Vol. 4 No. 2 2019 ISSN : 2550-1364

Asia Pacific Journal of Health Sciences & Research

ASIA PACIFIC JOURNAL OF HEALTH SCIENCES & RESEARCH

KDN : PP19116/11/2016(034639)

Vol.4 No.2 2019
ISSN : 2550-1364
KDN : PP19116/11/2016(034639)

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Address : **No. 9, Jalan Teknologi Taman Sains Selangor, Kota Damansara PJU 5, 47810 Petaling Jaya, Selangor Darul Ehsan, Malaysia**
Printer : **TED PRINT SDN BHD (965577-u)**
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Content

Original article

Functional activity of antisera against the recombinant Zika virus protein subunits expressed in Escherichia coli

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Abstract

Background: The recent global Zika virus (ZIKV) outbreak highlights the urgent development of ZIKV vaccines that offer rapid, precise and specific protection to those living in the high-risk regions. Despite many publications on *in vitro* development of ZIKV subunits as the vaccine candidates, due to the lack of knowledge on humoral and cellular immune responses against virus vaccines, a commercialized vaccine against Flavivirus in Philippines has been suspended due to a health scare in the public. Moreover, the close relationship between DENV and ZIKV has indicated serological cross-reactivity between both viruses. This has led to greater attentions to precautions needed during the design of ZIKV and DENV vaccines. **Materials and Methods**: We pre-selected, synthesized and expressed the domain III of ZIKV envelope protein (namely rEDIII) based on a previously established report (GenBank: AMC13911.1). The characteristics of purified ZIKV rEDIII was tested using SDS-PAGE, Western blotting, and LC-MS/MS. Then, we assessed the

possible outcome of pre-existing immunity against the rEDIII proteins by conducting dot-blotting assays using mice antisera pre-immunised with ZIKV particles (ZIKV strain: MRS_OPY_Martinique_PaRi_2015, GenBank: KU647676). **Results**: We were able to express ZIKV rEDIII protein in bacterial system with valid protein conformation. Also, it was interesting that the pre-immunised hamster antiserum was able to recognise the rEDIII, which was sourced from a different ZIKV strain (GenBank: AMC13911.1). **Conclusion**: Despite its great antigenicity in eliciting humoral and cellular immunity against ZIKV infection, our finding calls for greater attention to evaluate the details of ZIKV rEDIII as a stand-alone vaccine candidate.

Keywords: ADE, antiserum, recombinant rEDIII, Zika virus

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Introduction

Zika virus (ZIKV), a member of the *Flaviviridae* family, is transmitted between humans by its main mosquito vectors, *Aedesaegypti*or *Aedesalbopictus*[1]. ZIKV carries a single-stranded, positive-strand RNA genome of about 11 kb in length. Despite its first isolation from *Rhesus macaque* in 1947, limited reports on human infection is available. This is largely due to its self-limited illnesses including low-grade fever, headache, myalgia and arthralgia [2]. There are two lineages (African and Asian) and three genotypes (East African, West African, and Asian) of ZIKV circulated in tropical and subtropical regions. As

of 2018, the diagnosis assays for ZIKV comprised of 5 serological assays and 14 molecular assays with Food and Drug Administration Emergency Use Authorisation (FDA EUA), which have been well reviewed by in 2018 by Theel and Hata[3]. On the other hand, in terms of vaccine candidate discovery and development, a recent article by Alan Barett reported that over 45 vaccine candidates have been discovered, with at least 9 are currently in clinical evaluation [4]. Nevertheless, it is not plausible to develop an efficacious ZIKV vaccine in near future due to the serological cross reactivity of antibodies between Dengue virus (DENV) and ZIKV [5]. In addition, similarities in transmission process, disease manifestations and transmitting vectors between Zika fever and Dengue fever are often confused [6], which have further halted the development of ZIKV-specific vaccine candidate.

Since its declaration as Public Health Emergency of International Concern by World Health Organisation (WHO) in February 2016, Zika virus has been associated with microcephaly and neurological complications such as Guillain-Barré syndrome [7]. Since then, international attention has been brought towards the rapid chain of disease outbreak, which was spread throughout South, Central and part of North America, followed by Asia Pacific [8]. Vector transmission of Zika fever occurs mainly in tropical regions. However, cases in returning travellers have frequently been reported in locations including Europe, US, Australia, New Zealand, Japan, UK, and China [9,10].

Upon infection, ZIKV was reported to persist in body fluids, such as urine or saliva, for longer than that of in the blood [11]. This becomes an important consideration in the development of rapid and effective tools for ZIKV detection. Prior to 2018, several research groups reported various ZIKV detection strategies, including a newly developed strategy - liposome-based

immunoassay reported by Shukla *et. al*. [12], who reported the low sensitivity of 5 commercially available immunoassays to detect ZIKV infection [13]. Soon after, Powley *et. al.* reviewed the current methods of ZIKV detection and their limitations [14]. The authors highlighted a few restrictions including the need of expensive machineries, trained personnels, intensive laborious processes, viral RNA stability, lack of specific anti-ZIKV antibodies, and possibilities of false-positive results with current diagnostic techniques. In addition, Pawley *et. al.* also emphasised the importance of anti-ZIKV monoclonal antibodies in the development of novel point-of-care paper-based detection method [14]. This has again emphasised the importance of the domain III of ZIKV envelope protein, which carries a strong antigenicity and greatest power of discrimination from other members of Flavivirus[15], as an ideal protein candidate in ongoing and future development of point-of-care testing for active infection for ZIKV.

Recombinant domain III of ZIKV envelope protein (rEDIII) has been previously expressed and purified using different protein expression systems, including yeast [16], insect cells [17], plant cells [18] and bacterial cells [17]. Sylvia *et. al.* proved the integrity of rEDIII through SDS-PAGE, Western blot and immunoblotting [19], while Yang *et. al.* described the generation and immunogenicity of the ZIKV rEDIII as a protein subunit vaccine candidate, which was also demonstrated to elicit anti-rEDIII monoclonal antibody in pre-clinical studies [20]. In accordance with this, this study was designed not only to construct a protein-expression plasmid for recombinant ZIKV envelope protein (domain III, rEDIII) production, but also to assess the possibilities of the ZIKV rEDIII to cause antibody dependent enhancement (ADE) or serum sickness (SS) in the recipients, especially those who had exposed to ZIKV infection prior to

receiving vaccine which contains rEDIII as the vaccine candidate.

Materials and methods

Zika virus EDIII gene

Complete coding sequence of the domain III of Zika virus (strain: PRVABC59) envelope protein (EDIII) was retrieved from National Centre for Biotechnology Information (NCBI) (GenBank accession number: AMC13911.1) [20,21]. Gene block and primers were synthesised (Integrated DNA Technologies, IDT®) and stored in -20 ℃ until used.

Gene cloning and protein expression

ZIKV EDIII gene was synthesised and cloned in pUCIDT plasmid vector, namely pUCIDT-ZVEDIII. The plasmid was transformed into *Escherichia coli* DH5α strain for long-term storage at -80 ℃. After plasmid purification, ZIKV EDIII coding sequence was amplified using primers (forward: TCTGCAGCTGGTACCGCGTTCACATTCACCAAGATCCC GGCTG; reverse: TCAAGCTTCGAATTCTGCTTTTCCAATGGTGCTGCCAC TCCTG) with the following PCR conditions: 1 cycle of 94 ℃ (2 minutes); 35 cycles of 94 ℃ (45 seconds), 55 ℃ (45 seconds), 72 ℃ (1 minute); 1 cycle of 72 ℃ (10 minutes); on hold at 4 ℃ until use. PCR product was cloned in-frame into pRSET-B protein expression vector (Invitrogen, CA, USA) using In-Fusion® HD Cloning Plus (Takara Bio, USA). Recombinant plasmid was transformed into competent *E. coli* BL21 (DE3) strain for protein expression analysis.

For protein expression, an overnight culture of transformed BL21 (DE3) *E. coli* was diluted to 1:100 with Luria Bertani broth supplemented with ampicillin at final concentration of 75 μg/mL. Bacteria culture was incubated (37 ℃, 180 rpm) until OD_{600} of

0.50 was reached. Protein expression was induced by the addition of IPTG to the final concentration of 1 mM and incubation was further conducted for 3 hours (37 ℃, 180 rpm). Then, the cells were harvested by centrifugation (3000 *g*, 4 ℃, 2 minutes). Cell pellet was resuspended in SDS reducing buffer, aliquoted into 50 µL, heated at 99 ℃ for 10 minutes before loading into a 12% SDS acrylamide gel.

SDS-PAGE and Western blot

SDS-PAGE was conducted in vertical direction at 100 V in a 1x Tris-glycine running buffer (25 mMTris, 192 mM Glycine, 0.1% SDS, pH 8.3) [22]. After that, protein bands were stained with R-250 Coomassie Brilliant Blue stain. Another duplicated gel was subjected to Western blotting. Protein bands were transferred onto PVDF membrane [23], followed by blocking (5% BSA, 1 hour, 25 ℃), primary antibody (anti-Xpress monoclonal antibody, 1:5000 dilution, 1 hour, 25 ℃), and secondary antibody (anti-mouse IgG, 1:5000 dilution, 1 hour, 25 ℃). Protein bands were visualised by addition of substrate (BCIP/NBT).

rEDIII purification

ZIKV rEDIII was purified with gradual decrease of urea concentration (8 M, 6 M, 4 M, 2 M and 0 M) to progressively remove urea through dialysis. All buffers (except elution buffer) were supplemented with 20 mM imidazole to reduce nonspecific binding of unwanted protein to the HisTrap HP histidine-tagged protein purification columns (GE Healthcare). In brief, after protein expression, cell pellets of transformed BL21 (DE3) *E. coli* was suspended in dissolving buffer supplemented with 8 M urea. Mixture was incubated in HisTrap HP histidine-tagged protein purification columns at room temperature for 30 minutes, followed by washing steps using a series of buffers supplemented with 6 M, 4 M, 2 M and 0 M of urea. Lastly, rEDIII was eluted with elution buffer (supplemented with 0 M urea and 500 mM of

imidazole). Eluents were subjected to dialysis using 1x PBS buffer at 4 ℃ for 2 hours. Purified rEDIII was kept at 4 ℃ for further analyses.

LC-MS/MS

The rEDIII protein band was excised from polyacrylamide gel and the sample was prepared for *de novo* protein sequencing using in-gel digestion according to manufacturer's protocol (Agilent Technologies, Inc., 2015). Briefly, the excised gel slice was destained with 200 mM of ammonium bicarbonate (ABC) in 40% acetonitrile (ACN), followed by reduction and alkylation by DTT and IAA respectively. After that, gel slice dehydrated by 100% CAN (15 min, 37°C). The dehydrated gel slice was incubated with trypsin (16 hours, 37 ℃) and the reaction was stopped by addition of formic acid. The tryptic peptides were further extracted from the gel slices using 50% ACN and 100% ACN for 15 min each. The recovered peptides were analysed using Agilent 1200 HPLC-Chip/MS interface, coupled with Agilent 6550 iFunnel Q-TOF LC/MS. The *de novo* sequences was analysed and aligned using PEAKS 8.0 software [24].

rEDIII protein integrity test

Gold Syrian hamsters were bred and housed at the specific pathogen free (SPF) animal facilities, Monash University Malaysia. Ethics approval for animal housing and experimentation were obtained (Monash Animal Ethics: MARP/2017/060). Hamsters were administered subcutaneously with Zika virus (strain: MRS_OPY_Martinique_PaRi_2015, NCBI: KU647676) with TiterMax adjuvant at 10^7 pfu. After 35 days, serum sample were collected to determine its binding ability towards ZIKV rEDIII proteins.

The integrity of ZIKV rEDIII was determined through Dot Blot assay. First, purified rEDIII was immobilised on a PVDF

membrane at 1 µg per dot. rEDIII were dried at 25 ℃ before blocking (5% BSA, 1 hour, 25 ℃). After washing, mouse serum (1:500) were applied (1 hour, 25 ℃), followed by anti-mouse IgG (1:5000, 1 hour, 25 ℃) before visualisation using BCIP/NBT as the substrate. Control spots were also conducted concurrently using mock-infected mouse serum.

Results

rEDIII expression

The coding sequence of ZIKV rEDIII (GenBank accession number: AMC13911.1) was synthesised and cloned in-frame into pRSET-B protein expression vector for protein expression using *E. coli* BL21 (DE3). After SDS-PAGE, based on the molecular weight, the rEDIII was expressed at its expected size (total of 14 kDa) with the 11 kDa moiety carrying a 6x histidine tag at the N-terminal of the recombinant protein. Hence the total expected protein size of 14 kDa (Fig 1).

Western blotting

Western blot was conducted on PVDF membrane using anti-Xpress antibody as the primary antibody. The result showed that ZIKV rEDIII was expressed at the expected size (14 kDa) (Fig 2).

rEDIII purification

ZIKV rEDIII was purified using HisTrap HP histidine-tagged protein purification columns. rEDIII was mainly detected in insoluble inclusion bodies (Fig 3A, lane 2). Lane 2, 3 and 4 was loaded with eluents of washing buffers. The rEDIII was not detected these lanes (Fig 3B, lane 2, 3 and 4). Lastly, purified rEDIII were successfully eluted, which was shown in a single protein band (Fig 3B, lane 5 and 6).

LC-MS/MS

The sequences of tryptic digested peptides of ZIKV rEDIII were aligned with the protein database through PEAKS DB search and showed alignment with ZIKV polyprotein (A0A0U4ETI0) starting from position 601 to 699 (Fig 4).

rEDIII protein integrity test

Dot blot assay was conducted to test the integrity of purified rEDIII. Antisera derived from mock-infected and ZIKV - infected mice and were used. The results showed that antisera was able to recognise the purified rEDIII (Fig 5), which also explains the chances of administered ZIKV rEDIII being recognised by antibodies produced by recipients who were previously infected by ZIKV. On the other hand, no binding to ZIKV rEDIII was observed when the antiserum of mock-infected mice was used in the dot-blot assay.

Figures

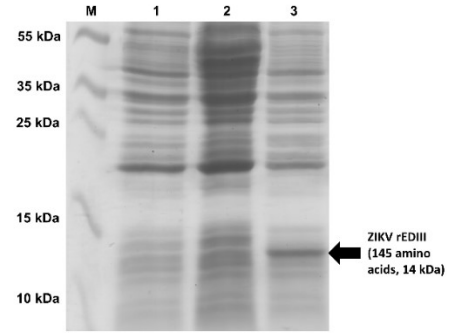

Fig 1. Expression of recombinant domain III of Zika virus (ZIKV) envelope protein (rEDIII) in *Escherichai coli* BL21 (DE3). (M) Protein marker; (1) Negative control: The cellular lysate of untransformed BL21 (DE3) *E. coli* ; (2) Supernatant of transformed and IPTG-induced BL21 (DE3) *E. coli* after sonication; (3) Pelleted inclusion body and cellular debri of

transformed and IPTG-induced BL21 (DE3) *E. coli* after sonication. The presence of ZIKV rEDIII is indicated by black arrow.

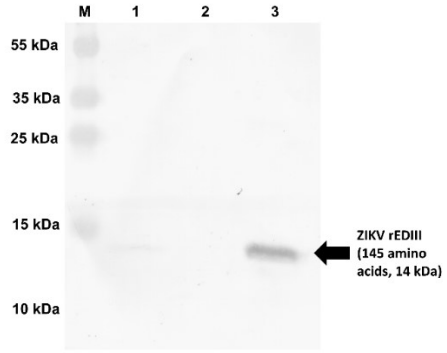

Fig 2. Western blotting analysis shows the presence of the recombinant domain III of Zika virus (ZIKV) envelope protein (rEDIII) at the expected position (14 kDa, indicated by black arrow). (M) Protein marker; (1) Negative control which contains the cellular lysate of untransformed *E. coli* BL21 (DE3); (2) Supernatant of transformed and IPTG-induced *E. coli*BL21 (DE3) after sonication; (3) Pelleted inclusion body and cellular debri of transformed and IPTG-induced *E. coli* BL21 (DE3) after sonication.

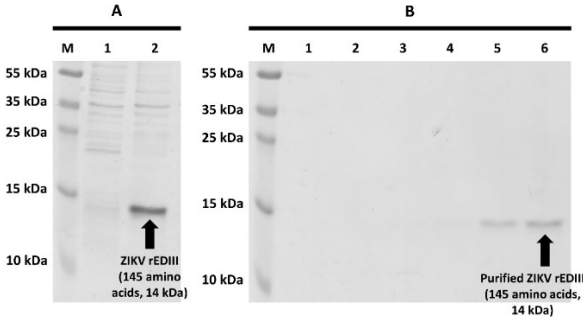

Fig 3A. SDS-PAGE analyses of different portions of bacterial cell lysate after IPTG induction. (M) Protein marker; (1) The

supernatant of cellular lysate after sonication and centrifugation; (2) The pelleted inclusion body and cell debri after sonication and centrifugation. The distinctive protein band (indicated by black arrow) shows the expected position of ZIKV rEDIII which present in the inclusion body of the *E. coli*.

Fig 3B. The replicated samples of lane 2 in figure 3A were directed to protein purification using Ni-charged resins. (1) The first flow-through of ZIKV rEDIII inclusion body dissolved in buffers supplemented with 8M urea; (2, 3 & 4) Flow-through of washing buffers supplemented with 6 M, 4 M and 2 M of urea, respectively; (5 & 6) First and second elution of ZIKV rEDIII from the Ni-charged resins using elution buffers supplemented with 500 mM imidazole. ZIKV rEDIII was successfully purified with the expected size of protein indicated by black arrow (14 kDa).

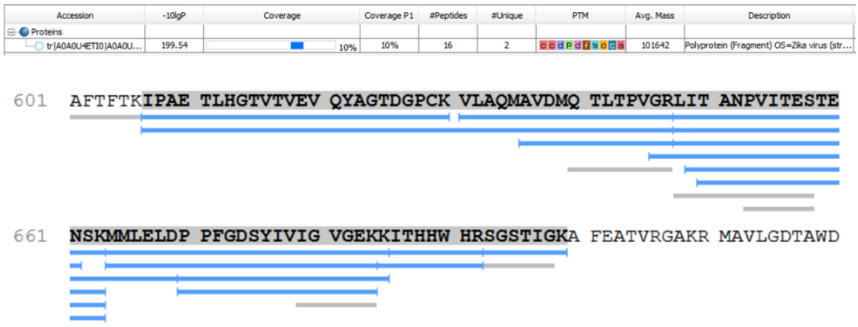

Fig 4. Alignment of tryptic-digested peptides of recombinant ZIKV rEDIII using PEAKS 8.0. The amino acids of subject sequence (domain III of ZIKV) are bold and highlighted in grey. All query sequences are illustrated in blue.

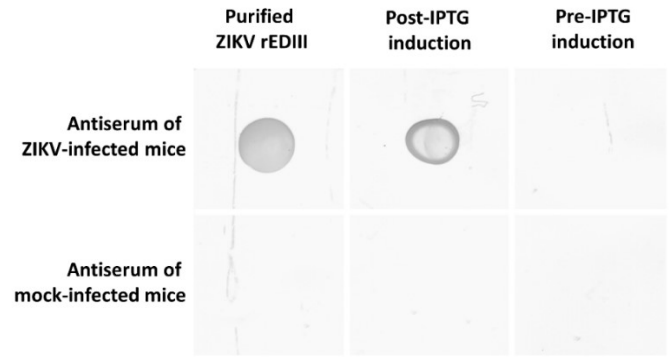

Fig 5. Dot-blot analyses of recombinant domain III of Zika virus (ZIKV) envelope protein (rEDIII). All 6 samples were loaded with either 1 μg of purified ZIKV rEDIII, or bacterial cell lysate of post- or pre-IPTG induction. All samples were air dried prior to incubating with antiserum derived from either ZIKV-infected or mock-infected mice.

Discussion

In this study, we employed the coding sequence of ZIKV rEDIII reported by Yang *et al*. (GenBank accession number: AMC13911.1) in their previous studies for immunogenicity assessments in mammals [20,21]. Yang et al. concluded that ZIKV rEDIII produced in either plants or *E. coli* had successfully induced immune response to confer sufficient protection against ZIKV infection in mice. However, being one of the most neglected diseases in tropical regions, Zika fever manifested in many individuals without medical attentions, which individuals have developed natural-active immunity against the rEDIII. In order to assess the specificity of these antisera against this ZIKV rEDIII, this study developed and obtained mice antisera containing natural-active antibodies for antibody specificity tests against the ZIKV rEDIII.

A number of concerns have been raised for marketed vaccines, including polio vaccine and measles vaccine which cause toxic shock syndrome [25,26]. Although some avoidable cases were reported to be human-caused [27], the major dengue vaccination programme in Philippines led to a theoretical elevated risk of dengue haemorrhagic fever (DHF) in seronegative vaccine recipients [28]. Although more seroepidemiological surveillance data is needed, adversed manifestations of Zika virus infection due to antibody-dependent enhancement (ADE) has been reported [29,30]. These data highlighted the possibilities of rEDIII to cause ADE in its recipients.

We infected mice with active virus particles to raise antiserum against ZIKV. In another parallel experiment, the rEDIII was expressed in BL21(DE3) *E. coli*, extracted and purified. The protein identity was confirmed with LC-MS/MS and PEAKS DB search, with the native structure of the rEDIII confirmed by dot blot assays. Meanwhile, the dot blot assay also proved the hypothesis that the antibodies produced by natural active immunity in mammals are able to recognise the our ZIKV rEDIII protein.

ADE caused by administration of vaccine is not uncommon. Understanding immune responses to viral infections is crucial in deciphering the molecular mechanisms behind the enhanced illness by pre-existing antibodies found in the serum of vaccine recipients. Usually, ADE is caused by type III hypersensitivity of the immune system against the vaccine candidate. Cases of ADE after vaccine administration were reported for several vaccine candidates including inactivated and purified influenza virus [31], recombinant Hepatitis B virus [32] and Dengue virus [33,34]. Since the knowledge and understanding of ADE caused by Zika virus infection is sparse, more attentions should be placed in the development of rEDIII into ZIKV vaccine, where the protein

subunit may develop ADE in the vaccine recipients. This is especially important when Dengue virus, the virus that is prevalent in causing ADE, and Zika virus are taxonomically close, with evidences showing serological cross reactivity of antibodies against both viruses in mammals [5,15,35–37].

Dejnirattisai *et al*. reported that most of the antibodies against DENV epitopes also bound to ZIKV, but unable to neutralise ZIKV and instead promoted ADE [36]. Recently, other researchers have discussed the risk-to-reward ratio of developing ZIKV vaccine, with regards to current controversial data and unknown interplay between members of flavivirus[30]. This important information must be taken into considerations especially during the development of any virus vaccine.

Several improvements can be made to enhance the bioavailability and effect of ZIKV vaccine using rEDIII as the vaccine candidate. This can be done through the optimisation of adjuvant, which has been thoroughly reviewed by Hogenesch*et al*. in 2018 [38]. HogenEsch*et al.* described the pharmacokinetics of aluminium-based adjuvants, characteristics of antigens, and formulations of vaccines with aluminium adjuvants. On the other hand, in light with its potential wide global distributions of ZIKV vaccine across different continents, the thermodynamic stability of ZIKV rEDIII can also be improved by molecular structural modifications or optimisation of subcellular protein expression [39].

In conclusion, although rEDIII can be the ideal protein candidate in the development of ZIKV vaccine, our results, in conjunction with several previous studies, call for a greater attention on the mechanisms of ADE in vaccine recipients. Our findings are also useful for ZIKV rEDIII applications in the field of virus diagnostics, vaccine developments and viral disease therapies.

Acknowledgements

The authors acknowledge the continuous support given by the Virus–Host Interaction Research Group (Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia), Biopharmaceutical Research Unit, Faculty of Pharmacy (SEGi University), and Research and Innovation Management Center (RIMC, SEGi University).

Conflicts of Interest

No potential conflict of interest was reported by the authors.

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Original Article

Knowledge, Attitude and Practice towards Self-Medication of Non-Steroidal Anti-Inflammatory Drug (NSAID) among First-Year Non-Health Science Undergraduates in Klang Valley, Malaysia

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Abstract

Background: Non-Steroidal Anti-Inflammatory Drugs (NSAID) are commonly used analgesics among the public, secondary to paracetamol. Despite of its wide usage, the knowledge, attitude and practice on NSAID is yet to be determined among the first-year non-health science undergraduates in Klang Valley, Malaysia. **Objective:** The objective of this study is to assess the level of knowledge, attitude and practice of NSAID usage among the first-year non-health science undergraduates regarding self-medication of NSAID.

Materials & Methods: A cross-sectional study design was adapted. A self-administered questionnaire was distributed to 402 first-year non-health science undergraduates from various universities located in Klang Valley, Malaysia. Undergraduates who were on long term usage of NSAID was excluded in the study and convenient sampling was incorporated. **Results:** A total of 56.47% of participants showed low knowledge level towards NSAID and an average level of attitude (mean score: 20.16 ± 2.674) was obtained from the study. Up to 58.95% of participants were adherent towards the usage of NSAID according to advices given. The result further showed significant association between the education stream and their attitude (p=0.012), as well as between the ethnicity and their practice

level (p=0.025). **Conclusion:** In conclusion, the level of knowledge of NSAID is low but a satisfying level of attitude and practice on NSAID usage are noticed among the first-year non-health science undergraduates.
Keywords: Non-Steroidal Anti-Inflammatory Drug, Self-Medication, Undergraduate
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Introduction

Non-steroidal anti-inflammatory drug (NSAID) is a group of analgesic and anti-inflammatory agent, or better known as pain reliever for the public. It inhibits the production of cyclooxygenase (COX), an enzyme in the body which is responsible for signalling pain and inflammation through production of prostaglandin, thus exhibiting the anti-inflammatory and analgesic effect[1]. NSAID is classified as part of the poison drug and it can only be obtained either through prescription or dispensed by the healthcare professionals due to its legality in Malaysia's setting[2]. Even though the side effects are serious, such as stomach ulceration and kidney failure upon misuse of NSAID in daily life, the usage of NSAID is still high among the public, especially for self-medication cases in relieving pain. Self-medication is defined as an act of treating a self-recognised disease without seeking any consultation from the healthcare professionals through the usage of medications[3]. The practice of self-medication with analgesic is getting more common among the public nowadays due to various ~~of~~ factors and this has become a worrying issue as the public might not be using the appropriate type or dose of medication for their

complaints, especially when they suffered from other health issue during the treatment period[4].

The knowledge on NSAID among the public is relatively low which further increases the risk of getting dependence and adverse drug reaction due to misuse of NSAID[5] and it was noted that in a study, more than half of the respondent were not aware on the side effects of NSAID as well as the possibility of drug dependence on NSAID after prolonged usage[6]. This is an alarming phenomenon especially in teenagers whom are common with the complaint of headaches due to studies or sports injuries, where the self-medication practice of analgesic is high[7,8]. On the other hand, studies have shown that higher education level, majoring in health sciences programmes did show a better knowledge towards NSAID, but it also portraited a higher practice rate of self-medication using it, even when they are aware on the adverse drug reaction[9,10,11]. Furthermore, public also showed a negative attitude towards NSAID where most of them were not aware on the toxic dose of NSAID and this further affected the safe use of NSAID among the public[12,13,14].

University undergraduates are always working under pressure to achieve their study goals, making use of their time to study and constantly exposed themselves towards minor illness, especially headaches. As there are numerous types of analgesics, which includes paracetamol and NSAID, which contains different types of active ingredients, there is a possibility that the undergraduates might chose the unsuitable medication for themselves[14]. In short, the aim of the survey is to assess the level of knowledge, attitude and practice of NSAID usage among users and non-users from first-year non-health science undergraduates regarding the self-medication of NSAID. As most of the research surveys target on various years of health-science undergraduates, this survey targeted the first-year undergraduates who are newly introduced

to their respective degree programme as their stress level might be lower compared to the higher education level undergraduates. Besides that, by focusing on the non-health science undergraduates, we would be able to obtain the raw result from population that had not been exposed to any health sciences related elements as this will affect the result in knowledge and attitude of NSAID.

Materials and Methods:

Study design, population and sample size

A cross-sectional study design was adopted. A self-administered questionnaire was distributed to the first-year non-health science undergraduates among the universities in Klang Valley, Malaysia from August 2018 to October 2018. Convenient sampling technique was used during the distribution of questionnaires to the 402 participants and a minimum sample size of 385 participants was needed to achieve 95% confidence interval. The minimum sample size was calculated using the Cochran's formula $n_0 = \frac{z^2 pq}{e^2}$, through the assumption of maximum variability (50% of total), 95% confidence interval and ±5% of precision (p=0.5;q=1-0.5;e=0.05;z=1.96; $n_0 = \frac{(1.96)^2(0.5)(0.5)}{(0.05)^2} = 385$)

Inclusion and exclusion criteria

First-year non-health science undergraduates, including both Malaysian and International students from the universities located in Klang Valley, Malaysia were included in the study. However, if the participant had been on long term usage of NSAID (defined as more than two weeks' usage for all types of NSAID except aspirin and more than twelve weeks for aspirin) in the previous twelve months, they were excluded from the study.

Study instruments

The self-administered questionnaire was prepared by adapting questions from different reference articles[7,15,16] and reconstructed into a total of four sections with six to seven questions respectively for each section for participants to answer. The questionnaire consisted of a total of twenty-eight questions and had been validated through phase study by distributing to eighteen participants who acted as respondent for pilot study of the survey. A table of common NSAID that is being sold in Malaysia was included in the questionnaire after the consent form for the participants to identify the respective analgesic used and paracetamol or acetaminophen was notified to the participants as not part of NSAID due to its minor anti-inflammatory effect and were excluded in the table provided.

The first section was targeting on the socio-demographic details of the participants, followed by the second section which contained questions related to the knowledge of NSAID. Each correct answer in this section was awarded with 1 point and a total of 7 points could be obtained in this section. The third section was statements related to the attitude of participants towards NSAID usage and participants were asked to answer through the usage of Likert Scale. Points were given according to the scoring of the statement excluding Q15 where a score of 1 was awarded with 5 points instead. A total of 30 points could be accumulated in this section.

The last section comprised of questions related to the practice towards NSAID usage among the participants and each 'No' answer was awarded with 1 point, excluding Q26 where a 'Yes' answer was awarded with 1 point instead and a total of 6 points could be obtained in this section. The final two questions were multiple answers question which served as extra information to

understand the sources of information related to NSAID and the reason of self-practising with NSAID among the participants.

Data Collection

The questionnaires were answered face-to-face and the participant was given a maximum of 15 minutes to answer the questionnaire and it was collected on the same day.

Statistical Analysis

The data collected was analysed using Statistical software SPSS version 23 and was interpreted using descriptive statistics and chi-square test. $p<0.05$ was considered significant.

Result

Socio-demographic

A total of 402 participants were included in the survey with a mean age of 20.26 ± 1.71 years old. Among the 402 participants, 12.9% (n=52 of 402) of participants were NSAID user during the past twelve months, which also known as current users in the article thereafter and 87.1% (n=350 of 402) of the participants were non-user as per recorded in Table 1.

Table 1: Socio-demographic of participants (n=402)

Characteristic	Total Participants (n=402)	User (n=52)	Non-User (n=350)
Age (y), mean (± SD)	20.26 ± 1.71	20.87 ± 2.02	20.17 ± 1.64
User of NSAID, n (%)			
Yes	52 (12.9)		
No	350 (87.1)		
Gender, n (%)			
Male	165 (41.0)	20 (38.5)	145 (41.4)

Female	237 (59.0)	32 (61.5)	205 (58.6)
Nationality, n (%)			
Malaysian	334 (83.1)	46 (88.5)	288 (82.3)
Other	68 (16.9)	6 (11.5)	62 (17.7)
Ethnicity, n (%)			
Chinese	208 (62.3)	15 (33.3)	193 (66.8)
Indian	50 (15.0)	12 (26.7)	38 (13.1)
Malay	63 (18.9)	18 (40.0)	45 (15.6)
Others	13 (3.9)	0 (0.0)	13 (4.5)
Education Steam, n (%)			
Art Stream	342 (85.1)	42 (80.8)	300 (85.7)
Science Stream	60 (14.9)	10 (19.2)	50 (14.3)

Knowledge

Based on the result showed in Table 2, it was noted that 47.8% of the total participants had answered correctly for Question 7, which the question asked about the indication and effects of NSAID and among the current users, a high percentage of 86.5%had the correct answer, compared to 42.0% from the non-user group.

Table 2: Participants' knowledge on NSAID

QN	Statement	Correct (%) (n=402)	User (n=52)	Non-User (n=350)
1	**NSAID medicines can relieve pain, reduce fever and contain anti-inflammatory effects.**	192 (47.8)	45 (86.5)	1472.0)

2	**NSAID medicine can reduce formation of blood clots.**	50 (12.4)	6 (11.5)	44 (12.6)
3	**NSAID medicines may cause harm to your kidney if they are misused.**	185 (46.0)	41 (78.8)	144 (41.1)
4	**NSAID medicines may cause stomach ulcer if they are misused.**	111 (27.6)	23 (44.2)	88 (25.1)
5	**All the NSAID medicines registered in Malaysia are totally safe to use without any side effects.**	38 (9.5)	7 (13.5)	31 (8.9)
6	**NSAID medicines can be consumed along with corticosteroid (i.e. Prednisolone) or herbal medicines such as Gingko Biloba and St. John's wort.**	35 (8.7)	8 (15.4)	27 (7.7)
7	**NSAID medicines are the same as antibiotic.**	42 (10.4)	9 (17.3)	33 (9.4)

***QN indicates the question number.**

***Data reported in brackets represents the percentage (%).**

According to the result showed in Table 3, there was no significant difference between the genders, nationality, ethnicity among the Malaysians and the education stream in the scoring of knowledge of NSAID, however significant difference was found between the users' group ($p<0.05$).

Table 3: *p*-value for each variable in knowledge section.

QN	*p*-value *				
	Gender	**Nationality**	**Ethnicity**	**Education Stream**	**Users**
1	0.258	**0.007**	**0.042**	**0.006**	**<0.001**
2	0.086	0.580	**0.030**	0.300	0.817
3	0.070	0.528	**0.010**	**0.031**	**<0.001**
4	0.090	0.387	**0.036**	0.175	**0.013**
5	0.933	**0.029**	**0.001**	**0.002**	**0.001**
6	0.109	0.618	0.726	0.801	**0.033**
7	0.142	0.126	**0.003**	0.301	**<0.001**

***p*-value which is in bold showed significant differences ($p<0.05$).**

However, when breaking down into each questions, Question 7 and Question 11 showed significant differences in all the variables except in the genders. Furthermore, when subject to each variables' group, it was noticed that there were significant differences among the ethnicity and users' group in all the knowledge questions except for Question 12 among the ethnicity group and Question 8 between the users' group.

Overall, according to Table 4, up to 227 participants (56.47%) of the participants scored 2 points and below, indicating poor knowledge on NSAID among this studies population, 163

participants (40.55%) scored between 3 points to 5 points, indicating for moderate knowledge level obtained on NSAID and 11 participants (2.98%) scored 6 points and above that portraited a good knowledge level of NSAID.

Attitude

Refer to Table 4, most of the participants showed neutral attitudes towards NSAID usage.

Table 4: Participants' attitudes towards NSAID

QN	Statement	Responses, n (%) (n=402)				
		Strongly disagree	**Disagree**	**Neutral**	**Agree**	**Strongly Agree**
1.	**Self-medication of NSAID will be harmful if they are taken for long period.**	4 (1.0)	16 (4.0)	151 (37.6)	147 (36.6)	84 (20.9)
2.	**The course of NSAID medicines must be completed although the symptoms are subsided.**	20 (5.0)	62 (15.4)	238 (59.2)	59 (14.7)	23 (5.7)
3.	**Pharmacists can provide**	6 (1.5)	27 (6.7)	160 (39.8)	132 (32.8)	77 (19.2)

	best advice or information regarding NSAID medicines.					
4.	**The medication leaflet shall be studied before consumption of NSAID to understand more on the usage and precautions.**	7 (1.7)	13 (3.2)	163 (40.5)	127 (31.6)	92 (22.9)
5.	**NSAID medicines can be purchased in any pharmacy upon request without prescription from doctor.**	46 (11.4)	82 (20.4)	179 (44.5)	62 (15.4)	33 (8.2)
6.	**NSAID medicines help to relieve the pain more effective**	3 (0.7)	30 (7.5)	267 (66.4)	71 (17.7)	31 (7.7)

	compared to other alternative medications.

***Data reported in brackets represents the percentage (%).**

There was a significant difference between the users and non-users regarding their attitude towards NSAID, as well as within the education stream and ethnicity ($p<0.05$) as shown in Table 5.

Table 5: *p*-value for each variable in attitude section.

QN	*p*-value *				
	Gender	**Nationality**	**Ethnicity**	**Education Stream**	**Users**
1.	0.989	**0.012**	0.329	**0.005**	**0.010**
2.	0.873	0.793	0.502	**0.009**	**0.007**
3.	0.287	0.290	**0.001**	**0.008**	**0.008**
4.	0.080	0.126	**0.029**	**0.004**	**0.022**
5.	0.978	0.599	**0.002**	0.255	**<0.001**
6.	**0.020**	**0.030**	0.054	0.152	**<0.001**

******p*-value which is in bold showed significant differences ($p<0.05$).**

The average attitude scoring among the participants were recorded at 20.16 ± 2.674, which was at the average level, thus it showed that the participants consists of a relatively satisfied attitude towards NSAID usage.

Practice

According to Table 6, 59 of the participants (15.5%; n=380) had taken less than or more than the recommended dose of NSAID, and 24 out of the 59 participants(40.68%) were current users of NSAID.

Table 6: Participants' Practice towards NSAID

QN	Statement	Yes (%) (n=380)	User (n=52)	Non-User (n=328)
1.	Have you ever taken less than or more than the recommended dose of NSAID medicine?	59 (15.5)	24 (46.2)	35 (10.7)
2.	Have you ever taken the next dose of NSAID medicine sooner than directed on the label?	47 (12.4)	19 (36.5)	28 (8.5)
3.	Have you ever taken more than the number of dosages for NSAID medicine per day as directed?	32 (8.4)	11 (21.2)	21 (6.4)
4.	Have you ever shared NSAID medicine with others who have similar illness?	86 (22.6)	27 (51.9)	59 (18.0)
5.	Have you ever taken NSAID medicine according to suggestions from people other than healthcare professionals (i.e. doctors, pharmacists)?	133 (35.0)	34 (65.4)	99 (30.2)

6.	Have you ever reused doctor's prescription when you get similar complaints?	120 (31.6)	24 (46.2)	96 (29.3)
7.	When your symptoms are relieved, do you discontinue NSAID medicine by yourself?	174 (45.8)	41 (78.8)	133 (40.5)
8.	Have you ever taken NSAID medicine according to suggestions from people other than healthcare professionals (i.e. doctors, pharmacists)?	133 (35.0)	34 (65.4)	99 (30.2)

*** QN indicates the question number.**

***Data reported in brackets represents the percentage (%).**

Based on the result obtained shown in Table 7, there was no significant difference between the genders relating to their practice of NSAID usage.

Table 7: *p*-value for each variable in practice section.

QN	*p*-value *				
	Gender	**Nationality**	**Ethnicity**	**Education Stream**	**Users**
1.	0.908	0.512	**<0.001**	0.828	**<0.001**
2.	0.182	0.632	**0.016**	0.930	**<0.001**
3.	0.407	0.008	**0.003**	0.057	**<0.001**
4.	0.109	0.531	**<0.001**	**0.022**	**<0.001**

5.	0.356	0.798	**0.020**	0.939	**<0.001**
6.	0.822	**0.046**	**0.039**	0.609	**0.015**
7.	0.585	0.381	**0.010**	0.089	**<0.001**

******p*-value which is in bold showed significant differences (p<0.05).**

A minor significant difference had been found between the nationality, relating to the reuse of old prescription when the participant re-experienced the similar symptoms and among the education stream where relating to the sharing of NSAID medication with others who had similar illness. Moving on to the users' group and ethnicity, there was a significant difference in their practice of NSAID usage where all the questions relating to the practice behaviour showed a significant difference (p<0.05).

Sources of information

Table 8: Sources of information relating to NSAID

Source	**No. of participants (n=380)**	**User (n=52)**	**Non-User (n=328)**	***p*-value ***
Media (i.e., radio, newspaper etc.) and textbooks	83 (21.8)	9 (17.3)	74 (22.6)	0.394
Internet	154 (40.5)	19 (36.5)	135 (41.2)	0.528
Professional parties (i.e., doctors, pharmacists etc.)	187 (49.2)	43 (82.7)	144 (43.9)	**<0.001**
Others	57 (15.0)	8 (15.4)	49 (14.9)	0.933

***_p_ value in bold showed significant association with the variable**

***Data reported in brackets represents the percentage (%).**

Reason(s) to practice self-medication of Non-Steroidal Anti-Inflammatory Drugs

Table 9: Reason(s) to practice self-medication of NSAID among participants.

Reason	No. of participants (n=380)	User (n=52)	Non-User (n=328)	*p*-value*
The symptoms will subside soon after consumption of NSAID	94 (24.7)	21 (40.4)	73 (22.3)	**0.005**
Prior experience	94 (24.7)	20 (38.5)	74 (22.6)	**0.014**
Lack of time to consult doctor	131 (34.5)	16 (30.8)	115 (35.1)	0.545
Recommendation from family/friends/Internet	158 (41.6)	28 (53.8)	130 (39.6)	0.053
Cost effectiveness	81 (21.3)	13 (25.0)	68 (20.7)	0.485
Others	25 (6.6)	0 (0.0)	25 (7.6)	**0.039**

***_p_-value in bold showed significant association with the variable**

***Data reported in brackets represents the percentage (%).**

Discussion

Paracetamol is the most common over-the-counter analgesic where it can be purchased everywhere, even in the convenient

shops. On the other hand, NSAID is categorized as prescribed medicine, where its usage is rising among the public due to the stronger analgesic effect compared to paracetamol[17]. This study targeted only on first-year non-health science undergraduates who were from the universities in Klang Valley, Malaysia and minority of the participants were International students (refer to Table 1).

Female participants were recorded higher than the male participants, regardless of the category of users or non-users of NSAID. This result had matched with a few studies where the female participants were dominating in their study results[7,18,19]. An explanation on this behaviour was due to factors such as stress and period pain[20]. However, all the variables that were recorded, such as the gender, nationality, ethnicity and education stream were not equally distributed as the results obtained were more dominating in one of the variables compared to the other variables due to the convenient sampling method used during the study's survey period.

Knowledge

Participants showed relatively low knowledge level regarding NSAID where more than half of the participants scored 2 points and below out of the total points of 7 in the knowledge section of the questionnaire. This result was in accordance with the study done among the public in Rawalpindi and Islamabad, Pakistan as well as among the undergraduates in Poland where even the health-science undergraduates who had learnt about NSAID in their studies had low knowledge in this group of medication, in support with other students from different fields of study[12,21] It had further been supported by a study among physiotherapists, a registered healthcare profession, and the result also showed that they had low knowledge on NSAID as well[22]. When the result was subjected for comparison between the users and non-users,

it showed significant differences between these two groups where all the *p*-values for each question were below 0.05, except for the question related to the low-dose aspirin's effect. Users showed to have better knowledge on NSAID compared to the non-users group. This might due to users' experiences were still new and intact as they used NSAID during the past 12 months compared to non-users who have used it more than 12 months ago or totally not being exposed to NSAID beforehand. One of the reasons behind this might be due to patient who had exposed to the medications not long ago tends to remember its usage and information clearer compared to those who had used it long ago, however study should be done on this assumption to understand the reliability on it. Next, to evaluate their knowledge on this question, we had further asked those who had answered correctly, to specify which NSAID contain this effect and they managed to answer it correctly, which is aspirin, but they were unaware on the dose which causes this effect. Upon further questioning, it was noted that the source of this information was either from their health-science friends or seniors, the family members who were exposed to it as well as from the Internet sources. As per result obtained, it was noted that there was a significant association between the ethnicity group in most of the questions in knowledge section.[23]

Attitude

Most of the participants showed neutral responses on their attitude towards NSAID and no favouritism towards NSAID usage in this study. Most of the participants were unsure of the harmful effects of NSAID when it was taken for long period, and when matching it with the additional supporting evidence in the knowledge section, where the rate of correct answer on the side effects of NSAID were less than half of the whole studied populations obtained in this study. This further supported the reason that up to 37.6% of the participants showed neutral

response on this statement. However, up to 36.6% of participants agreed on the availability of harmful effects brought by long term usage of NSAID, which in accordance with an article review done in 2015[24]. Next, most of the NSAIDs are to be taken when necessary upon pain and can be stopped when the symptoms are relieved thus it is unnecessarily for the patients to complete the whole course when there is no indication of pain relieve[25]. Unfortunately, more than half of the participants felt neutral on this statement. This might due to the participants' symptoms may not been relieved even though they had completed the whole course of NSAID or they had been asked to complete the full course of NSAID due to the seriousness of the injuries. Besides that, up to 39.8% of participants had neutral opinions on the pharmacists being the best source of information on NSAID. This was in accordance with study done in New Zealand where most of the respondents would seek information from other professional parties which including through a pharmacist[26]. Moving on to the medication leaflet, participants felt neutral on the process of studying the medication leaflet before the consumption of NSAID and a few reasons can be explained on this result. Firstly, some NSAID that had been prescribed might not be included with its medication leaflet during the dispensing process and second, some participants did not understand the jargons being used in the medication leaflet as they had not been exposed with the medical jargons. However, it showed differently in the study done on first-year medical students in Bahrain where up to 71.6% of the respondent read the package insert before consuming of the medication[27]. Regarding the availability of NSAID in pharmacy without any prescription, participants showed neutral response as most of the pharmacy will be dispensing NSAID regardless the availability of prescription upon request as NSAID is categorised as a Poison C drug which can be purchased without any prescription through

the pharmacist in the pharmacy[2]. Therefore, it was in accordance with the result obtained as the participants were unaware on this issue yet able to purchase NSAID upon request in the community pharmacy. There are different types of analgesic available in the market, which including the traditional remedies as well as the over-the-counter analgesic, paracetamol. Participants believed that any analgesic works equally well for them, thus they showed a neutral response for NSAID being the most effective analgesic. This result was in accordance with a study among teachers in a university in Pakistan, where the most common analgesic being prescribed or used by the public is paracetamol, followed by NSAID[28]. Moving on to the undergraduates' perspective, there are some of them would choose traditional regime such as Ayurveda as their choice of analgesic[24,29]

Practice

Based on the result obtained, most of the participants showed a satisfactory level of practice on NSAID. This was not in accordance with most of the studies where their results showed the participants had unsatisfied level of practice on NSAID where they favour the self-medicating practice without knowing the appropriate medication to be used nor the actual dose to be consumed[13,19,30]. This might due to the participants were non-health science based where they had not been exposed to the usage of NSAID unless they were previously being exposed to as part of their daily needs for analgesic purpose. However, in the study done among general public in Malaysia, it was reported that majority of the participants complied with the medication regimen which portraited a good practice phenomenon as similarly with our study's result. They further explained that the participants were aware of the dangerous of self-medicating without any doctor's advice or prescription, thus resulting on the medication adherence pattern[15]. Among the practice questions, the most common bad practice being maintained was reusing of

the medication according to the suggestions from people other than the healthcare professionals. This phenomenon had also been noticed among the recreationally trained college-aged students in 2014 where majority of the participants would take in other's suggestions on the medication, especially when they showed similar symptoms[8]. Another similar result was obtained from the study done in Penang, Malaysia on 2014 where the participants did obtain the medicines based on others' suggestions directly from the community pharmacy. Another common practice by the participants was reusing their old prescription or leftover medication during the remission of the similar pain. This was noted in the study among the public in Malaysia during 2016 where the participant with non-chronic diseases would reuse the old medication for similar illness. However, within the same study, for participants who suffered from chronic diseases, they would rather get the new medications as they believed new medications will be more effective[15]. On the other hand, the result on discontinuation of NSAID was in tie with the result obtained in the attitude section, where the participants would discontinue NSAID and did not complete the full course of NSAID upon relieve of their symptoms. This indicated that the participants were aware on the proper usage of NSAID but so do the reason of the common practice of public to not finish the whole course of medication once their symptoms had been relieved. This phenomenon was noted as well in the study done in Egypt on self-medication practices where the respondents would stop taking in the required medication once the symptoms had subsided (63.3%)[31].

Source of Information and Reason to Practice NSAID

To understand more on the sources of information on NSAID retrieved by the participants, it was noticed that the sources were in accordance with a study done in New Zealand and Taif Kingdom of Saudi Arabia where the most common source was

from the professional parties such as doctors and pharmacists[26,32]. The studies also showed that participants get to know NSAID during the dispensing process upon their visit to the clinic or community pharmacy, looking for analgesic. Furthermore, there is another study showing that the public managed to get the information on medication due to the advice from the physician, nurses (13.4%) and pharmacists (25.6%)[33,34]. Besides that, some participants mentioned that they get to know NSAID's existence through the widely used Internet which supported by a study in Saudi Arabia and India where up to 69.1% and 55.18% respectively of the participants in that study got the information from electronic and print media advertisement[7,35]. Other source of NSAID information includes the recommendation from their friends and family which similar to the result obtained from a study on Iranian University student in central Iran. It showed that recommendation from the friends and family being the commonest sources to obtain the information on medication, including NSAID group (54.7%)[36].

Moving on to the reason for self-medication using NSAID, the result showed that recommendation from their friends and family members being the top reason for self-medication practice, which was supported by the result in the study done on basic science undergraduate medical students in western Nepal[10]. Lack of time to consult doctor ranked the second top reason in this study, however it was the top reason for medical, pharmacy and nursing student in Karnataka to practice NSAID (86.54%). It further explained that due to the long waiting time in the clinic made the respondent to give up on consultation and seek for help in the community pharmacies. Besides that, their previous exposures towards NSAID usage and the belief of their symptoms were too trivial for consultation further encouraged the participants to take NSAID without any consultation[37]. It was reported similarly in the study done in Saudi Arabia and further supported by the study

done in Penang, Malaysia[15,38]. Some participants did agree that the self-medication with NSAID can help to reduce cost especially on the consultation fees, which was also found similarly in the studies done in Romania and Saudi Arabia[38,39]. However, on the other hand, there were still a minority of the participant in this study mentioned that they did not practice self-medication of NSAID at all. Overall, recommendation from family and friends, lack of time for consultation, prior experience with NSAID, belief of the minor symptoms of their illness and the cost effectiveness issues remain the mainstay of the reason for self-medication practice among the public.

Recommendation of Study

There is limited research studies done on the non-health science undergraduates in Malaysia, and since this research only targeting the undergraduates within Klang Valley, Malaysia, it is recommended to do more studies on the same populations in various areas of Malaysia to understand and assess the level of knowledge, attitude and practice of NSAID among this population more accurately.

Conclusion

In conclusion, the first-year non-health science undergraduates in Klang Valley, Malaysia portrait a lacking in their knowledge on NSAID, however they showed a relatively satisfying attitude and a good practice pattern towards this group of analgesic. However, it is still a major concern where the rate of self-medicating with NSAID is increasing among the undergraduates despite the lack of knowledge due to the numerous reasons issued in this study. This will further become an alarming issue as it might result in harmful adverse reactions among the users. Another major finding in this study was that it showed a significant association

between the level of knowledge, attitude and practice towards NSAID among the users and non-users of this group of medication. It showed that users will have a better knowledge level, relatively similar in the attitude level but poor practice pattern towards NSAID when compared with the non-users.

Acknowledgement

The author would like to acknowledge all the undergraduates who had participated in the study for their time and response as well as the university's authorities for granting the permission to conduct the survey within their campus.

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Case report

Dental findings in patient with brittle bone disease

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Abstract:
Osteogenesis imperfecta (OI) or brittle bone disease is genetically heterogeneous connective tissue disorder which is characterized by skeletal deformities due to fragile bone, reduced bone mass and frequent fractures. The genetic mutations in collagen genes, COL1A1 and COL1A2 are responsible for the pathogenesis of OI. The clinical and radiological features of OI manifest in different age groups and severity of the condition depends on the type of OI. The common clinical findings include recurrent and multiple fractures, laxity of the ligaments, blue sclera, growth retardation, and scoliosis. OI is commonly associated with Dentinogenesis imperfect; (DI) which is characterised by defective dentin formation. Here we report a rare case of OI type IV (Group A) with dental manifestations such as micrognathia, retained primary teeth, class III malocclusion, crossbite, multiple impacted teeth, and delayed eruptions.

Key words: Osteogenesis imperfect, Dental, Micrognthia, Primary teeth

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Introduction: Osteogenesis imperfecta (OI) is a hereditary congenital osteoporosis, characterized by bone fragility and low bone mass, and is linked to a mutation in the gene encoding type-I collagen. It exhibits a variety of clinical presentation ranging from intrauterine death to normal growth and low fracture incidence depending upon the severity. The incidence of OI varies between 6 and 20 in 100,000 new-borns and its prevalence is 4-10 in 100,000 individuals.[1]Sillence classified, OI based on clinical, genetically, and radiographic features into four groups. Type I is a mild form and Type II is the lethal form. Type III patients show progressive limb deformation. Patients with type IV shows moderate to severe phenotypes and do not fit into any of the first three categories. [2]Some cases of OI demonstrate heterogeneous features, which are not mentioned in Sillence classification. Patients affected by these types do not demonstrate DI and blue sclera;OI types V-VIII are called syndromes resembling OI. [3,4] Sillence's type IV is further subdivided into groups A and B, which can be differentiated based on the dental findings. Group B is characterized by the presence of Dentinogenesis imperfect (DI) and group A is associated with normal teeth. [5]

Dentinogenesis imperfecta (DI) is a hereditary disorder of dentin. The mutation of gene dentin sialophosphoprotein result in the formation of defective dentin that is prone for wear and fracture. Shields et al proposed three types of DI: type 1 is associated with OI. Type 2 has essentially the same clinical radiographic and histological features as DI type 1 but without OI; Type 3 is rare and is only found in the triracial Brandywine population of Maryland.[6]The most common clinical manifestations in teeth are teeth discoloration and enamel fracture. The enamel may be of normal thickness, but frequently is dislodged exposing the softer

dentin which may be attributed to the smooth dentinoenamel junction. We report a case of type IV Group A OI with normal enamel and dentin.

Case History

A 20-year-old female patient reported to SEGi oral health centre with the complaint of pain in left lower jaw region. Pain was mild, intermittent and aggravates during chewing. Her past medical history includes multiple fractures in long bones (femur and pelvic) since childhood and these fractures were treated by specialists. Both the parents and siblings did not have any bony diseases. On general physical examination, she had short stature (Figure 1), normal upper and lower limbs, normal gait, size of the head appeared smaller however, it was in proportionate to her body stature.

Figure 1: Photograph showing short stature

On extraoral examination, she had normal mandibular movements and adequate mouth opening. Intraoral examination showed micrognathia of both the arches, mixed dentition with retained 53,55,63,64,65, 73,74,75 and 85 (Figure 1 &2).

Mild brownish discoloration of deciduous teeth with conical shaped 23 and missing 14,15, 16, 17, 23, 24, 25, 26, 27 33, 34, 35, 37, 45 were noticed.Class III dental malocclusion, cross bite and mild to moderate attrition of retained teeth was evident.

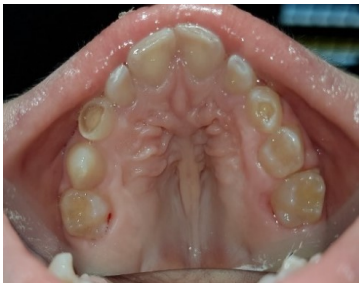

Figure 2: Photograph of maxillary arch

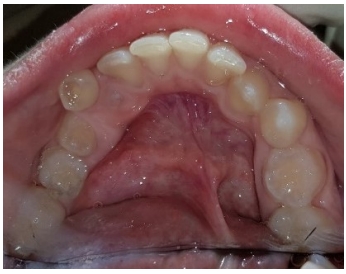

Figure 3: Photograph of mandibular arch

OPG revealed normal thickness and enamel and dentin. Size of the pulp chambers appeared normal. Multiple impacted 14,15, 16, 17 23, 24,25, 26,27, 34, 35, 45 and 47 were noticed. There were structural and morphological changes in right and left condylar head without any evidence of fractures (Figure 4).

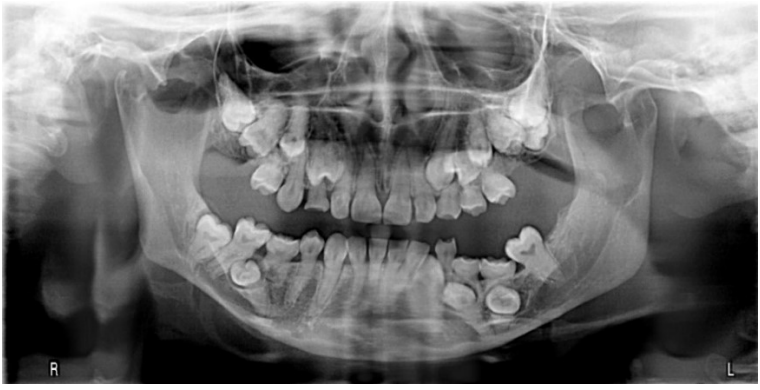

Figure 4: OPG showing multiple reatined primary teeth and impacted permanent teeth.

Based upon these clinical findings and previous medical history, a provisional diagnosis of osteogenesis imperfect (Type IV A) with normal dentin was made. Patient was explained regarding the dental condition and condyles. The preventive dental care was rendered and informed to come for periodic follow up.

Discussion:

Osteogenesis imperfecta, also known as "brittle bone disease", is a heterogeneous group of genetic connective tissue-associated disorders.[7]Most form of OI is due to mutations in the genes (COL1A1 and COL1A2) that encode the pro-alpha 1 and pro-alpha 2 polypeptide chains of type I collagen. Hence, tissues which have abundant Type I collagen like bone, dentin, sclera, and ligaments are usually affected most.[7]A reduced amount of structurally normal collagen results in OI Type I, whereas qualitative and quantitative alterations in the collagen synthesis result in OI Types II, III, and IV.[8]Three possible reasons that may cause a child to be born with OI. [9]

1. Direct inheritance from a parent: overall there are 50% chance that the disorder will be passed to next generation.

2. A new dominant mutation: spontaneous gene mutation of the sperm or the egg before the child's conception.

3. Mosaicism: clinically an unaffected parent may have more than one affected child. In this scenario mutation may have occurred during fetal development of the parent.

In our case, patient's clinical feature showed moderate form of OI, Sillence Type IV, which is characterized by brittle bones, growth retardation, pathologic fractures, without DI. Such moderate form has better survival rate compared to severe form which occurs at a very young age. [10]Sillence's Type IV OI is the

most diverse group as it contains all OI cases which cannot be categorized in Type I to Type III. Type IV is subdivided into A and B based on their dental findings.[9]Type IVA is associated with normal teeth whereas Type IV B has DI Type II and teeth may appear as opalescent greyish brown hue. Even though enamel of teeth in Type IV B will be of normal thickness, it easily gets dislodged due to soft dentin and smooth dentinoenamel junction. Ideally, diagnosis of OI should be achieved at earliest to provide adequate dental treatment to reduce the need for extensive and invasive procedures.[7] Sometime diagnosis of OI can be challenging, especially if the family history is negative. In such scenario clinical examination and genetic counselling can aid in diagnosis. [11]

Severe types of OI has abnormal craniofacial characteristics due to various malocclusion. In one report, class III malocclusions occurred in 70–80% of types III and IV OI cases, with a high incidence of anterior and posterior cross bites and open bites. [12] In our case, patient had class III malocclusion with cross bite. Her micrognathic jaws led to dental findings of impacted permanent teeth and retained deciduous teeth with no changes in enamel and dentin thickness.

The medical treatment of OI is focused on minimizing fractures, maximising mobility, independent function and general health. Pamidronate, a type of drug which increases bone density and regulates bone formation has thus far shown considerable success in the treatment of severe OI. Safe exercises such as swimming are encouraged to promote a healthy lifestyle. Orthopaedic surgery to implant rods may be recommended to increase support to bones.[10]

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Case report

Reactive Cavernous Haemangioma Of Tongue; An Unusual Presentation

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Abstract:

Haemangiomas are benign vascular tumor most commonly seen in the head and neck region. Oral cavity haemangiomas are mostly seen on cheeks and upper lip with tongue being a rare finding. We present a case of tongue cavernous haemangioma which appeared as pedunculated proliferative growth on the dorsum of the tongue. Unlike usual presentation of infantile or congenital haemangioma our case presented later in the childhood and had an insidious onset hence reported as a reactive variant.

Keywords: Haemangioma, Vascular malformation, Capillary malformation, Venousmalformation

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Introduction

Hemangioma is defined as "a benign tumor of dilated blood vessels." Haemangiomas are one of the common developmental vascular anomalies of infancy and childhood most often they are seen in head and neck region.[1] oral mucosal involvement is less frequent with tongue being rarely involved. Hemangioma of head and neck appears a few weeks after birth and they grow rapidly. It has varied terminology as port-wine stain, strawberry hemangioma, and Salmon patch. There is still uncertainty about this condition being neoplastic or a reactive state. This paper describes the management of cavernous haemangioma in a 10 year -old child with a unique proliferative presentation. Colour doppler imaging studies ruled out venous malformation but showed internal vascularity. The lesion was diagnosed as cavernous haemangioma through histopathology. Early detection and biopsy become important in this condition in order to determine the clinical behaviour of the tumour and to prevent potential complications.[2]

Case report

10-year-old child presented with history of growth over the dorsum of the tongue since one year. The growth was insidious in onset and was slowly increasing in size. The patient did not complain of any pain or bleeding. The patient had recently developed swallowing difficulty due to the increased size of the lesion. Clinical examination revealed a proliferative pedunculated soft tissue mobile growth over the middle third of the dorsum of the tongue of size 2cmx 3cm. The lesion had reddish appearance with no surface ulceration. The surrounding soft tissue was normal with no induration. (Figure 1) The growth showed blanching on application of mild pressure.

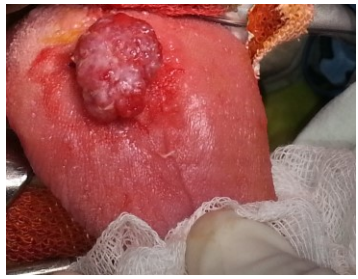

Figure 1: 3cm x 2cm pedunculated, mobile growth on the middle one third dorsum of tongue.

A provisional diagnosis of haemangioma was given and colored doppler imaging was done which ruled out any vascular malformation and internal calcification but showed presence of internal vascularity. (Figure 2) Based on the clinical and doppler findings decision was made to excise the growth under general anaesthesia with adequate haemostatic precaution with the use of electrocautery and suturing.

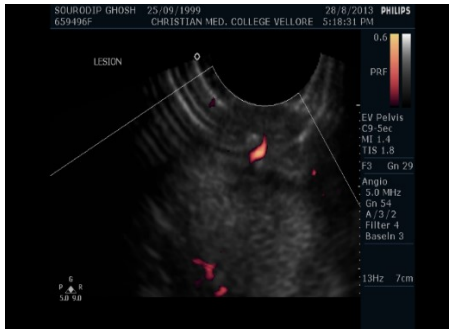

Figure 2: Color doppler showing internal vascularity

First a purse string suture was placed all around the base of the growth using 3-0 vicryl which helped to occlude any collateral blood supply. Following which electrocautery was used to dissect out the growth which helped in producing additional haemostasis. (Figure 3) The surgery was uneventful with normal

post-operative recovery. Surgical biopsy reported as cavernous haemangioma.

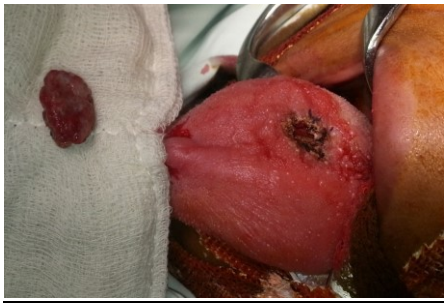

Figure 3: Surgical excision done with complete haemostasis

Discussion

Mulliken and Glowacki in 1982 proposed an effective classification of vascular anomalies [3] two distinct variants proposed included haemangiomas and vascular malformations. Based on the depth of tissue involvement haemangiomas were subdivided into superficial, deep and compound. Vascular malformation on the other hand was classified based on type of vessels involved like capillary, venous, arteriovenous, lymphatic or combined. Further based on the flow characteristics vascular malformations were divided into low flow, high flow or combined.[4]

Haemangiomas are benign tumors seen in infancy and early childhood with most of them associated with head and neck region. Infantile variant appears during the first weeks of life mostly as bluish pink macules or skin patches. The lesions enters a proliferative phase were the lesion becomes aggressive appearing elevated above the surrounding skin surfaces. Generally, this phase lasts up to fifth month hereafter involution occurs with 90% completion by age 4 years. Complete resolution is possible, but in many cases the cutaneous lesion still remains

mostly as telangiectasia.(5)Congenital hemangiomas however rare fully formed at birth but are clinically similar to infantile variant, these are mostly rapidly involuting type but may be non-involuting as well.The present case of cavernous haemangioma in a 10-year-old is exceptionally unusual. Haemangiomas are well known to be associated with infancy, however in our case the lesion was not present at birth but wasan acquired reactive form. The etiology of acquired reactive formhaemangiomais unknown, but hormone, inflammation and trauma are thought tobe the likely causes.[1]

Haemangiomas are characterized by hyperplasia of blood vessels, usually veins and capillaries, in a focal area of submucosal connective tissue. It is almost never encapsulated.Clinically they may have varied manifestation as seen in congenital form as strawberry patches to other infantile variants as compressible soft submucosal swelling. In our patient, the lesion involving the tongue had a proliferative growth not involving the deeper tongue musculature. Color doppler also ruled out vascular flow, henceforth it was possibleto treat the patient through surgical excision. Other modalities in treatment include corticosteroids, sclerosing agents, diathermy, electrocauterization, cryosurgery, laser, embolization, radiofrequency, radiation therapy, and interferon therapy. [6-8] The frontline choice of treatment is however decided based on age of presentation, stage of involution, size and extent of the lesion. These lesions often mimic as pyogenic granuloma and histopathology of the lesion often is helpful in confirming the diagnosis as was in our case which presented to be cavernous haemangioma, characterized by the presence of large dilated blood-filled sinusoidal spaces with thin walls showing an endothelial lining.

Conclusion

cavernous hemangioma of tongue is a rare occurrence. Clinical findings must always need to be supplemented with radiographic imaging and ultrasound color doppler to rule out the possibility of other forms of vascular anomalies. Suitable treatment modality needs to be planned based on the prognosis and characteristics of particular anomaly.

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Case report

Prosthodontic Management of a case with massive Torus Palatinus using Molloplast-B

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Abstract

The management of patients with largetorus palatinus in denture fabrication can be quite challenging for a dental practitioner. A large palatal torus usually affect the close adaptation of the denture base to the mucosa thereby leading to loss of retention and stability of maxillary denture. It is, therefore, very important in the ways of management of patients with large Torus palatinus. The purpose of this article is to provide an overview of the modified approaches during acrylic removable partial denture fabrication for a patient with large Torus palatinus.

Keywords: Torus palatinus, Denture fabrication

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Introduction

Torus and its plural term tori are benign developmental localised bony protuberances occurring within the oral mucosa. Tori, with its Latin origins explain the anatomical morphology resembling cushion or knots in an entangled rope[1]. Depending on its location intra-orally, tori can be further denoted as torus palatinus and

torus mandibularis[1]. Torus palatinus is a slow growth, non-malignant intra-oral hamartoma that arises from the median intermaxillary-interpalatine suture and extends inferiorly through the vault of the palate[2,3,4]. The oral mucosa surrounding the tori would be thin and relatively avascular. This contributes to the clinical appearance of paled or white mucosa enclosing the torus palatinus, whereas other areas in the palate region present a deeper red in shade.

While the exact pathogenesis of torus palatinus remains shrouded in mystery, researchers have identified that torus has a multifactorial etiology. Few studies have shed some insight regarding the role of genetics in the formation of torus palatinus[5,6,7].Cagirankayaet al[8] hypothesized that presence of torus palatinus serves as a bio indicator of an adequately developed maxillary arch with little establishment of intra-arch malocclusions. However more recent studies seem to disprove Cagirankaya et al study, instead alluding the formation of torus palatinus as a physio-mechanical process that continues throughout the dentate period[9,10,11]. It is imperative to note that the association between functional stresses within the oral cavity and development with 'maturation' of torus palatinus holds little water till date[12], although the converse is true for formation of torus mandibularis[13,14]. Certain medical conditions such as hypothyroidism, trauma, infection, use of certain drugs, environmental and nutritional disturbances could increase the chances for development of torus palatinus[15,16].

Worldwide prevalence of torus palatinus and torus mandibularis varies 8 to 51% and 6% to 32% respectively from region to region [16-30].Tori are found to be more common in females. In United States of America, prevalence of torus palatinus were found in 69.7% of women from all ethnic groups[18]. It is also noted that tori is prevalent in specific ethnic and racial groups, especially

Asians. Sing AK et al reported 33% of prevalence rate among Malaysian population [31]. Jainkittivong et al revealed a much higher prevalence of torus palatinus (60.5%)in Thai population [32].

Thoma and Goldman, in 1960 classified tori as: 1) flat; 2) nodular; 3) spindle and 4) lobular types[3,4,11,33]. Irrespective of its morphology, the mere presence of a large torus palatinus (diameter exceeding six centimetres) would greatly interfere with the placement of any dental prosthesis involving the palate, especially for removable partial and complete dentures requiring adequate palatal coverage to aid in its retention and stability[3,4,11,33]. The thin oral mucosa overlying the palatal tori could easily ulcerate without undue pressure from the denture owing to poor vascularisation. Repetitive traumatic loading by the rigid intaglio surface of denture against soft friable tissue during denture seating and masticatory functions could further degenerate oral mucosa covering the tori on long-term. Such discomfort and painful association would surely reduce the comfort and compliance of denture wearer in the long run, thus negatively affecting the quality of life thereon[3,4,11,33,34].

A cursory review of literature published on treatment and management options for large torus palatinus would fall back on surgical resection, a clinically-proven method, accepted and practiced internationally by oral and maxillofacial surgeons and operatory teams[3,4,11,33,34]. As with any elective maxillofacial surgery, the expected benefits of the surgery should be weighed carefully against the potential risks, especially in medically compromised patients, and often it is geriatric and potentially frail patients who undergo torus resection as a step toward better dental prostheses. Additionally, few authors have expounded the usage of softer yet resilient materials on the intaglio surface of dentures to minimise traumatic loading and occurrence of palatal

ulceration[36].Soft denture liners provide a cushion like effect between the intaglio surface of the denture and the palatal mucosa thereby help in even distribution of the functional load on the denture-bearing area. They are widely for improving the retention of the dentures by engaging undercuts thereby increasing patient comfort and compliance to denture application. This case report describes the use of Molloplast B on the intaglio surface of the denture in a patient with large torus palatinus as an adjunct therapy to improve the retention and stability of the removable denture.

Case report

A 60 years old male patient visited SEGi Oral Health Care with chief complain of difficulty in eating due to loss of teeth. Patient did not have any denture wearing experience and had been edentulous for 15 years. Patient had no significant medical history. Intraoral examination showed that edentulous maxillary arch with only left lateral incisor remaining. The maxillary arch was classified as Kennedy's Class I and mandibular arch as Class II with modification 2. Patient had a large torus palatinus of approximately 3cm in diameter. (Figure 1a, 1b) Patient was advised to undergo extraction of the remaining left lateral incisor in the maxillary arch to fabricate a complete denture but he insisted on retaining the tooth.

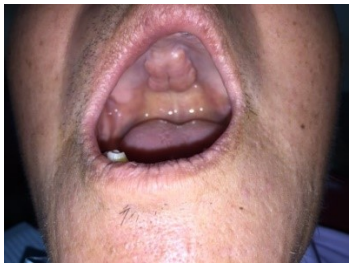

Figure:1a

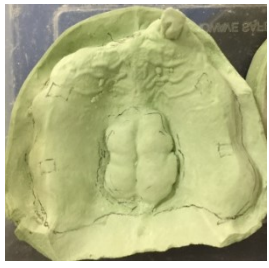

Figure:1b

After discussing the treatment options with the patient, which included implant supported overdenture, conventional removable denture and cast removable denture, the treatment plan was to fabricate a conventional removable partial denture for both maxillary and mandibular arch. This treatment option was devised based on financial concerns of the patient and time constraints. The maxillary and mandibular dentures were made following the standard clinical and laboratory protocols. The maxillary denture was modified to relief the torus palatinus. The posterior border of the maxillary denture was made following the contour and outline of the torus palatinus. (Figure 2). When the maxillary denture was inserted, it appeared to non- retentive due to the interference of the torus and failure to achieve a tight contact between the soft tissue and the denture base.

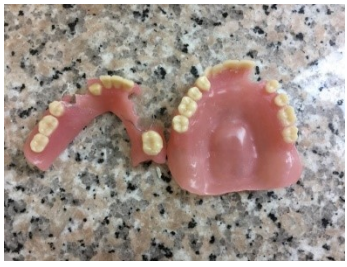

Figure 2

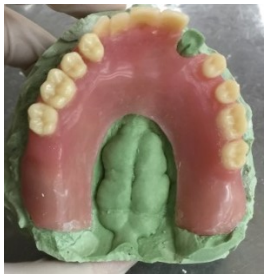

Figure 3

The maxillary denture was then planned to modify the posterior border by using Molloplast-B around the tori region to improve the retention and stability of the denture. First, the maxillary denture was trimmed to resemble a U-shaped denture (Figure 3) and approximately 2mm away from the tori region using diamond acrylic bur.

The acrylic portions that have been trimmed off were then replaced by Molloplast-B. (Figure 4).

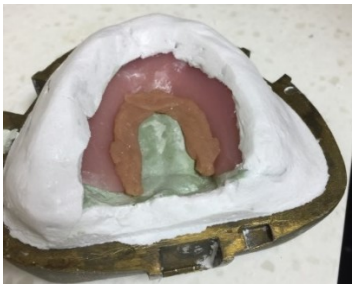

Figure 4

The maxillary denture was invested in the dental flask using dental plaster. Molloplast-B was compacted into the gap between the denture and the torus palatinus. The denture was then cold cured , excess flash of the Molloplast-B was removed using a lecron carver and acrylic diamond bur. The final maxillary denture was finished and polished.

The modified maxillary denture was then inserted in the patient's mouth. The denture border modified by Molloplast-B around the tori engaged into the undercut of the tori without causing any pain or discomfort. (Figure 5) The modified maxillary denture had good retention after the relining of the surrounding of the torus palatinus using Molloplast-B.

Discussion

Soft denture lining materials are widely used in the treatment of traumatized oral mucosa, ridge atrophy, bony undercuts, bruxism, xerostomia, edentulous arches opposing natural dentition, congenital oral defects requiring obturation to enhance the retention and comfort of the prosthesis. There are two types of denture intaglio lining surface material which are soft in consistency: 1) acrylic compounds and 2) silicone elastomers which are widely used in clinical dental practices worldwide[37]. In this case report the tori observed was of Nodular type

according to Thomas and Goldman classification, extending up to the border of the posterior palatal seal area. Hence, obtaining adequate retention with conventional technique was challenging since the denture seal would be jeopardized. We opted to use Molloplast-B (Regneri GmbH & Co., KG Karlsruhe, West Germany and distributed by Buffalo Dental Mfg. Inc., United States of America) a silicone elastomer because it can retain its 'elastic' dimensional stability for a greater length of time compared to soft acrylic compounds[38].Previously, Molloplast because of its unique properties has been used as a permanent liner under denture bases to prevent trauma to the tissues, but never attempted for fabricating a palate less denture. This technique was a novel approach to improve the retention of the maxillary denture using Molloplast to engage the undercuts around the tori. While the maxillary denture is being seated over the palatal surface, the mucosa overlying the extensive torus palatinus would be subjected to less traumatic force as the permanent soft silicone denture intaglio surface liner deforms readily and returns to its original intended dimensions after successful seating of denture[37-40] ·Also, the material would engage within the undercuts present surrounding the margins of the torus palatinus, further enhancing the retention of maxillary denture against gravitational and adhesive forces of macerated foods[37]. During mastication, the permanent soft silicone denture intaglio surface liner would undergo transient deformation under functional loading thereby ensuring forces are distributed evenly throughout the material before being transmitted into the underlying oral mucosa[37-40].

Limitations

One of the drawbacks of such design being incorporated into the fabricated maxillary denture would be iatrogenic-induced degradation of the permanent soft silicone denture intaglio

surface liner. Such degradation occurs when the patient fails to adhere proper hygiene instructions regarding maintenance and care of denture. The usage of abrasive toothpastes to clean the intaglio surface of denture and utilization of excessive brushing force are strongly discouraged. Not only the permanent soft silicone liner would lose its 'elastic' dimensional stability rapidly, the roughened and surface porosities present would greatly increase the chances of plaque maturation. Subsequent exposure of the intaglio surface of denture directly in contact with the oral mucosa overlying the torus palatinus would lead to bacterial invasion and infection of the friable tissues. This could compromise healing rate due to relatively poor vascularisation[3,4,33,34]As such, the manufacturers of Molloblast-B claimed to have developed specific oral plaque inhibitors within the material in attempts to negate such unfortunate occurrences. The efficacy of the plaque inhibitors within the permanent soft silicone denture intaglio surface liner after following the manufacturer's recommended heat-curing process has yet to be established via clinical means.

Another drawback of soft denture liners is the failure or poor adhesion between the soft denture liner and the denture base. Few studies [41,42,43]indicate that bond strength is higher when Molloplast-B was applied to unpolymerized polymethyl methacrylate (PMMA) and processed together while Kawano F et al report better boning on polymerized PMMA. In this case we have applied the Molloplast- B on the polymerized denture surface to improve retention since the treatment plan was modified after denture issue.

Conclusion

Torus palatinus present in patients can be quite difficult to manage using conventional methods and materials. In some

situations, patient may refuse to undergo surgery to remove their tori. Therefore, a combination of materials and methods may be required to effectively increase the function and retention of the prosthesis without the need of surgical procedures.

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- **Conclusion**

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