Original Research Article

Subclinical Malaria and Reticulocyte Count in Apparently Healthy Female Undergraduate Students in Rivers State University, Port Harcourt, Nigeria.

ABSTRACT

Aims: The study was aimed at determing subclinical malaria and estimating reticulocyte count in apparently healthy female undergraduate students of Rivers State University, Port Harcourt
Study design: This is a non-randomized, comparative case-control study.
Place and Duration of Study: The study was conducted female students residing at the hostels of Rivers State University, Port Harcourt. Analysis was carried out at the Haematology Laboratory, Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria, between July and August, 2018.
Methodology: A total of 32 students (32 %) were infected with *Plasmodium falciparum* and a total of 68 uninfected students (68 %) were used as control in the study. Thick and thin blood films examination using Giemsa staining technique was used to detect and calculate the malaria parasite density while a thin film examination using new methylene blue staining technique was used to evaluate the reticulocyte count in the blood.
Results: The reticulocyte count of the infected students was 0.15 ± 0.04 % and that of the uninfected students was 0.31 ± 0.08 %. The infected students had significantly lower reticulocyte count ($p < 0.0001$) than the uninfected students. The age bracket 15-19 years had the highest malaria parasite density 0.52 ± 0.18 % while 25-29 years had the least 0.33 ± 0.24. There was no statistical variation in malaria parasite density according to age brackets ($p = 0.13$, $p > 0.05$). However, the age bracket 15-19 years had the lowest reticulocyte count as most of the students within this age group were infected with malaria parasite.
Conclusion: This study revealed that reticulocyte counts of malaria infected individuals were significantly decreased and there was no statistical significant variation in malaria parasite density irrespective of age groups. Prophylaxis for malaria in such settings would be an efficient means of preventing infectious reservoirs and higher rates of subclinical malaria infection.

Comment [CY1]: Did you infect the 32 student with malaria?? Or what??

Comment [CY2]: The conclusion, though might generally be expected, as the authors have pointed out similar conclusions from literature, it cannot to drawn from this very manuscript. It's not that «White and Black» clear from this work to put out this assertion.

Keywords: Subclinical malaria, reticulocyte count, Plasmodium falciparum, female undergraduate.

1. INTRODUCTION

Malaria is a life-threatening disease of man caused by a parasite of the genus Plasmodium, which is transmitted through the bite of infected female Anopheles mosquitoes. This mosquito-borne infectious disease that affect humans can also affect animals. It is a killer and debilitating disease and remains a difficult health and socio-economic problem in the world [1]. In tropical and subtropical regions, malaria has been a common disease and it continues to be one of the most widely spread health hazards. An estimated 1.2 billion are at high risk of being infected with malaria and developing disease, and 3.3 billion people are at risk [2].

The second leading cause of death from infectious diseases in Africa is malaria after HIV/AIDS [3]. More than half of the world's population lives in the areas where they remain at risk of malarial infection. Malaria exacts a heavy burden on most vulnerable communities where the poorest are most severely affected, having the highest risks associated with malaria, and the least access to effective services for prevention, diagnosis and treatment [4].

Malaria parasites belong to the genus *Plasmodium*. Malaria is caused by *Plasmodium falciparum*, *Plasmodium malariae, Plasmodium ovale, Plasmodium vivax,* and *Plasmodium knowlesi* infect humans [5];[6]. According to Nadjm and Behrens, [7], *Plasmodium falciparum* is the most common species identified (~75 %) among those infected, followed by *Plasmodium vivax* (~20 %). *Plasmodium falciparum* traditionally accounts for the majority of deaths [8]. Recent evidence suggests that *Plasmodium vivax* malaria is associated with potentially life-threatening conditions about as often as with a diagnosis of *P. falciparum* infection [9]. However, *Plasmodium vivax* proportionally is more common outside Africa [10].

Malaria - stricken family spends an average of over one quarter of her profit on malaria treatment and can only harvest 40 % of crops harvested by healthy families [4]. It is estimated that about 132 billion Naira is lost to malaria annually in Nigeria in the form of treatment costs and prevention [11]. Malaria remains a major public health challenge where it estimates for more cases and death than any other country in the world [12].

In Nigeria, high prevalence of malaria parasitaemia has been reported [13]; [14]; [15]. The variation among other target groups is not much as prevalence of 28.0 % was recorded among blood donors in Port Harcourt [16] and 26.0 % prevalence was reported among pregnant women attending ante-natal clinic in Port Harcourt [17].

Malaria transmission in humans occurs through indirect, vector transmission and natural living reservoirs. In the transmission of malaria, 30 of the 400 different species of Anopheles mosquitoes are of significant importance [18]. Transmission begins when a female Anopheles bites an infected human and ingests protozoan gametocytes. The parasite incubates in the mosquito for 8-35 days before the infectious sporozoites are formed. The disease is transferred when the mosquito bites a human host and introduces the malarial sporozoites [19].

Malaria can be classified as uncomplicated or severe (complicated). Uncomplicated symptoms include tiredness, fever, vomiting and headache while in severe or complicated cases it can cause yellowing of the skin, coma, seizures or even death [20]. Ten to fifteen days after being bitten by infected mosquito, symptoms ensue. If not properly treated, people may have recurrences of the disease months later and in those who have recently survived an infection, reinfection usually causes milder symptoms if not properly treated reinfection usually causes milder symptoms in those who have recently survived an infection [21]. This partial resistance disappears over months to years if the person has no continuing exposure to malaria [20].

There is no standard definition for "subclinical" malaria infections but it is generally accepted to be malaria parasitaemia of any density, in the absence of fever or other acute symptoms, in individuals who have not received recent antimalarial treatment [22]. The vast majority of individuals with detectable malaria parasitaemia can be categorized as asymptomatic based on this definition, regardless of the level of malaria transmission. This definition includes early detection of rising parasitaemia that has yet to reach the pyrogenic threshold (that is, the density of parasitized erythrocytes that is sufficient to trigger innate immune responses and fever) [23], infections that are intermittently symptomatic but not severe enough to cause the person to seek health care and long-standing infections imperfectly controlled by the immune response [24].

Asymptomatic parasitaemia is prevalent in highly endemic areas of Africa, reaching over 90 % in children, with only a small percentage of individuals ever demonstrating clinical symptoms. The clinical consequences of asymptomatic malaria may differ across different epidemiological settings and are not fully understood. It is generally assumed that

asymptomatic parasitaemia is involved in the development of partial immunity in endemic areas and may protect against clinical disease from new infections [25]. Notwithstanding, asymptomatic parasitaemia provides a reservoir for transmission and may be a precursor in the progression to symptomatic disease [26].

Previously, it was believed that only areas of high endemicity are at risk of subclinical infection, but more recent studies from other malaria-endemic regions (for example, countries in Africa) have observed that communities living in low-transmission areas are also at the risk of asymptomatic parasitaemia [23]. In low-transmission areas, submicroscopic carriers can become the source of approximately 20-50 % of all transmission [27].

The prevalence of asymptomatic parasitaemia depends upon the high or low transmission area, period of residency in the endemic area, age, development of partial immunity by the previous repeated exposures to malaria, gender, use of bed nets, and the genetic back-ground [28]; [29]. Malaria transmission depends on two primary factors. These are location of mosquito breeding sites, and clustering of human habitations where people serving as reservoirs of parasites for mosquito infection live [30]. The presence of a large number of asymptomatic carriers in a population is a challenge and places an additional burden on malaria control programmes [22].

During malaria infection, anaemia is a common complication and it causes mortality and morbidity in patients. The pathophysiology of malarial anemia is said to be multifactorial and complex [31]. The preferential invasion of particular red blood cell age class is based on the characteristics of some species of human malaria parasites. *Plasmodium falciparum* has the capability of invading all red blood cell age classes, while *Plasmodium vivax* and *Plasmodium ovale* exhibit a strong preference for the youngest red blood cells (reticulocytes) and *Plasmodium malariae invades* the mature red blood cells [32]. The red blood cell invasion preferences for *P. knowlesi* are still to be identified [33]. There is obvious loss of infected erythrocytes through parasite maturation during infection but many uninfected cells are also destroyed due to antibody sensitization or other physiochemical membrane changes and increased reticuloendothelial activity in spleen. Additional factor contributing to worsening the condition has been said to be suppression of erythropoiesis. Some patients suffering from malaria develop severe anaemia while others retain near-normal hemoglobin levels which may be explained by the degree of red cell destruction during the period before normal bone marrow function returns [34].

Anaemia may either develop rapidly with severe haemolysis, or present as a relatively slow rate of red-cell destruction in the presence of persistent bone-marrow suppression. Some studies have showed evidence of hypoproliferative erythropoiesis and dyserythropoiesis for weeks' post-infection, while other researchers found that bone-marrow suppression reverses rapidly after treatment [35]. Reticulocytes which are non-nucleated immature red cells are delayed in release during acute malaria infection, indicating transient suppression of the normal erythropoietin (Epo) response [36]. The effect is probably controlled by an autologous serum factor that suppresses the growth of an early precursor red cell [37].

In sub-Saharan Africa over 90 % of all deaths is caused by malaria and about 85 % of deaths globally are in children under 5 years of age [38]. According to Chukwura *et al*., [39], *Plasmodium falciparum* malaria is the most prevalent and virulent in Nigeria and it is capable of causing mental apathy, weakness and generally slowing down economic development; accounting for up to 98 % of severe cases with significant mortality and morbidity [38].

The risk of malaria can be reduced by preventing mosquito bites through the use of insect repellents and mosquito nets, or with mosquito control measures such as spraying insecticides and draining standing water [20]. No effective vaccine exists, despite a need, although efforts to develop one are ongoing [4]. In patients with drug resistance malaria, treatment is made difficult and before designating the final treatment plan, drug resistance of the infecting parasite must be determined. For the treatment of uncomplicated malaria caused by *Plasmodium falciparum* Artemisinin-based combination therapy (ACT), ACT can be used for the treatment which is a combination of the drug artemisinin and another partner drug is recommended [40]. Chloroquine is recommended for infections caused by *Plasmodium vivax*, injectable artesunate, followed by a course of severe malaria [41]. Adjustments must be made accordingly if the infecting parasite is found to be resistant to the recommended treatment [19]. Treatment of these patients with anti-malarial drugs however increases reticulocyte numbers, as *Plasmodium falciparum* is one of the causes of dyserythropoiesis and ineffective erythropoiesis [35].

Since asymptomatic parasitaemia provides a reservoir for transmission and may be a precursor in the progression to symptomatic disease, it is pertinent to ascertain the level of subclinical malaria among undergraduate students leaving in the hostels with a view to proffering a better control and management strategies for malaria, hence this research.

2. MATERIAL AND METHODS

2.1. Study Area

The study area was female hostels (hostel NDDC, hostel C and hostel H) in Rivers State University (RSU), Nkpolu-Orowurokwo Port-Harcourt in South-South Nigeria from the month of July through august, 2018. Rivers State University, formerly called Rivers State University of Science and Technology is a university located in the Diobu area of Nkpolu-Orowurokwo Port-Harcourt, Rivers State in South-South, Nigeria. The university has a GPS coordinate of 4.7958^{0}N, 7.0246^{0}E, and female undergraduate student population in the hostel is about 1,000.

2.2 Study Population

The exercise was carried out among female undergraduate hostel students of the Rivers State University, Nkpolu-Orowurokwo, Port Harcourt, Nigeria. A total of 100 students were sampled that is 40 students from Niger Delta Development Commission (NDDC) hostel, 40 students from hostel C and 20 students from hostel H. Only Rivers State University undergraduate female students within the age of 15-29 years were included in this study population. Malaria symptomatic individuals experiencing fever, malaise and nausea, individuals that are not female undergraduate students of Rivers state university, individuals that are not within 15-29 years, individuals having health challenges whether genetically or pathological, those that have been on malaria medication within three weeks prior to sample collection and those that declined consent were excluded from this study

Comment [CY3]: ?/? This study exercise or The study execise??? Just writing «The exercise» carry the sense of some physical work-out ???

2.3 Collection of Blood Samples

Two millimeters of venous blood was collected from each student using a standard tevenepuncture technique in 0.5 mg ethylene diaminetetraacetic acid (EDTA) per ml of blood and promptly analyzed for malaria parasite and reticulocyte count.

Comment [CY4]:

Comment [CY5]: Whole sentence lack clarity? So it means the 2mm blood was mixed with 1.0 mg of EDTA??

2.4 Identification of Malaria Parasite

2.4.1 Principle of Giemsa stain

Giemsa solution is composed of eosin and methylene blue (azure). The eosin component stains the parasite nucleus red, while the methylene blue component stains the cytoplasm blue.

2.4.2 Procedure for Thin blood film Preparation

A drop of small blood was dropped near the end of a slide. The edge of the spreader was placed in front of the drop of blood at an angle of 30-45. The spreader was drawn back until it touched the drop of blood and the drop spread along the line of contact between the spreader and the slide on which the film was to be made. The spreader was pushed along the slide with a smooth movement. The film was allowed to air-dry at room temperature. The patient's identification number was directly written on the thick end, using a lead pencil.

Comment [CY6]: What is «drop of small blood»??? relative quantity of measure at least?

2.4.3 Procedure for thick Blood film preparation

Two small drops of fresh blood were placed on a clean glass slide. The drops of blood were mixed with a corner of another slide in a circular motion over an area about two centimeters in diameter. The blood film was allowed to air-dry at room temperature

Comment [CY7]: At least relative quantity, say 2um, 5um etc should be stated? What is two smalll drops???

2.4.4 Procedure for thin film staining using Giemsa stain

One in 10 dilution of Giemsa stain was made by mixing 1ml of stain and 9ml of buffered water. The film was prepared and fixed in absolute methanol for 1 minute. The film was allowed to air-dry. It was flooded with diluted Giemsa stain for 10 minutes and washed in buffered water at pH 7.2. The film was dried on a rack in a vertical position. The stained film was examined microscopically using oil immersion objective (100x).

Comment [CY8]: From which manufacturer??? Olympus or Karl Zeiss or who? Proper identification may be required??

2.4.5 Procedure for staining of thick film staining using Giemsa stain

One in 10 dilutions of Giemsa stain was prepared by mixing 1ml of stain and 9ml of buffered water. The film was dried for several hours and then covered with diluted Giemsa stain for 10 minutes. The film was washed in buffered water at pH 7.2 and dried in a vertical position. The stained film was examined microscopically using oil immersion objective (100x).

Comment [CY9]: What is several hours??? 10 hours or 100 hours ??

Comment [CY10]: From which manufacturer??? Olympus or Karl Zeiss or who? Proper identification may be required??

2.5 Reticulocyte Count

2.5.1 Principle of Reticulocyte Count

Reticulocytes are immature red blood cells that contain remnant cytoplasmic ribonucleic acid (RNA) and organelles such as mitochondria and ribosomes. Reticulocytes are visualized by staining with supravital stains (methylene blue or brilliant cresyl blue) that precipitate the RNA and organelles.

2.5.2 Procedure for Reticulocyte Count

Two drops of blood and two drops of the staining solution was mixed in a test tube and allowed to stand at room temperature for 20 minutes. The tube was gently tapped to remix the content and with a drop of the mixture, a blood film was prepared in the same manner as for peripheral smear. The film was allowed to air-dry completely. The stained film was examined microscopically using the oil- immersion objective (100x)

Comment [CY11]: At least relative quantity, say 2um, 5um etc should be stated? What is two smalll drops???

2.5.3 Calculation of Percentage Reticulocyte Count

The percentage reticulocyte count was calculated using the formula:

$$\% \text{ Reticulocyte count} = \frac{\text{Number of reticulocytes counted}}{\text{Number of red blood cells+reticulocyte counted}} \times 100$$

Reference range for reticulocyte count = 0.2- 2.0 % [42] (Ochei & Kolhatkar, 2007).

Comment [CY12]: This range is for what??? Healthy range or non-healthy range?

2.6 Reading of Slides and Counting of Malaria Parasites Density

The slides were microscopically examined using the low magnification (10x, 40x objective lens) to ascertain a definite field with even distribution of red blood cells before finally examining with 100x or (oil immersion) objective lens. For malaria, the thick blood film was used to indicate the presence of malaria parasites and thin film was used for the specie identification and quantification. Malaria parasite density was then calculated using the formula:

$$\text{Malaria parasite density (\%)} = \frac{\text{Number of parasitized red blood cells}}{\text{Red blood cells observed}} \times 100$$

Levels of parasitaemia as described by Manas *et al*., [43] are:

1. High parasitaemia (> 10 parasite/ 1 oil field)
2. Moderate parasitaemia (1-10 parasite/ 1 oil field)
3. Low parasitaemia (1-100 parasite/ 100 oil field)

2.7 Statistical Analysis

The data generated were analyzed using special package for social science (SPSS) version 22. 0. Comparison of reticulocyte count between malaria positive and malaria negative subjects was analysed with student independent t- test. Comparison of malaria parasite density according to age bracket was analysed with single factor analysis of variance (ANOVA). The reticulocyte count according to age bracket was presented on a bar chart and correlation between malaria parasite density and reticulocyte count was analysed using the t-test. Values below 0.05 were considered statistically significant

Comment [CY13]: Have not seen any bar chat yet in the manuscript???

Comment [CY14]: What values??? Correlation values or t-test value or what??

3. RESULTS

3.1 Comparison of Reticulocyte Count between Malaria Parasite (MP) Positive Subjects and Malaria Parasite (MP) Negative Subjects

The mean reticulocyte count for malaria parasite positive subjects was 0.15 ± 0.04 % while that of the malaria parasite negative subjects was 0.31 ± 0.08 %. The malaria parasite positive subjects had significantly lower reticulocyte count ($p < 0.0001$) than the malaria parasite negative subjects.

Comment [CY15]: Falls out of the range mentioned under sectiom 2.6. So, again, what's that range in sectin 2.6 for ??? and what's the meaning of the fall out in section 3.1 ??

Table 1: Comparison of Reticulocyte Count between Malaria Parasite (MP) Positive Subjects and Malaria Parasite (MP) Negative Subjects

Comment [CY16]: «»Comparison of mean.....» you discussing mean values or ????

Comment [CY17]: «Mean reticulocyte cout (%)»

Subjects	Reticulocyte Count (%)
MP Positive (32)	0.15 ± 0.04
MP Negative (68)	0.13 ± 0.08
p-value	<0.0001

3.2 Comparison between Reticulocyte Count and Malaria Parasite Density

The reticulocyte count has a significant negative correlation with malaria parasite density ($r = -0.39$, $p<0.0001$) as shown in Figure 1. In other words, when malaria parasite density increases reticulocyte count decreases and when malaria parasite density decreases reticulocyte count increases.

Comment [CY18]: Well, based this graph, for me it really difficult to make the assertion being made hear. It definately not that «White and Black» as the authours are saying here.

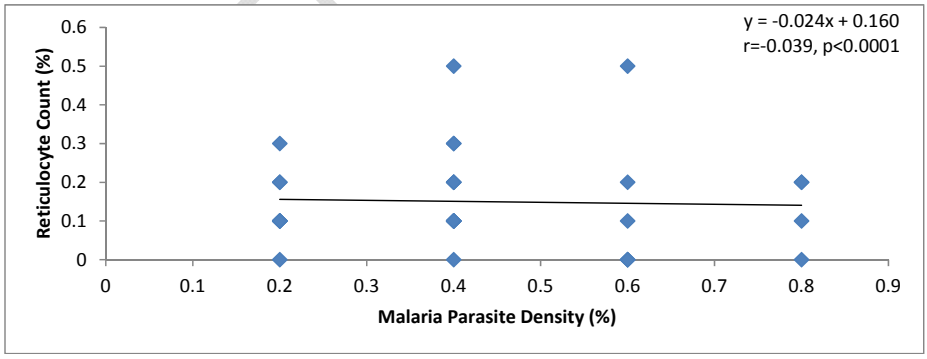

Figure 1:Scatter Diagram of Reticulocyte Count and Malaria Parasite Density

Comparison of Reticulocyte Count between Malaria Parasite Densities According to Age Brackets

The malaria parasite density for subjects in age bracket A (15-19) years was 0.52 ± 0.18 %, B (20-24) years was 0.40 ± 0.17 % while that of C (25-29) years was 0.33 ± 0.24 %. There was no significant difference in malaria parasite density according to age brackets (p = 0.13). Details are shown in Table 2.

Table 2:Comparison of Reticulocyte Count between Malaria Parasite Densities According to Age Brackets

Age (Years)	Malaria Parasite Density (%)
A (15-19)	0.52 ± 0.18
B (20-24)	0.40 ± 0.17
C (25-29)	0.33 ± 0.24
p - value	0.13

4. DISCUSSION

The study was done to evaluate subclinical malaria and reticulocyte count of apparently healthy female undergraduate students in rivers state university, port harcourt in south-south zone of nigeria. Malaria is indeed by far the most important tropical parasitic disease causing great suffering and loss of lives.

From this study, the prevalence of subclinical malaria was 32 %. This result is similar to the work done by Ezeigbo and Ezeigbo [44], who reported cases of 35.3 % malaria parasitic infection at the abia polythechnic, south eastern nigeria. The prevalence obtained from this study was at variance with work done in Southern Nigeria by omolade and his co-researchers in 2010 who reported malaria parasitic infection rate of 83.3 % and by Fernado and his colleagues [45] who reported prevalence of 77 % in Senegal. This study was conducted in a university environment among individuals of higher learning and understanding and may have contributed to the low level of asymptomatic or subclinical estimation obtained. High standard of education usually affect health awareness and therefore has a positive impact on health since they are probably better informed about vector control measures.

Comment [CY19]: This straight foward emphatic statement is quite far reaching from the content of this manuscript.

Interestingly, *Plasmodium falciparum* was the only *plasmodium species* encountered in this study which represents a major problem in nigeria. This collaborates with other researches carried out in Lagos state south west Nigeria [46], and in Port Harcourt metropolis, Rivers state Nigeria [47], where only infections of *Plasmodium falciparum* were reported. On the other hand, Matur and his colleagues [48], reported cases of *Plasmodium falciparum* and *Plasmodium malariae* while Sam and his co-researchers [49] reported cases of mixed infections of *Plasmodium falciparum* (95.6 %), *Plasmodium malariae* (3.3 %), *Plasmodium ovale* (0.7 %) and *Plasmodium vivax* (0.4 %) in Ogun state, Nigeria.

From the result of this study, the students (32 %) that were positive for malaria parasite had lower reticulocyte count of 0.15 ± 0.04 % when compared to the students (68 %) that were negative for malaria parasite with the reticulocyte count of 0.31 ± 0.08 %. This indicates that there was a significant difference in the reticulocyte counts of the positive and negative subjects. Also, in comparing the reticulocyte count with malaria parasite density, the reticulocyte count had a significant negative correlation with malaria parasite density ($r = -0.39$, $p < 0.0001$). In other words, when there is an increase in malaria parasite density, reticulocyte count decreases, and vice versa.

Conclusively, the significant difference in the reticulocyte counts of the positive and negative subjects and the significant negative correlation of reticulocyte count with malaria parasite density could be due to ineffective erythropoiesis. This collaborated with the findings of Thawani and his co-worker [50], who reported that dyserythropoiesis occurs in the bone marrow of patients with low parasitaemia. Roberts and his colleagues [51] also reported that during acute malaria infection reticulocyte release is delayed, depicting transient suppression of normal erythropoietin (epo) response, which is probably mediated by an autologous serum factor that suppresses the growth of an early-precursor red cell. Another researcher also collaborated by reporting that there could be reduced reticulocyte as a result of inhibition of haemopoiesis arising from the release of interferon gamma and tissue necrotizing factor (tnf) and bone marrow hypoxia, dysfunction of haemopoiesis as a result of sinusoidal obstruction of parasitized red cells. This depressed erythropoietic response may contribute to the development of anaemia, which is the primary clinical manifestation of malarial infections especially in severe cases causing mortality and morbidity.

From this study, the results of female undergraduate students of the age bracket a (15-19 years) were mostly affected with the malaria parasite (0.52 ± 0.18 %) followed by the age bracket b (20- 24 years) with 0.40 ± 0.17 and least affected age bracket c (25-29 years) with 0.33 ± 0.24 %. However, the result contrasted the report of Ebenezer and Eekpa [52], observed more infected rate in the age bracket 29-31 years (25.0 %)| followed by age bracket 17- 19 years (17.65 %) and least in the age bracket of 23-25 years (9.09 %) carried out among new intakes in the Isaac Boro college of education, Sagbama, Bayelsa state Nigeria. The variation of the malaria parasite density according to the age brackets from the study was not statistically significant ($p = 0.13$, $p > 0.05$). this insignificance in statistical variation could arise due to an equal chance of been infected irrespective of ages as long as they are confined to same environmental conditions and also the clustering of human habitations in the hostels where people are serving as reservoirs of malaria parasites.

The results obtained showed that that the age brackets 15-19 years had the lowest reticulocyte count when compared to the other age brackets 20-24 years and 25-29 years which had approximately equal reticulocyte counts. This decreased reticulocyte count seen in this age bracket 15-19 years could be as a result of the increased effect of malaria parasitaemia on this age group seen from the study. Esan [53], reported that the haematopoietic system is modestly affected by aging and these effects become particularly notable after age of 65years, where there is a continuous decrease in the volume of the haematopoietic marrow while it is markedly increased in children as the bone marrow is fully active and extremely cellular.

5. CONCLUSION

There is a moderately high estimation of subclinical malaria amongst the female undergraduates boarding in the hostel in Rivers State University as reflected in the study. This study has indicated that there will be reduced reticulocyte count with malaria parasitic infection due to the suppression of the bone marrow by the malaria parasites. This could impact

negatively on the health of the population as individuals gradually go into anaemic condition. Therefore, improving hygienic conditions, and periodic insecticides spray in and around the hostels can go a long way in reducing the mosquito population and treating of asymptomatic individuals with malaria thus to reduce transmission of malaria parasite.

CONSENT AND ETHICAL APPROVAL

Informed consent was obtained from apparently healthy subjects prior to enrolment upon ethical clearance by the Ethics Committee of the Department of Medical Laboratory Science, Rivers State University.

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