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Research Article

Molecular Detection of Malaria Co-Infections with Some Arboviruses in Pregnant Women Attending Ante-Natal in Hospitals within Bauchi State, Nigeria

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ABSTRACT
Malaria and arboviruses are public health menaces, causing major morbidity in developing countries and are more severe in pregnancy leading to adverse effects which can lead to fetal and maternal death, hence, this study determined the prevalence of dengue, zika and malaria infections among pregnant women attending antenatal clinics in some hospitals within Bauchi Local Government Area, Bauchi State, Nigeria. One hundred and seventy-seven blood samples of pregnant women were collected and screened for malaria using the Rapid Diagnostic test kits, while the diagnosis for dengue and zika infections was done using real time Polymerase Chain Reaction (RT-qPCR). From the blood samples tested, 50 (28.25%) were positive for malaria, 1 (2.00%) sample was positive for dengue and dengue-malaria co-infection, however, no sample was positive for zika virus (0%). Malaria was recorded across all the age groups with a high statistical significance, dengue was prevalent between age 15-25years (2.35%) and 26-35years (1.35%) while dengue-malaria co-infection was only seen in age group 15-25years (1.18%). Pregnant women with fever and the trimesters were significantly associated with the prevalence of malaria ($P<0.05$ respectively); Educational qualification and occupation were significantly associated with dengue infections ($P<0.05$ for both) as well as dengue-malaria co-infection ($P=0.05$ for both), whereas history of miscarriages was only statistically associated with prevalence of dengue infections ($P=0.02204$). Despite the low prevalence of dengue virus among pregnant women in this study, the result of this study gives an overview of dengue, zika and malaria in Bauchi, thereby providing the first baseline data.

Keywords: Dengue; Zika; Malaria; Pregnant; Women; Hospitals

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INTRODUCTION

The worldwide spread of vector-borne diseases, especially in tropical and subtropical regions has recently increased with vector-borne diseases representing 17% of all infectious diseases and generating over a million deaths worldwide (Padilla *et al*., 2017; Osarumwense *et al*., 2022). Malaria and other mosquito-borne arboviruses (such as Dengue and zika) are common vector-borne diseases of the tropical and sub-tropical regions and due to similarity in onset symptoms, they are mostly misdiagnosed (Nassar *et al*., 2019; Mubashir, *et al*, 2020; Miri *et al*. 2021; Osarumwense *et al*., 2022). Both diseases are transmitted through bites of mosquito of the genus *Anopheles* and *Aedes* respectively; and while the mosquitoes transmit these infections causing immense suffering to humans, they are not affected by these infections (WHO, 2022).

In recent years, Dengue has become a significant public health concern in Asia and Latin America, while in African, is gradually becoming a cause for alarm, especially with ubiquity of the *Aedes spp*. mosquito vectors within the region (Ogwuche *et al*., 2023). Isa *et al*. (2021) stated that over 3.9 billion people in 129

countries are at risk of DENV infection and 390 million dengue infections occur per year of which 96 million manifests clinically. Although this is of global public health significance, little attention has been given to Dengue or *Aedes*-borne arboviruses especially in Africa (Isa *et al*., 2021). The World Malaria Report in 2021 published by WHO (2022) revealed 241 million cases of malaria in 2020 marking a 14 million increase from the previous year. Also, malaria caused an estimated 627,000 deaths in 2020, with more than half of the deaths in Africa, and Nigeria had the highest death rate at 31.9% (WHO, 2022).

The risk of spontaneous abortions, premature births and stillborn children is 30% higher in pregnant women who contract malaria, because malaria parasites compete favourably with the foetus for nutrients in the mother's blood in the placenta. This leads to severe anaemia which may result in poor oxygen supply to the unborn baby causing, low birth weight and a reduced chance of survival for the child. Pregnant women can transmit the dengue virus to their foetuses during pregnancy or around the time of birth and this can lead to low birth weight, premature birth and foetal death (Centre for Disease Control, CDC, 2024).

In Nigeria, most cases of dengue are underdiagnosed or misdiagnosed as malaria, typhoid or referred to as fever of unknown cause (Oderindea *et al*., 2020; Isa *et al*., 2021), as such, greater than 70% of febrile illnesses are treated presumptively as malaria, often without a laboratory evaluation for other possible causes of fever (Onyedibe *et al*, 2018).

Dengue and malaria are prevalent in Nigeria due to the established presence of mosquito vectors that transmit them and published reports on their prevalence, mostly reporting them as single infections (Ayorinde *et al*., 2016; Omar *et al*., 2022; Osarumwense *et al*., 2022; Shiraz, 2023), therefore, there is the need to explore different localities for in-depth understanding of the infection rate. Generally, Infants, children under 5 years, pregnant women, travellers and people with HIV or AIDS are at higher risk of severe infection with malaria and worst with severe dengue (WHO, 2022) since dengue in pregnancy adversely affects maternal and foetal outcomes with high maternal mortality of about 15. 9% resulting in risks of prematurity and postpartum haemorrhage to mother and baby, hence, this study aims to investigate the prevalence of dengue, zika and malaria co-infections among pregnant women attending antenatal clinics in some major hospitals within Bauchi Local Government Area, Bauchi State, Nigeria.

MATERIALS AND METHODS

Study Area

Bauchi is one of the twenty Local Government Areas of Bauchi State, situated in the Nigeria's North-Eastern geopolitical zone created in 1976. The state is located between latitudes 9°30' and 12°30' North of the equator, and between longitudes 8°45' and 11°0' East of the Greenwich meridian (Waziri *et al.*, 2018). Bauchi LGA covers a landmass of 3,687kmsq and a population of 493,810 as in 2006 census.

Study Sites

The study sites for the collection of samples were Abubakar Tafawa Balewa Teaching Hospital (ATBUTH) (10.2784° N, 9.7968° E), Bauchi, General Hospital Bayara, Bauchi (10.2303° N, 9.7436° E) and the Specialist Hospital Bauchi (10.3220° N, 9.8320° E).

Ethical Approval

Ethical approvals were obtained from Abubakar Tafawa Balewa University Teaching Hospital, Bauchi, Bauchi State and the Bauchi State Ministry of Health. Consent forms were also duly signed by prospective participants after being given detailed information on the research.

Study population

The study population sampled include all pregnant women present as at the time of blood sample collection and only consenting pregnant woman were sampled. Questionnaires were also administered for demography data and other useful information.

Collection of Blood samples and Testing

5mls of venous blood were collected into a well labelled plain sample bottles by licensed nurses or any approved health worker from all consenting participants and placed in a cooling box. The box was then transported to the Molecular Genetics and Infectious Diseases research laboratory (MOGID lab), Abubakar Tafawa Balewa University, Bauchi, Bauchi State where the blood samples were centrifuged at 3000rpm for 5mins. The serum was carefully separated from the plasma using a Pasteur pipette and transferred into a clean collecting tube and stored in the refrigerator at -80^{o}C (WHO, 2018) for further diagnostic test.

Malaria Diagnostic Test

The malaria test was carried out immediately at point of blood collection using the Malaria Antigen *Plasmodium falciparum* rapid diagnostic test kits (HRP2). A drop of the blood was dropped on the designated point of the cassette directly from the syringe before transferring the remaining blood into a plain container. Buffer solution was dropped immediately onto the blood on the cassette and allowed to stand for about 20 minutes. The results were interpreted based on the manufacturer's guideline and recorded accordingly. The positive samples were further analysed for dengue and zika viruses to establish co-infection.

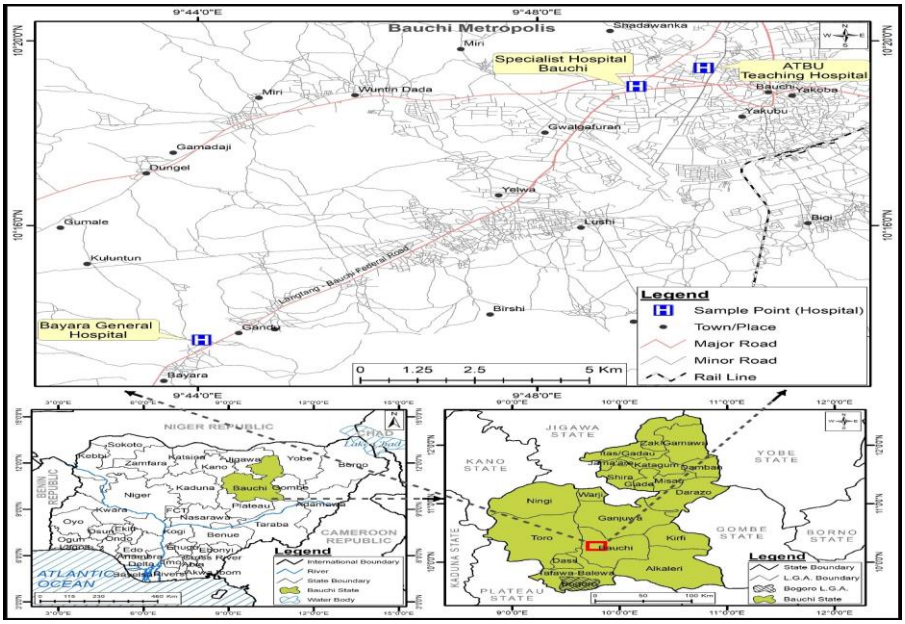

Figure 1: Map of Sampled Hospitals within Metropolis, Bauchi State
Source: https://www.openstreetmap.org

***RNA Extraction and* Real Time Polymerase Chain Reaction (RT-qPCR) for Dengue and Zika Viruses**

Total RNA was extracted from 190ul of the stored serum using Qiagen viral Extraction kits (Qiagen, Hilden, Germany) and in 40ul of elution buffer as directed by detection kits used for the RT-qPCR. One-step reverse transcriptase (RT) real-time (qPCR) was carried out to detect the pathogens in a single run for each of the viruses using Novaplex™ Tropical fever virus assay (RUO) (Seegene, South Korea) according to manufacturer's instruction to qualitatively screen blood samples for arboviruses. The assay involves three steps performed in a single tube which are the conversion of target and Internal Control RNA to cDNA through reverse transcription, PCR amplification of target and Internal Control cDNA and detecting the PCR amplicons using fluorescent dye each specific for its targeted virus. The amplification protocols involved a 20-minute reverse transcription at 50˚C, followed by 2 minutes activation of Taq polymerase at 95˚C, succeeded by 45 cycles of amplification consisting of heating at 95˚C for 10seconds, 60˚C for 15seconds, and extension at 72˚C for 10seconds. Positive samples for the dengue and zika were detected at ≤45 critical threshold (ct) values.

Data Analysis

Data were saved in Excel, and also transferred to R Console software version 3.2.2 which was used for statistical analysis. Pearson's Chi-square test was used to compare the frequencies at 95% confidence interval.

RESULTS

A total of 177 blood samples were screened from 3 major hospitals and 50 (28.25%) were positive with Malaria. The 50 samples positive with malaria were further screened for dengue and zika viruses and 1 (2.00%) was positive for dengue which was a case of dengue-malaria co-infection while none of the samples was positive with zika virus 0 (0%). However, the prevalence of the dengue, zika, malaria and the dengue-malaria co-infection were all not statistically significant across the sampled hospitals (Table 1).

Malaria was seen to be prevalent across all the sampling months with the highest prevalence in April 12 (33.33%) which was also the only month were dengue virus 1 (2.00%) and the only dengue-malaria co-infection 1 (2.00%) were detected. The prevalence of dengue and the dengue-malaria co-infection across the months were not statistically significant (P = 0.0916) (Table 2).

The highest prevalence with malaria was 26 (30.59%) and highest prevalence of dengue which was 1 (2.00%) were recorded in the 15-25years age group, However, only the prevalence of malaria was statistically significant across the age groups (P=0.0062) (Table 3). Also, the only positive dengue-malaria co-infection were seen in the 15-25years age group 1 (2.00%) but it is not statistically significant (Table 3)

Table 1: Prevalence of Malaria, Dengue and Zika in Pregnant Women Sampled from Some Hospitals within Bauchi Metropolis

Hospitals	No. Examined	No. Positive with Malaria (%)	No. positive with Dengue (%), n=50	No. positive with Zika (%); n=50	No. Positive with Dengue and malaria (%); n=50
ATBUTH	57	13 (22.81)	0 (0.0)	0 (0.0)	0 (0.0)
General Hospital Bayara	60	19 (31.67)	1 (2.00)	0 (0.0)	1 (2.00)
Specialist Hospital Bauchi	60	18 (30.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	**177**	**50 (28.25)**	1 (2.00)	0 (0.0)	1 (2.00)
P values		0.4552	0.1353		0.1353

Table 2: Prevalence of Dengue, Zika and Malaria in the Pregnant Women Sampled across the Months

Month	No. Examined	No. Positive with Malaria (%)	No. positive with Dengue (%)	No. positive with Zika (%)	No. Positive with Dengue and malaria (%)
January	36	10 (27.78)	0 (0.00)	0 (0.00)	0 (0.00)
February	36	11 (30.56)	0 (0.00)	0 (0.00)	0 (0.00)
March	34	6 (17.65)	0 (0.00)	0 (0.00)	0 (0.00)
April	36	12 (33.33)	1 (2.00)	0 (0.00)	1 (2.00)
May	35	11 (31.43)	0 (0.00)	0 (0.00)	0 (0.00)
Total	**177**	**50 (28.25)**	1 (2.00)	0 (0.00)	1 (2.00)
P values		0.243	0.0916		0.0916

Table 3: Age-Related Prevalence of Dengue and Malaria in the Sampled Pregnant Women

Age group	No. Examined	No. Positive with Malaria (%)	No. positive with Dengue (%)	No. positive with Zika (%)	No. Positive with Dengue and malaria (%)
15-25	85	26 (30.59)	1 (2.00)	0 (0.00)	1 (2.00)
26-35	74	22 (29.73)	0 (0.00)	0 (0.00)	0 (0.00)
≥36	18	2 (11.11)	0 (0.00)	0 (0.00)	0 (0.00)
Total	**177**	**50 (28.25)**	1 (2.00)	0 (0.00)	1 (2.00)
P value		0.0061	0.1353		0.1353

Malaria and dengue were prevalent in pregnant women in their second and third trimester while no infection was recorded in pregnant women that are in their first trimester (Table 4). Prevalence of malaria in regards to trimester was highly significant statistically (P<0.05) while the prevalence of dengue (P=0.1035) and the co-infection (P=0.1353) were statistically not significant (Table 4). Fever in the pregnant women was significantly associated with malaria (P=0.0001) but not statistically significant in relation to dengue infection (P=0.4598) and the co-infection (P=0.4028). In regards to the educational background of the pregnant women, there is a high statistical significance in the prevalence of dengue infection (P=0.0002) and the dengue-malaria co-infection (P=0.0074) in the pregnant women while the prevalence of malaria infection is not statistically significant. There was high prevalence of malaria infection (33.33%), dengue infection (11.11%) and the dengue-malaria co-infection (11.11%) recorded in the women that are tailors with a high statistical significance in the prevalence of dengue (P<0.05) and also the dengue-malaria co-infection (P<0.05), while there was no statistical significant difference with the malaria infection (P=0.4998) across the occupations. The highest prevalence of malaria was recorded in women that had 3 miscarriages (40.00%) while the three dengue and only dengue-malaria co-infection cases were observed in those that never had miscarriages (2.86% and 0.95% respectively). The prevalence of dengue infection is statistically significant with number of miscarriages (P=0.0220) (Table 4).

Table 4: Prevalence of Dengue and Malaria in the Sampled Pregnant Women in Relation to Some Risk Factors.

	No. examined	No. Positive with Malaria	No. Positive with Malaria and Dengue
Trimester			
1st trimester	4	0 (0.00)	0 (0.00)
2nd trimester	86	21 (24.42)	1 (4.76)
3rd trimester	87	29 (33.33)	0 (0.00)
P value		0.001	0.0086
Presenting with Fever			
Yes	34	15 (4.12)	0 (0.00)
No	143	35 (24.48)	1 (2.86)
P value		0.001	0.0908
Educational Qualification			
None formal/none	21	6 (28.57)	0 (0.00)
Primary	25	7 (28.00)	1 (14.29)
Secondary	91	29 (31.87)	0 (0.00)
Tertiary	40	8 (20.00)	0 (0.00)
P value		0.4221	0.001
Occupation			
Business/Trade	69	21 (30.43)	0 (0.00)
Civil Servants	14	3 (21.43)	0 (0.00)
Housewife	60	17 (28.33)	0 (0.00)
Tailoring	9	3 (33.33)	1 (33.33)
Others	25	6 (24.00)	0 (0.00)
P value		0.4998	0.001
Number of Miscarriage			
0	105	28 (26.67)	1 (3.57)
1	44	15 (34.09)	0 (0.00)
2	19	4 (21.05)	0 (0.00)
3	5	2 (40.00)	0 (0.00)
4	4	1 (25.00)	0 (0.00)
P value		0.0967	3.57

DISCUSSION

The results from this study revealed a low prevalence of dengue, zika and dengue-malaria co-infection although the prevalence of malaria is high. The low prevalence of dengue and zero prevalence of zika in this study is similar to the research of Shaibu et al. (2023) who reported zero prevalence of DenV, ZikV, RVFV and ChikV but only 3% positive for only Yellow fever virus from febrile patients in Delta State samples (Ct values: 32–38); from the samples screened from Lagos, Kwara, Ondo and Delta States (0.75% for the total samples) between 2018 and 2021 using real time RT-qPCR. However, the prevalence of this study is lower when compared to studies of Onyedibe *et al*., 2018 from Jos and Maiduguri (2.9% and 1.8% respectively; 2.3% overall prevalence), 2.2% by Dawurung (2010) in Jos and 1.8% by Idoko *et al* (2014) in Kaduna. Other higher prevalences of 17.2% DenV was reported in apparently healthy individuals in Ogbomosho by Oladipo, Amanetu *et al*. (2018), 19.3% in febrile patients within three locations in Adamawa State and 44.7% in febrile patients from hospitals within Abia, Nasarawa and Kaduna States of Nigeria (Mac *et al*., 2023).

Dengue-malaria co-infection was seen in only one subject (1.67%) in one of the hospitals, showing that indeed there is dengue-malaria co-infection and supports the report of Mac *et al*. (2023) on the coinfections of malaria and flaviviruses within Nigeria, with Tizhe *et al*. (2022) in Adamawa reporting higher prevalence of 19.40%. The co-infection with dengue and malaria may indicate the suitability of environmental conditions for *Anopheles* and *Aedes* mosquitoes to thrive and transmit diseases. The study of Gebremariam *et al*. (2023) showed a rise in the prevalence of malaria and dengue co-infection in Africa to be 0.9%, 3.8% and 5.5% in 2008-2013, 2014-2017 and 2018-2021 respectively, resulting in an overall prevalence of 4.2%, hence, the need for vigilance and more thorough investigation toward proffering solutions.

Malaria in the pregnant women was recorded in all the five months of collection while the dengue-malaria co-infection detected in this study was only in samples collected in the month of April which was contrary to

the report of Kazaure (2019) who reported a high prevalence of DenV in February (20.0%) with low prevalence in July (4.7%) in febrile patients in Jigawa State of Nigeria while Mac *et al.* (2023) reported high seroprevalence of flaviviruses in October from febrile patients in Abia, Nasarawa and Kaduna States.

The prevalence of the dengue-malaria co-infection in this study was observed in ages 15-25years which is similar to the report of Okonko *et al*. (2023) of 6.7% in Port Harcourt in pregnant women between 16-20years. Oladipo et al. (2018) in Ogbomoso reported highest prevalence of 25.0% in 16-30 years which is similar to the age group of this study and Dawurung reported 3.1% in ages 21-30years. However, Kazaure (2019) reported a high prevalence of 20.4% in age group 36 - 45 years in febrile patients from Jigawa showing a higher prevalence in older people in contrast to the result of this study.

The prevalence of dengue-malaria co-infection in pregnant women that are in their second trimester from this study is in agreement with the study of Ebrahim (2021) in Northern Province of India, where a 6-months pregnant woman admitted for suspected malaria, later diagnosed with malaria and dengue co-infection. It is not surprising that the pregnant women presenting with fever were highly significantly associated with the prevalence of malaria since fever is a symptom of malaria in most febrile patients.

The high prevalence of malaria and dengue-malaria co-infection in tailors from this study agrees with several other studies that reported an increased risk of dengue infection among manual laborers and tailors, and this can be attributed to their continuous movement that results in increased release of carbon dioxide, which is a natural attractant to mosquitoes. Also, their extended time outdoors or in an open environment increases their risks of mosquito bites and possible infections by mosquito-borne pathogens (Raji & DeGennaro, 2017; Wang *et al*., 2021; Okonko *et al*., 2023).

CONCLUSION

This study has established that malaria and dengue-malaria co-infection are prevalent of in pregnant women within Bauchi, indicating a significant issue and a potential threat. Due to the biological and clinical similarities of dengue and malaria, the suspicion of dengue-malaria co-infection should be highly considered and included in screening in pregnant women during antenatal. Moreover, there is need for further comprehensive research into the current situation of co-infections between malaria and arboviral diseases and the need for effective public health policies and investments in health education.

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