Background: Mosquito-borne parasites include the pathogenic protozoa and helminths that are transmitted by the insect vector which may co-infect with other organisms to elicit an immune response. Purpose: To determine the frequency of mosquito-borne parasites in patients newly infected with HIV in relationship with CD4 count and TNFα. Method: Thirty-one (31; aged 15-32 years; male-12; female-19) newly diagnosed HIV positive patients and fifty (50) age-matched HIV negative volunteers were recruited as a control subject for this study. All subjects were negative to anti-HCV/HBsAg ELISA, Plasmodium, Acid-Fast Bacilli (AFB) tests and the control subjects were also negative to HIVP24 Ag-Ab ELISA, Plasmodium spp. and Wuchereria bancrofti microscopy. Venous blood including Night blood samples and sputum samples were obtained from the participants for CD4 count by cyflowmetry, TNFα, HIVP24Ag-Ab, anti-HCV, HBsAg by ELISA and microscopic identification by Giemsa staining while Sputum sample was used for Ziehl Neelsen staining to demonstrate Acid Fast Bacilli (AFB). Result: A lower frequency of 25.8% (Rajan, 2008) Plasmodium spp. and 6.5% (James et al., 2015) W. bancrofti was obtained in newly infected HIV patients compared with 32% (Zeitlmann et al., 2001) Plasmodium spp. and 8% (WHO, 2019) W. bancrofti obtained in the non-HIV infected control subjects. Showed a significant decrease in CD4 count and increase in plasma TNFα in both HIV mono-infection and coinfection with Plasmodium spp. and W. bancrofti compared with the results obtained in the non-HIV infected control subjects (p<0.05) and the results obtained in the newly infected HIV patients without Plasmodium spp. and W. coinfection (p<0.05). Conclusion: There was a significant increase in plasma TNFα and a decrease in CD4 count in both HIV mono-infection and coinfection with Plasmodium spp. and W. bancrofti while a lower frequency of Plasmodium spp. and W. bancrofti was obtained in newly infected HIV patients compared with the results obtained in the non-HIV infected control subjects.

Latar Belakang: Parasit tular nyamuk meliputi protozoa patogen dan cacing yang ditularkan oleh serangga yang dapat ber-koinfeksi dengan organisme lain untuk menimbulkan respon imun. Tujuan: Untuk mengetahui frekuensi parasit tular nyamuk pada pasien baru terinfeksi HIV yang berhubungan dengan jumlah CD4 dan TNFα. Metode: 31 pasien HIV-positif yang baru didiagnosis (umur 15-32 tahun; Laki-laki-12; Perempuan-19) dan 50 sukarelawan HIV-negatif dengan usia yang cocok direkrut sebagai subjek kontrol. Semua subjek negatif terhadap tes ELISA anti-HCV/HBsAg, Plasmodium, BTA dan subjek kontrol terhadap HIVP24 Ag-Ab ELISA, Plasmodium spp. dan Wuchereria bancrofti. Darah vena termasuk sampel darah malam dan sampel sputum untuk penghitungan CD4 dan TNFα, TNFα, HIVP24Ag-Ab, anti-HCV, HBsAg dengan ELISA dan identifikasi mikroskopis dengan pewarnaan Giemsa sedangkan sampel dahak untuk pewarnaan Ziehl Neelsen dalam mendemonstrasi Acid Fast Bacillus (AFB). Hasil: Frekuensi lebih rendah dari 25.8% (Rajan, 2008) Plasmodium spp. dan 6.5% (James et al., 2015) W. bancrofti dibandingkan dengan 32% (Zeitlmann et al., 2001) Plasmodium spp. dan 8% (WHO, 2019) W. bancrofti. W. bancrofti dibandingkan hasil subjek kontrol yang tidak terinfeksi HIV menunjukkan peningkatan signifikan TNFα dan penurunan jumlah CD4 pada HIV monoinfeksi dan koinfeksi dengan Plasmodium spp. dan W. bancrofti dibandingkan hasil subjek kontrol yang tidak terinfeksi HIV (p<0.05) dan hasil pada pasien HIV yang baru terinfeksi tanpa koinfeksi Plasmodium spp. dan W. bancrofti (p<0.05). Kesimpulan: Peningkatan signifikan pada TNFα dan penurunan jumlah CD4 pada HIV monoinfeksi dan koinfeksi dengan Plasmodium spp. dan W. bancrofti sedangkan frekuensi lebih rendah pada pasien HIV yang baru terinfeksi dibandingkan pada subjek kontrol yang tidak terinfeksi HIV.
INTRODUCTION

Mosquito-borne parasites are parasites transmitted by a mosquito which include unicellular (protozoa) and multicellular (helminths) pathogenic organisms (Caraballo and King, 2014; James et al., 2015; WHO, n.d.; Nadjam and Behrens, 2012). Mosquito is an insect vector that transmits Plasmadium spp. (Protozoan) and W. bancrofti (Nematode) which can cause diseases are known as malaria and Lymphatic filariasis/Elephantiasis respectively. The mosquito injects the parasites into the bloodstream when taking a blood meal of a person (Caraballo and King, 2014; James et al., 2015; WHO, n.d.; Nadjam and Behrens, 2012).

Lymphatic filariasis is caused by W. bancrofti. Lymphatic filariasis could be asymptomatic and may also lead to medical signs and symptoms called elephantiasis characterized by severe swelling in the arms, legs, breasts or genitalia, thick skin and could be painful. The first-stage larvae of W. bancrofti (microfilariae) are found in the circulation (Junghanss et al., 2013; Ridley, 2012; Rajan, 2008; Melrose, 2002; Ramaiah and Ottesen, 2014). W. bancrofti is the major cause of lymphatic filariasis compare with B. malayi and B. timori, which infect the lymphatic system to cause lymphatic filariasis. W. bancrofti has two hosts which include humans as the definitive host and mosquitoes as the intermediate host (Junghanss et al., 2013; Ridley, 2012; Rajan, 2008; Melrose, 2002; Ramaiah and Ottesen, 2014). The adult parasites live in the lymphatic system of the human host. This may cause socio-economic problems (Junghanss et al., 2013; Ridley, 2012; Rajan, 2008; Melrose, 2002; Ramaiah and Ottesen, 2014). Plasmadium spp. is transmitted by the female anopheles’ mosquito to cause a mosquito-borne infectious disease known as malaria. Female Anopheles mosquito is the definitive host of Plasmadium that transmits the motile infective form of the parasite (sporozoite) to humans which is the secondary host (Caraballo and King, 2014; James et al., 2015; WHO, n.d.; Nadjam and Behrens, 2012).

Human immunodeficiency virus (HIV) is a viral infection that can destroy the human immune system, specifically white blood cells called CD4+ lymphocytes that may make such an individual susceptible to infections like tuberculosis and some cancers (WHO, 2018; Isobe et al., 1986; Ansari-Lari et al., 1996; Barber et al., 1989; Owens et al., 2013; Zeitlmann et al., 2001; Gorsk et al., 2004). This is because CD4 is an epitope of protein with which HIV binds to enter a cell or body. The virus replicates on CD4-bearing cells to produce millions of HIV (WHO, 2018; Isobe et al., 1986; Ansari-Lari et al., 1996; Barber et al., 1989; Owens et al., 2013; Zeitlmann et al., 2001; Gorsk et al., 2004). At the end of the replication the cell will burst to release new HIV that will infect other CD4 bearing cells on which the virus replicates again and finally cause the destruction of the cells. This process will continue as long as the cells are living, and this will eventually lead to the depletion of CD4-bearing cells (WHO, 2018; Isobe et al., 1986; Ansari-Lari et al., 1996; Barber et al., 1989; Owens et al., 2013; Zeitlmann et al., 2001; Gorsk et al., 2004).

Tumor necrosis factor (TNFα) is a pro-inflammatory cytokine involved in systemic inflammation and acute-phase reaction (Swardfager et al., 2010; Locksley et al., 2001; Victor and Gottlieb, 2002). It is synthesized majorly by activated macrophages and other cells like T helper cells, natural killer cells, neutrophils, mast cells, eosinophils and neurons. It regulates immune cells and acts as endogenous pyrogen to induce fever, apoptotic cell death, cachexia, inflammation, inhibit tumorigenesis and viral replication (Swardfager et al., 2010; Locksley et al., 2001; Victor and Gottlieb, 2002).

Owo is blessed with tropical rain forest is rich in indigenous tree species that can favor the survival of mosquitoes that may harbor parasites of medical importance. Mosquito-borne parasites can trigger inflammatory responses which may worsen the prognosis of HIV infection (Haastrup et al., 2019; Awoyemi and Ogundele, 2008). The prevalence of HIV among adults in Nigeria is relatively low (3.2%) (Haastrup et al., 2019; Awoyemi and Ogundele, 2008). This work was therefore designed to determine mosquito-borne parasites in patients newly infected with HIV in relationship with CD4 count and TNFα.

MATERIAL AND METHOD

• Study area

This work was carried out in Owo/Ose Federal constituent. It consists of Owo and Ose Local government areas that share a border with Edo state and are equidistant between Lagos and Abuja, Nigeria.

• Study population

Thirty-one (31; aged 15 - 32 years; Male-12; Female-19) newly diagnosed HIV-positive patients who visited the hospitals with signs and symptoms of HIV infection and fifty (50) age-matched apparently healthy HIV-negative volunteers were recruited as a control subjects for this study. All subjects were negative to anti-HCV/HBsAg ELISA, Plasmadium and Acid Fast Bacilli (AFB) tests. In addition the control subjects were also negative to HIV24 Ag-Ab ELISA, Plasmadium spp. and W. bancrofti microscopy.
**Sample collection**

Venous blood including night blood samples and sputum samples were obtained from the participants. The blood sample was used for CD4 count, TNFα, HIV, anti-HCV, HBsAg ELISA and identification of Plasmodium and W. bancrofti while the sputum sample was used for Ziehl Neelsen staining to demonstrate Acid Fast Bacilli (AFB).

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

Laboratory of Plasmodium spp., W. bancrofti was carried out by Microscopy using Geimsha-Thick film method while Acid Fast Bacilli was demonstrated in the sputum as described by Cheesbrough (Hastrup et al., 2019).

**Anti-HCV ELISA assay**

This was determined in the subjects using the Abcam kit. Anti-HCV Elisa is a “direct sandwich principle” as the basis for the assay to detect antibodies to Hepatitis C virus (anti-HCV).

**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.

**BsAg ELISA test**

This was assayed using the Biorad ELISA kit. It is an ELISA technique based on binding antibody with its specific antigen to give an observable reaction in form of color formation.

**TNF alpha ELISA**

Plasma TNF alpha was determined in the subjects using Abcam kit. Abcam TNF alpha Human in vitro ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of TNF alpha in supernatants, buffered solutions, serum and plasma samples. A monoclonal antibody-specific TNF alpha has been coated onto the wells of the microtitre strips provided. Samples, including standards of known TNF alpha concentrations, control specimens or unknowns are pipetted into these wells. During the first incubation, the standards or samples and a biotinylated monoclonal antibody specific for TNF alpha are simultaneously incubated. After washing, the enzyme StreptavidinHRP, which binds the biotinylated antibody is added, incubated and washed.

A TMB substrate solution is added which acts on the bound enzyme to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of TNF alpha present in the samples.

**CD4 Enumeration**

This was carried out on fresh blood samples using Partec CyFlow Counter and reagent.

**Ethical considerations and clearances**

The proposal of this work was presented and approved by ethical and research committee of Federal Medical Centre, Owo-Nigeria (FMCERC/VIII/21/189) 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.

**RESULT**

A lower frequency of 25.8% (Rajan, 2008) Plasmodium spp. and 6.5% (James et al., 2015) W. bancrofti was obtained in Newly infected HIV patients compared with 32% (Zeitlmann et al., 2001) Plasmodium spp. and 8% (WHO, 2019) W. bancrofti obtained in the non-HIV infected control subjects (Table1, Figure2).

The results obtained showed a significant increase in plasma TNFα in all the newly infected HIV patients; the newly infected HIV patients with Plasmodium spp. and W. bancrofti coinfection and also in the newly infected HIV patients without Plasmodium spp. and W. bancrofti coinfection compared with the results obtained in the non-HIV infected control subjects (p<0.05) (Table1 and 2, Figure 2).

There was a significant decrease in CD4 count in the all the Newly diagnosed HIV patients and also in the newly infected HIV patients with Plasmodium spp. and W. bancrofti coinfection compared with the results obtained in the non-HIV infected control subjects (p<0.05; Table1 and 2, Figure 1).

There was also a significant increase in plasma TNF and a significant decrease in CD4 count in newly infected HIV patients with Plasmodium spp. and W. bancrofti coinfection compared with the results obtained in the newly infected HIV patients without Plasmodium spp. and W. bancrofti coinfection (p<0.05; Table1 and 2, Figure2).
### Table 1. Frequency of Plasmodium spp., *W. bancrofti* blood CD4 count and plasma TNFα obtained in the subjects

<table>
<thead>
<tr>
<th></th>
<th>Plasmodium spp.</th>
<th>CD4 Count (Cells/µL)</th>
<th>W. bancrofti</th>
<th>TNFα (pg/ml)</th>
<th>Acid Fast Bacilli</th>
<th>Anti-HCV</th>
<th>HBsAg</th>
<th>HIV P24Ag-Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newly infected HIV patients (n=31)</td>
<td>25.8% (Rajan, 2008)</td>
<td>540 ± 31</td>
<td>6.5% (James et al., 2015)</td>
<td>4.8 ± 0.5</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Non-HIV infected control subjects (n=50)</td>
<td>32% (Zeitlmann et al., 2001)</td>
<td>664 ± 21</td>
<td>8% (WHO, 2019)</td>
<td>2.2 ± 0.2</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Newly infected HIV patients with Plasmodium spp. and <em>W. bancrofti</em> coinfection (n=10)</td>
<td>25.8% (Rajan, 2008)</td>
<td>430 ± 29</td>
<td>6.5% (James et al., 2015)</td>
<td>6.3 ± 0.4</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Newly infected HIV patients without Plasmodium spp. and <em>W. bancrofti</em> coinfection (n=21)</td>
<td>Negative</td>
<td>621 ± 30</td>
<td>4.0 ± 0.1</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Comparative analysis of the results obtained in the subjects

<table>
<thead>
<tr>
<th></th>
<th>Newly infected HIV patients (n=31) Vs Non-HIV infected control subjects (n=50)</th>
<th>Newly infected HIV patients with Plasmodium spp. and <em>W. bancrofti</em> coinfection (n=10) Vs Newly infected HIV patients without Plasmodium spp. and <em>W. bancrofti</em> coinfection (n=21) Vs Non-HIV infected control subjects (n=50)</th>
<th>Newly infected HIV patients without Plasmodium spp. and <em>W. bancrofti</em> coinfection (n=21) Vs Non-HIV infected control subjects (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 count (Cells/µL)</td>
<td>'t' value -3.35885, 'p' value 0.04*</td>
<td>'t' value 4.57756, 'p' value 0.02*</td>
<td>'t' value -6.5354, 'p' value 0.01*</td>
</tr>
<tr>
<td></td>
<td>('t' value -6.5354, 'p' value 0.01*)</td>
<td>('t' value 4.57756, 'p' value 0.02*)</td>
<td>('t' value -6.5354, 'p' value 0.01*)</td>
</tr>
<tr>
<td>TNFα (pg/ml)</td>
<td>'t' value 4.82808, 'p' value 0.02*</td>
<td>'t' value 5.57832, 'p' value 0.02*</td>
<td>'t' value 9.16788, 'p' value 0.006**</td>
</tr>
<tr>
<td></td>
<td>('t' value 4.82808, 'p' value 0.02*)</td>
<td>('t' value 5.57832, 'p' value 0.02*)</td>
<td>('t' value 9.16788, 'p' value 0.006**)</td>
</tr>
</tbody>
</table>

* Asterisk denotes statistical significance.** Asterisk denotes statistical significance.*
DISCUSSION

A lower frequency of 25.8% (Rajan, 2008) Plasmodium spp. and 6.5% (James et al., 2015) W. bancrofti was obtained in newly infected HIV patients compared with 32% (Zeitlmann et al., 2001) Plasmodium spp. and 8% (WHO, 2019) W. bancrofti obtained in the non-HIV infected control subjects. A lower frequency of Plasmodium spp., in newly infected HIV patients compared with HIV non-infected subjects is not consistent with those though were not newly infected as reported by Akinbo et al. (Awoyemi and Ogundele, 2008) who obtained an overall prevalence of 7.8% and 2% of malarial infection in HIV-infected patients on HAART and non-HIV participants, respectively. This might be due to the fact that the HIV subjects investigated by Akinbo et al. (Awoyemi and Ogundele, 2008) had a lower CD4+ T-cell count of <200 cells/μL than the least count of 430 ± 29 obtained in HIV coinfection. Plasmodium infection in the work of Akinbo et al. (Awoyemi and Ogundele, 2008) might be opportunistic considering the value of CD4+ T-cell count. On the frequency of W. bancrofti in this work Inge et al. (Cheesbrough, n.d.) reported a significantly increased risk of acquiring HIV by lymphatic filariasis-infected individuals which accounts for the coinfection obtained in this work.

The results obtained showed a significant increase in plasma TNFα in all the newly infected HIV patients; the newly infected HIV patients with Plasmodium spp. and W. bancrofti coinfection and also in the newly infected HIV patients without Plasmodium spp. and W. bancrofti coinfection compared with the results obtained in the non-HIV infected control subjects. These findings are because the Tumor necrosis factor is an inflammatory cytokine and acute phase reactant (Swardfager et al., 2010; Locksley et al., 2001; Dowlat et al., 2010; Victor and Gottlieb, 2002) which is majorly produced upon pathogenic infections by activated macrophages and other cells like T helper cells, natural killer cells,
neutrophils, mast cells, eosinophils and neurons to induce fever, apoptotic cell death, inflammation, inhibit tumorigenesis and viral replication (Swardfager et al., 2010; Locksley et al., 2001; Dowlat et al., 2010; Victor and Gottlieb, 2002). Therefore, the elevated plasma level might have been triggered by HIV, Plasmodium spp. and W. bancrofti infections.

There was a significant decrease in CD4 count in all the newly infected HIV patients and also in the newly infected HIV patients with Plasmodium spp. and W. bancrofti coinfection compared with the results obtained in the non-HIV infected control subjects.

CD4+ T helper cells are white blood cells that constitute an essential part of the human immune system. They are so called because they help other cells by sending signals to other types of immune cells such as CD8 killer cells to release cytoxin to destroy the infectious particle and virally infected cells. If CD4 cells are infected with HIV it will trigger the release of cytokine that will stimulate CD8 killer cells to release cytokotoxin that will kill HIV and HIV-infected cells like the CD4. The cytokine will also stimulate Blymphocyte for the production of a non-neutralizing HIV antibody. As the destruction of CD4 bearing cells continues CD4 count will reduce hence the significant decrease in CD4 count obtained in this work compared with the control.

There was also a significant increase in plasma TNF alpha and a significant decrease in CD4 count in newly infected HIV patients with Plasmodium spp. and W. bancrofti coinfection compared with the results obtained in the newly infected HIV patients without Plasmodium spp. and W. bancrofti coinfection. Above explanations also hold for this but the magnitude was increased by coinfections (WHO, 2018; Isobe et al., 1986; Ansari-Lari et al., 1996; Barber et al., 1989; Owens et al., 2013; Zeitlmann et al., 2001; Gorsk et al., 2004; Swardfager et al., 2010; Locksley et al., 2001; Dowlat et al., 2010; Victor and Gottlieb, 2002)

This work revealed the significance of Plasmodium spp. and W. bancrofti infection control and prevention in HIV management to prevent the suppression of immunity.

**CONCLUSION**

There was a significant increase in plasma TNFalpha and a decrease in CD4 count in both HIV mono-infection and coinfection with Plasmodium spp. and W. bancrofti while a lower frequency of Plasmodium spp. and W. bancrofti was obtained in newly infected HIV patients compared with the results obtained in the non-HIV infected control subjects.

**ACKNOWLEDGMENTS**

The authors state there is no conflict of interest with the parties involved in this study.

**REFERENCES**


