Pattern of Mosquito Borne Parasitic Infection in the Night Blood Samples of Patients with Elevated TNF-α of > 5.0 pg/ml

Mathew Folaranmi Olaniyan1*, Tolulope Busayo Ojediran1, Donatus F nochuruoke2

1Department of Medical Laboratory Science, Edo University Iyamho – Nigeria
2Education Department, Medical Laboratory Science Council of Nigeria, Abuja

Corresponding Author: Mathew Folaranmi Olaniyan, E-mail: olaniyanmat@yahoo.com

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.

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.

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 . 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 mosquitoes as the intermediate host for W. bancrofti while human is the
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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 activated by 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 curved 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 TNFα of 5.8 ±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 TNFα of 2.2 ± 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 TNFα, 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 ±0.7 pg/ml was 31.4%(22) as against a frequency of 18%(9) in subjects with plasma TNF-α of 2.2 ± 0.3 pg/ml. (n=50) (Table 1, 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 ±0.7 pg/ml (n=70) and TNF-α of 2.2 ± 0.3 pg/ml (n=50) respectively. (Table 1, 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 (Table 1, 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 (Table 1, Figure 1, 2).
The frequency of Plasmodium spp., and Wuchereria bancrofti in the subjects

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±0.7 pg/ml (Test; n-70) | TNF-α of 2.2±0.3 pg/ml. (Control; n-50) | Females n=60 | Males n=60 | Total n=120 |
| Plasmodium spp., | | | | | |
| 31.4%(22) | 18%(9) | 20%(24) | 13.3%(16) | 33.3% (40) |
| Wuchereria bancrofti | 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 ±0.7 pg/ml was 31.4%(22) as against a frequency of 18%(9) in subjects with plasma TNF-α of 2.2 ± 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 ±0.7 pg/ml (n-70) and TNF-α of 2.2 ± 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][23][23][20].
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.

**REFERENCES**

1. Caraballo, Hector. “Emergency Department Management Of Mosquito-Borne Illness: Malaria, Dengue, And West Nile Virus”. Emergency Medicine Practice. 2014: 16 (5).

2. WHO | Malaria”. www.who.int . Retrieved 2018-02-15.

3. American Mosquito Control Association. Mosquito-Borne Diseases - www.mosquito.org . Retrieved 2018-02-15.

4. Kerr, Peter “Viral Infections of Rabbits”. Veterinary Clinics of North America: Exotic Animal Practice. 2013: 16 (2): 437–468.

5. Hewitt, Kirsten; Whitworth, James AG . “Filariosis”. Medicine. 2005: 33 (8): 61–64. doi:10.1383/medc.2005.33.8.61.

6. Center for Disease Control and Prevention. “Lymphatic Filariasis”. Retrieved 18 July 2010.

7. Bockarie, Moses; Hoerauf, Achim; Taylor, Mark J. “Lymphatic filariasis and onchocerciasis”. The Lancet. 2010 : 376 (9747): 1175–1185. doi:10.1016/s0140-6736(10)60586-7. PMID 20739055.

8. Koella, J.C.; Sorensen; Anderson. “The malaria parasite, Plasmodium falciparum, increases the frequency of multiple feeding of its mosquito vector, Anopheles gambiae”. Proceedings of the Royal Society B. 1998: 265 (1398): 763–768. doi:10.1098/rspb.1998.0358. PMC 1689045. PMID 9628035.

9. Sinka, Marianne E; Bangs, Michael J; Manguin, Sylvie; Coetzee, Maureen; Mbogo, Charles M; Hemingway, Janet; Patil, Anand P; Temperley, Will H; Gething, Peter W; Kabaria, Caroline W; Okara, Robi M; Van Boeckel, Thomas; Godfray, H Charles J; Harbach, Ralph E; Hay, Simon I. “The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and biological précis.” Parasites & Vectors. 2010 : 3 (1): 117. doi:10.1186/1756-3305-3-117. PMC 3016360. PMID 21129198.

10. Gerald, N.; Mahajan, B.; Kumar, S. “Mitosis in the Human Malaria Parasite Plasmodium falciparum”. Eukaryotic Cell. 2011 : 10 (4): 474–482. doi:10.1128/EC.00314-10. PMC 3127633. PMID 21317311.

11. Junghanss, Jeremy Farrar, Peter J. Hotez, Thomas . Manson’s Tropical Diseases: Expert Consult - Online (23rd ed.). Oxford: Elsevier/Saunders.2013: pp. e49–e52. ISBN 9780702053061.

12. Ridley, John W. (2012). Parasitology for Medical and Clinical Laboratory Professionals. Clifton Park, N.Y.: Cengage Learning. pp. 103–104. ISBN 9781435448162.

13. Rajan, T.V. Textbook of Medical Parasitology. Bl Publications Pvt Ltd. 2008: pp. 73–77. ISBN 9788172253172.

14. van Hoegaerdens M, Ivanoff B. “A rapid, simple method for isolation of viable microfilariae”. Am J Trop Med Hyg. 1986: 35 (1): 148–51. doi:10.4269/ajtmh.1986.35.148. PMID 3456213.

15. Swardfager W,兰cott K, Rothenburg L, Wong A, Cappell J, Herrmann N “A meta-analysis of cytokines in Alzheimer’s disease”. Biol Psychiatry. 2010 : 68 (10): 930–941. doi:10.1016/j.biopsych.2010.06.012. PMID 20692646.

16. Locksley RM, Killean N, Lenardo MJ. “The TNF and TNF receptor superfamily: integrating mammalian biology”. Cell. 2001: 104 (4): 487–501. doi:10.1016/S0092-8674(01)00237-9. PMID 11239407.

17. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctôt KL. “A meta-analysis of cytokines in major depression”. Biol Psychiatry. 2010 : 67 (5): 446–457. doi:10.1016/j.biopsych.2009.09.033. PMID 20015486.

18. Victor FC, Gottlieb AB . “TNF-alpha and apoptosis: implications for the pathogenesis and treatment of psoriasis”. J Drugs Dermatol. 2002 : 1 (3): 264–75. PMID 12851985.

19. Monica Cheesbrough District Laboratory Practice in Tropical Countries 2006: Part 2 Second Edition. Cambridge University Press The Edinburgh Building, Cambridge CB2 8RU, UK Published in the United States of America by Cambridge University Press, New York www.cambridge.org

20. World Health Organization, Malaria Fact sheets 2020

21. Patricia N. Okorie,, George O. Ademowo, Yisa Saka, Emmanuel Davies, Chukwu Okoronkwo, Moses J. Bockarie, David H. Molyneux, and Louise A. Kelly-Hope. "A rapid, simple method for isolation of viable microfilariae". Am J Trop Med Hyg. 1986: 35 (1): 148–51. doi:10.4269/ajtmh.1986.35.148. PMID 3456213.

22. Mu’awiyaa Umar Ladan, Tukur Adamu, Aminu Yabo Bala and Muhtari Jangebe Ladan, 2019. Sero-prev-
23. NO Adekunle, SO Sam-Wobo, MA Adeleke, UF Ekpo, E Davies, AO Ladokun, E Egbeobauwaye, OA Surakat. Prevalence and distribution of Wuchereria bancrofti in Ose Local Government Area, Ondo State, Nigeria. Nigerian Journal of Parasitology. 2016: 137 (1), 96-100. http://dx.doi.org/10.4314/njpar.v37i1.19

24. Nas FS, Yahaya A and Ali M Prevalence of Malaria with Respect to Age, Gender and Socio-Economic Status of Fever Related Patients in Kano City, Nigeria. Greener Journal of Epidemiology and Public Health, 2017: 5(5): 044-049, http://doi.org/10.15580/GJEPH.2017.5.091017126