

Rare Familial Weak a Subgroup: A Case Report

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Introduction: The ABO blood group system was the first system discovered, a century ago, but occurrence of its weaker variants still poses as a challenge in transfusion medicine field. The commonest weak A subgroup i.e. A₃ subgroup shows a characteristic mixed-field (mf) agglutination following incubation with anti-A. We reported a case of a 62-year-old female, diagnosed with advanced left breast carcinoma who was admitted to our centre for her first cycle of palliative radiotherapy. She required 3 pints crossmatched-compatible packed red blood cells (RBC) to alleviate anaemic symptoms. However, forward ABO grouping demonstrated mf reaction with Anti-A antisera which resulted in ABO discrepancy. She had positive transfusion history with group O packed (RBC) in Hospital Bukit Mertajam a week prior. Further history revealed the earliest detection of mf reaction, which preceded the first packed (RBC) transfusion for patient in Hospital Kepala Batas three months previously. History of transplantation and twin siblings was negative.

Objective: To identify the cause of mf reaction during forward ABO grouping.

Material and methods: A batch of serological tests to ascertain weak subgroup was performed on patient's sample which include anti-A₁ lectin, anti-H lectin, crossmatching and Rh phenotyping. Saliva inhibition test was also performed. Family screening was undertaken to assess A₃ prevalence in her family.

Result: Forward ABO grouping showed 2+/mf reaction with anti-A and anti-A,B whereas reverse grouping demonstrated strong reaction (3+) with B cells. Testing patient's red cells with anti-A₁ lectin showed negative result. Patient's serum reacted strongly (4+) with anti-H lectin. Antibody screening and Direct Coombs test were negative. Saliva inhibition test exhibited presence of strong H substances and reduced A substances. Patient probable full Rh phenotype is CDe/cDE or R1R2. Patient serum was compatible with both O and A donor red cells. However, in view of unresolved ABO discrepancy at that time, patient was supplied with group O packed (RBC). Family screening was helpful because all serological tests of her healthy second offspring demonstrated similar reaction suggestive of A₃ subgroup. However, stronger reaction was detected (3+/mf) during ABO grouping.

Discussion: This case reinforces the importance of relevant history and family screening in resolving ABO discrepancy. Out-of-group transfusion, ABO-mismatched transplantation and twin chimerism were virtually ruled out by history alone. Not only the family screening reveals the inheritance of A₃ subgroup, but also it provides argument against malignancy as the cause of mf reaction in patient. It also highlights the probable malignancy effect towards weakened antigen expression on patient RBC. This case additionally demonstrates the need for more advanced tests such as ABO genotyping to confirm patient blood group.

Conclusion: A₃ subgroup is infrequently encountered in Malaysia, let alone in a familial pattern. Although weak A subgroups are mainly of academic interest in the textbook, we should consider A₃ subgroup in mf reaction during forward ABO grouping. The inaccessibility of more advanced blood grouping techniques can be offset by extensive serological tests, relevant history and detailed family screening

Keywords: ABO discrepancy, A₃ subgroup

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References:

1. Beenu Thakral, M.B., Karan Saluja, Neelam Marhawa, *Importance of Weak ABO Subgroup*. Laboratory Medicine 2005. **36**(32-34).
2. Pipatvanichkul, A., et al., *Mutation of ABO gene in Thai blood donors with A3 phenotype*. J Med Assoc Thai, 2011. **94**(3): p. 379-85.
3. Harmening, D., Baldwin, A. J., & Sohmer, P. R. (2012). *Modern blood banking and transfusion practices (6th edition)*. Philadelphia, F.A. Davis.
4. Nambiar, R.K., et al., *Blood group change in acute myeloid leukemia*. Proc (Bayl Univ Med Cent), 2017. **30**(1): p. 74-75.
5. Dabelsteen, E. and S. Gao, *ABO blood-group antigens in oral cancer*. J Dent Res, 2005. **84**(1): p. 21-8.
6. Novaretti, Mcz, Domingues, Ae, Pares. Rapid detection of 871G>A mutation by sequence specific PCR in ABO*A301 blood donors.2004. Blood (ASH Annual Meeting Abstracts) 104:4098.

A Pilot Clinical Audit on The Comparison of Thyroid Function Determination Using Conventional Neck-To-Thigh Ratio Versus Quantitative Planar Thyroid Scintigraphy. A HUSM Experience

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Introduction

Thyroid scintigraphy is a fairly common procedure performed in assessing the morphology and functional status of thyroid gland. Thyroid scintigraphy has been used in the evaluation of patient with hyperthyroidism. Traditionally, in Malaysia, function of the thyroid gland can be semi-quantitatively determine by performing the neck-to-thigh ratio with a commonly acceptable normal range of 5-10. However this value was coined in the 70s with no updated value nor a local value to support its continuous used in the current setting. Newer processing software now provide quantitative estimation of the thyroid function via the percentage technetium (^{99m}Tc) thyroid uptake (%TcTU) at 20mins via performing a simple planar thyroid scintigraphy post injection of ^{99m}Tc-pertechnetate. We aim to audit our practices in our centre by comparing the values and assessing the correlation of both the neck-to-thigh-ratio (NTR) and %TcTU. We also aim to assess their correlation to serum free T4 (FT4) and TSH levels.

Methodology

We performed a retrospective clinical audit of all thyroid scintigraphy performed in our department from June 2017 till July 2018. The referral documents, patients' case-notes and scintigraphic images were reviewed and the information were entered into a datasheet. The thyroid scintigraphy images were re-read, reprocess and reanalyse by a single investigator and the NTR as well as the %TcTU from planar thyroid scintigraphy were determined. Studies with ectopic thyroid tissue and non-visualization of thyroid gland were excluded from this study. Universal sampling was applied and 34 patients of various degree of thyroid status with complete dataset were recruited in this study and further analysis was performed. The demographic details were analysed and the correlation between NTR and %TcTU was determined using the Pearson Correlation. The correlations of free T4 and TSH with NTR and %TcTU were also measured.

Result

A total of 72 patients had undergone thyroid scintigraphy in our centre for various indications. The majority of patients referred for thyroid scintigraphy were for the evaluation of congenital hypothyroidism and assessment of suspicious nodule in multinodular goitre. Of these 72 patients, 34 patients with complete dataset were included in this study, comprising of 25 (73.5%) females and 9 (26.5%) males with 97.1% of them are of Malay descendants, consistent with the Malay majority population of at which our centre are located. The mean age of our patients are 33.62 (3 – 61, SD 19.327). Of the 34 patients, 27 patients were referred for evaluation of goitre and another 7 patients were referred for assessment of congenital hypothyroidism. We did not received any referral for evaluation of thyroiditis throughout this study period. The mean FT4 and TSH are 36.52 pmol/L and 0.76 mIU/L respectively. This study demonstrated that there is good correlation (0.957) with statistical significance at $p < 0.05$ between the NTR and %TcTU values. There are also good correlation between FT4 with both NTR and %TcTU values. However, there is negative correlation of statistical significance ($p < 0.05$) of TSH with %TcTU (-0.423) and NTR (-0.429).

Discussion

Nuclear medicine techniques have been used to assess thyroid activity via the thyroid uptake studies. The more widely accepted method of determining thyroid gland uptake is determination of radioiodine ^{131}I thyroid uptake at 24 hours (RAIU). However, as this method is not readily available, various authors have suggested the utilization of $^{99\text{m}}\text{Tc}$ -pertechnetate as a more convenient substitute. The NTR obtained from a planar $^{99\text{m}}\text{Tc}$ -pertechnetate thyroid scintigraphy was first described by Selby et al. (1975) and Schneider et al. (1979) and has been the preferred method used in many centres in Malaysia. However, quantitative method such as the %TcTU allows for a more objective and accurate estimation of thyroid gland function in few simple steps. Furthermore, the %TcTU has been widely investigated and its values validated extensively. Lee et al. (2016) and Szumowski et al. (2016) have shown good correlation between RAIU and %TcTU. In addition, Lee et al. (2016) have shown that %TcTU obtained from a scintigraphy approach may provide an even more accurate estimation of thyroid uptake as compared to the conventional RAIU determination using a gamma probe. Unfortunately, there is no available local data pertaining to the use of %TcTU in determining thyroid gland function. Our study have shown that the NTR value does represent an accurate estimation of the thyroid uptake and hence thyroid activity. This is proven with the good and near perfect correlation with the validated values of %TcTU. Likewise the NTR values also correlate well with serum FT4 and TSH level. We would like to point out that this study has several limitations such as the small sample size and skewed population distribution which may not represent the entire population. We aim to expand this study to include a larger sample size in the near future.

Conclusion

Our study showed that the age-old NTR method in determining thyroid gland activity is still valid and correlates well with the validated values of %TcTU.

Keywords: Neck-to-Thigh Ratio, Thyroid Uptake, Thyroid Function, Thyroid Scintigraphy, Radioiodine, Technetium

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References:

1. Lee, H., Kim, J., Kang, Y., Moon, J., So, Y. and Lee, W. (2016). Quantitative single-photon emission computed tomography/computed tomography for technetium pertechnetate thyroid uptake measurement. *Medicine*, 95(27), p.e4170.
2. Stokkel, M. P., Junak, D. H., Lassmann, M., Dietlein, M. & Luster, M. (2010). EANM procedure guidelines for therapy of benign thyroid disease. *European Journal of Nuclear Medicine and Molecular Imaging*, 37, 2218-2228.
3. Szumowski, P., Mojsak, M., Abdelrazek, S., Sykała, M., Amelian-Filonowicz, A., Jurgilewicz, D. and Myśliwiec, J. (2016). Calculation of therapeutic activity of radioiodine in Graves' disease by means of Marinelli's formula, using technetium ($^{99\text{m}}\text{Tc}$) scintigraphy. *Endocrine*, 54(3), pp.751-756.
4. Walraven, C., Naude, F., Venter, E. and Meyer, B. (2004). A17 Comparison of ^{131}I thyroid uptake versus $^{99\text{m}}\text{TcO}_4$ thyroid uptake. *Nuclear Medicine Communications*, 25(10), p.1058.
5. Selby, J., Caldwell, J., Magoun, S. and Beihn, R. (1975). The Use of the $^{99\text{m}}\text{Tc}$ -Pertechnetate Neck/Thigh Ratio as a Test of Thyroid Function. *Radiology*, 114(1), pp.107-110.
6. Schneider, P. (1979). Simple, rapid thyroid function testing with $^{99\text{m}}\text{Tc}$ -pertechnetate thyroid uptake .

Polymeric Nano-Encapsulation of Haemoglobin Isolated from Expired Human Red Blood Cells as Blood Substitute

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The need to attend aging population, patients with blood disorders and malignancies and other medical treatments have been causing blood scarcity in blood transfusion services globally. Additional challenges such as prevalence of transfusion transmitted infections have placed a great demand amongst scientific community to embark on vigorous scientific works in looking for possible red blood cells alternatives (Cohn and Cushing, 2009). Malaysia is no different from other countries. With our 2.5% donor to population ratio, we anticipate at least 20,000 of blood unit's shortage every year. In tandem with other promotional and awareness activities to drive better altruism amongst existing and potential donors, it is about time for us to take apparent step towards research and development (R&D) programme in formulating blood substitutes. In this study, we aim to isolate and purify haemoglobin (hb) from expired human red blood cells (RBC) upon successive steps which involved high-speed multiple washings, sonication, organic extraction phase and dialysis methods. Comparative UV-Vis spectra analysis between the hb and red blood cells (RBC) displayed that the tetrameric proteins have been isolated successfully from the cells. Subsequently, there are a few possible chemical modifications that could be manipulated to the harvested hb such as surface- modified hb, cross-linked hb, polymerised hb and hb liposomal capsule, mainly to ensure its stability for efficient therapeutic intervention (Jia, Duan and Li, 2016). Some approaches utilise intermolecular crosslinking between α and β subunits using a site-specific cross-linker to achieve better stability. In our case, we proposed a micelle system of a diblock copolymer known as methoxy poly (ethylene glycol)-b-poly (ϵ -caprolactone) (PEG-PCL). Upon determination of its critical micelle concentration (CMC) which is within 1-2 mg/ml, the polymer acted as host molecules to load the hb within its hydrophobic cavity in aqueous environment (PBS buffer). The residing encapsulated hb molecules within the polymer carrier was then further analysed for its half-life ($t_{1/2}$) upon air exposure, reversible oxygen capacity and its biocompatibility in cell-viability assays. The finding revealed that average encapsulation efficiency of hb within the polymeric micelle was 45%, indicating that at least two hb molecules were successfully 'trapped' within each host molecule. High hydrophilic nature of the hb might have limit its entry into the polymer but the odd was overcome by applying homogenisation technique during encapsulation phase rather than simple stirring during the encapsulation phase. Despite that, there was also an equal chance of losing out the hb once it was encapsulated especially when the system loses its equilibrium dynamic, favouring release of the guest molecule. Curve fitting of hb-micelle stability graph plots demonstrated a projected half-life ($t_{1/2}$) of 112,242 mins or almost 78 days as compared to 30 mins of the free hb control. Despite its good stability under continuous air exposure the hb-micelle system could only endure an average of six complete reversible oxygen cycles before the system faded off due to evaporation because of gas bubbling during oxygenation and deoxygenation process. Soret band shifts of between the two UV-Vis spectra is as illustrated in **Figure 1**. Only one approximate cycle was recorded by the free hb control during the same procedure. Should the experiment be conducted within enclosed system that mimics physiological condition, more cycles could have been recorded by the sample. Even with its US-FDA biocompatible approved status, the use of PEG-PCL polymer to encapsulate hb is a rather new approach hence cell compatibility investigation is a necessity. Hb solution at 1.89×10^{-5} M concentration was prepared for two micelle preparations at 1 and 2 mg/ml respectively, while free human hb and bovine hb at concentrations of 2.0 and 2.2×10^{-5} M were the analogue for similar preparations with polymer. All the samples and controls were tested on murine macrophage cells (RAW 264.7) and incubated for 24 hours before treatment. Treated cells were further incubated for 48 hours of which cell viability

analysis were taken at time 4, 8, 24 and 48 h time points. Relatively, the free hb demonstrated approximately 10% and 18% reductions in cell growth after 4h as compared to the hb-micelle which continued to exert no lethal effect on the cells up to 24h (**Figure 2**). This result indicated that isolation of the free hb might still contain traces of impurities such as lipid debris or other proteins. Without any coating, the chance of phagocytic activity by the cell to get rid of the hb became higher as compared the better tolerated hb encapsulated micelle that composed of PEG tail on its outer face. The presence of PEG not only improved hb stability but also its biocompatibility by preventing cell surface interaction such as opsonisation that could lead the protein early clearance. In general, the cell viability results exhibited great potential of the host-guest system as future blood substitute candidate. Further advancements such as improving hb loading into the carrier must be looked upon for next development.

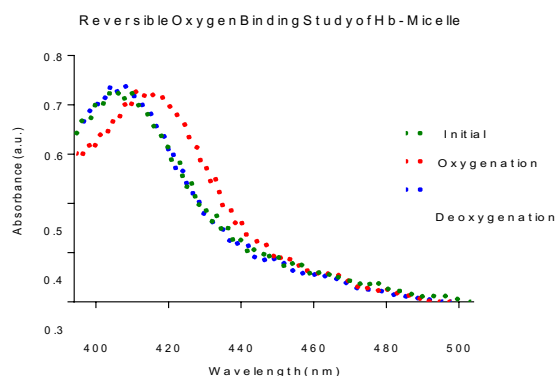


Figure 1: The Soret band shifts upon oxygenation and deoxygenation of hb-micelle

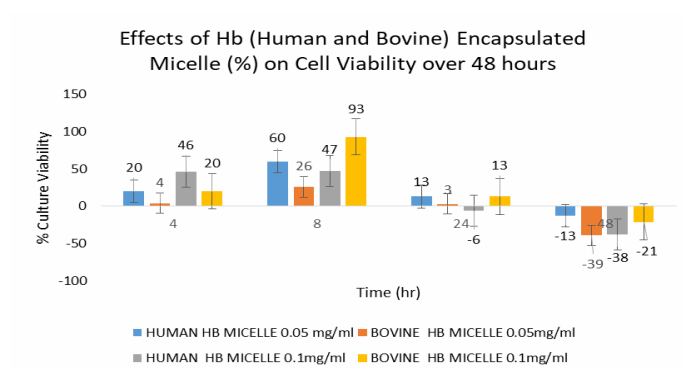


Figure 2: Effects of human and bovine haemoglobin encapsulated within PEG-PCL micelle on cell viability (%) over 48 hours duration taken at four time points (4, 8, 24 and 48 hours.)

Keywords: blood substitute, micelle, artificial oxygen carrier

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References:

1. Cohn, C. S. and Cushing, M. M. (2009) ‘Oxygen Therapeutics: Perfluorocarbons and Blood Substitute Safety’, *Critical Care Clinics*, 25(2), pp. 399–414. doi: 10.1016/j.ccc.2008.12.007.
2. Jia, Y., Duan, L. and Li, J. (2016) ‘Hemoglobin-Based Nanoarchitectonic Assemblies as Oxygen Carriers’, *Advanced Materials*, 28(6), pp. 1312–1318. doi: 10.1002/adma.201502581.

The Impact of Malocclusion Severity on Self-Esteem in Malay Teenagers

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Even though malocclusion is not considered as a disease and it is not a life-threatening condition, a high demand for treatment can be noticed. Malocclusion is considered an important health issue in all over the world (Petersen, 2003). Epidemiological studies of malocclusion reported a high prevalence of this condition in different countries (Thilander & Myrberg, 1973). It seems that malocclusion and orthodontic treatment need become a health care issue and subsequently a quality of life problem (Liu *et al.*, 2009).

Social relationship of people can be adversely affected by their dentation and facial morphology, and severe malocclusion can have a negative impact on self-esteem (Choi *et al.*, 2016). Self-esteem is defined as individual's subjective emotional evaluation of his or her own worth (Hewitt, 2009). According to Rosenberg individual with high self-esteem has self-respect and consider her or his self a person of worth, is aware of his or her faults and at the mean time appreciate his merit. In contrast lack of self-respect, feeling of being unworthy, inadequate and in severe case being deficient as a person is correspond with low self-esteem (Gray-Little *et al.*, 1997).

Studies on psychological aspect of malocclusion shed light on the effect of malocclusion and orthodontic treatment on self-esteem of adolescents (Jung, 2010). Even though malocclusion can cause difficulties in oral hygiene, chewing, swallowing, speech, breathing, and predisposition for oral habits that can result in pain and discomforts, in majority of cases the key motivator for orthodontic treatment is the cosmetic impairment caused by the malocclusion (Albino *et al.*, 1990).

There is little information about impact of malocclusion on self-esteem of school children in Malaysia, and if the use of measure such as Self-Esteem Scale can lead to a better understanding of need for orthodontic treatment which can end up with patient satisfaction.

By further understanding the impact of malocclusion on self-esteem, it will aid in planning a realistic treatment, as expected by patients. Therefore the aim of the present study is to determine the impact of the severity of malocclusion on self-esteem among Malay ethnic students. This study also evaluated the relationship of malocclusion severity with self-esteem.

This prospective cross-sectional study was conducted on 252 Malay students aged 13 to 16 years old. Malay version of Rosenberg self-esteem Scale was used for assessing the child's self-esteem. A five-point Likert scale is used in Malay version. Overall score can range from 10 to 50. The cut off score for Rosenberg self-esteem was 10-29= low self-esteem; 30-39= moderate self-esteem; 40-50= high self-esteem (Bakar & Ismail, 2009). Oral examination was performed by the use of IOTN ruler to examine the severity of malocclusion. Each student was examined for the most severe malocclusion trait using Index of Orthodontic Treatment Need-Dental Health Component. IOTN-DHC has five categories, defining mild malocclusion (grade 1) to severe malocclusion (grade 5). Malocclusion traits which has been assessed included; Missing teeth, Overjet, Cross bites, Displacement and Overbite.

A total of 252 Malay students participated in the present study. 139 (55.2%) of participants were male and 113 (44.8%) were female. 133 (52.8%) were aged 13-14 and 119 (47.2%) were 15-16 years old. According to IOTN-DHC 81.3% of the study sample had some degree of malocclusion which required orthodontic treatment, and only 18.7% of the student showed no need for treatment. There was no statistically significant difference in severity of malocclusion between genders and age groups. Mean scores and standard deviation of the Rosenberg Self-Esteem Score (RSES) was 33.64 (3.40)±

Mean score and SD of RSES individual items ranged between 1.85 for the item “All in all, I am inclined to feel that I am a failure,” and 4.38 for the item “I take positive attitude toward myself.”

The mean score and standard deviation of RSES for male participants was 33.97 (\pm 3.38), while mean score and standard deviation for female was 33.23 (\pm 3.39). There was no significant difference in mean score of RSE between male and female and age groups ($p > 0.05$). The ANOVA test showed a significant difference in mean score of RSE between grade one and grade three of IOTN-DHC ($P < 0.05$). There was no statistically significant difference in mean score of RSE among other grades ($P > 0.05$). Pearson correlation coefficient analysis showed no relationship between severity of malocclusion and RSES. In univariate and multivariate regression analysis no significant association was seen between age, gender and IOTN-DHC with overall score of $P > 0.05$.

Further studies are needed to evaluate the impact of malocclusion severity on self-esteem including all ethnic groups in Malaysian society. Furthermore, using IOTN-Aesthetic Component, can help to understand the need for orthodontic treatment from point of view of the study sample, as IOTN-DHC only assess the normative orthodontic treatment need.

Keywords: Malocclusion severity, Self-esteem, Malay Teenagers

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References:

1. Albino, J. E., Alley, T. R., Tedesco, L. A., Tobiasen, J. A., Kiyak, H. A. & Lawrence, S. D. (1990). Esthetic issues in behavioral dentistry. *Annals of Behavioral Medicine*, 12(4), 148-155.
2. Choi, S.-H., Kim, J.-S., Cha, J.-Y. & Hwang, C.-J. (2016). Effect of malocclusion severity on oral health-related quality of life and food intake ability in a Korean population. *American Journal of Orthodontics and Dentofacial Orthopedics*, 149(3), 384-390.
3. Gray-Little, B., Williams, V. S. & Hancock, T. D. (1997). An item response theory analysis of the Rosenberg Self-Esteem Scale. *Personality and Social Psychology Bulletin*, 23(5), 443- 451.
4. Hewitt, J. P. (2009). *Oxford Handbook of Positive Psychology*. UK: Oxford University Press
5. Jung, M.-H. (2010). Evaluation of the effects of malocclusion and orthodontic treatment on self-esteem in an adolescent population. *American Journal of Orthodontics and Dentofacial Orthopedics*, 138(2), 160-166.
6. Liu, Z., McGrath, C. & Hägg, U. (2009). The impact of malocclusion/orthodontic treatment need on the quality of life: a systematic review. *The Angle Orthodontist*, 79(3), 585-591.
7. Petersen, P. E. (2003). The World Oral Health Report 2003: continuous improvement of oral health in the 21st century—the approach of the WHO Global Oral Health Programme. *Community Dentistry and oral epidemiology*, 31(s1), 3-24.
8. Thilander, B. & Myrberg, N. (1973). The prevalence of malocclusion in Swedish schoolchildren. *European Journal of Oral Sciences*, 81(1), 12-20.

Toxicity and Selected Neuroenzymes Activity in Relation to Vulnerability of *Heterotrigena itama* (Cockerell, 1918) Worker Bees to Common Insecticides

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The importance of stingless bee farming to harvest its honey has become a recognized economy with the establishment of Malaysia Standards MS 2683:2017 and MS 2679:2017. The production of these farm breed stingless bee and their colony health are important to be protected from hazardous chemicals and any potential exposure to toxicant in agricultural and public health insecticide products, as adversely experienced by honeybee farming in Europe. The high use of insecticides to control vector mosquito at suburban residential areas and agrochemicals at farms appear to be the vital threats to the stingless bee farming industry in Malaysia. Thus, an early detection of the problem and rapid assimilation of the stingless bee vulnerability to insecticide is essential to prevent adverse impact, subsequently rational pesticide choices can be made. The study evaluates the toxicological effects of the common group of insecticide against the stingless bee in term of the behavioural response and biochemical response. The *Heterotrigena itama* (Cockerell, 1918) was selected for the evaluation in consideration of it as the main species being farmed. The behavioural response relates to the knockdown, moribund and mortality response shown by the stingless bee upon exposure to papers impregnated using the Interim Guidance for Entomologist in Monitoring and Managing Insecticide Resistance in Aedes Mosquito (WHO/ZIKV/VC/16.1) published by World Health Organization in 2016. In reference to the WHO recommended insecticide bioassay procedure, the worker bees were exposed to a selected insecticide impregnated papers, namely permethrin 0.75%, malathion 5%, propoxur 0.1% and DDT 4%, which represent the pyrethroids, organophosphate, carbamate and organochlorine, respectively. Whereas for the behavioural response, the neuroenzyme activity within the body of the stingless bee was quantified using the techniques to detect insecticide resistance mechanisms (field and laboratory manual) (WHO/CDS/CPC/MAL/98.6) described by World Health Organization in 1998. The method detects the activity of non-specific esterase, acetylcholinesterase (AChE) and mixed function oxidases (MFO) neuroenzymes. Neuroenzyme bioassay on the worker bees were expressed quantitatively as optical density representing the content of the neuroenzyme capable to withstand the neurotoxic effects of the insecticides. The worker bees were collected from the stingless bee farm located at MARDI, Serdang. In each replicate of experiment, 5 worker bees were exposed to an impregnated paper. The stingless bees were provided with cotton wick moist with 5% honey solution throughout the experiment. The 30 replicates of exposure resulted 0.0±0.0%, 100.0±0.0%, 100.0±0.0%, 0.0±0.0% and 88.7±21.5% of mean percentage moribund/mortality at the 48th hour observation for the exposure to blank (control), permethrin 0.75%, malathion 5%, propoxur 0.1% and DDT 4%, respectively. In comparison to blank ($P < 0.05$), the result significantly indicated that the worker bees were highly vulnerable to permethrin, malathion and DDT, but less vulnerable to propoxur (refer to Figure 1). Neuroenzyme activities resulted significant increase ($P < 0.05$) from blank and varied intensities among the esterase, AChE and MFO neuroenzymes (refer to Table 1) that shows the presence of target site for the insecticides action. Therefore, the study suggests that the residue of permethrin, malathion and DDT have the capability to cause toxic effects to the farmed *H. itama* stingless bee upon continuous exposure of 24 hours.

Keywords: Stingless bees, *Heterotrigona itama*, toxicity, insecticide residue, neuroenzyme bioassay

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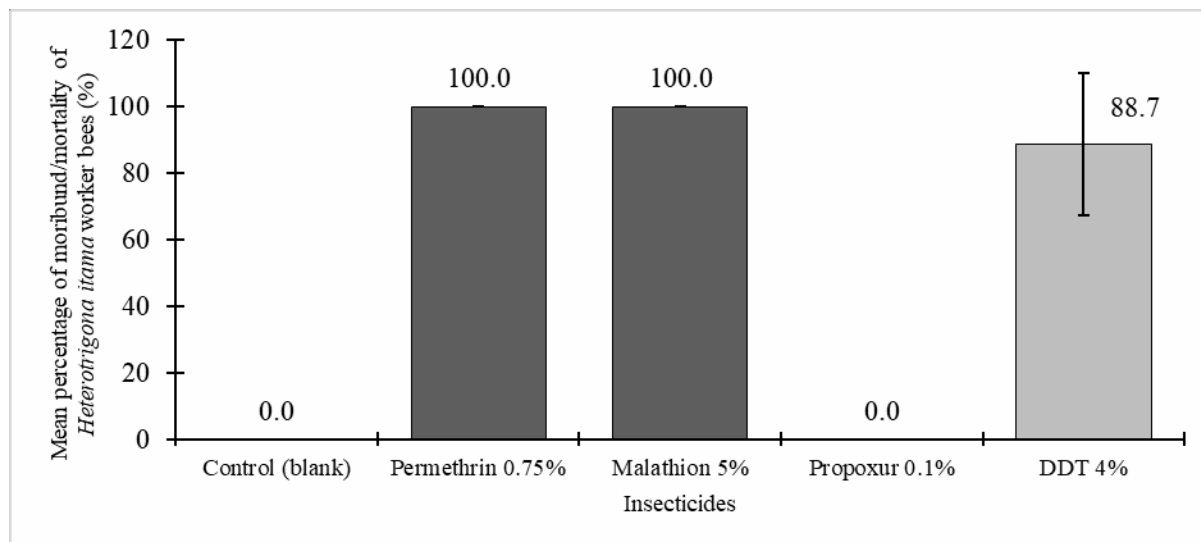


Figure 1. Mean percentage of moribund/mortality of *Heterotrigona itama* worker bees exposed to paper impregnated with permethrin 0.75%, malathion 5%, propoxur 0.1% and DDT 4% using the Interim Guidance for Entomologist in Monitoring and Managing Insecticide Resistance in *Aedes* Mosquito (WHO/ZIKV/VC/16.1) published by World Health Organization in 2016.

Table 1. Mean optical densities of non-specific esterase, acetylcholinesterase (AChE) and mixed function oxidases (MFO) neuroenzymes activity of the blank and the *Heterotrigona itama* worker bee whole body supernatant.

Supernatant	Esterase	Neuroenzyme	
		AChE	MFO
Mean optical density (\pm SD)*			
<i>Heterotrigona itama</i> whole body	2.87 \pm 0.19	1.36 \pm 0.02	1.68 \pm 0.09
Blank (without supernatant)	0.58 \pm 0.02	0.08 \pm 0.00	0.05 \pm 0.00

*Absorbance: OD at 570 nm (for esterase), OD at 410 nm (for acetylcholinesterase), OD at 630 nm (for mixed function oxidase).

References:

1. Chauzat, M.P., Faucon, J.P., Martel, A.C., Lachaize, J., Cougoule, N. & Aubert, M. (2006). A survey of pesticide residues in pollen loads collected by honey bees in France. *Journal of Economic Entomology*, 99(2), 253-262.
2. Cockerell, T.D.A. (1918). XXXV. Descriptions and records of bees. LXXX. The Annals and Magazines of Natural History, Zoology, Botany and Geology. 9(5), 384-390.
3. dos Santos, C.F., Otesbelgue, A. & Blochtein, B. (2018). The dilemma of agricultural pollination in Brazil: Beekeeping growth and insecticide use. *PloS one*, 13(7), e0200286.

4. Frazier, M. (2007). Protecting Honey Bees from Chemical Pesticides. *Senior Extension Associate. Penn State. Undated.*
5. Karunaratne, S.H. & Hemingway, J. (2001). Malathion resistance and prevalence of the malathion carboxylesterase mechanism in populations of mosquito vectors of disease in Sri Lanka. *Bulletin of the World Health Organization*, 79, 1060-1064.
6. Lee, C.Y., Loke, K.M., Yap, H.H. & Chong, A.S.C. (1997). Baseline susceptibility to malathion and permethrin in field-collected *Culex quinquefasciatus* Say from Penang, Malaysia. *Tropical Biomedicine*, 14, 87-92.
7. Liao, L.H., Wu, W.Y. & Berenbaum, M.R. (2017). Impacts of dietary phytochemicals in the presence and absence of pesticides on longevity of honey bees (*Apis mellifera*). *Insects*, 8(1), 22.
8. Franklin, E.N. (2016). Farmers Utilization of Insecticide Treated Bed Nets for Malaria Prevention in Ahoada East Local Government Area, Rivers State, Nigeria. *Agrotechnology*, 5(2): 150.
9. Renzi, M.T. (2013). Effects of pesticides on honey bees (*Apis mellifera* L.): study of a specific route of exposure and evaluation of biochemical-physiological changes in the assessment of the pesticides toxicity (Doctoral dissertation, Université d'Avignon).
10. Rosilawati, R., Lee, H.L., Nazni, W.A., Siti, F.F.F., Roziah, A., Khairul, A.M., Azahari, A.H., Liu, Q.Y. & Meng, F.X. (2018). Susceptibility Status and Resistance Mechanisms in Permethrin-Selected, Laboratory Susceptible and Field-Collected *Aedes aegypti* from Malaysia. *Insects*, 9(2), 43.
11. Sandrock, C., Tanadini, L.G., Pettis, J.S., Biesmeijer, J.C., Potts, S.G. & Neumann, P. (2014). Sublethal neonicotinoid insecticide exposure reduces solitary bee reproductive success. *Agricultural and Forest Entomology*, 16(2), 119-128.
12. Wan-Iryani, W.I. (2016). A review on beekeeping in Malaysia: History, importance and future directions. *Journal of Sustainability Science and Management*, 11(2), 70-80.
13. WHO. (1998). Techniques to detect insecticide resistance mechanisms (field and laboratory manual). World Health Organization, WHO/CDS/CPC/MAL/98.6.
14. WHO. (2016). Monitoring and Managing Insecticide Resistance in *Aedes* mosquito Populations: Interim guidance for entomologists. World Health Organization, Geneva, Switzerland, WHO/ZIKV/VC/16.1.
15. Yang, E.C., Wu, P.S., Chang, H.C. & Chen, Y.W. (2010). Effect of sub-lethal dosages of insecticides on honeybee behaviour and physiology. In Proceedings of International Seminar on Enhancement of Functional Biodiversity Relevant to Sustainable Food Production in ASPAC (pp. 9-11).

Effect of Salivary pH On Coating Durability of Aesthetic Archwire with Polytetrafluoroethylene (PTFE)

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In orthodontics, new generation of orthodontic archwires coated with tooth-coloured polymers have been marketed to address aesthetic concern of patients who undergo treatment. The coated archwire consists of core archwire of either NiTi or SS alloys coated with an outer layer of tooth-coloured polymers. The most widely used coating polymers are PTFE or commercially known as Teflon[®] and epoxy resin (Kravitz, 2013). The thickness of the coating layer varies from one manufacturer to another but is reportedly ranges about 20 to 25 μm (Choi et al., 2015). Apart from its excellent aesthetic property, PTFE also has ideal physical properties for clinical practice, as it is nonreactive heat resistant and hydrophobic (Tang et al., 2016). Clinically, the present coated archwires in the market have demonstrated partial or full coating loss following intraoral exposure as early as 21 days. This study evaluated the effect of pH on the durability of aesthetic archwire coating and observed the surface topography of the coating. NiTi archwires of 0.018" in diameter, which were readily coated with PTFE (Euroform[™] Cosmetic, Ortho-Care LTD, Yorkshire, UK) were sectioned into 20 mm segments. All specimens (N = 51) were divided into three groups; two groups treated with two different pH and control group. The pH of artificial saliva was adjusted to 6.75 and 3.5 using sodium hydroxide (NaOH) and lactic acid, respectively. Each specimen was immersed in individual test tubes containing 10 ml of modified solution, which were sealed and stored in a 37°C water bath for 28 days. After the time elapsed, the specimens were removed and subjected to mechanical stress using an electronic toothbrush for 210 seconds to mimic the recommended tooth brushing activity. Images were photographed using digital single lens reflex (DSLR) camera and coating loss measurement was done using Autodesk[®] AutoCAD[®] software. Surface topography of the specimens from each group was evaluated using scanning electron microscope (SEM).

PTFE treated groups showed that the most considerable changes were observed in the pH 3.5 group (**Fig 1A**), although these changes were not visible by the naked eye, but under SEM, the changes are manifested as a decomposition in the coating layer. PTFE 6.75 group (**Fig 1B**) has a change somewhat similar to those in the control group (**Fig 1C**), where relative roughness in both groups can be observed if compared to as-received specimen (**Fig 1D**).

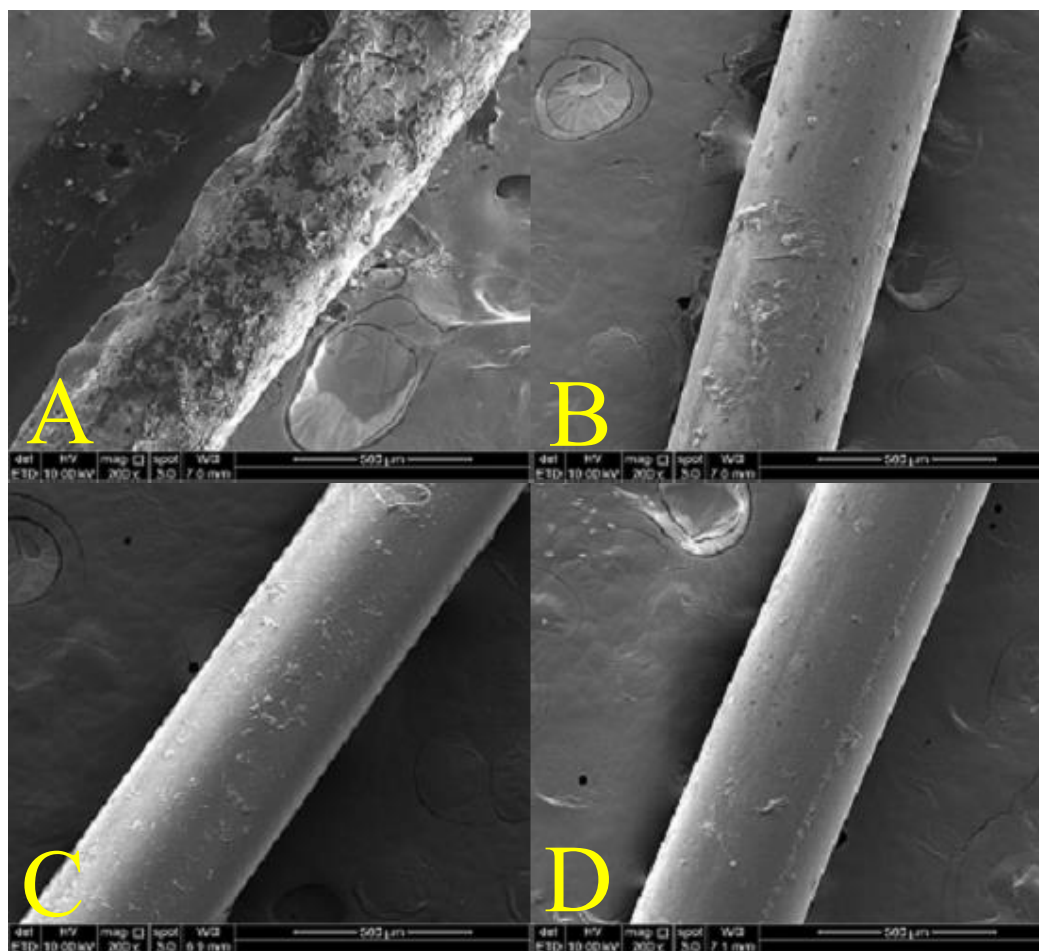


Fig 1: PTFE coated archwires at magnification 200x. **A**, treated with pH 3.5 followed by mechanical stress. **B**, treated with pH 6.75 followed by mechanical stress. **C**, treated with mechanical stress only. **D**, as received.

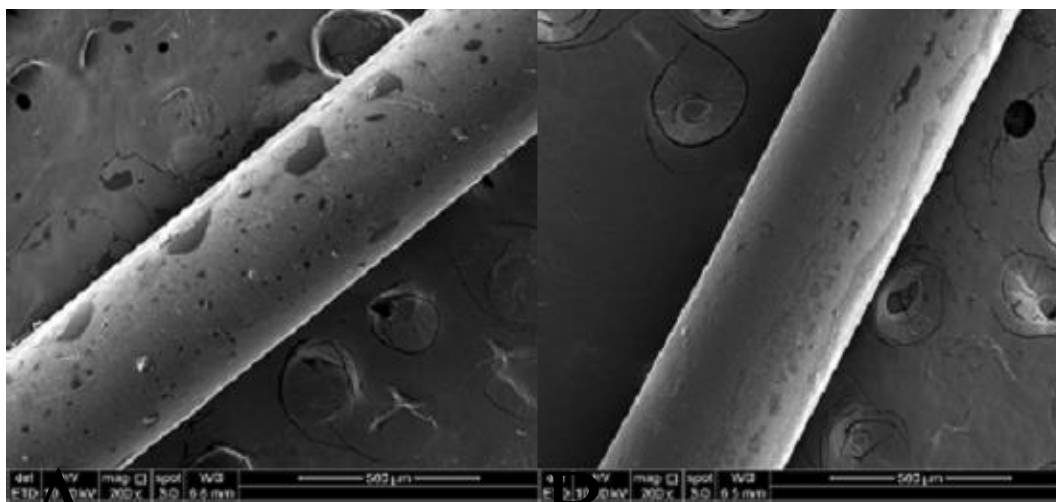


Figure 2: **A**, PTFE coated archwire treated with pH 3.5 only. **B**, PTFE coated archwire treated with pH 6.75 only.

When the coated archwires were treated solely in the two different acidic medium, the PTFE layer showed relative stability with no significant changes to its surface topography in both different pH values (Fig 2).

From this study, it was found that PTFE coating showed good stability with no coating loss. Despite this stability, PTFE coating layer has shown a sharp deterioration when it was treated with the pH 3.5 solution followed by mechanical stress test (**Fig 1A**). In addition, relative roughness was observed on the outer surface of the wire in the other treatments. This finding is consistent with the results reported by Ryu and his team (Ryu et al., 2015). In their study, they demonstrated that PTFE coating had less impact on the mechanical properties of the archwire, as well as less roughness and greater stability than the other wires types used in their study. However, Argalji et al. (2017) had another view in which they stated in their study that PTFE coated archwires showed a significant loss of the coating layer after being used in the oral cavity for 21 days. PTFE is a hydrophobic material (Kravitz, 2013). This property explains why the PTFE coating layer is stable. Argalji et al. (2017) proved that the thickness of the coating layer of as-received aesthetic archwires was in fact less than what was reported by the manufacturer, which is 0.002 inches and concluded that this could be the factor of poor stability of PTFE coating. The degradation of the surface of the PTFE coated archwire treated with pH 3.5 followed by mechanical stress (**Fig 1. A, B**) occurred as a result from the effect of the acidic medium and the mechanical stress. This is parallel with the finding by Giorgini et al. (2016) who concluded that there was an interaction between PTFE and different acids which varies in intensity and behaviour depending on the factors such as types of acid, manufacturing process and thickness of the coating layer. When comparing (**Fig 1. C**) with (**Fig 1. D**), it may able to notice the slight external difference but subject the wire to the mechanical stress after treatment with the acid solution led to this result of the destruction that appearance in (**Fig 1.A**). **Fig 1. D** shows the status of the as-received archwire when compared to **Fig 1. C** after being subjected to mechanical stress test, the changes in the coating layer is obvious. The result of this comparison supports the effect of the tooth brushing on the coated archwires. This result is similar to that of Yamodis (2011).

In conclusion, the results proved that different pH did not affected the PTFE coating polymers where there was no noticeable coating loss on the PTFE layer. This lead to the coating loss clinically is due to other factors like tooth brushing, salivary enzyme or oral bacteria.

Keywords: coated archwire, PTFE, Epoxy resin, pH

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References:

1. Argalji, N., SILVA, E. M. d., CURY-SARAMAGO, A. & MATTOS, C. T. (2017). Characterization and coating stability evaluation of nickel-titanium orthodontic esthetic wires: an in vivo study. *Brazilian Oral Research*, 31.
2. Choi, S., Park, D. J., Kim, K. A., Park, K. H., Park, H. K. & Park, Y. G. (2015). In vitro sliding-driven morphological changes in representative esthetic NiTi archwire surfaces. *Microscopy research and technique*, 78(10), 926-934.
3. Giorgini, L., Fragassa, C., Zattini, G. & Pavlovic, A. (2016). Acid Aging Effects on Surfaces of PTFE Gaskets Investigated by Fourier Transform Infrared Spectroscopy. *Tribology in Industry*, 38(3), 286-296.
4. Kravitz, N. D. (2013). *Aesthetic archwire*. Available at: <http://www.orthodonticproductsonline.com/2013/05/aesthetic-archwires/>
5. Ryu, S. H., Lim, B. S., Kwak, E. J., Lee, G. J., Choi, S. & Park, K. H. (2015). Surface ultrastructure and mechanical properties of three different white-coated NiTi archwires. *Scanning*, 37(6), 414-421.
6. Tang, Y., Peh, X., Zakaria, N. & Radzi, Z. (2016). Mechanical and Surface Properties of Initial and Working Aesthetic Orthodontic Archwires. *ANNALS OF DENTISTRY (ADUM)*, 23(1), 1-12.
7. Yamodis, S. A. (2011). "Effect of Toothbrush Abrasion on Frictional Resistance of Aesthetic Orthodontic Archwires". UNLV Theses, Dissertations, Professional Papers, and Capstones.