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EVALUATION OF COMMERCIALY AVAILABLE RAPID DIAGNOSTIC TEST KITS FOR THE DIAGNOSIS OF PLASMODIUM FALCIPARUM INFECTION IN NIGERIAN CHILDREN

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ABSTRACT:

The use of rapid diagnostic tests (RDT's) as an alternative to microscopy for the diagnosis of malaria has been adopted as an effective diagnostic tool in many endemic countries. However, the differential sensitivity and specificity of these commercially available RDTs is a major challenge that affects their diagnostic accuracy. This study was conducted to evaluate the performance of three commercially available RDTs for their abilities to detect *Plasmodium falciparum* in Nigeria children. A total of 184 (71 males, and 113 females) children presenting with fever together with signs and symptoms of malaria at the outpatient unit of Ekiti State Teaching Hospital, Ado Ekiti in Nigeria, were recruited into the study. Blood was aseptically collected by venipuncture into EDTA bottle and tested for malaria infection by three RDT, microscopic examination and nested Polymerase Chain Reaction (PCR). The sensitivity, specificity and predictive values of the methods were calculated using PCR as the gold standard. The prevalence of malaria by nested PCR and microscopy was 79.9% and 77.7% respectively, while for the RDTs it was 66.8%, 73.4% and 69.0% for SD Bioline, Carestart, and Micropoint respectively. The sensitivity and specificity of microscopy was 95.8% and 75.0% respectively. The corresponding values for the RDTs were: SD bioline 84.4% and 29.5%; Carestat 83.7% and 30.6%; and for Micropoint 85.8%. Using Kappa coefficient as a measure of agreement, microscopy showed a high measure of agreement ($k = 0.73$) while each of the RDTs showed poor measures of agreement. The study concluded that diagnosis of malaria cannot completely rely on RDTs in our study area.

Keywords: Malaria, Microscopy, RDT, PCR, Children, sensitivity, specificity.

INTRODUCTION:

Malaria still remain a major cause of morbidity and mortality globally, inflicting significant and increasing burden on the global economy, with sub-Saharan Africa accounting for about 90% of all cases reported [1,2]. It is caused by one or more infections with *Plasmodium falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* with infection of *P. falciparum* resulting in most severe form of malaria. Currently the control of malaria primarily relies on early and reliable parasite - based diagnosis and reliable treatment [3, 4]. In most endemic countries including Nigeria, laboratory diagnosis is based on inexpensive and sensitive examination of stained blood smears under light microscope [5]. However, there are a number of shortcomings of microscopy; it is time consuming, labor intensive, the need for good expertise and highly experienced microscopist among others [6].

Many other methods including Polymerase Chain Reaction (PCR), immunochromatographic assay, mass spectrometry, and flow cytometry have all being described for the diagnosis of malaria [6,7]. They all have the disadvantages which include the requirement of expensive and sophisticated equipment which is not affordable for use in the endemic malaria communities in resource limited countries. To address the limitations of microscopy and PCR-based techniques, other

methods are being explored. Fast diagnostic methods based on the detection of the parasite antigen using monoclonal antibodies incorporated into test strips called rapid detection tests (RDTs) have been introduced. Two major parasites antigens, histidine-rich protein 2 (HRP2) and the parasite lactate dehydrogenase (pLDH) are the target proteins for the numerous numbers of RDTs that are currently available commercially [8]. While quality RDTs are currently recommended as the standard diagnostic tools for routine malaria diagnosis, the sensitivity and specificity of these RDTs under different conditions and in different locations are still unclear [9]. RDTs have been shown to be useful in rapid diagnosis of malaria parasite in human blood when high quality microscopy is not readily available [10]. The world Health Organization (WHO) recommended that either RDT or microscopy should be used in diagnosing all suspected cases of malaria infection before treatment [11].

Generally, the HRP2 based RDTs have higher sensitivity for *P. falciparum* detection and they are also less expensive than the *Plasmodium* lactate dehydrogenase (pLDH) or Aldolase based RDTs which detects also the non-*falciparum* [12]. Since more than 80% of malaria in Nigeria is due to *P. falciparum*, it makes the HRP2 based RDT a more preferred test. One of the disadvantages

of the HRP2 based RDT is the persistence of the HRP2 protein in the blood for extended period after successful elimination of the parasite with an effective antimalarial treatment leading to false positive results and limited specificity [13,14]. In addition, the spread of HRP2 deleted parasites that produces false negative results may have a major negative impact on the sensitivity of PfHRP2-based RDTs in sub-Saharan Africa [15,16].

Many WHO recommended HRP2 RDT based kits are currently available and these kits are being used in hospitals and health centers where neither microscopic nor PCR test methods are available. In addition, home management of malaria (HMM) with treatment based on RDT results, is recommended to reduced unnecessary use of antimalarial as one of the strategies for improving access to prompt and effective malaria case management [17,18].

In this study, three HRP2-based commercially available RDTs; SD BIOLINE Malaria Ag Pf, (Standard Diagnostics, Seoul, Korea); CareStart™ Malaria HRP2 (*Pf*) (Access Bio, Inc, USA) and Micropoint *Pf*HRP-2 were assessed for their *P. falciparum* diagnostic performance. The RDTs were assessed in comparison with microscopy and PCR assay in order to provide information on the diagnostic performance of these commercially available RDTs in Nigeria.

METHODOLOGY:

This study was carried out at the Ekiti State Teaching Hospital, Ado-Ekiti situated in the south-Western geopolitical zone of Nigeria, with the city representing a typical urban setting in Nigeria. The study design was a double-blind clinical diagnostic assay of malaria using microscopy, three RDT kits which were purchased from a pharmaceutical shop at a city in Nigeria and nested PCR as the gold standard. The study was approved by the ethical committee of Ekiti State Teaching Hospital, Ado – Ekiti, and conducted in accordance with the Declaration of Helsinki of 1975.

Simple random sampling technique was used to recruited participants for the study. The study population comprised of children with clinical signs of malaria and for whom test for malaria parasite have been requested for by clinicians. Clinical diagnosis was based on fever (temperature = 37.5°C and above) or history of fever, alongside typical symptoms associated with acute malaria infection. Children whose parents/guardians willingly gave informed consent were recruited into the study.

Blood was aseptically collected into EDTA bottle from which RDT was performed with the three (SD- Bioline, CareStat, Micropoint) HRP2-based RDTs following the manufacturer's protocol [19]. Thick blood smears were prepared and

stained with 10% Giemsa solution (Sigma-Aldrich, USA) for 30 minutes, allowed to air dry and subsequently examined using oil immersion objective lens. All the fields were examined and parasites counted against 200 White Blood Cells (WBCs) [10, 20]. Genomic DNA was extracted from whole blood using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) and nested PCR method was used for the amplification of the *P. falciparum* 18sRNA gene using protocol previously described [21]. The PCR products were visualized under UV light on 2% agarose gel after electrophoresis in 0.5X Tris borate EDTA buffer and ethidium bromide staining.

All data were analyzed using descriptive statistics. Statistical group analysis was performed with SPSS, version 16.0 for windows.

The sensitivity, specificity and predictive values of each of the methods (RDTs, microscopy) were calculated using PCR as the standard. Sensitivity was defined as the probability that a truly infected individual will test positive and specificity as the probability that a truly uninfected individual will test negative. Cohen's kappa coefficient was used to compare the measure of agreements between microscopy and the RDTs versus nested PCR results as the reference standard. All statistical analysis was calculated at 95% level of significance.

RESULTS:

A total of 184 children comprising of 113 (61.4%) females and 71 (38.6%) males were recruited into the study. The age range of the children was 0.5 -12years with a median age of ± 6 years and an interquartile range (IQR) of 6.7 ± 0.3 years. The median weight and IQR was 21 ± 1.5 kg and 23.0 kg respectfully. The mean body temperature was 38.3 ± 0.5 °C while the mean PCV of the participants was 25 ± 1.0 % respectively.

The number of *P. falciparum* positive cases detected by microscopy was 77.7 % (143/184), while nested PCR detected 79.9 % (147/184). The three RDTs detected 66.8 % (SD-Bioline, 73.4% (Carestat) and 69.0 % (Micropoint) malaria positive cases (Table 1).

Table 2 shows the varied performance of each diagnostic test across the different age groups. The detection rate of all the diagnostic test was higher in younger children aged 0-5 years (Microscopy 78.4%; PCR 81.9%; SD Bioline 68.1%; Carestart 73.3%; Micropoint 70.7%) compared to older children aged 6-12 years (Microscopy 77.6%; PCR 76.5%; SD Bioline 64.7%; Carestart 75.3%; Micropoint 66.2%) but the differences were not statistically significant. Similarly, the prevalence based on gender detected by all the methods was not statistically significant though prevalence was higher among the females (Table 3).

Comparison of RDTs diagnostic methods with PCR and Microscopy

The comparison of the three commercially available RDTs selected for this study revealed a higher carestat result of 113 out of 147 detected by PCR when compared to the others. Both SD Bioline and Micropoint showed a higher negativity (17/37) rate agreement with PCR compared to Carestat (14/37). Table 4 shows the performance of the Three RDTs with respect to their positive and negative rates in comparison to PCR.

Sensitivity, specificity and the predictive values of each diagnostic method:

The sensitivity and specificity of RDTs and microscopy using PCR as the detection standard is shown in table 5. Microscopy had a higher sensitivity (95.8%), specificity (75.0%) Positive Predictive value (PPV) (93.2%) and Negative

Predictive Value (NPV) (83.3%) compared to the three tested RDTs.

Among the RDTs, Micropoint had the highest sensitivity of 85.8% followed by SD Bioline and Carestart with 84.4% and 83.7% compared to nPCR. All the three RDTs had very low specificity compared to Microscopy. The RDTs specificity in descending order was 33.3%, 30.6% and 29.5% for Micropoint, Carestart and SD Bioline respectively (Table 5). Carestart had the highest PPV of 76.9% while Micropoint had the highest NPV of 51.4%.

Kappa coefficient was used to compare the agreement between microscopy and RDTs using PCR results as the gold standard. This study found a good agreement between microscopy and PCR ($k=0.73$) while among the RDTs the agreement with PCR was poor (SD Bioline $k=0.16$; Carestat $k=0.16$; Micropoint $k=0.21$).

Table 1: Results for Giemsa microscopy, PCR and malaria RDTs for the detection of *Plasmodium falciparum* (N = 184)

Methods	Positive, (%)	Negative, (%)
Giemsa microscopy	143 (77.7)	41 (22.3)
Nested PCR	147 (79.9)	37 (20.1)
SD Bioline	123 (66.8)	61 (33.2)
Carestat	135 (73.4)	49 (26.6)
Micropoint	127 (69.0)	57 (31.0)

Table 2: Prevalence of *P. falciparum* by age group based on different diagnosis methods

Methods	0-5-year (%) N = 116	6-10 years (%) N = 68	p-value
Microscopy	91 (78.4)	52 (77.6)	0.52
PCR	95 (81.9)	52 (76.5)	0.24
SD Bioline	79 (68.1)	44 (64.7)	0.38
Carestart	85 (73.3)	50 (75.3)	0.55
Micropoint	82 (70.7)	45 (66.2)	0.32

Table 3: Prevalence of *P. falciparum* by gender group based on different diagnosis methods

Methods	Male (%) N = 113	Female (%) N = 71	p-value
Microscopy	85 (75.9)	58 (81.7)	0.23
PCR	87 (77.0)	60 (84.5)	0.15
SD Bioline	74 (65.5)	49 (69.0)	0.37
Carestart	80 (70.8)	55 (77.5)	0.21
Micropoint	78 (69.0)	49 (69.0)	0.56

Table 4: Performance of the different RDTs in comparison to PCR and Microscopy

	RDT	PCR Positive (N = 147)			PCR Negative (N = 37)		
		Positive	Negative	Total	Positive	Negative	Total
Microscopy							
SD-Bioline	Positive	99	5	104	5	14	19
	Negative	38	5	43	1	17	18
Carestat	Positive	108	5	113	5	17	22
	Negative	29	5	34	1	14	15
Micropoint	Positive	103	6	109	4	14	18
	Negative	34	4	38	2	17	19

Table 5: Comparison of the sensitivity, specificity, positive predictive value, negative predictive value of microscopy and RDTs versus PCR

Method	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95 % CI)	Kappa
Microscopy	95.8 (92.5-99.1)	75.0 (61.6-88.4)	93.2 (89.1-97.3)	83.3 (71.2-95.5)	0.73
SD Bioline	84.4 (78.0-90.9)	29.5 (18.1-41.0)	70.6 (63.2-77.9)	48.6 (32.5-64.8)	0.16
Carestart	83.7 (77.5-89.9)	30.6 (17.7-43.5)	76.9 (70.0-83.7)	40.5 (24.7-56.4)	0.16
Micropoint	85.8 (79.8-91.9)	33.3 (21.1-45.6)	74.2 (67.1-81.2)	51.4 (35.3-67.5)	0.21

DISCUSSION:

This study evaluated the diagnostic performance of three commercially available RDTs and microscopy using nested PCR as the gold standard method for the diagnosis of *P. falciparum* among children. The sensitivities obtained for microscopy, SD Bioline, Carestart and Micropoint were 95.8%, 84.4%, 83.7% and 85.8% respectively. This confirms previous report that RDTs can be very useful and reliable in the management of patients with suspected malaria, especially in rural health centers where microscopic diagnosis cannot be readily performed and in community case management of malaria where treatment is provided by trained community health workers [22,23,24]. Although the sensitivity of these diagnostic methods is good and comparable the same cannot be said of their specificity. The specificity obtained for microscopy was relatively high (75.0%) but those of the RDTs were very low (SD Biolone 29.5%,

Carestart 30.6% and Micropoint 33.3%). The implication of these results is that the true positive rate (sensitivity) of all the methods are good, the true negative rate (specificity) of the RDTs are poor. Approximately half of the samples that were negative were actually taken as positive by the RDTs. Studies have shown that HRP-2- based RDTs can be influenced by several factors, including antigenic variability of the target protein, antigen persistence in the bloodstream following elimination of parasites, parasite density below the RDT threshold of detection and parasites lacking *hrp2* genes [16,25]. In malaria endemic areas where transmission is perennial, studies have shown that HRP2 antigen could persist in the bloodstream for more than 5 weeks after successful treatment [26,27].

Another possibility that cannot be excluded is that many individuals carried low parasite density that may not be detected by microscopy despite

the quality control like using double reading of two experience microscopist. These findings point towards the possibility of over diagnosis when using HRP-2 tests for the management of malaria in children living in area of intense and seasonal transmission. Besides all the aforementioned factors, RDTs performance could be affected by storage, handling and weather [28]. Generally, in Nigeria, RDTs are sold in many places with little or no proper good storage facility. Many sellers do not strictly follow the storage and handling conditions stipulated by the manufacturers. The low specificity observed in this study may therefore not be the true performance of these RDTs as the RDTs used for the study were purposely bought from pharmaceutical stores in the city.

Another important finding from this study is the high rate of malaria infection observed in the study population. Although the study was conducted during high malaria transmission period, it is still an indication that there is still high transmission of malaria in this area despite different malaria intervention that has been introduced [10]. The malaria infection rate was higher among younger children (0-5 years old), compared to older ones (6-10-years-old) although the difference in the prevalence was not statistically significant. This observation is consistent with the finding of some studies in endemic areas where it is well known that

immunity to malaria increases with age [29,30]. In addition, male children were found to have a higher infection rate than their female counterparts. Several studies have reported similar findings, which may be due to female children being less biologically vulnerable to infectious diseases than male children [31,32].

In conclusion, our data showed high sensitivity of microscopy and commercially available RDTs but low specificity of the RDTs in the diagnosis of malaria in our study population. The implication of this is that overreliance of malaria diagnosis on RDTs may lead to misdiagnosis in the study area. There is need for a more coordinated malaria control approach by all stakeholders that will include making sure that available RDTs in the market are stored according to manufacturer's instructions. There must be a renewed interest from stakeholders to scale up the control effort in this area.

Conflict of interest: The authors declare that there is no conflict of interest.

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ASSESSING THE KNOWLEDGE AND AWARENESS OF GENDER BASED VIOLENCE (GBV) AMONG FEMALE STUDENTS IN UNIVERSITY OF PAPUA NEW GUINEA

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ABSTRACT:

Several studies have been carried out on the existence and prevalence of gender based violence (GBV) in Papua New Guinea (PNG). However there are no published data on the prevalence of GBV among female students in the universities in PNG. The purpose of this study was to assess the knowledge, awareness and prevalence of GBV among female students at the University of Papua New Guinea (UPNG). This was an institution-based, cross-sectional, observational, descriptive study. The target population was residential and non-residential female students. The study was carried out during the 2019 academic session. All registered female students in their second year or higher were eligible to participate in the study. Simple random sampling was used to select 450 consented students. A structured self-designed, pre-tested self-evaluating questionnaire with mostly close-ended questions was used for data collection. The completed questionnaires were checked, coded and entered into Excel Spreadsheet. A total of 217 questionnaires were completed and returned. The response rate was 48.2%. The age range of the respondents was 18 to 39 years. A total of 97.7% of respondents have heard about GBV and 89.9% knew about GBV. However, 92.2% did not know if UPNG has policy about GBV. Some of the respondents have personally experienced violence. Verbal violence (25.8%) was the highest, followed by psychological violence (19.8%), sexual violence (19.7%) and physical violence (11.0%). However, the sequence and prevalence were different with regards to their knowledge of the occurrence of violence against their colleagues. Physical violence (70.0%) was the highest, followed by verbal violence (62.7%), psychological violence (58.0%) and sexual violence (22.6%). Male students were the main perpetrators in all the forms of violence, with minimal involvement of the lecturers.

In conclusion, awareness workshops and campaigns should be carried out in the university to educate both female and male students about GBV and its negative impacts. The GBV policies in the university should be enforced. A GBV center should be established where students can seek medical attention, counseling and other related services. Risk-reduction and health-promoting programs should be developed and implemented to ensure safety on campus through strict non-tolerance policies and appropriate penalties for perpetrators of GBV.

Keywords: Gender based violence (GBV), Female students, University of Papua New Guinea.

INTRODUCTION:

Recognition of Gender Based Violence (GBV) or Violence Against Women (VAW) as a public health issue as well as a human right issue was strengthened by declarations and agreements at key international conferences during the 1990s [1-3]. These included the World conference on Human Rights in Vienna (1993) [1], the Fourth World conference on Women in Beijing (1995) [2] and the International Conference on Population and Development in Cairo (1994) [3].

Recognizing the urgent need for the universal application to women of the rights and principles with regard to equality, security, liberty, integrity and dignity of all human beings, the United Nations (UN) General Assembly proclaimed the UN Declaration on the Elimination of Violence against Women [4]. It was agreed that the declaration made up of six separate articles should become generally known and respected in all member states who are signatories to the declaration [4]. The first three of the six articles clearly stated the nature of these complex problems.

Article One: For the purposes of this Declaration, the term "Violence Against Women" means any act of Gender Based Violence that results in, or is likely to result in, physical, sexual or psychological harm or suffering to women, including threats of such acts, coercion or arbitrary deprivation of liberty, whether occurring in public or in private life.

Article Two: Violence against women shall be understood to encompass, but not be limited to, the following: (i) Physical, Sexual and Psychological violence occurring in the family, including battering, sexual abuse of female children in the household, dowry-related violence, marital rape, female genital mutilation and other traditional practices harmful to women, non-spousal violence and violence related to exploitation; (ii) Physical, Sexual and Psychological violence occurring within the general community, including rape, sexual abuse, sexual harassment and intimidation at work, in educational institutions and elsewhere, trafficking in women and forced prostitution; (iii) Physical, Sexual and Psychological violence perpetrated or condoned by the State, wherever it occurs.

Article Three: Women are entitled to the equal enjoyment and protection of all human rights and fundamental freedoms in the political, economic, social, cultural, and civil or any other field. These rights include, inter alia: The right to life; the right to equality; the right to liberty and security of person; the right to equal protection under the law; the right to be free from all forms of discrimination; the right to the highest standard attainable of physical and mental health; the right to just and favourable conditions of work; the right not to be subjected to torture, or other cruel, inhuman or degrading treatment or punishment [4].

According to recent modifications [5, 6], the acts of physical aggression include slapping, hitting, kicking and battering; psychological abuse include intimidation, constant belittling and humiliating whether in public or in private, controlling behaviors such as isolating the partner from his/her family and friends, observing and monitoring their movements and prohibiting their access to information as well as assistance from others around them. Verbal violence can be spoken words, written words, degrading comments, threatening, blaming or name calling. It encompasses a wide range of behaviors and although the damage cannot be physically seen, it affects a person mentally and psychologically, and reduces self-esteem and self-confidence [7, 8]. It is often confused with normal day-to-day talk among partners, friends or peers. As a result of the usual practice of using cuss words or telling a joke that is sarcastic and often belittling, it has already been instilled in the minds of people that it is actually condoned to verbally abuse a person [7, 8]. Verbal violence needs continuous check where every individual should be educated on what is considered to be unacceptable because only then will a person be responsible for their choice of words and thus not intentionally exercise verbal abuse [7, 8]. Despite the international collaborative efforts to combat GBV in countries around the world, recent reports indicate prevalence of various forms of violence against women in some UN member states. According to a recent

global report on the prevalence and health effects of intimate partner violence and non-partner sexual violence, about 35% of women worldwide have experienced some form of physical and/or sexual violence that was committed either by an intimate partner or a non-partner. In addition, about 7% of women on a global level have been sexually abused by a non-partner and almost 30% of all women who have been in relationships were physically and sexually abused [5].

Many of the developing countries are at different stages of implementing the UN Declaration on the Elimination of Violence against Women. They have taken various steps towards gender equality and have declared that both females and males can hold similar job aspirations as well as be compatible in working environments [3, 9]. One of these countries is Papua New Guinea (PNG). The estimated population of PNG is about 8.5 million of which about 49.0% are females [10]. Several reports including the reports compiled by the Law Reform Commission in PNG indicated that the most common type of GBV in the country is domestic violence [7, 11 – 14].

The major impact of these reports led to the promulgation of the PNG National Strategy on GBV 2016 – 2025. It is the Government's framework to prevent and respond to GBV, which is viewed as an endemic problem affecting the lives of many individuals and communities in the country [15]. The reports indicated that one of the reasons why men in

PNG are so violent is because of the traditional mentality surrounding male control and superiority. A small sign of disrespect or disloyalty by women was met with harassment and violence by men or spouses. At most times these domestic matters are left alone because they are deemed to be “private” matters that should be sorted out within the family [11, 13, 14]. It was also stated that women may think that they deserve to get a beating because of some wrongdoing, while others may not report these incidences in fear of their partners or some because of the embarrassment of being branded as a “battered woman” [7, 11, 13, 14]. In PNG, when a selection of informants were asked the question; “*is it alright for a man to hit his wife?*” three out of four females answered that “*yes, at some point it is alright for a man to hit his wife*”. Based on these responses, the majority of women in PNG may have the same view and due to these instilled views of society, young women in the generation may not be aware that some of these characters that are portrayed by men, whether physically, verbally, psychologically or sexually, are actually forms of GBV [14, 16].

A study carried out by PNG National Department of Health (NDOH) in 2015 states that, in 2007, 400 women at health clinics confessed that the main trigger for violence in their homes was the woman’s refusal to have sex or asking their partners to use condoms [17]. About 50% of the women that were interviewed admitted that they cannot refuse

having sex without being punished. Another study carried out by WHO mentions that in PNG, 67% of women in rural areas and 57% of women in urban areas were abused by their partners on a daily basis [17]. A study carried out at the Angau hospital in Morobe province in PNG reported that out of 239 survivors of sexual violence, 98% were females; of these 28% were raped by more than one man and 57% of them were under 20 years old [17].

Although there are several reports to confirm the existence of domestic violence in PNG and the occurrence of GBV in business sectors, no studies have been carried out amongst students in any of the universities in PNG [18].

One of the major strategies for reducing the rate of GBV is to educate female as well as male students in the higher institutions on the effects that GBV can have in the lives of affected individuals, the forms of violence and the nature of these acts, as well as the importance of gender equality. One of the findings in a recent study on the prevalence of sexual violence against female university students in Ethiopia is that it contributes to the establishment of a non-conducive and intimidating learning environment [19]. Similar deductions have been made from studies carried out in universities in South Africa [20] and Nigeria [21].

GBV is inclusive of different forms of violence; however the focus of our present study is on physical, sexual, verbal and psychological/emotional violence experienced

by female students in the UPNG. A major justification for this study is that the university is a microcosm of society, and the societal culture always finds expression on university campuses [20]. For this reason, it is of utmost importance that the experiences of female students in the UPNG are investigated and the data obtained be used to fill in the existing gaps on the prevalence of GBV in PNG.

The purpose of the present study is to assess the knowledge, awareness and prevalence of GBV among female students in the University of Papua New Guinea.

METHODOLOGY:

This study was carried out in the UPNG, which is the premier university in PNG. The university has two campuses; the Waigani and Taurama campuses. Students in the Taurama campus complete their foundation year in the Waigani campus before moving over to Taurama to pursue the degree in medicine, dentistry or health sciences.

This was an institution-based cross-sectional observational descriptive study. The target population was current female students whether residential or non-residential in both campuses (Waigani and Taurama) of the UPNG. The study was carried out during the 2019 academic session. All registered female students in their second year or higher were eligible to participate in the study. Simple random sampling was used for selection of the participants.

The sample size was calculated using a design effect of one, relative precision of 10% and confidence level of 95% [22]. As there was limited data on likely prevalence of GBV in UPNG, a prevalence rate of 25% was used. With a predicted non-response rate of 15%, the total sample size of about 450 female students was obtained. This sample size was considered adequate for a mini-survey with limited resources.

A structured self-designed questionnaire with mostly close-ended questions was used for data collection. The questionnaire was pretested among 20 randomly selected female students that were not included in the final cohorts of participants in the study. Suggested changes and modifications were made, after which a second pre-test was done among another group of 15 female students. The final questionnaire was approved after the additional changes and modifications were made. The questions were categorized under the following subsections; socio-demographic characteristics of the respondents, experience and awareness of physical, psychological, verbal, sexual violence, and gender stereotypes. Apart from the questions on the socio-demographic characteristics, the questionnaire contained a total of 33 closed-ended and two open-ended questions. Some questions were used to elicit perceived causes of violence and also to suggest ways of preventing GBV in the university.

A consent form containing a brief background of the problem and explaining the purpose of the study was attached to each of the questionnaire. A total of 450 questionnaires were randomly distributed. In order to ensure confidentiality, each of the participants was requested not to include their name, student identification number and degree program in the questionnaire. They were however, requested to complete the questionnaire honestly and truthfully, and then drop it in one of the collection boxes placed at different sites on campus including the university libraries. This precaution was taken because of anecdotal evidence that female students may be afraid to answer questions related to violence against women.

All female students in the foundation year and those that matriculated in 2019 were excluded from the study.

The questionnaires were collected checked, coded and entered into Excel Spreadsheet. Statistical Package for Social Sciences (SPSS) software (version 20) and the Microsoft Excel Data Pack 2010 were used for statistical analyses of the data. Categorical variable were presented as frequencies and percentages. Bivariate analysis involving the use of Chi-square tests was used as appropriate. A p-value of less than 0.05 was considered as statistically significant.

The ethical clearance for this study was obtained from the Ethics and Research committee in the SMHS UPNG. Informed

consent was obtained from each of the female students that participated in this study. Their participation was completely voluntary.

RESULTS:

In the present study, a structured self-administered anonymous questionnaire was used for data collection. A total of 217 questionnaires were completed and returned. The response rate was 48.2% (217/450). The age range of the respondents was 18.0 to 39.0 years, mean age was 21.7 ± 2.56 years (Mean \pm standard deviation), the 95% confidence interval (95% CI) was 21.4 to 22.0 years. Distribution of the respondents according to age groups show that 18.0% (39/217) were below 20 years of age, 73.7% (160/217) were between 20 to 25 years and 8.3% (18/217) were above 25 years of age. Of the 217 respondents 177 (81.6%) were resident on-campus and 40 (18.4%) were living in accommodations off-campus. A total of 51.2% (111/217) of respondents were currently in a relationship but not married, 29.5% (64/217) were not married but had past relationships, 12.4% (27/217) had never been in any relationship and 6.9% (15/217) were currently married.

The responses to the 33 closed-ended questions are presented in Table 1. A total of 97.7% (212/217) of respondents have heard about GBV and 89.9% (195/217) knew about GBV. This indicated good knowledge about the

existence of GBV among the female students. However, 92.2% (200/217) did not know if UPNG has any policy about GBV. This raises questions about the information sharing and communication procedures in relation to policies on GBV within the UPNG campuses.

With regards to physical and psychological abuses, 70.0% (152/217) were aware of female students that have been physically abused by their partners on campus. However, 88.5% (192/217) said that they have not been abused or punished by their partners; although 19.8% (38/192) of them said that they have experienced continuous ridicule directed at them by their partners; furthermore, 33.0% (30/91) of those that have not been ridiculed directly said that their partners have prohibited them from seeing their friends on campus. When asked if their partners have emotionally threatened them into doing what they do not want to do, 80.6% (175/217) responded in the negative. When answered if they are aware of other female students that have been psychologically abused, 58.1% (126/217) said yes. In relation to verbal abuse, 25.8% (56/217) of respondents have been verbally threatened by their partners; 36.4% (79/217) have partners that have used foul/inappropriate language against them either in public or in private.

A larger percentage (62.7%; 136/217) of respondents knew of other female students that have been verbally abused.

When asked if the male students usually treat them respectfully, 43.8% (95/217) answered in

the affirmative, and 55.3% (120/217) said sometimes but not always. When asked if the male students allow them the opportunity to share their opinion in the university public forum, 29.5% (64/217) said “always”, but 59.9% (130/217) said “sometimes”.

A majority of the respondents (77.0%; 167/217) said that their opinions were sometimes respected and valued; but in response to another question, 71.9% (156/217) said sometimes they were made to feel inferior to the male students.

In relation to sexual violence, 35.0% (76/217) of respondents had experienced sexual intercourse; of these 19.7% (15/76) had not given their consent at that time. A total of 22.6% (49/217) of respondents are aware of female students who were forced to have sex by someone in the university, and 6.5% (14/217) said that they have been forced to have sex by someone in the university. In addition, 13.3% (29/217) of respondents knew female students from whom male staff demanded sex in exchange for something. The respondents knew female students (39.2%; 85/217) who have been sexually harassed on campus; but they do not know if any formal complaint was made by any of the female students.

Additional questions indicated that 30.4% (66/217) of the respondents were also sexually harassed on campus, but only 6.0% (13/217) were in relationships that have been sexually abusive.

The responses to the two open-ended questions that required the opinions and recommendations of the respondents were analyzed into themes and presented in Tables 2 and 3.

In response to the first open-ended question (Table 2), 56.7% (123/217) said the male students were respectful / well-mannered and knew a bit about GBV; 16.6% (36/217) said male students were ignorant of issues related to GBV and 12.4% (27/217) said they tend to express male superiority on campus.

In response to the second open-ended question (Table 3), 29.0% (63/217) suggested that the university should “*enforce/ implement the current GBV policy*”; 22.1% (48/217) suggested “*Improve awareness /empowerment /educate students on issues of GBV*”. A total of 20.7% (45/217) of the respondents did not answer this question. This strongly suggests the need for more effective awareness campaign, education and enhanced communications among the female students in the University of Papua New Guinea.

Table 1: Distribution of the frequency (%) of the responses to questions in the questionnaire

QUESTIONS	RESPONSES	RESPONDENTS n = 217 (%)
Knowledge and awareness of GBV		
Q1. Have you ever heard about GBV?	Yes	212 (97.7%)
	No	5 (2.3%)
Q2. Do you know about GBV?	Yes	195 (89.9%)
	No	22 (10.1%)
Q3. Do you know if UPNG has any policy about or on GBV?	Yes	17 (7.8%)
	No	200 (92.2%)
Physical violence		
Q4. Do you have someone that you call / have called your partner, in UPNG or outside of UPNG?	Yes	128 (59.0%)
	No	89 (41.0%)
Q5. Are you aware of any female(s) in UPNG who has been physically abused by a partner?	Yes	152 (70.0%)
	No	65 (30.0%)
Q6. Have you ever been physically punished or abused by any of your partner/partners?	Yes	24 (11.0%)
	No	192 (88.5%)
	No response	1 (0.5%)
Psychological violence		
Q7. If No to Q6, have you experienced continuous ridicule directed to you by your current/previous partner(s), in public or in private? [N = 192]	Yes	38 (19.8%)
	No	91 (47.4%)
	No response	63 (32.8%)

Q8. If No to Q 7, has your current/previous partner(s) ever prohibited you from seeing friends/family? [N = 91]	Yes	30 (33.0%)
	No	60 (65.9%)
	No response	1 (1.1%)
Q9. Has your current/previous partner(s) ever intentionally ignored you or dismissed you from his social activities?	Yes	59 (27.2%)
	No	157 (72.3%)
	No response	1 (0.5%)
Q10. Has your current/previous partner(s) ever emotionally threatened you into doing something that you did not want to do?	Yes	42 (19.4%)
	No	175 (80.6%)
Q11. Have you ever been in a situation where your current/previous partner(s) imposed suicidal threats on himself if you did not comply with his request?	Yes	42 (19.4%)
	No	175 (80.6%)
Q12. Do you know of any female students at UPNG who have experienced any of the actions described in Q8-11?	Yes	126 (58.1%)
	No	91 (41.9%)
Verbal violence		
Q13. Have you ever been verbally threatened by any of your current/previous partner(s)?	Yes	56 (25.8%)
	No	161 (74.2%)
Q14. Have you been insulted & shouted at by your current/previous partner(s) in public or in private?	Yes	65 (30.0%)
	No	152 (70.0%)
Q15. Has any of your current/previous partner(s) ever used foul /inappropriate language against you in public or in private?	Yes	79 (36.4%)
	No	138 (63.6%)
Q16. How do you usually handle / respond to the situations in Q14 and 15?	Reply in a similar manner	23 (10.6%)
	Walk out or away while he is talking	56 (25.8%)
	Sit still and listen	29 (13.4%)
	Ignore him	109 (50.2%)
Q17. Do you know of any female UPNG students who have experienced any of the actions described in Q13 to 15?	Yes	136 (62.7%)
	No	81 (37.3%)
Q18. Do male students in UPNG treat you with respect?	Yes	95 (43.8%)
	No	2 (0.9%)
	Sometimes	120 (55.3%)
Q19. Do the male students allow you the opportunity to share your opinion in the public forum?	Always	64 (29.5%)
	Sometimes	130 (59.9%)
	Never	21 (9.7%)
	No response	2 (0.9%)
Q20. Do you think that your opinion is respected, valued and considered when in discussion with male students?	Always	45 (20.7%)
	Sometimes	167 (77.0%)
	Never	5 (2.3%)
Q21. Have you ever been made to feel inferior by the	Always	6 (2.8%)

male students around you?	Sometimes	156 (71.9%)
	Never	55 (25.3%)
Sexual violence		
Q22. Have you already experienced sexual intercourse?	Yes	76 (35.0%)
	No	140 (64.5%)
	No response	1 (0.5%)
Q23. If Yes (to Q22), how old were you when you first engaged in sexual intercourse? Please give approximate age: [N = 76]	14-19.9 years	37 (48.7%)
	20-24.9 years	37 (48.7%)
	25-29.9 years	1 (1.3%)
	No response	1 (1.3%)
Q24. If Yes (to Q22) did you give your consent at that time? [N = 76]	Yes	61 (80.3%)
	No	15 (19.7%)
Q25. Do you know of any female students who have been forced to have any form of sex by anyone at school in UPNG?	Yes	49 (22.6%)
	No	166 (76.5%)
	No response	2 (0.9%)
Q26. Have you ever been forced to have any form of sex by anyone in the UPNG?	Yes	14 (6.5%)
	No	201 (92.6%)
	No response	2 (0.9%)
Q27. Do you know of any female students from whom a male staff in UPNG demanded sex in exchange for anything?	Yes	29 (13.3%)
	No	187 (86.2%)
	No response	1 (0.5%)
Q28. Has any male staff in UPNG ever demanded sex from you in exchange for anything?	Yes	1 (0.5%)
	No	215 (99.0%)
	No response	1 (0.5%)
Q29. Has any male student in UPNG ever demanded sex from you in exchange for anything?	Yes	5 (2.3%)
	No	211 (97.2%)
	No response	1 (0.5%)
Q30. Do you know of female students who have been sexually harassed in the UPNG campuses?	Yes	85 (39.2%)
	No	130 (59.9%)
	No response	2 (0.9%)
Q31. If yes to Q 30, do you know if any formal complaint was made by any of the female students? [N = 85]	Yes	0 (0%)
	No	85 (100%)
	Not sure	0
Q32. Have you ever experienced sexual harassment or was at the receiving end of sexual comments when in UPNG campuses?	Yes	66 (30.4%)
	No	150 (69.1%)
	No response	1 (0.5%)
Q33. Does any of the relationships that you have been in sexually abusive?	Yes	13 (6.0%)
	No	203 (93.5%)
	No response	1 (0.5%)

Table 2: Responses to the open ended question Q 34:
What is your general opinion of most of the male students around you?

Answer theme	Frequency (%)
Respectful/well-mannered and knows a bit about GBV	123 (56.7%)
Ignorant of issues related to GBV	36 (16.6%)
Tends to express male superiority	27 (12.4%)
Disrespectful when intoxicated	17 (7.8%)
No opinions	14 (6.5%)

Table 3: Responses to open ended question: What suggestions, if any, would you like to make to the university authorities about GBV? Q 35

Answer theme	Frequency (%)
Enforce/ implement the current university GBV policy	63 (29.0%)
Improve awareness/empowerment/educate students on issues of GBV	48 (22.1%)
Increase security in female dormitories; encourage female students to report cases of violence on campus	24 (11.1%)
Engage more counselors to help female students on campus	17 (7.8%)
Establish the GBV center/ Support groups in university campus	12 (5.5%)
Prohibit drinking of alcohol by strongly implementing the zero tolerance policy on campus, especially in the Waigani campus	8 (3.7%)
No recommendations	45 (20.7%)

DISCUSSION:

The non-response rate of 51.8% (233/450) obtained in the present study was significantly higher than the predicted 15.0% non-response rate used in calculating the sample size. One of the major reasons for the very low response rate was because it was optional for each of the consenting respondents to complete and return the questionnaire. It indicates one of the difficulties encountered in carrying out research involving obtaining information about issues considered as private and personal to the respondents in such studies.

The low response rate also indicates the lack of interest on issues related to GBV among female students in the UPNG. This is not unique to university students in PNG, because low response rates have been reported by other authors that conducted similar studies in other countries [19, 20, 21]. This strongly indicate the need to carry out intensive advocacy and awareness campaign among female students in UPNG about the need to be pro-active in supporting the implementation of the United Nations policy to reduce the negative impact of GBV [4] and the PNG National Strategy on GBV 2016 – 2025 [15].

The very low percentage (7.8%) of respondents that are aware of the UPNG policies should be of concern to the authorities. It indicates the need for effective communication strategies with the students. The importance of awareness and advocacy is to ensure that female students are able to recognize the different forms of GBV and can readily report these acts to the appropriate authorities in the university without fear of intimidation.

In the present study 11.0% of respondents said that they have experienced physical violence; this result was lower than the 22.8% reported among female university students in Northern Nigeria [21], and 28.1% among female students in Kenya [22]. It was higher than the 8.9% among female students in Wolaita Sodo University in Ethiopia [23].

The respondents in the present study were aware of a higher percentage (70.0%) of their colleagues that have been subjected to physical violence. It is therefore possible to assume that in the present study, the 11.0% may be due under-reporting because of the low response rate. The fear of physical abuse by their partners may have prevented some of the respondents that collected the questionnaires from completing and returning them. Physical violence or threat of physical violence is one of the methods used in the university campuses to intimidate and manipulate female students [21, 22, 23].

Although 19.4% of the respondents had experienced psychological or emotional violence, they are aware of a higher percentage (58.1%) of their colleagues that have experienced this form of violence. This further strengthens the earlier suggestion that intimidation may have prevented some of the students that collected the questionnaires from completing and returning them. A similar study in Kenya [22], states that threats from partners are one of the major types of psychological abuse that is seen among university students; it shows that students can be psychologically intimidated to keep them from participating in research which may be regarded as an invasion of private matters. Alcohol consumption pattern was not included in our present study. However, alcohol consumption is a risk factor for emotional violence on university campuses [22, 24]. Thus it can be considered as a contributing factor to emotional violence in our present study.

Over 25.0% of the respondents have experienced verbal violence on campus and they are aware of about 63.0% of their colleagues with similar experience. This should be of great concern to the authorities in UPNG, because of the negative impact on the moral and self-esteem of the female students. It is a violation of the fundamental rights and privileges of these students; it strips them of their right to freedom of speech as well as

expression and hinders their ability to openly communicate with others. Verbal violence is as intimidating as physical violence; it is a secret weapon that causes depression, mood changes and low self-confidence that can affect the academic performance of female students.

The 6.5% of respondents in the present study that have been forced to have any form of sex by anyone in the university was lower than the 22.2% reported for female students in Northern Nigeria [21], and the 24.4 to 36.1% reports for female students in Ethiopia [19]. Several other studies in universities have presented higher prevalence of sexual violence [24-27]. The 6.5% may be due to under-reporting because of the low response rate in our present study. However, 22.6% of the respondents were aware of their female colleagues that have been forced to have sex by someone in the university. In addition, 30.4% of the respondents and 39.2% of their colleagues have been sexually harassed on campus. Having a regular boyfriend where there is intimacy involved can increase the risk of sexual violence. The marital status of respondents indicate that a higher percentage were currently in a relationship but were not married. These are strong evidence indicating high rate of sexual violence on campus that urgently needs the attention of the appropriate authorities in the University of Papua New Guinea. Sexual violence is one of the worst

forms of GBV and leaves a person mentally and emotionally scarred [24, 25].

The results show that male students were the main perpetrators in most cases of all the forms of violence, with minimal involvement of the lecturers in the present study. In addition, about 43.3% of the male students were said to be often disrespectful. There was also indication of alcohol consumption by male students on campus. These findings were different from other studies in which some lecturers were involved in sexual violence. Studies in South Africa, Zimbabwe, Ghana, Nigeria, Malawi reported that male students and lecturers were involved in abuse and sexual harassment of female students [20-23; 25-27].

Verbal violence (25.8%) was the highest, followed by psychological violence (19.8%), sexual violence (19.7%) and physical violence (11.0%) that the respondents have experienced personally in our present study. These values were lower than the corresponding values reported for Northern Nigeria, Ethiopia, Kenya, Uganda and Sub-Saharan Africa in general [21-27]. However, the sequence and prevalence were different with regards to their knowledge of the occurrence of violence against their colleagues. Physical violence (70.0%) was the highest, followed by verbal violence (62.7%), psychological violence (58.0%) and sexual violence (22.6%).

According to Beyene et al [24] studies on GBV are highly heterogeneous because of the definitions used, sample size, tools used and data collection methods. This makes it difficult to compare and to generalize the results in the various studies. In a recent systematic review and meta-analysis by Beyene et al [24], the overall prevalence of GBV ranged from 42.3% in Nigeria to 67.7% in Ethiopia. The prevalence of sexual violence ranged from 4.3 to 76.4%, physical violence ranged from 7.4 to 66.1%, and emotional violence prevalence ranged from 26.1 to 50.8%. The results obtained in our present study are within these ranges reported by Beyene et al [24].

Although the university can be considered as a microcosm of society, the results obtained in our present study should not be extrapolated to the general population in the other universities and in PNG as a whole. This is because of the highly selective nature of our study population. Apart from the small sample size the female university students are highly educated and may not be representative of the cross-section of females in the different social classes in PNG.

Our present study has a number of limitations. The actual non-response rate was higher than the predicted value used in the calculation of the sample size. A self-reporting questionnaire was used for data collection.

CONCLUSION:

The results indicate the prevalence of GBV among female students in the UPNG. Verbal violence was the highest, followed by psychological violence, sexual violence and physical violence that the respondents have experienced personally. The sequence and prevalence were different with regards to the knowledge of the occurrence of violence against the colleagues of the respondents in that physical violence was the highest, followed by verbal violence, psychological violence and sexual violence. Male students were the main perpetrators in most cases of all the forms of violence, with minimal involvement of male lecturers. Proper awareness workshops and campaigns should be carried out in the university to educate both female and male students about GBV and its negative impacts. The GBV policies in the university should be enforced. A GBV center should be established where students can seek medical attention, counseling and other related services.

It is recommended that risk-reduction and health-promoting programs should be developed and implemented to ensure safety on campus through strict non-tolerance policies and appropriate penalties for perpetrators of GBV.

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ANTIOXIDANT ACTIVITY AND TOXICOLOGICAL IMPLICATIONS OF THE AQUEOUS EXTRACT OF *Phyllanthus amarus* (Euphorbiaceae) LEAVES IN FEMALE WISTAR RATS

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ABSTRACT:

The aqueous extract of *Phyllanthus amarus* leaves (AEPAL) have been used in the treatment of female sexual dysfunction without recourse on its safety. Hence, the need to ascertain the safety of this plant by establishing the toxicological implication of consuming the plant and increasing their acceptability. Twenty-eight female Wistar rats (159.25 ± 3.32 g) were randomized into four groups (A - D) of seven animals each and orally administered 0.5 mL of distilled water, 20, 40 and 80 mg/kg body weight of *P. amarus* leaves extract respectively once daily for 21 days. The toxicological effects of the AEPAL were evaluated by assessing selected parameters in the liver, kidney and antioxidant system. All parameters measured were analyzed using standard procedures. The levels of liver total protein, calcium ion, catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase increased significantly ($p < 0.05$), whereas the activities of glucose-6-phosphate dehydrogenase and concentration of reduced glutathione, urea, creatinine and sodium ion decreased significantly ($p < 0.05$). There were no significant changes in the activities of lactate dehydrogenase, liver and serum alkaline phosphatase as well as the levels of malondialdehyde and total antioxidant capacity. The results obtained in the present study indicate that the AEPAL is nephroprotective but may cause microvesicular steatosis in the liver at a dose of 80 mg/kg body weight.

Keywords: Antioxidant; Euphorbiaceae; hepatotoxicity; histoarchitecture; *Phyllanthus amarus*; medicinal biochemistry

INTRODUCTION:

Medicinal plants, either as extracts, polyherbal mixtures, pure compounds or derivatives offer unlimited opportunities towards the discovery of new drugs [1]. The biological activities of various medicinal plants used in folklore medicine have been validated or refuted with scientific evidence. The general acceptability of herbal medicines has been limited by a lack of defined chemical characterization, dose regimen and adequate toxicity data to evaluate their safety [2]. Hence, there is a dearth of information on the toxicological implications of most of these plants majority of which are anecdotally perceived as safe. One of such plant is *Phyllanthus amarus* Schum Thonn (Euphorbiaceae) commonly known as *eyin olobe* (Yoruba – Western Nigeria), *geeron tsutsaayee* (Hausa – Northern Nigeria) and *ngwu ite kwowa nasu* (Igbo – Eastern Nigeria) [3]. It is a small, erect, tropical, annual herb found in Philippines, Cuba, India, Malaysia and Nigeria. *P. amarus* can grow about 30 – 40 cm high and has slender leaves-bearing branchlets, anchored by distichous leaves which are sessile, elliptically oblong, obtuse and rounded base. It is widely used in the treatment of various diseases such as diarrhoea, hepatitis, chronic dysentery, gonorrhoea, diabetes, appendix inflammation, intermittent fever, scabies, gallstones, ulcers and prostate problems [4 – 6].

Nurudeen and Yakubu [7] reported the restoration of sexual competence in fluoxetine-induced female sexual dysfunction rats treated with the aqueous extract of *P. amarus* leaves at 80 mg/kg body weight. The acute and chronic toxicity study of *P. amarus* leaves revealed that there was no mortality at 5000 mg/kg body weight as well as the absence of functional and structural toxicity at 100, 400 and 800 mg/kg body weight respectively [8]. The extracts of *P. amarus* (aqueous, methanolic and hydromethanolic) and *P. fraternus* (hydroethanolic) had no toxic effect on WBC and RBC counts as well as the enzymes used for liver function tests (ALT, AST and ALP) [9]. The hepatotoxic assessment of the methanolic extract of *P. amarus* leaves at 1000, 2000 and 5000 mg/kg body weight revealed that the extract was relatively safe for consumption [6]. Despite the avalanche of studies on the toxicological evaluation of *P. amarus* leaves extract in open scientific literature, the efficacy and toxicity of plants vary by geographical location and soil composition. Hence, this study was designed to evaluate the toxicological implication of the aqueous extract of *P. amarus* leaves grown in Ilorin, Nigeria at 20, 40 and 80 mg/kg body weight on the functional capacity of the liver, kidneys and antioxidant system.

MATERIALS AND METHODS:

Collection and authentication of the plant material:

Fresh *Phyllanthus amarus* leaves were collected behind the Administrative building of Al-Hikmah University, Ilorin, Nigeria (Latitude 8° 29' 47.90" N; Longitude: 4° 32' 31.70" E) and authenticated at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. A voucher specimen was deposited under UIH 001/1109.

Experimental animals:

Twenty-eight healthy, female Wistar rats (*Rattus norvegicus*) weighing 159.25 ± 3.32 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were contained in their respective cages placed in a well-ventilated Animal House and maintained at a temperature of $25 \pm 3^{\circ}\text{C}$, 12 hours light and dark cycle, relative humidity of 45-50%. The animals were maintained on rat pellets (Premier Feeds, Ibadan, Nigeria) and tap water ad libitum. All the animals were strictly handled in conformation to the Declarations of Helsinki in 1995 (as revised in Edinburgh 2000) and the University's guidelines on Care and Use of Laboratory Animals (approval number – UERC/ASN/2015/210).

Reagents and assay kits:

The assay kits used for the determinations of Albumin, Total and Conjugated Bilirubin, Alanine

Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), Glucose-6-phosphate Dehydrogenase (G6PDH), Catalase (CAT), Glutathione Reductase (GRc), Glutathione Peroxidase (GPx), Reduced Glutathione (GSH), Urea, Creatinine, Electrolytes (Na, K, Ca, Cl and PO₄), Superoxide Dismutase (SOD), Malondialdehyde (MDA) and Total Antioxidant capacity (TAC) were products of Randox Laboratory Ltd, Co-Atrim, UK. All other reagents used were of analytical grade and prepared using distilled water and stored in air-tight reagent bottles except otherwise stated.

Preparation of plant extracts:

Fresh leaves of *P. amarus* were removed from the stalks and rinsed in distilled water. Thereafter, the leaves were oven-dried at 40°C (Quincy Laboratory Oven, Model 30 GC, Chicago, USA) for 48 hours and pulverized in a blender (Master Chef Blender, Model MC-BL 1980, China). The powdered leaves (50 g) were extracted in 1000 ml of distilled water for 48 hours at room temperature with intermittent shaking. The resulting filtrate was concentrated in a rotary evaporator (Model RE 52A Zhengzhou, Henan, China) to give a yield of 6.97 g corresponding to a percentage yield of 13.94 %. This was reconstituted in distilled water to give the required doses of 20, 40 and 80 mg/kg body weight of the rats. The selected doses were

adopted from the previous study by Nurudeen and Yakubu [7] on the efficacy of AEPAL in the management and/or treatment of female sexual dysfunction.

Animal grouping and administration of plant extracts:

A total of 28 female rats were acclimatized for 2 weeks and randomly assigned into four groups (A-D) of seven animals each. The rats in group A were orally administered 0.5 ml of distilled water only (control group) whereas those of groups B, C and D (experimental groups) received equal volume of AEPAL corresponding to 20, 40 and 80 mg/kg body weight respectively for 21 days.

Preparation of serum and tissue supernatants:

The procedure described by Yakubu and Salimon [10] was adopted for the preparation of serum and tissue supernatants. Twenty-four hours after the last administration (Day 22), the rats were anesthetized in diethyl ether fumes to render them unconscious, and then weighed. The jugular veins were then cut for the collection of blood samples into clean, dry centrifuge tubes. The samples were allowed to clot for 15 minutes and then centrifuged (Biobase Laboratory Centrifuge, Model LC-4KA, Jinan, China) at $894 \times g$ for 10 minutes. The sera were aspirated using Pasteur's pipette and kept frozen for 12 hours prior to biochemical analysis. The animals were then quickly dissected, and the liver and kidney were carefully removed, blotted and

stored in ice-cold 0.25M sucrose solution. The organs were separately homogenized in ice-cold 0.25 M (1:5 w/v) sucrose solution and subsequently, the homogenates were centrifuged at $1789 \times g$ for 10 minutes. The supernatants were frozen for 12 hours before being used for the determination of biochemical parameters.

Biochemical Analysis and Histological Examination:

The procedures described by Gornal *et al.* [11], Doumas *et al.* [12], Jandrassik and Grof [13], Tietz [14], Veniamin and Verkirtzi [15], Bartels and Bohmer [16] were adopted for the determination of total protein, albumin, bilirubin (total and conjugated), globulin, urea and creatinine respectively. The concentrations of electrolytes (Na^+ , K^+ , Ca^{2+} , Cl^- and PO_4^{2-}) were determined as described by Tietz [14] while the levels of GSH, MDA and TAC were determined following the procedures described by Ellman [17], Ohkawa *et al.* [18] and Miller *et al.* [19] respectively. The activities of ALT, AST, ALP, CAT, G6PDH, GRe, GPx, LDH and SOD were determined using standard procedures as described by Reitman and Frankel [20], Wright *et al.* [21], Aebi [22], Beutler [23], Goldberg and Spooner [24], Prabhu *et al.* [25], Wroblewski and La due [26] and Fridovich [27] respectively. Histological examination was carried out following procedures described by Krause [28].

Data Analysis:

Data were expressed as means \pm standard error of mean (Mean \pm SEM) of seven determinations. The Statistical Package for Social Sciences, Version 20.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis and further subjected to Duncan Multiple Range Test. Differences were considered statistically significant at $p < 0.05$.

RESULTS:

There was dose dependent increase in the levels of liver total protein and calcium ions whereas the levels of serum total protein, albumin, globulin and conjugated bilirubin were not significantly ($p > 0.05$) altered by the aqueous extract of *P. amarus* at all doses investigated compared to the controls (Table 1).

Similarly, there was a significant ($p < 0.05$) increase in the activities of ALT and AST of both the liver and serum (Table 2). The activity of G6PDH was significantly ($p < 0.05$) increased in a dose-dependent manner when compared to the control group that received distilled water only, whereas the activities of LDH, liver-ALP and serum-ALP were not significantly ($p > 0.05$) altered (Table 2). There was a significant dose-dependent decrease in the concentrations of urea, creatinine and sodium ion (Table 3).

The levels of phosphate ion were significantly ($p < 0.05$) increased at 20 and 40 mg/kg body weight only, whereas there was no alteration in the levels of potassium and chloride ions at all doses investigated (Table 3). Furthermore, the activities of CAT, SOD, GPx and GRe were significantly ($p < 0.05$) increased in a dose-dependent manner (Table 4). There was a significant decrease in the levels of GSH at 40 and 80 mg/kg body weight of the extract while the levels of TAC and MDA were not significantly ($p > 0.05$) altered at all doses investigated (Table 4). The histo-architecture of the liver of rats treated with the aqueous extract of *P. amarus* at all doses investigated revealed a normal sinusoid with no hepatocellular infiltration (Plates 1 – 4). However, there was a mild, moderate and severe microvesicular steatosis in rats on 20, 40 and 80 mg/kg body weight of the extracts respectively (Plates 2, 3 and 4). Compared with the control that showed normal glomeruli, mesangial cells, tubules and interstitial space (Plate 5), there was no histoarchitectural changes in the kidneys of rats administered 20 and 80 mg/kg body weight of the extract (Plates 6 and 8). However, there was mild tubular necrosis in rats administered 40 mg/kg body weight of aqueous extract (Plate 7).

Table 1: Effects of the aqueous extract of *Phyllanthus amarus* leaves on the liver function indices of female Wistar rats

Parameters/Dose (mg/kg body weight)	Control	20 mg/kg body weight	40 mg/kg body weight	80 mg/kg body weight
Liver total protein (g/l)	8.92 ± 0.37 ^a	10.75 ± 0.45 ^b	10.25 ± 0.31 ^b	11.28 ± 0.23 ^c
Serum total protein (g/l)	12.86 ± 1.55 ^a	13.20 ± 1.20 ^a	13.99 ± 1.98 ^a	13.72 ± 1.45 ^a
Albumin (g/l)	1.19 ± 0.06 ^a	1.20 ± 0.03 ^a	1.20 ± 0.00 ^a	1.22 ± 0.02 ^a
Globulin (g/l)	11.67 ± 1.01 ^a	12.00 ± 0.15 ^a	12.79 ± 0.75 ^a	12.50 ± 1.33 ^a
Total bilirubin (mg/ml)	2.94 ± 0.10 ^a	2.93 ± 0.00 ^a	2.89 ± 0.01 ^a	2.75 ± 0.03 ^c
Direct bilirubin (mg/dl)	2.38 ± 0.05 ^a	2.35 ± 0.10 ^a	2.35 ± 0.02 ^a	2.29 ± 0.04 ^a
Calcium ion (mg/dl)	6.88 ± 0.03 ^a	7.68 ± 0.11 ^b	8.92 ± 0.04 ^c	9.32 ± 0.08 ^d

Values are mean of 7 determinations ± SEM, values with different superscripts (a-d) across the rows for each parameter are considered to be significantly different at $p < 0.05$.

Table 2: Effects of the aqueous extract of *Phyllanthus amarus* leaves on some liver enzymes in female Wistar rats

Parameters/Dose (mg/kg body weight)	Control	20 mg/kg body weight	40 mg/kg body weight	80 mg/kg body weight
Liver alanine amino-transferase (U/l)	41.87 ± 0.27 ^a	42.75 ± 0.19 ^b	44.13 ± 0.25 ^c	44.53 ± 0.30 ^c
Serum alanine amino-transferase (U/l)	29.67 ± 0.99 ^a	37.17 ± 1.02 ^b	41.83 ± 0.67 ^c	43.17 ± 0.82 ^c
Liver aspartate amino-transferase (U/l)	65.50 ± 2.19 ^a	74.67 ± 0.89 ^b	75.83 ± 0.75 ^b	75.00 ± 1.03 ^b
Serum aspartate amino-transferase (U/l)	35.40 ± 0.11 ^a	38.40 ± 0.50 ^b	39.40 ± 1.00 ^b	37.73 ± 1.15 ^b
Liver alkaline phosphatase (U/l)	11.96 ± 0.25 ^a	10.12 ± 1.08 ^a	10.92 ± 0.73 ^a	10.46 ± 0.39 ^a
Serum alkaline phosphatase (U/l)	1.84 ± 0.02 ^a	1.86 ± 0.05 ^a	1.79 ± 0.03 ^a	1.92 ± 0.10 ^a
Lactate dehydrogenase (U/l)	201.57 ± 12.45 ^a	209.12 ± 13.33 ^a	193.57 ± 12.75 ^a	235.42 ± 10.33 ^b
Glucose-6-phosphate dehydrogenase (U/l)	11.22 ± 0.33 ^a	22.43 ± 1.35 ^b	23.10 ± 0.88 ^b	33.65 ± 3.02 ^c

Values are mean of 7 determinations ± SEM, values with different superscripts (a-d) across the rows for each parameter are considered to be significantly different at $p < 0.05$.

Table 3: Effect of the aqueous extract of *Phyllanthus amarus* leaves on the kidney function indices of female Wistar rats

Parameters/Dose (mg/kg body weight)	Control	20 mg/kg body weight	40 mg/kg body weight	80 mg/kg body weight
Urea (mm/l)	30.20 ± 0.81 ^a	18.49 ± 1.05 ^b	15.55 ± 0.15 ^c	11.09 ± 0.33 ^d
Creatinine (mg/ml)	2.96 ± 0.05 ^a	2.34 ± 0.09 ^b	1.79 ± 0.04 ^c	1.23 ± 0.12 ^d
Sodium ion (mEq/l)	129.55 ± 5.11 ^a	124.86 ± 3.08 ^a	118.04 ± 3.90 ^b	106.11 ± 10.88 ^b
Potassium ion (ppm)	6.10 ± 0.03 ^a	5.79 ± 0.25 ^a	5.55 ± 0.74 ^a	6.40 ± 0.32 ^a
Chloride ion (mg/dl)	111.25 ± 0.08 ^a	112.54 ± 0.19 ^a	112.11 ± 0.70 ^a	111.67 ± 0.82 ^a
Phosphate ion (mg/dl)	2.98 ± 0.02 ^a	3.53 ± 0.20 ^b	3.55 ± 0.05 ^b	3.07 ± 0.10 ^a

Values are mean of 7 determinations ± SEM, values with different superscripts (a-d) across the rows for each parameter are considered to be significantly different at $p < 0.05$.

Table 4: Effect of the aqueous extract of *Phyllanthus amarus* leaves on enzymatic and non-enzymatic antioxidant parameters

Parameters/Dose (mg/kg body weight)	Control	20 mg/kg body weight	40 mg/kg body weight	80 mg/kg body weight
Catalase × 10 ² (U/l)	20.00 ± 0.01 ^a	22.50 ± 0.05 ^b	22.55 ± 0.15 ^b	27.50 ± 0.50 ^c
Superoxide dismutase (U/l)	24.88 ± 0.58 ^a	49.75 ± 6.05 ^b	99.50 ± 7.55 ^c	149.75 ± 10.12 ^d
Glutathione peroxidase (U/l)	185.77 ± 0.39 ^a	132.63 ± 4.11 ^b	80.02 ± 3.89 ^c	35.54 ± 1.88 ^d
Reduced glutathione (U/mg)	8.75 ± 0.73 ^a	8.78 ± 1.05 ^a	8.05 ± 0.34 ^{ab}	7.05 ± 0.72 ^b
Glutathione reductase (U/mg)	569.71 ± 7.51 ^a	334.61 ± 8.23 ^b	105.68 ± 5.07 ^c	76.87 ± 2.29 ^d
Total antioxidant capacity (mg/dl)	82.80 ± 1.17 ^a	80.93 ± 0.91 ^a	84.85 ± 2.50 ^b	84.62 ± 1.85 ^b
Malondialdehyde × 10 ⁻⁵ (U/l)	2.30 ± 0.02 ^a	2.31 ± 0.02 ^a	2.33 ± 0.02 ^a	2.34 ± 0.01 ^a

Values are mean of 7 determinations ± SEM, values with different superscripts (a-d) across the rows for each parameter are considered to be significantly different at $p < 0.05$.

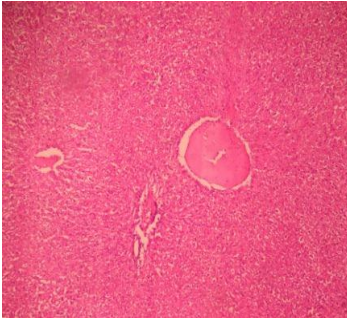


Plate 1: Cross section of the liver of control rat treated with distilled water (Mag. × 100; H & E)

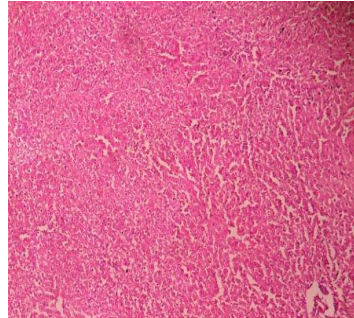


Plate 2: Cross section of the liver of rat treated with 20 mg/kg body weight of *P. amarus* leaves extract (Mag. × 100; H & E)

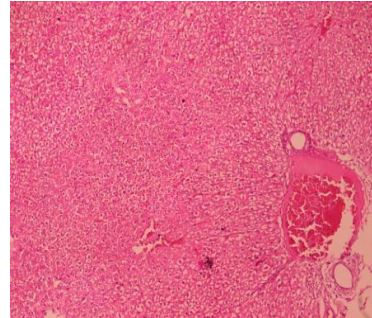


Plate 3: Cross section of the liver of rat treated with 40 mg/kg body weight of *P. amarus* leaves extract (Mag. × 100; H & E)

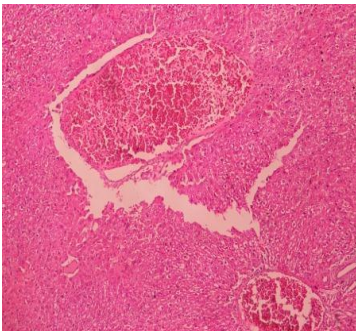


Plate 4: Cross section of the liver of rat treated with 80 mg/kg body weight of *P. amarus* leaves extract (Mag. × 100; H & E)

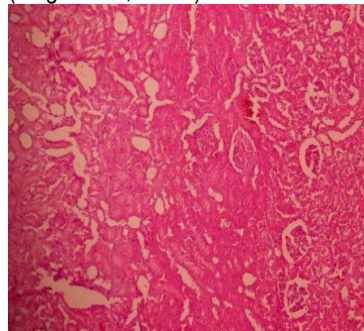


Plate 5: Cross section of the kidney of control rat treated with distilled water (Mag. × 100; H & E)

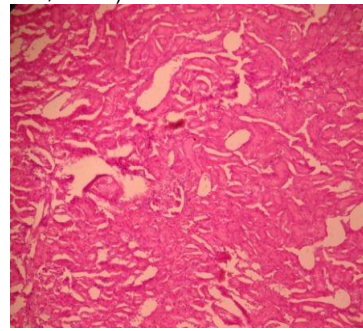


Plate 6: Cross section of the kidney of rat treated with 20 mg/kg body weight of *P. amarus* leaves extract (Mag. × 100; H & E)

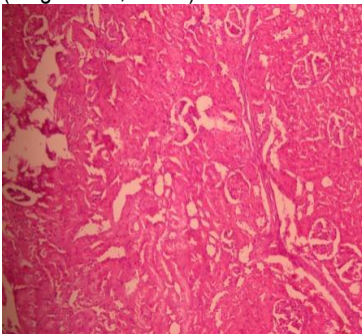


Plate 7: Cross section of the kidney of rat treated with 40 mg/kg body weight of *P. amarus* leaves extract (Mag. × 100; H & E)

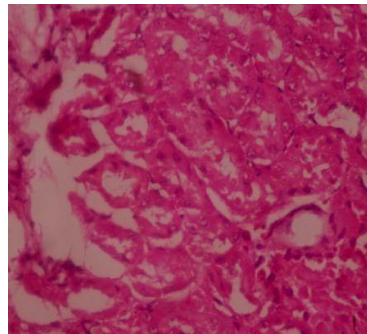


Plate 8: Cross section of the kidney of rat treated with 80 mg/kg body weight of *P. amarus* leaves extract (Mag. × 100; H & E)

DISCUSSION:

There is an ascendance in the rate of formulation and/or consumption of herbal products by the general populace due to the misconstrued belief that they are relatively safe unlike orthodox medicine with various known complications [29]. However, to declare a drug safe, it is imperative to determine the possible side effects, such as, the structural and functional integrity of the liver and kidney (the major organs involved in detoxification/biotransformation) following the use/consumption of the drug.

In the present study, total protein, albumin, globulin and bilirubin (total and direct) were monitored to assess the secretory and excretory functions of the liver [10]. The absence of significant changes in the levels of albumin, globulin and bilirubin implies that the functional capacity of the liver was not affected by any of the components in the extract. The dose-dependent increase in the levels of protein and calcium ions can be attributed to the abundance of essential amino acids and minerals in *P. amarus* leaves [30], which could result in improved bone density, muscle development, organ and tissue repair. Our findings were consistent with those of Oduola *et al.* [6] and Sirajudeen *et al.* [9] where the aqueous extract of *P. amarus* leaves was found to be relatively safe for consumption.

Tissue and serum enzyme activities were evaluated to monitor toxicity at cellular level [31]. The enzymes ALT and AST are important markers for hepatocellular impairment or necrosis [10]. The elevation in the levels of ALT and AST in both liver and serum might imply enhanced *de novo* synthesis [32]. This was further corroborated by the absence of changes in the activity of liver and serum ALP, a plasma membrane enzyme that characterizes the leakage of cytosolic content (ALT and AST) in the biliary tract to the external milieu [31].

The activity of LDH often increases during tissue degeneration, a catabolic process occurring due to increased anaerobic respiration [33]. The absence of alterations in the activity of LDH at 20 and 40 mg/kg body weight suggests that the extract is safe at these doses whereas the significant increase in activity at 80 mg/kg body weight might be an indication that the extract have caused tissue breakdown or increased hypoxic events in the experimental animals. G6PDH is a cytoplasmic enzyme in the Pentose phosphate pathway; it is involved in the biosynthesis of Ribose-5-phosphate (a precursor for the biosynthesis of Purines and Pyrimidine) and the production of Reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH) which is required for the maintenance of GSH [34]. Various intracellular processes as well as

antioxidant system requiring reduction depends on the adequate supply of NADPH. The upregulation in the expression of G6PDH at all doses of the extract can be attributed to the protective role of some components in the plant against oxidative stress. The dose-dependent decrease in the concentrations of urea and creatinine in plasma is an indication of the nephroprotective effect of the plant as it stimulates the excretion of nitrogenous waste products. This was in corroboration with the absence of alterations in the concentrations of potassium, calcium and chloride ions. This study contrasts previous findings by Yakubu and Salimon [10] where the aqueous extract of *Mangifera indica* adversely affected the glomerular and tubular function in female rats. The reduction in the activities of GRe and GPx could be related to the superfluous mobilization of antioxidant enzymes towards the mop up of oxidative stress [31]. The decrease in the level of GSH might be consequence of the depletion of GRe and GPx [35]. Elevation of TAC at 40 and 80 mg/kg body weight as well as the lack of alteration in MDA levels at all doses investigated confirms the aqueous extract of *P. amarus* leaves as an antioxidant modulator as the extract was able to mitigate oxidative damage and protect membrane lipids [31].

The absence of gross distortion in the histo-architecture of the kidney implies that there was

no treatment related structural toxicity. However, the presence of microvesicular steatosis at 80 mg/kg body weight might have resulted from the accumulation of lipids which can be attributed to impaired synthesis and/or elimination of triglycerides in the hepatocytes. Chemical profiling of AEPAL revealed the presence of anti-nutrients such as cyanide, oxalate, phytate and polyphenols in trace amounts [30]. Hence, the structural toxicity reported in this study might be attributed to the presence of these constituents due to accumulation over the duration of the study. This result was in tandem with the findings of Adebayo et al. [36] where the administration of Hepacare at 750 and 2500 mg/kg body weight inflicted mild – severe microvesicular steatosis in rat liver.

CONCLUSION:

The present study has demonstrated that the aqueous extract of *P. amarus* leaves may not induce significant toxic effects when administered at 20, 40 and 80 mg/kg body weight and thus may be safe for use at the recommended therapeutic dose. However, intake of higher doses should be avoided by consumers, especially patients with protracted liver diseases to avoid additional complications.

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Conflict of interest: We declare that we have no conflict of interest.

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DOES THE AGE OF ONSET OF LEARNING ENGLISH (AO) AFFECT STUDENTS' ACADEMIC PERFORMANCE IN THE UNIVERSITY OF PAPUA NEW GUINEA?

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ABSTRACT:

Our previous studies established a negative correlation between students' Age at Onset of learning English (AO) / Age at Literacy (AGELIT) and their academic performance in all of the six National High Schools (NHSs) of Papua New Guinea, as well as in the University of Papua New Guinea (UPNG). Lower academic achievement was also associated with Early Learning Languages (ELLs) other than English, such as Tok Pisin or Tok Ples. The current study widens the scope of enquiry in terms of university-wide survey coverage and greater sample size (N = 2001). The relationship between UPNG students' language education backgrounds and their 2018 GPAs was analysed, using SPSS 20 and the Microsoft Excel Data Pack 2010. Our findings showed a significant negative relationship between AO and students' academic achievement – a year's increase in AO reduced the GPA by 0.208 grade points ($p < 0.001$). ELL Tok Pisin (ELL TP) reduced the students' GPA by 0.343 points, while ELL TPENG (Tok Pisin + English) – by 0.297 grade points, compared to ELL English ($p < 0.001$). Multiple regression analysis also showed that the academic achievement of students with ELL TP, ELL V, and ELL TPENG was significantly lower than that of students with ELL ENG. We conclude that these results corroborate our previous findings, and highlight the malleability of the developing brain during the critical 'windows of opportunity' in early childhood. We contend that early childhood education initiatives can yield enormous educational and social benefits for Papua New Guinea.

Keywords: Second Language Acquisition (SLA), Critical Period Hypothesis (CPH), age factor, Early Childhood Education (ECE), sensitive periods, biological foundations of language, Vernacular and English (VENG), Vernacular and Tok Pisin (VTP), Vernacular, Tok Pisin and English (VTPENG)

INTRODUCTION:

Half a century after Lenneberg [1] first linked "L2 learning difficulties in adulthood with hemispheric functional specialization and declines in plasticity that constrain primary language acquisition," the effect of age on

language acquisition has been examined in hundreds of studies [2]. (L2 = second language). In recent years, neuroimaging technologies, and particularly functional magnetic resonance imaging (fMRI), have yielded remarkable insights into the workings of

living and developing human brains. As revealed by fMRI studies, “language processing is impossible without an efficient transfer of information between various language-supporting regions in separate parts of the brain” [3]. Postnatal development gradually ‘wires’ these separate language-relevant brain regions into one system, the ‘language network’ [4]. Robert C. Berwick and Noam Chomsky, referencing Perani’s diffusion tensor imaging MRI (dMRI) study [5; 6], elaborated on how these fiber tracts, absent at birth, mature over time, until thick bundles of myelinated axons form a complete ‘ring,’ enabling syntactic processing and other forms of efficient Prefrontal Synthesis (PFS) [4].

Myelination and the formation of perineuronal nets (co-incident with the closure of critical periods) rapidly increase the brain size, particularly in the first three years of life [5; 7; 8; 9]. By twelve months, the primary sensory and motor areas are myelinated, which enables the integration and processing of sensory (visual and auditory) signals and motor functions, necessary for language acquisition. Shaped by early experience, the neural circuits are laid in place in the course of a “cascading sequence of multiple, overlapping periods of plasticity that enable the development of phonetic perception in the native language, starting with discrimination of linguistic sounds in the first few months of infancy through the structuring of word forms and phonological categories as children approach 20 months of age” [10; 11].

Early experiences “wire” the brain, literally building the infrastructure required for further development – Donald Hebb, whose phrase “Neurons that fire together, wire together” succinctly describes how pathways in the brain are formed and reinforced through repeated stimulation [12].

The existence of biological constraints with regard to First Language Acquisition (FLA) is indisputable; however, debate still rages over whether age is a significant factor in Second Language Acquisition (SLA). A leading authority in SLA and bilingualism, David Birdsong, has given one of the most comprehensive accounts of current Age at Onset of learning English (AO) in SLA research in his review article “Plasticity, Variability and Age in Second Language Acquisition and Bilingualism” [2]. In it, he outlines two neurobiological explanations for plasticity deficits over age:

- The “use it, then lose it” model: “after adolescence, the circuitry that is required for language learning is dismantled, because in adulthood there remains no selection pressure on humans to keep learning languages and the metabolically greedy neural systems that subserve language learning” [13; 14; 2].
- Myelination, which “insulates axons for efficient transmission of electrical impulses, but does so at the cost of

reducing the synaptic plasticity required for new learning" [15;16; 2].

These two explanations are, of course, interrelated and equally valid for FLA – in the absence of ‘wiring’ (a product of myelination) to integrate audio-visual signals and motor functions into one ‘language circuit,’ no human language is possible.

After a brief account of current research findings on the nature and mechanisms of the cascading and overlapping critical periods in FLA, Birdsong focuses on SLA, and the variability in SLA outcomes, as reported in a number of studies [17; 18]. On the question of age-conditioned plasticity in L2 learning, many of these studies reported significant AO effects on L2 attainment (i.e., Verissimo et al. [18], who observed native-like priming for inflected German participle forms only when the study participants’ learning began in early childhood, before the age of 5 years). So, while admitting that AO “is commonly employed as a predictive factor for learning outcomes in training studies,” Birdsong looks at “variability and plasticity with respect to their underlying sources, and at age as a modulating factor in variability and plasticity.” In terms of ultimate attainment (UA) in SLA classroom context, AO is indeed merely a ‘meta-variable,’ with a multitude of other important dimensions to explore (i.e., “critical period effects vs. bilingualism effects, early and late bilingualism, native-like and non-native-like L2 attainment, cognitive aging, individual

differences in learning, and linguistic dominance in bilingualism”).

Birdsong’s review of current research into AO effects on L2 UA cites insights gleaned from studies in the context of classroom foreign language instruction/immigration into Western countries. Very little research has been done in multilingual developing nations where the so-called “foreign” languages (English, French, or Spanish/Portuguese) are no longer foreign - they are “owned” and used as the medium of instruction. The future socio-economic progress of these nations depends on their ability to produce home-grown quality human resources (HR). This, in turn, is predicated on the students’ ability to learn in a so-called “foreign” language. This important practical distinction in SLA research has little relevance in the Western world and has, therefore, been of no concern to Western researchers, as is evident from Birdsong’s review. That is why his concluding statement totally ignores this perspective:

”By demonstrating the connectedness of non-uniform outcomes with age and plasticity, the research reviewed here has shown that such variation is neither unexplainable nor unexpected. From this understanding, emerges heuristic guidance for further explorations of the richness of L2 acquisition and bilingualism” [2]. The findings in our studies so far have established a robust inverse correlation between students’ age at onset of learning

English (AO) and their academic achievement in high school and university:

In 2015, the study conducted among students in the UPNG [19] revealed that, despite Vernacular Education practiced at the time, ELL Vernacular (ELL V) students were disproportionately underrepresented in all five schools, while ELL Tok Pisin (ELL TP) students' performance was far below that of ELL English and ELL V students.

In 2016, the study among students in the Port Moresby National High School (POMNATH) [20] established a significant inverse correlation between high school students' AO/AGELIT and their academic scores. ELL English students constituted the most numerous group of POMNATH students, and their mean academic grades were higher than those in other ELL groups.

In 2017, our survey of all six National High Schools (NHSs) of Papua New Guinea [21] found that AO increase by one year corresponded to a 1.37% decrease ($p < 0.001$) in students' academic scores.

Our studies in 2017 and 2018 among students in the School of Humanities and Social Sciences (SHSS) UPNG [22; 23] also established the negative impact of AO on the GPA of students.

These findings show that, in PNG, those who start learning English younger, fare better in high schools and university. The present study, which is a follow-up on our first UPNG survey in 2015, aims to give resonance to Hensch's

recommendation, made over fifteen years ago: "An understanding of the neural basis of critical/sensitive periods of brain development should inform not only educational policy, but also clinical therapy and strategies for improved learning into adulthood" [8].

Research Questions and Hypotheses:

This study aimed to explore the impact of AO and ELLs on students' academic performance in UPNG. Our research questions addressed the relationship between students' AO/ELL and their academic performance, measured by their 2018 GPAs.

Our null hypotheses assumed no relationship between these factors and the students' GPAs.

METHODOLOGY:

The detailed methodology is presented in earlier studies [19, 20, 21]. During the 2019 academic year, a survey, using purposive cross-sectional sampling method, was carried out in all five schools of UPNG: SHSS, School of Natural and Physical Sciences (SNPS), School of Business and Public Policy (SBPP), School of Law (SOL), and School of Medicine and Health Sciences (SMHS). All full-time registered students were eligible to participate in the study. A self-designed, pretested, self-administered questionnaire yielded data on students' language education backgrounds (AO and ELL). The GPA for each of the students for the 2018 academic year was obtained by special request and permission from the

appropriate authorities in the UPNG Administration.

Students' responses were entered into Excel spreadsheets, coded, and matched with their respective GPAs, forming our final dataset. The SPSS software version 20 for Windows was used to conduct quantitative analysis. Normality of the data was assessed by the Shapiro-Wilks tests. Independent-Samples Mann Whitney U was used to determine differences between groups. Descriptive statistics, comparisons of means, nonparametric correlations, Ordinary Least Squares (OLS) and multiple regression models with bootstrapping were used as appropriate.

RESULTS:

Of the 3,918 questionnaires distributed, 2001 (51.1%) were completed and returned. This gave a non-response rate of 48.9%, which is lower than the 61.0% non-response rate obtained in our earlier study [19]. The high non-response rate was due to voluntary participation on the part of the students, and to some logistical and administrative issues.

Gender distribution:

Of the 2001 students that consented to participate in this study, 798 (39.9%) were female, and 1203 (60.1%) were male. The Gender Parity Index (GPI) was 0.66 (789/2001), which is a slight improvement on the GPI of 0.60 obtained in earlier study [19].

Location of students' elementary schools:

The students were separated according to the location of the elementary schools they went to. The results are illustrated in Figure 1. Out of the 2001 students, 392 (19.6%) received their elementary schooling in Port Moresby (POM), 234 (11.7%) in Western Highlands Province (WHP), 177 (8.8%) in Morobe, 137 (6.8%) in Enga, 134 (6.7%) in Eastern Highlands Province (EHP), 121 (6.0%) in Southern Highlands Province (SHP), and 104 (5.2%) - in East Sepik Province (ESP). A few students received their elementary schooling outside of PNG: 13 schooled in the Solomon Islands (SI), 12 in Samoa, 7 in Australia (AUS), 5 in Indonesia and one in the USA. They represent only 1.9% of our sample population.

Home Language (L1) distribution:

The 2001 students were distributed according to their home language (L1). L1 for 958 (47.9%) students was Tok Pisin (TP); Vernacular was L1 for 538 (26.9%) students; both Tok Pisin and English (TPENG) were home languages for 188 (9.4%) students; L1 for 35 (1.7%) students was English (ENG); 30 (1.5%) students spoke both Vernacular and English (VENG) at home. 142 (7.1%) used both Vernacular and Tok Pisin (VTP), and 110 (5.5%) used a mix of all three languages (TPVENG).

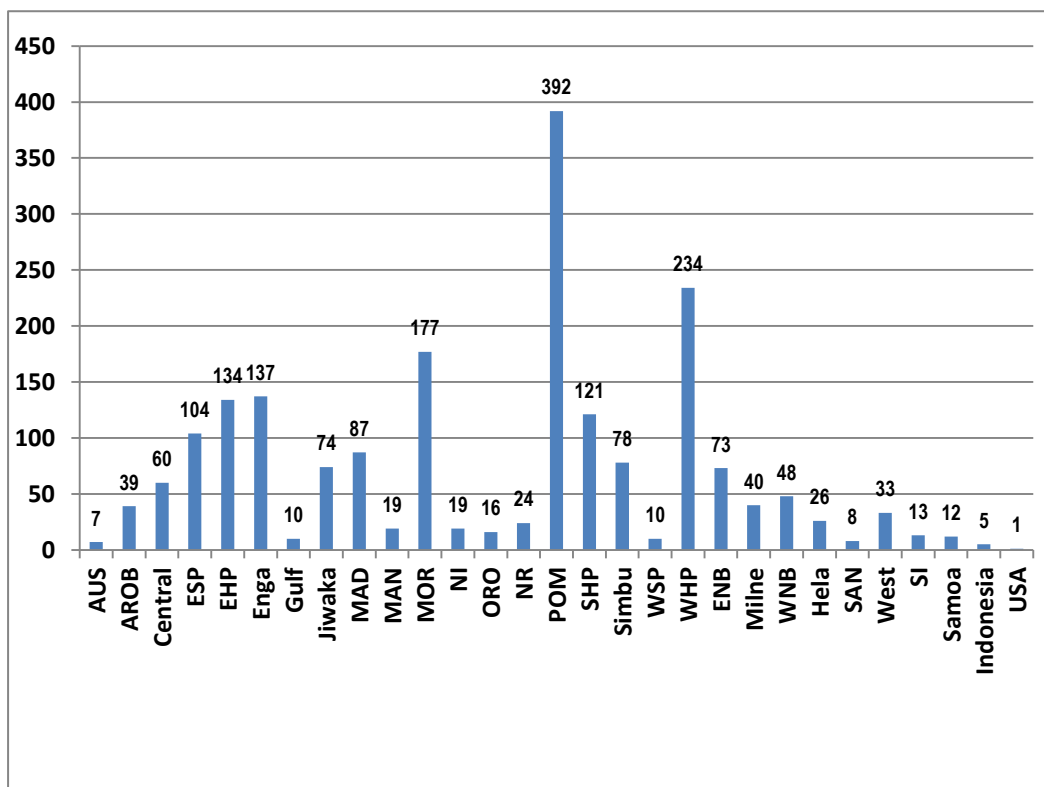


Fig. 1. Locations of students' elementary schooling

AROB = Autonomous Republic of Bougainville; MAD = Madang; MAN = Manus; MOR = Morobe; NI = New Ireland; NR = No Response; WSP = West Sepik; ENB = East New Britain; Milne = Milne Bay; WNB = West New Britain; SAN = Sandaun; West = Western.

Early Learning Language (ELL) distribution:

The frequency of language use in early childhood education changes dramatically, despite the predominantly Vernacular Education practiced in PNG at the time. The 2001 students were distributed according to their ELL. The results show that the ELL for 704 (35.2%) students was English; 835 (41.7%) were taught in both Tok Pisin and English (TPENG); the ELL for 219 (10.9%) was Tok Pisin (TP); for 163 (8.1%) students, the ELL was Vernacular (V); both Vernacular and

English (VENG) were used as ELL for 73 (3.6%) students; only 7 (0.3%) of all the students in our sample reported combinations of Vernacular and Tok Pisin (VTP) or Tok Pisin, Vernacular and English (TPVENG) as their ELLs.

Thus, in the present study of the 2001 students, a total of 1539 (76.9%) listed ENG (704) and TPENG (835) as their Early Learning Language (ELL) in the first two years of their formal education.

Age of Onset of learning English (AO):
 According to Shapiro-Wilks tests of normality, our AO data for all the students and for the female and male students were not normally distributed ($p = 0.000$). This was further confirmed by the Box-plots (Fig 2) of the AO data for the female and male students.

Thus, bootstrapping was used for the descriptive statistics presented in Table 1. The Mean AO for all the students was 6.88 years and the 95% Confidence interval was 6.79 – 6.98 years.

Table 1 Descriptive statistics of the Age of Onset (AO) of learning English for the female and male students

Parameters	Females	Males	All
N	798	1203	2001
Mean (years)	6.29	7.27	6.88
Standard Deviation (SD)	1.89	2.08	2.07
95% Confidence Interval (95% CI) (Bootstrapping)	6.16 – 6.43	7.16 – 7.39	6.79 – 6.98
Range	1.0 – 14.0	1.0 – 21.0	1.0 – 21.0
Median	6.0	7.0	7.00



Fig. 2. Box-plots of the distribution of the data for Age of Onset of learning English (AO) for Female (N=798) and Male (N=1203) students.

For the female students, the mean AO was 6.29 years and 95% CI was 6.16 – 6.43 years.

For the males, the mean AO was 7.27 years and the 95% CI was 7.16 – 7.39 years.

The Mann-Whitney U test and Kruskal-Wallis test indicated statistically significant difference ($p = 0.001$) in the mean and median AO values between the female and male students.

Another interesting observation was made with regard to general AO distribution across four schools of UPNG (SHSS, SNPS, SBPP and SOL) with mean AO of 6.90 years, compared to the School of Medicine and Health Sciences (SMHS), where the MBBS students' mean AO was 5.66 years ($p = 0.01$). This may be attributed to the rigorous selection criteria for admission into the MBBS program (after their

Foundation Year in the School of Natural and Physical Sciences (SNPS), students with younger AO were more successful in meeting the GPA 3.00 selection requirement for the MBBS program).

GPA variable

The Shapiro-Wilk test for normality also indicated that the GPA data were not normally distributed ($p = 0.000$). The descriptive statistics of the GPA for all the students is presented in Table 3. The Mean GPA was 2.51 and 95% CI (bootstrapping) was 2.48 – 2.54.

Table 3: Descriptive statistics of the GPA for all students

N	2001
Mean	2.51
SD	0.73
95% CI (Bootstrapping)	2.48 – 2.54
Range	0.0 – 4.8
Median	2.50

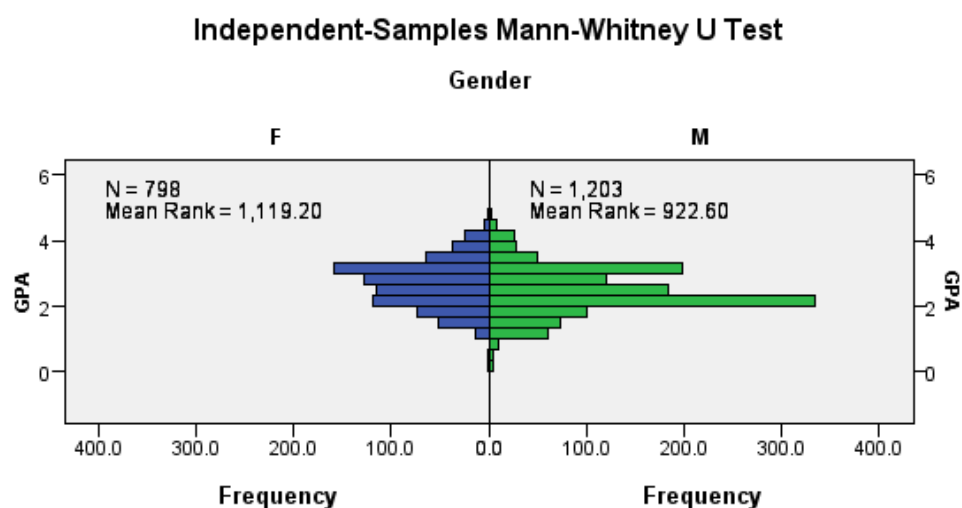


Fig. 3 GPA Frequency distribution of the GPA for the female and male students.

A statistically significant difference was observed when the distribution of the GPA data for the female and male students was compared using Mann-Whitney U Test (Fig 3). The results show that both data were not normally distributed. The GPA data were analysed statistically, using bootstrapping. For the female students, the mean GPA (\pm STD) was 2.657 ± 0.713 and the 95% CI (Bootstrapping) was 2.61 - 2.71; the median was 2.75. For the male students, the mean GPA was $2.416 \pm .726$ and the 95% CI

(Bootstrapping) was 2.375-2.459, the median was 2.40. These results were confirmed by the independent samples Mann-Whitney U and Wilcoxon tests ($p = 0.01$, 2-tailed).

A significant association ($\eta^2 = 0.423$) was observed between the mean AO and the mean GPA for all the students.

Comparison of Means of the ELL and GPA:

Our data were not normally distributed; thus, we used bootstrapping to assess the Mean GPA in all seven ELL groups. The results are presented in Table 4.

Table 4. Descriptive statistics of the GPA for the students in the different ELL* groups

ELL groups	N	Mean	SD	95% CI (Bootstrapping)	ANOVA F value	p-value	η^2
English	704	2.72	0.78	2.67-2.79	95.296	0.000	0.046
Tok Pisin (TP)	219	2.38	0.72	2.29-2.48	7.994	0.005	0.004
Vernacular (V)	163	2.20	0.58	2.11-2.89	33.087	0.000	0.016
TP + English (TPENG)	835	2.43	0.68	2.38-2.47	20.105	0.000	0.010
V + English (VENG)	73	2.55	0.58	2.43-2.67	0.199	0.656	0.000
TP + V (TPV)	3	2.13	0.32	1.90-2.50	0.808	0.368	0.000
TP + V + Eng (TPVENG)	4	2.83	0.56	2.20-2.50	0.735	0.391	0.000

Comparison of Means revealed highest performance amongst students with ELL English, followed by those with ELL TPENG. The Mean GPA for ELL V students, despite the Vernacular Education policy then in place, was significantly lower (2.20). At first glance, ELL VENG students seemed to have done well, even better than ELL TPENG students - but their numbers were insufficient to yield statistically significant results. ELL TP students, however, consistently show statistically significant low performance (Mean

= 2.38). ELL TPV and ELL TPVENG results were not statistically significant due to insufficient representation in the student population (this fact in itself is remarkable, as Vernacular Education policy was law at the time of these students' elementary education).

For further statistical analysis of the data, Correlation, Ordinary Least Squares (OLS) and multiple regression analyses were performed.

The results for the correlation analyses are presented in Table 5. A strong negative

statistically significant correlation ($\rho = -0.626$, $p = 0.000$) was observed between the AO and GPA. A direct statistically significant correlation ($\rho = 0.222$, $p = 0.000$) was obtained between

the GPA and ELL English. Negative statistically significant correlations were also obtained between GPA and TPENG, V, and TP.

Table 5. Relationship between GPA and Age of Onset (AO) and between GPA and ELL

	GPA	AO	English	TPENG	V	TP
Correlation Coefficient (ρ)	1.00	-0.626	0.222	-0.108	-0.131	-0.061
Sig. (2-tailed)		0.000	0.000	0.000	0.000	0.006
N	2001	2001	2001	2001	2001	2001

* The sample size for ELL VENG, ELL TPV and ELL TPVENG were too low to yield statistically significant results

A statistically significant negative correlation ($\rho = -0.167$, $p = 0.000$) was also observed between male gender and GPA, compared to female gender and GPA ($\rho = 0.167$, $p = 0.000$). This result further indicates better performance by female students, compared to that of their male counterparts.

Linear Regression analyses: Various linear regression (OLS and Multiple) models were used to examine the relationship between several factors (AO, ELLs) and students' academic performance.

OLS linear regression AO and GPA:

The R Squared regression coefficient was 0.346 ($R^2 = 0.35$; $SE = 0.59081$). This means that AO explains 35% of the variation in GPA, the dependent variable in our sample.

The F-Statistic measures the effectiveness of the regression model in assessing the variation between the predictor (AO) and the outcome variable (GPA); $F = 1057.036$ ($p = 0.000$) indicates very high variation in mean GPA values between the two gender groups.

The results in Table 6 show that the relationship between AO and GPA was negative and statistically highly significant:

- The unstandardized beta coefficient of -0.208 ($p = 0.000$) means that a year's increase in AO reduces the GPA by 0.208 grade points.
- The standardized beta coefficient of -0.588 indicates a higher-than-medium effect size of AO on GPA, according to Cohen's (1988) classification.

Table 6. OLS Regression Coefficients a

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
1 (Constant)	3.943	0.046		85.809	0.000
AO	-0.208	0.006	-0.588	-32.512	0.000

a. Dependent Variable: GPA

To assess the combined effects of AO and gender on students' academic performance, we ran a multiple linear regression model which includes student level control variable (i.e.

gender). The model below was used for the analysis of the data. The results obtained are presented in Table 7.

$$\text{GPA} = \beta_1(\text{AO}) + \beta_2(\text{X}') + e_i,$$

where β is the constant, X' is the student level control variable, and e_i the error term.

Table 7. Estimated coefficients from AO+GENDER*GPA regression model

R	0.589	
R ²	0.347	
Adj. R ²	0.346	(SE = 0.5906797)
F value	529.69787	Sig. 0.000
Bootstrap for Coefficients (95% confidence interval)		
	B	Sig (2-tailed)
(Constant)	-3.952	0.001
AO	-0.206	0.001
GENDER	-0.038	0.171
Pearson's correlation coefficient (r)		
AO	r = -0.588	Sig (1-tailed) 0.000
GENDER	r = -0.162	0.000

The relationship between AO and GPA was negative and statistically significant for AO, even after controlling for gender. Adjusted R² = 0.346 explains 35% of the outcome variable (GPA), the F statistic indicates significant variation in mean GPA values between the two gender groups. Each year's delay in AO causes

a drop in GPA by 0.206 points - which may account for the lower academic performance observed amongst male students (their median AO was 7, as opposed to AO = 6 for female students). Pearson's correlation coefficients confirmed these findings (Table 8).

Multiple Regression: assessing the relationship between ELL variables and GPA

To assess any additional effects of the various ELLs on students' academic performance, we

$$GPA = \delta_1(TPENG) + \delta_2(TP) + \delta_3(V) + \delta_4(VENG) + \delta_5(TPV) + \delta_6(TPVENG) + e_i,$$

where δ is the constant and e_i is an error term.

Since our data are not normally distributed, bootstrapping was used to satisfy the assumptions, associated with a linear regression model (i.e., normalcy of distribution, homoscedasticity, absence of outliers, linearity, etc.).

ELL is a categorical variable with 7 ELL subcategories (ENG, TP, V, TPENG, VENG, VTP and TPVENG). Therefore, in order for our ELL variable to be usable in a multiple linear regression prediction model, it was re-coded

ran a multiple linear regression model, using the equation below:

into 7 dichotomous "dummy" variables. English (ENG) was taken as the reference level, followed by all other "dummy" variables – TPENG, VENG, V, TPV, and TPVENG. Each one of the "dummy" variables has its level in relation to the one that is taken as the reference level (in this case, ENG), and the model was adjusted, taking into account all reference levels. Our results are presented in Table 8.

Table 8. Estimated coefficients from linear regression models ELL*GPA

R	0.234			
R ²	0.055			
Adj. R ²	0.052	(SE = 0 .71114)		
F value	19.218	Sig. 0.000		
Bootstrap for Coefficients			Collinearity statistics	
	B	Sig (2-tailed)	Tolerance	VIF
(Constant)	-2.724	0.001		
TP	-0.343	0.001	0.856	1.168
V	-0.524	0.001	0.884	1.131
TPENG	-0.297	0.001	0.785	1.274
VENG	-0.174	0.017	0.940	1.063
VTP	-0.590	0.001	0.997	1.003
TPVENG	0.101	0.625	0.996	1.004

These results indicate that students' Early Learning Language (ELL) contributed significantly to the AO impact on their 2018

GPA, even though the coefficient of determination, R² and Adjusted R² values are relatively low at 0.055 and 0.052, respectively.

The high F coefficient (19.218, $p=0.000$) indicates considerable variation between sample means. Thus, as can be seen from Table 7, the academic achievement of students with ELL TP, ELL V, and ELL TPENG was significantly lower, in comparison to that of students with ELL ENG. Notably, as can also be seen from Table 8, there was no collinearity between ELL variables, which further strengthens the validity of our results.

Thus, the correlation analyses, nonparametric tests and regression models in this study have provided clear evidence that students' AO and ELLs have a significant effect on their academic performance, measured by their GPAs. Based on this evidence, we reject our null hypotheses (that there was no significant AO/ELL effect on UPNG students' 2018 GPAs), and accept the alternative hypotheses, listed below:

1st H_1 = AO has a significant effect on UPNG students' GPAs.

2nd H_1 = ELL has a significant effect on UPNG students' GPAs.

DISCUSSION:

The results obtained in the present study established a robust negative (inverse) statistically significant correlation between students' AO and their academic achievement. While academic performance is, undoubtedly, a function of the totality of one's biology and socio-cultural experience, this complexity was beyond the scope of our study. However, the understanding of the mechanisms of neurobiological development and the advantages of using the "windows of opportunity" it provides for effective learning may help in developing optimal strategies for quality education. During development, brain structures are formed over time, in 4 waves of myelination progressing from the bottom up, and from the caudal to anterior areas. "In the first few years of life, more than one million new

neural connections are formed every second. After this period of rapid proliferation, connections are reduced through pruning, so that brain circuits become more efficient. Sensory pathways like those for basic vision and hearing are the first to develop, followed by early language skills and higher cognitive functions. Connections proliferate and prune in a prescribed order, with later, more complex brain circuits built upon earlier, simpler circuits" [24]. Early childhood education (ECE) captures these windows of opportunity while they are open, allowing for most effective learning and laying stronger foundations for later learning. Practical, factual evidence of the negative impact of any increase in students' age of onset of learning English (AO) on their academic performance in Papua New Guinea should be factored into government education policy.

Here, as in several other multiethnic and multicultural developing nations, English is the medium of formal education; therefore, students' ability to learn in all post-elementary education is predicated on their English proficiency. We contend that preserving linguistic and cultural diversity, on the one hand, and pursuing government objectives of developing quality human resources (HR) to advance sustainable socio-economic development, are not mutually exclusive propositions. Both goals are achievable, if strategy, grounded in science, makes use of the biologically 'sensitive' periods in early childhood development.

Disparity in access to early education not only accelerates social class division, but also prevents the development of human resources - that is why governments around the world increasingly turn their attention to maximizing the benefits of ECE. Many publications have come out in recent times on studies in this area [27; 28; 29]. For example, Chen et al. [25] describe the remarkable success of one such intervention, launched in 2009 by the China Development Research Foundation (CDRF) - the One Village One Pre-school (OVOP) project. This government-supported public intervention established over 2300 centers in central and western rural China, providing over 170,000 disadvantaged rural and minority 3–6-year olds with access to ECE, free of charge for all.

OVOP uses the “windows of opportunity” in children's cognitive development, addressing a range of biological, public health and socio-cultural issues. OVOP provided every village with a pre-school, trained and employed high school leavers/ local volunteers to deliver a standardized curriculum, and dispensed nutritional supplements to needy children. Chen et al. [25] present a comprehensive analysis of the impact these measures have had on the children's long-term academic achievement, providing convincing evidence of the value of using those critical "windows of opportunity" while they are still open in early childhood. Their analysis of longitudinal data on the academic achievement of 1962 “OVOP” children over the first five years of their primary school and compared their performance with that of those who had had no ECE. Their results revealed that children who had attended OVOP centers scored much better than those who had not, and even better than others who had attended private centers. The OVOP study has demonstrated the remarkable efficacy of ECE intervention; it provides empirical support for the value of investing in low-cost ECE, which benefits the entire society long-term. Our earlier studies [19; 20; 21; 22; 23] also lead to the same conclusions, providing evidence of better performance amongst those high school and university students who started their education earlier, particularly with regard to learning English (which is the medium of

instruction in all post-elementary education in Papua New Guinea).

CONCLUSIONS AND RECOMMENDATIONS:

To conclude, our results show that students' AO and ELLs have a significant effect on their academic performance, measured by their GPAs. Increase in AO correlated with lower GPAs, as did ELLs other than English. This highlights the need for children to be sufficiently proficient in English before they embark on their educational journey.

To move the quality of education forward, efforts must be made to utilize the “critical periods” in children's cognitive development, and provide them with the tool they need for learning at later stages of their education - English proficiency.

To achieve sustainable national development, a comprehensive multifaceted strategy must be developed - a strategy, based on language education policy grounded in the science of human brain development and understanding of socio-economic needs of the country, ensuring effective teacher training, and addressing public health and infrastructure development issues.

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GERIATRIC NURSES HOME VISITS: CONSIDERATIONS FOR EVALUATING IMMOBILE OLDER PATIENTS IN THE COMMUNITY

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ABSTRACT:

The increase in ageing requires the planning and provision of medical services to older people across functional abilities, ranging from independent to immobile patients. The considerations for evaluating immobile older patients during geriatric nurse's home visits are provided. Home visits have been shown to be effective in reducing functional decline, reduce admission to residential care and improve survival in younger-old patients. It requires a comprehensive geriatric assessment approach, with interventions provided for issues identified during the home visit. The home visit assessment requires systematic handover and discussions between healthcare professionals. The Identification, Situation, Background, Assessment and Recommendation (ISBAR) tool is recommended for handover. Screening for common geriatric conditions including malnutrition, delirium, falls, pressure injuries and pain should occur during the home visit. The Malnutrition Universal Screening Tool (MUST) can be used to identify patients who are malnourished. The Confusion Assessment Method (CAM) is a useful tool to identify delirium during home visits, which requires further clinical evaluation to diagnose the underlying trigger or illness. Risk factors from the Braden scale should be identified to minimise risk of pressure injuries, as well as routinely checking the buttock, sacrum and heels of dependent older people during home visits. Pain should be assessed and treated. Medication reconciliation should also be performed to assess for compliance.

Keywords: Geriatric Assessment; Geriatric Nursing; House calls; Malnutrition; Pain

INTRODUCTION:

The increase in ageing requires health services to consider provision of medical services to older people across functional abilities, ranging from independent to immobile patients. A tertiary hospital in Brunei identified that older

patients admitted under geriatric medicine had a high burden of co-morbidities, dementia and poor functional status [1]. Patients discharged from hospital who are highly dependent for activities of daily living have a high one-year mortality rate of up to 43% [2].

Comprehensive geriatric assessment and multidisciplinary interventions are necessary, in addition to community support services to manage dependent patients. Palliative and supportive care services should also be provided through a continuum of care; from primary health care, specialist services, as well as community and home-based care [3]. Due to strong family values locally, family members are often involved in care of these patients; hence caregiver support interventions are also required.

In Brunei, other than primary care services, community-based services are nurse-led, consisting of Geriatrics Home Visits (GHV), Palliative Home Visits (PHV) and Home Based Nursing (HBN). GHV focus on immobile older patients who require comprehensive geriatrics assessment. PHV visits provides palliative and supportive care at home, with an emphasis on symptom assessment and treatment. HBN are mainly involved in management of nasogastric feeding tubes, indwelling catheters and wounds, particularly pressure injuries.

A meta-analysis showed that home visits were effective in reducing functional decline, preventing the need for nursing home admission and improved survival in the younger-old patients. This survival benefit was not seen in the older patients aged 80 years and older [4]. Interventions during the preventive home visits required multi-dimensional geriatric assessments and multiple follow-up home visits. A stratified randomized

trial in Switzerland confirmed the benefit of home visits, with delayed onset of disability in older people, reduced nursing home admissions and health cost savings for patients aged 75 years and older [5]. These interventions consisted of annual multi-dimensional assessments, quarterly follow-up home visits by nurses who collaborated with geriatricians to provide recommendations, as well as nursing health education and monitoring of adherence to recommendations. However, beneficial effects were related to the home visitor's performance in conducting the visits. In this review, an assessment approach for evaluating immobile older patients is provided, with a focus on nursing home visits for geriatrics patients.

Comprehensive Geriatric Assessment:

A comprehensive geriatric assessment (CGA) is the mainstay for management of complex older people. It enables the clinician to identify as many risk factors and reversible causes for functional decline, then proceed to formulate a management plan for the problem list. In a randomized controlled trial, gerontologic nurse practitioners provided annual in-home CGA for people living in the community aged 75 years and older. This intervention led to delayed development of disability, required assistance with basic activities of daily living and admission to residential care; at a cost of USD\$6000 for each year of disability-free life gained [6]. For community-living frail older

people, CGA-based individually targeted interventions improved mobility and the ability to walk 400 metres [7]. In older people discharged from hospital, applying CGA in combination with a post-discharge home intervention by an interdisciplinary team was associated with shorter hospital readmissions, reduced nursing home placements, improved functional capacity and reduced health care costs without improvement in survival [8]. Thus, CGA is essential for improved outcomes for community living frail older people, or those recently discharged from hospital.

However, there are challenges in applying CGA-based interventions in the community. CGA, formulation of problem lists and providing interventions are time consuming, requires multidisciplinary expertise and patient compliance to interventions. A randomized-controlled trial on frail community dwelling people applied CGA and interventions including medication adjustment, exercise prescription, nutrition support, physical rehabilitation, referrals to social workers and medical specialties. Although there was some benefit with CGA and subsequent intervention, the inability to complete CGA and poor compliance with intervention in participants contributed to less favourable outcomes [9].

Community programs that provide CGA annually resulted in a constant number of therapeutic and preventive recommendations made annually (Mean 11.5 per subject annually). Adherence to intervention depended

on type of recommendations, referrals to physicians compared to non-physicians or community services or recommendations involving self-care activities [10]. It also requires support and buy-in from primary care physicians to improve effectiveness of community based CGA programs. A study showed that primary care physicians who cooperated with nurse practitioners for CGA and interventions had fewer years in practice, were more likely to discuss the program and see benefits from patients. This was also more likely to predict patient adherence to intervention recommendations [11].

Assessment Approach for CGA in Dependent Geriatrics Patients in the Community:

The geriatrics nursing assessment form incorporating CGA used in Brunei is included as an Appendix in this paper. It incorporates use of the ISBAR approach to support handover by geriatrics nurses to geriatricians in a weekly case conference to develop a comprehensive management plan for dependent geriatrics patients in the community. ISBAR stands for Identification, Situation, Background, Assessment and Recommendation, which is a tool that facilitates systematic handover of the correct information between healthcare professionals. It has been shown to be a reliable and effective communication tool to promote patient safety, and found to be acceptable and easy to use [12, 13].

A problem list should be formulated prior to the home visit based on the patient's clinical notes so that the visiting geriatrics nurses can consider and prioritise aspects of assessment to plan interventions. In addition to asking regarding presenting complaints and concerns from patients and caregivers, the mnemonic SPICES can be applied as a screening tool. SPICES stands for Sleep, Problem with eating and drinking, Incontinence, Confusion, Evidence of Falls and Skin breakdown. A cohort study of frail hospitalized older adults found that almost all patients met at least one SPICES criteria; with the sum of SPICES significantly correlated to age and other measures of vulnerability, comorbid conditions, depression and predictive of adverse events [14]. For a home visit assessment, it serves as an aide memoire for review of systems, which if affected, will require further evaluation to consider underlying causes and contributors. For example, if an older person has sleep issues, it would be worthwhile to systematically enquire regarding contributing factors. For example, a patient may need to be treated for cardiac failure if a person wakes up with breathlessness or frequency of passing urine at night, resulting in poor sleep. Pain affecting sleep is of sufficient severity to warrant further investigations. Positive somatic complaints should be followed-up with assessments towards a diagnosis and a management plan, which should include a non-pharmacological component, such as sleep hygiene practices.

Evaluation of an older person in the community should also screen for the following common problems: malnutrition, delirium, falls, pressure injuries and pain.

Malnutrition:

Malnutrition is common among older people, with the majority of older people who are malnourished living in the community (93%) [15]. Malnutrition may be a cause or consequence of illness, and has a detrimental effect on disease risk, progression, prognosis, complications, and delayed recovery [16]. Once identified, early intervention with oral nutritional supplements and dietary counselling resulted in increased dietary intake and improved quality of life.

A study demonstrated the importance of malnutrition screening among older people, where implementation of the Malnutrition Universal Screening Tool (MUST) showed a high incidence of malnutrition, with more than half of geriatrics inpatients having a body mass index (BMI) below 20 kg/m² [16]. The MUST has been shown to be easy to use and applicable to different patient groups in hospital, outpatient clinics and in the community [17]. Patients are flagged as at risk for malnutrition if they have a low BMI, unplanned weight loss in the past three to six months or if they have acute illness and will likely have minimal nutritional intake for more than five days. These patients should be

proactively identified and interventions planned to avoid worsening functional decline.

Delirium:

Delirium is an acute state of confusion that represents a medical emergency. It is a manifestation of acute illness, including infections, and requires diagnosis and treatment of the underlying problem. The Confusion Assessment Method (CAM) is a useful approach for identifying delirium across multiple settings [18]. It usually involves scoring a person's cognitive function during formal cognitive testing. The CAM criteria for delirium are: acute onset and fluctuating course, inattention and either disorganized thinking or altered level of consciousness.

However, education and bedside training is required, with the diagnosis to be confirmed according to formal Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria [19]. A study found that when nurses were assessed on applying CAM for assessing delirium, sensitivity was poor but had high specificity, with nurses successfully diagnosing patients without delirium in more than 90% of their observations. The main difficulties encountered were recognizing features of acute onset, fluctuation and altered level of consciousness [20].

Falls:

Falls are associated with injuries, functional decline, morbidity and mortality. Each

community visit should be used as an opportunity to screen for falls. Screening questions for falls include whether they have fallen within a year, if they feel unsteady and whether there is a fear of falls [21]. However, for dependent older people requiring assistance with mobility and transfers, falls indicate the need for caregiver training and evaluation of equipment involved during the fall. If a patient had a previous fall, further assessment is also required to identify if any injuries occurred.

Pressure Injuries:

A study among medical inpatients indicated a high prevalence of pressure injuries up to 20.4%, necessitating actions to improve risk assessment, preventive interventions and management of pressure injuries [22]. The most common sites were the buttock, sacrum and heels, which should be routinely checked during community visits. Risk factors for developing pressure injuries should be identified and minimized. The Braden scale is a useful tool that may be used during patient assessment, and considers the following risk factors: sensory perception, moisture, activity, mobility, nutrition, as well as friction and shear. A multicenter study evaluating the validity and reliability of the Braden scale showed that the original Braden scale was reliable with sufficient sensitivity and specificity [23]. Age was also an important risk factor, with sensory

perception, friction and shear as highly predictive risk for developing pressure injuries. For community visits, a study assessing the validity of the Braden scale found that a cut-off score of 19 provided the best sensitivity. It was recommended that initial assessment of pressure injury risk for older people should begin on entry into home health care, weekly reassessments for the first four weeks, and then alternate week reassessments until day 62. Reassessment may be appropriate at each 62-day recertification period for active caseload patients, depending on their condition and frequency of home visits [24]. For patients discharged from hospital, pressure injury assessment and management from hospital to the community should be integrated, with community nursing follow-up and caregiver training to emphasise prevention of pressure injuries [25].

Pain:

Pain is another common complaint among older people, which is associated with poor outcomes. Pain assessment should be performed routinely during community visits; as dependent older people are vulnerable to under-treatment of pain. There are multiple tools available to assess pain severity, including the Numeric Rating Scale (NRS), Visual Analogue Scale (VAS), Pictorial Pain Scale (PPS), and Verbal Descriptor Scale (VDS). These tools have been shown to be valid and acceptable for use in older people

[26]. For patients with severe cognitive deficits, other approaches to assessment, particularly observational methods may be required.

In our clinical practice, the Face, Legs, Activity, Cry and Consolability (FLACC) tool and Faces Pain Scale Revised (FPS-R) tool appear applicable for non-communicative patients. The FLACC tool grades five criteria with scores from zero to two, with the scale scored between zero to ten (0 – no pain, 10 – severe pain). For example, a person's face with occasional grimacing or frowning scores one point, while a constant quivering chin with a clenched jaw scores two points for the Face criteria. This tool has been shown to be useful in non-communicative patients in intensive care and patients unable to self-report their pain [27-28]. This tool also appears to have test-retest reliability and validated in non-English countries, including Korea [29].

The FPS-R requires patients to choose which face represents their level of pain. In a study of cognitively impaired minority adults, African-Americans, Hispanics and cognitively impaired participants preferred the FPS-R over other pain assessment tools [30]. However, this scale is often used incorrectly, where nursing staff do not ask patients regarding the intensity of their pain when the patient is capable of self-reporting [31].

Medication Review:

The community visit is an opportunity for medication reconciliation. Geriatrics nurses

should have a medication list from clinical records and compare with medications available at the person's home. Patients may take over-the-counter medications, herbal or complementary and alternative medications, that may not be volunteered during consultations. Based on the amount of medications available at home, it is also possible to identify non-compliance with treatment. For patients with recurrent admissions, there is a risk of medication error, or accumulation of medications at home due to the tendency to prescribe regular medications on discharge [32]. It is also useful to reiterate to patients the indications for medications and any planned changes to medication prescriptions.

CONCLUSION:

For older patients who are immobile and fully dependent, geriatrics home visits are essential for assessment and management of these patients. These visits should incorporate a CGA approach, and screening for malnutrition, delirium, falls, risk of pressure injuries and pain.

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APPENDIX: Geriatric Medicine Nursing Home Visit Assessment Form

Home Visit By:			
Referred by:			
Date / Time of Visit:			
Address:			
Contact Number:			
INTRODUCTION			
Patient Name:			
Patient Identifier:			
Date of Birth:			
SITUATION			
Reason for Home Visit:			
BACKGROUND			
Problem List:			
NG tube / PEG:	Yes / No	Date Changed:	
Urinary Catheter / Suprapubic:	Yes / No	Date Changed:	
Wound	Yes / No		

Medication List:				
No	Name of Medication	Dose	Route	Check with Patient Supply (Comments)
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

ASSESSMENT			
History:			
Fulmer SPICES Assessment	Yes	No	Remarks
Sleep Disorder			
Problem with Eating and Drinking			
Incontinence (Bladder / Bowel)			
Confusion			
Evidence of Falls			
Skin Breakdown (Wounds)			

Pain Assessment: Verbal Descriptor Scale / FLACC Behaviour Assessment Scale					
0	2	4	6	8	10
No Pain	Mild	Moderate	Severe	Extreme	Worse Pain
Examination:					
Vital Signs	Temp:	BP:	HR:	Sats:	Glucose:
Examination Findings: (muscle tone, wounds, swallowing)					
Medication Review: (Compliant / Non-Compliant)					
RECOMMENDATIONS:					
Advice Given:					
Health Education:					
Medications:					
Follow-Up:					
Carer / Family in Attendance:					
Signature:					

PATIENTS' PERCEPTIONS ON FACILITATORS AND BARRIERS OF UTILIZATION OF DENTAL SERVICES: SUGGESTIONS FOR PACIFIC NATIONS

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ABSTRACT:

Oral disease is a worldwide public health issue including Pacific nations. The aim of this study was to review patient's perception on the main factors affecting utilization of dental services among patients and to provide a suggestion for Pacific countries. The literature search was done using Medline, Embase, Scopus, and Proquest databases, and relevant keywords were applied to find studies which have been conducted in the field of dentistry specifically looking at patient's perception on utilization of dental services. All the studies pertaining to the topic, published between 2000 and 2020 in English language were reviewed and the main themes were identified. The results showed that patient's perceptions are primarily based on their experience with dentists and the way services are delivered in the clinical setting. Most patients prefer their dentists to display professionalism and possess good communication and clinical skills. Negative perceptions arise mainly in terms of the cost of dental services, poor accessibility, dental anxiety and fear of pain endured during treatment. This study concluded that there is no research done on factors influencing utilization of dental services in Pacific. Tailored interventions by considering the factors identified in this study may improve oral health among Pacific people.

Keywords: Patients' Perceptions, Facilitators, Barriers, Dental Services, Oral Health, Pacific

INTRODUCTION:

Oral disease affects 3.9 billion people worldwide [1], with untreated dental caries (tooth decay) impacting nearly half of the world's population (44%), making it the most prevalent of all the 291 conditions included in the Global Burden of Disease Study (GBDS) [2]. Oral health has also been identified as a key indicator of overall health, wellbeing and quality of life [3]. Apart from its effects on general and oral health, oral diseases also have huge impacts on people's daily lives and economic development, with the loss of millions of school and work hours yearly around the world [4].

The Pacific region is no exception to this, and the people of this area face oral health problems of varying magnitudes [5]. This is because, industrialization and urbanization of Pacific Island nations, in particular those which lie on trade routes, has brought a transformation in lifestyle, which encompasses a diet high in refined sugar, resulting in deterioration in oral health [6]. The unique environment of the Pacific Islands and its diverse population, with an array of ethnic backgrounds, also poses a complex problem in both collection and interpretation of epidemiological data. As a result, very few oral health studies have been conducted over the years. Most Pacific Island nations have collected some information on dental caries (toothache), but

this is inadequate and not done on a regular basis [5]. According to the World Health Organization (WHO), oral health surveys must be conducted every 5 – 6 years in the same community or setting, to help identify changing patterns of disease [7]. Regardless, some of the data available shows the following: high Decayed Missing Filled Teeth (DMFT) found in 12-year-old - Tongan children, in a survey conducted in the year 2000 by the South Pacific Medical Team (SPMT) and Tongan dental team. The survey showed the mean DMFT for boys being 4.40, and girls being 5.81 [8]. The 2017 National Oral Health Survey (NOHS) of Vanuatu highlighted that almost 70% of all children aged 5 -7 years had dental caries (tooth decay) [9]. In addition, just below 69% of adults between 30 – 49 years of age and over 76% of people 60 years or more have decayed, missing or (rarely) filled teeth with a very high caries experience. Lastly, over 50% of adults reported of suffering toothache in the last 12 months and around 25% responded of being in pain just a month ago [9]. The 2004 National Oral Health Survey (NOHS) of Fiji, revealed that 88% of 6 year olds showed signs of dental caries (tooth decay) in their primary teeth. Furthermore, only 4.3% of the study population had healthy periodontal status (gum health). The report also stated, that the periodontal status of the population had remained unchanged in the last 30

years, therefore, periodontal disease continued to be, one of Fiji's biggest public oral health problem [10]. Thus, it could be said that oral diseases are prevalent in the Pacific region, and needs to be addressed through all means. So far, the approach towards addressing any disease has been via preventive and curative intervention. These 2 factors are concerned with the clinician's level of skill and expertise.

An aspect that is now gaining attention globally is patient's perception towards utilization of dental services and factors affecting it. This is because, many factors are responsible for influencing health seeking behaviors and these can be perceived as deterring or enabling professional health care [11]. Research on patient's view point is very important because the healthcare system is moving towards a consumer oriented approach, whereby dentists are seen as service providers and the patients as customers [12]. Assessment of healthcare quality has emerged as a noteworthy matter in the care process and it has been acknowledged that patient feedback is an essential factor of these assessments [13]. While it has been recognized that patients cannot evaluate the medical competency of the dentist, their experience towards the procedure of care accentuates their perception regarding the quality of care and its enhancement [14].

Since no research has been conducted to assess the factors affecting the utilization of dental

services in the Pacific; the aim of this study was to identify facilitators and barriers of utilization of dental services and to suggest, Pacific Nations on further research in this area.

METHODOLOGY:

This literature review focused on several aspects related to patient's perception on dental services utilization. Four databases were used to search for publications on relevant studies: Medline, Embase, Scopus, and ProQuest. The keywords used included: (Perceptions OR belief OR Opinions) AND (Dentist* OR "Oral health") AND (Patients OR "Care giver"), AND ("Dental Services" OR "Oral Health"). The focus of the search was studies published between 2000 and 2020 and in English language. The titles of all the studies were scanned by two independent researchers and those not relevant were excluded. The abstracts of the remaining studies were reviewed and the full text of the manuscripts that met the study inclusion criteria were printed for future review and to formulate the themes that are discussed below.

Theme 1 – Dentist Characteristics:

In the last few years, patients have become critical consumers that search for better priced services. Moreover, patients' overall requirements and expectations are higher, which results in greater levels of disappointment when treatments were

not up to their expectations [15]. Dental care is expensive and low income individuals have the perception that dentists are selfish, greedy, and uninterested in public health services [16]. Despite this, patients believe that an “ideal” or excellent dentist should have the following qualities: professionalism, a good communication skill, trust, support, friendliness, and state of the art equipment [15]. According to McKenna, Lillywhite and Maini, “Professionalism” is an image that promotes a successful relationship with the patient and thus enables the foundation of effective patient care [17]. A cross - sectional descriptive study conducted by these authors, on new patients attending an outpatient dental facility in Edinburg, revealed, that majority of the patients would prefer dental professionals to wear a name badge, particularly if it’s the first time the patient is seeing the dentist.

In addition, majority of respondents (56%) preferred their dentist to wear safety glasses, and 72% preferred their dentist to wear a mask. Lastly, 62% of the patients felt very strongly on the way dentists would dress, with dental consultants and specialists to wear formal clinical attire and white coat, as it portrayed the qualities of cleanliness, professionalism and authority. Another factor that influences a patient’s attitude towards the dentist is the clinicians’ age. In regards to patient preference for different age groups in dentists, studies reveal that patients prefer older dentists,

as they consider that they have better clinical and interpersonal skills, together with more years of experience than the younger professionals [18]. On the contrary, in a questionnaire survey conducted by Swami et al, involving 161 British participants, it was found that patients have better perceptions of younger dentists, as they use new methods and technology [19].

In some regions, another relevant factor that influences patient’s perception is the ethnicity of the dentist. It has been shown that some patients prefer their dentists to be of the same ethnic origin or similar culture, as it improves patient – doctor relationship, and the patients understanding of the reasoning’s provided by their dentist [20, 21]. However, other studies conducted show that patients are now more open to getting themselves treated by professionals that come from different ethnic groups [19].

Lastly, since dentists inject drugs and perform invasive surgical procedures, patients have expectations that their dentist is knowledgeable and capable of dealing with medical emergencies [22]. A study done by Vaughan et al [22] showed that 50% of the public would expect dentists and dental surgeries to be required to stock equipment involved in the management of medical emergencies.

The study also noted that patients have a belief that dentists undergo continuing training in medical emergencies.

Theme 2 – Dental Service Characteristics:

Waiting time on health treatment has been reported as one of the factors that increase the level of frustration in patients and is regarded as an obstacle in one's activities [23]. The length of time patients spend in a waiting room has been associated with having negative effect on patient's satisfaction [24-26]. In a quasi-experimental study conducted by Inglehart et al., [24] on 399 adult patients who regularly visited a dental school clinic in United States of America, it was found that letting patients wait for their appointments and not being on time affects their satisfaction negatively. The authors also concluded that longer waiting time not only affects the patient's response, but also lowers the satisfaction of the provider; therefore, longer waiting times affect the dynamics of the patient provider relationship.

A study conducted by Akbar et al., [23] on 114 adult patients in Bantaeng Regency, Indonesia, revealed that first visit patients with a late service provider had worse satisfaction and patient/provider relationship, but overall, there was no significant influence. In cases where patients had made multiple visits to the dentist, they continued to show high satisfaction despite waiting for a long time. Similar results were obtained by Tuominen and Eriksson [27], where it was found that waiting for dental treatment was well tolerated, as long as the waiting time was reasonable.

Cost of services:

Most people do not seek dental care due to the high cost associated with it. In a study conducted by Wallace and MacEntee [28], involving low income earners, dentists and social service providers in Canada, it was found that low income participants along with dentists and other healthcare providers identified the cost of dentistry and the inadequacy or inaccessibility of public insurance schemes as major impediments to dental services for low income people. In a similar study conducted by Kadaluru, Kempraj and Muddaiah [29], involving 246 adults aged 18 -55 attending community outreaches in Bangalore, it was found that 22% of the respondent's stated that high cost of oral health care was one of the major barriers for seeking oral health care. In this matter, public hospitals provide dental services at a lower cost compared to private clinics. As such, many people choose public hospitals over private clinics for their dental needs. According to a study done by Luo, Liu and Wong [30] with 30 participants attending the teaching dental hospital of the University of Hong Kong, cost was mentioned by almost all participants in the consideration of the satisfaction level towards the dental care provided by the hospital. They agreed that the price of the dental care services provided by the hospital was much lower compared with private clinics. Despite the reduced cost in public sector, some individuals still find the cost of dental

treatment as a barrier to accessing dental care. Some parents' voice out that they do not take their children to the dentist as they find it very expensive [31]. Some patients cannot afford treatment options, and they reach a point where their oral health is so compromised that they simply allow their teeth to be extracted/ lost, instead of treating the problem and taking protective measures [16].

Ease of accessibility:

Accessibility in dental care refers to how easily patients can utilize the dental services provided. Research shows that access to general dental services is highly problematic for people and needs to be improved [32]. According to a study done by Marshman et al [33], from a postal survey involving 10864 adults in United Kingdom, it was seen that perceived difficulty accessing a dentist was a predictor of oral health outcomes and influenced dental service utilization indirectly through perceived need. Similarly, another aspect concerning access is the expectations between the providers and recipients of dental services. According to Wallace and MacEntee [34], cognitive and physical disabilities, compounded by substance use and homelessness, can be serious impediments to accessing treatment in the traditional dental practice. In addition, there are also reports that dentists find difficulties managing patients in wheelchairs or long-term care facilities,

or who need sedation. Apart from issues in logistics and accessibility, some studies show that the fear of dental treatment and associated anxiety was identified by many low – income participants as reasons for avoiding dentists, even when public health dental benefits were available.

Privacy and Confidentiality:

Dentists must ensure that they perform their duties such that privacy and confidentiality is upheld. When patients attend a dental practice or clinic, they expect that their data or what they regard as private information will be handled with care by those who might get to know some (or all) of that information [35]. However, there are instances when this is not maintained, a study in Brazil showed that although most doctors (91.43%) claimed that the cabinet auxiliary staff has been trained to respect the confidentiality of patient data, 44.29% of those surveyed act otherwise, discussing clinical cases with people outside the dental cabinet [36]. In addition, use of social media is becoming more prevalent amongst clinicians and patients, which can also result in violation of privacy and confidentiality. Thus it is important to ensure that both social media and internet are used appropriately and not result in breach of patient information. An example of a severe privacy and confidentiality breach, involved a nursing student taking the photo of a young pediatric patient when his mother was not present,

and then, without permission, posting it on Facebook [37]. According to Brennan [35], confidentiality is not an optional extra in dental practice, but essential to good patient care and treatment.

Range of treatment/ services provided:

It is important that patients are given all options for their oral health needs; this can be achieved by spending enough time with the patient and communicating. According to Aldosari et al., [38] giving the patients enough time to express their concerns will open a two-way communication that makes patients feel heard and part of the decision-making process. It will not only lead to higher satisfaction but also help the dental staff to meet the patients' needs and expectations. Communication and development of respectful health care relations is also very important. A study done by Sbaraini et al., [39], concerning 16 adult patients in Australia, found that even when patients were uncertain about the value of a recommended treatment, a perception that their dentist cared about their problems persuaded them towards compliance. This suggests that even the most "uncooperative" patient may have the potential to be more cooperative in the context of such a relationship. Furthermore, most patients' based complaints arise because of an imperfect relationship, further accentuating the importance

of communication between professionals and their patients [15].

In hospital based setting, apart from dentists, even dental therapist/ hygienist can provide services to patients such as cleaning, fillings and dental extractions; it is not uncommon to find patients feeling dissatisfied when they are not seen by dentists, but by other dental professional. According to Dyer, Owen and Robinson [40], despite some patients being happy with care provided by therapists/hygienists, some reported negative experiences on being treated by them, which appeared to emerge from a lack of communication both before and during an appointment. According to the authors [40], good communication by a dentist to the patient on the delegation of duties to a therapist/hygienist will reduce dissatisfaction and increase cooperation.

Dental infrastructure and Occupational Health and Safety (OHS):

A dental clinic must be well equipped and safe, as patient safety-related accidents at healthcare facilities range from minor problems to permanent damage or death [41-43]. Most dental services typically are performed on an outpatient basis, but many dental procedures use potentially dangerous drugs and complex equipment [41]. According to a study done by Hiivala et al., [44] which looked at dentistry related complaints from 2000 to 2011 in Finland, it was highlighted that patients can in

many instances assess safety risks related to their own dental care, such as many treatment injuries, poor hygiene or practitioner impairment, fairly well. Furthermore, a cross – sectional - questionnaire – based epidemiological study including 384 patients across 52 dental offices in Romania by Barlean et al., [45], found that majority of patients, especially men and high educated subjects were concerned by the risk to get infected during the dental treatment. In the same time men were more confident in safety measures but women were more active and implicated in assessing the implementation of infection control protocols. In a research done by Aldosari et al., [38], patients from each of Brazil's 16202 oral health teams were interviewed, and it was seen that patients' perception towards the physical environment had a positive association with the experience and the overall satisfaction. Conversely, a worse perception about the physical environment and cleanliness of the dental office may result in a negative view of primary health care, with dissatisfaction as a consequence. Last but not the least, a dental chair in good working condition is, at least, essential for dental care in primary health care. In addition, dental infrastructure and technology can also be a contributor to dental anxiety, which is a serious health issue. In a study conducted by Mak, Wong and Xu [46], 230 dental students of University of Hong Kong and 230 non –dental students from other Hong Kong

universities were interviewed regarding dental infrastructure, it was noted that amongst the 230 non-dental students, 42.3% reported that they did not easily adapt to environmental noise and 98% reported that they would feel more comfortable during dental treatment if the volume of the dental drill was lower. 15.5% of the non-dental students did not perceive the sound of the dental drill to be unpleasant, while 25.9% of them regarded it as extremely unpleasant. While these findings involve dental students, similar sentiments may also be experienced by patients.

Waiting area and child friendliness:

Dental fear is a normal emotion, a reaction to one or more specific threatening stimuli in a dental care situation, while dental anxiety refers to a state of uneasiness that something dreadful is going to happen as a result of dental treatment [47]. It has been estimated that about 11% of children and adolescents suffer from dental anxiety [48]. One of the environmental factors that can cause anxiety prior to dental treatment includes the waiting room experience, especially the environment of the waiting room and time spent [49]. In a study conducted by Pandiyan and Hedge [50], 65% of parents agreed that pleasantness of dental setup (color, decoration) and friendliness of staff affects the child's behavior in the dental clinic. Among the results, 80% of the parents claimed that dental setting plays an

important role on child's behavior in the dental clinic. However, in a case control study conducted by Fux et al., [51], involving 122 children in Hadassah University Hospital, Israel, no significant difference was found in the anxiety of children waiting for dental treatment in a multisensory waiting room or conventional waiting room. A quasi-experimental study was conducted by Pati and Nanda involving 158 pediatric patients in United States of America, comparing distraction clinic and non – distraction clinic, to assess if introduction of positive distractions in a waiting area would be associated with any changes in the behavior and activities of waiting pediatric patients [52]. The findings revealed that when the TV was switched on there was 26% increase in distraction. The authors concluded that introduction of distraction conditions was associated with higher calm behavior and less fine and gross movement, suggesting significant calming effects of the distraction conditions. In turn, this suggests that the use of positive distractions can affect the stress and anxiety associated with the waiting experience.

Application for Pacific Nations:

Based on these findings, it can be said that patient's perception can greatly influence the utilization of dental services. Factors that have been found to facilitate the utilization of dental services include dentists who display great

professionalism, safe clinical settings, and good dental services in terms of short waiting time and accessibility. On the other hand, dental anxiety and high cost of dental services are barriers for people to access dental care. Overall, the impact of oral diseases in the Pacific cannot be ignored, and Pacific Island Countries (PICs) must conduct oral health surveys in a timely manner to ascertain disease patterns and establish a base line for future comparison. In addition, the Pacific Islanders perception towards these factors must also be investigated. The combination of both these studies will provide the ideal foundation from which oral health policies and strategies can be implemented and enforced. The ultimate goal of oral health surveys and analyzing perception, will be to bring in reduction of oral diseases in the Pacific and to improve the quality of life.

CONCLUSION:

Patient's perception on dental services utilization is influenced by a number of factors that depend on the dentist themselves, the services they provide, and the clinical settings in which these services are delivered. Patients who have poor perception of dentists or unable to access dental care, have poor oral health and consequences of it is irreversible. This literature review, mainly looked at developed countries, since no data or studies were available for Pacific Nations, concerning patient's perceptions. Thus, the aim of

this literature review was to highlight this grey area, and build a foundation on which further research can be conducted by Pacific Nations on this matter. Furthermore, the results of such a research could be used to modify treatment approaches and strategies which will encourage people to seek dental care for improved oral health.

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SODIUM CHLORIDE 0.9% INTRAVENOUS SOLUTION IS ACCEPTABLE AS A PRE-ANALYTICAL PREPARATION SOLUTION FOR FINE NEEDLE ASPIRATES PRIOR TO GENEXPERT TESTING FOR THE DETECTION OF MYCOBACTERIUM TUBERCULOSIS COMPLEX IN PORT MORESBY GENERAL HOSPITAL, PAPUA NEW GUINEA.

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Running title: 0.9% NaCl is acceptable as pre-analytical sample preparation solution for Xpert testing of fine needle aspirates.

Keywords: mycobacterium tuberculosis complex, fine needle aspiration, tuberculous lymphadenitis, Genexpert, sodium chloride.

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Dear Editor,

Conventional laboratory confirmation of tuberculous lymphadenitis (TBLN) is by fine needle aspirate biopsy (FNA) and identifying features of granulomatous inflammation (caseous necrosis, epithelioid cells and granuloma) with or without the presence of acid fast bacilli (AFB) using a microscope. This method requires interpretation of the smear by a trained cytologists or pathologist. The turn-around-time is long causing delay in treatment. Genexpert test

(Xpert) has been shown to detect mycobacterium tuberculosis complex (MTB) in FNA specimens [1] and is now routinely used for TBLN diagnosis in some settings [2]. The assay is fully automated and results are available in two hours.

The Xpert diagnostic system (Cepheid, Sunnyvale, CA, USA) was originally developed for rapid detection of anthrax for use by the United States postal service following mail contamination in sorting offices [3]. As described by Lawn et al [3], it is a cartridge-based system

that incorporates microfluid technology and automated nucleic acid analysis to purify, concentrate, detect, and identify targeted nucleic acid sequences from unprocessed clinical specimens. The assay uses molecular beacon technology to detect deoxyribonucleic acid (DNA) sequences amplified in a heminested real-time polymerase chain reaction (PCR) assay. The assay uses single use cartridges with several chambers that are preloaded with liquid buffers and lyophilised reagent beads necessary for sample processing, DNA extraction and PCR [3]. Sample reagent included in the assay is used for decontamination of specimen prior to processing. Pre-analytical sample preparation of FNA aspirates for Xpert testing is an important procedure because poor sample preparation can affect the accuracy of the results. Various types of solutions have been used for mixing FNA aspirates to generate a suitable aspirate suspension which can be used for further analysis. For example N-acetyl-L-cysteine/sodium hydroxide (NALC/NaOH) [4] or phosphate buffered saline (PBS) [5] have been used.

In a study evaluating the performance of Xpert in detecting MTB in FNA aspirates [6] we used 0.9% sodium chloride (NaCl) intravenous solution as the pre-analytical sample preparation liquid for suspending FNA aspirates for Xpert testing. Patients clinically diagnosed with probable TBLN

and referred to the Port Moresby General Hospital (PMGH) for FNA were recruited.

Those that gave consent were clinically examined and FNA samples obtained from enlarged lymph nodes that were more than two centimeters in diameter. Where multiple sites and glands were involved, the largest palpable node and or the most superficially enlarged glands were chosen for sampling. The procedure for FNA sampling including the results of that study has been published [6,7].

The FNA aspirate suspensions were generated by rinsing the needle in 2.0ml of 0.9% NaCl in a standard urine specimen container. Although any suitable container can be used, we chose urine container because it was readily available in our laboratory. Rinsing was done by drawing 2.0ml of 0.9% NaCl completely into the syringe and expressing the aspirate-NaCl mixture back into the container. This process was repeated several times to maximize aspirate material in the needle hub to be mixed with NaCl. Care was taken not to create visible aerosols. Biohazard and safety protection measures were implemented throughout the procedure. The aspirate-NaCl suspension was transported in room temperature to the Central Public Health Laboratory (CPHL) housed within PMGH and stored at two to eight degree Celsius for Xpert assay. On the day of Xpert testing the aspirate-NaCl mixture underwent further pre-analytical sample

preparation following an in-house protocol as outlined:

- One milliliter of the aspirate-NaCl mixture was pipetted into 1.0ml 0.9% NaCl to make up to 2.0ml.
- Then manufacturer supplied Xpert sample buffer was added at 2:1 ratio.
- The mixture was vortexed and incubated at room temperature for 10 minutes.
- Samples with high mucoid matrix were vortexed again and incubated at room temperature for further 5 minutes. The additional 5 minutes of vortexing was required to lyse pus cells, increase yield and reduce viscosity to prevent test failure. The time interval and length were divided in this manner to allow the laboratory scientist to determine whether an extra 5 minutes was needed to lyse the sample further.
- Finally 2.0 ml of the whole solution from the final mixture was analyzed using Xpert.
- Results were reported as positive if MTB detected, negative if MTB not detected and invalid if there was test failure. Invalid tests were repeated using fresh sample. Instrument settings were established following manufacturer's guidelines [8]. The results of this study were used to establish standard

operating procedures at CPHL for TBLN Xpert testing [9].

A total of 107 FNA samples were processed. Mycobacterium tuberculosis complex was detected in 66 samples (61.7%; 66/107). Of the 66 positive cases, rifampicin resistance was detected in 19 samples (28.8%; 19/66). There were no invalid results. The remainder of the aspirate-NaCl mixture was used for culture. Samples for culture were sent to Australia due to unavailability of MTB culture facility within CPHL at the time of this study. Culture was completed on 24 samples (22.4%, 24/107) with a culture yield of 58.3% (14/24).

Xpert detected MTB in all culture positive samples (100%, 24/24). Drug susceptibility testing pattern of the MTB isolates have been published [7].

Previous studies evaluating diagnostic performance of Xpert in detecting MTB in FNA aspirates used NALC/NaOH [4], PBS [5] and other bactericidal reagents [9] for mixing FNA aspirates to suspend the aspirate material in the liquid. The rate of MTB detection in FNA aspirates using Xpert is variable and the type of fluid used for suspending aspirate material may affect test accuracy. For example, MTB detection rate with NALC/NaOH is 60% [4] and 64.6% with PBS [5]. Biadglegne et al [10] used a bactericidal reagent and the MTB detection rate in their study

was 39.0%. Fantahum et al [11] directly inoculated aspirate material into the manufacturer provided buffer solutions for Xpert testing and obtained a detection rate of 49.3%. The Xpert MTB detection rate in the present study was 61.7%. However, other factors could also influence Xpert MTB detection rates. Some of these factors may include quantity of material aspirated, immune status of the patient, whether the patient is on anti-tuberculous drugs or not, storage temperature and length of time sample is stored before Xpert testing. Further studies would need to be conducted to determine if these factors have any influence on the Xpert test outcome.

Although the culture yield of 58.3% is high compared to Wright et al [12] who used a MTB specific transport media, the present study is small and a larger study will need to be conducted at PMGH to confirm these findings. Culture yield of patients infected with Human Immunodeficiency Virus (HIV) is also higher compared to HIV negative patients [13]. The design of the present study did not permit investigating the HIV status of the patients.

In conclusion, the results obtained in this study demonstrated that 0.9% intravenous NaCl is suitable for pre-analytical sample preparation for Xpert testing for detecting MTB in FNA aspirates at PMGH. The laboratory protocol used for pre-analytical FNA aspirate preparation for Xpert

testing of FNA samples is suitable for use at PMGH and can be adopted by laboratories for use in PNG. It is hoped that widespread use of Xpert testing of FNA samples in PNG will reduce the result turn-around time ensuring prompt commencement of anti-tuberculous chemotherapy.

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Acknowledgements:

The following should be acknowledged: Research or other financial grants; Material support, Contributions of Institutions, Colleagues, and other relevant participants.

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Book:

Gillett JE. The health of women in Papua New Guinea. PNGIMR: Kristen Press, 1991

Chapter in a Book:

Chaney SG. Principles of nutrition II: Micronutrients. In: Delvin TM, editor. *Textbook of Biochemistry with Clinical Correlations*, 4th ed. Brisbane: Wiley-Less, 1997: 1107– 36.

Published proceedings paper:

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