Research Article

Immunoglobulin Levels in *Plasmodium falciparum* Malaria Infected Subjects in Port Harcourt, Nigeria

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Abstract

This study assessed the immunoglobulin levels (IgG, IgM and IgE)in patients with acute *Plasmodium falciparum* malaria infection in Port Harcourt, Nigeria, reporting to the participating clinics and compared with apparently healthy non-parasitemic persons from the same population. A total of 226 malaria parasitaemia positive subjects comprising of 136 males and 90 females and 47 malaria parasitaemia negative subjects (control) comprising of 25 males and 22 females were recruited. QBC malaria parasite specie identification and quantification was used to determine the malaria infection status of the subjects. Immuno-turbidimetric assay was used to analyze for immunoglobulin G (IgG) and immunoglobulin M (IgM) levels while analysis for immunoglobulin E (IgE) concentration was by Microwell enzyme immunoassay (EIA). The IgG and IgM did not show any observable variation with malaria infection. There was a negative correlation between IgG antibody concentration and parasitemia. IgM had a strong positive correlation with parasite density (P<0.01) among both male and female infected subjects. Persons with parasitemia tended to have significantly higher IgE than those who were malaria-negative (P<0.05). There was a strong positive correlation between IgE and parasite density (P<0.01) with over 600% increase in the mean value of IgE from moderate to very heavy infection. This study supplements previous literature on the immunologic effects of malaria and helps define those alterations for an endemic population. Abnormally elevated IgE was identified as a key indicator of malaria in these febrile patients.

Introduction

The scourge of malaria has continued to devastate many parts of Sub-Saharan Africa, including Nigeria. Although the Federal Government has been paying attention to the scourge through the introduction of the Roll Back Malaria (RBM) initiative in 1999 to combating malaria through United Nations Millennium Development Goals (MDGs) initiatives with 2015 as the target year and now through sustainable Development Goals (SDGs), many Nigerians are still living at the mercy of the disease. The incidence of malaria in Port Harcourt city, a typical coastal zone in the Niger Delta of Nigeria located between latitudes $4^o\ 2^/$ North and $4^o\ 47´$ North and longitudes $6^o\ 55´$ East and $7^o\ 08´$ East, has continued to increase despite all government efforts to reduce the malaria burden in Nigeria. Some of the reasons are the mean annual temperature of 26° C and rainfall of about 2,405mm (Gobo, 1988) which favours *Plasmodium species* and its vector.

Malaria caused by the protozoan parasite *Plasmodium falciparum* is a major burden of disease globally,

causing an estimated 225 million illness episodes and around 800,000 deaths per year (World Health Organization, 2010). Young children are at highest risk of developing malaria, with *P. falciparum* being a leading cause of mortality among children under 5 years (Elliott & Beeson, 2008).

Malaria is holoendemic in Nigeria with *Plasmodium falciparum* as the dominant strain (Lesi and Adenuga, 1996).The most severe form of malaria is caused by *P. falciparum.* The severity of the disease depends largely on the species and strain of the infecting parasite, and the immunological status of the person who is infected.

The symptoms and clinical complications of malaria are caused by the erythrocytic stage of infection, and the majority of the acquired immune response is against these blood-stage parasites (Beeson *et al*., 2008; Doolan *et al*,** 2009). The capacity for immune evasion enables *P. falciparum* to cause repeated and chronic infections; after repeated exposure to malaria, individuals eventually develop effective immunity that controls parasitemia and prevents severe and life-threatening complications.

Activation of the immune system in malaria infection plays an important role both in defense against the parasite and the pathogenesis of the disease (Peter *et al*., 1997). Acute malaria in man appears to induce a temporary state of reduced immune-competence and increased susceptibility to concomitant infections, accompanied by changes in many parameters of immune function (Weidanze, 1982, Ivan and Delves, 2001).

Where malaria transmission is "stable" (where populations are continuously exposed to a fairly constant rate of malarial inoculations) and if the inoculation rates are high (entomological inoculation rate (EIR) >10/year), then partial immunity to the clinical disease and to its severe manifestations is acquired early in childhood (Weidanze, 1982; William and Phillip, 2003). In such situations, which prevail in most of sub-Saharan Africa and part of Oceania, the acute clinical disease is almost always confined to young children who suffer high parasite densities and acute clinical disease. If untreated, this can progress very rapidly to severe malaria. In stable and high-transmission areas, adolescents and adults are partially immune and rarely suffer acute clinical malaria, although they continue to harbour low blood-parasite densities (Weidanze, 1982). Immunity against malaria infection is reduced in pregnancy, and can be lost when individuals move out of the transmission zone (Weidanze, 1982).

In areas of unstable malaria, the situation prevailing in most of Asia and Latin America and the remaining parts of the world where malaria is endemic, the rates of inoculation fluctuate greatly over seasons and years(EIRs are usually <5/year and often >1/year). This retards the acquisition of immunity and results in people of all ages, adults and children alike, suffering acute clinical malaria, with a high risk of progression to severe malaria if untreated. Epidemics may occur in areas of unstable malaria when inoculation rates increase rapidly.

The precise targets of protective immunity against malaria remainunknown, although present evidence implicates about 20 candidateantigens, most of them polymorphic surface proteins (Berzins and Anders, 1999; Mahajam *et al*., 2004). Structurally different antigens are expressed during each partof the parasite's life cycle and hence, naturally acquired immunity ismostly stage specific (Berzins and Anders, 1999). Both antibodies and T cells arerequired for naturally acquired immunity. CD4$^+$ and CD8$^+$ T-cellresponses are particularly effective against intracellular liver-stageparasites, while antibodies may block host cell invasion bysporozoites and merozoites. In addition, antibodies to variantantigens expressed on the surface of RBCs may facilitate phagocytosisof infected cells and inhibit or reverse their adhesion to endothelialreceptors. T-cell helper is essential for an effective antibodyresponse, but relatively little is known about the additional roles of cell-mediated immunity in the clearance of blood-stage malaria parasites (Berzins and Anders, 1999).

Achidi *et al*., (2005) carried out a study on *Plasmodium falciparum* isotypic antibodies in paired maternal cord blood from South West Cameroon and reported that the mean ELISA OD values of neonates born from positive placentas, or whose mothers had peripheral malaria parasitemia were higher than those who were parasite negative. The Fulani group in Nigeria and Mali had significantly higher levels of IgG and IgE against crude malaria antigen than the Dogon group in Mali. This is suggesting a role of anti-malaria antibodies in the immune protection seen in these groups (Bryceson *et al.,* 1976; Greenwood *et al.,* 1987).

Malaria parasitemia has been reported to have effects on some immunlogical parameters in many parts of the world (Mahajam *et al* 2004; Joana *et al*., 2007).

People living in malaria endemic areas have elevated levels of immunoglobulin E (IgE) in their blood (Biswas *et al.,* 1990; Perlmann *et al*., 1994).

This study examined the immunoglobulin levels (IgG, IgM and IgE*)* onmalaria infected subjects in Port Harcourt Nigeria. Specifically, the immunoglobulin profiles of persons infected with *P. falciparum* were compared with expected normal values. Additionally, correlation analysis was employed to ascertain the level of relationship between parasite density and immunoglobulin levels. Also,immunoglobulin type most predictive of malaria in this population was identified.

Methodology

The laboratory study was carried out for one year during which a total of two hundred and twenty six (226) malaria patients comprising of 136 males and 90 females and forty-seven control subjects comprising of 25 males and 22 females were recruited. The inclusion criteria were out-patients to the participating clinic within the age of 1- 60 years queried for malaria infection with the presentation of at least one of the following: an oral temperature of 38°C, headache, or a history of fever within the past 72 hours and who must not have commenced any treatments for malaria. Exclusion criteria were out-patients with pathological conditions outside malaria such as protozoan or helminthes infection, typhoid fever and HIV/AIDS, congenital manifestations such as sickle cell disease, physiological manifestations such as pregnancy and history of allergy.

Control subjects were selected from among apparently healthy male and female subjects aged 1 - 60 years without QBC detectable parasitemia. Such subjects must also not have had recent malaria attack/ treatment or evidence of congenital or any recent pathological or physiological manifestations.All enrolled patients and controls were interviewed for information on current symptoms and previous malaria episodes and treatments.

Blood Collection

A volume of 2.6 ml of venous blood sample was drawn into monovette tubes containing the anticoagulant potassiumethylenediamine-tetraacetate (EDTA) for QBC malaria parasite specieidentification and quantification and finally spun to obtain the plasma for the immunoglobulin assays. Daily quality assurance checks were performed and recorded.

Malaria Parasite Detection Analysis

The centrifuged tube was examined under a fluorescence microscope in the region between the light red blood cells and granulocytes and lymphocytes/monocytes, where the parasites are most abundant (Benito *et al*., 1994). Examination of the centrifuged blood under a fluorescence microscope readily permits the detection of malaria parasite in the infected cells and plasma. Since the parasites contain DNA which takes up the acridine orange stain, they appear as bright specks of light in the dark background of non-fluorescing red cells (Rickman *et al*., 1989)

QBC Malaria Parasite Species Identification

At magnification of 600X, all parasites in the red blood cells were easily visualized and their morphologies identified. Species identifications were made based upon the size and shape of the various stages of the parasite and the presence of stippling and fimbriation. Plasmodium parasites are always intracellular, and they demonstrate, if stained correctly, blue cytoplasm with a red chromatin dot. The parasite densities were obtained by multiplying the average number of parasites in 10 QBC fields by a factor of 10.5 (QBC operator's manual, 2006)

Immuno-Turbidimetric Assay

Immuno-turbidimetricassayusing IgG and IgM Randox reagents was used to quantify IgG and IgM respectively. The Autolab machine was used and was first calibrated with the standard reagent (Randox Liquid Assayed specific protein calibrator). The controls were equally diluted in the same ratio as in calibration and placed in the machine control position. Samples containing human IgG and IgM were suitably diluted and then reacted with specific antiserum to form a precipitate which was measured turbidimetrically at 340 nm.

After the reagent, sample and control information have been programmed into the machine, the assemblage was allowed to carry out the analysis automatically and have the results displayed on the screen and then finally printed out. Proper quality control measures were adopted.

Microwell Total-IgE EIA Employment for IgE Quantification

Microwell Total Immunoglobin E (IgE) enzyme immunoassay was employed for IgE quantification.

The microwell total-IgE EIA is a solid-phase enzyme immunoassay based on the "sandwich" principle. Two separate antibodies directed against distinct antigenic determinants of the IgE molecule were utilized in the assay. During the first incubation, the IgE present in the test samples reacts and binds with anti-IgE antibody immobilized on the microwell surface. During the second incubation, the bound IgE reacts with and to another anti-IgE antibody conjugated to horseradish peroxidase enzymes (HRP). So an Ab-Ag-Ab-enzyme complex is formed on the microwell surface.

The unbound conjugate was then removed by washing and the colour development reagents (substrates) are added. Upon exposure to the enzyme, there was a colour change. The intensity of the colour reflects the amount of bound anti-IgE enzyme conjugate and is proportional to the concentration of IgE in the specimen within the dynamic range of the assay. After stopping the reaction, the resulting colour was measured using a spectrophotometer at 450nm. Using a standard curve obtained by plotting the IgE concentration of reference standards versus the corresponding absorbances, the IgE concentration of the concurrently run test samples and controls were then determined.

The data obtained were grouped into males and females and each sex group further divided into four parasite densities to determine the effect of sex and parasite density on the immunoglobulin levels of the malaria infected subjects. Independent-sample t-test was used to compare the immunoglobulin levels of the malaria infected groups with their corresponding sex specific control groups. The mean and standard deviation (SD) and correlation of the Immunoglobulin levels were carried out using Excel Statistical Analysis Package. The correlation was performed at 99% confidence level of significance. The significance of the correlation was tested to determine the probability (P) of chance occurrence. The type and degree of association of sex and parasitemia with immunoglobulin levels was assessed using the results of correlation analysis.

Results

Immunoglobulin levels of *P. falciparum* malaria infected subjects were compared with those of the sex-specific control group. There were observable variations in the Mean±SD of Immunoglobulin levels of the infected male subjects and the control males on one hand and females infected subjects and the control females on the other hand as shown in Tables 1 and 2.

The difference between the mean values of IgG and IgM in the male and female malaria infected subjects were not statistically significant from their corresponding control group ($P > 0.05$) as shown in Tables 1 and 2 respectively. However the difference between the mean values of IgE in both infected sexes and their corresponding controls were statistically significant ($P < 0.05$) (Tables 1 and 2)

Table 1: Immunoglobulin levels in Malaria infected and non- infected Males

Immunoglobulin levels	Male control subjects	Male infected subjects	P-value
IgG (g/dl)	14.59±0.57	13.29±0.78	P>0.05
IgM (g/dl)	1.77±0.08	2.23±0.58	P>0.05
IgE (IU/ml)	35.39±36.19	613.03±295.22	P<0.05

Table 2: Immunoglobulin levels in Malaria infected and non- infected Females

Immunoglobulin levels	Female control subjects	Female infected subjects	P-value
IgG (g/dl)	15.32±0.48	13.25±0.92	P>0.05
IgM (g/dl)	1.67±0.10	2.35±0.64	P>0.05
IgE (IU/ml)	40.05±40.87	639.24±315.44	P<0.05

The variations of immunological parameters with changes in parasitemia are shown in Tables 3 and 4. The mean value of IgG decreased gradually from 14.45±0.51g/l in scanty infection (1+) to 12.35±0.57 g/l in very heavy infection (4+) in the male malaria infected subjects. In the female malaria infected subjects the same parameter ranged from 14.75±2.25g/l in scanty infection to 12.27±0.84 g/l (Table 4). IgM increased only gradually with parasitemia in both males and females (Tables 3 and 4). Mean IgM value in the malaria infected male subjects ranged from 1.76±0.11 g/l in scanty infection to 3.04±0.56 g/l in heavy infection while the corresponding mean values in the malaria infected female subjects were 1.70±0.11 g/l to 3.11±0.66 g/l. IgE increased appreciably with parasitemia. IgE mean value in scanty infection among the male infected subjects was 158.15±97.91IU/ml while the mean value in very heavy infection was 1039.45±64.70IU/ml. Female infected subjects had a mean value of 130.64±109.10IU/ml in the scanty infection and 1046.75±125.58IU/ml in very heavy infections.

Table 3: Mean±SD of Immunoglobulin Levels of Males of Different Parasitaemia

Parameters	1+	2+	3+	4+
IgG (g/l)	14.45 ± 0.51	13.59 ± 0.68	13.16 ± 0.41	12.35 ± 0.57
IgM (g/l)	1.759 ± 0.11	1.95 ± 0.32	2.20 ± 0.33	3.04 ± 0.56
IgE (IU/ml)	158.2 ± 97.91	411.44 ±102.25	692.69± 152.38	1039.45 ± 64.70
PD(Parasite/µl)	4.9 ± 2.85	49.25 ± 25.06	377.97 ± 256.23	1359.95±309.19

Table 4: Mean±SD of Immunoglobulin Levels of Females of Different Parasitaemia

Parameters	1+	2+	3+	4+
IgG (g/l)	14.75 ± 0.25	13.6815 ± 0.51	13.09 ± 0.43	12.27 ± 0.84
IgM (g/l)	1.70 ± 0.11	1.9615 ± 0.35	2.36 ± 0.42	3.11 ± 0.66
IgE (IU/ml)	130.64± 109.10	415.5 ± 96.45	699.21± 150.78	1046.75 ± 125.56
PD (Parasite/µl)	3.64 ± 2.50	47.05 ± 20.59	377.37± 305.71	1447.70 ± 319.07

The correlation of immunoglobulin levels with parasite density in malaria infected subjects is shown in Table 5. IgM and IgE each had a strong positive correlation with parasite density among both male and female infected subjects. The correlation coefficients of IgM among the malaria infected male and female subjects were 0.9935 and 0.9739 respectively while the corresponding correlation coefficients of IgE are 0.9295 and 0.9142. Immunoglobulin G showed a strong negative correlation of -0.897 and -0.86389 in the male and female malaria infected subjects respectively.

Table 5: Correlation of Immunoglobulin Levels with Parasite Density in Malaria Infected Subjects

Parameters	Male		Female	
Immunoglobulin levels	Correlation coefficient	Probability	Correlation coefficient	Probability
IgG (g/l)	-0.8970	P<0.01	-0.8638	P<0.01
IgM (g/l)	0.9935	P<0.01	0.9739	P<0.01
IgE (IU/ml)	0.9295	P<0.01	0.9142	P<0.01

Discussion

When the mean IgG value of the malaria infected subjects and the control subjects were compared, it was discovered that malaria infection did not have any significant influence on the IgG of the infected subjects. The individual IgG values of all the infected males and females were all within the normal range of 8-18 g/l. A slight decrease in the mean IgG values was observed across the quartiles of parasite density for male and female subjects. This is in agreement with the findings of Desowitz et al., (1993).

The negative correlation between IgG antibody concentration and parasitemia described here supports the findings of Bouhaouron-Tayoun*et et al*., (1990) that antibodies are important for protection by reducing parasitemia and alleviating clinical illness in *P.falciparum* malaria.

A slight increase from the IgM normal range of 0.6-2.5 g/l for men and 0.7-2.8 g/l for women was observed across the quartiles of parasite density for both malaria infected sexes.

The mean values of IgE in both male and female controls were all within the normal range of 100 IU/ml. The effect of malaria infection on these values is evident from the sharp increase in the rate of abnormal values in the malaria infected subjects. Elevation of IgE values of malaria patients have also been reported by Jarrett *et al*., (1982); Desowitz *et al*., (1993); Helmby *et al*., (1996); Perlmann *et al.*, (2000) and Joana *et al*., (2007).

In each of the cases there was over 600% increase in the mean value of IgE from moderate to very heavy infection. This suggests that IgE antibodies may be contributing to the pathogenesis of malaria infection. This is similar to the results obtained by Craig *et al*., (2004) and Desowitz *et al*., (1993).

The negative correlation between IgG antibody concentration and parasitemia observed supports the findings of Bouhaouron-Tayoun *et al*., (1990) that antibodies are important for protection by reducing parasitemia and alleviating clinical illness in *P.falciparum* malaria. IgM and IgE showed a significant direct positive relationship with parasitemia in both malaria infected male and female subjects.

Conclusion

In this malaria-endemic area, an acutely febrile patient with abnormally high IgE values irrespective of QBC malaria or smear report should always be thoroughly re-evaluated for malaria. This study used very sensitive QBC procedures, which should minimize misclassification of malaria status. Daily quality control checks of the machines used maintained the accuracy and precision of the studied parameters.

Although these basic immunological changes associated with malaria are not new to the subject, our data add more detailed information to the limited body of knowledge. This study implies that malaria must always be a key differential diagnosis in acutely febrile patients with abnormally elevated IgE levels from this endemic area. This may afford means to improve diagnosis and alleviate the clinical severity of or accelerate recovery from this disease.

References

Achidi, E. A., Anchangb, J. K., Minangb, J. T., Mokube, J. A. & Troye-Blombergd, M. (2005). Studies on *Plasmodium falciparum* isotypic antibodies and numbers of IL-4 and IFN-secreting cells in paired maternal cord blood from South West Cameroon. *International Journal of Infectious Diseases,* 9, 159-169.

Beeson, J.G., Osier, F.H.A. & Engwerda, C.R. (2008). Recent insights into humoral and cellular immune responses against malaria.*Trends Parasitology*, 24(12), 578–584.

Benito, A., Roche, J., Molina, R., Amela, C. & Alvar, J. (1994). Application and evaluation of QBC malaria diagnosis in a holoendemic area. *Applied Parasitology,* 35, 266-272.

Berzins, K. & Anders, R. F. (1999). The malaria antigens. In: Wahlgren, M. & Perlmann, P. (eds.), *Malaria: Molecular and Clinical Aspects*. Harwood Academic Publishers, Amsterdam.

Biswas, S., Saxena, Q. . & Roy, A. (1990). The natural occurrence of circulating antibodies in populations of endemic malarious areas. *Indian Journal of Malariology,* 27, 139–148.

Bouhaouron-Tayoun, H., Attanath P., Sabchareon, A., Chongsuphajaisiddhi, T. & Druilhe P. (1990). Antibodies that protect humans against *Plasmodium falciparum* blood stages do noton their own inhibit parasite growth and invasion *in vitro,* but act in cooperation with monocytes. *Journal of Experimental Medicine*, 172, 1633–1641.

Bryceson, A. D. M., Fleming, A. F. & Edington, G. M. (1976). Splenomegaly in Northern Nigeria. *ActaTropica,* 33, 424–426.

Craig, S. B., Weinberg, J.B., Baker, J., Bockarie, M. J., Mgone, C. S., Cheng, O. & Anstey, N. M. (2004). Nitric Oxide Production and Nitric Oxide Synthase Activity in Malaria-Exposed Papua New Guinean Children and Adults. *Infection and Immunology*, 72, 6932–6938.

Desowitz, R. S., Elm, J. & Alpers, M. P. (1993). *Plasmodium falciparum* specific immunoglobulin G (IgG), IgM, and IgE antibodies in paired maternal-cord sera from East Sepik Province, Papua New Guinea. *Infection and Immu**nology***, 61, 988–993.

Doolan, D.L., Dobaño, C. & Baird, J.K(2009). Acquired immunity to malaria. *Clinical Microbiology Review*, 22(1),13–36.

Elliott, S. R. & Beeson, J.G. (2008). Estimating the burden of global mortality in children aged <5 years by pathogen-specific causes. *Clinical Infectious Diseases*,46(11):1794–1795.

Gobo, A. E. (1988). Relationship between rainfall trends and flooding in the Niger-Benue River Basins. *Journal of Metereology*, 13, 220-224.

Greenwood, B. M., Groenendaal, F., Bradley, A. K., Greenwood, A. M., Shenton F., Tulloch, S. & Hayes, R, (1987). Ethnic differences in the prevalence of splenomegaly and malaria in The Gambia. *Annual Tropical Medicine and Parasitology*, 81, 345–354.

Guerin, P. J., Olliaro, P., Nosten, F., Druilhe, P., Laxminarayan, R., Binka, F., Kilama, W. L., Ford, N. & White N. J**.** (2002). Malaria: current status of control, diagnosis, treatment, and a proposed agenda for research and development. *Lancet***,** 2, 564-573.

Helmby, H., Perlmann, H., Troye-Blomberg, M. & Perlmann, P. (1996). Immunoglobulin E elevation in *Plasmodium chabaudi* malaria. *Infection and Immunology*, 64, 1432–1433.

Ivan, M. R. & Delves, P. J. (2001). Activation of the immune system in malaria infection. *Essential Immunology*, 10th Edition. Blackwell Scientific Publications, Oxford.

Jarrett, E. E. E. & Miller, H. R. P, (1982). Production and activities of IgE in helminthes infection. *Progress in Allergy*, 3**,** 178–233.

Joana, D., Prakash, D., Vincent, G., Salah, M., Constantin, F., Pierre-André C., Gyan, C. M., Maryvonne, K. & Sylviane P. (2007). Total and functional parasite specific IgE responses in *Plasmodium falciparum*-infected patients exhibiting different clinical status. *Malaria Journal*, 6, 1-7.

Lesi, F. E. A. & Adenuga, A. O. (1996). Malaria in Childhood: An overview. *Nigeria Journal of General Practice*, 1, 9-12.

Mahajam, R. C., Ganguly, N. Y., Sharma, B. K., Chandnani, R. E., Singh, S. & Prasad, R. N. (2004). Immunological status in human plasmodium infection. *Indian Journal of Medical Research***,** 45, 123-128.

Perlmann, H., Helmby, H., Hagstedt, M., Carlson, J., Larsson, P. H., Troye-Blomberg, M. & Perlmann, P. (1994). IgE elevation and IgE antimalarial antibodies in *Plasmodium falciparum* malaria: association of high IgE levels with cerebral malaria. *Clinical Experimental Immunology*, 97, 284–292.

Perlmann, P., Perlmann, H., Looareesuwan, S., Krudsood, S., Kano, S., Matsumoto, Y., Troye-Blomberg M. & Aikawa, M. (2000). Contrasting functions of IgG, IgM & IgE antimalarial antibodies in uncomplicated and severe *Plasmodium falciparum* malaria. *American Journal of Tropical Medicineand Hygiene*, 62, 373–377.

Peter, P., Hedvig, P., Birgitta, W. F., Margareta, H., Gehad, E., Shewangizaw, W., Victor, F., Acleus, S. M. R. & Troye-Blomberg, M. (1997). Immunoglobulin E: a pathogenic factor in *Plasmodium falciparum* Malaria. *Infection and Immunity*, 65, 116–121.

QBC operator's/service manual (2006). QBC Diagnostic Incorporated, USA.

Rickman, L. S., Long, G. W., Oberts, R., Caraban, A., Sangalang, R., Smith, J. I., Chulay, J. D. & Hoffman S. L. (1989). Rapid diagnosis of malaria by acridine orange staining of centrifuged parasites. *Lancet*, 334, 68-71.

Weidanze, W. P. (1982). Malaria and alterations in immune reactivity. *British Medical Bulletin*, 38, 167-172.

William, S. & Philip, R. (2003). Diagnosis and Treatment of Malaria in Children. *Clinical Infectious Diseases*, 37, 1340–1348.

World Health Organization (2010). Global Malaria Programme. *World Malaria Report 2010.* Geneva, Switzerland.

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