Asian Journal of Immunology

2(1): 64-71, 2019; Article no.AJI.53159

Interleukin-12 (P70) Concentrations in Malaria Patients Attending Some Hospitals in Zaria, Kaduna State, Nigeria

Gideon Yakusak Benjamin[1*], B. E. Moses[2], E. D. Jatau[1] and C. M. Z. Whong[1]

[1]Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of General Studies, School of Agricultural Technology, Nuhu Bamalli Polytechnic, Zaria, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author GYB designed the study, wrote the protocol and performed the laboratory experiments and statistical analysis. Author BEM read and contributed to literature searches. Authors EDJ and CMZW co-supervised the research. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Tania Mara Pinto Dabes Guimaraes, Associate Professor, Faculty of Pharmacy, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.
(2) Dr. Cynthia Aracely Alvizo Baez, Professor, Laboratory Immunology and Virology, Faculty of Biological Sciences, Autonomous University of Nuevo Leon, Mexico.
(3) Dr. Darko Nozic, Professor, Higher Medical School in Belgrade, University of Belgrade, Serbia.
Reviewers:
(1) Muhammad Ghafoor Ali, Pakistan.
(2) Fadia M. Attia, Suez Canal University, Egypt.
Complete Peer review History: http://www.sdiarticle4.com/review-history/53159

Original Research Article

Received 15 October 2019
Accepted 19 December 2019
Published 25 December 2019

ABSTRACT

Background and Aim: Malaria is the most important parasitic disease of man, and it remains one of the major threats to public health and economic development in Africa. Interleukin-12 is a heterodimeric cytokine which has potent effects on innate and adaptive immunity. This study was aimed at determining Interleukin-12 (p70) concentrations among malaria patients attending some hospitals in Zaria, Kaduna State.
Methods: A cross sectional hospital based study was conducted on consenting participants in Zaria. Four hundred blood samples were collected, from which Giemsa-stained thick and thin blood films were prepared and examined for the presence of *Plasmodium* species by microscopy. Enzyme Linked Immunosorbent Assay (ELISA) was used to determine concentrations of interleukin-12 (p70) in malaria positive samples and control samples.

**Corresponding author: E-mail: gideonbenjamin.y@gmail.com;*

Results: Males had higher malaria prevalence (37.2%) than females (24.7%). The difference was statistically significant (*P* = 0.01). Pregnant women had a prevalence of 17.8% which was lower than the 27.9% obtained in non-pregnant women (*P* = 0.07), and the highest malaria prevalence (20.0%) was found in pregnant women in their third trimester (*P* = 0.65). Interleukin-12 (p70) was present at a significantly (*P* = 0.00) higher level in the plasma of participants in the malaria positive group than in the control group (those who tested negative for malaria).
Conclusion: Gender was significantly associated with malaria in this study. The prevalence of malaria was higher in males than females; males are therefore encouraged to take more precautions to prevent malaria. Despite the fact that the exact role of cytokines in malaria pathogenesis is unclear, we can infer from the findings of this study that more interleukin-12 (p70) is produced during malaria infection.

Keywords: Interleukin-12(p70); plasmodium; prevalence; malaria; cytokine.

1. INTRODUCTION

Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female *Anopheles* mosquitoes. It is preventable and curable [1]. Malaria is the most important parasitic disease of man. Approximately 5% of the world's population is infected. It remains one of the major threats to public health and economic development in Africa. It is estimated that three million deaths result from malaria throughout the world, with Africa having more than 90% of this burden [2]. About half of the world's population is at risk of malaria [3]. In 2016, an estimated 445 000 malaria deaths occurred worldwide from an estimated 216 million reported cases of malaria; 90% of these cases were in the World Health Organization (WHO) African Region. Of the 91 countries reporting indigenous malaria cases in 2016, 15 countries – all in sub-Saharan Africa, except India – carried 80% of the global malaria burden [1]. In 2017, *Plasmodium falciparum* accounted for 99.7% of estimated malaria cases in the WHO African Region, as well as in the majority of cases in the WHO regions of South-East Asia (62.8%), the Eastern Mediterranean (69%) and the Western Pacific (71.9%) [1].

According to the level of malaria transmission and immunity acquisition, vulnerable populations differ in endemic areas. In highly endemic settings, children under five years and pregnant women are the most affected, constituting the main target population of new malaria control strategies as recommended by the World Health Organization [4]. There are now a large number of regular prevalence surveys of childhood parasitemia [5], as most malaria deaths occur in children. However, the prevalence of parasitemia in adults remains of scientific interest, not only because clinical attacks in adults remain an important cause of death in adults [6,7], as well as of morbidity and health service use [8], but also because adults form a community reservoir of infection for children. With the current sustained implementation of malaria control and prevention strategies across most African countries and the consideration of elimination in some settings [9,10], the impact of this adult reservoir in these control strategies needs to be assessed. In pregnancy, there is a transient depression of cell-mediated immunity that allows fetal allograft retention which in the other hand interferes with resistance to various infectious diseases such as malaria [11]. On the top of host, pregnant women, immunossupression; studies showed that immunological interactions between protozoan and helminths infection can intensify the impact of parasitic infection when they co-exist [12]. In addition, as the epidemiology of malaria changes across Africa there are likely to be changes in the disease pattern with adults becoming susceptible to severe disease and this trend should be monitored.

While there are many studies that associate patterns of cytokines to disease, results may be different depending on the cohort population. Thus, there is an association between elevations in certain cytokines and disease outcomes, but it is hard to generalize these associations to different patient populations. IL-12 acts on antigen stimulated CD4+ T cells, promoting the differentiation of T cells into the Th1 subset [13], which acts on macrophages not only to stimulate their microbicidal functions, but also to increase their production of IL-12. The elevated levels of IL-12 also modulate the macrophage activity, which is associated with the increased erythrocyte destruction, bone marrow dyserythropoiesis [14] and thrombocytopenia [15]. During the intraerythrocytic life cycle of

Plasmodium falciparum, macrophages avidly phagocytize parasite specific products, leading to the impairment of macrophage functions [16] and cytokine production [17].

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in four selected hospitals in Zaria Nigeria; Major Ibrahim B. Abdullahi Memorial Hospital Zaria, Hajiya Gambo Sawaba Hospital Kofan Gaya Zaria, Salama Hospital and St. Luke's Hospital Wusasa Zaria. Zaria is a city found in Kaduna state, Nigeria. It is located at 11.11 latitude and 7.72 longitude and it is situated at elevation 644 meters above sea level. Zaria has a population of 975,153 making it the second largest city in Kaduna [18].

2.2 Sample Size

The sample size was determined using a prevalence of 23.45% [19] and the following formula as described by Naing, et al. [20]:

$$n = \frac{z^2 p(1-p)}{d^2}$$

n= number of samples

p=prevalence rate of previous study = 23.45% = 0.2345

z=standard normal distribution at 95% confidence limit = 1.96

d=absolute desired precision of 5% = 0.05
z=1.96

$$n=\frac{1.96^2 * 0.2345(1-0.2345)}{0.05^2}$$

n=275 samples

Four hundred (400) blood samples were however collected for this study.

2.3 Administration of Structured Questionnaire

A structured questionnaire was used to collect data from consenting participants.

2.4 Sample Collection

A total of 400 blood samples were collected from Major Ibrahim B. Abdullahi Memorial Hospital Zaria (former Limi Hospital), Hajiya Gambo Suwaba Hospital Kofan Gaya Zaria, Salama Hospital and St. Luke's Hospital Wusasa Zaria (100 samples from each hospital). Venipuncture technique was used for blood sample collection. A soft tubing tourniquet was fastened to the upper arm of the patients to enable the index finger to feel a suitable vein. The puncture site was then cleansed with Methylated spirit (methanol) and venipuncture was made with the aid of a 21 G needle attached to a 5 ml syringe. When sufficient blood (3 ml) was collected, the tourniquet was then released and the needle removed immediately while the blood was transferred into an EDTA bottle [21].

2.5 Determination of Malaria Parasitemia

The malaria parasitemia was determined using a grading scheme of + =1-10 parasites, ++ = 11-20 parasites, +++ = more than 20 parasites per microscopic field was used to establish the levels of parasitemia [19].

2.6 Determination of Interleukin-12 (p70) Concentrations

The concentrations of interleukin-12 (p70) were determined in eighty eight (88) serum samples, using Boster's interleukin-12 ELISA kit purchased from Boster Biological Technology Co. Ltd. (Fremont, CA USA). The samples were divided into two groups; the malaria positive group (59) and the control group; without parasitemia (29). The interleukin-12 concentrations were determined according to manufacturer's instruction.

2.7 Statistical Analysis

The data obtained were analyzed using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL,USA). Chi square and odds ratio were used to check for association. $P \leq 0.05$ was considered significant.

3. RESULTS

Table 1 shows malaria prevalence in relation to gender. Out of the 113 males examined; 42 (37.2%) were positive, while out of the 287 females screened; 71 (24.7%) were positive. Therefore males had a higher prevalence (37.2%) than females (24.7%). The *P* value and Odds ratio showed significant statistical association (*P* = 0.01, Odds ratio=1.800, Confidence interval= 1.129-2.869, Chi square = 6.180).

The age related prevalence of malaria among the study population is shown in Table 2. The prevalence rate in the table reveals that the highest prevalence of 50.0% was found in the age group 66 years and above, followed by the age group 56-60 years with 44.4% prevalence, 51-55 years with 40.0% prevalence, 11-15 years with 37.5% prevalence, 41-45 years with 33.3% prevalence and 16-20 years with 32.9% prevalence. The lowest prevalence was in the age group 46-50 years (0.00%). The age group 31-35 years also had a low prevalence of 13.3%. Statistically, there was no significant difference among the age groups (χ^2=11.620, *P* value=0.56).

Table 3 shows the prevalence of malaria in relation to pregnancy status. Pregnant women had a prevalence of 17.8% which was lower than the 27.9% obtained in non-pregnant women. The difference was not statistically significant (*P*>0.05).

Table 4 shows the distribution of malaria according to pregnancy trimester. Pregnant women in their third trimester had the highest prevalence (20.0%), followed by those in their second trimester (19.6%) and those in their first trimester (10.5%). The difference was not statistically significant (*P*>0.05).

Table 5 shows the mean concentrations of IL-12 (P70) among malaria positive individuals and control group. The malaria positive group had a mean concentration of 28.31 pg/ml which was high compared to the 19.23 pg/ml mean concentration of the control group (those without malaria infection). The statistical difference was not significant (*P*=0.06).

Fig. 1 shows the mean concentrations of IL-12 (p70) in relation to malaria parasitemia. The ++ parasitemia group had the highest mean concentration of 39.39 pg/ml. The + parasitemia group had 22.59 pg/ml mean concentration of IL-12 (p70) which was lower than the 27.67 pg/ml mean IL-12 (p70) concentration found in the +++ parasitemia group. The mean IL-12 (p70) concentration in each of the three parasitemia groups (+,++,+++) was more than the mean IL-12 (p70) concentration in the control group (19.23 pg/ml). The difference was statistically significant (df=3, *P* value=0.00).

Table 1. Malaria prevalence in relation to gender

Gender	No. examined	No. positive	% Prevalence	χ^2	*P* value	OR	95%CI
Male	113	42	37.2	6.180	0.01*	1.800	1.129-2.869
Female	287	71	24.7				
Total	400	113	28.3				

*Key: No=Number, OR=Odds ratio, CI=Confidence interval, *=Significant*

Table 2. Age-related prevalence of malaria among the study population

Age(years)	No. examined	No. positive	% prevalence	χ^2	*P* value
0-5	43	11	25.6	11.620	0.56
6-10	28	9	32.1		
11-15	24	9	37.5		
16-20	70	23	32.9		
21-25	74	20	27.0		
26-30	61	17	27.9		
31-35	30	4	13.3		
36-40	26	7	26.9		
41-45	18	6	33.3		
46-50	8	0	0.00		
51-55	5	2	40.0		
56-60	9	4	44.4		
61-65	2	0	00.0		
66>	2	1	50.0		
Total	400	113	28.3		

Key: No=Number, χ^2=Chi square

Table 3. Malaria prevalence in relation to pregnancy

Pregnant	No. examined	No. positive	% Prevalence	χ^2	*P* value
Yes	90	16	17.8	3.412	0.07
No	197	55	27.9		
Total	287	71	24.7		

Key: No=number χ^2= Chi square

Table 4. Distribution of malaria according to pregnancy trimester

Trimester	No. examined	No. positive	% prevalence	χ2	*P* value
First	19	2	10.5	0.867	0.65
Second	56	11	19.6		
Third	15	3	20.0		
Total	90	16	17.8		

Key: No=number χ^2= Chi square

Table 5. Mean concentrations of IL-12 (P70) among malaria positive individuals and control group

Malaria	No.	Mean(pg/ml)	SE	*P* value
Positive	59	28.31	2.003	0.06
CG	29	19.23	2.125	
Total	88	25.32	1.576	

Key: CG= control group, SE=Standard error, No.= number of samples

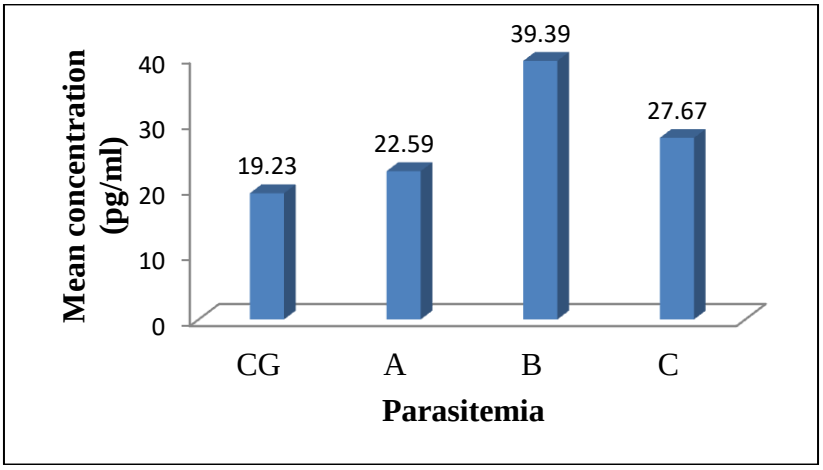

Fig. 1. Mean concentrations of IL-12 (p70) in relation to malaria parasitemia

(P value<0.05)

KEY: CG= control group, A= +, B=++, C=+++, (+=1-10, ++=11-20, +++=20> parasites per microscopic field)

4. DISCUSSION

In the current study, we found a high prevalence of malaria in males than females. The difference observed was statistically significant (p<0.05). This agrees with the findings of Muntaka and Opoku-Okrah [22] who reported the percentage of males with malaria to be higher than females; 19.4% and 10.7% for males and females respectively. Reza and Taghi [23] also reported a similar finding. Our finding is in contrast to that of Otajevwo [24] who reported a higher infection rate in females than males. The high prevalence in males may be due to the fact that men are less likely to sleep under the insecticide treated bed nets than females [4]. According to Olapeju, et al. [25], ITN use tends to be higher among females than males especially in households without sufficient ITNs. In some societies, men have a greater occupational risk of contracting malaria than women if they work in mines, fields or forests at peak biting times. Leisure activities and

sleeping arrangements may also be contributing factors, as men are more likely to sleep outdoors or be found outdoors during the active biting hours of *Anopheles* mosquito [26,27]. This can increase the human-vector contact, and consequently lead to *Plasmodium* infection [26].

Interleukin-12 (p70) was present at a significantly (p=0.000) higher level in the plasma of those in the malaria positive group than in the control group (those who tested negative for malaria). This is in agreement with the findings of Lyke, et al. [28]. However, Adrian, et al. [29] reported that concentration of IL-12 (p70) was significantly higher in the plasma of those with mild malaria than in the plasma of those with severe malaria. On comparing the different parasitemia groups and their mean interleukin-12 (p70) concentrations, the control group still had the lowest mean concentration of IL-12 (p70). The + and +++ parasitemic group had mean IL-12 (p70) concentrations which were less than the IL-12 (p70) concentrations in the ++ parasitemia group. This is possible because low interleukin-12 (p70) activity has also been associated with severe *Plasmodium falciparum* malaria [30]. Our finding is however in contrast to that of Adrian, et al. [29] who reported that the acute-phase, pretreatment plasma IL-12 and alpha interferon (IFN-a) levels, as well as the acute-phase mitogen-stimulated whole-blood production capacity of IL-12, were significantly lower in children with severe rather than mild malaria. It has been reported that early events in the cell-mediated immune response required for protection against malaria are initiated by the release of interleukin-12 (IL-12) from monocytes/macrophages, B cells, and perhaps other cell types [31,32]. In *Plasmodium falciparum* infection, IL-12 has immunoregulatory functions with effects on the immune response to the blood stage of disease, but also induces protection and reduces malarial anemia [33,34]. IL-12 has been shown to be involved in protective immunity against malaria by regulating gamma interferon. Pro-inflammatory cytokines like IL-12 are thought to be critical for controlling the erythrocytic and hepatic stages of *Plasmodium* infection [34] this may be one of the reasons why we had higher concentrations in the parasitemia group than the control group in this study.

5. CONCLUSION

This study found a significant association between the gender of participants and malaria. The prevalence of malaria was higher in males than females; males are therefore encouraged to take more precautions to prevent malaria. Although the concentrations of interleukin-12 (IL-12 (p70) in this study differed with parasitemia, its concentrations reflect the role it plays in malaria infection. And despite the fact that the exact role of cytokines in malaria pathogenesis is unclear, the findings of this study suggest that more IL-12 (p70) cytokine is produced during malaria infection.

CONSENT

As per international standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The research protocol was read and approved by the Ethics and Research Committee (ERC) of Kaduna State Ministry of health, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization. World Malaria Report; 2017.
2. Breman JG, Alilio MS, Mills A. Conquering the intolerable burdine of malaria; what is new, what is needed. American Journal of Tropical Medicine and Hygiene. 2004; 71(2):1-15.
3. World Health Organization (WHO). World Malaria Report. Geneva: Licence: CC BY-NC-SA 3.0 IGO; 2016.
4. World Health Organization. Guidelines for the Treatment of Malaria; 2010.
5. WHO. The world malaria report. WHO, Geneva; 2013. Available:http://www.who.int/malaria/publications/ world_ malaria_report
6. Adjuik M, Smith T, Clark S, Todd J, Garrib A, Kinfu Y. Cause specific mortality rates in sub Saharan Africa and Bangladesh. Bull World Health Organ. 2006;84:181–188.
7. Murray CJ, Rosenfeld LC, Lim SS, Andrews KG, Foreman KJ, Haring D. Global malaria mortality between 1980 and 2010: A systematic analysis. Lancet. 2012; 379:413–431.
8. Snow RW, Craig M, Deichmann U, Marsh K. Estimating mortality, morbidity and

disability due to malaria among Africa's non-pregnant population. Bull World Health Organ.1999;77:624–640.

9. Alonso PL, Brown G, Arevalo-Herrera M, Binka F, Chitnis C, Collins F. A research agenda to underpin malaria eradication. PLoS Med. 2011;8:e1000406.
10. Snow RW. Global malaria eradication and the importance of *Plasmodium falciparum* epidemiology in Africa. BMC Medicine. 2015;13:23.
DOI:10.1186/ s12916-014-0254-7
11. Trowsdale J, Betz AG. Mother's little helpers: Mechanisms of maternal fetal tolerance. National Immunology. 2006; 7:241–246.
12. Mwangi TW, Bethony J, Brooker S. Malaria and helminths interactions in humans: An epidemiological viewpoint. Annals of Tropical Medicine and Parasitol. 2006; 100:551–570.
13. O'Garra A, Arai N. The molecular basis of T helper 1 and T helper 2 cell differentiation. Trends in Cell Biology. 2000;10:542–550.
14. Philips RE, Pasvol G. Anemia of *Plasmodium falciparum* malaria. Baillieres Clinical Haematology. 1992;5:315–330.
15. Lee SH, Looareesuwan S, Chan J. Plasma macrophage colony-stimulating factor and P-selectin levels in malaria-associated thrombocytopenia. Journal of Thrombosis Haemostasis. 1997;77:289–293.
16. Schwarzer EF, Turrini F, Ulliers G, Giribaldi HG, Ginsburg H, Arese P. Impairment of macrophage functions after ingestion of *Plasmodium falciparum* infected erythrocytes or isolated malarial pigment. Journal of Experimental Medicine. 1992;173:1033–1041.
17. Scholfiewd L, Hackett F. Signal transduction in host cells by lycosylphosphatidylinositol toxin of malaria parasites. Journal of Experimental Medicine. 1993;177:145–153.
18. World Atlas. Where is Zaria, Nigeria? 2015.
Available:http://www.worldatlas.com/af/ng/kd/where-is-zaria.html
19. Bechemagbor A. Studies on malaria prevalence, drug treatment regimes and intensity of chloroquine-resistant *Pasmodium falciparum* in Zaria, Nigeria; 2010.
Available:http://hdl.handle.net/123456789/4048
20. Naing L, Winn T, Rusli BN. Practical issues in calculating the sample size for prevalence studies. Archives of Orofacial Sciences. 2006;1:9-14.
21. Epidi TT, Nwani CD, Ugorji NP. Prevalence of malaria in blood donors in Abakaliki Metropolis, Nigeria. Scientific Research and Essay. 2008;3:162-164.
22. Muntaka S, Opoku-Okrah C. The Prevalence of malaria parasitemia and predisposition of abo blood groups to *Plasmodium falciparum* malaria among blood donors at a ghanaian hospital. Assumption University Journal of Technology. 2013;16(4):255-260.
23. Reza YM, Taghi RM. Prevalence of malaria infection in Sarbaz, Sistan and Bluchistan province. Asian Pacific Journal of Tropical Biomedicine. 2011;1(6):491–492.
DOI: 10.1016/S2221-1691(11)60107-X
24. Otajevwo FD. Prevalence of malaria parasitemia and its association with ABO blood grouping among students of Igbinedion University Okada, Nigeria. British Journal of Medicine and Medical Research. 2013;3(4):1:164-77.
25. Olapeju B, Choiriyyah I, Lynch M, Acosta A, Blaufuss S, Filemyr, et al. Age and gender trends in insecticide-treated net use in sub-Saharan Africa: A multi-country analysis. Malaria Journal. 2018;17:423.
26. World Health Organization. Gender, Health and Malaria; 2007.
27. Monroe A, Mihayo K, Okumu F, Finda M, Moore S, Koenker H, et al. Human behaviour and residual malaria transmission in Zinzibar: Findings from in-depth interviews and direct observation of community events. Malaria Journal. 2019; 18:220.
28. Lyke KE, Burges R, Cissoko Y, Sangare L, Dao M, Diarra I, et al. Serum levels of the proinflammatory cytokines Interleukin-1 Beta (IL-1β), IL-6, IL-8, IL-10, tumor necrosis factor alpha, and IL-12(p70) in malian children with severe *Plasmodium falciparum* malaria and matched uncomplicated malaria or healthy controls. Infection Immunity. 2004;72(10):5630-5637.
DOI: 10.1128/IAI.72.10.5630-5637.2004
29. Adrian JFL, Douglas JP, Bertrand L, Ruprecht S, Leopold GL, Doris L, et al. Low Interleukin-12 activity in severe *Plasmodium falciparum* malaria. Infection and Immunity. 2000;68(7):3909.

30. Nnaemeka CI, Okafor CMF, Balogun HA, Idowu A, Yusuf O, Jan-Olov P, et al. Cytokine profiles and antibody responses to *Plasmodium falciparum* malaria infection in individuals living in Ibadan, southwest Nigeria. African Journal of Health Science. 2009;9(2):66–74.
31. Crutcher JM, Stevenson MM, Sedegah M, Hoffman SL. Interleukin-12 and malaria. Research in Immunology. 1995;146:552–559.
32. Mosmann T, Coffman R. Th1 and Th2 cells: Different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology. 1989;7:145–173.
33. Malaguarnera L, Imbesi RM, Pignatelli S, Simpore J, Malaguarnera M, Musumeci S. Incereased levelsof interleukin-12 in *Plasmodium falciparum* malaria: Correlation with the severity of disease. Parasite Immunology. 2002;24(7):387-389.
34. Song Y, Aguilar R, Guo J, Manaca MN, Berthoud TK, Khoo S, et al. Cord blood IL-12 confers protection to clinical malaria in early childhood life. Scientific Reports. 2018;8:10860.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/53159