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Malaria Parasite Density and B Lymphocyte Quantitation among Students in Owo, Southwest NigeriaMuhibi, M.A.¹, Isah, K.², Adedokun, K.A.³, Akerele, O.I.⁴, Jeremiah, Z.A.⁵

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Abstract

Malaria is one of the greatest public health problems of the world. It is a parasitic disease of major global health significance that causes an estimated 2.7 million death each year. There is a school of thought which hypothesize that there is development of immunity in malaria endemic area because *Plasmodium* infection interferes with B cell development and maintenance of memory responses. The aim of this study was to evaluate the effect of malaria parasite density on B lymphocytes in Owo. Eighty-two blood samples collected from consenting undergraduate students of Achievers University, were analysed for malaria parasitaemia and B lymphocyte count using microscopy and immunophenotyping respectively. Giemsa-stained thick films and Leishman-stained thin films were assessed microscopically for the presence of *Plasmodium specie* and densities of the parasite were recorded. B lymphocytes were counted with cyflow Cube 6 from Partec, Germany. Statistical analysis was done with SPSS and mean \pm SD values were compared using Pearson's chi square test. Majority of the participants were females (73.2%) with the mean age and standard deviation of 19.939 ± 3.048 years respectively. A great number of the participants (95.1%) were unmarried. Thirty-eight (46.3%) of the participants were free from malaria parasite as at the time of testing, while 40.2% and 13.4% were lightly (+) and moderately (++) infected respectively. The mean \pm SD of the B lymphocyte counts was 70.19 ± 3.706 while p values obtained for Pearson's chi square test were greater than 0.05. The study revealed that the malaria parasitaemia and

parasite density has no effect on absolute B lymphocyte counts of undergraduate students in Owo. Additional researches are advocated on symptomatic patients, and health care system should be strengthened with adoption of diagnostic technology like immunophenotyping.

Keywords: Immunophenotyping, malaria parasitaemia, B lymphocytes, students.

Introduction

Malaria is a life-threatening disease, with nearly half of the world's population being vulnerable to this infection. Malaria accounts for an estimated 2-3 million deaths annually and it is also responsible for the untold morbidity in approximately 300-500 million people annually (Mishra *et al.*, 2002). Malaria, which predominantly occurs in tropical areas, is caused by infection with different species of *Plasmodium* transmitted by an infective female *Anopheles* mosquito. These species are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and rarely *Plasmodium Knowles* (Mueller *et al.*, 2007). At risk for contraction of malaria are persons living in or traveling to areas of Central America, South America, Hispaniola, sub-Saharan Africa, the Indian subcontinent, Southeast Asia, the Middle East, and Oceania. Among these regions, sub-Saharan Africa has the highest occurrence of *P falciparum* transmission to residents and visitors; especially from less endemic zone of the United States (Marchand *et al.*, 2011).

Malaria caused by *Plasmodium falciparum*

continues to cause over a half million deaths each year, with children and teenagers being disproportionately affected (*WHO, 2013*). Children suffer the greatest morbidity and mortality from malaria since immunity to malaria takes years to develop, increases with age and exposure. One manifestation of acquired immunity to malaria is control of blood stage parasites, resulting in lower parasite densities and lack of febrile symptoms of disease. Antibodies have been shown to be an important mediator of this blood stage immunity. Effective B cell and antibody responses to *Plasmodium* infection generally develop only after years of repeated exposure, presumably due to immune immaturity of the host and antigenic variation of parasites (*Cohen et al., 1961*). Another hypothesis for the slow development of immunity is that malaria infection may interfere with B cell development and maintenance of memory responses. After initial maturation in the bone marrow, B cells pass through a series of developmental differentiation stages, many of which can be detected in the peripheral blood. Transitional B cells emerge from the bone marrow and mature into naïve B cells prior to antigen exposure. After antigen exposure, B cells in secondary lymphoid organs differentiate into class-switched classical Memory B Cells (MBCs), non-class switched 'innate-like' MBCs and antibody-secreting plasmablasts; these cells can be detected in blood as they migrate to other secondary lymphoid organs and tissues (*Weiss et al., 2009*).

Exposure to *Plasmodium* alters the distribution of these B cell sub-sets, and has been associated with an expansion of 'atypical' MBCs in individuals living in malaria-endemic areas. Atypical MBCs are class-switched but lack the classical MBC marker (CD27), and unlike classical MBCs, do not appear to readily produce antibodies. This functional difference has led to the hypothesis that atypical MBCs may be 'exhausted' and may interfere with development of effective immunity. On the other hand, higher circulating proportions of atypical MBCs and immunity to malaria are both associated with increasing age and *P. falciparum* exposure (*Portugal et al., 2015*). Thus, the relationship between atypical MBCs and immunity to

malaria remains unclear. B cell sub-sets generated during malaria episodes may indicate which B cells are associated with developing immunity. Various studies have described multiple B cell sub-sets in people exposed to varying levels of malaria, but the kinetics of B cell responses following malaria have not been well described in humans. One study tracked the kinetics of transitional B cells following malaria infection and found that the relative proportion of these cells increased following malaria infection. Studies of experimental infection of mice with *Plasmodium chabaudi* have found that newly differentiated plasmablasts only circulate in the blood for a short time following primary or secondary infection while other sub-sets such as transitional, naïve B cells and MBCs fluctuate greatly but remain readily detectable in the peripheral blood. It was suggested that there are likely to be dynamic changes in the composition of the B cell pool both during and following acute malaria in humans, and that these changes may be reflected in the peripheral blood (*Portugal et al., 2015*). It was equally established that potent markers of B cell in bone marrow and peripheral blood include monoclonal CD 20 antibody (Murphy, 2012; Pelanda *et al.*, 2012). Alterations in lymphocyte sub-populations are one of the general immunological consequences of malaria infection. Depletion of B cells and T cells, with an associated increase in null cells, has been associated with malaria infections (Krettl and Nussenzweig, 1974). In this study, we determined the relationship between malaria parasitaemia and absolute CD20+ lymphocytes (B lymphocytes) in the population studied.

Materials and Methods

Study Area

This study was carried out among undergraduate students of Achievers University, Owo, Owo Local Government Area of Ondo State in Nigeria. Owo is a town situated at the South-Western Nigeria, latitude 7°11'46.32" N and longitude 5°35'12.52" E at an altitude of 304 meters. It is at the southern edge of the Yoruba hills, and at the intersection of roads from Akure, Kabba, Benin City and Siluko. The community has a population of 276593 according to national population in the year 2006 census (NPC, 2007).

Study participants

Eighty-two undergraduate students living in well laid out hostels environment with provisions of window net and inconsistent use of insecticide-treated bed net. All participants were apparently healthy, with no sign of malaria fever. Both males and females were included in this study and all subjects were adult between the age of 15 and 30 years. Students with sickle cell anaemia and others who harbor any chronic health challenges including immunosuppression were excluded from the study.

Sample processing

Five milliliters of venous blood were collected aseptically, via the median cubital vein into EDTA tube. Thick blood films were made from the samples and stained with 10% dilution of Giemsa stain stock at Ph of 7.0, for 30 minutes. Also, thin films were made at an angle of 45°C and stained with Leishman stain diluted with buffer distilled water (Ph=6.80) for 10 minutes, after fixing the films in undiluted stock of Leishman stain for 2 minutes. Procedures were adhered to, as earlier validated (Cheesbrough, 1998). The malaria parasite densities were determined by examining a thick blood film and thin films were used for speciation and confirmation only. Classification of parasitaemia was done in accordance with the method earlier validated (Cheesbrough, 1998).

Also, B lymphocytes were stained, sorted and quantitated by immunophenotyping using CD 20 monoclonal antibody (PE), red cells lysis solution and leucocytes fixing solution (Sysmex) for dilution, fixation and staining; and Cyflow Cube 6 Immunophenotyping Machine (Partec, Germany) sorting and counting (Sysmex-Partec, 2015).

Statistical Analysis

The results obtained in this study were analysed

statistically using SPSS version 20. The mean and standard deviation values were determined in each case and the Pearson's chi square tests were employed for comparison of the mean values. A p-value equal or less than 0.05 ($p \leq 0.05$) was considered statistically significant at 95% confidence level.

Results

Table 1 shows that majority of the participants were females (73.2%) with participants in the age bracket 18-20 who were 36 in number being the majority (43.9%) in the age group categorization. The mean age and standard deviation were 19.939 and 3.048 respectively. A great number of the participants (95.1%) were unmarried.

Table 2 depicts the prevalence and densities of malaria parasitaemia among young undergraduate students in Owo. Thirty-eight (46.3%) of the participants were free from malaria parasite as at the time of testing, while 40.2% and 13.4% were lightly (+) and moderately (++) infected respectively.

Table 3 reflects the absolute B lymphocyte count of the participants with 69 (84.1%) having counts of 100cells per cubic milliliter and below. The mean \pm standard deviation of the counts was 70.19 ± 3.706 , reflecting that dispersion of this parameter among the undergraduate youth was minimal.

Table 4 depicts the distribution of parasitaemia among age groups, while table 5 revealed no relationship between malaria parasitaemia and B lymphocyte counts, when the variables were subjected to Pearson's chi square test where all p values are higher than 0.05. The B lymphocyte count was also independent of the various socio-demographic variables ($p > 0.05$).

Table 1: Socio-demographic characteristics of participants

Parameter	Frequency	Percent
Age range (years) Mean age = 19.939 ± 3.048 years		
15-17	19	23.2
18-20	36	43.9
21-23	20	24.4
24-26	3	3.7
27-29	2	2.4
30-32	2	2.4
Total	82	100.0
Gender		
Female	60	73.2
Male	22	26.8
Total	82	100.0
Marital Status		
Married	4	4.9
Single	78	95.1
Total	82	100.0

Table 2: Malaria parasite densities of the participants

Parasite density	Frequency	Percent
Negative	38	46.3
Positive (+)	33	40.2
Moderate density (++)	11	13.4
Total	82	100.0

Table 3: Absolute B lymphocytes count among undergraduate youth in Owo

B Lymphocyte (cells/cmm)	Frequency	Percent
1-100	69	84.1
101-200	13	15.9
Total	82	100.0

Mean = 70.19 ± 3.706

Table 4: Malaria parasite densities among participants according to age group

Age Groups (Years)	Malaria Parasite			
	N	P	PP	Total
15-17	6	9	4	19
18-20	17	16	3	36
21-23	11	7	2	20
24-26	1	0	2	3
27-29	1	1	0	2
30-32	2	0	0	2
Total	38	33	11	82

Key:

N = Negative

P = Positive (+)

PP = Positive (++)

Table 5: Effect of some variables and prevalence of malaria parasitaemia on B lymphocyte count

Parameters	B lymphocyte count	
	p-value	Comment
Age	0.291	Not significant
Gender	0.465	Not significant
Marital status	0.386	Not significant
Malaria Parasitaemia	0.462	Not significant

Discussion

In this study, we observed a malaria prevalence of 53.7% among asymptomatic students in Owo, Southwest Nigeria. This finding further confirms that most asymptomatic individuals in Nigeria harbors malaria parasite. This is particularly challenging since blood donors are not routinely screened for malaria parasitaemia. This observation tallies with reports of other researchers on this subject matter (Owosu-Ofori *et al.*, 2010).

High sensitivity of microscopy is equally established here, as earlier observed by other researchers to be as high as 90% (Wilson, 2012). Although, these participants live in apartment with window nets, their inability to adhere consistently to use of insecticide treated net rendered them susceptible to mosquito bite; and such attitude is documented elsewhere to be counter-productive in roll back malaria programme (WHO, 2006).

Profiling of B lymphocytes was reported elsewhere to have important diagnostic and

prognostic implications in parasitic infection and bodily interaction with such infection (Virtaneva *et al.*, 2001) although, this has not been proven in our study. Our report shows that B lymphocyte counts in youth are independent of their malaria parasitaemia status. It is not impossible. However, symptomatic infections may reveal otherwise. Our findings here actually corroborate the documentation elsewhere on how lymphopoiesis in malaria infested children and young adults might not defer from uninfected age and gender matched groups (Weiss *et al.*, 2009).

Recent work by Portugal and others only demonstrated that *Plasmodium chabaudi* causes elevation of B lymphocyte in non-human primate (Portugal *et al.*, 2015). Incidentally, *Plasmodium chabaudi* does not parasitize human beings. Hence, the findings from this work remain justifiable, as it is comparable with reports on humans (Weiss *et al.*, 2009).

Conclusion

We observed that majority of participants have counts below 100cell/cmm; and no relationship between the B lymphocyte count and malaria parasitaemia. Malaria is largely asymptomatic in endemic zones like Nigeria and may not manifest any alteration in haemopoietic system of an asymptomatic patients, who having had repeated exposures to the parasite in the past, would have developed tolerance to low and moderate densities of malaria parasite.

Literature also revealed paucity of data on application of immunophenotyping in Nigeria. It is therefore recommended that this technology should be employed to advance the course of providing quality health care which becomes achievable with application of right diagnostic technology. Quests to know the trend in symptomatic, malaria patients should be explored by further research on the B cell counts among symptomatic youths and other age groups. Blood donors who are asymptomatic for fever should not be subjected to laboratory assessment for malaria parasite, or else, we lose the battle of striving for sufficiency and safety of blood in transfusion medicine.

Conflict of interest

None declared.

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