Seroprevalence of Yellow Fever Virus and Plasmodium Falciparum in Patients Clinically Diagnosed of Malaria in Delta State, Nigeria

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Abstract: Mosquitoes are carriers of various disease causing agents that can cause diseases both in humans and animals. Malaria is a parasitic disease transmitted by infected female Anopheles mosquitoes while arboviruses are viral disease causing agents also transmitted by Aedes mosquitoes. It is recently considered a global threat to humans and has caused widespread morbidity in the world, especially in Africa. The study was carried out to determine the co-infection of Yellow fever virus and Plasmodium falciparum among residents of Delta North, Delta State, Nigeria. The study adopted a crosssectional survey and was carried out at Ogwashi-Uku central hospital, Kwale central hospital, and Agbor central hospital. Out of the 300 samples analyzed it was found that 62% were positive for *Plasmodium falciparum* using *PF* rapid diagnostic test (RDT) kit while 66.7% were positive on microscopy. The study revealed that Agbor central hospital had the highest prevalence rate of *P. falciparum* (Pf) representing 29% followed by Kwale central hospital 19.7% and then Ogwashi-Uku central hospital 13.3%. PCR technique was used to confirm P.falciparum at 205bp. Yellow fever (YF) immunoglobulins (IgG and IgM) were determined using enzyme linked immunosorbent assay (ELISA) technique. The study revealed the prevalence of yellow IgG and Plasmodium falciparum co-infection in Ogwashi-Uku central hospital as 4.8% and yellow fever IgM and *Plasmodium falciparum* co-infection as 1.1%. In Kwale central hospital the seroprevalence of yellow fever IgG and Pf was 7% while that of yellow fever IgM and Pf co-infection was 2.2%. Similarly, in Agbor central hospital the study reveals seroprevalence of yellow fever IgG and Pf as 9.1% while that of yellow fever IgM seroprevalence with Pf was 3.2%. This present study suggests that co-infections of yellow fever in malaria patients exists Delta North but in varying degrees. The extent of complications of the co-infection was beyond the scope of this present study.

Keywords: Seroprevalence, Immunoglobulins, Aedes Anopheles and Yellow Fever.

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I. INTRODUCTION

Arboviruses have been recently considered a global threat to human and public health. They have caused widespread morbidity in the world, especially in Africa (WHO, 2004). Arboviral diseases especially dengue viral disease, are among the most important of the emerging infectious diseases (WHO, 2008).

Malaria is one of the leading causes of acute febrile illness (AFI) in Africa and is transmitted by the bite of infected female anopheles mosquito (WHO, 2010). These vectors responsible for the transmission of Malaria parasite are basically nocturnal and indoor biters. Human malaria is commonly caused by five plasmodium species: *Plasmodium vivax, P. malariae, P. falciparum, P. ovale* and *P. knowlesi* each with their geographical location and varied incubation periods (IP), from infection to manifestation of symptoms with P. falciparum causing 80% of infections and 90% of deaths worldwide (Ito *et al.,* 2014). The wide spectrum of malaria morbidity and mortality is dependent largely on the complex pathogenesis of this parasitic infection.

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Micronutrients are known to influence the disease progression in man (Nmorsi *et al.*, 2007).

Mosquitoes are carriers of various pathogens that cause diseases in human population. Among these diseases are Malaria, Yellow Fever (YF), Dengue Fever (DEN), Chikungunya (CHIK) and West Nile virues. (CDC, 2007). It is estimated that over two billion people worldwide live in regions where these diseases are endemic (WHO, 2010). On the other hand, the vector responsible for Yellow fever virus transmission is an infected female *Aedes* mosquitoes (mostly, *Ae.aegypti* and *Ae. albopictus*) and are principally diurnal and outdoor biters. Cases of Yellow fever have been recently reported in various parts of Nigeria (Akaninyene *et al.*, 2019).

Co-infection among these diseases is possible in geographical locations where the respective vectors co-exist (Mohaptra et al., 2012). In 2009, Ayorinde and co proposed that co-infection of Yellow fever virus and Plasmodium falciparum is possible in localities where the vectors coinhabit (Ayorinde et al., 2009). Arboviral infections are often times misdiagnosed and treated as malaria due to their very similar clinical presentations (Senn et al., 2011). Arbovirus and malaria parasite co-infections have previously been reported in Nigeria (Senn et al., 2011), Senegal and in European travellers in Senegal, Guinea and Sierra Leone (Charrel et. al., 2005). Epelboin et al., (2012) opined that, concurrent dengue fever, yellow fever and malaria infection tends to be more severe than single infections as they were characterized by haematologic abnormalities, such as thrombocytopaenia and anaemia, which are known risk factors of severe yellow fever and/or malaria.

These fever causing diseases (malaria and yellow fever) have become significant public health diseases worldwide, especially in Africa due to their endemicity and similarities in signs and symptoms such as fever, severe joint and muscle pains, headache, sore throat, malaise, nausea, an irritating rash etc, (Baba et.al., 2009). Owing to these similarities in symptomatic presentations, there is possibility that persons infected with any of these microbial agents could be misdiagnosed when using clinical considerations alone without recourse to laboratory investigations. Most patients presenting with febrile conditions are often times treated with anti-malaria chemotherapy, especially in the developing countries like Nigeria where malaria is highly endemic; and viral diagnosis scarce and highly expensive. But it is possible that many of the febrile conditions could be of arboviral origin but due to lack of proper diagnosis are often not reported or reported as pyresia of unknown origin (PUO) in most hospitals.

Arboviruses are not systematically investigated and are generally only considered by clinicians, at best, when samples test negative for malaria and presumptive anti-malaria chemotherapy proves ineffective. Consequently, this has resulted to total absence of early identification and report of possible arboviral disease outbreak and potentially high morbidity and mortality may ensue (Baba *et. al.*, 2013; Monlun *et. al.*, 1993 and WHO, 2010). Management of malaria may be complicated due the coexistence of other disease causing agents that can also cause febrile symptoms. Treatment may be prolonged and drug resistance possible due to different levels of drug interactions.

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II. MATERIALS AND METHODS

Management of dengue virus disease is mainly by supportive

Study Area:

therapy (Domingues, 2009.).

The study was carried out in all the major hospitals within Delta North senatorial zone of Delta State. The phlebotomy unit of each of the hospitals was used as the sample collection point. Delta State lies roughly between longitudes $5^{\circ}00$ and $6^{\circ}45$ 'E and latitudes $5^{\circ}00$ and $6^{\circ}30$ 'N. It has a total land area of 16,842 sq. km. The states bordering Delta State are Edo State to the north, Ondo State to the northwest, Anambra State to the east and Bayelsa and River States to the southeast. On its southern flank is 160 km of the coastline of the Bight of Benin.

Study Population:

The study adopted a cross-sectional survey among patients visiting Agbor central hospital, Ogwashi-Uku Central hospital and Kwale Central hospital all in Delta North, Delta State. Both those clinically diagnosed of malaria and the apparently healthy patients were recruited for the study. Since the vectors for dengue virus and Yellow fever transmission are majorly outdoor biters, children below the ages of 2 years were excluded from the study are naturally meant to spend most of their times indoors.

Sample Size:

A total of 300 blood samples were collected from the volunteered participants, hundred and twenty (120) samples from Agbor central hospital, a hundred (100) samples from Kwale central hospital and eighty (80) samples from Ogwashi-Uku central hospitals selected. The sample size was be calculated using the formula:

Sample size (n) =
$$\frac{Z^2P(1-P)}{d^2}$$

Z = is standard normal variate (at 5% type 1 error (P<0.005) it is 1.96 and at 1% type 1 error (P<0.001) it is 2.58). As in majority of studies P values are considered significant below 0.005 hence 1.96 is used in the formula.

P = Expected proportion in population based on previous studies or pilot studies.

d = Absolute error or precision - Has to be decided by researcher.

Study Design:

The study adopted a cross-sectional design. A wellstructured questionnaire was used as a tool to extract demographic information of the patients and also to collate other relevant information concerning the study. Volume 10, Issue 4, April – 2025

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> Ethical Consideration:

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Ethical approval was gotten from Delta State Ministry of Health, Asaba. Ethical clearance was also obtained from Faculty of Science ethical committee, Delta State University, Abraka. Also, an informed consent of the recruited patients was sort before sample collection. The intent of the study was clearly made known to the interested participants in the language he/she understands. In any situation where the interested participants cannot speak for himself or herself, may be due to speech or ear defect, the patients' relative or any other person appointed by the participant was duly consented before sample collection.

Sample Collection:

Three millilitres (3 ml) of whole blood was collected aseptically through venopuncture using sterile 5 ml syringe and transferred into a sterile EDTA container already properly labeled. The samples were taken to National Arbovirus and Vectors Research Centre (NAVRC) Enugu for sample processing and analysis.

Sample Processing/Analyses:

• Microscopy

A thick blood film was made on a dry clean grease-free glass slide and stained with Giemsa stain. The stained slide was allowed to air-dry and then viewed under a light microscope to estimate the malaria burden of the sample. All the slide positive samples were further analyzed using rapid diagnostic test for *Plasmodium falciparum*.

• *Rapid Diagnostic Test (RDT):*

At the NAVRC Laboratory, rapid diagnostic testing for malaria parasite (*P. falciparum*) was done following standard operating procedures for the test according to the manufacturer's instruction.

• Serology Assay (Rapid Diagnostic Test and ELISA)

A rapid diagnostic testing (RDT) technique was used to detect Plasmodium falciparum antibody using а commercially prepared serology test kit. The plasma was harvested from each sample that were positive for Malaria microscopically and stored frozen at -20°C. The malaria positive samples were analyzed using Enzyme Linked Immunosorbent Assay (ELISA) to determine the ones that are also seropositive to Dengue Virus both Imunnoglobulin G (IgG) and immunoglobulin M (IgM). The kits for the ELISA analysis were purchased commercially from Inqaba Biotec West Africa. The manufacturers' protocol for the analysis was strictly followed and the optical density read out from the ELISA reader (Biobase 10, ELISA reader). Internal controls included in the commercial kits were Positive controls (PC), Negative controls (NC) and Cut-off value (COV). The NC, the OD value of each specimen and the COV was used to calculate the real values of the specimen to determine seropositivity and seronegativity of the specimen to dengue virus.

• Dry Blood Spot (DBS):

Seventy microlitre (70μ) of the whole blood was placed on DBS card and properly labeled. It was allowed to air-dry at room temperature inside a hood for 24hrs, then packaged inside a clean zip-lock bag and stored at -20°C until time for use. The samples preserved on DBS card are only the samples that are malaria-positive for both microscopy and RDT.

• Molecular Assay:

The samples on DBS card was used for molecular confirmation of *Plasmodium falciparum* from the positive RDT samples. *P. falciparum* was detected using conventional PCR technique. The primer sequences used for the *Plasmodium* detection are; forward primer (*Pf1*) 5'-agc gtg atg aga ttg aag tca g-3' and the reverse primer (*Pf2*) 5'-ccc taa acc ctc taa tca ttg tc-3'. The primers was designed from NCBI sequence data base and synthesized at Inqaba Biotec West Africa.

The DNA was extracted from the mosquitoes using ZymoResearch kit purchased from BioLabs England. The manufacturers' protocol was strictly followed for the DNA extraction. The following amplification conditions were adopted during amplification process; initial denaturation @95°C for 5min, denaturation @95°C for 30 sec, annealing @58°C for 30 sec, elongation @72°C for 1min, final elongation @72°C for 5 min and final hold @4°C for for 7min. The protocol was adopted and modified from Mohanty *et al.*, 2009.

After amplification, the amplicons were stained with ethidium bromide and run on 2% agarose gel for 1hr30mins at 120V. The stained DNA bands were visualized under an ultraviolet transilluminator.

III. RESULTS

Out of the 300 samples analyzed 65 % Of them were female while 35% were males. It was also shown that participants within the age bracket 0f 31 - 40 had the highest percentage of 20% followed by age group of 21 - 30 with 19.7%. Similarly, traders participated mostly in the study representing 26% followed by civil servants 25.3% and the least were drivers 5% only (Table 1). it was found that 62% were positive for Plasmodium falciparum using PF rapid diagnostic kit but 66.7% were positive for malaria on microscopy. Also, Plasmodium falciparum were more predominant amongst female participants 32.7% than their male counterparts 24%. (Table 2). Comparatively, across the hospitals, Agbor central hospital had the highest prevalence rate of *P. falciparum* representing 29% followed by Kwale central hospital 19.7% and then Ogwashi-Uku central hospital 13.3% using rapid diagnostic testing kit. Table 3. Majorly, the participants in this study were traders 58(31.2%), followed by civil servants 52(28%), students 41(22%), Farmers 28 (15.1%), Drivers 4 (2.2%) and those with unspecified occupation (others) 3(1.6%).

Molecularly, the *Plasmodium spp* was confirmed molecularly using conventional PCR technique as *Plasmodium falciparum* at 207bp **Fig 1.**

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Table 1 Demographic Information of the Participants

Variable	Number recruited (N)	Percentage (%)			
Gender		0 、 /			
Males	105	35			
Females	195	65			
Total	300				
Age Group					
5-10	35	11.7			
11-20	46	15.3			
21-30	59	19.7			
31-40	60	20.0			
41-50	56	18.7			
50&aboe	44	14.7			
Total	300				
Occupation					
Farmer	47	15.7			
Civil Servant	76	25.3			
Student	61	20.3			
Trader	78	26.0			
Driver	15	5.0			
Others	23	7.7			
Total	300				

Table 2 Prevalence of Malaria Among Gender and Age Groups

Variables	Number Tested	+Slides (%)	+RDT (%)	
Gender				
Male	105	86 (28.7)	72 (24)	
Female	195	124 (65)	98 (32.7)	
TOTAL	300	210 (70)	170 (56.7)	
Age groups				
5 - 10	35	10 (3.3)	8 (2.7)	
11 - 20	46	15 (5.0)	11 (3.7)	
21 - 30	69	38 (12.7)	30 (10)	
31 - 40	60	45 (15)	38 (12.7)	
41 -50	56	23 (7.7)	19 (6.3)	
50 & above	44	12 (4.0)	4 (1.3)	
TOTAL	300	143 (47.7)	110 (36.7)	

Table 3 P	revalence	of Malaria	Parasites	Across	the	Hospitals

Hospital	No. of Samples (%)	+Slides (%)	+RDT (%)
Ogwashi-Uku Central hosp.	80	47 (15.7)	40 (13.3)
Kwale central hosp.	100	62 (20.7)	59 (19.7)
Agbor central hosp	120	91 (30.3)	87 (29)
Total	300	200 (66.7)	186 (66)



Fig 1 Molecular Confirmation of *Plasmodium Falciparum* from The Three Hospitals Within Delta North Senatorial District. The *Pf* Is Confirmed at 205bp as Shown Above. L1 and L7 Are The 100bp DNA Ladder. L3, L4, And L5 Are The

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Hospitals	No. of Samples	+Pf/IgG (%)	+Pf/IgM (%)
Ogwashi-Uku Central hospital	40	9 (4.8)	2 (1.1)
Kwale central hospital	59	13 (7.0)	4 (2.2)
Agbor central hospital	87	17 (9.1)	6 (3.2)
Total	186	39 (20.9)	12 (6.5)

Table 4 Prevalence of Yellow Fever Immunoglobulins and *Plasmodium falciparum* Across the Hospitals.

IV. DISCUSSION

Plasmodium infection can be complicated when it coexists with other viral pathogens like arboviruses. Yellow fever outbreak has been recently reported in almost all the States of Nigeria. Confirmed cases of yellow fever with varying degree of complications have also been reported by Nigeria centre for Dissease Control and Prevention (NCDC). This study was targeted at determining the prevalence of Yellow fever virus infection in patients that have Plasmodium infection. In this present study it was found that 66.7% was positive for malaria and 62% of it was confirmed to be Plasmodium falciparum using molecular technique. The high prevalent rate contradicts an earlier report of 21.1% by Tadesse et al., 2022. This discordant may be due to seasonal variation, age difference and geographical location.

In this present study, female participants were more in number (65%) than their male counterparts (35%). Microscopically, malaria prevalence according to gender was found to be male (28.7%) and female (65%), while using rapid diagnostic technique, Plasmodium falciparum prevalence was still more among females (32.7%) than males (24%). This finding is similar to an earlier report by (Teck-Hui *et al.*, 2018). This could be partially as a result of the population of females being higher than that of males in Delta State. Also, females seem to be more exposed to the bite of Anopheles mosquitoes due to their dressing pattern (sleeveless gowns and short skirts) especially during evening hours while doing domestic activities in the house. The finding contradicts the previous report by Ayorinde, *et al.*, 2009, they opined that males stay late outside during the night and are more prone to mosquito bites than female counterparts (Ayorinde, *et al.*, 2009).

Considering the age groups in this present study, it was found that those within the age bracket of 31 - 40 years had more prevalence of *Plasmodium falciparum* (12.7%) followed by those within the age of 21 - 30 years than every other age group. This observation is similar to previous reports by Ayorinde *et al.*, 2009 and Sow, *et al.*, 2016; who independently reported high prevalence rate amongst people in the same age bracket.

The study also showed that Agbor central hospital had the highest prevalent rate of plasmodium falciparum 29% followed by Kwale central hospital 19.7% and then Ogwashi-Uku central hospital 13.3%. This outcome could be as a result of the strategic locations of the three different hospitals. Agbor central hospital is located around a thickly populated environment and has more sick patients visiting the hospital. Also, because of the population of the area, more breeding sites are possibly available for Anopheles mosquitoes. It is important to sensitize the inhabitants of the environment about the breeding sites of mosquitoes around their vicinity. Integrated training of the inhabitants on larval source management will go a long way to reduce the burden of malaria in Agbor and its environs. The Plasmodium prevalence rate in Kwale central hospital was second to what was observed at Agbor central hospital. The high prevalence ISSN No:-2456-2165

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rate observed in Kwale was attributed to the environmental hygiene of the area that possibly encourages the breeding of *Anopheles* mosquitoes. This observation was similar to an earlier report by Adeleke *et al.*, 2016 who also attributed the high prevalence rate they observed to poor environmental sanitation.

This present study shows that there is a significant difference between the positive and negative yellow fever IgM seroprevalence (p-value = 0.119), and there is a significant difference between the genders (p-value = 0.460). The ANOVA table suggests that there is a significant difference between the positive and negative seroprevalence groups, but no significant difference between the genders. Similarly, the study also reveals that there is no statistically significant difference in the means of IgG levels between females and males (p-value = 0.4386 > 0.05) and there is no statistically significant difference in the means of IgG levels between negative and positive groups (p-value = 0.1190 >0.05). This implies that irrespective of the gender, yellow fever virus infection is possible once exposed to the bite of an infected female Aedes mosquito. This finding contradicts an earlier report by Akaninyene et al., 2019. However, the data shows that the majority of the studied participants had negative results for yellow fever IgM, with a higher total count, average, and variance compared to positive results. This suggests that the seroprevalence of yellow fever IgM is low in the study population. Furthermore, the data shows that the seroprevalence of yellow fever IgM was highest in the age group of 31 to 40, Age may be a significant factor in explaining the variation in IgG levels in the context of yellow fever seroprevalence. The average IgG levels vary significantly among different age groups, indicating that age may play a role in the immunity and seroprevalence of yellow fever. Further research may be needed to explore the underlying mechanisms and implications of these findings which suggests that there may be age-related factors influencing the seroprevalence of yellow fever IgM.

Also, the study shows that the seroprevalence of yellow fever IgM was highest among traders, with a total count of 58, an average of 29, and a variance of 800. The seroprevalence was lowest among drivers and others, with total counts of 4 and 3, respectively. This suggests that occupation may be a potential factor influencing the seroprevalence of yellow fever IgM, with traders potentially being at a higher risk. The reason could be that traders spend most of their time outside during the biting peaks of yellow fever vectors and more exposed to mosquito bites. This finding is in discordant with an earlier report by Chukwuma *et al.*, 2018 and (Teck-Hui *et al.*, 2018). They independently reported that students had a higher prevalent rate of arbovirus infection probably because they found more outside during the day and at the biting peaks of *Aedes* mosquitoes.

However, the findings from the ANOVA suggest that occupation may be a significant predictor of Yellow fever IgG seroprevalence in the study population. This indicates that different occupations may have varying levels of exposure to Yellow fever, which could be due to differences in occupational activities, geographical location, or personal behaviors. For example, traders and civil servants may have higher exposure to mosquitoes, which are the vectors for transmitting the Yellow fever virus, due to their outdoor activities or travel patterns, resulting in higher seroprevalence rates. On the other hand, drivers and individuals in other occupations may have lower exposure due to different work environments or lifestyles, resulting in lower seroprevalence rates

Similarly, co-infection of yellow fever and Plasmodium falciparum was higher amongst patients from Agbor central hospital (3.2%). This high prevalent co-infection rate observed at Agbor could be attributable to the dense population of the area and the poor environmental sanitation that supports the breeding sites of both Anopheles and Aedes mosquitoes, the vectors for malaria and yellow fever respectively. Kwale central hospital and Ogwashi-Uku central hospital showed a significant co-infection rate of 2.2% and 1.1% respectively. This general implies that the vectors for transmission of yellow fever virus and Plasmodium falciparum co-inhabit in Delta State. It is also suggestive that complications of malaria infection in Delta State could be as a result of arbovirus co-infection. Diagnosis of malaria cases should be done alongside arboviral screening to detect possible co-infection and inform the clinicians of the best treatment option to adopt. Early detection of the co-infection will reduce the complication burden associated with arbovirus and Plasmodium falciparum co-existence in a febrile patient.

V. CONCLUSION AND RECOMMENDATIONS

A significant proportion of our subjects had yellow fever Immunoglobulins. Yellow Fever-Malaria co-infections were confirmed amongst citizens of Delta North senatorial zone, Delta State. This is a threat to rational anti malaria drug use and may be promoting anti malaria resistance since yellow fever are not routinely detected among febrile patients in Nigeria. Based on our findings, we recommended inclusion of arboviral screening among patients presenting with febrile illness in Delta State. Findings from this study confirmed the assumption that Aedes species (potential vector of Yellow fever virus) exists in Delta State and perhaps, yellow fever viruses are in circulation in the State. Future entomological study in Delta State is also recommended to confirm this assumption. For better understanding of the circulating viral strain, further molecular characterization of yellow fever virus is recommended.

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