Molecular Identification and Speciation of Human Non-Falciparum Plasmodium Species in Pregnant and Non-Pregnant Patients visiting Federal Medical Centre (FMC) Owerri, Imo State, Nigeria

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ABSTRACT

This study evaluated the prevalence of malaria parasite among pregnant and non pregnant women attending Federal Medical Centre (FMC) Owerri Imo State Nigeria within the age range of 21-50 years. The molecular identification and speciation of the malaria parasites were carried out using different diagnostic techniques. The blood samples were analyzed using microscopy, Rapid Diagnostic Test (RDT) and Polymerase Chain Reaction (PCR). Results using the microscopy for pregnant women was 181(90.5%) and for non pregnant women, it was 185(92.5%) within the age range of under 21, 41 and above. The result of RDT for pregnant women was 58(32.0%) and for non pregnant women were 46(24.9%). However, the age group of pregnant women that had the highest occurrence of malaria using microscopy was (21-25yrs) with 100% of persons tested positive and (41-45yrs) with 78.8% had the lowest occurrence. For non pregnant women, the age group that had the highest occurrence of malaria parasite was (31-35yrs) with 95.3% of persons reporting tested reporting positive while, (21-25yrs) with 84.6% had the lowest occurrence. Polymerase Chain Reaction (PCR) was used for the speciation of the parasites and the result revealed that Plasmodium falciparum in pregnant women within the ages of 46-50yrs had the highest (96.0%) prevalent followed by Plasmodium vivax for women within the ages of 21-25yrs had the highest (13.6%) occurrence, Plasmodium malariae for women within the ages 21-25yrs and had the highest (9.4%) occurrence. In this study, Polymerase Chain Reaction was very sensitive, takes more large samples at a time and specific for both P. falciparum and non falciparum Plasmodium infections and has many diagnostic advantages over microscopy. Out of 400 venous blood samples collected from both pregnant and non-pregnant women, 27 blood samples had mixed malaria parasite infections. Therefore, following this study, it will be recommended that we urge the pharmaceutical companies to improve on the manufacturing of broad-spectrum antimalarial drugs to cover all species of Plasmodium as it's done with antimicrobials not only concentrating on the drugs for the treatment of P. falciparum alone.

Keywords: Malaria, Plasmodium, Prevalence, Molecular identification, Speciation.

Introduction

Malaria is a mosquito-borne infectious disease of humans caused by unicellular microorganism of parasitic protozoans of the genus *Plasmodium*. Malaria infection during pregnancy is common, which can result in fetus low birth weight, still birth, premature delivery, spontaneous abortion and decrement in intrauterine fetal growth in addition to maternal death from severe malaria or indirectly due to severe anemia. Beside, malaria infection has the greatest impact on the survival of mothers (Agomo and Oyibo, 2013). The factor behind the high burden of malaria during pregnancy could be the increase in body surface and specific odor secretions during pregnancy which may expose them to increased mosquito bites (Babin *et al.,* 1983). Malaria entails a significant burden for many developing countries in the world. As per the WHO report in 2015, 91 countries had active malaria transmission and approximately 212 million people were affected with malaria disease worldwide (WHO, 2016). Reliable diagnostic tools are necessary for early confirmation of malaria, especially with the current efforts to decrease transmission and increase interventions, as pregnant women are an important reservoir of parasites in the community. (Menard *et al.,* 2010). Malaria is a major societal health challenge in many region of the world. (Bassey *et al.*, 2017). Ndiok *et al.* (2016) reported that malaria is among the major cause of morbidity and mortality in the Sub-Sahara Africa. Authors have reported that 3.28 - 3.4 billion world populations are at risk of malaria infections in 92 countries. WHO (2019) reported that 1.1 billion people are at high risk of malaria infection.

According to World Malaria report 2018, 203-262 million and 435 000 cases and deaths, respectively were recorded in 2017, which showed a decline of 18% (for malaria cases) and 28% (of death resulting from malaria infection) in 2010. World Health Organization reported that 212 million and 429,000 malaria cases and deaths, respectively were recorded in 2015. The authors reported that there was a decline of 29% in fatality cases due to prevention and control. Malaria cases are higher in Africa with prevalence rate of 81 – 93% of total global cases (NMFS, 2011). Malaria in children has higher fatality compared to adults. Reports have shown that about half of global malaria death occurs in Nigeria, Democratic Republic of the Congo, Uganda and Ethiopia (NMFS, 2011). The prevalence of malaria in Nigeria differs according to the geopolitical zone. Nigeria malaria Fact Sheet reported a prevalence of 41 – 50% (North-West, North-Central and South-West), 31 – 40% (North-East and South-South) and 21 – 30% (South-East). In Nigeria, a substantial number of outpatient consultation cases are often treated with malaria. Malaria has been reported among pregnant women, infants and elderly. Nkuo *et al.* (2002) reported that 60% and 30% of outpatient visits and hospitalizations respectively of <5 year children are often treated of malaria.

During pregnancy several physiological changes occurs in women. If not properly managed it could result to other heath concern to both the foetus and mother. Luxemburger *et al.* (2013) reported that malaria associated with *P. falciparum* and *P. vivax* during pregnancy increases neonatal mortality by lowering birth weight. Malaria is a parasitic infection caused by a protozoan of the genus *Plasmodium*. Several species of Plasmodium exist but five (*P. falciparum, P. vivax, P. ovale, P. knoweski* and *P. malariae*) have been reported to cause malaria (Eledo *et al.,* 2019). The occurrence of malaria caused by this species of *Plasmodium* differs based on geographical locations. In Nigeria, most cases of malaria cases are caused by *P. falciparum* and *P. vivax*. Basically, mosquito is iniquitous dipteran fly. Like malaria, several disease conditions (Chikungunya, lymphatic filariasis, Japanese encephalitis and dengue fever) has mosquito as their vector (Izah *et al.,* 2019). Malaria parasite is transmitted through an infective bite of the female anopheles mosquito. Malaria transmission is initiated when the sporozoites of the *Plasmodium* is inoculated by female anopheles' mosquitoes into the human blood stream, sporozoites disappear and invade hepatic cells to establish the liver stage. During the next red blood stage, the gametocytes are taken up by mosquito to ensure the survival of the species. Malaria is conventionally identified with microscopy approach. With research in science and technology, Enzyme Linked Immunosorbent Assay (ELISA) and polymerase chain (PCR) reaction were developed for the detection of malaria parasites in human. These methods are applied in several medical laboratories, but their application is still quite low when compared to microscopy method especially in areas of malaria endemicity like Nigeria (Nkuo *et al.,* 2002).

This study reports on the molecular identification and speciation of human non *falciparum Plasmodium* species and to ascertain the reliability of microscopy, rapid diagnostic test (RDT) and PCR used in the detection of malaria parasites in pregnant and non pregnant patients visiting Federal Medical Centre (FMC) Owerri, Imo state, Nigeria.

Materials and Methods

Study Area

The research was carried out in Federal Medical Center (FMC) located in Owerri Municipal council, Imo State. It has an area of 58km square and a population of 127,213 according to the 2006 National population census. The city

sits at an intersection of roads from Onitsha, Port Harcourt, Aba and Umuahia. Temperature and weather condition is seasons.The study was carried out during wet warm season when there is less rainfall and early dry season. Mosquito prefer warm weather at the temperature consistently below 50°C.

Sample Size

Four hundred (400) blood samples randomly selected from women with clinical symptoms of malaria were collected. The sample size was determined by the CDC software epiinfo^{TM} with a population size of 35,600, at 95% confidence level, 5% confidence limit which was generated on an expected frequency of 49%.

Blood Sample Collection

Venous blood samples were collected aseptically to ensure test results are reliable. Protective gloves were worn during the collection and handling blood samples. Lancets, needles and syringes used are sterile and dry. Blood collecting materials were discarded safely to avoid injury from needles and lancets (WHO 2010).

For PCR molecular identification and RDT tests, the anticoagulants used is EDTA (ethylenediamine tetra-acetic acid) also called sequestrene. This chemical prevent blood from clothing by removing calcium.

Giemsa Staining of Malaria Parasites Using Thick and Thin Films for Microscopical Analysis

A malaria parasite in thick and thin blood films requires staining at pH7.1- pH7.2. Romanowsky stain (containing azure dyes and eosin) was used (Tijitra *et al.,* 1999).Before used, the giemsa stain was diluted in 1 in 10 in buffered pH 7.1 water, and 45 ml of buffered water pH 7.1 was measured in a 50ml cylinder. 5 ml of giemsa stain was added to 50ml mark and was mixed gently.

Slides of the thick films were placed in a staining rack. The diluted Giemsa stain was used to flood the blood film area and was allowed to stand for 30mins. The stains were then washed using clean water, while the back of the slides were wiped with dry cotton wool and placed in a stainless draining rack to air dry.

The blood films were examined microscopically using the 40x and 100x objectives (USAID. Nigeria, 2013).

Leishman Staining Technique

Method: The dried blood film was covered with undiluted stain by counting the number of drops required to cover the film (The undiluted stain acts as a fixative and also partially stains the smear). It was allowed to stand for 2mins. Twice the volume of pH 6.8 buffered water was added (twice the number of drops as stain).

The diluted stain was not allowed to overflow but allowed to stand for another 8mins which brings the total staining time to 10mins. The stain was washed off with tap water and the back of the slide was wiped clean and was placed in a draining rack for the smear to air dry. The smear was examined microscopically by using 40x and 100x objectives and immersion oil (Barat *et al.,* 1999).

Parasitemia:A smear was considered to be negative when no parasites were detected in the total area of the microscopic objective field, where 200 WBCs were observed (WHO, 2017).

Thick blood smears were used to calculate parasitemia, Parasites/microliter of blood as described by WHO. (2017).

$$Parasites\,/\,\mu l = \frac{Number\ of\ parasites\ counted\ \times\ 8000WBC}{Number\ of\ white\ blood\ cell\ WBC}$$

The above formula was used to calculate the significant presence of malaria parasite in the blood (WHO 2016).

Molecular Identification

Isolation of DNA from 200 μl whole blood

Deoxyribonucleic acid (DNA) was extracted using the DNA extraction kits. Gene amplification was carried out after the extraction in a NORMAL PCR MACHINE.

PCR Program

Denaturation, annealing, elongation and final elongation (extension) ran at temperature of 94 degree Celsius for 1min, temperature of 58 degree Celsius for 2mins, temperature of 72 degree Celsius for 2mins and temperature of 72 degree Celsius for 5mins with a total of 25 cycles respectively.

The species-specific primers used were:-

*P. falciparium***:**

   Forward:

   TTAAACTGGTTTGGGAAAACCAAATATATT

   Reverse:

   ACACAATGAACTCAATCATGACTACCCGTC

P. vivax:

   Forward:

   CGCTTCTAGCTTAATCCACATAACTGATAC

   Reverse:

   ACTTCCAAGCCGAAGCAAGAAAGTCCTTA

P. malariae:

   Forward:

   ATAACATAGTTGTACGTTAAGAATAACCGC

   Reverse:

   AAAATTCCCATGCATAAAAAATTATACAAA

PCR Products Analysis by Gel Electrophoresis

Wataya (1993) method was adopted and modified. The PCR products were confirmed and analysed on a 1.5% agar rose gel electrophoresis with 5µl of ethidium bromide. The electrophoresis gel was ran for 35 mins at 80 volts on a

horizontal electrophoresis tank submerged in TBE buffer solution. The DNA amplification product were examined under ultraviolet (UV) transilluminator and ran alongside the DNA ladder with the control strains after which different base pairs of the genes were compared with the DNA ladder for base pairs of (205, 800 and 1100) respectively.

Results

Malaria parasite test of blood samples collected from 200 pregnant women were examined microscopically. Out of 200 pregnant women whose blood samples were tested microscopically for the presence of malaria parasites, 181(90.5%) were positive whereas 19(9.5%) were negative. The highest occurrence was found among the women of the age range of 21 – 25yrs with 100% persons tested positive as shown on Table 1.Out of 200 non-pregnant women blood samples were tested microscopically185(92.5%) were positive whereas 15(7.5%)were negative. The highest occurrence was found among the women of the age range of 31 – 35yrs with 95.3% persons tested positive as shown on Table 2.

Table 1. Microscopic examination of blood samples collected from 200 pregnant women

Age Group (Yrs.)	Number of Pts. Examined Microscopically	Number of Positve Cases (%)	Number of Negative Cases (%)
21-25	22	22 (100)	0
26-30	32	27 (84.4)	5 (15.6)
31-35	38	35 (92.1)	3 (7.9)
36-40	50	47 (94.0)	3 (6.0)
41-45	33	26 (78.8)	7 (21.2)
46-50	25	24 (96.0)	1 (4.0)
	200	**181 (90.5%)**	**19 (9.5%)**

Table 2. Microscopic examination of blood samples collected from 200 non-pregnant women

Age Group (Yrs.)	Number of Pts. Examined Microscopically	Number of Positive Cases (%)	Number of Negative Cases (%)
Under 20	42	38 (90.5)	4 (9.5)
21-25	13	11 (84.6)	2 (15.4)
26-30	38	36 (94.7)	2 (5.3)
31-35	43	41 (95.3)	2 (4.7)
36-40	44	40 (90.9)	4 (9.1)
41 & Above	20	19 (95.0)	1 (5.0)
	200	**185 (2.5%)**	**15 (7.5%)**

Out of 200 pregnant women whose blood samples were tested using PCR for the speciation of malaria parasites, 14(7%) were positive for *P. vivax,* 9(4.5%) positive for *P. malariae* and non-detected for *P. ovale.* The highest occurrence of *P. vivax* was found among the women of the age range of 21 –25yrs with 13.6% whereas the highest

occurrence of *P. malariae* was found among the women of the age range of 26 – 30yrs with 9.4% of persons tested positive as shown in Table 3. The results for non-pregnant women of same population of PCR for speciation as shown in Table 4 is 8(4.0%) positive for *P. vivax,* 7(3.5%) positive for *P. malariae* where 0% for *P. ovale*. The highest occurrence of *P. vivax* was found among the women of the age range of 21 – 25yrs with 15.4% whereas the highest occurrence of *P. malariae* was found among the women of the age range of 36 – 40yrs with 6.8% of persons tested positive.

Table 3. PCR results of blood samples collected from 200 pregnant women

Age Grp.	No. examined	PCR No. of samples with +ve (%)	No. of samples with *PF* (%)	No. of samples with *PV* (%)	No. of samples with *P.M* (%)	No. with *P.O*
21– 25	22	22 (100)	18 (81.8)	3 (13.6)	1 (4.5)	0
26– 30	32	32 (100)	27 (84.4)	2 (6.3)	3 (9.4)	0
31 – 35	38	38 (100)	35 (92.1)	2 (5.3)	1 (2.6)	0
36– 40	50	50 (100)	47 (94.0)	2 (4.0)	1 (2.0)	0
41– 45	33	33 (100)	26 (78.8)	4 (12.1)	3 (9.1)	0
46 – 50	25	25 (100)	24 (96.0)	1 (4.0)	0 (0)	0
	200	**200 (100%)**	**177 (88.5%)**	**14 (7%)**	**9 (4.5%)**	

Table 4. PCR results of blood samples collected from 200 non-pregnant women

Age Grp.	No. examined	PCR No. of samples with +ve (%)	No. of samples with *PF* (%)	No. of samples with *PV* (%)	No. of samples with *P.M* (%)	No. with *P.O*
Under 20	42	42 (100)	38 (90.4)	2 (4.8)	2 (4.8)	0
21 – 25	13	13 (100)	11 (84.6)	2 (15.4)	0 (0)	0
26 – 30	38	38 (100)	36 (97.7)	1 (2.6)	1 (2.6)	0
31 – 35	43	43 (100)	41 (95.3)	1 (2.3)	1 (2.3)	0
36 – 40	44	44 (100)	40 (90.9)	1 (2.3)	3 (6.8)	0
41and abov	20	20 (100)	19 (95.0)	1 (5.0)	0 (0)	0
	200	**200 (100%)**	**185 (92.5%)**	**8 (4.0%)**	**7 (3.5%)**	

PCR Amplified Agarrose Electrophoresis

PCR Agarrose electrophoresis of amplified *P. malariae* bands on well 1 to 6, 9 and 10 with 1100bp, the second well below is amplified *P. falciparum* bands on well 3, 6 and 9 with 205bp while ladder lane L shows 100bp. This indicates mixed malaria parasite infection of *P. malariae* and *P. falciparum* as shown in Plate 1. PCR Agarrose electrophoresis of amplified *P. falciparum* bands on well C, F and I with 205bp while L lane shows 100bp is shown in Plate 2. PCR Agarrose electrophoresis of amplified *P. vivax* bands on well 3, 6 and 9 with 800bp while L lane shows 100bp as is shown in Plate 3.

Plate 1: PCR Agarrose electrophoresis of amplified *P. malariae* and *P. falciparum* bands
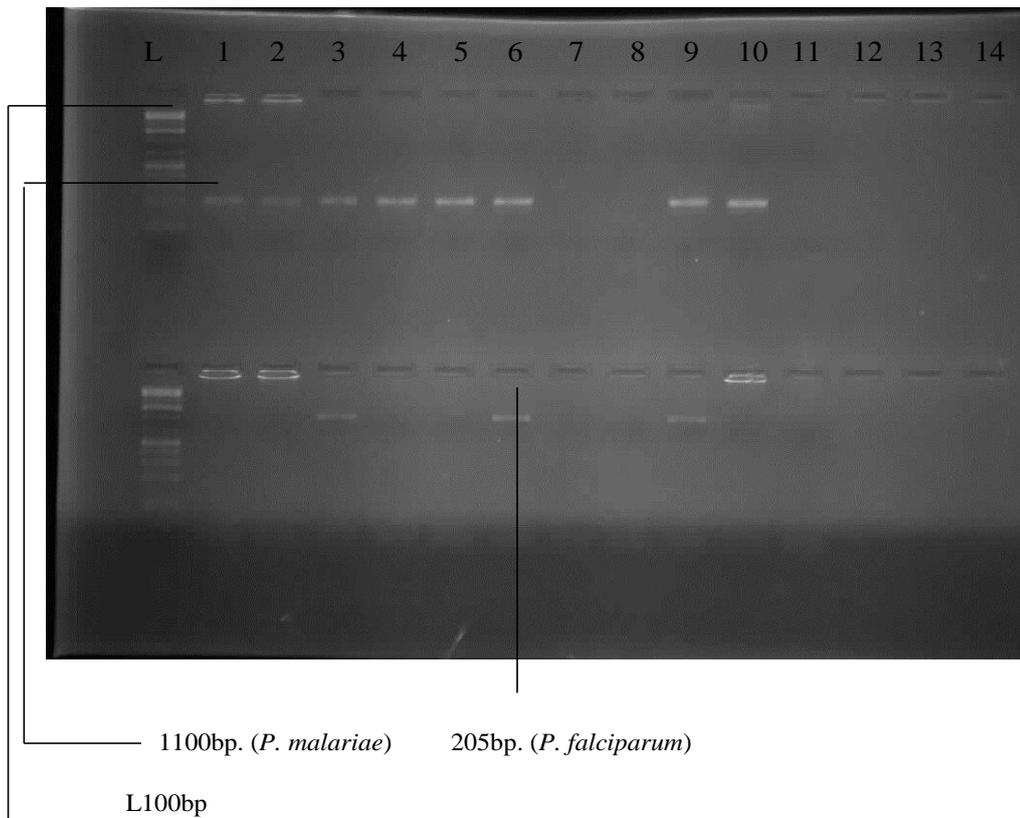

1100bp. (*P. malariae*)   205bp. (*P. falciparum*)

L100bp

Plate 2: PCR Agarrose electrophoresis of amplified *P.falciparum* bands

L100bp   L100bp

205bp (*P. falciparum)*

Plate 3: PCR Agarrose electrophoresis of amplified *P.vivax* bands

L100bp

800bp (*P.vivax*)
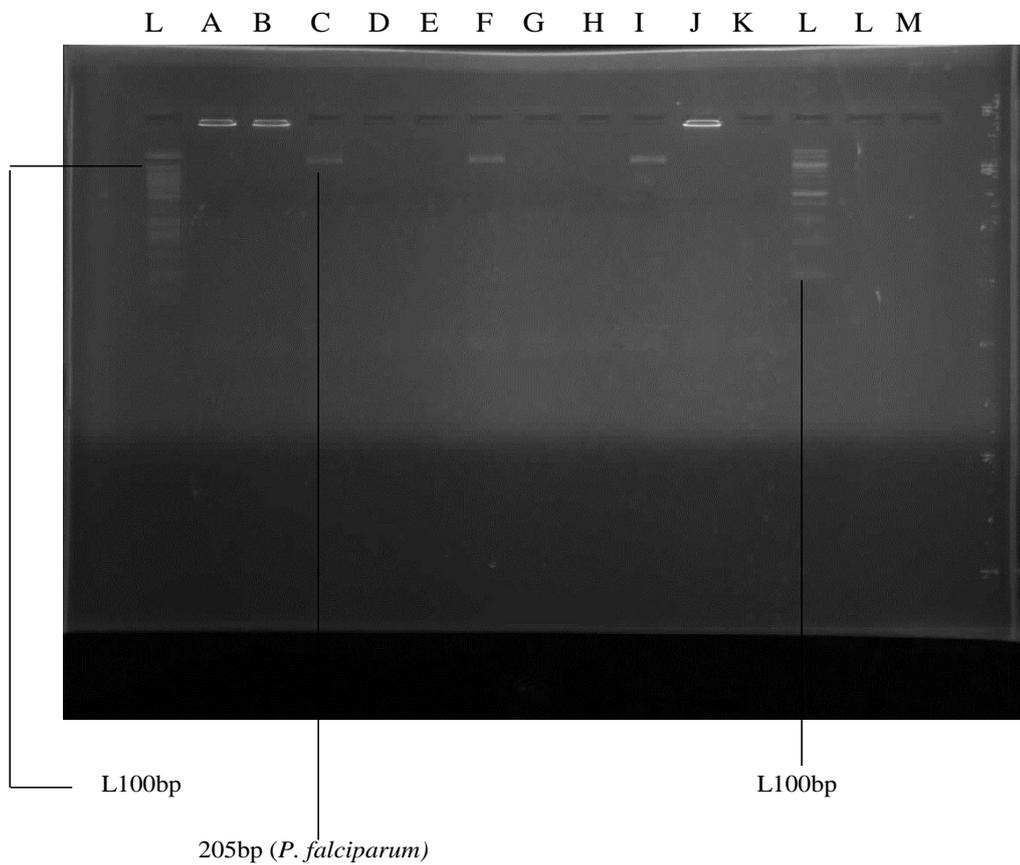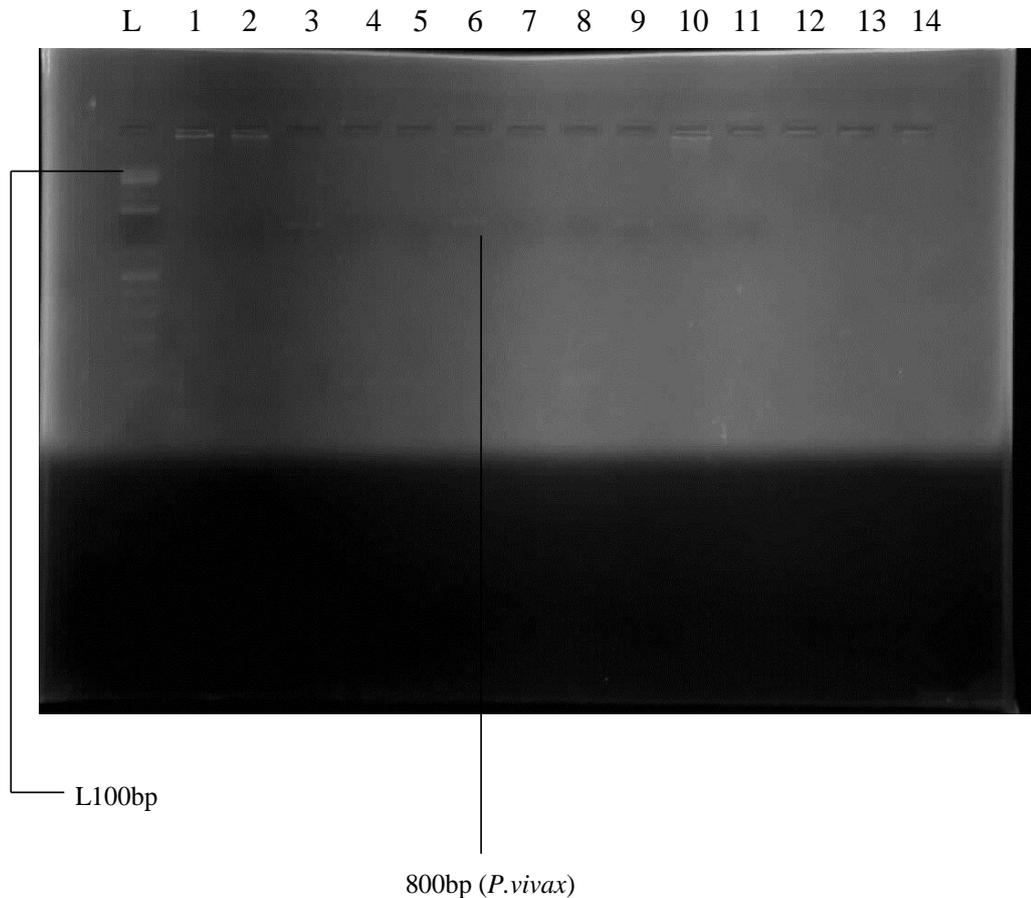
Discussions

Malaria is a mosquito borne irresistible disease of human *Plasmodium* species, while malaria in pregnancy is characterized by the accumulation of *Plasmodium* species in infected erythrocytes in placental intervillous spaces (Brabin, 1983). This infection is caused by a nibble from an infected female anopheles mosquito, which presents the life forms from its spit into an individual's circulatory framework where the parasite travels to the liver to develop and instate. In the past decade, the world has experienced a steady increase in antimalaria multi – drug resistance in pregnant and non pregnant women who were diagnosed of malaria infection (WHO, 2017).

In this study, a total of 400 (four hundred clinical blood samples) were collected.

Out of four hundred (400) clinical blood samples analysed for molecular identification and speciation of human non- *falciparum Plasmodium* species from pregnant and non-pregnant women visiting Federal Medical Centre Owerri, Imo State, Nigeria, 181(90.5%), 19 (9.5%) positive and negative microscopy examination of 200 pregnant and 185(92.5%), 15(9.5%) positive and negative microscopic examination of 200 non-pregnant women respectively where p<0.05. Statistically, there was significant increase among the prevalence of different parasites (Moody *et al.,* 2000) with different age groups. Age group 21 – 25yrs age with recorded 100% positive microscopic testing among pregnant women. Others represented as 21-25, 26-30, 31-35, 36- 40, 41- 45, 46-50 recorded 22(100%), 27(84.4%), 35(92.1), 47(94.0%), 26(78.8%) respectively while in non- pregnant women 31-35 age group with 41(95.3%) recorded the highest microscopy positive test against other groups.

The prevalence of malaria parasite by rapid diagnostic techniques (RDT) reveals that, out of 181 positive microscopic examined pregnant women, 58(32%), 123(68%) were positive and negative respectively while out of 185 non-pregnant women 46(24.9%) and 139(75.1%) were positive and negative in RDT respectively. This indicates that both in specificity and sensitivity microscopic analysis remains the gold standard against Rapid Diagnostic Technique (RDT). The failure of RDT to detect these samples as positive could be due to low level of target pf(HRp2 and HRp3) antigen or even a deletion of it entirely from the isolate (Koita *et al.,* 2012; Kumar *et al.*, 2013) and (Starzengruber *et al.,* 2014) identified as negative, contrary to this study. The microscopic prevalence of *P. falciparum* of other studies was quite lower than what was obtained here (White, 2008) and (Golassa *et al.,* 2013). This however, could be due to the experience and expertise of the microscopist that read the slides. Difference in transmission intensity as well as intervention implementation could both influence the burden of parasite carriage in various epidemiological settings.

PCR study reveals the dominance of *P. falciparum* with *P. malariae*, *P. vivax* and *P. ovale* when subjected to 200 pregnant women. The prevalence of *P. falciparum* in pregnant women within the ages of 46 – 50years had the highest (96.0%), followed by *Plasmodium vivax* (13.6%) with the age group 21 – 25years having the highest occurrence, age group 26-30 having the highest occurrence in *Plasmodium malariae* with (9.4%). as seen in the table 3. (WHO, 2016, WHO, 2017). This seems to substantiate the focused attention of malaria control strategies towards this species when compared with other research findings in Nigeria where PCR method is applied. This method is sensitive and specific for both *P. falciparium* and human non- *falciparum Plasmodium* species. The diagnostic advantages of the PCR methods outweighs the microscopic method. In general, PCR shows mixed infections of *P. falciparium and P. malariae* in patients. Some of the benefits of the PCR methods is that it is less laborious, performed in a close system which minimizes the risk of post-amplification contamination, results can be obtained within a short period of time and the analysis is automated and suitable for multi-purpose studies (Perandin *et al*., 2004; Purfield *et al*., 2004; Wilson *et al*., 2005). Microscopy testing is the most routine diagnostic technique used in endemic areas, but its use as a standard may generate misleading results in clinical trials (Perandin *et al*., 2004; Mens *et al.,* 2007; Boonma *et al.,* 2007).

The prevalence rate recorded in this study is higher than the previous study in other parts of East and Western Nigeria. Obianumba (2012) reported malaria prevalence rate of 53.9% among pregnant women attending antenatal clinic in Ozubulu, in Anambra State, Nigeria. Agomo and Oyibo (2013) reported malaria prevalence rate of 7.7% among pregnant women in Lagos State, Nigeria. Of these *P. falciparum*, *P. malariae* and mixed infection of *P. falciparum* and *P. malariae* were 91.6%, 4.8% and 3.6% respectively. Nwonwu *et al*. (2009) reported prevalence rate of malaria parasitemia during pregnancy in Abakaliki, Ebonyi State, Nigeria as 29%. Enoch and Ethel (2018) reported a high prevalence of malaria among pregnant women from two hospitals in Port-Harcourt, River State, Nigeria between 27.5 – 35.0%. Akinboro *et al.* (2010) reported high prevalence in malaria associated with *P. falciparum* as 63.5% among pregnant women in a secondary hospital and tertiary hospital in Osogbo, South – Western Nigeria. Omoya and Atobatele (2017) reported the prevalence of malaria among pregnant women attending primary healthcare centre in Ojo Local Government Area (LGA) in Lagos State, Nigeria as 65.88% (112 of 170 samples).

Table 4: Shows PCR testing subjected to 200 (Two hundred non pregnant women) who were initially tested using microscopy and RDT respectively, when distributed based on their age groups, therefore, there is need for country-wide survey to ascertain the burden of *P. vivax* and *P. malariae* as well as mixed-malaria infection. Just order to put in place adequate control measures, this co-infection of malaria with other disease (Bassey and Izah, 2017) the difference associated with the malaria prevalence in this study when compared to previous study could be associated to the medical conditions of the individuals, environmental condition and human status (pregnancy, blood group, rhesus factor, age or educational status).

This study reveals that microscopy did not detect other non- *falciparum Plasmodium* infections which could lead to mistreatment of the malaria infection using a wrong anti-malaria drug, the PCR analysis remains the gold standard for further research in identification and speciation of other human non-falciparum species and microscopic testing is the best when compared to RDT we use for diagnosis these days for faster result.

Conclusion and Recommendations

The molecular identification and speciation of malaria parasite using different diagnostic techniques among pregnant and non pregnant women attending Federal Medical Centre, Owerri Imo State Nigeria was evaluated. The results found that the prevalence of malaria parasites was high 181(90.5%). The parasite prevalence was compared using the three diagnostic techniques: Microscopy, Rapid Diagnostic Test (RDT) and Polymerase Chain Reaction (PCR). The polymerase chain reaction revealed better method for the detection of malaria parasite in the blood samples used due to its specificity, intake of large samples at the same time and sensitivity. The prevalence from the PCR result showed that the highest prevalence of the parasites were 96.0% (*P. falciparum*) for the age groups of 46 – 50 yrs, 13.6% (*P. vivax*) for the age groups 21 – 25 yrs and 9.4% (*P. malariae*) for the age groups of 26 – 30 yrs respectively. However, this research made us to understand that, out of 400 blood samples examined, 27 samples had mixed malaria infections; that is one patient having both *P. falciparum* and *P. malariae* at the same time or another patient harbouring both *P. falciparum* and *P. vivax* at the same time which means, that most times we are treating the wrong malaria parasite and that is one of the reasons why we treat malaria and the symptoms persist.

Be that as it may, it will be recommended that the Government should try and equip most of our Tertiary Health Institutions in Nigeria with PCR machines for molecular identifications. More so, we urge the Pharmaceutical companies to also improve in the manufacturing of broad-spectrum anti-Malaria drugs to cover all the species of *Plasmodium* as it is done with antimicrobials and not concentrating on drugs for treatment of *P. falciparum* alone.

Competing Interests Statement

The authors declare no competing financial, professional and personal interests.

Ethical Approval

Ethical approval for this research was based on institutional guidelines.

Consent for publication

Authors declare that they consented for the publication of this research work.

Availability of data and material

Authors are willing to share the data and material according to relevant needs.

Authors' Contributions

Amadi, E. S., Umeh, S. I. supervised the entirety of this study, while Anumudu, B.E conducted the sampling, Interview, analysed the data and wrote the paper. All authors read and approved the final manuscript.

References

Agomo C.O., and Oyibo W.A (2013). Factors associated with risk of malaria infection among pregnant women in Lagos, Nigeria, Infectious Diseases of Poverty, 19.

Akinboro R.A., Ojurongbe O., Akindele A.A., Adefioye O.A., Bolaji O.S., and Olaniran O., (2010). Plasmodium falciparum parasitemia in pregnancy in relation to maternal anaemia, African Journal of Clinical and Experimental Microbiology, 11:164-169.

Babin B.J., (1983). "An analysis of malaria in pregnancy in Africa" Bulletin of World Health Organization, 61(6):1005-1016.

Barat, L., et al. (1999). Does the availability of blood slide microscopy for malaria at health centres improve the management of persons with fever in Tanzania? American Journal of Tropical Medicine Hygiene; 60(6):1024-30.

Bassy S.E., and Izah S.C., (2017). Some determinant factors of malaria prevalence in Nigeria, Journal of Mosquito Research, 7:48-58.

Boonma P., Christensen P., Suwanarusk R., Price R., Russel B., and Lek-Uthai U., (2007). Comparison of three molecular methods for the detection and speciation of Plasmodium vivax and Plasmodium falciparum. Malaria Journal, 6:124.

Eledo B. O., Allagoa D. O., Egwugha C. T., Dunga K. E., and Izah S. C., (2019). "Some haemostatic indicators among Malaria Infected Adolescents Attending a Nigerian University Teaching Hospital," Open Access Blood Research and Transfusion Journal, 3:555-602.

Enoch A.S., and Ethel K.O. (2018). Malaria in pregnancy a comparative study of pregnant women in two hospitals, European Journal of Pharmaceutical and Medical research, 5:40-45.

Golassa L, Enweji N, Erko B, Aseffa A, Swedberg G. (2013). Detection of substantial number of sub-microscopic Plasmodium falciparum infection by polymerase chain reaction: a potential threat to malaria control and diagnosis in Ethopia. Malar. J. 12:352.

Izah S. C., (2019)."Activities of crude, acetone and ethanolic extracts of Capsicum frutescens var. minima fruit against larva of anopheles gambiae," Journal of Environmental Treatment Techniques, 7:96-200.

Koita O.A., Doumbo O.K., Ouattara A., Tall L.K., Konare A., and Diakite M. (2012). False-negative rapid diagnostic tests for malaria and deletion of the histiden-rich repeat region of the HRP2 gene. Am J Trop Med HYg. 86:194-8.

Luxemburger C., McGready, R., Kham A. M., Morison L., Cho T., and Chongsuphajaisiddhi T., (2013) "Effects of malaria during pregnancy on infant mortality in an area of low malaria transmission," American Journal of Epidemiology, 154:459-465.

Menard D., Barnadas C., Bouchier C., Henry-Halldin C., and Gray L.R (2010). Plasmodium vivax clinical malaria is commonly observed in Duffy-negative Malagasy people. Proceedings of the National Academy of Sciences of the United State of America; 107:5967-5971.

Mens P., Spieker N., Omar S., Heijnen M., Schalling H., and Kager P.A. (2007). Is molecular biology the best alternative for diagnosis of malaria to microscopy? A comparison between microscopy, antigen detection and molecular tests in rural Kenya and urban Tanzania. Tropical Medicine of International Health, 12:238-244.

Ndiok E. O., Ohimain E. I., and Izah S. C., (2016). "Incidence of Malaria in Type 2 Diabetic patients and the effect on the liver: a case study of Bayelsa state," Journal of Mosquito Research, 6:1-8, Nig. Malaria Fact Sheet. (2011).

Nkuo A. T. K. Ajame, E. A., and Achidi E. A. (2002). "An investigation of symptomatic malaria parasitaemia and anaemia in nursery and primary school children in Buea District Cameroon," The Central African Journal of Medicine, 48:1-4.

Nwonwu E.U., Ibekw P.C., Ugwu J.I., Obarezi H.C., and Nwagbara O.C. (2009). Prevalence of malaria parasitemia and malaria related anaemia among pregnant women in Abakiliki. Southeast Nigeria, Nigerial Journal of clinical practice, 12:182-186.

Obianumba S.N., (2012). Prevalence of malaria among pregnant women attending antenatal clinic and knowledge and attitude of caregivers to malaria diagnosis in Ozubulu, Anambara State. M.Sc thesis submitted to the department of parasitology and entomology, faculty of biosciences Nnamdi Azikiwe University Awka.

Omoya O.F., and Atobatele O.O. (2017). Co-infection of malaria and typhoid fever among pregnant women attending primary health care centre, Ojo Local Government, Lagos, Nigeria, Health Sci. J, 11:2.

Perandin F., Manca N., Calderaro A., Piccolo G., Galati L., and Ricci L. (2004). "Development of real-time pcr assey for detection of Plasmodium falciparum, Plasmodium vivax and Plasmodium ovale for routine clinical diagnosis. Journal of clinical microbiology," 42:1214-1219.

Purfield A., Nelson A., Laoboonchai A., Congpuong K., McDaniels P., and Miller R.S (2004). "A new method for detection pfmdr1 mutations in Plasmodium falciparum DNA using real time PCR" Malaria Journal, 3.

Starzengruber P., Fuehrer H., Ley B., Thriemer K., Swoboda P., and Habler V.E. (2014). High prevalence of assymptomatic malaria in South-Eastern Bangladesh. Malar.J, 13:16.

Tjitra, E., Suprianto, S., Dyer, M., Currie, B. and Anstey, M. (1999).Field evaluation of the ICT malaria Pf/Pv immunochromatographic test for detection of Plasmodium falciparum and Plasmodium vivax in patients with presumptive clinical diagnosis of malaria in eastern Indonesia. J. Clin. Microbiol. 37:2412–2417.