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Comparison and Evaluation of Asymptomatic Malaria Parasitaemia among Pregnant Women Attending Specialists Hospital, Sokoto, North-Western, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KM and UA designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors THIS, KKI and EII managed the analyses of the study. Authors SUN and MKG managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to compare and evaluate asymptomatic malaria parasitaemia among pregnant women attending Specialists Hospital, Sokoto, North-western, Nigeria.
Study Design: This was a cross-sectional, descriptive study designed to compare and evaluate asymptomatic malaria parasitaemia among pregnant women that were recruited during their ante-natal clinic visit in specialist hospital Sokoto.
Place and Duration of Study: This study was conducted in Specialists Hospital sokoto between March to June, 2016.
Methodology: A total of 205 apparently healthy, confirmed pregnant women within the age of 15 –

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45 years attending Specialists Hospital Sokoto, and had not been on any sort of malaria treatment, history of malaria or fever within the last 4 weeks were recruited for this study. Thick and thin blood films were performed for all the women. PCV estimation was also done using microhaematocrit centrifuge method and comparison was made for women with parasitaemia and those without parasitaemia.

Results: Of the two hundred and five pregnant women recruited for this study, 48 (23.4%) had malaria parasitaemia while 157 (76.6%) had no infection. The mean PCV of the women positive to malaria parasitaemia was 30.52 ± 3.71 against 34.30 ± 3.76 for those without parasitaemia ($P < 0.05$).

Conclusion: The prevalence of asymptomatic malaria parasitaemia in the study group was high compare to those without infections and there was associated anaemia in those with parasitaemia. The use of intermittent preventive treatment is recommended for all pregnant women including those who are asymptomatic to forestall complications like maternal anaemia.

Keywords: Comparison; malaria; asymptomatic; pregnant women; Sokoto.

1. INTRODUCTION

Malaria is caused by protozoan parasites of the genus plasmodium and is the most deadly tropical infectious disease affecting mostly poor children under the age of 5 years and pregnant women. The main forms of malaria parasitaemia are caused by five species important to humans and these include; *Plasmodium falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi* [1].

About 148-304 million people worldwide are infected with malaria each year, and more than 429,000, mainly children, die from the disease. Most cases occur in developing countries, particularly in Africa (25). There were an estimated 216 million episode of malaria worldwide in 2010 of which approximately 81% or 174 million cases were in the African region [1]. Globally, the annual mortality due to malaria is estimated to be between 0.5-2.5 million people. The transmission rate and degree of severity are worse in *P. falciparum* malaria [2].

Public health burden of malaria is due to a combination of factors, which include increasing resistance of malaria parasite to chemotherapy, increasing resistance of Anopheles mosquito to insecticide, ecologic and climate changes and increasing travel to malaria endemic areas by non-immune travellers [2].

Malaria infection during pregnancy is a major public concern with significant risk for the pregnant woman and her foetus [3]. Annually, about 125 million women (25 million pregnant women) are at risk of malaria around the world. Over 90% of the world's malaria related deaths in the world occur in the sub-saharan Africa. It has been reported that in this region malaria can cause as many as 10,000 cases of malaria-related deaths in pregnancy per year, usually due to severe maternal anaemia [3]. Pregnant women are three times more likely to develop severe malaria than non-pregnant women from the same area. Pregnant women have reduced immune response to malaria infection. Therefore, the infection can be in its severe form with more complications in the mother and her fetus. In addition, malaria parasite distain and replicate in the placenta. This parasite replication presumably reduces nutrient transport across the placenta and allows for passage of parasitized red blood cells to the fetus that may compromise fetal growth and infant survival. [4].

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Specialists Hospital Sokoto and and samples were analyzed in Faculty of Medical Laboratory Sciences (FMLS), Usmanu Danfodiyo University Sokoto (UDUS). Sokoto is located in the extreme North Western Nigeria. It has a population of approximately 4 million [5]. It has latitude of 13º4´0´´ N and a longitude of 5º13´60´E. Sokoto State occupies 25,973 square kilometers, shares borders with Niger Republic to the North, Zamfara State to the East, Kebbi State to the South-East and Benin Republic to the West. The major ethnic groups in the state are the Hausa, Fulani and Dakarkari, and other minority groups such as Zabarmawa and Tuareq of which also speak Hausa as a common language. Most Sokoto State residents are Sunni Muslims, with Shia minority. Sokoto State is in the dry Sahel, surrounded by sandy Savannah and isolated hills. With an annual average temperature of 28.3ºC (82.9℉), Sokoto

is, on the whole, a very hot area. However, maximum daytime temperatures are for most of the year generally under 40℃ (104.0℉) and the dryness makes the heat bearable. The warmest months are February to April when daytime temperatures can exceed 45℃ (113.0℉). The rainy season is from June to October during which showers are a daily occurrence. The showers rarely last long and are far from the regular torrential rain known in wet tropical regions. From late October to February, during the cold season, the climate is dominated by the Harmattan wind blowing Sahara dust over the land. The dust dims the sunlight thereby lowering temperatures significantly and also leading to the inconvenience of dust everywhere in houses

2.2 Study Population

The study population consists of 205 pregnant women attending Specialists Hospital, Sokoto, North-Western, Nigeria.

2.3 Target Population

The target population consists of all pregnant women attending Specialists Hospital, Sokoto.

2.4 Study Design

This was a cross-sectional study designed to compare and evaluate asymptomatic women with malaria infestation during pregnancy between March to June, 2016 at Specialists Hospital, Sokoto.

2.5 Selection of Study Area

The area was chosen based on the fact that:

a) The area was not on regular or current malaria treatment.
b) The area has stable resident population with median duration longer than 12 months.
c) The residents are mostly of low socio-economic class.

2.6 Sampling Method

Simple random sampling technique was used to recruit 205 pregnant women into the study. The number of women that attend ANC every week was approximately 152 as obtained from the register. Therefore 152 pieces of paper were numbered and folded. They were then put into a bowl and were mixed. 100 pieces were randomly chosen. The chosen pieces of paper were unfolded and the numbers written were recorded. So the patients that bear the numbers on the piece of the papers were recruited into the study. Same procedure was repeated on the second week where the remaining 105 patients were recruited.

2.7 Inclusion Criteria

The study included all consented, apparently healthy, confirmed pregnant women within the age range of 15 – 45 years that were attending Specialists Hospital, Sokoto, those that have not been on any sort of malaria treatment and have no history of malaria or fever within the last 4 weeks.

2.8 Exclusion Criteria

All women that did not meet the inclusion criteria were excluded from the study; women on anti-malarial therapy, those that had malaria or fever within the last 4 weeks, those that did not consented, those that were less than 15 years or greater than 45 years of age.

2.9 Sample Size Determination

Sample size determination for this research was based on the findings of 12.5% obtained from the previous study of 200 asymptomatic patients that were screened for malaria [6]. Number of sample size was determined using the formula;

$$n = Z^2 P Q/d^2$$

Where:

n = Minimum sample size
Z (standard deviation of normal) = 1.96
P (prevalence rate) = 12.5% (0.125) [6].
Q (1-P) = (1 - 0.125) = 0.875
d = confidence interval = 5% (0.05)
$n = (1.96) \times 0.125 \times 0.875/ (0.05)^2$
n = 168

Due to attrition, 20% of 168 were added to the sample size

168 + 34 = 202

Therefore the minimum sample required was 202

2.10 Ethical Consideration

Ethical clearance was obtained from the Ethical committee of Specialists Hospital, Sokoto in accordance with the code of Ethics for Biomedical Research involving Human subjects.

The relevance and benefit of the study was explained to all of the subjects to ensure their voluntary participation and a written informed consent was taken from each subject.

2.11 Sample Collection

Three millilitres (ml) of venous blood was collected aseptically from the cubital fossa using a sterile needle and syringe after cleaning the site of venipuncture with methylated spirit (70%) and allowed to dry. The blood sample was dispensed into EDTA specimen bottle, then mixed gently and kept at room temperature (25-30ºC).

2.12 Laboratory Test

Two glass slides were labelled for each participant. A drop of blood was then placed on the clean, grease free glass slide and allowed to dry. Precaution was taken to maintain a constant volume as much as possible. The thin smear was made to spread on the glass slide so that newsprint could be read through it. This was immediately fixed in absolute methanol for 5 seconds and allowed to air dry completely before staining. The dried slides were then placed on a rack in preparation for staining. Two capillary tubes were filled with the blood and one end sealed with plasticin gum for each patient for determination of packed cell volume at booking.

2.13 Packed Cell Volume Estimation

Using two heparinized capillary tube, 4-5cm column of blood was obtained from blood already collected. This was to ensure that the average of the two values obtained is used for calculation. One end of the capillary tube was sealed with plasticin, several samples were assembled in the Centrifuge (haematocrit machine) and spinned at 12,000 revolution per minute for 5 minutes. PCV was read using Hawksleys micro haemotocrit reader. Anaemia was diagnosed when packed cell volume was below 33%, according to World health Organization recommendation.

2.14 Staining Technique for Thick Blood Film

The thick blood smear was allowed to dry completely under a drier before staining. Giemsa staining technique was used for staining the slides. A staining time of 45 minutes in a 3% volume/volume dilution was used. The air –dried thick blood film was stained in a trough containing the 3% Giemsa stain for 45 minutes. The slides were then removed with the aid of a forceps, rinsed in buffer water and the back wiped clean with dry wool. The slides were then placed vertically on the staining rack to air–dry before examination.

2.15 Staining Technique for Thin Film

The Giemsa staining technique was also used. The thin film already fixed in absolute methanol for 5 seconds was allowed to air dry completely on the staining rack. The slide was then immersed in a trough containing 3% Giemsa for 30 minutes. The stained slides were removed and rinsed in buffer water (PH 7.2). The back side of the slide was wiped with dry cotton wool, kept vertically on the rack to air- dry before examination [7].

2.16 Reading of Slides

When the slides were completely dried, a drop of oil immersion was placed on each slide and examined using a compound microscope with x100 objective magnification. Properly stained areas were selected and observed for malaria parasites.

2.17 Research Tools

2.17.1 Questionnaire

Data collection was carried out using questionnaire in order to obtain socio-demographic in of the respondent. During data collection, research investigator ensures that the data were collected accurately and correctly.

2.17.2 Validation of questionnaire

After the questionnaire was designed, it was sent to 3 experts in order to seek for their opinion as part of expert review panel to evaluate questionnaire content validity.

2.17.3 Domain of the questionnaire

The questionnaire survey consists of items socio-demographic characteristics of the participants as well as the risk factors associated with malaria infection in pregnancy. The questionnaire has 3 domain which includes socio-demographic domains in section A consisting of age, gender, ethnicity and religion. Section B socio- economic data consisting of occupation, type of family, gravidity, parity, trimester etc. section C

Laboratory investigation results consisting of PCV and result of blood film.

2.18 Statistical Analysis

Chi-square test was used to determine the prevalence of malaria among the study group.. Student t test was used to compare the mean PCV of malaria infected and non infected women.

3. RESULTS

The study involved two hundred and five (205) pregnant women attending Specialists Hospital, Sokoto State. Forty-eight (23.4%) of the pregnant women studied were positive for malaria parasite against one hundred and fifty seven (76.6%) that were negative for malaria parasite.

Table 1 also shows the prevalence of malaria parasitaemia based on presence of stagnant water, socio-economic status and gestational age. There was a higher prevalence of the infection (44.4%) in those who live where there is presence of stagnant water compared to the other group (22.4%) there was no statistically significant difference (p- value = 0.128, this might be due to sample size being so small). With regards to socio-economic class, a higher prevalence of infection was recorded (25.0%) among those in the higher class compared to (22.0%) in the lower socio-economic class (p-value =0.897). Table 2 shows malaria parasitaemia prevalence with respect to age group. The result of our study shows that prevalence of infection was higher among those in the age group 21-25 years (33.3%) compared to age group 41-45 years (2.1%) p-value= 0.779. Table 3 shows the prevalence of malaria parasitaemia with respect to packed cell volume (PCV). There was higher prevalence of the infection in those with PCV within the range of 31-35 (47.9) compared to other PCV with the lowest prevalence in those within the range of 36-50 and 15-20 (2.1%) (P< 0.05).

Table 4 shows the comparison between mean PCV of malaria positive (30.52 ± 3.71) and malaria negative women (34.20 ± 3.76) (P < 0.05).

Table 1. Prevalence of malaria parasitaemia with respect of presence of stagnant water, socio-economic classes and gestational age

Variables	Malaria Parasitaemia		Total	*p*-value
	Negative	Positive		
Stagnant water	**n (%)**	**n (%)**	**n (%)**	
No	152 (77.6)	44 (22.4)	196 (100.0)	0.128
Yes	5 (55.6)	4 (44.4)	9 (100.0)	
Socio-economic class				
Lower class	78 (78.0)	22 (22.0)	100 (100.0)	0.897
Medium class	76 (75.2)	25 (24.8)	101 (100.0)	
Higher class	3 (75.0)	1 (25.0)	4 (100.0)	
Gestational age				
First trimester	7 (87.5)	1(12.5)	8 (100.0)	0.745
Second trimester	63 (76.9)	19 (23.1)	82 (100.0)	
Third trimester	87 (75.7)	28 (24.3)	115 (100.0)	

Table 2. Prevalence of malaria parasitaemia with respect to age group

Age group	Malaria Parasitaemia		Total	*p*-value
	Negative	Positive		
	n (%)	n (%)	n (%)	
15-20	28 (17.8)	7 (14.6)	35 (17.1)	0.779
21-25	43 (27.4)	16(33.3)	59 (28.8)	
26-30	46 (29.3)	13 (27.1)	59 (28.8)	
31-35	26 (16.6)	9 (18.8)	35 (17.1)	
36-40	13 (8.3)	2 (4.2)	15 (7.3)	
41-45	1 (0.6)	1 (2.1)	2 (2.0)	
Total	157 (76.6)	48 (23.4)	205 (100.0)	

Table 3. Prevalence of malaria parasitaemia with respect to PCV

PCV range	Malaria	Parasitaemia	Total	*p* –value
	Negative	Positive		
	n (%)	n (%)	n (%)	
15-20	0 (0.0)	1 (2.1)	1 (0.5)	0.001
21-25	1 (0.6)	3 (6.2)	4 (2.0)	
26-30	22 (14.0)	20 (41.7)	42 (20.5)	
31-35	86 (54.8)	23 (47.9)	109 (53.2)	
36-40	40 (25.5)	1 (2.1)	41 (20.0)	
41-45	5 (3.8)	0 (0.0)	5 (2.4)	
Total	157 (76.6)	48 (23.4)	205 (100.0)	

Table 4. Comparisons between mean PCV of malaria positive and malaria negative women

Malaria	n	Mean PCV	std	T	df	*p*-value
Positive	48	30.5208	3.713	56.949	47	0.001
Negative	157	34.2994	3.759	114.314	156	
Total	205					

4. DISCUSSION

In this study, two hundred and five (205) pregnant women were examined for asymptomatic malaria parasitaemia, out of which forty eight (48) were positive giving an overall prevalence of 23.4%. The high prevalence of asymptomatic malaria in this study is similar to the findings of Nwaneri [8], who reported a prevalence of 25.9% in pregnant women in rural of Ondo- south district. The findings of this study were also similar to that of Iriemenam [9], who reported a prevalence of 27.4% among pregnant women attending antenatal care clinic in metropolitan Lagos, Nigeria. Interestingly, another study conducted in south-eastern Nigeria by Nwonwu and his colleagues [10] reported a prevalence of 29% among group of urban asymptomatic pregnant women which is also similar to the findings in this study.

The findings in this study were however in contrast to the reports obtained elsewhere. It showed a higher prevalence when compared to the findings of Wasif [11] who reported a prevalence of 2.3% among asymptomatic pregnant women in Chittagong hill district of Bangladesh. It also showed a higher prevalence when compared with 13% reported from Abuja in north central Nigeria [12].

Lamikanra [13] reported a prevalence of 2.3% parasitaemia in a group of pregnant women in Lagos while a study in Sokoto, north western Nigeria reported a prevalence of 11% by Aliyu and co-workers, 2011, which is similar to another findings in the same state by Abubakar [6] all of which shows a lower prevalence when compared to the findings in this study.

The findings from this study was however lower than the 58% reported in urban pregnant women as reported by Nwagha and co-worker [14] and 59.9% reported by Ogbodo [15] for some rural women in south-eastern Nigeria. It was reported that a prevalence of 39% was obtained among pregnant attending primary health care centre in Kano, north-western Nigeria [16] and a more recent report from Keffi, in Abuja, north central Nigeria reported a prevalence of 38.8% [17] both of which are higher than the prevalence obtained from this study.

There is therefore a wide range of variation in reported prevalence even within the same region. Reasons for these wide variations could be attributed to differences in the group of pregnant women that were studied (all pregnant women or attending a clinic or only healthy pregnant women who had no complaints). In this study, very strict inclusion criteria were used recruit women who were asymptomatic. Also in this study, simple random sampling was employed in the recruitment process, while other studies such as that of Aliyu [14], recruited pregnant women consecutively. It is therefore possible that sampling method of recruitment could also affect the results. Another reason for these variations may be attributed to sample size. This study had a smaller sample size when compared to all other previous studies.

Finally, I postulate that pregnant women in the southeast region of the country are perhaps better prepared to "accommodate" malaria parasite without manifestation of symptoms than their counterpart in the northwest. This same hypothesis may also apply to pregnant women in the southwest of Nigeria where 89% malaria prevalence in asymptomatic women was reported [18]. The probable cause of this "relative intolerance" by our women to the parasite can only be speculative and might be a subject for another research.

Primigravidae and multigravidae constituted 27.8% and 72.2% of the women studied respectively where 28.8% of the primigravidae were positive whereas 23.8% of the multiparae were positive. This shows that primigravidae were more at risk of infection than to multiparae. This is similar to the report of Anorlu [19] who reported a prevalence of 44% among the primigravidae compared to 33% for multiparae. Opare Addo [20] have observed that primigravidae alongside those women in their second pregnancy were more vulnerable to malaria parasitaemia. Ogbodo and co-workers also found an inverse relationship between parity and parasitaemia [16]. This might probably be explained that the protective immunity of the African women to malaria parasitaemia might not be as a result of parity.

The age range of the subjects in this study was between 16 – 45 years which was similar to earlier findings in Kano [17] and Abuja [18]

In this study, there was no statistically significant association between malaria parasitaemia and the risk factors (age, parity and use of LLIN). This is also similar to findings of Marielle et al. 2003 and Desalange [21]. This however is contrast to the findings of Bouyou et al. 2003, which shows significant association between malaria with age, gravidity, parity and use of LLIN. This contrast might be due to differences in the sample size, sampling technique, physiologic and biochemical factors of pregnant women and the study setting such as geographical differences, altitude, temperature, and age categorization scheme.

The mean PCV of the study group was 32.41 ± 3.74. Malaria parasitaemia often causes anaemia, increased uterine activity and low birth weight, among others [22]. The presence of anaemia in those who were MP positive in this study was demonstrated by the significant difference between the mean PCV of women with malaria parasitaemia (30.52 ± 3.71) and those without parasitaemia (34.20 ± 3.76) ($P<0.05$). This similar to other studies conducted by Isah [23] and Gadija [17]. Despite the presence of anaemia in this group, they were asymptomatic for malaria. Chemoprophylaxis during pregnancy has been shown to significantly reduce the risk of malaria infection in pregnant women [24].

5. CONCLUSION

Malaria in pregnancy is a common and serious public health problem in our environment as large proportion of the asymptomatic pregnant women had malaria parasitaemia. Malaria infestation during pregnancy affects more primigravidae and teenage mothers than those of higher gravidity and older age group. Anaemia is also a serious problem especially among pregnant women with asymptomatic parasitaemia. Therefore this study has indicated that malaria is a contributor to anaemia among these pregnant women as the mean PCV among the MP positive women was lower than those without the parasite.

6. RECOMMENDATION

Public enlightenment on malaria among women and girl child education are recommended so as to reduce the proportion of women who present for antenatal booking with parasitaemia and anaemia. There should also be a Federal Government policy to aimed at screening all pregnant women for malaria parasitaemia and anaemia especially primigravidae at booking so that appropriate antimalarial therapy is instituted to clear the parasitaemia and this will lead to reduction in anaemia. There is a need for a comprehensive strategy including intermittent preventive treatment of malaria in pregnancy, good nutrition and effective use of insecticide treated bed nets among pregnant women in this endemic region.

7. LIMITATIONS

Inability to record the parasite density is a major drawback to this research. Future studies integrating the parasite density are advocated. Only microscopies of thick and thin blood films were carried out. While this is definitive for diagnosis, it is suggested that other method of diagnosis such as immunodiagnosis using rapid

malaria strip test and concentrating parasites in venous blood by centrifugation should be employed in order to make comparison in level of malaria parasitaemia.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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