Original Article

Relationship between Parasitic Infection of *Toxoplasma gondii*, IL-2, TNF-α Tryptophan and CD4+ Count

Mathew Folaranmi Olaniyan, Temitayo Afolabi[1], Nwachi Ogbona Idume[2]

Department of Medical Laboratory Science, Edo University, Iyamho, Edo, [1]Department of Medical Laboratory Science, Achievers University, Owo, Ondo, [2]Department of Education, Medical Laboratory Science Council of Nigeria, Abuja, Nigeria

Abstract

Study Background: *Toxoplasma gondii* a protozoan and zoonotic infection can stimulate innate and adaptive immune responses. Aims and **Objectives:** This work was designed to determine relationship between parasitic infection of *T. gondii,* interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-α), and Tryptophan and CD4+ count. **Materials and Methods:** A total of 150 individuals aged 21–73 years (male: 100 and female: 50) were recruited from Saki-West, Saki-East, and ATISBO local governments of Nigeria. Plasma TNF-α, IL-2, Tryptophan, anti-hepatitis C virus (anti-HCV), hepatitis B surface antigen (HBsAg), anti-HIV, *Plasmodium*, and *T. gondii* infections were determined in each of the subjects. The results were used to group the subjects into: Control (n = 104; individuals not infected with *T. gondii* noninfected, *Plasmodium spp.,* HIV or HCV)*; T. gondii* mono-infected patients (n = 9) and patients with *T. gondii* co-infection with *Plasmodium spp.,* HIV, HBV, or HCV. Plasma TNF-α, IL-2, anti-HCV, HBsAg, and anti-HIV were determined by enzyme-Linked Immunosorbent Assay. *Plasmodium* spp., was identified by thick blood film-Giemsa staining technique. Plasma tryptophan was determined by fluorometry. **Results:** Of 150 subjects recruited for the work, the results obtained showed a frequency of occurrence of 69.3% (104) *T. gondii* noninfected control not infected with *Plasmodium* spp*.,* HIV, HBV, and HCV; 8.0% (9) *T. gondii*-infected patients not infected with *Plasmodium* spp*.,* HIV and HCV; 22% (33) were infected with at least one of the *Plasmodium* spp*.,* HIV, HBV, and HCV; 2.7% (4) *T. gondii* patients co-infected with at least one of the *Plasmodium* spp*.,* HIV, HBV, and HCV. There was a significant decrease in plasma tryptophan in *T. gondii* mono- and co-infection including *T. gondii* noninfected individuals but infected with at least *Plasmodium* spp., HIV, HBV, and HCV compared with the control [$P < 0.05$]. There was a significant decrease in CD4 count in *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV and *T. gondii* co-infection compared with *T. gondii* mono-infection; controls and also in *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV compared to *T. gondii* confection ($P < 0.05$). There was a significant increase in plasma TNF-α in *T. gondii* mono-infected patients compared with the controls; *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV compared to controls; *T. gondii* confection compared to controls; *T. gondii* mono-infected patients compared to *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV; *T. gondii* mono-infected patients compared to *T. gondii* co-infection and *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV compared to *T. gondii* confection ($P < 0.05$). **Conclusion:** *T. gondii* and its coinfection with HIV, HCV, and HBV caused a significant immunological alterations in the plasma values of IL-2, TNF-α, Tryptophan, and blood CD4+ count.

Keywords: CD4+ count, interleukin-2, *Toxoplasma gondii,* tryptophan, tumor necrosis factor-alpha

Introduction

Toxoplasma gondii is a protozoan parasite that causes a disease known as toxoplasmosis. Infected individuals with strong immunity can carry the parasite as a carrier without any illness.[1,2,3,4] Some infected individuals may have "flu-like illness" with swollen lymph glands or muscle aches and pains that may last for a month or more, damage to the brain, eyes, or other organs, can develop from an acute *Toxoplasma* infection, reduced vision, blurred vision, pain, redness of the eye, and sometimes tearing.[5] Most infants who are infected *in utero* have no symptoms at birth, but they may develop symptoms later in life.[6,7]

Access this article online

Quick Response Code:

Website: www.jnsmonline.org

DOI: 10.4103/JNSM.JNSM_50_18

Address for correspondence: Dr. Mathew Folaranmi Olaniyan, Department of Medical Laboratory Science, Achievers University, Owo, Ondo, Nigeria.
E-mail: olaniyanmat@yahoo.com

How to cite this article: Olaniyan MF, Afolabi T, Idume NO. Relationship between parasitic infection of *Toxoplasma gondii,* IL-2, TNF-α Tryptophan and CD4+ count. J Nat Sci Med 2018;XX:XX-XX.

T. gondii could be contracted by: eating undercooked contaminated meat (pork, lamb, and venison), eating food, drinking water contaminated with *T. gondii,* accidentally swallowing the parasite through contact with cat feces that contain *Toxoplasma*, ingesting anything that has come in contact with cat's feces with *Toxoplasma,* ingesting contaminated soil and vegetables.[5] Mother-to-child (congenital) transmission can also occur.[6] It can also occur through blood transfusion and organ transplant though this is rare**.** *Toxoplasma* cannot be absorbed through intact skin.[7]

Tumor necrosis factor-alpha (TNF-α), is a proinflammatory cytokine involved in systemic inflammation and one of the cytokines that make up the acute phase reaction. CD4 bearing cell upon infection produce cytokines that stimulate CD8 bearing cells/Natural killer cells to produce cytotoxin and also stimulate B-lymphocytes for production of antibodies. Interleukin-2 (IL-2) is a cytokine that controls the activities of white blood cells (leukocytes, especially lymphocytes) that are responsible for immunity. It constitutes part of the body's natural response to microbial infection, and in discriminating between foreign ("nonself") and "self." IL-2 mediates its effects by binding to IL-2 receptors, which are expressed by lymphocytes. It elicits a CD4+ and CD8+ T-cell-mediated immune response.[8]

Tryptophan is an essential amino acids, and is mainly used for protein synthesis.[9] It serves as a building block for several metabolites such as kynurenine, serotonin, tryptamine, melatonin, niacin, and NAD/NAPD. Tryptophan exists in two forms: In blood as bound (BTRP) and free (FTRP) tryptophan.[10] Changes in tryptophan concentrations are directly related to a number of physiological and behavioral processes which include: sleep, memory, depression, motion sickness, bipolar disorders, and schizophrenia. In general, tryptophan is the least abundant amino acid in humans. Food sources of tryptophan include chicken, tuna, bananas, cheese, and chocolate.[10]

This work was designed to relate mono- and co-infections of *T. gondii,* IL-2, TNF-α, Tryptophan and CD4+ count in Saki-West, Saki-East, and ATISBO local government areas of Oyo state.

Materials and Methods

Materials

Study area

Individuals were recruited from Saki-West, Saki-East, and ATISBO local governments constituting a Federal constituency in Nigeria located at the Northern part of Oyo state. It shares a border with Burkina Faso and Kwara state.

Study population

A total of 150 individuals aged 21–73 years (male: 100 and female: 50) were recruited from Saki-West, Saki-East, and ATISBO local governments constituting a Federal constituency in Nigeria. Plasma TNF-α, IL-2, Tryptophan, anti-hepatitis C virus (anti-HCV), hepatitis B surface antigen (HBsAg), anti-HIV, *Plasmodium,* and *T. gondii infections* were determined in each of the subjects. In the results, the studied subjects were grouped into Control; *T. gondii* mono-infected and co-infected patients.

Methods

Plasma interleukin-2 enzyme-linked immunosorbent assay

Plasma IL-2 Assay was carried out using Abcam's IL-2 Human Enzyme-Linked Immunosorbent Assay (ELISA) kit.

Principle

This assay employs an antibody specific for Human IL-2 coated on a 96-well plate. Standards and samples are pipetted into the wells and IL-2 present in a sample is bound to the wells by the immobilized antibody. The wells are washed, and biotinylated anti-Human IL-2 antibody is added. After washing away unbound biotinylated antibody, horseradish peroxidase (HRP)-conjugated streptavidin is pipetted to the wells. The wells are again washed, a tetramethyl-benzidine (TMB) substrate solution is added to the wells and color develops in proportion to the amount of IL-2 bound. The stop solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Tryptophan assay using abcam Kit (fluorometric)

Principle

This assay detects BTRP and FTRP in serum. It is based on a nonenzymatic reaction that uses tryptophan as a building block, producing an intermediate product that reacts with a catalyst in order to generate a fluorophore that can be detected at Ex/Em = 370/440 nm. The reaction is specific for tryptophan and other amino acids do not interfere with the assay.

Anti-hepatitis C virus enzyme-linked immunosorbent assay

This was carried out using Anti-HCV Core Antigen-antibody (ab50288) Abcam kit. The HCV core protein is a viral structural protein; it also participates in some cellular processes, including transcriptional regulation. HCV core protein is thought to contribute to HCV pathogenesis through its interaction with various signal transduction pathways. In addition, HCV core antigen is a recently developed marker of hepatitis C infection. The HCV core protein has been previously shown to circulate in the bloodstream of HCV-infected patients and inhibit host immunity through an interaction with gC1qR.

HIV enzyme-linked immunosorbent assay test

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

Principle

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. The reaction is stopped and absorbances are read using a spectrophotometer at 450/620–700 nm. The absorbance measured on a sample determines the presence or absence of HIV Ag or HIV-1 and/r HIV-2 antibodies.

Assay procedure

The assay procedure includes the following reaction steps:

1. Conjugate 1 (biotinylated polyclonal antibody to p24 HIV-1 Ag) is added into the microplate wells
2. Serum/plasma samples to be assayed and controls are pipetted and added into the wells. If present, HIV antigens bind with the monoclonal antibody bound to the solid phase and the conjugate 1. HIV-1 and/or HIV-2 antibodies, if any, bind to the antigens immobilized on the solid phase. Deposition of conjugate 1 and sample is validated through a color change, from yellow-green to blue
3. After incubation at 37°C then washing, conjugate 2 is added: Streptavidin-peroxidase will react with biotinylated Ab-Ag-Ab complexes Peroxidase-labeled purified HIV-1 and HIV antigens bind in turn to the IgG, IgM, or IGA antibodies captured on the solid phase
4. After incubation at 18°C–30°C, the unbound conjugate 2 fraction is removed by washing. After incubation in presence of the substrate at room temperature (18°C–30°C), the presence of the complex conjugate is shown by a change of color
5. The reaction is stopped and absorbances are read using a spectrophotometer at 450/620–700 nm. The absorbance measured on a sample determines the presence or absence of HIV Ag or HIV-1 and/or HIV-2 antibodies. Every reactive result (in accordance with the interpretation criteria of Genscreen™ ULTRA HIV Ag-Ab test) should be confirmed with an appropriate method.

Hepatitis B surface antigen enzyme-linked immunosorbent assay test for HBV infection

This was carried out using Diagnostic Automation/Cortez Diagnostics, INC kit by ELISA method.

Principle

The HBsAg ELISA Test kit employs an antibody sandwich ELISA technique where monoclonal antibodies unique to HBsAg, are pre-coated on polystyrene microwell strips. The serum or plasma sample is added together with a second antibody, the HRP conjugate, (HRP) and directed against a different epitope of HBsAg. Throughout the time of incubation, specific immunocomplex that may have formed (indicating the presence of HBsAg) is captured on the solid phase. After washing, to eliminate serum proteins and unbound HRP-conjugate, chromogen solutions containing TMB and urea peroxide are added to the wells. Next, the colorless chromogens are hydrolyzed by the bound HRP-conjugate to a blue-colored product while in the presence of the antibody-antigen-antibody (HRP) sandwich immunocomplex. Halting the reaction with sulfuric acid, the blue color then turns yellow. The color intensity can be gauged proportionally to the amount of antigen captured in the wells, and to the amount in the sample, respectively. The wells remain colorless if the HBsAg result is negative.

Identification of Plasmodium parasite

This was carried out as described by.[11] by thick blood film-Giemsa staining technique.

Thick blood films

1. A thick film of the blood sample was prepared and air-dried
2. The thick film was Stained with diluted Methanol based Giemsa stain (1:20, vol/vol) for 20 min
3. It was washed by placing the film in buffered water for 3–5 min
4. The stained film was air dried in a vertical position and examined under microscope using oil-emersion objective.

Ethical consideration

The proposal of this study was reviewed and 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 patients and controls.

Method of statistical analysis

The results obtained were subjected to statistical analysis using SPSS 18.0 (IBM, Armonk, New York, USA) to determine mean, standard deviation, probability, student "*t*"-test and statistical significance is calculated using a *P* value level of statistical significant at 0.05.

Results

Of 150 subjects recruited for the study, 69.3% (104) considered *T. gondii* noninfected control not infected with *Plasmodium* spp., HIV, HBV, and HCV; 8.0% (9) *T. gondii*-infected patients not infected with *Plasmodium* spp., HIV and HCV; 22% (33) were infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV and 2.7% (4) *T. gondii* patients co-infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV [Tables 1 and 2].

There was no significant difference in plasma tryptophan in *T. gondii* mono-infected patients compared with *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV. This was also found in *T. gondii* confection and *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV compared with *T. gondii* confection [$P > 0.05$; Tables 1 and 2]. There was no significant difference in blood CD4 count in *T. gondii* mono-infected patients compared with the controls [$P > 0.05$; Tables 1 and 2]. There was also no significant difference in plasma IL-2 in *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV compared with *T. gondii* confection [$P > 0.05$; Tables 1 and 2].

There was a significant decrease in plasma tryptophan in *T. gondii* mono- and co-infection including *T. gondii* noninfected individuals but infected with at least *Plasmodium* spp., HIV, HBV, and HCV compared with the control [$P < 0.05$; Tables 1 and 2]. There was a significant decrease in CD4 count in *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV and *T. gondii* co-infection compared with *T. gondii* mono-infection; controls

Table 1: Frequency of *Toxoplasma gondii, Plasmodium* spp., HIV, hepatitis B virus and hepatitis C virus infection and CD4, plasma interleukin-2, tryptophan and tumor necrosis factor-alpha mean and standard deviation of among each group tested

	Total number of subjects recruited	Controls	*T. gondii* mono-infection	*T. gondii* noninfected subjects but infected with at least *Plasmodium* spp., HIV, HBV and HCV	*T. gondii* confection
n	150	69.3% (104)	8.0% (9)	22% (33)	2.7% (4)
Plasma tryptophan (nmol/mL)	-	49±7.0	21±5.0	18±4.0	13±2.0
CD4 (cells/mm^3)	-	653±21.0	623±19.0	518±11.0	382±10
IL-2 (pg/ml)	-	3.5±0.4	6.2±0.3	8.2±0.5	11±1.0
TNF-α (pg/ml)	-	2.3±0.3	4.5±0.1	6.0±0.4	9.2±0.3

T. gondii: Toxoplasma gondii, IL: Interleukin, HCV: Hepatitis C virus, HBV: Hepatitis B virus, TNF: Tumor necrosis factor

Table 2: Comparative analysis of the mean and standard deviation of CD4, plasma interleukin-2, tryptophan, and tumor necrosis factor-alpha obtained in the subjects

	T. gondii mono-infected patients versus controls	*T. gondii* noninfected subjects but infected with at least one of the *Plasmodium* spp., HIV, HBV and HCV versus controls	*T. gondii* confection versus controls	*T. gondii* mono-infected patients versus *T. gondii* noninfected subjects but infected with at least one of the *Plasmodium* spp., HIV, HBV and HCV	*T. gondii* mono-infected patients versus *T. gondii* co-infection	*T. gondii* noninfected subjects but infected with at least one of the *Plasmodium* spp., HIV, HBV and HCV versus *T. gondii* confection
Plasma tryptophan (nmol/mL)						
t	3.255	3.845	4.945	0.469	1.486	1.1180
P	0.041*	0.031*	0.02*	0.34	0.138	0.1899
CD4 (cells/mm^3)						
t	1.059	5.695	11.651	4.7823	11.225	9.148
P	0.20	0.015*	0.004*	0.021*	0.004*	0.006*
IL-2 (pg/ml)						
t	5.4.	7.340	6.96358	−3.43	−4.598	−2.5044
P	0.02*	0.009*	0.010*	0.038*	0.022*	0.065
TNF-α (pg/ml)						
t	−6.957	−7.4	−16.26	−3.64	−14.86	−6.4
P	0.01*	0.009*	0.002*	0.034*	0.002*	0.01*

*Significant. *T. gondii: Toxoplasma gondii*, IL: Interleukin, HCV: Hepatitis C virus, HBV: Hepatitis B virus, TNF: Tumor necrosis factor

and also in *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV compared to *T. gondii* confection [$P < 0.05$; Tables 1 and 2].

There was a significant increase in plasma TNF-α in *T. gondii* mono-infected patients compared with the controls; *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV compared to controls; *T. gondii* confection compared to controls; *T. gondii* mono-infected patients compared to *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV; *T. gondii* mono-infected patients compared to *T. gondii* co-infection and *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV compared to *T. gondii* confection [$P < 0.05$; Tables 1 and 2].

Discussion

Of 150 individuals recruited for the work, the results obtained showed a frequency of occurrence of 69.3% (104) *T. gondii* noninfected control not infected with *Plasmodium spp.,* HIV, HBV, and HCV; 8.0% (9) *T. gondii* infected patients not infected with *Plasmodium spp.,* HIV and HCV; 22% (33) were infected with at least one of the *Plasmodium spp.,* HIV, HBV, and HCV; 2.7% (4) *T. gondii* patients co-infected with at least one of the *Plasmodium spp.,* HIV, HBV, and HCV This result could be attributed to the possibility of evidence of co-infection between HIV, HBV, HCV, and *Plasmodium* as reported by Rotman and Liang,[12] and Gasim and Adam.,[13] Furthermore, *T. gondii* infection was lower than 23.9% and 24% reported in Maiduguri by Kamani *et al.,*[14] and Gyang *et al.,*[15] respectively possibly because of the environment and the number of individuals as it affects interaction with cats.

There was a significant decrease in plasma tryptophan in *T. gondii* mono- and co-infection including *T. gondii* noninfected individuals but infected with at least *Plasmodium* spp., HIV, HBV and HCV compared with the control. Possibly, this could be due to the loss of tryptophan to the parasite by

the hosts as tryptophan is an essential amino acid for *T. gondii,* which it scavenges from host cells.[8]

There was a significant decrease in CD4 count in *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV and *T. gondii* co-infection compared with *T. gondii* mono-infection; controls and also in *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV compared to *T. gondii* confection which may be due to depletion in CD4 level as a result of infection such as HIV/AIDS.[8] *T. gondii* infection can elicit a CD4+ and CD8+ T-cell-mediated immune response. CD8+ T-cell is a natural killer cell a type of cytotoxic lymphocyte critical to the innate immune system. The role NK cells play is analogous to that of cytotoxic T cells in the vertebrate adaptive immune response.[16,17]

There was also no significant difference in plasma IL-2 in *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV compared with *T. gondii* confection. There was also a significant increase in plasma TNF-α in *T. gondii* mono-infected patients compared with the controls; *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV compared to controls; *T. gondii* confection compared to controls; *T. gondii* mono-infected patients compared to *T. gondii* noninfected individuals but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV; *T. gondii* mono-infected patients compared to *T. gondii* co-infection and *T. gondii* noninfected subjects but infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV compared to *T. gondii* confection *T. gondii* infection stimulates production of IL-2 and IFN by the innate immune system to stimulate a CD4+ and CD8+ T-cell-mediated immune response continuous and excessive utilization of these cytokines might be responsible for a significant decrease in *T. gondii* infected patients and patients infected with at least one of the *Plasmodium* spp., HIV, HBV, and HCV.[18]

Conclusion

There was an evidence of *T. gondii* co-infection with *Plasmodium spp.,* HIV, HBV, and HCV and a significant decrease in the plasma IL-2 and TNF-α due to *T. gondii* mono- and co-infections and a decrease in CD4 in plasmodia and viral infections possibly due to HIV mono- or co-infections. The evaluation of plasma IL-2 and TNF-α CD4+ cells, *Plasmodium* spp., HIV, HBV, and HCV *T. gondii* infection will provide useful direction in the management of the parasitic infection.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Pappas G, Roussos N, Falagas ME. Toxoplasmosis snapshots: Global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. Int J Parasitol 2009;39:1385-94.
2. Berdoy M, Webster JP, Macdonald DW. Fatal attraction in rats infected with *Toxoplasma gondii*. Proc Biol Sci 2000;267:1591-4.
3. CDC. Toxoplasmosis – Biology. CDC; 17 March, 2015. [Last retrieved on 2015 Jun 14].
4. Cook TB, Brenner LA, Cloninger CR, Langenberg P, Igbide A, Giegling I, *et al.* "Latent" infection with *Toxoplasma gondii*: Association with trait aggression and impulsivity in healthy adults. J Psychiatr Res 2015;60:87-94.
5. Dubey JP. Toxoplasmosis of Animals and Humans. 2nd ed. CRC Press; 2010. DOI: 10.1186/1756-3305-3-112. Available from: https://parasitesandvectors.biomedcentral.com/articles/10.1186/1756-3305-3-112. [Last retrieved on 2013 Mar 12].
6. Dardé ML, Ajzenberg D, Smith J. Population Structure and Epidemiology of *Toxoplasma gondii.* In: Weiss LM, Kim K, editors. *Toxoplasma gondii*: The Model Apicomplexan. Perspectives and Methods. Ch. 3. London: Academic Press/Elsevier; 2011. p. 49-80.
7. CDC. Toxoplasmosis – Biology. CDC; 17 March, 2015. Available from: https://www.cdc.gov/parasites/toxoplasmosis/biology.html. [Last retrieved on 2015 Jun 14].
8. Miller CM, Boulter NR, Ikin RJ, Smith NC. The immunobiology of the innate response to *Toxoplasma gondii*. Int J Parasitol 2009;39:23-39.
9. Radwanski ER, Last RL. Tryptophan biosynthesis and metabolism: Biochemical and molecular genetics. Plant Cell 1995;7:921-34.
10. Slominski A, Semak I, Pisarchik A, Sweatman T, Szczesniewski A, Wortsman J, *et al.* Conversion of L-tryptophan to serotonin and melatonin in human melanoma cells. FEBS Lett 2002;511:102-6.
11. Cheesbrough M. District Laboratory Practice in Tropical Countries. Part 2, 2nd ed. New York: Published in the United States of America by Cambridge University Press; 2006. Available from: http://www.cambridge.org. [Last retrieved on 2009].
12. Rotman Y, Liang TJ. Coinfection with hepatitis C virus and human immunodeficiency virus: Virological, immunological, and clinical outcomes. J Virol 2009;83:7366-74.
13. Gasim GI, Adam I. Hepatitis B, Hepatitis C, virus and malaria co-infection. Int J Vaccine Immun 2015;1:1-3.
14. Kamani J, Mani AU, Egwu GO, Kumshe HA. Seroprevalence of human infection with *Toxoplasma gondii* and the associated risk factors, in Maiduguri, Borno state, Nigeria. Ann Trop Med Parasitol 2009;103:317-21.
15. Gyang VP, Akinwale OP, Lee YL, Chuang TW, Orok A, Ajibaye O, *et al. Toxoplasma gondii* infection: Seroprevalence and associated risk factors among primary schoolchildren in Lagos city, Southern Nigeria. Rev Soc Bras Med Trop 2015;48:56-63.
16. Roitt I, Brostoff J, Male D. Immunology. 6th ed. St. Louis: Mosby; 2015. p. 480.
17. Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, *et al.* Innate or adaptive immunity? The example of natural killer cells. Science 2011;331:44-9.
18. Boyle JJ. Macrophage activation in atherosclerosis: Pathogenesis and pharmacology of plaque rupture. Curr Vasc Pharmacol 2005;3:63-8.