear for both biological and pathological concentrations. The comparison with the reference technique did not reveal a significant difference for analytical performance however, FIA-ESI-MS/MS produced faster results. The detection and quantification of GAA and Cr by FIA-ESI-MS/MS method is highly reliable and recommended for the biochemical diagnosis of PCD.

Keywords: *Primary creatine disorders; Guanidinoacetate; Creatine and FIA-ESI-MS/MS*

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In silico protein binding analysis between HLA-Cw*03:02 and HLA-Cw*04:01 with EBNA-1 associated with nasopharyngeal carcinoma

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Abstract

The development of nasopharyngeal carcinoma (NPC) has been associated with the genetic susceptibility of the human leukocyte antigen (HLA) genes and the interaction with Epstein Barr virus (EBV). Latent infection of EBV allows the manifestation of an antigenic protein, EBV nuclear antigen (EBNA-1), that helps to escape recognition by the host immune system. Meanwhile, the genetic diversity of certain HLA genes encoded by major histocompatibility complex (MHC) class I may have influenced the emergence of NPC. In silico binding assays were utilized to predict the binding properties between the proteins. The bioinformatics tools used were accessed through open-source online resources: IMGT/HLA Database, RCSB PDB, IEDB, EMBOSS (Needle), Jalview, IntFOLD, DiscoTope, PatchDock, and PyMOL. The nature of the interaction mechanism between certain HLA-C alleles and EBNA-1 was also predicted in this work. It was found that HLA-Cw*03:02 and HLA-Cw*04:01 have 97.0% similarity and 94.8% identical with 0.0% gaps. Meanwhile, protein docking indicated that the selected EBNA-1 antigen did not attach to the HLA-Cw*03:02 and HLA-Cw*04:01 predicted binding sites, preventing both HLA-Cs from interacting with it. The vulnerability to NPC of HLA class I genes, particularly HLA-C alleles, has been investigated. Findings from this study indicated that both HLA-Cw*03:02 and HLA-Cw*04:01 may be susceptible genes for the malignancy. The prediction showed that the genetic susceptibility of specific HLA types played a significant influence on NPC development.

Keywords: *Nasopharyngeal carcinoma, HLA-C; EBV; bioinformatics and susceptibility*

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Virgin coconut oil (VCO) an alternative for xylene as clearing agent for serous fluids in Sarawak General Hospital

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Abstract

Clearing process in cytology is the process of removing alcohol from the cell to increase the refractive index of the cell and to make the cell transparent for microscopic evaluation. Xylene is usually used as a clearing agent in cytology, but it was proven to be harmful and carcinogenic. To substitute xylene in routine processing of serous fluid samples in the cytology laboratory, virgin coconut oil (VCO) was studied for its clearing ability on serous fluid smears. The objective of this study is to study the clearing ability of virgin coconut oil as an alternative for xylene as clearing agent in the processing of cytology serous fluids samples. In this study, serous fluid samples (n=50) were processed into smears and stained with Papanicolaou's stain and May Grunwald Giemsa stain. Next, the clearing process was done by using VCO and xylene before mounting. To compare the clearing ability of VCO and xylene, microscopic evaluation was done focusing on nuclear features, cytoplasmic features, and clarity of smear by using a scoring-based method. The result was then analysed by using SPSS version 22. From the findings, it showed that there was no significant difference on nuclear features, cytoplasmic features, and clarity of smear ($p>0.05$) between VCO and xylene as clearing agents for both Papanicolaou's stain and May Grunwald Giemsa stain. As a conclusion, VCO can be used as an alternative for xylene as a clearing agent for serous fluid smears.

Keywords: *Virgin Coconut Oil (VCO); clearing agent and serous fluid*

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Neonatal Nav1.5 expression in syngeneic mouse model of an orthotopic-induced mammary tumour

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Abstract

Neonatal splice variant of Nav1.5 (nNav1.5) is a novel tumor associated antigen (TAA), linked to aggressiveness in human breast cancer cells in vitro and metastasis in patients with advanced breast cancer (positive for lymph node metastasis). Hence, the molecule now emerged as a potential target to control breast cancer progression or to prevent metastasis. Anti-metastatic agents targeting nNav1.5 need to be evaluated in an animal model validated for nNav1.5 expression. In this study, a syngeneic mouse model of an orthotopic-induced mammary tumor was generated. Twelve BALB/c female mice (age 11-12 weeks) were assigned into two groups, normal (PBS) (n=6) and tumor induced (n=6). For tumor induction, 1 X 10^5 cells (0.1ml) of 4T1 cells were inoculated into the animal's mammary fat pad. The animals were monitored for 30 days for changes in body weight and tumor development. On day 30, all animals were euthanized, and serum was collected. Real-time PCR and indirect ELISA were performed to evaluate nNav1.5 gene expression in the extracted tumor tissue and serum antibody against nNav1.5, respectively. Whilst tumor volume increased gradually in the tumor induced group, body weight was significantly reduced compared to the normal group. Although the anti-nNav1.5 levels in the two groups were comparable, the tumor induced group's relative nNav1.5 mRNA expression in tumor tissues was slightly higher than the PBS group's normal tissue. A syngeneic animal model expressing nNav1.5 has been established and could be used in future research exploring therapeutic value of nNav1.5 to combat metastasis.

Keywords: *Advanced breast cancer; metastasis; neonatal Nav1.5 and orthotopic mouse model*

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In silico protein binding analysis between HLA-A*24:02 and HLA-A*11:01 with LMP1: relevance for nasopharyngeal carcinoma

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Abstract

Infection of Epstein Barr virus (EBV) is common in Southeast Asian populations. Although most infections are asymptomatic, it has also been associated with the etiology of nasopharyngeal carcinoma (NPC). Latent membrane protein 1 (LMP1) is the antigenic protein of EBV that plays a role in the carcinogenesis of NPC. Human leukocyte antigen-A (HLA-A) gene has also been associated with the susceptibility of NPC. To find out the relations and interaction prediction of HLA-A*11:01 and HLA-A*24:02 with LMP1, computational methods was conducted using bioinformatics tools available online with open-source access such as International ImMunoGenetics Database (IMGT), Immune Epitope Database (IEDB), UniProt or Universal Protein Resource, IntFOLD5, PyMOL and PatchDock. Amino acids of both A*11:01 and A*24:02 have 92.1% identity and 94.5% similarity with 0% gaps. Both protein-protein bindings were found outside the binding groove but the affinity of the protein binding between A*11:01 with selected LMP1 antigenic peptides are better than A*24:02 with selected LMP1 antigenic peptides as it binds more at predicted binding sites. These two HLA-A are likely to have the same structure. It can be interpreted that both A*11:01 and A*24:02 do not induce proper immune reaction against the antigenic peptides of LMP1. The high affinity of the binding can induce an immune reaction that will prevent the development of NPC in individuals. This experiment provides understanding on the carcinogenesis of NPC in regards to the binding between the antigenic protein of EBV and the recognition of the infectious agent by HLA-A.

Keywords: *HLA-A; LMP1; nasopharyngeal carcinoma and in silico*

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Antioxidant level of green tea mixed with honey and calamansi

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Abstract

Green tea, honey, and calamansi antioxidant-rich ingredients combined into a beverage with multiple nutritional benefits. This study was carried out to determine the antioxidant level of a green tea infusion with honey and calamansi by using Ferric Reducing Antioxidant Power (FRAP) assay. Nine formulations of green tea mixed with honey and calamansi drinks were created based on the percentage generated by the Design Expert software. Then, the antioxidant level of each basic ingredient and all nine mixed drinks formulations were determined using the FRAP assay. Next, five formulations with higher antioxidant levels were chosen from nine formulations (A to I) to proceed with sensory evaluation testing to determine the best formulation. An analysis of nine formulations showed that formulation B has the highest antioxidant level (23.55 ± 1.019 μM/ml) when compared to others ($p < 0.05$). The basic ingredients were then compared to the mixed green tea infusion and the results showed that the antioxidant level of the mixed green tea infusion was significantly ($p < 0.05$) higher. In the sensory evaluation test, formulation B was chosen as the most preferred formulation by a significant ($p < 0.05$ margin. Based on the findings of this study, a combination of two or more components produces combination drinks with multiple nutritional values that can serve as an alternative drink for consumers' market. Overall, formulation B from the mixed green tea can be used as a reference for creating mixed drinks with higher antioxidant content that could be commercialized in beverage industry.

Keywords: *Green tea; infusion; honey; calamansi and antioxidant*

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Benzimidazole and its derivatives in managing the severity of cardiovascular disease (CVD)

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Abstract

Cardiovascular disease (CVD) affects the cardiovascular system and is currently the topmost leading cause of death worldwide. Previous studies reported that benzimidazole derivatives prevent blood clot. Their effectiveness as an anticoagulant in treating CVD were determined by the disease severity. This study aims to analyze the efficacy of benzimidazole as an anticoagulant to treat CVD and to compare their effectiveness among low, medium and high-risk CVD patients. A systematic review was performed for studies related to benzimidazole and CVD using five databases - PubMed, Scopus, Cochrane Library, Emerald Insight and Wiley Online Library. These databases were searched using keywords to provide information. All of the articles related to this study were then screened for eligibility to remove duplicates and only the articles that matched the inclusion study criteria were selected. The risk of bias assessment was conducted to determine whether the risk of bias was low, unclear or high for each article. Lastly, all extracted data that has been tabulated in a table was further analyzed and synthesized. The compound benzimidazole is effective as an anticoagulant in adult patients with CVD for all risk scores - low, medium and high. Benzimidazole might be an effective anticoagulant used to treat adult patients with low, medium and high CVD risk. Besides that, oral drug administration is believed to be an effective method for drug delivery in CVD patients. This study concludes that benzimidazole via oral route administration is efficient for drug delivery and in treating CVD patients.

Keywords: *Cardiovascular disease (CVD); benzimidazole; anticoagulant; adult and risk of bias assessment.*

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Supplementation of antioxidant vitamin C protects vascular endothelium in REM sleep deprivation animal model

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Abstract

REM sleep deprivation is associated with oxidative stress. Endothelial dysfunction, an early sign of cardiovascular disease (CVD) is linked to oxidative stress. Antioxidants have been shown to lower the risk of CVD. This study aimed to investigate the effects of REM sleep deprivation on the endothelium and determine the protective effects of vitamin C in the REM sleep-deprived animal model. Twenty-one male Sprague-Dawley (SD) rats were randomly divided into three groups (n=7): free-moving control rats (FMC), 72-hour REM sleep-deprived rats (REMsd) and 72-hour REM sleep-deprived rats (pre-treated with 100 mg/kg vitamin C daily for four weeks (RVC)). Rats were deprived of REM sleep using the inverted flowerpot technique. The descending thoracic aorta was isolated for *in vitro* functional study, oxidative stress markers measurement and histology examination. Systolic blood pressure (SBP) was significantly higher in the REMsd group compared to other groups. REMsd group showed impaired endothelium-dependent vasodilator responses to acetylcholine (ACh) compared to other groups. Malondialdehyde (MDA) levels were significantly increased, whereas superoxide dismutase (SOD) activity, levels of catalase (CAT) and total antioxidant capacity (TAC) were significantly decreased in REMsd compared to other groups. Endothelial damage was observed in the REMsd rat on scanning electron microscope (SEM). REM sleep deprivation induced endothelial dysfunction and increased SBP. Vitamin C supplementation is beneficial because it protects against the deleterious effects of REM sleep deprivation. Vitamin C may retard the development of endothelial dysfunction that may lead to CVD.

Keywords: *Sleep deprivation; endothelial dysfunction; oxidative stress and vitamin C*

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Evaluation of sigma value and quality goal index of Therapeutic Drug Monitoring in Hospital Kuala Lumpur

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Abstract

Sigma used to measure how far a given process deviates from perfection. Quality goal index (QGI) acts as an effort on sigma quality improvement, provides easy insight into where improvement is required. The aim of this study is to evaluate the performance of individual Therapeutic Drug Monitoring (TDM) parameters in terms of sigma and QGI, using Bias% sources from IQC monthly performance and RIQAS. IQC data, Bias% and coefficient of variant (CV) of nine TDM parameters were extracted between January 2020 and October 2020 from Biorad Unity QC Software. The Allowable Total Error (TEa) was obtained from RCPA and CLIA. Sigma and QGI for each individual TDM parameters were calculated using Bias% sources from IQC monthly performance and RIQAS. For control level 1, 8 parameters except for Phenobarbital achieved six sigma. Phenobarbital and Phenytoin at control level 2 showed <six sigma, and others showed ≥six sigma. For level 3 control, 6 parameters except for Amikacin, Phenobarbital and Theophylline achieved six sigma. There is no significant difference of sigma value measured in using Bias% from RIQAS and IQC monthly performance. Phenobarbital failed to meet six sigma performance at all three controls level, and QGI (<0.8) indicating imprecision problem. Analytes with a high Bias% gives a high QGI value, showed at analytes Amikacin (IQC level 1), Theophylline (IQC level 2) and Vancomycine (IQC level 2 and 3) which indicates inaccuracy and imprecision. This study shows that analysis of sigma metric and QGI provides a benchmark to address poor assay performance and assess the area need to be improved.

Keywords: *Internal Quality Control (IQC); Randox International Quality Assessment Scheme (RIQAS); Royal College of Pathologist of Australasia (RCPA) and Clinical Laboratory Improvement Amendments (CLIA)*

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Intestinal hookworm infection in a young immunocompetent patient

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Abstract

Hookworm infection is common in endemic areas. It can be prevented by good sanitation and personal hygiene practices. The manifestation is rarely seen in immunocompetent individuals unless in prolonged exposure to the parasite or heavy infection. In this case study, we reported a case of hookworm infection in a young immunocompetent male patient, presented with gastrointestinal symptoms after exposure to contaminated soil and cats at home. He suffered generalised abdominal pain associated with foul-smelling loose stool for 10 days. He was initially prescribed home with an oral antibiotic for five days, however, the condition did not improve. Subsequently, he was referred to hospital and was admitted. During admission, the laboratory examination showed leukocytosis with eosinophilia, whilst hookworm ova was seen in stool ova and cyst examination. The patient was treated with oral albendazole and covered for bacterial infection with oral ciprofloxacin. He was discharged well with the medications with no subsequent follow-up. This case suggests the possibility that hookworm infection can occur in a healthy person with prolonged exposure to the parasite and it can happen all over the places either in the remote or urbanized regions.

Keywords: *Parasite; hookworm; infection; immunocompetent; albendazole*

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Association of phylogroups and virulence profile of asymptomatic bacteriuria *Escherichia coli* isolates during pregnancy

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Abstract

Escherichia coli (E. coli) is a predominant agent causing asymptomatic bacteriuria (ASB) during pregnancy. The unavailability of genotypic data coupled with disparity in treatment becomes a grave situation of inappropriate management practices, which could potentially lead to the development of multidrug resistance (MDR). This study aimed to identify the association of *E. coli* phylogroups with virulence genes to analyse the prognostic likelihood of development of symptomatic UTI (urinary tract infection) in pregnant women. 160 *E. coli* isolated from 1315 midstream urine sample of pregnant women without symptomatic UTI were subjected to detection of phylogroups and prevalence of uropathogenic *E.coli* (UPEC) associated virulence genes using polymerase chain reaction (PCR). The majority of the *E. coli* isolates in this study belonged to phylogenetic group B2 (41.3%) followed by group A (25.0%), Group B1 (16.9%) and group D (16.3%). The most identified virulence genes in descending order were *fimH* (75.6%), *chuA* (58.1%), *kpsMTII* (38.1%), *usp* (34.4%), *fyuA* (31.9), *hlyA* (27.5%), *iroN* (24.4%), *cnf* (22.5%), *papC* (20.6), *sfa* (16.9%), *ompT* (15.0%) and *sat* (7.5%). *E.coli* isolated from ASB among pregnant women are mostly from phylogroup B2 and harbour virulence genes associated with UPEC causing symptomatic UTI. Therefore, determining the nature of the *E. coli* through genotyping and virulence profiling should be explored to ensure the utility and accuracy of the treatment decision for ASB cases in pregnancy. Such an approach will go a long way in reducing the use of antibiotics and the development of drug resistance.

Keywords: *Asymptomatic bacteriuria; urinary tract infection; pregnancy; phylogroups and Escherichia coli*

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Dengue infection and various serotypes identification in Sandakan

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Abstract

Dengue is mosquito-borne viral infection caused by dengue virus known as DENV. We aimed to study the distributions of dengue case by age and gender together identifying positive dengue cases in a relation FBC parameters. This is retrospective cross-sectional study based at Pathology Department,HDOK,Sandakan. Patient's details were collected form compiled database in microsoft access. Blood investigation for dengue serology were performed via rapid serological tests. Other data such as FBC parameters (Hb, HCT, PLT) and demographic profile (gender, age and race) were traced from the mentioned database. Follow-up with dengue serotype testing for all cases with positive dengue serology were carried out at Virology Unit,MKA Kota Kinabalu, Sabah. Total sample received were 3011 with 344(11.4%) positive and 2667(88.6%) negative. Majority of the samples are by male gender group with 1589(52.8%). Age group 0-10 years with 1330(44.2%) were the most detected dengue serology together with dengue serotypes 23 (63.9%). DENV-1 serotype is the most common with 14 (38.9%). DENV-1 with FBC; Hb,g/dL(10.7-17.6), HCT,%(24.4-48.6) and PLT, 10^3/µL(9-1556) may important markers in monitoring dengue patients. These findings could help for better clinical management and reduce risk of mortality.

Keywords: *Full blood count; dengue virus; dengue serology; dengue serotype and hospital duchess of kent sandakan*

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Transcriptional profiling of alpha giardins in H_2O_2-treated *Giardia intestinalis*

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Abstract

Giardiasis, a gastrointestinal disease caused by *Giardia intestinalis*, leads to childhood morbidity and mortality in developing countries. *G. intestinalis* is an anaerobic protozoan that is highly vulnerable to reactive oxygen molecules due to the lack of conventional ROS-scavenging enzymes. Continued exposure to ROS leads to cellular damage and oxidative stress. The information on how *G. intestinalis* survives in the oxidative stress intestinal environment still remains scarce. Herein, we sought to examine the role of alpha giardins in response to hydrogen peroxide (H_2O_2) treatment in *G. intestinalis*. Our findings showed that *G. intestinalis* treated with increased concentration of H_2O_2 demonstrated a significant reduction in cell viability and significant increase in the production of reactive oxygen species. The level of caspase-3, caspase-8 and caspase-9 were not detected in H_2O_2-treated *G. intestinalis* suggesting that the mode of cell death in *G. intestinalis* is of caspase-independent pathway. We demonstrated that the transcriptional level of alpha-1, alpha-2, alpha-3, alpha-7.3 and alpha-11 giardin increased significantly in H_2O_2-treated *G. intestinalis* when compared to untreated cells. However, there was no significant difference in the expression level of alpha-7.2 giardin between H_2O_2-treated and untreated *G. intestinalis*. Together these findings suggest that alpha giardins play a modulatory role in the antioxidant defence system of *G. intestinalis*, which could serve as a potentially promising target for future drug design and vaccine development.

Keywords: *Giardia intestinalis; alpha giardin; giardiasis; hydrogen peroxide and oxidative stress*

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A research paper: detection of genetic mutation in a family with Marfan Syndrome

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Abstract

Marfan Syndrome (MFS) is a genetic disease where a mutation in certain genes occurs resulting in abnormal fibrillin-1 protein expression causing defective connective tissues (weakened aorta, blood vessels and eye problems). Approximately 90% of patients diagnosed with MFS have mutations within the FBN1 gene, whereas 10% of MFS cases have mutations in other genes such as TGBR1 and TGBR2. Blood samples of a family with MFS history were collected into EDTA tubes. DNA was extracted using the spin column technique. Genetic mutation was determined using Whole Exome Sequencing (WES). Allele Specific-Polymerase Chain Reaction (AS-PCR) will be employed to analyse genetic mutations that might be present in other family members. WES analysis showed mutation at point 48741078 in *FBN1* gene. Forward Common and Reverse Common employed in the analysis were successfully amplified in the correct region of the *FBN1* gene consisting of a 48741078 mutation point. This amplified region was then sent for sanger sequencing for confirmation of the mutation point. The results for WES from a patient with Marfan Syndrome showed a mutation at point 48741078 on the FBN1 gene, which does not occur on negative control (normal) subjects. This region will then be optimised using the AS-PCR method and tested on other family members of the patient. AS-PCR consisting Forward Common, Reverse Common, Allele Specific Forward and Allele Specific Reverse Primer will be further optimised in order to provide cost-effective genetic testing and used to genotype all family members and the control group (200 subjects).

Keywords: *Marfan Syndrome (MFS); FBN1; Allele Specific-Polymerase Chain Reaction (AS-PCR) and Whole Exome Sequencing (WES)*

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Assessment of greenness in morphine analysis

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Abstract

Green analytical chemistry (GAC) was proposed by Namiesnik in 1999. In the same year, Anastas brought attention among chemists to develop green analytical methodologies in the research field. The milestone of GAC can be traced back to 1974. The basic goal of the GAC is to reduce the use of chemical substances, manage the waste properly, decrease energy consumption, and increase operator safety. The method for morphine analysis by urine test strip, immunoassay, gas chromatography-mass spectrometry (GC-MS), and liquid chromatography hyphenated to tandem mass spectrometry (LC-MS/MS) was compared using Analytical Eco-Scale, and Green Analytical Procedure Index (GAPI). Scoring was made for sample collection, sample preparation, the reagent used, and instruments for each method for greenness and appraisal. Greening the analytical principles shall find the balance with figures of merit in morphine analysis. Although GC-MS and LC-MS/MS methods are superior to the test strip and immunoassay method in terms of analytical characterization, it lacks greenness. Derivatization, extraction, large usage of chemical volume, use of toxic reagents, and solvents should be avoided and replaced by the green process. Method development in drug abuse analysis is a continuous process with the emerging of new psychoactive substances in the market. Scientists and researchers should spearhead the search for cheaper, efficient, accurate, greener, and miniaturized alternatives of green technology to decrease the negative impact on the environment and public health.

Keywords: *Green analytical chemistry; morphine; LC-MS/MS; GC-MS and immunoassay*

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Modulation of microrna regulating self-renewing and differentiation of lineage-committed mouse hematopoietic stem/progenitor cells by 1,4-benzoquinone

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Abstract

Hematopoietic stem/progenitor cells (HSPCs) are the major targets for benzene-induced hematotoxicity and leukemogenesis. However, studies concerning the epigenetic toxicity of benzene affecting microRNAs profiles in differential lineage-committed HSPCs remain understudied. Here, we investigated the epigenetic toxicity of a benzene metabolite, 1,4-benzoquinone (1,4-BQ), in HSPCs focusing on the self-renewing (miR-196b and miR-29a) and differentiation (miR-181a) – controlling microRNAs (miRNAs). Mouse bone marrow cells (MBMCs) were exposed to 1,4-BQ (1.25–5 μM) for 24 h. Then, the epigenetic toxicity of 1,4-BQ in lineage-committed progenitors comprising myeloid, Pre-B lymphoid, and erythroid lineages were evaluated following 7–14 days of colony forming cell assay. Expressions of miR-196b were significantly downregulated ($p < 0.05$) in MBMCs upon exposure to 1,4-BQ at 1.25, 2.5 and 5 μM, but upregulated in HSPCs starting at 2.5 μM. As for miR-181a, a significant downregulation ($p < 0.05$) was notable in MBMCs and HSPCs at all concentrations; exceptionally for Pre-B lymphoid progenitors which showed significant upregulation at 2.5 and 5 μM of 1,4-BQ. Myeloid progenitors and MBMCs showed significant upregulation ($p < 0.05$) of miR-29a expression at 2.5 and 5 μM 1,4 BQ; whereas miR 29a was downregulated at all concentrations for erythroid and Pre-B lymphoid progenitors. 1,4-BQ induces aberration of miRNAs expression in self-renewal and differentiation pathways of HSPCs via a lineage-driven mechanism. The role of cell lineage in governing the epigenetic toxicity of 1,4-BQ in HSPCs deserves further investigation.

Keywords: *Benzene; 1,4-Benzoquinone; Lineages-specific; epigenetic and microRNA*

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Development and optimisation of olmesartan medoxomil for intranasal application using box-behnken statistical design

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Abstract

Dementia is a chronic neurological disorder in which there is deterioration in memory, thinking abilities accompanied by behaviour changes. Available therapeutics have limited application due to their side effects and developing resistance. The potential benefit of angiotensin receptor blockers (ARBs) against demented conditions has paved our current research. Olmesartan medoxomil (OM) is a non-brain penetrating (ARB). Thus, the present research aimed to develop an oil-in-water nanoemulsion of OM (OMNE) intended for intranasal application using Box-Behnken statistical design. The optimised OMNE formulation was further coated with chitosan (COMNE) to achieve improved retention at the application site in the nasal mucosa. Developed COMNE containing OM would facilitate penetration of the drug to the brain for its effective role. OMNE was developed using Sefsol oil, Tween 80 (surfactant) and Transcutol P (co-surfactant). Different OMNE formulations were characterised for their droplet size, size distribution, zeta potential and viscosity. The droplet size, PDI and zeta potential of the optimised OMNE formulation was found to be 102.7 nm, 0.362 and -14.1 mV, respectively. The size of the nanodroplets was increased upon coating with chitosan, whereas the zeta potential was changed to +33.5mV, which could facilitate the stability of the formulation. The rate of drug release was also decreased due to the fabrication of COMNE, which indicated the entrapped OM would release the drug for a longer period following their mucoadhesion at the nasal environment. Therefore, the obtained results demonstrated that COMNE was successfully developed and optimised using a conducive low-energy method.

Keywords: *Dementia; olmesartan medoxomil; chitosan; nanoemulsion and intranasal*

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Effect of 1,4-benzoquinone exposure on transcription factors controlling hematopoietic stem/progenitor cells fate

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Abstract

Hematopoietic stem/progenitor cells (HSPCs) are the major targets for benzene-induced hematotoxicity and leukemogenesis. However, the molecular mechanism of benzene toxicity concerning transcription factors controlling hematopoietic stem/progenitor cells fate remains understudied. Here, we investigate such a mechanism using a benzene metabolite, namely 1,4-benzoquinone (1,4-BQ) which is exposed to bone marrow derived-HSPCs for 24h. The transcription factors associated with the self-renewing (Bmi-1, HoxB4, and Wnt3) and differentiation (GATA1, GATA2, and GATA3) pathways responsible for governing stem cell fate were studied for gene and protein expressions at non-cytotoxic concentrations (1.25, 2.5, 5) and all ranges of concentrations (1.25, 2.5, 5, 7, and 12 μM) of 1,4-BQ, respectively. Significant upregulation of HoxB4 expression level was observed at all concentrations. GATA3 and Bmi-1 expressions were also significantly upregulated at 2.5 and 5 μM 1,4-BQ, respectively. No significant effect was noted for the expression of Wnt3, GATA1 and GATA2 genes. Meanwhile, expression of HoxB4 protein was significantly increased ($p < 0.05$) at all 1,4-BQ concentrations compared with BMI-1 and GATA3 which were only significantly ($p<0.05$) elevated starting at higher concentration (2.5 μM) of 1,4-BQ. In conclusion, 1,4-BQ could modulate the fate of HSPCs by altering the self-renewal and differentiation-related transcription factors; with HoxB4 being the most affected transcription factor.

Keywords: *Benzene; 1,4-BQ; Hematopoietic Stem / Progenitor Cells and transcription factors*

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Metabolic shifts leading to key mutations regulating bacterial motility

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Abstract

Flagellum (FliC) expression is regulated by bacterial metabolism. Deletion of alcohol/aldehyde dehydrogenase (Δ*adhE*) in the pathogenic enterohemorrhagic *Escherichia coli* (EHEC) showed a pleomorphic phenotype - overexpression of FliC and lack of motility. Understanding the molecular mechanism of this phenotype will provide a better understanding of bacterial metabolism and motility. Motility assays for Δ*adhE* were conducted at 37°C for 18 hours on motility agar. FliC expressions were measured using pGFP-reporter assays. Whole-genome sequencing (WGS) of the Δ*adhE* and escape mutants was analysed using the single-nucleotide-polymorphism (SNP) calling workflow. Structural modelling was visualised using PyMOL. WGS showed an A46V-mutation in FliC of *adhE.* Complementation of the Δ*adhE* with pBAD-harbouring the AdhE rescued the motility by 50%. Motility assays of Δ*adhE* over 24 hours gave escape mutants with 100% recovered motility and continuous FliC overexpression. WGS showed that all escape mutants retained the A46V mutation and gained a secondary SNP in FliC. Acetate build-up from Δ*adhE* has increased the selective pressure and proposed to induce the A46V mutation. Structure modelling showed that A46V might induce polymorphic transition, which led to the lack of motility. In the escape mutants, the selective pressure from extended periods in motility assays has increased and proposed to deduce the second mutation in the flagellin structure to recover the motility. The shift in bacterial metabolism from Δ*adhE* have induced SNPs leading to the lack of motility. Treatments focused on regulating bacterial metabolism could thus potentially be used to disarm bacterial motility and virulence.

Keywords: *Motility; single nucleotide polymorphism; bacterial virulence and bacterial metabolism*

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Microarray gene expression analysis of epithelial differentiation of dental stem cells

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Abstract

Epidermal stem cells are regenerated during wound healing. Typically, a large number of epithelial is required for the process and not possibly available. To retain skin homeostasis for the treatment, stem cells can be used. This study aims to identify the differentially expressed genes (DEGs) involved in epithelial-like cells differentiation of stem cells from human exfoliated deciduous teeth (SHED). Using the in-direct method, SHED were co-cultured with keratinocytes in the epithelial microenvironment. The cells were harvested on day-5 and subjected to RNA extraction and, subsequently, microarray gene expression. The data obtained were bioinformatically analysed. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using the Database for Annotation, Visualisation, and Integrated Discovery (DAVID). The most upregulated DEGs were determined and validated using Real-Time PCR. A total of 2033 DEGs were identified. The DEGs that were mainly enriched are cell division, but they were downregulated. Among the highest upregulated DEGs were *SUSD2*, *CXCL1*, *GGT5 HEY1*, *TXNIP*, *PDGFRB*, *PAMR1*, and *COL6A2*, which were then validated. The highest gene upregulated, 1200-fold, *SUSD2* is involved in cell proliferation inhibition. Meanwhile, possible pathways were narrowed down towards inflammation, TGF-beta, PI3K-Akt, p53, and TNF signalling based on KEGG findings. DEGs related to cell division were found to be downregulated, which indicates cell differentiation. The DEGs identified during SHED-epithelial differentiation at day-5 indicate inhibition of cell division, suggesting initiation of cell differentiation.

Keywords: *Co-culture; epithelial-like; microarray; stem cell and SHED*

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A meta-analysis on the worldwide prevalence of intermediate *leptospira* spp. in human samples

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Abstract

Leptospirosis has been a public health threat for many years but is still recognized as one of the bacterial neglected tropical diseases. This is due to the broad spectrum of symptoms caused by the leptospires with different pathogenicity such as pathogenic, intermediate, and saprophytic. Intermediate *Leptospira* spp. can either be pathogenic or non-pathogenic. They have been reported to cause mild to severe forms of leptospirosis in several studies, contributing to the disease burden. This study aimed to estimate the worldwide prevalence of intermediate *Leptospira* spp. in humans via meta-analysis method with region-wise stratification. The articles were searched based on PRISMA guidelines, from PubMed, ScienceDirect, and Scopus. Records obtained were subjected to screening and quality of the pertinent studies that fit the criteria were assessed using modified Critical Appraisal Checklist recommended by Joanna Briggs Institute. Seven studies among 469 records were selected for quantitative analysis, consisting of two regions according to the United Nations geo-scheme regions. RevMan software was utilized for the analysis. The overall prevalence of intermediate *Leptospira* spp. in humans was 32%, and the pooled prevalence for the American and Asian regions were 62% and 17%, respectively. The findings also showed that *Leptospira wolffii* (n=223/225) was the most predominant species as compared to *Leptospira broomii* (n=1/225) and *Leptospira inadai* (n=1/225). This study revealed that intermediate *Leptospira* spp. are indeed contributing to the disease burden. The prevalence estimates from this study could be used to develop better control and intervention strategies in combating human leptospirosis.

Keywords: *Intermediate Leptospira; human leptospirosis; prevalence and meta-analysis*

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Selection of T-Cell Receptor (TCR) like antibody for cervical cancer diagnostics and therapeutics

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Abstract

Cervical cancer causes death among women and is caused by human papillomavirus (HPV) where 70% of all cervical cancer cases are linked to HPV 16 and 18. Recent advancement in science and technology has enabled the development of various cervical cancer diagnostic and therapeutic approaches. In this study, a novel antibody class, known as T cell receptor (TCR)-like antibodies, specific to HPV 16 and 18 E7 oncopeptides, has been hypothesised to enhance cervical cancer diagnostic and therapeutic potentials. The target for HPV 16 and 18 E7 peptides specific to HLA-A2 were selected via bioinformatics analysis. The HPV peptides were refolded with the HLA-A2 heavy chain and β2m light chain to form a peptide-MHC complex. The pMHC complexes were subjected to biopanning against an antibody phage display library to generate the potential TCR-like antibodies. The monoclones were analysed with monoclonal and comparative ELISA before sequence analysis. The potential TCR-like antibody clones were selected via biopanning against the domain antibody library. For HPV 16 E7 and HPV 18 E7 after analysis 10 and 21 clones were sent for sequencing, respectively. Phage display technique made T cell receptor like (TCR) antibody generation possible. TCR-like antibody is suitable for cervical cancer diagnosis because it sandwiches both cell mediated and humoral immunity in a single platform. Upon recognizing the antigenic peptides on MHC complex, the TCR-like antibody can also elicit various antibody defence mechanisms. Further investigations are required for cervical cancer diagnostics and therapeutics based on finding from this study.

Keywords: *T cell receptor (TCR)-like antibody; cervical cancer; human papillomavirus (HPV); diagnostics and therapeutics*

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Network pharmacology-based prediction on the combination of *Moringa oleifera* and gemcitabine against pancreatic cancer

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Abstract

Gemcitabine (GEM) is known as the first-line chemotherapy drug in pancreatic cancer treatment. *Moringa oleifera* (MO), which is used in Ayurveda, has exhibited various biological activities including an anti-cancer role. However, the effectiveness of their combination against pancreatic cancer has yet to be explored. This study evaluates the effect of MO and GEM combination against pancreatic cancer through network pharmacology. TCMSP, TCMID and PubMed were used to identify and screen the bioactive compounds in MO. Target genes of MO and GEM were predicted through DGIdb, CTD, and DrugBank. Pancreatic cancer genes were collected from OMIM and MalaCards databases. Protein-protein interaction (PPI) and compound-target-pathway network were established via STRING and Cytoscape, respectively. Gene ontology (GO) and pathway enrichment analysis were conducted using DAVID Bioinformatic Tools. MO was found to contain 7 potential bioactive compounds that met the drug screening requirement, which include catechin, kaempferol, quercetin, epicatechin, glucomoringin, glucoraphanin and moringinine. Catechin was found to be the main hub compound in MO. *TP53, AKT1, VEGFA, CCND1, CASP3, MAPK3, JUN, BCL2L1, MAPK1* and *TNF* were discovered via PPI network as hub genes that have biological importance in pancreatic cancer. GO and pathway analysis revealed that the combination of MO and GEM to be mainly associated with cancer including pancreatic cancer through regulation of apoptosis. This study has revealed the multi-compounds, multi-targets and multi-pathways of MO and GEM combination against pancreatic cancer. The bioinformatics analysis provides insights on the potential use of MO and GEM combination for pancreatic cancer treatment.

Keywords: *Moringa oleifera; pancreatic cancer; bioactive compound; gemcitabine and network pharmacology*

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Functionalized gallic acid-loaded graphene oxide (GAGO) accelerates wound healing in *in vivo* model

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Abstract

Gallic acid (GA) is a well-known agent that promotes wound healing, while graphene oxide (GO) nanocarrier is known for its high dispersibility, hydrophilicity, large specific surface-to-volume ratio, and low production cost. Encapsulation of GA with GO, known as gallic acid-loaded graphene oxide (GAGO), is believed to possess a synergistic effect that enhances wound healing activities. This study aims to investigate the acceleration of wound healing potential of nanoformulated GA. Rats were assigned into 6 groups and a cutaneous wound was created on the dorsal area of an individual rat. Each group was topically treated with 250 µM and 500 µM of GAGO, daily for 14 days. Native GA and GO, solcoseryl gel and no treatment group were used as comparisons. Tissue samples were then isolated for further analyses. Topical application of GAGO improved the wound closure when compared to other groups. Wounds in rats treated with 500 µM and 250 µM of GAGO closed at day 9 and day 12 post-treatment, respectively. In contrast, wounds in the control groups were still not fully closed at day 14 post-treatment. The histological analysis also revealed the effectiveness of GAGO in improving wound contraction and scar area. These results demonstrate that treatment of GAGO nanoformulation accelerates wound healing compared to its native compounds, GA and GO. It also indicates the potential of GO as a carrier for nanoformulation. However, further study is still warranted to understand the effect of GAGO and GO nanocarrier on wound healing properties at molecular level.

Keywords: *Wound healing; nanoparticles; graphene oxide and gallic acid*

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The role of metallic-based drugs on fighting the *Staphylococcus aureus* biofilm

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Abstract

Bacterial adhesion has become a serious problem in the multidisciplinary sector of life, such as industry or clinical setting. In general, bacteria exist in two forms, planktonic cells and sessile cells form a biofilm. The *Staphylococcus aureus* contributes to becoming a problematic pathogen in both community and health settings. *Staphylococcus aureus* biofilm is the main reason for significantly increased morbidity and mortality especially when associated with indwelling medical devices. By using RTCA technology for first time in our lab to test bacterial biofilm, micrograph examinations (CLSM and Fluorescent Microscope) and quantitative RT-PCR to evaluate the anti-biofilm activity of Gold (I) based compound against Methicillin-resistant Staphylococcus aureus biofilm. Interestingly, we observed significant inhibitory and eradication activity of MRSA biofilm greater than 70%. The RTCA results showed low cell index (CI) less than 0.5 at five hours after treatment exposure, however, addition of proteinase K enhanced anti-biofilm activity by decreasing the CI to 0.2 after five hours of treatment exposure. qRT-PCR showed notable altering in gene expression of biofilm associated genes (*ica , fin eno epbs*). Our finding might shed novel light on the application of metallic based drugs in combat against pathogenic bacteria especially biofilm formation bacteria as they are considered more resistant and stubborn to drugs. Our finding also implies that using RTCA (xCelligence) technology to evaluate and screening drugs against bacterial biofilm gave better results and ease hands-on comparing to the traditional methods.

Keywords: *Metallic drug; Staphylococcus aureus; Biofilm and xCelligence*

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Identification of uniparental disomy on chromosome 15 in prader-willi syndrome by microsatellite linkage analysis

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Abstract

Prader-Willi Syndrome (PWS) is a rare neurodevelopmental genomic imprinting disorder due to the absence of paternally expressed genes in the PWS critical region on chromosome 15 by either a paternal deletion, maternal uniparental disomy or an imprinting defect. Identifying the genetic mechanism is important for recurrence risk assessment as the risk to sibs is less than 1% if the affected child has a deletion or uniparental disomy, and up to 50% in imprinting defect cases. A six-month-old female baby was presented with global developmental delay, hypotonia and feeding difficulties. MS-MLPA analysis showed normal gene dosage and hypermethylation at the *SNRPN* & *MAGEL2* genes thus establishing the diagnosis of PWS due to uniparental disomy or imprinting defect (UPD/ID). This method could not distinguish between UPD/ID, therefore to determine the molecular cause of PWS, a microsatellite linkage analysis using eight microsatellite markers was carried out. PCR products were genotyped using capillary electrophoresis, and analyzed using GeneMarker software. The microsatellite linkage analysis in our patient revealed the inheritance of two identical chromosomes 15 from the mother and none from the father. Inheritance of two maternal copies were present within and outside the typical PW deletion region, hence implying isodisomy. This result confirmed the clinical diagnosis of PWS due to maternal uniparental disomy (upd(15)mat), therefore the recurrence risk is very low. The use of microsatellite markers enabled the identification of the molecular cause of PWS which is crucial for genetic counselling, patient's management and improvement of prognosis.

Keywords: *Genomic imprinting; Maternal uniparental disomy (upd(15)mat); Rare genetic disorder and Microsatellite marker.*

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Pre-analytical specimen rejections in an outpatient laboratory in Kuala Lumpur Hospital

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Abstract

The outpatient laboratory of Kuala Lumpur Hospital provides phlebotomy services and serves as receiving counter for all specimens from outpatient specialist clinics. This laboratory conducts basic routine biochemistry, full blood count (FBC) and urine test (UFEME and UPT). One of the common problems that occur is rejection of specimens which may implicate/delay the diagnosis and treatment of patients. A prospective study was conducted in the Specialist Clinics and Ambulatory Care Centre (SCACC) Pathology Laboratory from January to December 2020 to understand the causes of specimen rejections. The pre-analytical rejections were categorized based on criteria determined by the Pathology Department of Kuala Lumpur Hospital. The main reason for rejection was no specimens for the requested tests and this contributed to 70% (650 specimens) of the total pre-analytical rejections. Urine specimens contributed largely to the rejections, followed by blood specimens missed tube by the phlebotomy section. Urine specimens rejected were mainly for patients from Nephrology Clinic, Physician Clinic and Urology Clinic. The missed tubes were mainly for FBC, ESR, FBS, RP, LFT and VBG tests. The findings from this study enable the laboratory to identify and implement appropriate measures to reduce rejection of specimens.

Keywords: *missed tube; outpatient laboratory; pathology; pre-analytical and specimen rejections*.

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Bisphenol F reduced the sperm quality via testosterone hormone alterations in male Sprague Dawley rats

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Abstract

Bisphenol F (BPF) has been used widely in manufacturing industries as a replacement for Bisphenol A which is known to cause a systemic toxic effect. BPF has been found in human biological samples such as urine and has been identified as estrogenic and antiandrogenic agents leading to male reproductive toxicity. However, the effect of BPF on oxidative stress status on sperm and testes is under-reported. Hence, this study was done to evaluate the effect of BPF on plasma testosterone, sperm characteristics and oxidative stress status of sperm and testis of male Sprague-Dawley (SD) rats. Adult male SD rats (n= 20) weighing between 230-250g were randomly divided into four groups: control (received 1 ml/kg of normal saline and BPF1, BPF5 and BPF10 which received 1, 5, and 10 mg/kg/bw of BPF, respectively for 28 days. Plasma was obtained for testosterone analysis while sperm and testis evaluated for oxidative stress. The plasma testosterone in BPF1 group was insignificantly decreased when compared to the control, BPF5 and BPF10 groups. However, BPF5 and BPF10 significantly ($p<0.05$) increased the abnormal sperm morphology as compared to the control group. No significant differences were recorded for superoxide dismutase activity as well as glutathione, protein carbonyl and malondialdehyde levels in the sperm and testis of experimental rats. BPF did not induce oxidative stress in the sperm and testis however it decreased the sperm quality and testosterone level in male SD rats.

Keywords: *BPF; male reproductive system; oxidative stress and testosterone*

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Transcriptomic profiling of triple negative breast cancer unveils Annexin A1 mediated immunomodulation

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Abstract

Triple negative breast cancer (TNBC) is a clinically heterogeneous and highly aggressive disease with no targeted therapies. Recent studies have reported that AnxA1 is associated with the aggressive nature of TNBC. This study aims to develop comprehensive gene co-expression networks correlated with tumour microenvironment analysis on transcriptome-based AnxA1 and associated gene expression. A comparative transcriptomic analysis between MDA-MB 231(TNBC) and MCF-7 (non-TNBC) was performed. The functional enrichment analysis of the differentially expressed genes (DEGs) was performed using Ingenuity Pathway Analysis (IPA) and Gene Set Enrichment Analysis (GSEA). The connectivity network between enriched gene sets was constructed with Cytoscape. The expression status of AnxA1 in the cancer cells was validated with RT-PCR. The transcriptomic profiling showed that AnxA1 gene signatures were highly expressed in MDA-MB 231, but not MCF-7. A total of 8647 differentially expressed genes (DEGs; 4100 upregulation; 4547 downregulation) were identified. The functional enrichment analysis of the DEGs revealed a predominant role of high AnxA1 expression in immunomodulation, including immune cell response and cytokine & chemokine signaling in TNBC cells. Metastatic behaviour such as extracellular matrix remodeling and HIF-1α signaling were implicated in MDA-MB 231 compared to MCF-7. Taken together, based on the transcriptomic profiles, AnxA1 could exert a profound regulatory role in onco-immunity, tumour-stromal crosstalk, and epithelial-mesenchymal transition in the tumour microenvironment of the TNBC cell line. Thus, this result will be a foundation for the development of new therapeutic strategies in TNBC patients.

Keywords: *Annexin A1; triple negative breast cancer; RNA sequencing; RT-PCR; immunomodulatory and tumour microenvironment*

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Effects of different incubation times toward RNA recovery in plasma RNA extraction

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Abstract

RNA in plasma samples are known to exist in marginal amount, contributing to difficulties in obtaining favourable RNA results for downstream applications. Thereby, numerous plasma RNA extraction protocols that utilized different solutions were implemented. These protocols are frequently comparable with slight differences in the parameters especially on the incubation time. Hence, this paper will focus on the implications of different incubation periods in plasma RNA extraction protocol to identify the dependency of plasma RNA towards incubation times and how they are affected. Plasma RNA were extracted using miRNeasy Serum/Plasma (Qiagen, Germany). To test the effects of incubation time, the RNA extractions were repeated with reduced and extended incubation periods for several minutes gaps at certain steps, for instance, the extractions were done distinctly with three- (reduced), five- (default) and seven- minutes (extended) incubation period after TRIzol reagent addition and the results were compared. Our results demonstrated positive correlations when the incubation times were increased during sample homogenization and RNA precipitation, yielding around 14.30 ng/µL to 17.50 ng/µL, and 13.43 ng/µL to 20.05 ng/µL respectively compared to the average of 10.37 ng/µL on default duration. Generally, longer period of incubation might allow sufficient RNA recovery to occur. Steps like sample homogenization and RNA precipitation require longer incubation time for complete RNA recovery since plasma RNA are considerably dignified than DNA or other RNAs due to their low level and encapsulated within exosomes. Thus, the incubation time within extraction protocol play an important role for efficient plasma RNA recovery.

Keywords: *incubation time; plasma; RNA and optimization*

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Demographic data on red cell alloimmunization among a multi-transfused hepatobiliary patient at Hospital Selayang

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Abstract

Red cell transfusion is a frequent procedure for surgical patients such as hepatobiliary patients. Despite being a choice to increase hemoglobin level, it still exhibits significant risks. This multiple transfusion could risk the development of alloantibodies that lead to alloimmunization. Unexpected formation of red cell alloantibodies can cause hemolytic transfusion reaction and could delay the transfusion due to the challenge to find a compatible blood for the patient. There is no research done in Malaysia, specifically on red cell alloimmunization indices in hepatobiliary patients. Thus, this preliminary study aimed to determine the prevalence of red cell alloimmunization based on demographics data such as gender, age, number of transfusions, and blood group system. A cross-sectional study was done from January 2021- June 2021which involved hepatobiliary patients in Hospital Selayang. Descriptive analyses were carried out based on demographic data. From the 145 patients, 105 (72.4%) were alloimmunized with respective alloantibodies such as Anti-E (18.1%) followed by anti-Mia (12.4%). The occurrence of alloimmunization was higher in females (77.6%) and all those who received more than ten transfusions were susceptible to produce alloantibodies (100%). The prevalence of alloimmunization was higher in Chinese (82%) and mostly associated with O positive blood group (35.2%). Production of alloantibodies were higher among 50 years old and above (77%). The current findings show a trend of alloimmunization development in multi-transfused individuals which will be worth to be further investigated to provide beneficial information on the blood product management and supply especially among hepatobiliary patients.

Keywords: *Alloantibodies; alloimmunization; blood group; hepatobiliary and red cell transfusion*

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The blowfly *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) as an indicator for a decapitated case in Malaysia

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Abstract:

The first aim of forensic entomology is to assist investigators estimate the time of death. A bolster containing a severed human head was discovered in an apartment in Kuala Lumpur, Malaysia. A case is presented in which fly species were used to determine the time of decapitation. Entomological specimens were collected during autopsies and analysed in the laboratory for species identification and obtaining the insect developmental data. The minimum postmortem interval was calculated by relating the entomological evidence to data available for Diptera species and to our knowledge of the development of flies used as forensic indicators in Malaysia. The entomological evidence collected at the cut surface of the neck consisted of Calliphoridae (*Chrysomya megacephala, Ch. rufifacies, Ch. nigripes, Lucilia cuprina*), Muscidae (*Hydroteae spinigera*) and Sarcophagidae. For this case, the developmental data of *Chrysomya megacephala* was used, indicated that the decapitation occurred five days before the discovery of the body. To the best of our knowledge, this is the first reported case of decapitation related to forensic entomology in Malaysia. The roles of *Ch. megacephala* in this case strengthen the facts that this species is the main indicator for PMI estimation for forensic entomology cases in Malaysia.

Keywords: *Forensic entomology; Chrysomya megacephala; postmortem interval and decapitated*

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Evaluating the effect of lemongrass and gemcitabine combination treatment on pancreatic cancer through network pharmacology

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Abstract

Pancreatic cancer is mostly incurable. Southeast Asia and Africa have lower incidence than in other regions worldwide. Lemongrass found in those regions has a therapeutic value for pancreatic cancer. Gemcitabine is a first-line chemotherapy drug for pancreatic cancer. Lemongrass and gemcitabine are assumed to effectively manage pancreatic cancer; however, their combined effect still unclear. This study aims to evaluate the combined effect of lemongrass and gemcitabine on pancreatic cancer through network pharmacology. Lemongrass bioactive compounds were screened from Traditional Chinese Medicine Systems Pharmacology and Traditional Chinese Medicine Integrated Database. Potential targets of the compounds and gemcitabine were predicted through Disease Gene Interaction database, DrugBank, Comparative Toxicogenomics Database, Swiss Target Prediction, and Chemmapper databases. Pancreatic cancer genes were collected from MalaCards and Online Mendelian Inheritance in Man databases. STRING database and Cytoscape software were used to construct protein-protein interaction and compound-target-pathway network, respectively. DAVID bioinformatics database was utilized to conduct gene ontology (GO) and pathway analysis. 27 compounds in lemongrass with luteolin met the drug screening requirements selected as the bioactive compound. TP53, CASP3, BCL2L1, AKT1, CCND1, PTEN, MAPK3, STAT3 genes were identified as the hub genes which have high biological relevance in pancreatic cancer. The GO and pathway analysis indicated that the combination treatment mainly acts on several cancer-related pathways, including pancreatic cancer, through apoptotic and cell proliferation regulation. Hence, this *in silico* study might provide an insight into lemongrass and gemcitabine combination treatment against pancreatic cancer. However, further experimental validation is essential to verify these computational findings.

Keywords: *Bioactive compound; gemcitabine; lemongrass; network pharmacology and pancreatic cancer*

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Association between family history and cigarette smoking with increased risk for nasopharyngeal carcinoma in Pahang state of Malaysia

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Abstract

Nasopharyngeal carcinoma (NPC) is among common cancer in Malaysia, with prominent distribution among Chinese and Bidayuh natives. Although NPC is common in Malaysia, its risk factor studies in the country NPC is scarce and mostly obsolete. Thus, this study was designed to identify the risk factors associated with NPC in Pahang, which is the largest state in peninsular Malaysia. NPC cases diagnosed within 2012 to 2017 in two main referral hospitals in Pahang were included. Interviews were conducted with NPC patients using adapted questionnaires, including demographic data, family history of cancers, smoking status, alcohol drinking status and dietary intake of preserved foods. Gender-, ethnicity- and age-matched control subjects were recruited among those with no history of cancer. Simple Logistic Regression (SLR) analysis was used to determine the odds ratio (OR) of tested variables that significantly associated with NPC. Multiple Logistic Regression (MLR) analysis was used to determine the adjusted OR (AOR) of significant variables from the SLR analysis. The SLR analysis showed that family history of NPC (OR=6.96) and current smokers (OR=2.62) were significantly associated with the risk for NPC (p<0.05). Further analysis using MLR analysis showed that the family history of NPC and current smokers remained significant (p<0.05) with AOR of 7.9 and 3.01, respectively. The significant association of family history and smoking with NPC were consistent with the previous study. This was the first study that showed the significant association of family history and smoking with the increasing risk of NPC in Pahang.

Keywords: *Nasopharyngeal carcinoma; risk factors; family history and cigarette smoking*

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Establishing a rat model for diabetic cardiomyopathy development and progression

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Abstract:

The characteristics of early and advanced stages of DCM in clinical setting are well-understood, however the time points by which these stages developed in animal model is not fully determined. To determine the time points of early and late stages of DCM development from the induction of diabetes mellitus and to identify the functional and histological changes in both stages. Type 1 diabetes mellitus was induced with single injection of streptozotocin intraperitoneally (65 mg/kg body weight) before being divided into 4-week and 8-week diabetic groups and were left untreated for the whole respective study period. Cardiac functions measurement (Langendorff's reperfused heart method) and structural changes were analysed. The 4-week diabetic group exhibited significant reduction in cardiac function showed by reduced left ventricular developed pressure and relaxation rate (-dP/dt) ($p<0.05$) compared to normal rats, along with unaffected contraction rate (+dP/dt), exhibiting characteristics of early stage of DCM. In 8-week diabetic group, all cardiac functions deteriorated significantly ($p<0.05$), with prominent decline in +dP/dt, hence signify the advancement of DCM into the later stage. Cardiomyocyte size was significantly increased ($p<0.05$) in the 4-week diabetic rats. Both cardiomyocyte size and myocardial collagen deposition were elevated significantly ($p<0.05$) in 8-week diabetic group. Cardiac dysfunction and structural alterations were significantly more prominent ($p<0.05$) in 8 weeks as compared to 4-week diabetic group. At 4 weeks of untreated diabetes, early stage of DCM had already developed, and this condition deteriorated towards the advanced stage within the next following 4 weeks.

Keywords: *diastolic dysfunction; fibrosis; hypertrophy; systolic dysfunction and type 1 diabetes mellitus*

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Supplementation of antioxidant vitamin C protects vascular endothelium in REM sleep deprivation animal model

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Abstract

REM sleep deprivation is associated with oxidative stress. Endothelial dysfunction, an early sign of cardiovascular disease (CVD) is linked to oxidative stress. Antioxidants have been shown to lower the risk of CVD. This study aimed to investigate the effects of REM sleep deprivation on the endothelium and determine the protective effects of vitamin C in the REM sleep-deprived animal model. Twenty-one male Sprague-Dawley (SD) rats were randomly divided into three groups (n=7): free-moving control rats (FMC), 72-hour REM sleep-deprived rats (REMsd) and 72-hour REM sleep-deprived rats (pre-treated with 100 mg/kg vitamin C daily for four weeks (RVC)). Rats were deprived of REM sleep using the inverted flowerpot technique. The descending thoracic aorta was isolated for *in vitro* functional study, oxidative stress markers measurement and histology examination. Systolic blood pressure (SBP) was significantly higher in the REMsd group compared to other groups. REMsd group showed impaired endothelium-dependent vasodilator responses to acetylcholine (ACh) compared to other groups. Malondialdehyde (MDA) levels were significantly increased, whereas superoxide dismutase (SOD) activity, levels of catalase (CAT) and total antioxidant capacity (TAC) were significantly decreased in REMsd compared to other groups. Endothelial damage was observed in the REMsd rat on scanning electron microscope (SEM). REM sleep deprivation induced endothelial dysfunction and increased SBP. Vitamin C supplementation is beneficial because it protects against the deleterious effects of REM sleep deprivation. Vitamin C may retard the development of endothelial dysfunction that may lead to CVD.

Keywords: *Sleep deprivation; endothelial dysfunction; oxidative stress and vitamin C*

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The prevalence of vulvovaginal candidiasis among Libyan women attending antenatal clinic at Tripoli Maternity Hospital, Tripoli-Libya

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Abstract:

Vulvovaginal candidiasis (VVC) is estimated to be the second most common cause of vaginitis in women after bacterial vaginitis. Estimates indicate that 75% of them may experience at least one vaginitis episode during their reproductive years and 40-50% will have recurrence (RVVC). This study aimed to determine the prevalence of *Candida albicans* among Libyan females attending antenatal clinic, and to compare the prevalence of VCC between pregnant and non-pregnant women, and also diabetic and non-diabetic women. This study was conducted at antenatal Clinic in Tripoli, Libya between Jan 1st - July 30th, 2020. A semi-structured questionnaire was administered and total of 211 women (18-59 year-old) selected. Most patients were below 40 years (mean age of 32.7± 7.8). 38.9% of the women were pregnant and 12.3% were diabetic. Our results showed that 80.6 % (n=170) of the infection were yeast infections while the remaining 19.4% were bacterial origin. As expected, 82.9% of the patients reported having vaginal discharge and 62.6 % reported itchiness. Among the 170 patients with yeast infection, majority (51.8%) were having *C. Albicans,* followed by 53.3% *C. glabrata,* and then 12.9% were *C. tropiclas.* The result showed a significant sassociation between pregnancy status, diabetic status and type of infection where pregnant women as well as diabetic women were prone to have more VCC (p=0.004 and p=0.007. respectively. Candidiasis with *C. albicans* was the most common among all age groups. Hence, Candida screening as neonatal follow up is advised to minimize unnecessary use of antibiotics.

Keywords: *Vaginal infection; Candida albicans; antenatal; diabetic and Libya.*

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From serology to genotype: updates in Immunohematology testing

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Abstract

Since the discovery of the ABO system, numerous important innovations have contributed to continuous, rapid evolution in the diagnostic methods for in vitro measurements of the antigen-antibody reaction, allowing a significant improvement in the compatibility between blood from donors and the recipients. Patient antigen typing is now routinely available as an adjunct to antibody identification. In addition, the availability of monoclonal antisera has allowed for routine phenotyping for patients and donors. This provides the potential to match red cell antigens for patients and donors. Genotyping with the prediction of phenotype is an extension of this strategy, allowing for antigen prediction and donor unit matching, even for patients who have been recently or transfusion dependent. The aim of this review is to illustrate the principles and practical applications of these emerging techniques used in our laboratory to identify antigens and antibodies, in cases of red cell or platelet immunisation. In conclusion, genotype testing has been a powerful tool for aiding Immunohematology testing in serological techniques.

Keywords: *Antigen typing; phenotype and genotype*

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Paternal exposures to fenitrothion caused histomorphometric changes in selected organs of Sprague Dawley rats' progeny

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Abstract

Paternal exposure to fenitrothion (FNT) in agriculture and public health has been reported to affect sperm quality including DNA damage; however, this DNA damage consequences on the histomorphometry of progeny are still unknown. This study aimed to evaluate the effects of paternally FNT administration on histomorphometry of progeny. Fertile male *Sprague-Dawley* rats were randomly divided into three groups (n=8/group): Control - receiving corn oil (1 ml/kg); FNT-10 and FNT-20 receiving 10 mg/kg and 20 mg/kg of FNT, respectively. Prior to mating with untreated female rats, FNT was administered via oral force feeding for 28 days. The rats were sacrificed after mating to collect sperm for assessing sperm characteristics and DNA damage. Meanwhile, the rat's progeny namely *p*Control, *p*FNT-10 and *p*FNT-20 were allowed to grow until postnatal day 70 before being sacrificed to obtain the matured organs for histomorphometric analysis. FNT-10 and FNT-20 significantly reduced sperm quality and induced DNA fragmentation in paternal rats ($p<0.05$). The number of Leydig cells, as well as the diameter in glomerulus of the renal and the seminiferous tubules in the testis, were significantly reduced ($p<0.05$) in the *p*FNT-20 group compared to the *p*Control group. When compared to the *p*FNT-10 and *p*Control groups, the *p*FNT-20 group's Bowman space was significantly increased ($p<0.05$). The sperm DNA damage due to FNT paternal exposure might pass to the progeny proven by histomorphometric changes in certain organs of progeny. Paternal exposure to FNT caused histomorphometrical changes in certain organs of the *Sprague-Dawley* rats' progeny.

Keywords: *Histomorphometry; infertility; organophosphate; progeny; reproductive toxicity and sperm DNA damage*

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Microarray approach for gene profiling of postoperative cognitive dysfunction

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Abstract

Condition of neurocognitive decline after surgery is also known as post-operative cognitive dysfunction (POCD). POCD may happen due to several risk factors either preoperative, intraoperative or postoperatively. This condition causes many disadvantages as it burdens healthcare system by prolonged hospitalization and rehabilitation, poor quality of life and may be life-threatening. Neuropsychological assessment of this condition is very important, yet up to date the assessment is solely based on clinical assessment. The diagnosis of POCD is still vague as studies regarding the occurrence and pathway involves are limited. Microarray analysis is a genome analysis that can be used for gene profiling for deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or microRNA. This technique is efficient, rapid and high-throughout. This application has been used to diagnose diseases especially in chromosomal abnormalities, mutations, genetic disorder and disease-relevant biomarkers. Microarray approach can be utilized to understand the mRNAs involved and its pathways in POCD development, unleashing its potential to be considered as a diagnostic marker for POCD. This study identifies and summarizes research that use microarray-based approaches for POCD analysis. Since the application of microarray in POCD is expanding, there is a need to review the current knowledge of this approach.

Keywords: *Cognitive decline; gene expression; microarray and RNA*

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Effects on sperm quality of male ICR mice fed *Annona muricata* leaves ethanol extract (AMLE)

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Abstract

The increase in population is a global concern and regulation of fertility may be required. Medicinal plants have the potential to curb fertility by reducing certain reproductive parameters with little or no side-effects. This study aimed to determine the anti-fertility effects of AMLE in male mice. Twenty sexually mature male mice (ICR strain) were divided into four groups; control (distilled water) while treatment groups given AMLE at dose levels of 100 mg/kg (low), 200 mg/kg (medium) and 300 mg/kg (high) respectively for 35 days or one spermatogenic cycle. All mice were euthanized on day 36 and cauda epidydimal sperm processed for sperm viability and morphology using Eosin-Nigrosin staining, while concentration measured using Makler Chamber. The results showed that sperm concentration of treatment groups, low, medium and high were significantly reduced at 79.71 ± 1.931, 58.32 ± 4.707 and 41.78 ± 2.925, respectively as compared to control 146.8 ± 2.577 ($p<0.05$). Sperm motility of treatment groups were also significantly reduced at 70.36 ± 4.079, 57.17 ± 3.194 and 26.73 ±2.731, respectively, as compared to control (91.92 ± 3.5920) ($p<0.05$). In addition, sperm viability percentage for treatment groups were also significantly reduced at 86.57 ± 2.932, 57.96 ± 1.896 and 42.10 ± 3.264, respectively, as compared to control (92.93 ± 2.024) ($p<0.05$). The significant reduction of sperm parameters were in a dose dependent manner. Treatment of AMLE to male mice for 35 days may have a detrimental effect on sperm parameters by reducing sperm concentration, motility and viability.

Keywords: *Anti-fertility; Annona muricata leaves ethanol extract (AMLE); sperm concentration; sperm Motility; sperm viability*

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Etlingera elatior improved the structural damage of diabetic kidney

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Abstract

Diabetes mellitus (DM) is a global public health concern that leads to multiple complications, including diabetic nephropathy. Diabetic nephropathy (DN) can lead to morbidity and mortality. Improving glycaemic control in DN may improve the damage to the kidney. Etlingera elatior flower (Bunga kantan) has been shown to have anti-hyperglycaemic properties. This study aimed to evaluate the effects of E. elatior flower aqueous extract (EEAE) on structural damage to the kidney in Type-2 DM (T2DM)-induced DN. Twenty-one male Sprague-Dawley (SD) rats were used in this study and divided into three groups equally: normal rats, untreated-T2DM-DN and T2DM-DN on 1000 mg/kg EEAE. T2DM was induced by a high-fat diet and low-dose streptozotocin (35 mg/kg). EEAE was orally gavage daily for 6 weeks. The presence of DN was confirmed by microalbuminuria in 24 hours urine sample. The kidney tissue was studied using Haematoxylin and eosin (H&E), Periodic acid Schiff (PAS) and Masson's trichrome (MT) staining. In untreated-T2DM-DN, the glomerular basement membrane thickened with a mild segmental increase of mesangial cells on H&E. Similarly, with PAS stain, glomerular sclerosis and tubular atrophy were demonstrated. In addition, there was collagen deposition in the glomerulus in the damaged untreated-T2DM-DN kidney on the MT stain. EEAE 1000 mg/kg for 6 weeks significantly improved these structurallydamaged. DN caused structurally damage in the diabetic kidney. Treatment with EEAE was demonstrated to revert the damage. EEAE can be used as an anti-diabetic agent and prevent the progression of DN.

Keywords: *Etlingera elatior; kidney, type-2 diabetes; nephropathy; histology staining*

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Vascular endothelium-dependent relaxation effects of Syzygium polyanthum

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Abstract

Hypertension independently increases the risk of CVDs. The pathophysiology of hypertension is closely related to endothelial dysfunction, which demonstrated as vascular hypersensitivity (hypercontractility). Syzygium polyanthum (Serai Kayu) has been shown to reduce systolic blood pressure SBP in the conscious and unconscious hypertensive rat (SHR). This study aimed to demonstrate in vitro endothelium-dependent relaxation of S. polyanthum leaves aqueous extract (AESP). The descending thoracic aorta was isolated from male Wistar-Kyoto (WKY) rats. The aorta was cut into 3 mm-length rings. The endothelium was left intact or mechanically denuded by rolling the intimal surface against a piece of roughened metal wire. The aortic rings were mounted on a 4-channel myograph, bathed in Krebs's Henseleit solution, and aerated with 95% O2/5% CO2 at 37°C. The resting tension was set at 2 gm. The artery was pre-contracted with 1 μM phenylephrine/Phe (EC60). MESP was then cumulatively added from 1 nM to 30 mM. In another set of experiments, 100 μM L-NAME (non-selective nitric oxide synthase (NOS) inhibitor) were pre-incubated for 30 minutes before Phe contraction. AESP full relaxed the aortic rings. In contrast, AESP did not relax the denuded aortic rings (<75% at 30 mM). In addition, L-NAME inhibits the relaxations of AESP on intact endothelium aortic rings. Endothelium produces endothelium-dependent relaxant factor (EDRF), including nitric oxide through NOS. Endothelium dysfunction leads to a reduction in EDRF, hence impaired vascular relaxation. L-NAME inhibit endogenous NOS, thus inhibit the relaxation by AESP. The vascular relaxation of AESP is endothelium dependent.

Keywords: *Sygyzium polyanthum; hypertension, endothelium; relaxation; myograph*

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Transposition of forensic age estimation methods onto computed tomographic images: review

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Abstract

Age estimation is an integral aspect of the role of the forensic anthropologist, both in the identification of skeletal remains and living individuals. When direct access to human skeletal repositories is not possible, imaging modalities act as an alternative source of contemporary skeletal data to answer the need for formulating population-specific standards. This review presents a literature relative to recommended adult age estimation methods, originally developed from direct inspection of physical bones, later applied in conventional radiographs, and now frequently evaluated in computed tomographic scans. It considers the reliability and accuracy of this method as transposed into a 'virtual anthropological' platform. Also considered is the overall utility of such an approach towards developing population-specific forensic age estimation standards.

Keywords: *Forensic age estimation; computed tomography; forensic anthropology*

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