Journal of Advances in Medicine and Medical Research

32(23): 222-230, 2020; Article no.JAMMR.62705
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Malaria Status in Relation to Risk Factors among Attendees of Two Health Facilities in Port Harcourt, Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author GNW designed, wrote the protocol, supervised the project and wrote the manuscript. Author AME managed the analysis of the study and literature review while author VCW handled the statistical analysis and the final manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2020/v32i2330737
Editor(s):
(1) Dr. Sevgul Donmez, Mugla Sitki Kocman University, Turkey.
Reviewers:
(1) Tabe Franklin Nyenty, University of Yaounde 1, Cameroon.
(2) Hazem Mohammed Ebrahem Shaheen, Damanhour University, Egypt.
(3) Majid Mohammed Mahmood, Mustansiriyah University, Iraq.
Complete Peer review History: http://www.sdiarticle4.com/review-history/62705

Original Research Article

Received 15 September 2020
Accepted 19 November 2020
Published 26 December 2020

ABSTRACT

A cross sectional study was conducted among subjects from two health facilities in Port Harcourt to assess malaria status and its health determinants. Seven hundred subjects of different ages and both sexes were investigated after ethical approval was obtained from Rivers State Ministry of Health, Port Harcourt, Nigeria. Written consent of the subjects was obtained before questionnaire administration to obtain the demographic data. The uninfected subjects were used as control. Four (4) mls of blood was taken from each subject by vein-puncture into separate EDTA bottles for haematological profile tests and malaria parasite identification using standard haematological and parasitological techniques. The overall prevalence of malaria was 27%. The males had slightly higher prevalence (27.8%) than the females (26.5%), though the difference was not statistically significant (P> 0.05). The highest prevalence occurred among females (48.4%) of age group <1-10yrs and followed by males (40.7%) of same age group, and followed by males of 11-20 yrs with

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36.4%. From risk groups related prevalence the School children (45%) were the most infected, followed by Blood donors (35.7%) and pregnant women (26.5%). Only the Packed Cell Volume was significantly affected adversely by malaria (P<0.0001), of all the haematological Parameters tested.

Keywords: Plasmodiasis; prevalence; risk-factor; haematological-profile; Nigeria.

1. INTRODUCTION

Plasmodiasis also known as malaria, is a very common widespread parasitic protozoan disease transmitted by the bite of infested female *Anopheles gambiea gambiea* mosquitoes, which is predominant in the tropical and sub-tropical regions [1,2,3]. It could be said that malaria is as old as the world itself. The five species of medically importance are *Plasmodium falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi* [4,5]. The transmission of malaria parasite in Nigeria is almost constant because of the constant rainfall especially in the southern part of the country where the rain falls all through the year, [1,6,7]. The severe form of malaria in Nigeria is caused by *P. falciparum* [1] while *P. knowlesi* which is prevalent in Southern Asia, zoonotic to Macaques monkeys, has been reported as gaining prominence as medically important species in Asia [8,4].

All ages and both sexes of humans are affected by malaria [9,10,11]. Reports have shown that over 200 million people are exposed globally, with 5 million cases occurring each year and over 2 million deaths being recorded annually. Children below five years of age and the pregnant women are said to have the greater risk globally [1,12].

According to WHO [13], there were about 212 million cases of malaria and 429, 000 deaths in 2015 globally. It was also noted that about 6.8 million deaths have been prevented since the year 2001 resulting in decreased rates of incidence and mortality. WHO [13], also reported that malaria killed about 303, 000 under five globally and that a child dies every two minutes as a result of plasmodiasis.

Everyone in Africa experiences at least an episode of malaria in a year, [13]. Malaria is an economic burden both on the individuals and the world at large. Socio-economic activities are also affected by it. In 2015, about 65% of malaria deaths occurred among the under-five globally. Also there was a reduction in malaria prevalence among the under-five children form 42% in 2010 to 27% in 2015 [14].

It was reported that about 1 million children die in Africa every year as a result of malaria infection [10]. Nigeria which has an estimated population of 160 million has about 97% of the population at risk for malaria. There are about 51 million cases and above 2 million deaths annually [15]. According to studies, it was also estimated that about 60% out patients in Nigerian hospitals are infected by malaria, with almost 30% hospitalization, 11% maternal mortalities, 30% under-five mortalities and 25% infant mortality [15,14]. Nigeria is said to have more reported cases of malaria and associated deaths than other countries of the world [16]. This research is aimed at the assessment of malaria status and it's health determinants in Port Harcourt, Nigeria, using the attendees of the two biggest Government health facilities in the area.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out at Rivers State University Teaching Hospital (RSUTH) and Modern Primary Health Centre (MPHC), Eneka, all in Port Harcourt. RSUTH is a State Government owned Teaching Hospital. It is located in old Government Reserved Area (GRA), in Port Harcourt Local Government Area and is operated by Rivers State Hospital Management Board. MPHC, Eneka is also Rivers State Government owned and located in Obio-Akpor Local Government Area of Rivers State.

Rivers State has a tropical wet climate with a lengthy rainy season and a shorter dry season, which occurs within the months of December and January mostly. It has a relatively constant temperature range of 25°C to 28°C with an average rainfall of 367mm annually and an evergreen mangrove vegetation.

2.2 Study Population

This study was carried out among attendees of RSUTH in Port Harcourt and MPHC, Eneka. The

study population included; children, pregnant women, blood donors and health workers of both sexes and various age groups.

2.3 Determination of Sample Size

The minimum sample size required for this study was calculated using the formula by Goyal [17] at 95% confidence level and a reported prevalence of 69.4% [3].

Using the formula:

$$N = \frac{Z^2 P(1-P)}{d^2}$$

Where
N= sample size
P= Prevalence = 69.4% [3].
Z = Statistic is 1.96 at 95% confidence level.
d = Margin of sample error to be desired; = 5%, (d = 0.05).
q = 1-p.

$$N = \frac{1.96^2 \times 0.136 \times (1-0.694)}{0.05^2}$$
$$= \frac{3.8416 \times 0.136 \times (1-0.694)}{0.0025}$$
$$= \frac{0.5224576 \times 0.306}{0.0025}$$
$$= \frac{0.6374144256}{0.0025}$$
$$= 254.97$$
$= 255$ (Minimum sample size usable was two hundred and fifty five subjects).

2.4 Inclusion and Exclusion Criteria

All subjects of any age and gender willing were included. Patients on admission and those with the signs and symptoms suggestive of malaria were excluded.

2.5 Sample Collection

2.5.1 Blood sample collection

Four (4 mls) of blood was collected from each subject using vein puncture technique [16]. The puncture site was then cleansed with methylated spirit and vein puncture made with the aid of a seized needle attached to a 5 ml syringe. The tourniquet was released and the needle removed immediately after collecting sufficient blood; 2 mls of it was immediately transferred into EDTA bottle for film preparation and some haematological profile analysis.

2.5.2 Quality assurance

The blood films were examined by two different trained microscopists as quality control measure to ensure accuracy.

2.6 Sample Analysis

2.6.1 Blood films preparation

Thick and thin films were prepared from blood sample in the EDTA bottles.

2.6.2 Thin film

Two (2 µl) of blood was placed very close to one end of the slide. With spreader positioned at an angle of 45° looping backwards, the blood was touched and allowed to flow across the edge of the spreader. The spreader was pushed forward quickly but steadily without stopping. The films were air dried [18].

2.6.3 Thick film

Six (6 µl) of blood was placed at the center of the slide. A smear of about 2cm in diameter was made with the aid of a spreader. The film was air dried in a flat level position [18].

2.7 Staining of the Blood Films

The blood films were stained with Giemsa staining slow (3%) method as described by WHO [18]. The slides were placed horizontally on a staining rack. A small drop of absolute methanol was applied on the blood film and precaution was taken to ensure that the methanol applied did not spread to the thick blood film as this will fix the red cells and prevent lysis of the red blood cells thereby making thick film unreadable. Thin film was allowed to fix for 2 minutes. With the aid of a pipette, the prepared 3% buffered (pH 7. 2) solution of Giemsa stain was flooded on the films and was allowed to stain for 45 minutes, and stain was gently washed off with clean water and then placed in a draining rack to dry.

2.8 Microscopy

The blood films were examined, *Plasmodium falciparum* stages in Giemsa-stained thin and thick blood films were identified as described by WHO [18]. A definitive diagnosis of the presence of *P. falciparum* parasite in peripheral blood was made when a reddish chromatin dot with a purple or blue cytoplasm of the parasites were seen

together. A slide was regarded negative when 100 high power fields have been examined microscopically, using x100 oil immersion objective without the presence of parasite.

2.9 Haematological Parameters

Analysis of haematological parameters like packed cell volume (PCV), total white blood count (tWBC), neutrophils, lymphocytes and mixed cells consisting of monocytes, basophils and eosinophils were done using Automated Full Blood Count Machine (Sysmex XP-300) with serial number A2675, manufactured in Japan, used according to the manufacturer's prescription.

3. METHODOLOGY

The blood sample was allowed to mix for at least 10 minutes on the blood mixer. The power switch was turned on, self check was done, auto rinse and background check was automatically performed and "Ready" (ready for analysis) appeared. The samples and control samples were introduced into the instrument using a probe. A quality control check was done on the samples with the provided control blood (Eight check- 3WP), X control or L-J control program was used. Whole blood mode was selected and sample numbers were imputed. The plug was removed from the vacutainer tube; care was taken to avoid scattering blood. Sample or control was introduced through the sample probe. The start button was pressed for the sample or control as aspired. The sample tube was removed when the buzzer made a "beep beep" sound and the LCD screen displayed "Analyzing". The instrument automatically executed the analysis and the result was displayed on the LCD screen. The unit turned to "Ready", this indicated the device was ready to analyze the next sample.

The shut down key was press on the ready status at the end of the day. Shut down screen appeared. The machine prompted for the rinse (cell clean) solution. The cell clean solution was set to the sample probe and start button was pressed while the cell clean was held in the same state. The buzzer made a "beep beep" sound, this indicated the completion of the aspiration.

The cell clean solution was removed from the sample probe. After this the shutdown was executed automatically.

3.1 Precaution

The instrument was inspected by checking the connection of tubing and cords and it was ensured that there was no broken tubes and the power cord properly plugged into the outlet.

Reagents needed for the number of samples to be processed for a day were ensured to be available. A background count was made for the reagents before sample analyses to ensure the availability of enough reagents for the analyses. Reagent replenishment was done before the day's work to prevent the instrument from stopping during analyses. After replenishing a reagent, background count was ensured to be low before starting sample analysis. The reagent was brought to room temperature for more than 24hrs before use. Care was taken during replacement of reagent bottle, to prevent dust from adhering to the container's spout kit.

Error message was attended to, to increased the life span of the machine. Bending the sample probe was avoided. The sample probe was held when the LCD as A displaying "Aspirating", to avoid wrong analysis of result.

4. RESULTS

4.1 Malaria Prevalence by Location

A total of seven hundred (700) subjects were examined for malaria parasites. Four hundred (400) subjects were from RSUTH, Port Harcourt, while three hundred (300) were from MPHC, Eneka. Out of the 400 subjects from RSUTH, 115 (28.8%) were positive for malaria parasite, while out of the 300 subjects of Eneka 74 (24.7%) were positive for malaria. A total of 189 (27.0%) subjects were positive for malaria, with p-value ≥ 0.05 using one-way ANOVA.

Table 1. Location related prevalence of malaria in the study population

Location	No. Examined	No. Infected n (%)
RSUTH	400	115(28.8)
MPHC ENEKA	300	74(24.7)
Total	700	189(27.0)
P-value		0.23

Legend: RSUTH- Rivers State University Teaching Hospital, and MPHC- Modern Primary Health Centre

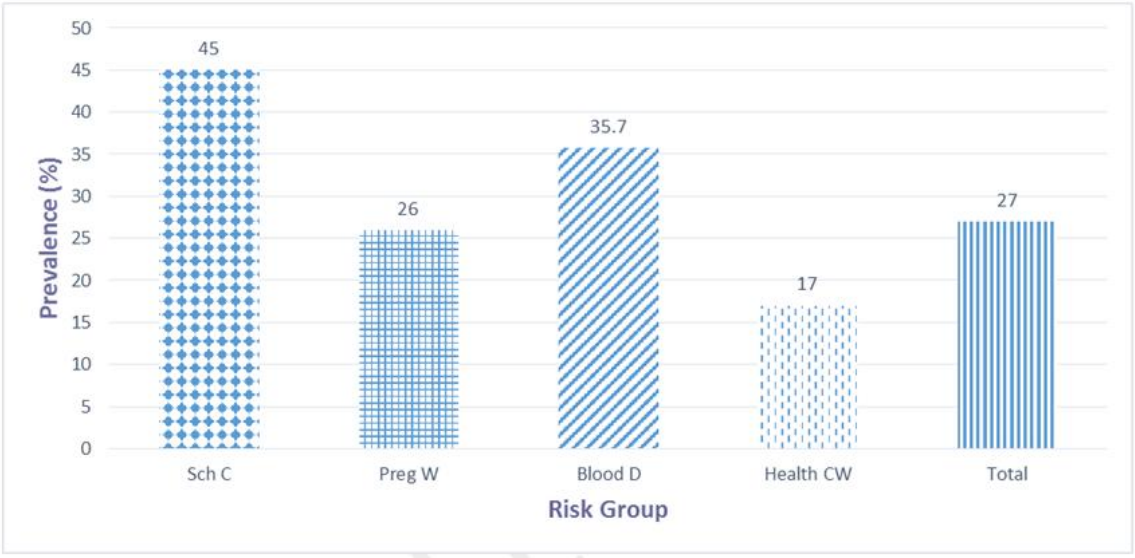

Fig. 1. Risks groups related prevalence of malaria in the study population

Legends: Sch C stands for school children, Preg W means pregnant women, Blood D is blood donors while Health CW stands for healthcare workers

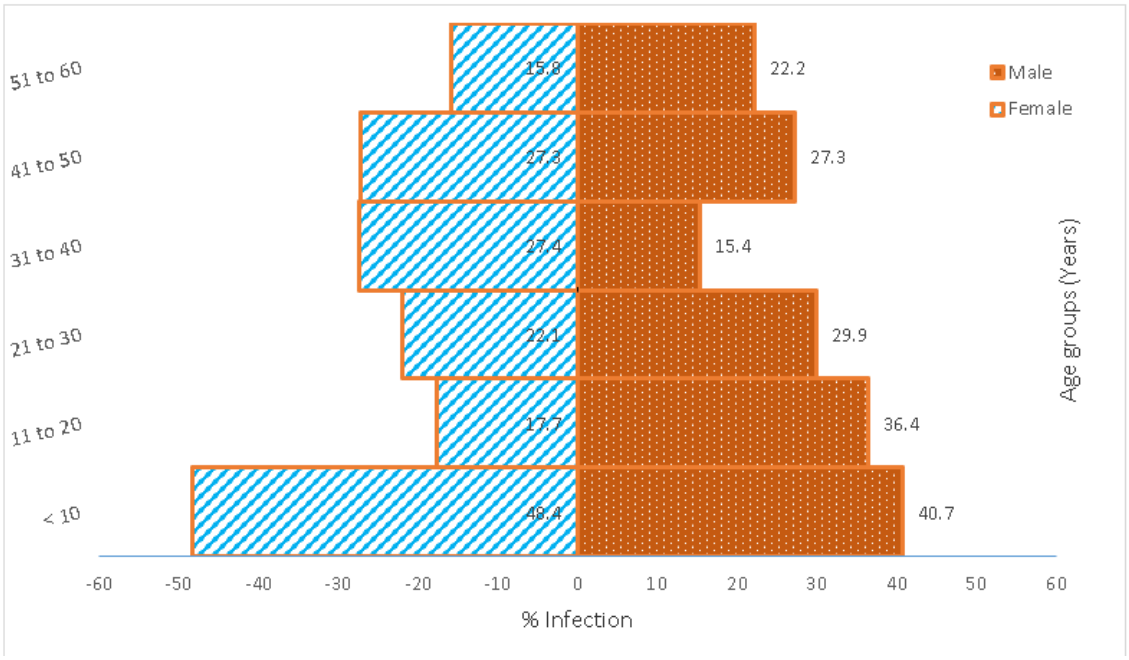

Fig. 2. Age-sex prevalence of malaria in the study population

4.2 The Risk Groups Related Prevalence of Malaria

Out of the 100 school children that were screened, 45(45.0%) were positive for malaria. A total of 200 pregnant women were also tested, 52(26.5%) were positive. Seventy blood donors were also tested, out of which 25(35.7%) were positive for malaria. Among the 100 healthcare workers screened, 17(17%) had malaria. The other 231 subjects whose complete details were not taken, had 50(21.7%) positive for malaria.

4.3 The Age-sex Related Prevalence of Malaria

The results showed that children of age brackets <1-10 years, females had 48.4% while the males had 40.7%, the difference was not statistically significant (P>0.05); whereas among age groups 11-20 years and 21-30 years malaria prevalence rates of 36.4% and 29.9% were for the males with females having lower prevalence rates of 17.7% and 22.1% correspondingly. Age group 41-50 years had 27.3% for both males and females. The least prevalence (15.4%) observed was among males of age group 31-40years while females of same age group had 27.4%. While sex showed no statistical significant difference, age did show statistical significant difference at P = 0.05.

Out of the 277 males that were screened in the study, 77(27.8%) were positive for malaria whereas for the females, out of the 423 subjects screened, 112(26.5%) were positive for malaria. There was no statistical significance in their prevalence with P-value ≥ 0.05(0.11) using one way ANOVA.

The means of haematological parameters of 110 controls that were compared with that of 189 malaria positive subjects, showed that only PCV had statistically lower difference by malaria infected subjects (P < 0.05) other parameters though lower in diseased subjects but were not statistically different.

5. DISCUSSION

The overall prevalence of malaria in this study was 27% which contrasts the earlier studies with higher prevalence rates of 73.1% by [19] in Anambra State, 68% in Calabar [20] and 69.4% in Port Harcourt, Rivers State by [3] using a population of 1,000 School children, all from Southern Nigeria. The differences in their population sizes, the study techniques and improved health awareness campaign may have contributed to the differences observed. Although, the result agrees with those of earlier researchers [21,7], who reported prevalence rates of 26% and 31.3% respectively in Port Harcourt, Rivers State, Nigeria.

The lower malaria prevalence observed in this study indicates appreciable decline in malaria transmission which could be attributed to the enlightenment campaign and the regular distribution of free insecticides-treated bed nets which are found in almost every household in Port Harcourt, Nigeria. However, the city is still having the problem of improper waste disposal management, un-kept drainage system, overpopulation which enhance malaria transmission dynamics, even though, the least family is cautious about mode of malaria transmission.

The malaria prevalence in males and females were 28.7% and 26% respectively with no statistically significant difference (P ≥ 0.05) observed. This confirms that malaria is not sex dependent; however, occupation, housing patterns and clothing habits could influence the exposure rates of healthy subjects to infested female *Anopheles gambiae* mosquitoes bites. Children of age ≤10 years of both sexes were the most infected as shown in the results. Their immature immune system and the habit of playing outdoors predisposed them more readily than others.

Table 2. Comparison of means of haematological parameters among study population

No. of Subject studied (n)	Haematological Parameters				
	PCV (%)	Lymph (%)	Neut. (%)	Mixed (%)	WBC ($X10^9$/L)
Control(C), n=110	38.27±7.69	35.68±16.09	55.35±2.94	9.33±2.94	7.92±2.89
P. f., n=189	32.23±6.24	35.50±12.35	56.00±12.66	8.30±3.33	7.38±3.39
P-value	<0.0001	0.987	0.866	0.133	0.435
C Vs P. f.	S	NS	NS	NS	NS

Legends: Vs =Versus, P. f.= Plasmodium falciparum, NS = No Significance, PCV = Packed Cell Volume, Lymph = Lymphocyte, Neut. = Neutrophil, WBC = White Blood Cell, C = Control, Mixed = Monocyte, Basophil and Eosinophil, C Vs f = Control versus Plasmodium falciparum

In the risk groups related prevalence of the studied, School children were the highest risk bearers of malaria which agrees with results of most work done elsewhere in the globe like Abah & Temple [2] with high prevalence of 64%, 52% in Abuja [10] and 65.3% in Kano [14] these confirmed the age group result outcome observed. The reasons for the higher prevalence of malaria among the children may include; tropical high temperature of the environment which they respond to by going bare body most playing periods, their playful habits outdoor which exposes them to the bites of infected female Anopheles mosquitoes, their low immunity due to their ages and lack of care on the parts of parents about clothing types. Although, there were other studies in Abia, Imo and Ogun States where the prevalence rates were as high as 80.4%, 85.5% and 80.5% respectively [22], however, Port Harcourt had a lower average prevalence of 45% malaria among children in this study. Blood donors had higher prevalence than pregnant women contrary to report of Barituuka et al. [23] in Port Harcourt where pregnant women recorded as high as 63% of 162 pregnant subjects. Good antenatal care, use of insecticides impregnated bed nets, bigger sample size, proper prophylactic use of anti malaria and quality health campaign in promotion to malaria control would have contributed to declined infection observed in the present work.

The study also showed that in age-sex related prevalence of malaria, ages <-10 years had the highest prevalence rates of 48.4% and 40.7% for females and males respectively. Ages above 18 years had lower prevalence rates comparatively. This agrees with most researchers reports, showing that premunition immunity in malaria increases with age for people living in endemic areas like Rivers State, Nigeria. Antibody levels have been noted to increase with age and exposure to malaria parasite in hyper endemic areas. It was also noted that most cases of severe clinical malaria occurred among children [1,3] while constant exposure confers some levels of partial immunity in terms of clinical symptoms and parasitaemia burden [3]. Though malaria is found in all the age groups, most adults showed only mild clinical symptoms as a result of the acquired immunity especially among older adults.

The comparison of means of the various haematological parameters among test subjects and control, showed that the only significant result in the control versus test was packed cell volume (PCV) with a P- value ($P< 0.0001$) meaning that PCV was negatively affected by malaria. Although other parameters like lymphocytes and WBC were lower while neutrophils were slightly higher when compared to control but they were not statistically significant ($P>0.05$). These results are in agreement with the findings of Wokem et al. [24, 3] who made similar observations.

6. CONCLUSION

Malaria parasite transmission has reduced significantly in Port Harcourt which reflected on the declined prevalence rates comparatively. More awareness campaign and health promotion policies like free distribution of insecticides impregnated bed nets should be sustained while their uses could be outlawed in schools and our health facilities. Again the Government Agencies and health facilities should implement free medical treatment for under five children. Blood donors must be disqualified if found infected with malaria parasiteamia after screening.

CONSENT AND ETHICAL APPROVAL

Ethical approval was gotten from the Rivers State Government Hospital Management Board, Port Harcourt. The informed consent of the subjects was obtained from each participant using consent forms and questionnaires.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/62705