Molecular Detection of Plasmodium Falciparum from Malaria Diagnosed Patients attending Mater Hospital

Egwurochi, WI[1]; Uchendu, DO[2]; Ngobidi, KC[3*]; Ogudu, KO[4]; Egbule, CU[5]; Ugwu, CN[6]; Ugwu, MN[7]; Anuna, CN[8]; Igwe, A[9]; Ukwuoma, H[10]; Ugwuanyi, CC[11]; Osigwe, AO[12]; Amadi, UB[13]; Maduekwe, AN[14]

[1,2,5-10,14]Microbiology Research Unite, Science Laboratory Technology Department, Akanu Ibiam Federal Polytechnic, Unwana Afikpo, Ebonyi State Nigeria

[3,11]Biochemistry Research Unite, Science Laboratory Technology Department, Akanu Ibiam Federal Polytechnic, Unwana Afikpo, Ebonyi State Nigeria

[4]National Arbovirus and Vector Research (NAVRC), Enugu Nigeria.

[13]Chemistry Research Unite, Science Laboratory Technology Department, Akanu Ibiam Federal Polytechnic, Unwana Afikpo, Ebonyi State Nigeria

[12]Biology Research Unite, Science Laboratory Technology Department, Federal Polytechnic, Isuochu Abia, State Nigeria

*Corresponding Author

Received:- 05 March 2024/ Revised:- 13 March 2024/ Accepted:- 18 March 2024/ Published: 31-03-2024

Abstract— *This research was aimed at evaluating the prevalence and molecular detection of Plasmodium falciparum from malaria diagnosed patients attending Mater Hospital. Venous blood samples (5ml) of 75 patients attending Mater Hospital were collected for preparation of thick blood firms for parasite screening and Nested PCR (nPCR) for Plasmodium falciparum (P. falciparum) gene detection. While thick film was prepared on microscopic slid, air dried, stained in field stain A and B., and viewed under the light microscope with x100 objective lens, 10µl each of whole blood sample used for PCR Plasmodium falciparum gene detection using the nested Polymerase Chain Reaction (nPCR) as a diagnostic tool. The PCR products were analyzed in ethidium bromide stained 2% agarose gel. Microscopic examination of the stained blood film showed the presence of rig form trophozoites, schizonts and gametocytes of P. falciparum confirming high prevalence of malaria in Afikpo within the sampled population as 50 persons out of the 75 collected blood samples showed positive for malaria parasite. Age distribution of the samples shows that the most affected were those within the age brackets of 10 – 15 years with a prevalence rate of 26 (52%) followed by those within the age bracket of 16 – 21 years 11(22%). However, the nPCR analysis showed the presence of Plasmodium falciparum gene that resolved at 250bp and 270bp in all the 50 samples. Thereby indicating that Plasmodium falciparum was the prevalent specie that responsible for malaria in Afikpo. Following this result, it is recommended that PCR be included as part of the diagnostic tools for screening of the causative specie of malaria as this will go a long way in ensuring effective treatment to prevent drug resistance*.

Keywords— malaria, molecular detection, PCR, plasmodium, prevalence.

I. INTRODUCTION

In this part of the world, malaria has constituted itself the most fearful ailments all year round. Its prevalence has been such that no single day passes without a reference to it as a main cause of an illness. Its devastating health impact has been recognized to be transmitted by species of female anopheles mosquitoes. In 2003, Mbogo *et al*. opined that of the over 400 Anopheles species, only 30 – 40 can transmit malaria with Anopheles gambiae being the principal vector. Malaria is the world's most deadly parasitic disease and is caused by infection with single-celled parasites of the genus *Plasmodium* belonging to the apicomplexan phylum. *Anopheles* mosquitoes transmit these parasites from one person to another in their bites (Microsoft Encarta, 2009).

In Nigeria and rest of endemic Africa, the bulk of malaria episodes are attributable to *P. falciparum*. With an estimated 28 million cases and 38 000 deaths in 2011, malaria remains a significant public health problem in Sub-Saharan Africa (Olawole *et al.,* 2014). The parasite destroys the red blood cells, leading to the clinical signs and symptoms such as fever, flu-like, chills, headache, muscle aches, tiredness, nausea, vomiting, diarrhea, and anemia and jaundice due to loss of red blood cells unless treated quickly the disease can kill within 24 hours: children under the age of five are particularly at risk (Wells *et al*., 2009).

Malaria treatment has defied many known antimalarial drugs and so there is need to actually ascertain the exact parasite responsible for malaria transmission in Afikpo, Ebonyi State Niger. It is based on this that this study is on molecular detection of *Plasmodium falciparum* from malaria diagnosed patients attending mater hospital was conceived.

II. METHOD

2.1 Study Area

The study was conducted in Afikpo Local Government Area in Ebonyi State of Nigeria. Afikpo Local Government Area is about 140KM South of Ebonyi State and is host to Akanu Ibiam Federal Polytechnic, Mater Misericordae Hospital and other private health institutions.

2.2 Sample Collection

Blood (5ml) samples of 71 patients attending Mater Hospital were collected. From the samples collected, thick blood firms were prepared for malaria parasite screening.

2.2.1 Parasitological preparation and Examination for Malaria Parasite

Thick blood films were made by using the end of a pipette to apply a large drop of blood on the slide to produce a thick smear. An area of about 15 mm × 15 mm was covered by the film. The blood films were air-dried and the slide placed on a horizontal position.

2.2.2 Thick film staining

Field stains A and B were used for staining. The slides were placed face downwards on a slide rack to air dry.

2.2.3 Microscopic Examination of Thick Film

Immersion oil was added by the edge and it spread to cover an area of about that equivalent to the diameter of the film. The blood films were examined under ×100 objective and malaria parasites recorded. The trophozoites, schizonts and gametocytes were looked for.

A smear will be considered negative for malaria parasites if no parasites was seen after examining at least 100 microscopic fields (Cheesbrough, 2005).

2.3 Result Interpretation

The presence of ring forms and Trophozoites of *Plasmodium* indicate positive results. The following plus sign scheme was used to report parasite numbers as described by Cheesbrough, (2005):

- 1 – 10 parasites per 100 high power fields +
- 11 – 100 parasites per 100 high power fields ++
- 1 – 10 parasites in every high power field +++
- More than 10 parasites in every high power field ++++

2.4 nPCR screening for *P. falciparum*

Blood samples of 50 microscopically malaria diagnosed and confirmed positive patients were collected and screened for the presence of *P. falciparum* gen using the nested PCR (nPCR) technique.

2.5 PCR Amplification

The two set of primer sequences used for the *Plasmodium* detection are; forward primer *(Pf1) 5[1]-agc gtg atg aga ttg aag tca g-3[1]* and the reverse primer (*Pf2*) *5[1]-ccc taa acc ctc taa tca ttg tc-3[1]*. The primers was designed from NCBI sequence data base and synthesized at Inqaba Biotec West Africa. A commercially prepared Master mix (Solis master mix) was purchased from Reddint Scientific, Lagos Nigeria. (The Solis master mix contains 2.5mM dNTPs, 5mM of $MgCl_2$, DNA polymerase enzyme, and 5X Go Taq buffer). The master mix Mixture for PCR comprises of 4µl of the master mix, 0.6µl each of the primers (Pf1 and Pf2) 5µl of the extracted DNA template, nuclease free water was used to make up the volume to 20µl in a 0.2ml PCR tube.

The following amplification conditions were adopted during amplification process; initial denaturation @95ºC for 5min, denaturation @95ºC for 30 sec, annealing @56ºC for 30 sec, elongation @72ºC for 1min, final elongation @72ºC for 5 min and final hold @4ºC for for 7min. The protocol was adopted and modified from Mohanty *et al.,* 2009.

2.6 Gel Electrophoresis

A 2% agarose gel was prepared by measuring 2g of the Agarose powder into 100ml of TAE buffer and microwaved until the powder dissolves completely. It was allowed to cool to cheek temperature before 10µl of ethidium bromide (DNA stain) was added into the liquid agarose gel. The gel was poured into the electrophoretic tray with the comb in place and allowed to solidify. The comb was gently removed to create a well on the gel where the amplicons and the DNA ladder are loaded. The gel with the loaded amplicons and ladder were run electrophoretically at 120V for 1hr30mins. A 100bp ladder was used which separates according to sizes of the DNA bands. The stained DNA bands were visualized under an ultraviolet transilluminator.

III. RESULT

TABLE 1
PERCENTAGE PREVALENCE OF POSITIVE PATIENTS

Result	Number	Percentage (%)
Positive	50	66.7
Negative	25	33.3
	75	100

Percentage distribution of malaria in the samples 75 patients indicates that 50 people making a prevalence of 66.7% were confirmed to be positive for malaria while 25 patients making a percentage of 33.3% were negative for malaria infection (tab. 1).

TABLE 2
DISTRIBUTION OF MALARIA POSITIVE PATIENTS ACCORDING TO AGE

Age bracket	Number positive	Percentage (%)
10-15	26	52
16-21	11	22
22-27	9	18
28-33	4	8
	50	100

Result of distribution of infection indicates that those within the age brackets of 10 – 15 years had the highest prevalence of infection of 26 (52%). This was followed by those within the age bracket of 16 – 21 years 11(22%). Those with age brackets of 22 – 27 years and 28 – 33 years had percentage infections of 18% and 8% respectively (tab. 2)

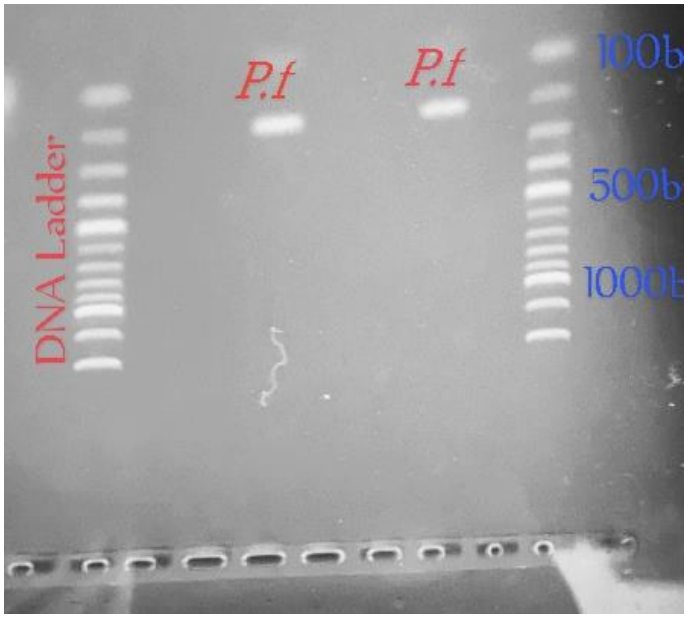

FIGURE 1: PCR RESOLUTION OF *PLASMODIUM FALCIPARUM (PF)*

Following the agarose gel electrophoresis of the amplicon extracted, *Plasmodium falciparum* (*Pf*) loaded was seen to resolve at 250bp (base pair) and 270bp (base pair) (tab. 1).

IV. DISCUSSION

This study is aimed at Molecular detection of *Plasmodium falciparum* from malaria diagnosed patients attending Mater Hospital Afikpo in Ebonyi State.

The high prevalence of malaria infection among patients within the age brackets of 10 – 15 years of age must not be unconnected with the fact that these people at this age brackets tend to expose themselves to mosquito bites without appropriate measure like sleeping under mosquito net. The high prevalence a of malaria among the age brackets of 10 – 15 years in this present study corroborates the work of Bawa and Auta (2014), had recorded higher percentage prevalence of 45.7% among those aged 10 to 19 years in Katsina. However, Bawa and Auta (2014), buttressed our position on the possible reasons for high rate of malaria infection when they remarked their study that the results indicated that some of the interviewed subjects do not sleep under mosquito nets. While the low prevalence observed among those at age brackets of 28 – 33 in this present study must not be unconnected with the ability of these people to access antimalarial drugs and sensitization about malaria infections.

The application of Polymarase Chain Reaction (PCR) in the detection of *Plasmodium falciparium* in all the 50 samples confirms the assumption that actually the major cause of malaria transmission in Nigeria including Afikpo is *Plasmodium falciparum.* The use of PCR in confirming the presence of *Plasmodium falciparum* in this present study gives a high level of understanding into the actual cause of malaria in Afikpo since PCR seems to be highly specific. Our position here is buttressed by the remark of Umeh *et al.* (2020). In their study on "molecular identification of *Plasmodium falciparum* isolates in Owerri municipality using nested polymerase chain reaction (nPCR)," Umeh *et al.* (2020), opined that polymerase chain reaction offers an alternative to microscopy having shown to have superior sensitivity and specificity.

It is possible that attitudes of people determine the transmission of this disease and many other diseases of human. WHO (2000), buttressed this point when it argued that the human behavioural pattern is a major epidemiological factor that impacts on disease transmission and progression in Africa and there is growing evidence that with appropriate awareness, education, attitude, attention to and chemotherapy of, the key symptoms of malaria, the incidence of severe malaria can be drastically reduced especially in the rural and urban areas where most of the estimated 2 to 3 million deaths per year from malaria occur.

The high occurrence of *Plasmodium falciparum* in all the blood samples in this present study, agrees with the of Uneke *et al.* (2005) in Jos who noted that in their study that *Plasmodium falciparum* was identified in all the cases.

The detection of *P. falciparum* by nPCR as applied in this present study might not just prevent misdiagnosis, incorrect treatment, false positives, false negatives reults, but also the emergence and spread of drug resistance, and the transmission of parasites from a malaria-endemic region to other parts as the drugs in use must be parasite specific.

V. CONCLUSION

This study was aimed at Molecular detection of *Plasmodium falciparum* from malaria diagnosed patients attending Mater Hospital Afikpo in Ebonyi State. Out of the 75 samples analyzed 50 samples showed the presence of *Plasmodium falciparum* which was confirmed with application of Nested Polymerase Chain Reaction. The result indicated that *P. falciparum* was responsible for the transmission of malaria in Afikpo and therefore for adequate measures in preventing the spread of malaria in this area such as sleeping under mosquito nets and use of appropriate and efficient diagnostic tools such as PCR for precise identification of causative organisms so as to enable appropriate recommendation of antimalarial drugs to reduce or eliminate malaria resistance to drugs.

PCR should be used in confirming the actual causes of malaria illnesses so as to be specific in treatment:

(i) People should adhere to the prescribed drugs for effective malaria treatment

(ii) All breeding sites for mosquitoes should be eliminated so as to terminate the mosquitoes-malaria transmission.

REFERENCES

[1] Bawa, J. A and Auta, T. (2014). Prevalence of malaria: Knowledge, attitude and cultural practices of pregnant women in Katsina Metropolis, Nigeria. *European Scientific Journal* **10**(21): 148 – 167

[2] Mbogo, C. M., Mwangangi, J. M., Nzovu, J., Gu, W., Yan, G., Gunter, J. T., Swalm, C., Keating, J., Regens, J. L., Shililu, J. I., Githure, J. I. and Beier, J. C. (2003). Spatial and temporal heterogeneity of Anopheles mosquitoes and *Plasmodium falciparum* transmission along the Kenyan coast. *American Journal of Tropical Medicine Hygiene,* **68**(6): 734 – 742.

[3] Cheesbrough, M. (2005). *District Laboratory Practice in Tropical Countries; Part.1,* UK: Cambridge University Press. 245-51.

[4] Olawole, O., Ozokonkwo, O. and Mokuolu, O. (2014). Prevalence of *plasmodium falciparum* malaria among patients attending University of Ilorin Teaching Hospital, Ilorin, Nigeria. *Animal Research International* **11**(3): 2063 – 2070,

[5] Wells, T. N., Alonso, P. and Gutteridge, W. (2009). New medicines to improve control and contribute to the eradication of malaria. Nature Reviews Drug Discovery, **8**(11): 879 – 891.

[6] Microsoft Encarta (2009). [DVD]. "Malaria." Redmond, WA: Microsoft Corporation, 2008.

[7] Umeh, SI., Nwamara, SA, Nwachukwu, IN. and Udensi, JU. (2020). Molecular Identification of Plasmodium falciparum isolates in Owerri Municipal using Nested Polymerase Chain Reaction (nPCR). *Int. J. Adv. Res.* **8**(08), 819-839.

[8] Unekea, CJ, Ogbua, O, Inyamab, PU and Anyanwu, GI (2005). Malaria infection in HIV-seropositive and HIV-seronegative individuals in Jos-Nigeria. *J Vect Borne Dis 42, December.* 151–154

[9] World Health Organization (2020). World Malaria Report; WHO: Geneva, Switzerland, Available online: https://www.who.int/ publications/i/item/9789240015791 (accessed on 8 December 2021).