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Evaluation of Heterozygous Hb E and its Interaction with Deletional Alpha Thalassaemia in Kelantan

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Purpose: Haemoglobin E (HbE) is a variant of structurally abnormal haemoglobin while alpha thalassaemia is reflected by the absence or decrease in production of alpha globin peptides. Here, we report the evaluation study on the haematological parameters using automated blood counters, morphology of red cells, Hb separation and quantitation of Hb fractions using CE and molecular analysis for alpha thalassaemia. The cut off point of HbE level in heterozygous HbE patients with concurrent deletional alpha thalassaemia by CE was also investigated. Methods: This cross sectional study involved 59 blood samples in EDTA tubes sent for investigation of anaemia, hypochromic microcytic red cells and family screening. Each sample was tested for full blood count, blood smear, CE and DNA analysis. Data were analysed using one way ANNOVA. Results: Total of 59 samples were observed with mean age of 18.76 (13.68) years old and majority were female patients (67.8%). Majority were malays (88.1%) while the rest were non-Malays (Chinese, Siamese and aborigines (Orang Asli)). 16 samples (27.1%) were HbE heterozygote with 3.7 gene deletion, 13 samples (22.0%) were HbE heterozygote with double gene deletion (South East Asian (SEA) type) and 1 sample (1.7%) was HbE heterozygote with compound heterozygous 4.2 and 3.7 gene deletion. The haematological parameters (RBC, Hb, MCV, MCH, RDW and HbE) were compared and showed no significant difference between the variables except for HbE (p <.001). Mean for HbE level in group HbE heterozygote with 3.7 gene deletion and double gene deletion SEA type is 21.42 (1.45) and 16.37 (0.74) respectively. Conclusion: Thus, molecular analysis are recommended to confirmed the variance in alpha thalassaemia and provides great help in the patients diagnosis and management.

Keywords: HbE, Alpha Thalassaemia, Capillary Electrophoresis, DNA

http://apps.amdi.usm.my/journal/
Haemoglobin G Makassar (Codon 6 GAG>GCG) Cases in Malaysia: Molecular Identification and Characterisation

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Purpose: Haemoglobin G Makassar was identified in Makassar, Sulawesi (Celebes), Indonesia in 1969 and has been reported in a family of Thai origin in 2002. Haemoglobin G Makassar was found to share identical properties with haemoglobin S in routine haemoglobin separation by cation-exchange HPLC. It is therefore, patients with Haemoglobin G Makassar and Haemoglobin S may sometimes be mistakenly identified for each other. The main focus of the study was to identify and characterize the Haemoglobin G Makassar. Methods: Five cases have been identified from 2015 to 2016 in Peninsular Malaysia by Molecular Genetics Laboratory, Institute for Medical Research. All patients were asymptomatic with mild hypochromic microcytic anaemia. Analysis by Capillary Electrophoresis showed that these patients had 39.9 to 44.0% of haemoglobin variant in zone S. Alpha and Beta globin gene analysis were performed on these samples. Results: DNA sequence analysis, revealed a single nucleotide substitution GAG to GCG at codon 6 of the beta-globin gene (Glu>Ala), indicating of Haemoglobin G Makassar. Multiple Amplification Refractory Mutation System (MARMS) PCR for Haemoglobin S was negative in all cases. However alpha-globin gene analysis showed that two of them had single alpha deletion (α3.7). Conclusion: The screening method may mistakenly identify Haemoglobin G Makassar as Haemoglobin S. Thus, identification and characterization of Haemoglobin G Makassar by several molecular methods is necessary for confirmation of the diagnosis.

Keywords: Haemoglobin G Makassar, Haemoglobin S, Molecular Identification, MARMS-PCR
β-Globin Gene Mutation in β-Thalassaemia and Haemoglobinopathies Patients in Hospital Universiti Sains Malaysia

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Purpose: β-thalassaemia and haemoglobinopathies are common genetic disorders worldwide. Each ethnic population has its own unique type of mutations. The aim of this study was to characterize type of β-globin gene mutation among β-thalassaemia and haemoglobinopathies patients in Hospital Universiti Sains Malaysia, Kelantan. Methods: A polymerase chain reaction (PCR) involving a multiplex amplification refractory mutation system (MARMS) was performed on 102 patients who was suspected with β-thalassaemia and/or haemoglobinopathies by high-performance liquid chromatography (HPLC) and haemoglobin (Hb) electrophoresis. Results: Twenty two (21.6%) were β-thalassaemia trait, 2 (2.0%) were β-thalassaemia/Hb E, 1 (1.0%) were β-thalassaemia/Hb Malay, 2 (2.0%) compound heterozygous Hb E/Hb Malay, 6 (5.9%) were homozygous Hb E, 40 (39.2%) were heterozygous Hb E, 4 (4%) were heterozygous Hb Malay while 25 (24.5%) patients were not detected with any β-globin gene mutation. Overall, 11 types of β-globin gene mutation were detected with the commonest mutation was codon 26 (G-A), followed by IVS-I-5 (G-C), codon 19 (A-G), codon 8/9 (+G), codon 41/42 (-TCTT), codon 17 (A-T), IVS I-1 (G-T), Poly A, IVS I-2 (T-C), -28 A-G and IVS 2-654 (C-T). Conclusion: The heterogeneity of the disease was observed among the patient’s population. Coupled with full blood count and haemoglobin analyses, Multiplex ARMS-PCR has permitted an accurate and straightforward β-thalassaemia genotyping for patients in Malaysia.

Keywords: Multiplex ARMS-PCR, β-globin Gene, Thalassaemia, Mutation
Detection of Beta Thalassaemia Mutations by Multiplex Amplification Refractory System and Gap-PCR

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Purpose: β-thalassaemia is characterized by a quantitative deficiency of β-globin chains production as consequences of heterogenous molecular defects. Good molecular laboratory services with the ability of simultaneously detection of common mutations are very important in a country like Malaysia where β-thalassaemia is common. This study aimed to optimize a simple and cost-effective molecular method for the detection of common β-thalassaemia globin chains mutations seen in Malaysian population. Methods: Four hundred and thirty-three peripheral blood samples of patients with raised Haemoglobin A2 (HbA2) (>3.5%) and/or Haemoglobin F (HbF) (>0.5%) were selected for molecular analysis. DNA extractions were subjected to Multiplex (ARMS and Gap) PCR analyses to detect 20 types of β-thalassaemia mutations and eight types of β-thalassaemia deletions. Results: A total of 494/866 alleles from 433 samples examined showed β-gene abnormalities by Multiplex (ARMS and Gap) PCR methods. Codon 26(G-A) was the most commonest mutation detected (46.2%), followed by IVS 1-5(G-C) (16.0%), Codon 41/42(-TTCT) (13.0%), IVS 2-654(C-T) (6.5%), IVS 1-1(G-T) (6.1%), Codon 19(A-G) (3.4%), -28(A-G) (2.0%), 45kb deletion (1.4%), Poly-A(A-G) (1.4%), Hb Lepore (1.2%), Codon 8/9(+G) (1.0%), Cap+1(A-C) (0.6%), δβ Siriraj I (0.4%), Codon 71/72(+A) (0.2%), -88(C-T) (0.2%), Initiation Codon (ATG-AGG) (0.2%) and δβ-Thai (0.2%). Malays showed highest rate with Cd 26 (HbE) whereas Cd41/42 was most prevalent among the Chinese. Conclusion: Multiplex ARMS-PCR and Gap-PCR methods proved to be useful in detecting β-thalassaemia with almost 96.1% (416/433) cases has been detected in our patient population in UKMMC.

Keywords: β-thalassaemia, Multiplex ARMS-PCR, Multiplex GAP-PCR
Detection of XMN1-Gγ Polymorphism Among Patients with HbE/Beta Thalassaemia in Northeast Malaysia

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**Purpose:** HbE/beta thalassaemia is widely known to have a diverse phenotypic spectrum despite having same primary genetic background leading to the unnecessary treatments. Present studies showed Xmn1-Gγ polymorphism at HBG2 promoter cause milder disease severity. However, in Malaysia, no study has been carried out to investigate the association between Xmn1-Gγ polymorphism and HbE/beta thalassaemia. Here, we report the use of Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique for detection of Xmn1-Gγ polymorphism, determine its frequency in HbE/beta thalassaemia patients and find its association with HbE/β thalassaemia severity. **Methods:** This hospital-based cross-sectional study was performed using 58 archived genomic DNAs with their respective research pro formas. Patients were classified into 3 disease severity groups using a scoring system. Genomic DNAs were genotyped employing PCR-RFLP technique. The genotypes were categorised into homozygous variant, heterozygous and homozygous wild type and validated by DNA sequencing. Appropriate statistical analysis were used to determine the association of Xmn1-Gγ polymorphism with disease severity. **Results:** The PCR-RFLP technique was successfully optimised. Majority of the subjects showed Xmn1-Gγ polymorphism (69%), where 66% (n=38) were heterozygous (CT) and 3% (n=2) homozygous variant (TT). Homozygous wild type (CC) were detected in 31% (n=18) subjects. No significant association of Xmn1-Gγ polymorphism with HbE/β thalassaemia severity were found for both genotype (p=0.65) and allele (p=0.58). **Conclusion:** We concluded that Xmn1-Gγ polymorphism is not associated with milder disease severity in HbE/β thalassaemia patients. This could be due to small sample size and possibly other genetic factors that interact with Xmn1-Gγ polymorphism. Future study with larger sample size and inclusion of other genetic factors is encouraged which may have potential for direct clinical application.

**Keywords:** Xmn1-Gγ, HbE, Thalassaemia, Beta
Genetic Modifiers of HbF and Phenotypic Severity Among Malaysian β-Thalassaemia Patients

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Purpose: Clinical manifestations of β-thalassaemia is portrayed by the types of β-globin gene mutations, as well as co-inheritance of α-thalassaemia and polymorphisms associated with HbF production. Mutational analysis of β and α globin genes are routinely analysed; however detection of polymorphisms associated with HbF is limited. Thus, we aimed to identify the polymorphisms and its association with disease severity using a clinical severity score and patients’ age of first transfusion. Methods: DNA samples from 100 β-thalassaemia patients were genotyped for 34 SNPs in HBB, HBS1L-MYB, BCL11A and olfactory receptor gene using the Agena MassARRAY®. HBG2:g.-158C>T was genotyped using Restriction Fragment Length Polymorphism PCR (RFLP-PCR). The genotyping results were analysed using the Hardy Weinberg (HWE) test to ensure that there was no genotyping error. The association between the polymorphisms and clinical severity was analysed using SPSS version 23. Results: All 35 types of polymorphism were detected. Statistical analysis using the severity score revealed that rs2210366 (HBS1L-MYB) and rs2071348 (HBBP) were identified as the most highly significant SNP (p<0.05). Whereas, association analysis using age of first transfusion showed that allele G from rs388623 (olfactory receptor) and allele C from rs4895441 (HBS1L-MYB) are significantly associated with a later age of blood transfusion (p<0.05). Conclusion: All 35 type of SNPs identified in our population may either increase or reduce the phenotypic severity of β-thalassaemia patients. These data may be used to further evaluate the severity of our β-thalassaemia patients, aiming for a better management of thalassaemia.

Keywords: β- thalassaemia, Modifiers, HbF, SNP
Genetic Variants in HBS1L-MYB Rs9399137 and Rs11759553 Associated with Elevated HbF Level Among Filipino β°-Deletion Carriers

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Purpose: Sabah population constitutes the most number of β-thalassaemia cases ranging from asymptomatic to transfusion dependent. Filipino β°-deletion has been reported as the predominant mutation in Sabah. Despite having the same primary mutation, co-inheritance of genetic variants at HbF quantitative trait loci of HBS1L-MYB intergenic region may cause variability in clinical features by affecting the haemoglobin (Hb) subtypes level. Elevation of HbF levels by HBS1L-MYB can act as an ameliorating factor in the clinical presentation of β-thalassaemia patients. This study aimed to elucidate the association of Hb subtypes levels with three HBS1L-MYB variants among 134 Filipino β°-deletion carriers.

Methods: PCR-RFLP analysis was done for HBS1L-MYB rs4895441 (A→G) while tetra-primers ARMS PCR analysis was done for HBS1L-MYB rs9399137 (T→C) and rs11759553 (A→T).

Results: Through the genotyping analysis, two HBS1L-MYB variants (rs9399137, MAF=0.18 and rs11759553, MAF=0.190) were found with significant minor allele frequency (MAF) which is greater than 0.05. HBS1L-MYB rs4895441 showed no influential effect on Hb subtypes level. However, rs9399137 and rs11759553 showed significant different in HbF level. HbF level was elevated when Filipino β°-deletion carriers co-inherited with HBS1L-MYB rs9399137 or rs11759553.

Conclusion: In conclusion, HBS1L-MYB rs9399137 and rs11759553 are significantly in elevating HbF levels which are not seen in rs4895441, making it a potent therapeutic target for gene therapy. The significant difference in Hb subtypes levels across the genotype variants had suggested the importance to include the detection of HBS1L-MYB rs9399137 and rs11759553 among Filipino β°-deletion patients in order to provide proper patient management.

Keywords: HBS1L-MYB Variants, Filipino β°-deletion
An Alternative Method for Rapid Detection of Alpha Thalassaemia Variants in Malaysia using Droplet Digital PCR

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Purpose: Globally, \(\alpha\)-thalassaemia is a highly prevalent disease. In Malaysia, this disorder is a well-known public health problem. The three most common deletional \(\alpha\)-thalassaemia found in this region include -- SEA deletion, \(-\alpha3.7\) and \(-\alpha4.2\) deletions. The prevalence rate of triplication alpha cases such as \(\alpha\alpha\alpha\)anti3.7 and \(\alpha\alpha\alpha\)anti4.2 is unknown in Malaysia although it plays a pivotal role in exacerbating the clinical phenotypes in beta thalassaemia carriers. Thus, the purpose of the study was to design an assay for the detection of triplications and common deletional alpha thalassaemia using droplet digital PCR (ddPCR).

Methods: Copy number changes were analysed using Quanta-Soft\textsuperscript{TM} software version 1.6.6 after performing ddPCR. Sensitivity and validation analysis were also performed on the DNA samples. Results: The changes in copy number changes (common deletions, duplications and triplications) in the alpha globin gene has been quantitatively detected using ddPCR. For the samples validation as determined by ddPCR, the mean copy number values for \(\alpha\alpha/\alpha\alpha\) are 2.0275±0.0177 (HS-40), 1.8175±0.0389 (HbA\textsubscript{2}), 2.0450±0.0848 (Hb 3.7), 2.0050±0.0000 (HbA\textsubscript{1}). For \(-\alpha3.7~/--\text{SEA}, the mean copy number values are 2.0225±0.2180 (HS-40), 0.9325±0.1213 (HbA\textsubscript{2}), 0 (Hb 3.7), 0.9984±0.1333 (HbA\textsubscript{1}). As for \(-\alpha4.2~/--\text{SEA}, the mean copy number values are 1.9350 (HS-40), 0 (HbA\textsubscript{2}), 0.7945 (Hb 3.7), 0.8480 (HbA\textsubscript{1}). The mean copy number values for --SEA/\(\alpha\alpha\) samples are 1.9067±0.1327 (HS-40), 0.8164±0.0364 (HbA\textsubscript{2}), 0.8920±0.0434 (Hb 3.7), 0.9148±0.0338 (HbA\textsubscript{1}) respectively. Conclusion: In a nutshell, ddPCR showed to be an alternative method that can be employed for rapid detection of alpha thalassaemia variants in Malaysia.

Keywords: ddPCR, Alpha Thalassaemia, Rapid Detection
Haematological Parameter Evaluation in Different Types of Deletional Alpha-Thalassaemia in Hospital USM

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**Purpose:** To evaluate the correlation of haematological parameters (Hb,RBC,MCV,MCH,RDW) with various types of deletional α-thalassaemia among patients in Hospital USM. **Methods:** A retrospective study on 211 samples sent to Molecular Unit, Department of Haematology, Hospital USM. Permission from Director of Hospital USM was obtained. The data was analysed to look for correlation of red cell indices (Hb, RBC, MCV, MCH, RDW) and platelet level with the presence of various types of deletional α-thalassaemia. **Results:** There were significant differences of Hb (p = 0.002), MCV, MCH and RDW levels (p<0.001) in patients with various types of deletional α-thalassaemia groups. Patients with one-gene deletion showed normal to mild anaemia (12.4±2.5g/dL) while two-genes deletion patients showed mild anaemia (11.3±1.5g/dL). The MCV and MCH values were lower in two-genes deletion (64.4±10.5fL and 20.3±2.4pg respectively) compared to one-gene deletion (73.3±11.1fL and 24.2±3.5pg respectively). While RDW level was higher in two-genes deletion compared to one-gene deletion (16.5±3.4% and 14.2±2.4% respectively). **Conclusion:** There is correlation between severities of anaemia, microcytosis, and degree of anisocytosis with number of genes deletion in α-thalassaemia. These data support the necessity using various haematological parameters as a screening measure before proceed for the molecular study in detecting α-thalassaemia deletion in developing countries.

**Keywords:** α-thalassaemia, Haematological Parameters, Screening
Endocrine Complications of Beta Thalassaemia Major Patients: Cross Sectional Survey

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Purpose: Endocrine complications act as a major cause for high mortality and morbidity in beta thalassaemia major patients in developing countries. The objective of this study was to evaluate the prevalence of endocrine complications in beta thalassaemia major patients in one of the thalassaemia treatment unit in Sri Lanka. Methods: The patients (n=40) aged >2 years admitted to the Teaching Hospital Peradeniya during the period of December, 2013 to December, 2014, under the treatment of iron chelation drugs were recruited for the study. The patients were interviewed for the socio-demographic variables and the data regarding endocrine complications were gathered from medical records. Anthropometric (Height and weight) measurers and pubertal status were assessed by qualified medical officer. The data were statistically analysed by SPSS version 21. Results: The mean age of the patients was 10.97±5.9 years (range 2-20). The median age at the first transfusion, the mean transfusion requirement, mean pre transfusion haemoglobin concentration, mean deferasirox dose and mean ferritin concentration of the patients in the study group were 6 months, 265.01ml/Kg/year, 8.15±1.21g/dl, 22.5± 4.5 mg/kg/day and 2992.2±1575.35ng/ml respectively. The most common endocrine complication was pubertal delay (66.7%). The prevalence of short stature, hypothyroidism, diabetes mellitus and hypoparathyroidism were 50%, 10%, 5% and 2.5% respectively. All the patients with endocrine complications were older children (>10 year old). Conclusion: Endocrine complications were common among the studied group of beta thalassaemia major patients and it is related with progression of age. Regular assessment of endocrine function is imperative in proper management of beta thalassaemia major patients.

Keywords: Endocrine Complications, Beta Thalassaemia Major
Concomitant Inheritance of Alpha Thalassaemia in Severe Beta Thalassaemia Intermedia: A Case Report

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Purpose: Co-inheritance of α-thalassaemia with β-thalassaemia is known to ameliorate thalassaemia clinical phenotype by reducing the number of unmatched α-globin chains, leading to a more balanced α- to β-globin ratio. A considerable number of patients present later in life due to this factor. Methods: We report a case of a Malay boy who was diagnosed as β-thalassaemia at two years of age, from a family screening. Case report: His haemoglobin (Hb) at presentation was 66g/L with a mean cell haemoglobin of 24.0 pg and a mean cell volume of 76.6 fl. His peripheral blood smear showed hypochromic microcytic red, severe anisopoikilocytosis, numerous target and pencil-shaped red cells. Quantitation of haemoglobins by high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) revealed raised Hb F and Hb A₂ levels. DNA analyses by multiplex Gap polymerase chain reaction, multiplex amplification refractory mutation system (MARMS) and DNA sequencing revealed co-inheritance of α°-thalassaemia (--SEA /αα) with compound heterozygous β⁺/β+-thalassaemia (βCd41/42/βPoly A). His younger brother also carried the same βCd41/42/βPoly A mutations but lacked the --SEA deletion leading to β-thalassaemia major clinical phenotype. Both his father and sister were β-thalassaemia trait (β/βPoly A) with concomittant α°-thalassaemia (--SEA /αα) while his mother was β-thalassaemia trait (β/βCd41/42). Initially, his transfusion requirement was only once in every three to four months but recently it was increased to once in every two months. Conclusion: In summary, this case highlighted that co-inheritance α°-thalassaemia in compound heterozygous β-thalassaemia may also present as a severe clinical phenotype despite the potential amelioration by two α-globin gene deletion.

Keywords: α, β, Thalassaemia Intermedia
Increased Ferritin Level Correlated with MAIT Cells Population in Pediatric Major β-Thalassaemia Patients

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Purpose: In the state of hyperferritinemia, the risk of infection is raising due to immune system interference. Mucosa-associated invariant T cell (MAIT cell) is a unique type of lymphocyte cells, involved in body defense against bacteria. This study aims to reveal the MAIT cells profile in the increased ferritin level of pediatric major β-thalassaemia patients.

Methods: Forty two pediatric β-thalassaemia patients routinely underwent blood transfusion in thalassaemia clinic were recruited in this cross-sectional study. The monoclonal antibody of CD3, Vα7.2, and CD161 were used in flowcytometry to identify CD3\textsuperscript{+}, Vα7.2\textsuperscript{+}, CD161++ lymphocyte as MAIT-cell from subject’s lysed-erythrocyte heparinized whole blood. Cell percentage and median fluorescent intensity (MFI) of Vα7.2 and CD161 of CD3\textsuperscript{+},Vα7.2\textsuperscript{+},CD161++ were measured. The TIBC, ferritin and serum iron level were examined as iron status. A correlation analysis was evaluated. Results: All thalassaemia patients (100%) had high ferritin level, ranging from 653,7-9718 ng/mL. Increase ferritin level in thalassaemia patients was positively correlated with MAIT cells population (r = 0,345; P = 0,025). Conclusion: This study showed that increased ferritin level may affect the population of MAIT cells.

Keywords: Ferritin; MAIT-cell; Thalassaemia
Effect of Transfusion, Iron Chelation and Splenectomy Therapies in HbE/β-Thalassaemia Individuals in Malaysia

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Purpose: HbE/β-thalassaemia is a compound heterozygous mutation with a vast clinical phenotype. To improve quality of life, HbE/β-thalassaemia individuals receive different treatment strategies, either individually or in combination with therapies, including transfusion, iron chelation and splenectomy. Thus far, there are limited studies conducted regarding the effect of treatments in HbE/β-thalassaemia individuals.

Methods: We hereby investigated the effect of treatments with respect to red blood cell indices, haemoglobin subtypes and gene expressions among 30 HbE/β-thalassaemia individuals.

Results: As compared to single therapy (transfused only individuals) and double therapies (transfused-chelated only individuals), individuals receiving triple therapies (transfused-chelated-splenectomised individuals) showed significantly high mean cell volume (MCV), mean cell haemoglobin (MCH) and reticulocytes count. These findings suggested that triple therapies was the most effective in ameliorating the severity of the disease in terms of microcytosis and hypochromia. The high reticulocyte count in triple therapies also allows the bone marrow to actively produce red blood cells suggesting that these therapies have clinical benefits by suppressing the ineffective erythropoiesis and improving the erythropoietic environment significantly among HbE/β-thalassaemia individuals in our studied group.

Conclusion: The effectiveness of these treatments were different among each HbE/β-thalassaemia individual whereby clinical variabilities among them could be a contributing factor.

Keywords: Transfusion, Iron Chelation, Splenectomy, HbE/β-thalassaemia
A Study on Haemoglobin Analysis and Factors Associated with the Sending of Molecular Analysis for Confirmation of Diagnoses in Penang, Malaysia

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**Purpose:** For accurate genetic counselling, molecular analysis may be required to provide a definitive thalassaemia diagnosis (e.g. in $\alpha$-thalassaemia, or $\beta$-thalassaemia with borderline HbA\textsubscript{2}). Failure to define carrier status accurately may be one obstacle to an effective control programme. The objective of this study was to evaluate the effectiveness of communication between laboratory and clinicians, particularly with respect to sending repeat samples for molecular analysis. **Methods:** This retrospective study reviewed all requests for haemoglobinopathy screening from government healthcare institutions in Penang state, between January and June 2015. We determined whether samples for molecular analysis were sent (within the following 12 months) when this had been suggested on the initial report. **Results:** A total of 1028 haemoglobinopathy reports were issued with the request to send a further sample for molecular analysis for diagnosis confirmation. However, such samples were received from only 385 cases (37%). The rate of sending samples for molecular analysis was positively associated with patients being: of reproductive age; female; Chinese; or pregnant. It was not associated with the district of origin of the sample. **Conclusion:** This study shows that many cases of potentially important haemoglobinopathy are not being confirmed by molecular analysis. Healthcare providers appear to target patients from important groups such as females of reproductive age, while perhaps neglecting males from the same age group. However, the exact reasons for 63% failing to have follow-up samples for DNA analysis should be studied further before attempting to devise effective strategies and educational programmes to remedy this situation.

**Keywords:** Haemoglobinopathy Screening
Case Series of Homozygous and Compound Heterozygosity of Hb Malay, its Diagnostic Features and Transfusion Requirements

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Purpose: Hb Malay was first described in 1989 following an investigation of a 22-year-old Malay gentleman, who was anaemic and found to be homozygous for this β chain variant. The mutation occurs at codon 19 (AAC→AGC) resulting in the substitution of serine for asparagine. This study aimed to describe the diagnostic findings of homozygous Hb Malay and its combinations with other thalassaemia/haemoglobinopathy and to determine the transfusion requirement in homozygous and compound heterozygous Hb Malay. Methods: A retrospective analysis was carried out at Hospital Ampang on thalassaemia and haemoglobinopathies database from 2012 to 2015. A total of 12 cases of confirmed heterozygous, homozygous or compound heterozygous of Hb Malay was collected. The diagnostic workups in this centre include CBC, blood smear, haemoglobin analysis and molecular study for confirmation of diagnosis. Their clinical presentations and transfusion requirement were also reviewed and compared. Results: There were two cases of homozygous Hb Malay with an average haemoglobin of 7.6g/dL, characterised by raised HbF level of 47% and 30% respectively. In heterozygous Hb Malay, Hb A₂ level was raised at 4.8%, which is similar to the classical heterozygous beta thalassaemia findings. Clinically, homozygous Hb Malay, does not require regular transfusions and in heterozygous state they are usually asymptomatic. Whereas, compound heterozygous of Hb Malay with beta thalassaemia, Hb S or HPFH presented as thalassaemia intermedia or major depending on the type of mutation and clinical phenotype. Conclusion: Hb Malay may be under-reported in Malaysia as the definitive diagnosis is by DNA analysis as it is indistinguishable from Hb A in haemoglobin analysis. The clinical phenotypes are heterogeneous and in homozygous state they are usually non-transfusion dependent.

Keywords: Hb Malay, Beta Variant
Exploring The Willingness for Carrier Screening Among Extended Family Members of a Thalassaemia Carrier Individual: A Lesson Learned

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Purpose: Although Indonesia harbors 6-10% thalassaemia carriers, screening of thalassaemia carrier is not mandatory. Carrier screening is simultaneously introduced among various community groups, especially in a ‘direct-related family’ of thalassaemia patients. This study aimed to explore the willingness for carrier screening in ‘extended family’ members of a thalassaemia carrier individual, both from father and mother sides.

Methods: Thalassaemia carrier individual was identified during regular medical check-up prior student admission into university. The family was approached in their family gathering, thalassaemia story was shared and a family tree was drawn to identify thalassaemia patients within the family. Thalassaemia screening was offered to all family members, especially to the unmarried children, above 15 years old. Results: The family tree identified a suspected thalassaemia major patient who died 30 years ago; at the age of 5 years old. None of the ‘direct-related’ and ‘extended’ family members knew about the disease. The thalassaemia patient had 2 sisters of whom later migrated to Australia and admitted that she was carrier, detected during her first pregnancy. All her three children were also carriers, detected during a regular medical check-up. The other sister in Indonesia didn’t know about the disease and both sisters never discussed about this disease. Haematology result of unmarried family members (n=16) found 3 boys had normal Hb; and 3 girls were anemic, all with MCH<20pg and HbA₂>3.5%, indicating β-thalassaemia carriers. Conclusion: Campaign and education for community carrier screening, especially among ‘extended’ families of carrier individuals, need to be increased targeting for zero thalassaemia. Further DNA analysis would be important to explore mutation type.

Keywords: Carrier Screening, Extended Family, Indonesia
Co-Inheritance of Southeast Asian Ovalocytosis and Hb E: Does It Affect Red Blood Cells Indices?

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Purpose: The complete blood count (CBC) is used broadly to screen individual's general health status. Some of the inherited red blood cell (RBC) disorders do have influenced on RBC parameters. Mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) are among the important RBC parameters used in thalassaemia-haemoglobinopathy screening. Globin chain disorders and Southeast Asian Ovalocytosis (SAO) are common RBC disorders in Southeast Asian countries. The aim of this study was to evaluate the RBC parameters in patients with co-inheritance of those disorders. Methods: A total of 33 patients’ samples sent for thalassaemia screening to Hospital Kuala Lumpur (HKL) were randomly selected. Their blood samples were subjected for full blood count (FBC), full smear, haemoglobin (Hb) analysis, and serum ferritin. DNA analysis was performed for respondents with co-inheritance of HbE and SAO (presence of stomatocytes in blood film). Results: Three respondents were confirmed to have co-inheritance of SAO and HbE trait. Their means RBC count, Hb, MCV, MCH, MCHC and RDW were 4.5 x10⁶/µL, 10.33g/dL, 66.33fl, 22.5pg, 33.96 g/dL, and 18.3%, respectively. The mean MCV and RDW were significantly lower in co-inheritance cases as compared to respondents with only HbE trait alone. Conclusion: Co-inheritance of SAO and HbE in Malay population reduces the MCV values, however it does not significantly give an effect on the cut-off value of parameters that are fundamental for screening haemoglobinopathies.

Keywords: Southeast Asian Ovalocytosis, Hb E
A Cross Sectional Assessment of Health-Related Quality of Life Among Patients with Thalassaemia in Malaysia

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Purpose: The study aims to assess the Health Related Quality of Life (HRQoL) among thalassaemia patients and identify the significant factors that contribute to HRQoL in thalassaemia patients in Malaysia. Methods: A cross sectional study was conducted at Kedah Thalassaemia Society Club in Kedah, Malaysia. The HRQoL was measured using a Short form survey version 2 (SF-36). Descriptive study was used to describe the demographic and disease related to the thalassaemia patients. The HRQoL was compared using the Mann-Whitney and Kruskal-Wallis test. The analyses were performed using the Quality Metric Health Outcomes Scoring software for SF-36 and SPSS v 22. Results: Three hundred and ninety thalassaemia patients were enrolled in the study. Majority of the participants (n = 221, 58.5%) were categorized in the age group of 18-27 years (25.40 ± 10.2). The HRQoL measure of less than 50 for the physical component summary (PCS) and mental component summary (MCS) among thalassaemia patients were rated as poor. Patients with higher education levels were significantly associated with PCS (p=0.002) and showed higher mean scores for PCS (52.0) compared to the others. Age, marital status, employment status, monthly income, health check-ups before screening of thalassaemia and medical insurance was associated with PCS levels compare to the others. Thalassaemia type, medical treatment received and side effects of the conventional treatment were significantly associated with p-values of less than 0.001 and PCS and MCS scores of below 50. Conclusion: This study identified many demographic and disease related factors which may contribute to the HRQoL of thalassaemia patients. The life quality assessment is necessary to improve understanding of the impact on thalassaemia patients and allow them to get the necessary support.

Keywords: HRQoL, Thalassaemia, SF-36, Malaysia
Globin Gene Defects in Normal and Borderline HbA2 Levels: An Institute For Medical Research (IMR) Experience

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Purpose: HbA2 level is an important parameter in Thalassaemia diagnosis. High HbA2 level detected with Hb analysis, points to the diagnosis of beta thalassaemia and other haemoglobinopathies. However, in some cases, the HbA2 levels are apparently normal or borderline high despite abnormal haematological profile. In these cases, further testing is required to confirm the diagnosis. The aim of this study is to determine any abnormality at molecular level in these cases. Methods: In the year 2015, 1890 samples were sent to Thalassaemia Molecular Genetics Lab, IMR for DNA analysis. Out of all samples, 299 had a normal and borderline HbA2 levels (3.3-3.9). The haematological parameters (haemoglobin, MCV, MCH, RDW and platelets) were analysed. Subsequently, multiple molecular techniques which included but not limited to beta-MARMS, beta-MGAP, alpha-MARMS, alpha-MGAP, beta-MLPA and beta-sequencing were employed for detection of any gene defects. All data were tabulated and analysed using Microsoft Excel. Results: Out of the samples analysed, 11% (33/299) has no globin gene defects. A majority of samples (58%; 174/299) has β gene defects, and α gene defects were detected in 15 cases. The remaining 77 samples (26%) were detected to have co-existence of α and β gene defects. From the 222 cases with gene defects, 31% (69 cases) has normal HbA2 level (3.3-3.5). Conclusion: This study showed that there were several cases with normal or borderline HbA2 levels but present of has globin gene defects. Hence we concluded that HbA2 range value which is indicated for further molecular testing should be revised.

Keywords: Thalassaemia, Globin Gene Defect, HbA2
CD16 Expression of Neutrophil Associated with Higher Iron Status of Paediatric β-Thalassaemia Major

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Purpose: Activation of neutrophils, vital innate immune cells widely contributed in battling pathogen invasion, may mediate excessive inflammation and damaged the surrounding tissues. Regular blood transfusion as a lifetime therapy for β-thalassaemia patients yet make a new issue by disrupting their immune system through iron overload condition. Present investigation found an association between activated neutrophils based on FcγRIII (CD16) expression and iron status of pediatric β-thalassaemia major patients. Methods: A cross sectional study involving lysed-erythrocyte in heparinized whole blood of 50 pediatric major β-thalassaemia patients treated with monoclonal antibodies i.e. CD16, CD14, and HLA-DR, applied to be dissected into CD16+ and CD16++ neutrophil population using flow cytometry. Expression of CD16 was measured as Median Fluorescent Intensity (MFI). Haematology and iron status were measured. A correlation study was done. Results: Positive correlation was found, \((r = 0.4, P = 0.007)\), between MFI of CD16 of neutrophil \([509.5 (371 – 796.5)]\) and ferritin level \([3209 \mu g/L, 1862 – 4564]\). Segmented neutrophils were found negatively correlated respectively with ferritin and serum iron \((r = -0.3, P = 0.02; r = -0.3, P = 0.02)\). Conclusion: Change in CD16 expression may implicate preliminarily neutrophil activation as a response of iron overloaded tissue and resulted in chronic inflammation in paediatric β-thalassaemia major patients. However, maturity of this cell may be altered. Future study in understanding of neutrophil-mediated inflammation, particularly related with immune complexes and functionality, is imperative to be explored.

Keywords: CD16, Neutrophil, Iron, β-thalassaemia
Molecular Identification of a Rare Haemoglobin Variant: Hb G Coushatta in Malaysia

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**Purpose:** Thalassaemia screening programme was conducted to reduce the burden of the disease. Here, we describe one unexpected discovery in a 33-year-old gentleman and also the importance of DNA analysis in detecting the globin gene mutation. **Case report:** Patient was screened for haemoglobin (Hb) variant after his wife was noted to have beta thalassaemia trait during her antenatal checkup. Otherwise, he was asymptomatic. He had normal Hb (16.09 g/L), increased red blood cell (RBC) count (5.91x10⁶/μL) with a borderline mean corpuscular volume (80.7 fl) and low mean corpuscular haemoglobin (27.1 pg). His RBCs appeared hypochromic microcytic. A prominent band was seen at the S region on Hb electrophoresis (alkaline), which was not showed in the high performance liquid chromatography. Instead, there was a significant increase in Hb A₂/E (42.7%), a great reduction in Hb A (45.6%) and normal Hb F value (0.3%). In capillary electrophoresis, an abnormal peak was observed in Hb D zone (40.8%) with normal Hb A₂ (2.6%) The screening methods would indicate Hb E, Hb D or Hb S. But none of these were shown by at least two of the methods. Therefore, beta-globin gene sequencing was carried out, which revealed Hb G Coushatta mutation [β22(B4)(GAA→GCA)]. **Conclusion:** Hb analysis may be useful in quantifying the Hb variant. However, definitive diagnosis by molecular analysis is required for identifying the rare mutation like Hb G Coushatta. Though the variant carries no significance in clinical manifestations, it is still important to identify as it can be passed on to the next generation and may have evolve to different haplotypes.

**Keywords:** Haemoglobin Variant, Hb G Coushatta, Molecular Analysis.
The Preliminary Study on The Prevalence and Demography of Alpha- and Beta- Transfusion Dependant Thalassaemia Encountered in Sarawak General Hospital Blood Bank from 2014 to 2015

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Purpose: Thalassaemias are genetic disorder due to reduced production of one or more of Alpha (α)- and Beta (β) - globin chains; phenotypically they manifest as mild-to-severe form of disease. The severe form of thalassaemia requires blood transfusion throughout their lifetime. Thalassaemias are commonly found amongst countries worldwide, including Malaysia; which consist of Peninsular and Borneo region. Although there are epidemiological studies on those thalassaemia forms for peninsular Malaysia, local study in the borneo state of Sarawak is still lacking. The objective of this study was to identify prevalence of both Alpha- and Beta- transfusion dependent thalassaemias (TDTs) cases encountered at Sarawak General Hospital (SGH) that occurred from 2014 to 2015. Following that: to characterise the profile of those cases including gender, race and phenotype. Methods: In this study, the 2 years (2014 to 2015) data of thalassaemias were extracted using extraction form and matched to the Laboratory Information System (LIS) in the Blood bank Unit of the SGH. Results: From a total of 568 possibly thalassaemia cases there were 309 cases possibly-TDTs and 53.1% (164/309) were TDT. Amongst those TDTs case, Alpha-Thalassaemia (271 cases) was more common than Beta-Thalassaemia (38 cases). Most of those TDT cases were female, Chinese, blood group O, Rhesus R1R1 and age within 31 to 40 years old. Conclusion: This study described the general profile of TDTs in Sarawak. Findings of this study will be useful as baseline to further investigate on more detail aspect of TDTs.

Keywords: Alpha- and Beta- Transfusion Dependent Thalassaemias (TDTs)
Isolation of Peripheral Blood Nucleated Red Blood Cells from β-Thalassaemia Patients Using CD71 Magnetic Activated Cell Sorting

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Purpose: The molecular biology knowledge in β-thalassaemia is limited due to the involvement of various erythropoietic processes where the genetic information is lack due to nucleus ejection throughout the maturation of red blood cell activities. Nucleated red blood cells (NRBCs) are typically found in peripheral blood (PB) of β-thalassaemia transfusion dependent patients and abundant in post splenectomy. The presence of NRBCs will provide further understanding on the molecular aspect of ineffective erythropoiesis in β-thalassaemia patients. Therefore, the objectives of this study were to isolate the NRBCs using CD71 magnetic beads from PB of β-thalassaemia patients and to compare the quantity of NRBCs enriched between non-splenectomised transfusion dependent and post-splenectomised β-thalassaemia patients. Methods: NRBCs were isolated from 6mL PB of both groups after the isolation of mononuclear cells (MNCs) based on density gradient. Magnetic activated cell sorting (MACs) with anti-CD71 was used to enrich isolated NRBCs. Cell count for NRBCs positive for CD71 was determined by trypan blue using haemocytometer (Weber,UK) and flow cytometry analysis (Becton-Dickson,USA) was performed for method validation. Results: NRBCs were successfully isolated from the peripheral blood of 15 non-splenectomised transfusion dependent and 7 post-splenectomised β-thalassaemia patients with >90% specificity. The median number of NRBCs (×10⁴) enriched were 58.5 (283) in non-splenectomised transfusion dependent and 340 (338) in post-splenectomised β-thalassaemia patients. Higher NRBCs were isolated from the post-splenectomised compared with non-splenectomised patients (P = 0.012). Conclusion: These findings suggest that MACs method is convenient, simple, and can be efficiently applied in isolating NRBCs from both non-splenectomised and post-splenectomised β-thalassaemia patients.

Keywords: NRBCs, CD71, MACs, β-thalassaemia
Assessing Knowledge about Thalassaemia among Reproductive Age Population after Video Media Education

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Purpose: Knowledge on thalassaemia becomes an important concern for Indonesian people, especially West Java, as the region has the highest prevalence of thalassaemia. One effort to convey the information can be through health education program such as using a video media. This research aimed to determine the knowledge improvement on thalassaemia after education intervention using video media among the reproductive age population.

Methods: This study was a quasi-experimental design. Data collection tool was a self-administered questionnaire that included 20 items. The subjects in the study consisted of 56 students at high school in Jatinangor sub-district, West Java, Indonesia. The study was conducted from April to May 2017. The subjects were selected by convenience non-probability sampling method. The instrument used for data collection in this research was validated questionnaire, given before and after video intervention, and two weeks later. Data analysed using statistical tests ANOVA and level of significance was considered on α < 0.05. Results: A total of 56 students participated in this research and 14 did not meet the inclusion criteria. The research findings showed the score of knowledge in the pre-test ranged from 8 – 15 (mean 11.53, SD 1.968). The average score after the intervention with video media was significantly increased (mean 14.95, SD 1.463). Subsequently two weeks later, second post-test scores were slightly decreased indicating reduced knowledge retention (mean 13.98, SD 1.933). There was a significant statistical relationship between video intervention and increasing knowledge (p <0.001). Conclusion: Knowledge about thalassaemia and its preventive measures is adequate using video media delivery. This intervention is an optional method for public health education programs concentrating on high risk/targeted population.

Keywords: Thalassaemia, Video Media Education, Reproductive Age, Prevention

http://apps.amdi.usm.my/journal/
Association between Oxidative Stress Level and β globin Gene Mutations in β-Thalassaemia Patients at Dr. Hasan Sadikin Bandung General Hospital

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**Purpose:** Ineffective erythropoiesis and multiple blood transfusions caused iron overload in β-thalassaemia patients and increased in oxidative stress. The oxidative stress occurs if amount of Reactive Oxigen Species (ROS) higher than antioxidant. The superoxide dismutase (SOD) and glutathione peroxidase (GPx) are the intracellular enzymatic antioxidants, and their activities indicate oxidative stress level in β thalassaemia patients. The common mutations of β-globin in Thalassaemia patients at Hasan Sadikin Hospital consist of IVS1nt5 homozygote (β₀) and compound heterozygote IVS1 nt5/HbE (β⁺). The aim was to explore the oxidative stress level by measuring SOD and GPx activities in β- thalassaemia patients.

**Methods:** Blood was collected from patients with homozygous IVS1nt5 and IVS1nt5/HbE mutation, recruited from Thalassaemia Clinic at Dr. Hasan Sadikin General Hospital, Bandung. SOD and GPx activity was measured using spectrophotometry method and compared between homozygous IVS1nt5 and IVS1nt5/HbE. Kruskal-Wallis analysis was conducted to assess association between both enzymatic antioxidants and the type of β thalassaemia mutations.

**Results:** Forty five patients with homozygous IVS1nt5, age ranges between 1 -18 years showed SOD activity 167.43 U/l and GPx activity 355 U/l, whereas 13 patients with IVS1nt5/HbE, age ranges between 2-26 years, showed 163.28 U/l and 482.12 U/l, respectively. No significant difference between the two groups (p<0.05).

**Conclusion:** Oxidative stress level tends not to be associated with the types of β thalassaemia mutations in this study.

**Keywords:** β-thalassaemia, IVS1nt5/HbE, SOD, GPx, Oxidative Stress
Is Transfusion Dependent Beta Thalassaemia is More Severe than HbE Beta Thalassaemia?

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Purpose: Transfusion dependent thalassaemia patients are defined as patients who require lifelong regular blood transfusion. In Malaysia, the most common types of thalassaemia are beta thalassaemia and HbE beta thalassaemia with estimated carrier rate 4.5% for beta thalassaemia and 3-40% for HbE. Hence, the aim of this study was to identify the clinical disease presentation of transfusion dependent beta thalassaemia syndrome in Malaysia.

Methods: About 388 transfusion dependent thalassaemia patients were recruited in this study. The parameters retrieved from patients’ folder were clinical diagnosis, haemoglobin level (g/dl), growth chart assessment, age of disease presentation (years), age receiving blood transfusion (years) and spleen size (cm). The scoring system was defined and Pearson Chi-square test was used for analysis. P-value <0.05 was considered as statistically significant.

Results: Out of 388 patients, 234 (61.1%) and 151 (38.9%) patients were classified as beta thalassaemia and HbE beta thalassaemia respectively. 27% of them have spleen size less than 3 cm. About 46% and 49% patients presented at age less than 2 years old and have stunted growth respectively. Only spleen size and height were significantly different between transfusion dependent beta thalassaemia and HbE beta thalassaemia (p-value <0.05).

Conclusion: Transfusion dependent beta thalassaemia syndromes are heterogeneous in their clinical manifestations. The disease is more severe in beta thalassaemia as compared to HbE beta thalassaemia. The underlying mechanisms explaining the differences need to be elucidated.

Keywords: Beta Thalassaemia, HbE Beta Thalassaemia, Scoring, Transfusion Dependent
Stunted Growth of Transfusion Dependent β-Thalassaemia Patients and the Association with TaqI SNP of VDR Gene and Vitamin D Levels

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**Purpose:** Vitamin D receptor (VDR) gene has been suggested as one of the candidate genes to control bone mass. TaqI Single Nucleotide Polymorphisms (SNPs) of VDR gene has been reported responsible for stunted growth phenotype. Objective: This study was conducted to determine the association of the TaqI SNP of the VDR gene and serum Vitamin D level with growth development among transfusion dependent β thalassaemia patients. **Methods:** Transfusion dependent β thalassaemia patients were categorized into 2 groups; stunted and non-stunted growth groups based on height percentile on growth chart assessment. Subsequently, 25-hydroxy-Vitamin D was measured and TaqI polymorphism was detected using Restriction Fragment Length Polymorphism-Polymerase Chain Reaction (RFLP-PCR). The association of vitamin D levels and the genotypes of TaqI VDR gene were determined against the growth chart and by statistical analysis using SPSS version 22. **Results:** Out of 33 samples analysed, genotype distribution of TT and Tt were 24.2%(8) and 36.4%(12) in stunted growth group and 36.4%(12) and 3%(1) in non-stunted group respectively. A significant higher frequency of heterozygous TaqI was observed in the stunted growth of thalassaemia patients. However no significant association between growth development and vitamin D levels. **Conclusion:** A significant association might be due to functional SNPs in the vitamin D receptor (VDR) gene which could be responsible for up to 60.6 % of stunted growth cases in this β thalassaemia cohort. Despite not statistically significant, low vitamin D is more prevalent in stunted group which may be attributed to defective 25 hydroxylation of Vitamin D in the liver from iron overload and subsequent liver dysfunction.

**Keywords:** Stunted Growth Thalassaemia, TaqI, VDR, Vitamin D level
Mutation Analysis of Beta Thalassaemia Mutations in Nepalese Population

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Purpose: Beta (β) thalassaemia is a common genetic disorder which passes the gene from parents to their offspring in autosomal recessive inheritance pattern. This disease is directly related to the haemoglobin chemical anatomy and functioning of red blood cells (RBC). This disease is found in almost all over the world. It is a great health related problem in developing nation like Nepal. There is no specific data on beta thalassaemia in Nepal. Hence, this study aimed to reveal the spectrum mutation of beta thalassaemia among Nepalese population.

Method: A total of 107 subjects were selected of which 61 were already clinically distinguished cases and the remaining were one of their immediate family members as unaffected thalassaemia according to the clinical data. DNA was extracted and evaluated by Multiplex ARMS-PCR. The results then were validated by Sanger sequencing.

Results: Nine different mutations were identified. Unaffected family members were analysed to find the link among them. The most common mutations were compound heterozygous CD 26 (G-A) and IVS 1-5 (23%) followed by 619 deletion (20%), CD 89 (+G) (12%), CD 16 (-C) (8 %), CD 41/42 (-TTCT) (6%), IVS 1-1 (G-T) (4%), CD 19 (A-G) (3%) and CD 17 (A-T) (1%).

Conclusion: This is one of the first molecular studies carried out in β thalassaemia patients in Nepal. The pattern of mutations detected is similar to South Asian countries. The findings obtained from this study may represent the prevalence of the different mutations in Nepalese population.

Key words: Thalassaemia, Mutation, Nepalese
A Pilot Study on the Willingness of Premarital Malays on Premarital Thalassaemia Screening

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Purpose: Thalassaemia carrier status is prevalent in nearly 15% of our population. Currently, there is no policy on thalassaemia-carrier screening for couples prior to marriage in Malaysia. Other countries such as Iraq and Saudi Arabia have established a policy for thalassaemia prevention by conducting premarital thalassaemia screening. Zero thalassaemia cases in new-born child in Cyprus have proven that thalassaemia can be prevented. This study aimed to investigate the willingness of premarital Malays on premarital thalassaemia screening. Methods: A set of questionnaire was distributed to 57 persons at premarital course sites and wedding fairs held in Kuala Lumpur and Selangor. Components in the questionnaire included: 1) demographic 2) knowledge about thalassaemia and screening method 3) attitudes towards thalassaemia premarital screening 4) practices of premarital thalassaemia screening. Analysis for the questionnaire was performed using IBM SPSS Statistics 23.0. Results: Our data showed that 75.41% participants have never heard of thalassaemia disease. In addition, 56.14% participants agreed that premarital screening is needed. Widely covered campaign about the facilities of the screening test is also one of the factors that can encourage people to do premarital thalassaemia screening. Eventhough premarital thalassaemia screening is not an obligation, 50.88% of participants agreed that it is important. The main reason people are refused to do premarital thalassaemia screening is because they are afraid to know the test result. Conclusions: Our study demonstrates that knowledge level on thalassaemia among Malaysian is low which may contribute to the low awareness of premarital thalassaemia screening. However, most participants agreed that it is beneficial to do premarital thalassaemia screening despite low knowledge and awareness of the disease. Several factors are needed to be taken into consideration if premarital thalassaemia screening will be implemented in Malaysia as a national policy.

Keywords: Knowledge, Attitude, Practice, Premarital Screening, Thalassaemia
A Study of Craniofacial Morphology and Dental Age in Transfusion Dependent Thalassaemia of 6-16 Years Old Patients

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Purpose: Thalassaemia diseases are common in Southeast Asia. However, information about the craniofacial morphology and dental age development are deficient in this area. The aims of this study are to investigate the craniofacial and dental characteristics, as well as estimated dental age in transfusion dependent thalassaemia patients (TDTP). Methods: A comparative cross sectional study was carried out to compare the craniofacial skeletal, dental and soft tissue morphology and dental age in TDTP age 6 to 16 years old and the control group. A cross sectional descriptive study was employed to determine the prevalence of incisal and molar classification in TDTP. Results: A total number of 43 (22 males, 21 females) Malay TDTP participated in this study. Majority of TDTP (81.4%) presented with Class II Angle malocclusion and Class II skeletal pattern. There was no difference in protrusion of the maxilla in both groups. However, the mandible showed to be reduced (smaller) in TDTP. The mandibular maxillary plane angle (MMPA˚) and the lower anterior facial height (LAFH) in TDTP were increased. Soft tissue analysis revealed protrusion of the upper and lower lips and the nasolabial angle was acute in TDTP. There was a significant association between Angle malocclusion and skeletal pattern in TDTP. TDTP showed delayed in dental age estimation compared to control group. Conclusion: TDTP have high prevalence of Class II Angle malocclusion with Class II skeletal pattern. There is also evidence of delayed dental maturation in TDTP.

Keywords: Craniofacial, Transfusion Dependent Thalassaemia
Coinheritance of Beta Thalassaemia (β0 Filipino) and Delta Variant (HbA2 Deventer) in Sabahan Population: A Case Series

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Purpose: Coinheritance of beta and delta thalassaemia is not uncommon. However, as beta thalassaemia diagnosis is mainly based on elevated HbA2 level, it may lead to diagnostic difficulty because this coinheritance may cause HbA2 level to be lowered. Here, we report 5 unrelated cases of compound heterozygous β0 Filipino ~45 kb deletion and codon 67 (GTG>ATG) HbA2 Deventer in Sabahan population. Methods: Genomic DNA was extracted. Multiplex ARMS and Gap PCR were done to detect common point mutations and deletions for both alpha and beta globin genes. Sanger sequencing was performed to detect mutations in delta globin gene. Results: Patients haemoglobin level ranges between 10.8 – 12.8g/dl. Hb analysis findings of HbA2 and Hbf level ranges between 2.9 – 4.0 and 2.2 – 9.4 respectively. Molecular findings revealed compound heterozygous β0 Filipino ~ 45 kb deletion and codon 67 (GTG>ATG) HbA2 Deventer. Conclusion: Detection of 5 unrelated cases of HbA2 Deventer may suggest that this delta variant is common in Sabahan. Since beta thalassaemia is also common in the population, more attention should be paid during diagnosis. Identification of delta variant in beta thalassaemia carrier is important because coinheritance of beta and delta thalassaemia results in a less elevated HbA2 level. Therefore, molecular testing of thalassaemia carrier state in the case of borderline HbA2 is warranted to avoid misdiagnosis of beta thalassaemia carriers.

Keywords: Coinheritance, Delta Variant, β0 Filipino, HbA2 Deventer
Alpha-Thalassaemia among Beta Thalasaemia Patient’s Relatives in Bandung

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Purpose: Research on alpha thalassaemia distribution in Indonesia, particularly in Bandung has never been done. This led us to study the distribution of thalassaemia alpha in thalassaemia transfusion dependent patients relatives in Bandung, since this population is the most likely population to reach. Methods: In this descriptive study, immunochromatographic (IC) strip test was used to detect alpha thalassaemia carrier among participants. Relatives of beta thalassaemia patients who underwent blood transfusion at hospitals in Bandung, joined the study for blood sample collection (n=88). The specimens were then taken for screening by IC strip test to specify alpha-thalassaemia carriers. Results: There was one case found as positive for alpha-thalassaemia using the strip test but was found negative either for alpha thalassaemia nor beta thalassaemia carrier using capillary electrophoresis. Conclusion: Only one case found as positive for alpha-thalassaemia using the strip test among relatives of B thalassaemia patients.

Keywords: Alpha-thalassaemia, Epidemiology, IC Strip Test
Attention and Executive Function Impairment in Children with Beta-Thalassaemia Major

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Purpose: Cognitive function plays a key role in academic achievement and future productivity in children. Thalassaemia is a common inherited blood disorder worldwide, including Indonesia. Children with thalassaemia may suffer from cognitive deficits such as attention and executive function impairment. Cognitive deficits need early detection for better management. The aim of this study is to determine the characteristics of attention deficits and executive function in children with beta thalassaemia major. Methods: One hundred beta-thalassaemia subjects, age 8-14 years old participated in this study. All subjects performed a vigilance, verbal fluency, and block design test. Results: The mean age of the subjects in this study were 10.94 ± 1.72 years and was predominantly male (52.0%). Most of the subjects had elementary education (78.0%) with the mean frequency of transfusions was 1.30 ± 0.52 times per month. Most of subject using deferiprone iron chelation (78.0%). Attention and executive function impairment was found in 26% and 23% of children respectively. Impaired attention and executive function were found in 21% of the children with the following characteristics: haemoglobin levels of <7 g/dL (66.7%), serum ferritin > 3000 µg/L (69.6%). The mean age (SD) was 9.63 (1.29) years, the frequency of transfusion was 1.42 (0.74) times per month, and time from the first diagnosis was 9.87 (1.3) years. Conclusion: There is impairment of attention and executive function in children with Thalassaemia. Monitoring of cognitive function should be applied as early as possible to detect any kind of abnormalities in children with beta thalassaemia major.

Keywords: Attention, Thalassaemia, Cognitive, Executive Function
Haemoglobin Lansing: A Rare Haemoglobin Variant Causing Falsely Decreased Oxygen Saturation by Pulse Oximetry

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Purpose: Haemoglobin (Hb) function can be altered by single nucleotide substitution in the globin gene. It may either cause a little clinical effect or haemolytic anaemia, polycythaemia, unstable Hb or abnormal oxygen affinity Hb. We report a rare Hb variant that was detected in a 10-year-old Malay girl. Case Report: Patient was admitted with mild pneumonia. Her vital signs and physical examinations were normal, except for oxygen saturation reading of 88% under room air. She had no obvious cyanosis and no signs of finger clubbing. Her arterial blood gases showed a good partial pressure of oxygen. Most of her blood counts were normal [Hb 12.6 g/dl, red blood cell (RBC) count 4.9x10^{12}/l, mean corpuscular volume 80fl] with borderline mean corpuscular haemoglobin of 26 pg. The RBCs were morphologically mildly hypochromic and there was no features of haemolysis. Hb analysis using high performance liquid chromatography method demonstrated within normal limit of Hb A_{2} (2.7%) and Hb F (0.3%). The index case has a brother, nephew and nieces with similar problem. All subjects were healthy, did not have any history of haemolysis or receiving blood transfusion. DNA sequence analysis of the alpha globin gene was therefore performed on index case and her brother; which revealed a CAC to CAG mutation at codon 87 of the alpha 2 gene, resulting in histidine to glutamine substitution. Conclusion: This report illustrates the importance of understanding the limitations of pulse oximetry and awareness that low SpO2 in otherwise healthy patients could be secondary to Hb variants. Early identification of the Hb variants can spare the patients of unnecessary investigations.

Keywords: Haemoglobin Langsing, Variant, Oxygen Saturation
An Unstable Haemoglobin Variant Hb Genova (β28 CTG>CCG) found in a Proposita which gives rise to Haemolytic Anaemia: A Case Report of Spontaneous Mutation


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Purpose: Different types of mutations in β-globin gene result in different properties of β-globin chains being produced such as the unstable haemoglobin which lead to haemolytic anaemia. We report a case of a 9-year old girl who presented with chronic non-immune haemolytic anaemia. Hb analysis finding was suggestive of β-thalassaemia trait with Hb A₂ and Hb F values of 3.3% and 3.7% respectively which did not correlate with her clinical phenotype. Hb analysis for her father only showed mildly raised Hb F value while her mother was normal. Methods: DNA sequencing of α-globin and β-globin gene were done to check for presence of any mutation for the family members. Results: DNA sequencing of the β-globin gene revealed a mutation at Codon 28 (CTG>CCG) (HBB:c.86T>C) Hb Genova for the proposita’s sample. This mutation causes an amino acid changes from Leucine into Proline. Hb Genova (β28 CTG>CCG) is a Hb variant with unstable properties. Individuals heterozygous for this variant have haemolytic anaemia and for our case it occurred as a spontaneous mutation to the proposita. Conclusion: In conclusion, we confirmed the presence of the genetic mutation giving rise to Hb Genova as variant haemoglobin with unstable properties and caused haemolytic anaemia in this patient.

Keywords: Hb Genova, Unstable Haemoglobin, DNA Sequencing, Beta Globin Gene
Occurrence of Delta Globin Gene Defects in Three Families


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**Purpose:** Mutations on the HBD gene are not clinically important. However, co-inheritance of β- and δ-globin defects decreases HbA₂ levels, which may lead to diagnostic misinterpretation. Unusually low HbA₂ levels were found in three families receiving molecular diagnosis of α- and β-thalassaemia at Institute for Medical Research, Malaysia. **Methods:** Molecular diagnosis of α-, β-, and δ-globin genes were performed to determine the thalassaemia status leading to such HbA₂ levels. **Results:** Here we report the complex diagnosis in which δ-variants and thalassaemia defects associated with the common α- and β-thalassaemia defects among members of the families. Two variants HbA₂-Indonesia and HbA₂-Deventer; and a δ-thalassaemia, -68 (C>T) were observed. **Conclusion:** The results of this single center study suggested that the mutations in the HBD gene among Malaysians are heterogeneous and DNA analysis of δ-globin have been included in the national thalassaemia prevention and control program.

**Keywords:** Thalassaemia Diagnostic, δ-globin Gene
Haemoglobin G Makassar Mimicking Haemoglobin S-DNA Analysis is Mandatory for HbS-Like Variants

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Purpose: Haemoglobinopathies are highly prevalent in Malaysia. DNA analysis to detect causative mutations is required for identifying rare haemoglobin variants, especially those that elute at the same position of common variants. Methods: The abnormal Hb fractions were eluting in the HbS window on HPLC, our ARMS HbS test was however negative. DNA sequencing of the β-globin gene revealed a single nucleotide substitution GAG>GCG at codon 6. The haemoglobin variant was Hb G Makassar (HBB:c.19G>C). Results: Here we report 5 unrelated cases detected with Haemoglobin G Makassar, and a proband from a cascade screening with compound heterozygous HbE and Hb G Makassar. Conclusion: This variant might be erroneously diagnosed as HbS unless molecular diagnostic tests are carried out. Since both variants have been reported in our population, fractions eluted like HbS on HPLC or on any other separation methods should always be confirmed by DNA analysis.

Keywords: Hb G Makassar, Haemoglobinopathies
Association of -582 A>G HAMP-P Polymorphism and Iron Status of Javanese Beta Thalassaemia Carriers

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Purpose: Thalassaemia is a genetic disorder most commonly found in Indonesia. Iron absorption in thalassaemia carrier has doubled compared to individuals with normal haemoglobin levels, causing an increase in iron accumulation. Polymorphism of -582 A> G HAMP-P gene is tertiary modifier on the severity of iron overload. The aim of this study is to identify the presence of -582 A>G HAMP-P gene polymorphism and association of the polymorphism with iron status of Javanese ethnic β thalassaemia carriers. Methods: -582 A>G HAMP-P gene polymorphism and iron status were assessed in Javanese β thalassaemia carriers. Thirty six subjects (23 females and 13 males) were involved in this study. Gene polymorphism analysis was done by PCR and RFLP method. Results: Genotype frequency of -582 A>G HAMP-P is 50 % AA (wildtype), 47.2 % AG (heterozygous mutant) and 2.8% GG (homozygotes mutant). There was no difference in serum iron levels between the wildtype and mutant genotype on female (p = 0.240) and male (p = 0.206) subjects. There was no difference in transferrin saturation between wildtype and mutant genotype on female (p = 0.228) and male (p = 0.260) subjects. There was no difference in serum ferritin levels between wildtype and mutant genotype on female (p = 0.806) and male (p = 0.754) subjects. Conclusion: -582 A>G HAMP-P gene polymorphism is commonly found in Javanese ethnic β thalassaemia carriers. However -582 A>G HAMP-P gene polymorphism does not modify iron status of β thalassaemia carriers.

Keywords: β-thalassaemia Carrier, HAMP, Iron Status
Hb E Syndrome with Co-inheritance of Alpha Thalassaemia and Compound Beta Thalassaemia: A Diagnostic Challenge


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**Purpose:** Haemoglobin (Hb) E is one of the world’s most common and important gene mutation. It is important to distinguish Hb E disorders diagnostically because of the marked difference in clinical course among different genotypes. In the condition of compound heterozygosity for Haemoglobin E and beta (β) thalassaemia, the clinical feature varies from thalassaemia minor through thalassaemia intermedia to thalassaemia major. However, it is known that the co-inheritance of both alpha (α) and β thalassaemia would improve the phenotype and clinical symptoms of the patients. In known case of concomitant β thalassaemia trait, Hb H disease is milder than it would be. **Case report:** We report here an-11-month-old girl with accidental findings of pale and hepatosplenomegaly. She is the last child of three siblings from a non-consanguineous marriage, whom the father and the mother are Hb E trait and Hb Constant Spring trait respectively by haematological tests. Clinically the child is small for age but active, mild pallor, mild frontal bossing but no jaundice. Palpable mild hepatosplenomegaly was found on abdominal examination. The cardiorespiratory examination was unremarkable. Full blood picture showed moderate hypochromic microcytic anaemia with marked anisopoikilocytosis. Quantitation of haemoglobin by using High Performance Liquid Chromatography (HPLC) revealed raised Hb A2/E and Hb F with presence of pre-run peak. Gel Electrophoresis by using agarose gel at alkaline pH discovered prominent A2 band and fast band to the left of Hb A band. H inclusions were positive. A diagnosis of combination HbE/β/α was entertained. **Conclusion:** Interpretation difficulty may be seen in compound β and α Thalassaemia using routine investigation. Definitive diagnosis by molecular test is required to confirm the haematological findings.

**Keywords:** HbE, Alpha Thalassaemia, Beta Thalassaemia
Economic Hardship, Catastrophic Health Expenditure and its Associated Factors Among Households with Thalassaemia

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Purpose: To study economic hardship, catastrophic health expenditure (CHE) and its associated factors among households with thalassaemia patients. Methods: This study had 200 respondents from thalassaemia households taken from three hospitals that provide thalassaemia outpatient services; Hospital Tuanku Ampuan Afzan, Kuantan, Hospital Wanita dan Kanak Kanak Likas, Sabah and Hospital Sultanah Nur Zahirah, Kuala Terengganu. The household’s economic hardship, CHE and its associated factors were captured through health economic proforma given to these respondents. Results: Economic hardship in households with thalassaemia was found to be manageable, with only 40% finding the disease a financial burden. Thirty one percent of the sample respondent received subsidies, while 63% claimed that their household income is not affected by thalassaemia. A quarter of the sample population were found to be experiencing CHE. A multiple logistic regression model found that two factors were significantly associated to CHE; presence of an elderly person above the age of 60 in the house [Adjusted OR 3.38 (1.56, 7.13), p-value<0.05] and middle income status [Adjusted OR 0.18 (0.05, 0.64), p-value<0.05]. Conclusion: Thalassaemia is a financial burden to households with the disease. This study shows that 25% of all households with thalassaemia suffer from CHE, and it is associated with the presence of an elderly person above the age of 60 and middle household income. However, the free basic thalassaemia treatment for all Malaysians alleviates the burden significantly with almost 60% of the population claiming thalassaemia is not a burden.

Keywords: Catastrophic Health Expenditure, Economic Hardship, Thalassaemia
Characteristic of Commonest Mutation among Transfusion Dependent HbE/β-Thalassaemia Malay Patients

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Purpose: The clinical phenotypes of HbE/β-thalassaemia patients are very diverse. The underlying pathophysiology is due to mutations of β-globin gene itself resulting in reduced (β+) or absence (β0) of β-globin chain production. While defect on the βE-globin gene decreased production of βE-globin chain. Identification of the mutation is therefore very important. The association of the severe with the specific mutation of β-globin and human disease has been known, but the wide scope and association of the mutation with others factor such as clinical manifestation has been only recently fully appreciated. The aims of this study were to characterize the HbE/β-thalassaemia mutations and to correlate the mutations with clinical manifestation. Methods: 152 HbE/β-thalassaemia subjects were randomly selected from Hospital USM and general hospitals in Malaysia. Genotyping was done using MARMS-PCR and CSGE methods. DNA sequencing was performed to confirm the results. Results: The commonest compound heterozygous mutations observed in this study were CD 26 (G-A) with IVS 1-5 (G-C) 36.2%, CD 41/42 (-TTCT) 26.9%, and IVS 1-1 (G-T) 11.8%. Two rare mutations which are CD 26 (G-A)/IVS 1-2 (T-C) 2.6% and CD 26 (G-A)/IVS 1-2 (T-A) 0.7% were discovered. No signification association was found between types of the mutations and clinical manifestation. This could be due to the small sample recruited in this study. Conclusion: To our knowledge this is the first reported molecular basis of compound heterozygous Hb/E β thalassaemia mutation and its association with clinical manifestation among transfusion dependent Hb/E β thalassaemia patients in Peninsular Malaysia. However further analysis will be carried out to recruit more patients.

Keywords: Mutation, Thalassaemia, Clinical, Severe
α-Globin Gene Cluster Haplotype in Genotyping Analysis of Non-Deletional α-Thalassaemia

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Purpose: α-thalassaemia is an inherited haemoglobin production abnormality caused by a decrease or absent α-globin synthesis. The most common causes of α-thalassaemia is usually due to deletion of one or both α-globin genes. However, non-deletional α-globin gene mutations are not uncommon causes of α-thalassaemia mutations in Indonesia and are usually manifested more severe phenotypes compare to the deletion types. One of the common non-deletional α-globin gene mutations is Codon 59 in α2-globin gene (GGC⁸→GAC¹⁸) or Hb Adana. The aim of this study is to evaluate α-globin gene cluster haplotype in genotyping analysis of non-deletional α-thalassaemia. Case Report: We found combination of the highly unstable α-haemoglobin variant Hb Adana and a regulatory single nucleotide polymorphism (rSNP) in a non-genic region between the α-globin genes and their upstream regulatory, known as Vanuatuan or Papua New Guinea (PNG) mutation in patient with Bugis ethnic background. PNG mutation creates new promotor-like element that subsequently disturb normal synthesis of α-globin chain. The α-thalassaemia patient with this genotype showed severe α-thalassaemia phenotype since childhood and had routine blood transfusion. The α-globin gene cluster is a highly polymorphic region that may contain several informative SNP for genotyping analysis. We used nine of the most common polymorphisms sites in α-globin gene cluster to confirm the mutant alleles inheritance in this patient. Conclusion: The α-globin gene cluster haplotype is very useful in genotyping analysis of non-deletional α-thalassaemia. The result suggest that this approach will also give benefit in association studies of unknown mutant alleles.

Keywords: Non-deletional, α-thalassaemia, α-globin gene cluster, Hb Adana, PNG
DNA Methylation of *IGSF4* gene as an Epigenetic Modifier in HbE/β-Thalassaemia

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**Purpose:** The large clinical spectrum of Haemoglobin E (HbE)/β-thalassaemia leads to identification of modifiers that cause the complexity. *IGSF4*, a member of the immunoglobulin superfamily 4 is known as a thalassaemia-related gene that play an important role in globin synthesis. Methylation of *IGSF4* was reported to interrupt the process of globin synthesis through its interaction with other genes in the regulation network of globin expression. The objective of this study was to describe the pattern of DNA methylation at the promoter region of *IGSF4* gene that may involve in the alteration of globin synthesis in HbE/β-thalassaemia patients. **Methods:** Nucleated red blood cells (NRBCs) were isolated from 6mL peripheral blood of thalassaemia patients after the isolation of mononuclear cells (MNCs) based on density gradient. Magnetic activated cell sorting (MACs) with anti-CD71 was used to enrich isolated NRBC and validated with flow cytometry (Becton-Dickson, USA). DNA was extracted from the samples and subjected for bisulfite modification using EZ DNA methylation-gold Kit. Methylation specific polymerase chain reaction (MS-PCR) and DNA sequencing were employed to screen and detect the methylation status targeting 10 CpG sites within the promoter region of *IGSF4* gene in HbE/β-thalassaemia patients with CD26/IVS1_1. **Results:** One HbE/β-thalassaemia patient with CD26/IVS1_1MS-PCR showed visible bands in both methylated and unmethylated primer sets for *IGSF4* gene, indicating that the M1 region (-696 to -582 relative to the ATG) of *IGSF4* promoter is partially methylated. DNA sequencing confirmed that all CpGs in the amplified regions are methylated. **Conclusion:** The mechanism of abnormal β-globin chain production could be due to the aberrant DNA methylation of *IGSF4* gene in HbE/β thalassaemia patient with CD26/IVS1_1. This finding may contribute to the potential of *IGSF4* as an epigenetic modifier in HbE/β-thalassaemia.

**Keywords:** HbE/β Thalassaemia, Methylation, *IGSF4*
Haemolytic Disease of Foetus and Newborn & Haemolytic Transfusion Reaction due to Kidd Antibody in Hospital Umum Sarawak, Malaysia

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Purpose: The aim of this study is to determine whether Kidd alloimmunization causes haemolytic disease of foetus and newborn (HDFN) & haemolytic transfusion reaction (HTR) in Hospital Umum Sarawak. Methods: Records of alloimmunisation cases from 2011 to 2014 were retrieved from patients’ medical records. Secondly, to determine the prevalence of Kidd phenotype, two hundred and fifty (250) regular blood donors in HUS from 1st to 10th September 2015 were recruited. Blood samples were phenotyped for Kidd blood group using Diamed-ID gel card system. Results: There were 1109 cases of alloimmunisation recorded and 4.0% of the cases were due to Kidd antibody. Ten (10) out of 44 (22.7%) cases of alloimmunisation were due to Kidd antibody resulting in HDFN whilst 4 out of 44 cases (9.1%) resulting in HTR. These results were not statistically significant (p> 0.05). Meanwhile, the results of Kidd phenotype showed the presence of Jk(a+b+) phenotype in 110 out of 250 (44.0%) and Jk(a-b-) phenotype in 7 out of 250 (2.8%) blood donors. The other Kidd phenotypes detected were Jk(a+b) in 60 out of 250 (24.0%) and Jk(a-b+) in 73 out of 250 (29.2%) blood donors. Kidd phenotype was detected in four (4) ethnic groups; Chinese, 127 out of 250 (50.8%), Malays, 96 out of 250 (38.4%), Bidayuh, 25 out of 250 (10.0%) and Iban, 2 out of 250 (0.8%). The results also showed that Jk(a-b-) phenotype is present only in the Malays 7 out of 250 (2.8%) but not found in the other ethnic groups, and this is statistically significant (p<0.05). Conclusion: There is low prevalence of Kidd antibody causing HDFN and HTR and Kidd blood group system was successfully characterised in regular blood donors in HUS.

Keywords: HDFN, HTR, Kidd, Ethnicity
Factors that Motivate and Hinder Temporarily Deferred First Time Donors

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Purpose: To identify factors motivate and hinders first time temporary deferred donors. In order to improve donor recruitment, retention, to ensure stable and safe blood supply and achieve WHO target of developing voluntary non-remunerated blood donors in Malaysia.

Methods: 480 first time temporary deferred whole blood donors from National Blood Centre, KL had participated. Donors randomly extracted from Blood Bank Information System Based on the inclusion and exclusion criteria, the donors were categorized into 2 groups each blood donor was contacted via telephone and questionnaire asked personally.

Results: Donors who return were significantly younger than donor who did not return (29.5 years versus 35 years, p < 0.001). Socio-demography also significantly differs between return and did not return. Donors who lived in urban areas were more likely to return for donation compared to donors who lived in rural areas (34.6%). Among factors that motivate donors to return for donation are self-satisfaction (25.8%), social contribution (24.6%), peer influence (16.7%), altruism (13.3%), family encouragement (13.3%) and others (6.3%). Factors that hinder donor from return for donation were no time (34.2%), loss of interest (20.8%), scarred to be deferred (17.9%), don’t know donation site (14.2%), no incentive (7.9%) and others (5%). Conclusion: In this study, clearly showed that younger generation, single and professionals from urban areas contributed to higher rate of return donor. Thus, focusing on this category of donors to become regular donors will enable us to enhance and improve transfusion service in Malaysia.

Keywords: Motivating, Hindering, First, Temporary Deferred
Study of von Willebrand Profiles of the Different ABO Blood Group among Malay Population

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Purpose: In 2010, 0.002% von Willebrand patients were reported among 28,000,000 of Malaysia population; 63% were from the Malay ethnicity. The objective of this research is to obtain the vWF profiles data of the different ABO blood type among Malays and to observe the association of demographic characteristics and smoking habit with the profiles.

Methods: One hundred and forty Malay blood donors were involved in the research administered in the National Blood Centre Kuala Lumpur. FVIII, vWF antigen, RiCof and CBA levels were measured by coagulometric clot detection, latex agglutination and ELISA methods. Results: Majority of the donor (59.3%) were aged between 30-49 years, male (81.43%), non-smoker (74.3%) and, overweight/obese (71.4%). The Malays vWF profiles were slightly higher compared to Caucasians, Indian, Thais and Chinese but average ratio of vWF activity: antigen was slightly lower compared to other populations. The highest level of vWF was among B blood group, followed by A and O. The prevalence of low (<50 IU/dl) vWF antigen and CBA were not common among the Malays. It was observed that the CBA levels were significantly interrelated with the age group. Conclusion: The higher levels of vWF profiles and lower vWF activity: vWF antigen in Malays may suggest the impact of ethnicity on the plasma vWF levels, and variation in the interaction of the vWF with collagen and platelet. These findings warrant for larger scale population-based study among Malays.

Keywords: vWF, FVIII, RiCof, CBA
Azacytidine Enhances Sensitivity Response to Imatinib in BCR/ABL Positive CML Cell Line

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Purpose: Azacytidine (5-Aza) is a chemotherapeutic drug used for DNA-de-methylation resulting in re-expression of silenced tumor suppressor genes (TSG). Epigenetic silencing of TSG such as involved in the development and progression of cancers. Re-expression of SHP-1 is inversely proportionate with STAT3 signaling pathways. Majority of CML patients treated with imatinib, a BCR/ABL inhibitor would develop resistance under prolonged therapy. Here we evaluated the expression of SHP-1 gene and its methylation status with sensitivity response of resistant CML cells to imatinib before and after treatment with 5-Aza.

Methods: BCR/ABL positive CML cell lines, K562 and K562-R, an imatinib resistant cell lines were treated with 5-Aza. Cytotoxicity of imatinib and apoptosis were determined by MTS and annexin-V, respectively. Gene expression analysis was detected by real time-PCR; STAT3 activity was examined using Western blot and methylation status of SHP-1 gene was determined by pyrosequencing analysis. Results: There was a significant higher in the expression of SHP-1 in K562-R+5-Aza cells compared to K562 and K562-R (p=0.001). Methylation of SHP-1 gene was significantly decreased in K562-R+5-Aza cells compared to others (p=0.003). STAT3 was inactivated in K562-R+5-Aza cell lines which showed higher sensitivity to imatinib. Conclusion: In conclusion, 5-Aza could enhances efficacy of imatinib on BCR/ABL CML cells through re-expression of SHP-1 gene and inhibition of STAT3 signaling.

Keywords: 5-Aza, Resistance, SHP-1, STAT3
Down Syndrome in one of a set of Dizygotic Twins: A Case Report

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Purpose: Studies have suggested that the risk of Down syndrome (DS) in twins is significantly lower than in singletons and the risk of DS in monozygotic twins is lower than dizygotic twins. This is due to early fetal loss of DS in multiple pregnancies, particularly in pregnancies concordant for DS. Here, we report a rare case of DS in one of a set of dizygotic twins. Case Report: The dizygotic twins were born healthy vaginally after 36 weeks of uneventful pregnancy. The mother was 25 while the father was 32 years when the twins were born. Both parents are non-consanguineous and of average intelligence. No history of DS or mental subnormality was discovered on inquiry into the family history. The male twin was noticed to have features of DS with minor congenital heart defect at birth which resolved spontaneously. The diagnosis of DS was confirmed by conventional cytogenetic analysis which revealed 47, XY, +21 (non-disjunction type). Whereas, the unaffected female twin showed normal female karyotype with 46,XX karyotype pattern. Conclusion: In dizygotic pregnancy, each co-twin has an individual risk of being DS. The production of trisomy 21 gamete in this case most probably could be due to sporadic event which might have resulted from meiotic errors in only one pair of the gamete during gametogenesis of either paternal or maternal origin. We believe this is the first case of DS in one of a set of dizygotic twins being reported in Malaysia.

Keywords: Dizygotic Twins, Discordance, DS
Complex Chromosomal Rearrangement der(5)ins(5;3)(q31;q25q29), t(3;12)(q24;p12.2) in a Dysmorphic Child: A Case Report

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Purpose: Complex chromosome rearrangements (CCRs) are structural aberrations involving at least three chromosomes or more chromosomal breakpoints. It may be balanced or unbalanced. Balanced CCRs can lead to an unbalanced condition of gametes during meiosis. The present study aimed to investigate the genetic cause underlying a 3-year-old Malay girl, of non-consanguineous parents who presented with abnormal features. She is short, has speech delay, triangular facies, strabismus and short neck. Methods: Peripheral blood lymphocytes were cultured and chromosome preparation were made as per procedures. Karyotype analysis was carried out based on ISCN (2016). FISH technique was carried out using Whole Chromosome Painting Probes (WCP) for chromosome 3, 5 and 12 as per standard procedures. Results: Cytogenetic analysis on 32 GTG banded metaphases showed 46,XX,der(5)ins(5;3)(q31;q25q29),t(3;12)(q24;p12.2) complex karyotype pattern. This abnormal karyotype showed derivative chromosome 5 resulting from an insertion of a segment 3q25q29 from the long arm of chromosome 3 into the long arm of chromosome 5 at band 5q31. The segment is replaced by translocation of a segment 12p12.2 from the short arm of chromosome 12 to the chromosome 3 at band 3q24. This three way translocations has been confirmed by FISH technique using WCP for chromosome 3, 5 and 12. Conclusion: This case is presented as CCRs are very rare events in the human population. Abnormal phenotypes observed in individuals who harbour apparently balanced chromosome rearrangements result from disruption of a gene(s) at chromosome breakpoint(s), cryptic genomic imbalance undetected by routine karyotyping or position effect. Parental karyotyping was advised.

Keywords: CCR, Dysmorphic, Child
Four Nucleotide Deletions of Exon 47 in Dystrophin Gene: A Case Report of a Kelantanese Duchenne Muscular Dystrophy Patient

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Purpose: Duchenne Muscular Dystrophy (DMD) is an X-linked recessive disease caused by alterations in the dystrophin gene at Xp21.2. Cases of DMD in Malaysia are still scarcely reported. Hence, we report a case of an 11-year-old Kelantanese Malay boy who has progressive muscle weakness since 5 years old with positive Gower sign. He has a strong family history of DMD and musculoskeletal problems. Methods: Genomic DNA was extracted from 3ml of whole blood and subjected to multiplex ligation-dependent probe amplification (MLPA) and Direct DNA sequencing. Results: MLPA and Direct DNA sequencing result showed deletion of four nucleotides in exon 47: c.6803delACAA, p.K2268NFsX2269 leading to premature stop codon. These findings demonstrate the genetic pathology of DMD in this patient. Conclusion: Deletion accounts for 60% of the mutations within the 79 exons of the dystrophin gene. Deletions of exons 49, 50 and 51 were the most frequent (71.43%) in Malaysian population. We believe this is the first case reported in Malaysia involving nucleotide deletions leading to shifting of normal reading frame within exon 47. This case represents a frameshift deletion whereby the 4-base deletion lead to the appearance of termination codon prematurely at exon 47, 32 exons short of the normal transcript. It results in the formation of a truncated dystrophin protein and in consequence obliterates the production of dystrophin in muscles. MLPA does improve the diagnostic technique, especially in detecting small mutations. Parental genetic screening is advised as risk for phenotypic abnormalities differs between familial or de novo mutation.

Keywords: DMD, MLPA
The Effect of Administration of Sappan Wood (*Caesalpinia sappan*, L.) on Iron Profile in Iron Overload Rats (*Rattus Norwegicus*, L.)

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**Purpose:** The purpose of this study was to determine the dose of sappan wood extract that effectively improve the iron profile. **Methods:** Research was performed using a completely randomized design (CRD) to 27 rats female wistar. Research is divided into 9 treatments with 3 repetitions, that is rats were given aquades as negative control 1 (KN1), CMC as a negative control 2 (KN2), iron dextran dose of 60 mg / kg bw (KP), iron dextran with deferipron dose of 75 mg / kg bw (P1), iron dextran with sappan wood extract dose 100 (P2), 200 (P3), 300 (P4), and 400 (P5) mg / kg bw, iron dextran with a dose of 400 mg / kg bw (satellite) (P6), and, administered orally for 28 days treatment. **Results:** The results showed that the optimum dose of 200 mg / kg/bb is an effective dose as iron chelator in rats under conditions of iron overload through a decrease of 24.91% ferritin levels, liver iron content of 54.08%, serum iron levels by 38.94% and amounted to 78.22% transferrin saturation, and increase levels of 102.27% transferrin and TIBC (Total Iron Binding Capacity) amounted to 112.55%. **Conclusion:** Sappan wood extract at doses of 200 / mg / kg bb effectively improves the iron profile.

**Keywords:** Iron Profile, Sappan Wood, Iron Overload, Ferritin
The Alteration of Cognitive Function in Iron Overload Mice

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Purpose: Routine blood transfusions is a lifetime treatment for blood disorder disease such as thalassaemia and can lead to iron accumulation in organs. Iron accumulation in brain can induce toxicity by increasing reactive oxygen species (ROS) and altering apoptotic signal. However, the impact of excessive iron in cognitive function is still unclear. The purpose of this study is to investigate the effect of iron overload to the cognitive function of mice.

Methods: Three groups of mice were injected with a specified dose of iron (0, 0.1, and 0.3 mg/mice) respectively. Iron was injected intraperitoneally for 19 days. A special experimental maze was used to assess cognitive function. The test was conducted three times; before injection, 6\textsuperscript{th} day of injection, and 11\textsuperscript{th} day of injection. After 19 days of injection, the mice were sacrificed and brain weight was measured by scale. Results: Our results showed cognitive function was disturbed after iron injection. The recognizing time in iron injected groups was almost 2 times higher compare to control group. There was no difference of brain weight amongst all those groups. Conclusion: Excessive of Iron, can reduce cognitive function in mice.

Keywords: Iron Overload, Cognitive Function
The Effect of Excess Iron on the Impairment of Glucose Metabolism in Mice

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Purpose: Excess iron in the body can trigger pathological conditions through production of reactive oxygen species (ROS) and its deposition in various organs. This condition can also disrupt whole metabolism process, including glucose metabolism. However, the mechanisms between iron and glucose metabolism remain unclear. The study was to investigate the effect of excess iron content with glucose metabolism disorder by assessing glucose tolerance test and gluconeogenesis rate in white mice through Intraperitoneal Glucose Tolerance Test (IPGTT) to determine insulin resistance, Intraperitoneal Pyruvate Tolerance Test (IPTT) to measure hepatic gluconeogenesis, and to assess pancreatic histology for routine histological examination. Methods: Eighteen male mice, were assigned to 3 equal groups. Mice were divided by the saline injected group (control) and the dose of iron dextran injection 0.1 mg/mice (group 2) and 0.3 mg/mice (group 3). Iron dextran was injected daily intraperitoneally. After 14 days of treatment IPGTT, IPTT and pancreas histology were examined. Results: IPGTT results showed glucose level was lower 39,85 % in group 3 compared to the control group mice. The results of IPTT showed that glucose level in mice treated with iron dextran were significantly lower in dose dependent manner. Furthermore, pancreas histology showed normal tissue without inflammation condition. Conclusion: Low dose iron injection increases glucose tolerance and supresses hepatic gluconeogenesis in mice.

Keywords: Excess Iron, Glucose Metabolism