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Prevalence of Falciparum Malaria in Conjunction with Age, Gravidity, Abo Blood Group/Rhesus Factor, and Genotype Among Gravid Women in South-eastern Nigeria

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ABSTRACT

Background and aim: The present research was done to determine the prevalence of falciparum malaria in relation to age, gravidity, blood group/rhesus factor, and genotype among gravid women attending Antenatal Clinic in 2 Primary Health Centres in Atani, Nigeria.

Materials and methods: This study was carried out from December 2020 to January 2021. A venous blood sample was collected from 150 gravid women selected by random sampling. Then, we prepared the thin and thick film, used Giemsa stain to stain it, and viewed it under the light microscope. ABO blood group and Hemoglobin genotype were obtained using standard methods. Moreover, statistical analyses were done by SPSS 23.

Results: The overall prevalence of malaria in our research samples equaled 59.4%, and the age group between 28-31 years exhibited the maximum prevalence of 33.0%, whereas the age group 16-19 years recorded the least prevalence with 2.3%. The prevalence of malaria in relation to gravidity showed that primigravida has the highest prevalence of 61.4%, while multigravida has the least prevalence of 38.6%. The blood group/rhesus factor demonstrated the greatest pervasiveness of the blood group O+ of 54.7%, while B- has the least prevalence of 0.0%. The prevalence of malaria in relation to genotype showed that HbAA has the highest prevalence of 62.6%, while the least prevalence was HbSSwith 4.5%. Prevalence values were statistically insignificant ($p>0.05$).

Conclusion: This study showed that malaria in pregnancy is endemic in Atani, Nigeria. The observed increased prevalence among pregnant women in this study could probably be due to the location of the study area in the riverine, favoring the breeding of Anopheles mosquito, a vector for malaria parasites. There is a need for serious advocacy and enlightenment of the populace on the prevalence and control measures of malaria transmission to curb the increased prevalence of malaria among gravid women.

1. Introduction

According to the studies, malaria has been introduced as one of the mosquito-borne infectious diseases due to the genus Plasmodium parasitic protozoa, and include the following species, vivax, ovale, malaria, and falciparum, and affects both humans and other organisms.[1] Of the four malaria species infecting humans, it has been noted that Plasmodium falciparum caused the bulk of serious diseases and consequences, due partly to its capability for adhering to endothelium and sequestering in the blood cells.[2] Research has also shown P. falciparum as a predominant species in the tropics, particularly Sub-Saharan Africa, due to the almost complete absence of a Duffy blood group with an affinity to P. vivax P. ovale. The malaria infection is usually transmitted by an infected female Anopheles mosquito.[2] The pregnant population is among the high-risk groups in danger of contracting malaria and developing severe disease manifestation. The risks of foetal and maternal anaemia, neonatal death, stillbirth, low-birth weight, and spontaneous abortion are increased by malaria in pregnancy.[3] The prevalence rate of malaria in pregnancy has been high in several regions of Nigeria, ranging from 19.7% to 72.0%.[4] The major devastating consequences of malaria in pregnancy have been noted to include miscarriages, anaemia, and low birth weight, and these have been attributed to 11% of maternal mortality

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in parts of Nigeria.[5] The majority of the consequences seen in malaria in pregnancy are attributed to Plasmodium falciparum infection.[3] Some reports have shown that malaria in pregnancy is more prevalent in primigravida than multigravida,[6] and the transmission pattern of malaria in Nigeria is strong and stable, probably because the infection stays fairly constant throughout the year. The ABO blood group system consists of carbohydrate antigens B, H, and A capable of regulating the proteins' activity in the course of infections. These antigens are formed when glycoproteins and glycolipid chains present on cell surfaces are glycosylated at the terminal end.[7, 8] The parasite experienced several developments of evading the host immune system while in the erythrocytic stage of its life cycle. However, binding the infected red blood cells (RBCs) to the un-infected RBCs, called Rosetting, is a virulence phenomenon that Plasmodium falciparum employs to cause infection. It has been reported to cause severe malaria in African children.[9] Studies have shown that rosetting parasites would not multiply superior to the non-rosetting clones in vitro.[9, 10] Some studies have shown that Rosetting is reduced in people with the blood group O. Although the rosetting capacities of the blood groups A, B, and AB have been still debated, studies are showing that Rosetting would be more significant in individuals with the blood group A.[10, 11] According to a case-control study carried out,[12] the blood group O confers protection on its host against severe malaria through reduced Rosetting. Two haemoglobinopathies-HbS and HbC (with genotypes AS and AC, respectively), have been shown to protect against severe malaria compared with their HbAA counterparts. Proposed hypotheses to explain the protection conferred by haemoglobin genotypes AS and AC against malaria includes, but are not limited to, impaired development of the parasite at the blood stage, reduced cyto-adherence of infected RBCs, and enhanced acquisition of immunity.[13, 14] Despite the nature of the malaria burden in Nigeria and with a majority of the population being at risk of infection, quite a few studies have been carried out to evaluate the role that age, gravidity, ABO/Rhesus blood groups, and haemoglobin genotypes play on the distribution of P. falciparum malaria among the pregnant populace. Hence, this study was designed to evaluate these concerns among gravid women in South-Eastern Nigeria to enhance efficacy for interventions against malaria.

2. Materials and methods

Study Area

We performed the present research in Atani, South-Eastern Nigeria. Atani is a town on the eastern bank of the Niger River populated by early fishermen and migrant settlers. They are known to produce most rice, fish, yam, and cassava sold in Anambra and Delta markets. The population has grown to an estimated 230,000.[15] The location of Atani in the tropical rainforest provides it with an ecological basis for the production of a wide range of tropical agricultural products with a widespread industrial convention potential.[15] The climate is the rainy and dry season in the study area; the dry season lasts from November to March, while every year from April to October, the rainy season lasts.

Study Population

Blood samples were collected from one hundred and fifty pregnant females of 15 to 45 years referred for the ante-natal clinic at two Maternity Centres in Atani and were used for the research. We chose the participants randomly without any previous information about their medical background. Structured questionnaires were given to pregnant women who came to the Maternity Centre to get the essential data needed for the study.

Study Design

As mentioned earlier, we conducted a cross-sectional study consisting of 150 gravid females from 2 Primary Health centers in Atani, Nigeria, between December 2020 to January 2021.

Sampling Method and Sample Size Calculation

Using malaria prevalence from a previous study by Oluwagbemiga et al., 2018, adequate sample size was calculated.[16] We employed Yamen formula for obtaining the total size of the samples because of the finite population.

$$n = \frac{N}{K + N[e]\hat{}2}$$

n: represents the sample size.
N: refers tp the statistical population.
e: degree of the expected error = 10%.
Therefore,

$$n = \frac{548}{1 + 548[0.1]\hat{}2}$$

n = 84.567901235

Anticipating non-response of 10% (f).
Adjusted Sample Size; ns= n/(1-f).
ns =86/0.9= 95.5 ~ 100.
However, 150 samples were collected.

Inclusion Criteria

Gravid women who reside within Atani, Nigeria, and within the age bracket of 15-45 years.

Exclusion Criteria

Gravid women not residing within Atani, Nigeria, and those below 15 years or above 45 years who reside within Atani, Nigeria.

Informed Consent

According to the research design, voluntary informed consent was gotten from the participants after adequate data concerning the aim of the study and guarantee of confidentiality were given. Our recruitment script illustrated the objectives of the study, its significance, implications, and probable risks. Moreover, it specified anonymity and voluntarity of participation, and thus non-participation did not impose any consequence whatsoever, and they were free to exclude from the research whenever they are no longer comfortable with the study.

Ethical Approval

The Scientific and Ethical Review Boards of the Nnamdi Azikiwe University Teaching Hospital (NAUTH) in Nnewi, Anambra State, South-East Nigeria, approved our study protocol with an ethical approval numberNAUTH/CS/66/VOL.13/VER III/98/2020/027. It was approved on 17th December 2020. The clearance was on the understanding that patient anonymity must be maintained; optimal laboratory practices and information got must be treated with the utmost confidentiality and for the research purpose only.

Data Collection and Blood Samples Examination

As mentioned earlier, we provided thin and thick blood films on different slides and labeled them properly to recognize malaria parasites and detect the Plasmodium species present, respectively.[17] Then, we employed a ten percent Giemsa stain for staining the blood films. Before that, the thin blood film was fixed using methanol for 2 minutes and then positioned the diluted stain on the slides until it enclosed thin and thick blood films. This was allowed to stand for 30 minutes and was washed in running water and air dried.

We observed the thin and thick blood films using a light microscope at x100 oil immersion objective lens. The thick blood film was observed first to check for the existence of the malaria parasite. Next, we performed examination of the thin blood film to detect the Plasmodium species existing with the use of the routine methods.[16] For this study, only Plasmodium falciparum was used. Blood samples were collected from the pregnant women by venepuncture through strict adherence to safety procedures. To increase blood pressure in the veins, we tied a tourniquet around the upper arm, and its area of the venipuncture was thoroughly cleaned with methyl spirit-soaked cotton wool and allowed to dry. The collected venous blood was transferred into a sample tube of Ethylene Diamine Tetra-acetic Acid (EDTA) and labeled accordingly, and was sent to Dozzy-Link Laboratory Centre, Atani, Nigeria. Thick and thin film studies were done on them to detect the presence of Plasmodium falciparum.

Thick Film Method

According to Fleck et al. (1988), the thick film was prepared. Two drops of blood were placed on a slide and spread to about 15 mm in diameter, and allowed to dry. It was subsequently stained with Giemsa solution 1:20, diluted with distilled water for 15 minutes. The slide was then washed gently with a few drops of distilled water, cleaned the back of each slide, and put in a draining rack to dry it. In order to detect the presence of Plasmodium, it was subsequently examined under an x100 oil immersion objective lens. If the ring form of trophozoites or any other blood stage of erythrocyte schizogony was detected, a film was considered positive for the malaria parasite. A film was considered negative when, after scanning at least 100 fields, no parasites were seen.

Thin Film Method

According to Fleck et al. (1988), the thin film was also prepared. Two drops of blood were placed on a slide and allowed to rest on a flat, firm surface. Another slide was used as a 'spreader,' and the blood allowed its edge to run along. After that, at an angle of 450, the spreader was pushed along the slide away from the largest drops. The thin film was subsequently fixed for 2 minutes in absolute methanol and stained for 10 minutes with Giemsa solution 1:20 diluted with distilled water at pH 7.2. The slides were then washed, dried, and examined under the microscope with an x100 oil immersion objective lens to identify the Plasmodium species in running water.

ABO Blood Group Typing and Genotype Determination

The ABO blood grouping was carried out on the collected blood samples using the routine agglutination test method using agglutinating A, B, AB, and Rhesus-D monoclonal antisera.[18] We finally utilized the allele-specific polymerase chain reaction (ASPCR) to determine genotypes.

Statistical Analyses

In this step, we pooled the collected information and employed the Chi-square test for analyzing them. Moreover, we considered P less than 0.05 for statistical significance and applied SPPSS 23 for all the analyses.

3. Results

Out of the 150 pregnant women examined, the overall prevalence in relation to age, gravidity, blood group, and genotype was 59.4%, respectively.

Study Flow Chart

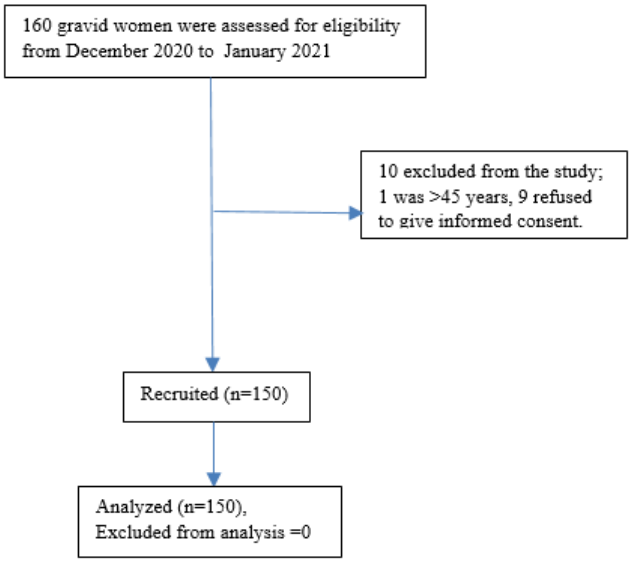

Table 1. Prevalence of malaria in conjunction with age among gravid females attending antenatal clinic at two Maternity Centres in Atani, Anambra State, South-eastern Nigeria.

Age group (Years)	Number Examined (N=150)	Number of age group infected (%)	Number Infected (%) (N=89)	Chi-Square (%)	df	P-value
16-19	5	2(40.00)	2(2.30)	7.656	10	0.662
20-23	16	9(56.30)	9(10.20)			
24-27	46	27(58.70)	27(31.70)			
28-31	48	29(60.40)	29(33.00)			
32-35	25	13(52.00)	13(14.80)			
36-39	10	9(90.00)	9(10.20)			

This table shows the prevalence according to age groups indicated that the age group 28 – 31years had the highest prevalence of malaria (33.0%), followed by 24 – 27 years which had 31.7%, while the age group 16 – 19 years recorded the least prevalence of malaria 2.3%. The differences between malaria prevalence in different age groups were not significant statistically (P>0.05).

Table 2. Prevalence of malaria in conjunction with gravidity among gravid women attending antenatal clinic at two Maternity Centres in Atani, Anambra State, South-eastern Nigeria.

Gravidity	Number Examined (N=150)	Number of gravidities Infected (%)	Number Infected (%) (N=89)	Chi-Square (%)	df	P-value
Primigravida	91	55(60.40)	55(61.40)	0.736	2	0.692
Multigravida	59	34(57.63)	34(38.60)			

This table shows the malaria prevalence in relation to gravidity recorded that the primigravida has the highest prevalence of malaria, 61.4%, while multigravida had the least prevalence of 38.6%. The prevalence of malaria was dependent on gravidity but with insignificant differences (P greater than 0.05).

Table 3. Prevalence of malaria in relation to blood group among gravid women attending antenatal clinic at two Maternity Centres in Atani, Anambra State, South-eastern Nigeria.

Blood Group	Number Examined (N=150)	Number of blood group Infected (%)	Number Infected (%) (N=89)	Chi-Square (%)	df	P-value
A-	2	1(50.00)	1(1.1)	-----	-----	-----
A+	12	8(66.67)	8(9.10)			
AB-	7	5(71.43)	5(5.70)			
AB+	11	4(36.36)	4(4.50)			
B-	4	0(0.00)	0(0.00)			
B+	8	4(50.00)	4(4.50)			
O-	26	19(73.08)	19(21.60)			
O+	80	48(60.00)	48(54.70)			

This table shows the prevalence of malaria in association to ABO/Rhesus blood group showed that those with the highest prevalence was O+ and O- (54.7% and 21.6% respectively), while blood group A- and B- had the least prevalence (1.1% and 0.0% respectively). The differences between malaria prevalence among the blood groups showed that they are not significant statistically (P less than 0.05).

Table 4. Prevalence of malaria in conjunction with genotype among gravid women attending antenatal clinic at two Maternity Centres in Atani, Anambra State, South-eastern Nigeria.

Genotype	Number Examined (N=150)	Number of genotype Infected(%)	Number Infected (%) (N=89)	Chi-Square (%)	df	P-value
AA	84	55(65.48)	55(62.60)	7.752	4	0.101
AS	62	30(48.39)	30(34.10)			
SS	4	4(100)	4(4.50)			

This table shows the prevalence of malaria in relation to genotype showed that genotype AA had the highest prevalence of (62.60%), while the least prevalence was seen among SS genotype(4.50%). The differences between the prevalence of malaria among the genotypes showed that they are insignificant statistically (P > 0.05).

4. Discussion

This research outcome showed P. falciparum is prevalent in Atani, Anambra State. P. falciparum was prevalent among gravid women in this study, which is also in tandem with the work of Ozougwu et al.[19] The prevalence of malaria changed substantially between age group, gravidity, blood group/rhesus factor, and genotype of the respondents. In relation to the age group, the result showed that the age group between 28-31 years (33%) had the highest prevalence of malaria, followed by the age group 24-27 years (31.7%), while the age group 16-19 (2.3%) had the least prevalence of malaria. Our findings are in line with the work done by Susanna et al.[20] in Port Harcourt, Nigeria, but not in agreement with Ozougwu et al.'s[19] finding in River State, Nigeria, as well as Amala et al.[21] in Anambra State, Nigeria where they documented the maximum prevalence in Age group below 21 years. The prevalence in relation to gravidity showed that primigravida had the highest prevalence of 61.4% than multigravida, which had a prevalence of 38.6%. This could probably be due to immunological variations throughout pregnancy and more enlightenment on malaria among multigravida. This contrasts with Ozougwu et al.[19] and Susanna et al.[20] which recorded that multigravida had more malaria prevalence than primigravida.

The pervasiveness of malaria in conjunction with the blood group showed that the blood group/rhesus factor with the highest prevalence was O+ (54.7%) followed by O^- (21.6%), and the least prevalence was seen in B- (0.0%). Evidence has shown people with blood groups B and A have a higher susceptibility to malaria infection than that of the blood group O individuals. Nevertheless, plasmodiasis varies in accordance to host immunity.[22] It is a known fact that any blood group antigens have been not found on the exterior of blood group O red cells; thus, more quantity of receptors and increased chance of malaria parasite attachment whereas in the blood groups B, AB, and A, individual blood group antigens enclose the red cells, leaving a fewer number of receptors for malarial parasites attachment. Therefore, there is less probability for attachment of the parasites to these red blood cells.[23]

The prevalence of malaria in relation to genotype showed that the HbAA genotype had the highest prevalence of 62.6%, followed by HbAS 34.1%, while having HbSS with 4.5% to be the least. This matches Taylor et al.'s[24] and Susanna et al.'s[20] findings where HbSS had the least prevalence, followed by HbAS with the HbAA showing the most prevalence. This may be attributed to point mutation of the beta-globin chain at the 6th position where

valine replaced glutamate resulting in the polymerization of HbSS cells.[25] These result in the generation of reactive oxygen species.

Strengths and Limitations of the Study

This study was the first of its kind in Atani, Nigeria. The study is relevant because it was conducted among pregnant women, which helped some of them be aware of their genotype to reduce the incidence of haemoglobinopathies. It is a cross-sectional study involving only pregnant women. Therefore its generalizability to the whole community may not be fair.

5. Conclusion

This study showed that malaria in pregnancy is endemic in Atani, Ogbaru Local Government Area, Anambra State, Nigeria. The observed increased prevalence among pregnant women in this study could probably be due to the location of the study area, which is riverine, hence favours breeding ground for Anopheles mosquito, a vector for malaria parasites. There is a need for serious advocacy and enlightenment of the populace at large on the prevalence and control measures of malaria transmission to curb the increased prevalence of malaria among gravid women.

Conflict of Interest

The authors declared that there is no conflict of interest.

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