

Original Article

Pattern of Mosquito Borne Parasitic Infection in the Night Blood Samples of Patients with Elevated TNF- α of > 5.0 pg/mlMathew Folaranmi Olaniyan^{1*}, Tolulope Busayo Ojediran¹, Donatus Fn Ozuruoke²¹Department of Medical Laboratory Science, Edo University Iyamho – Nigeria²Education Department, Medical Laboratory Science Council of Nigeria, Abuja

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ABSTRACT

Study Background: *Plasmodium spp.*, (Protozoan) and *Wuchereria bancrofti* (Nematode) are transmitted by mosquitos to cause insect borne diseases known as malaria and Lymphatic filariasis/Elephantiasis. Apart from the social implication of these parasitic infections the infections can also elicit immune responses. **Aim and Objective:** This work was therefore designed to determine the pattern of mosquito borne parasitic infection in the night blood samples of patients with elevated TNF- α of > 5.0 pg/ml. **Materials and Methods:** Seventy (70; aged 31 – 76 years; Male- 35; Female-35) volunteers with plasma TNF α of 5.8 \pm 0.7 pg/ml including age-matched control participants (n= 50 ; TNF α of 2.2 \pm 0.3 pg/ml). All participants were negative to Acid Fast Bacilli, anti-HCV, HBsAg and HIV tests were recruited for the study. Night blood samples and sputum samples were obtained from the participants. Blood sample was used for determination of TNF α , HIVp24ag-Ab, anti-HCV, HBsAg by ELISA and identification of *Plasmodium* and *Wuchereria* by Geimsha thick blood film staining while sputum samples were used for the demonstration of Acid Fast Bacilli by Ziehl Neelsen staining. **Results:** The results showed a frequency of *Plasmodium spp.*, in individuals with plasma TNF- α of 5.8 \pm 0.7 pg/ml as 31.4%(22) as against a frequency of 18%(9) in subjects with plasma TNF- α of 2.2 \pm 0.3 pg/ml. The results also showed a frequency of 5.71%(4) and 2%(1) *Wuchereria bancrofti* in subjects with plasma TNF- α of 5.8 \pm 0.7 pg/ml and TNF- α of 2.2 \pm 0.3 pg/ml. respectively. The overall frequency of parasitic infection obtained in both test and control subjects include: 33.3% (40)*Plasmodium spp.*, and 4.2%(5) *Wuchereria bancrofti*. The overall results from both test and control subjects also showed a gender distribution of 20%(24) and 13.3%(16) *Plasmodium spp.*, in female and males respectively while a distribution of 1.7%(2) and 2.5%(3) *Wuchereria bancrofti* in females and males respectively. **Conclusion:** This work revealed increase in the frequency of *Plasmodium spp.* and *Wuchereria bancrofti* infections with increase in plasma TNF- α while the overall frequency of parasitic infection obtained in both test and control subjects was found to be 33.3% (40)*Plasmodium spp.*, and 4.2%(5) *Wuchereria bancrofti* with possible variations in regional and gender distributions. Mosquito borne parasitic infection of *Plasmodium spp.*, was found to be more prevalent in patients with elevated TNF- α of > 5.0 pg/ml.

INTRODUCTION

Mosquito while taking a blood meal can transmit unicellular and multicellular parasites that causes malaria fever, dengue fever, West Nile virus, chikungunya, yellow fever, filariasis (*W. bancrofti*), tularemia, dirofilariasis, Japanese encephalitis, Saint Louis encephalitis, Western equine encephalitis, Eastern equine encephalitis, Venezuelan equine encephalitis, Ross River fever, Barmah Forest fever, La Crosse encephalitis, and Zika fever, Keystone virus and Rift Valley fever [1][2][3][4].

Malaria and filariasis are common diseases transmitted by mosquito in rural communities. Giemsa stained thick blood film smears is the “gold standard” (finger prick

test) for the identification of *Plasmodium* and *Microfilaria (W. bancrofti)*. Night blood sample is preferred for the identification of *W. bancrofti* [5][6][7].

Immune system of the mosquito has not been proven to destroy *W. bancrofti* or *Plasmodium falciparum* though the parasites especially *Plasmodium falciparum* alters the mosquito vector’s feeding habit by increasing frequency of biting in infected mosquitoes, thus increasing the chance of transmitting the parasite [8]. The life cycle of *Plasmodium spp.*, that causes malaria and *Wuchereria bancrofti* the major cause of lymphatic filariasis takes place in human and mosquito. Humans is the definitive host and mosquitos as the intermediate host for *W. bancrofti* while human is the

intermediate hosts in which asexual reproduction takes place and female anopheline mosquito is the definitive host in which sexual reproduction occurs^{[9][10][11][12][13][14]}.

Parasitic infection of *Plasmodium spp.*, and *Wuchereria bancrofti* can elicit innate and adaptive immune responses including inflammatory responses^{[9][10][11][12][13][14]}.

Tumor necrosis factor alpha(TNF- α) is an inflammatory cytokines (cell signaling protein) and one of the cytokines that make up the acute phase reaction. It is synthesized by activated macrophages, CD4+ lymphocytes, NK cells, neutrophils, mast cells, eosinophils, and neurons TNF- α primarily regulates the immune cells. It is an endogenous pyrogen that induces fever, apoptotic cell death, cachexia, inflammation in response to parasitic infection^{[15][16][17][18]}.

This work is therefore designed to determine the pattern of mosquito borne parasitic infection in the night blood samples of patients with elevated TNF- α of > 5.0 pg/ml in a rural community.

MATERIALS AND METHODS

Study Area

Atisbo was carved out of the old Ifedapo local government area which has been split to 3 local governments. It is located in Okeogun the Northern part of Oyo State in Nigeria with its headquarters in Tede. Atisbo local Government was created by former Head of State Late Gen. Sanni Abacha in 1996. It is dominated by communities and their major occupation is farming.. It shares boundaries with Orire Local Government, Republic of Benin, Saki East Local and Itesiwaju and Iwajowa Local Governments. ATISBO is an acronym for Ago-Are, Tede, Irawo , Sabe , Baasi, Ofiki and Owo communities.

Study Population

Seventy (70; aged 31 – 76 years; Male- 35; Female-35) volunteers with plasma TNF α of 5.8 \pm 0.7 pg/ml including 10 participants from the 7 major communities (Ago-Are, Tede, Irawo , Sabe , Baasi, Ofiki and Owo). Control participants included 50 individuals with TNF α of 2.2 \pm 0.3 pg/ml. Only participants who were negative to Acid Fast Bacilli, anti-HCV, HBsAg and HIV tests were recruited for the study.

Sample Collection

Night blood samples and sputum samples were obtained from the participants. Blood sample was used for TNF α , HIV, anti-HCV, HBsAg ELISA and identification of *Plasmodium* and *Wuchereria bancrofti*. Sputum sample was used for Ziehl Neelsen staining to demonstrate Acid Fast Bacilli (AFB).

Laboratory Identification of *Plasmodium spp.*, *Wuchereria bancrofti* and Acid Fast Bacilli

Laboratory of *Plasmodium spp.*, *Wuchereria bancrofti* was carried out by Microscopy using Geimsha-Thick film

method while Acid Fast Bacilli was demonstrated in the sputum as described by Cheesbrough^[19].

Anti-HCV ELISA Assay

This was determined in the subjects using Abcam kit.

HIV ELISA Test

HIV test was carried out using Genscreen™ ULTRA HIV Ag-Ab Biorad Kit.

The Genscreen™ ULTRA HIV Ag-Ab is an enzyme immunoassay based on the principle of the sandwich technique for the detection of HIV antigen and of the various antibodies associated with HIV-1 and/or HIV-2 virus in human serum or plasma.

HBsAg ELISA Test

This was assayed using Biorad ELISA kit.

TNF alpha ELISA

Plasma TNF alpha was determined in the subjects using Abcam's kit. Abcam's.

ETHICAL CONSIDERATIONS AND CLEARANCES

This work was approved by ethical and research committee of Baptist Medical center Saki-Nigeria before the commencement of this work. Informed consent was also obtained from each of the patient and control subjects.

METHOD OF STATISTICAL ANALYSIS

The results obtained were subjected to statistical analysis using IBM SPSS 18.0 to determine mean, standard deviation and frequency.

RESULTS

The frequency of *Plasmodium spp.*, in individuals with plasma TNF- α of 5.8 \pm 0.7 pg/ml was 31.4%(22) as against a frequency of 18%(9) in subjects with plasma TNF- α of 2.2 \pm 0.3 pg/ml. (n-50)(Table1, Figure 1)

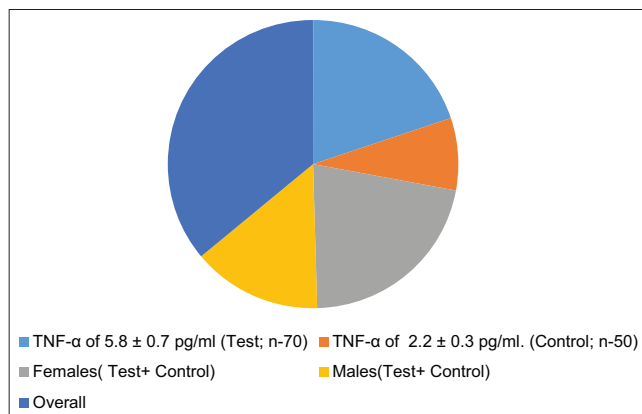
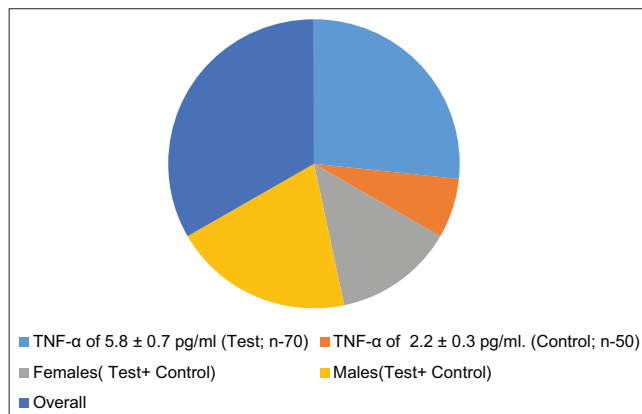
The results also showed a frequency of 5.71%(4) and 2%(1) *Wuchereria bancrofti* in subjects with plasma TNF- α of 5.8 \pm 0.7 pg/ml (n-70) and TNF- α of 2.2 \pm 0.3 pg/ml. (n-50) respectively. (Table1, Figure 2)

The overall frequency of parasitic infection obtained in both test and control subjects include: 33.3%(40)*Plasmodium spp.*, and 4.2%(5) *Wuchereria bancrofti* (Table1, Figure 1,2).

The results in both test and control subjects also showed a gender distribution of 20%(24) and 13.3%(16) *Plasmodium spp.*, in female and males respectively while a distribution of 1.7%(2) and 2.5%(3) *Wuchereria bancrofti* in females and males respectively (Table1, Figure 1,2).

Table 1. Frequency of *Plasmodium spp.*, and *Wuchereria bancrofti* in the subjects

	Subjects based on Plasma TNF- α		Subjects (Test+Control) based on gender		Overall (Test+Control)
	TNF- α of 5.8 \pm 0.7 pg/ml (Test; n-70)	TNF- α of 2.2 \pm 0.3 pg/ml. (Control; n-50)	Females n=60	Males n=60	Total n=120
<i>Plasmodium spp.</i> ,	31.4%(22)	18%(9)	20%(24)	13.3%(16)	33.3% (40)
<i>Wuchereria bancrofti</i>	5.71%(4)	2%(1)	1.7%(2)	2.5%(3)	4.2%(5)
Acid Fast Bacilli	Negative	Negative	Negative	Negative	Negative
Anti-HCV	Negative	Negative	Negative	Negative	Negative
HIVp24Ag-Ab	Negative	Negative	Negative	Negative	Negative

**Figure 1.** Frequency of *Plasmodium spp.*, in the subjects**Figure 2.** Frequency of *Wuchereria bancrofti* in the subjects

DISCUSSION

The frequency of *Plasmodium spp.*, in individuals with plasma TNF- α of 5.8 \pm 0.7 pg/ml was 31.4%(22) as against a frequency of 18%(9) in subjects with plasma TNF- α of 2.2 \pm 0.3 pg/ml. (n-50). The results also showed a frequency of 5.71%(4) and 2%(1) *Wuchereria bancrofti* in subjects with plasma TNF- α of 5.8 \pm 0.7 pg/ml (n-70) and TNF- α of 2.2 \pm 0.3 pg/ml. (n-50) respectively.

The frequency of *Plasmodium spp.*, and *Wuchereria bancrofti* was higher in subjects with elevated plasma TNF- α than the results obtained in those with lower (normal) plasma TNF- α . This is attributable to the bioactivities of TNF- α as a pro-inflammatory cytokine as *Plasmodium spp.*, and *Wuchereria bancrofti* can elicit inflammatory responses

to regulate immune cells and induce fever including cell death^{[12][15][16][17][18]}.

The overall frequency of parasitic infection obtained in both test and control subjects include: 33.3% (40) *Plasmodium spp.*, and 4.2%(5) *Wuchereria bancrofti*.

The frequency of *Plasmodium spp.*, reported in this study was higher than the report of WHO^[20] because World Health Organization^[20] in 2018 reported a prevalence of *Plasmodium spp.*, (malaria) infection of 25% in Nigeria. this difference might be due to the fact that the WHO report was an overall prevalence in Nigeria considering all regions whereas this work was carried out in a local government area in South West-Nigeria.

Prevalence of *Wuchereria bancrofti* found in this study was lower than the reports of previous studies in Nigeria because Okorie *et al.*,^[21] in 2013 found the prevalence of, Lymphatic Filariasis in Nigeria and reported that the mean prevalence of circulating filarial antigen (CFA) was 14.0% (in 134 locations), and by microfilaria (Mf) was 8.2% (in 162 locations). Okorie *et al.*,^[21] concluded that Nigeria has the highest burden of lymphatic filariasis (LF)/elephantiasis caused by *Wuchereria bancrofti* which is transmitted by mosquitoes; Mu'awiyya *et al.*,^[22] carried out a sero-prevalence of Lymphatic Filariasis in Six Communities of Talata Mafara Local Government Area, Zamfara State, Nigeria and found an overall sero-prevalence of 37.8%. with highest prevalence of 43.3% in farmers than other occupational groups and Adekunle *et al.*,^[23] reported that 27%(291) out of 1,090 blood specimens examined were positive for infection with *W. bancrofti* in Ose Local Government Area, Ondo State, Nigeria. They reported a ge frequency of 27%(108 out of 394) in males and 26%(183 out of 696) in females using Immunochromatographic Test (ICT) for the detection of *W. bancrofti*.

Generally, variations in the prevalence of these two parasitic infections considering the results obtained from some parts of Nigeria might be as a result of differences in vegetation, level of hygiene and major occupation favoring the habitation, multiplication of the transmitting mosquitoes and the transmission of the parasites^[12].

In addition this work targeted test subjects with elevated TNF- α and generally test and control participants who are free of HIV, HCV, *M. tuberculosis* and HBV infections which might account for the variation in the prevalence of the two parasitic infections compared with the previous reports^{[12][20][21][22][23][24]}.

The overall results in both test and control subjects also showed a gender distribution of 20%(24) and 13.3%(16) *Plasmodium spp.*, in female and males respectively while a distribution of 1.7%(2) and 2.5%(3) *Wuchereria bancrofti* in females and males respectively which is consistent with the reports of^[23] that reported gender difference in *Wuchereria bancrofti* infection and Nas *et al.*,^[24] who investigated frequency of malaria considering age, gender and socio-economic status of fever related patients in Kano City, Nigeria and found that *Plasmodium* prevalence was 84% which included 54% females and 46% males.

CONCLUSION

This work revealed increase in the frequency of *Plasmodium spp.* and *Wuchereria bancrofti* infections with increase in plasma TNF- α while the overall frequency of parasitic infection obtained in both test and control subjects was found to be 33.3% (40)*Plasmodium spp.*, and 4.2%(5) *Wuchereria bancrofti* with variations in gender distribution.. Mosquito borne parasitic infection of *Plasmodium spp.*, was found to be more prevalent in patients with elevated TNF- α of > 5.0 pg/ml.

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