Interleukin-8 (IL-8) profile in Nigerians with *Plasmodium falciparum* infection

[1]Nmorsi, O. P.G., [1]Isaac, C., [2]Ukwandu, N.C.D., [3]Ekundayo, A. O. and [3]Ekozien, M. I, Eifediyi R.A[4] .

1. Tropical Disease Research Unit, Department of Zoology, Ambrose Alli University, Ekpoma, Edo State, Nigeria
2. Department of Medical Microbiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria
3. Department of Microbiology, Ambrose Alli University,Ekpoma, Edo State, Nigeria
4. University Health Centre, Ambrose Alli University, Ekpoma, Edo State, Nigeria

nmorsiopg@yahoo.com

ABSTRACT: The profile of serum interleukin-8 (IL-8) among 96 volunteers infected with *Plasmodium falciparum* was investigated. Volunteers with severe malaria infection had mean IL-8 level of 580.0±101.5 pg/ml, moderate (245.8±32.4 pg/ml) and mild (102.0±56.2 pg/ml). The difference in the IL-8 levels of severe, moderate and mild infections was statistically significant (χ^2 = 388.8, p<0.05). The IL-8 profile of volunteers of age groups <16 years and >16 years with mean IL-8 concentrations of 466.1.5±62.5 pg/ml and 154.7±120.4 pg/ml, respectively, was observed and the difference in the mean IL-8 levels between these children and adults was statistically significant (χ^2 = 156.2, p<0.05). The relationship between IL-8 levels and age was positively correlated (r = 0.9). We deduce that IL-8 can be used as biomarker of intensity of malaria especially in severe malaria infection in our locality. [Report and Opinion. 2009;1(2):73-77]. (ISSN: 1553-9873).

Keywords: Interleukin-8 (IL-8); Nigerians; *Plasmodium falciparum;* infection

INTRODUCTION

About 300-500 million people suffer from malaria and 1.5-2.7 millions die annually (Sturdler, 1989). In sub-Saharan Africa, malaria exerts great influence on human health where is poses the greatest impact of morbidity and mortality among infectious diseases (WHO, 2000). Malaria infection has been implicated to be associated with immune responses, including elevated inflammatory cytokine levels (Moormann *et al*., 1999).

Interleukin-8 (IL-8) belongs to a class of cytokines referred to as chemokines. Chemokines are small proinflammatory peptides (8 to 17 KDa) which regulate innate and adaptive immunity (Luster, 2002). Some chemokines, including IL-8 have been identified as biomarkers of cerebral malaria mortality in Ghanaian children (Armah, *et al.,* 2007). IL-8 serum concentration among patients suffering from severe malaria has been shown to be highest at a time when no parasite was detected in the blood smear (Burgmann *et al.,* 1995). Also, report from Thailand revealed elevated IL-8 levels in falciparum-infected patients (Friedland *et al.,* 1993). Furthermore, a study conducted in Gabon, observed that IL-8 among other cytokines was significantly higher in severe malaria than in uncomplicated malaria (Kremsner *et al.,* 1995).

Many studies have been carried out concerning changes in serum cytokine levels in malaria-infected people in different part of the globe as it relates to the age of individuals (Linton and Thomas, 2000; Pawelec *et al.,* 1999; Pettiford *et al.,* 2002; Marie *et al.,* 2000). For example, Pettiford *et al.* (2002) documented that the percentages of both $CD8^-$ and CD8* T-cells producing IL-8 decreased with age. Conversely, IL-8 concentrations may increase with age (Pettiford *et al.* 2002) due to proinflammatory shift to type 2 patterns which leads to production of anti-inflammatory cytokines (e.g., interleukin-10) that inhibits the production of IL-8 (Pettiford *et al.,* 2002; Marie *et al.,* 2000).

In spite of the impact of malaria infection on our health and the role inflammatory cytokines play in regulating immune responses during protozoan infections (Brenier-Pinchart *et al.,* 2001); there is dearth of information on the profile of IL-8 in malaria-infected individuals in our locality. In this communication

therefore, we evaluate the profile of IL-8 with the intensity of malaria infection and establish the relationship between IL-8 levels and age.

MATERIALS AND METHODS

This study was carried out in Ihieve-Ogben; a rural community in Owan East local government area of Edo State. It is located at latitude 6˚N and longitude 6˚E. Agriculture especially farming and hunting is their predominant activities, while a few of them, mostly women, are traders. There is rainy season period of April to October which is followed by a dry season of November to March. Malaria transmission is perennial but highest during the rainy season.

Ninety six individuals who had malaria attack based on *P. falciparum* parasitaemia in their thick blood smears stained by Geimsa stain participated in the study. The malaria parasitaemia was categorized as mild (< 1000 asexual form of parasite/µL), moderate (1000-10,000 parasite/µL) and severe (>10,000 parasite/µL). They also had fever (axillary temperature of >37.5˚C) and clinical symptoms such as headache, vomiting, diarrhea, prostration, respiratory distress and other symptoms and signs of severe malaria as documented by WHO (2000). They were recruited after informed consent was obtained following thorough explanation of all procedures and the objective of the investigation. Ethical permission was obtained from Edo State Ministry of Health, Benin City, Nigeria. Volunteers with other detectable diseases such sickle cell anaemia, viral hepatitis B were excluded in the investigation using standard procedures and kits.

Whole venous blood (3 ml) was collected from a peripheral vein by venipuncture in the sterile EDTA bottle. Blood was processed by the centrifugation and the serum was immediately subjected to cytokine assays. The serum IL-8 concentration was determined by a standard enzyme-linked immunosorbent assay (ELISA) from kits obtained from Abcam plc, Cambridge, United Kingdom according to the manufacturer's instructions. From the information supplied by the manufacturer, the upper limit of normal serum IL-8 concentration is 76pg/ml with the mean serum IL-8 level of 44pg/ml.

The data obtained in this study were subjected to statistical analysis, namely, correlation and chi-square tests using Microsoft Excel statistical package.

RESULTS

Table 1 shows the intensity of malaria infection and mean serum IL-8 concentrations. Ninety six malaria positives were categorized based on their parasite load. Severe infections were observed in 32 patients with mean serum IL-8 level of 580.0±101.5 pg/ml. Fourty four volunteers had moderate infection with mean IL-8 concentration of 245.8±32.4 pg/ml, while 20 patients had mild infection with mean IL-8 level of 102.0±56.2 pg/ml. The difference in the IL-8 levels of severe, moderate and mild infections was statistically significant ($\chi^2 = 388.8$, $p<0.05$).

The IL-8 profile of 96 malaria-infected volunteers of which 53 volunteers were of age group <16 years while 43 patients of the age group >16 years with mean IL-8 concentrations of 466.1.5±62.5 pg/ml and 154.7±120.4 pg/ml, respectively, were documented (Table 2). The difference in the mean serum IL-8 level for age group <16 years and >16 years was statistically significant ($\chi^2 = 156.2$, $p<0.05$). The relationship between IL-8 levels and age was positively correlated ($r = 0.9$).

Table 1: Intensity of malaria infection and mean IL-8 levels

Intensity of infection/µL	Mean IL-8 (pg/ml)	No. infected
Mild <1000	102.0±56.2	20
Moderate 1000-10,000	245.5±32.4	44
Severe > 10,000	580.0±101.5	32

Table 2: Mean IL-8 levels with age group

Age group (years)	Mean IL-8 (pg/ml)	No. infected
>16	102.0±56.2	43
<16	245.5±32.4	53

DISCUSSION

We reported a significantly higher level of IL-8 among malaria positive participants in severe infection than both mild and moderate parasitaemia levels. This accords the report of Kremsner *et al.* (1995) and it implicates IL-8 in the immunopathogenesis of *P. falciparum* infection in our locality. Our investigation is proved valid by the report of Suguitan *et al.* (2003) who asserted that cytokines are secreted in response to *P. falciparum* infection. Furthermore, cytokines are implicated in the pathogenicity of malaria by the unique ability of *P. falciparum* to adhere to capillary and postcapillary venules endothelium during the second half of 48-h life cycle, a process called cytoadherence (Luse and Miller, 1971; MacPherson *et al.*, 1985). Cytoadherence confers survival of the parasite by ensuring microaerophilic venous environment which is better suited for parasite maturation; and the parasite adhesion to the endothelium allows the parasites to escape clearance by the spleen (Cranston *et al.*, 1984; Looareesuwan *et al.*, 1987; Ho *et al.*, 1990). Chemokines are chemotactic agents that enhance cytoadherence *in vivo* (Neote *et al.*, 1993). Human erythrocytes as well as postcapillary venular endothelial cells express the promiscuous Duffy antigen receptor (DARC) that binds to chemokines of the C-X-C (e.g., IL-8) class with high affinity (Neote *et al.*, 1993). So the presence of DARC on erythrocyte suggests that falciparum-infected erythrocyte may respond to chemotactic gradient. This probably explains our observation of significantly elevated level of IL-8 in patients with severe malaria infection.

We observed that a higher level of IL-8 for age group <16 years than age group >16 years. This pattern of age-related IL-8 profile of malaria-infected individuals supports the findings of (Archibald *et al.*, 2001; Pettiford *et al.*, 2002). This demonstrates the role age play in conferring immunity on malaria-infected individuals. It has been reported that a higher percentage of lymphocytes in children with malaria, produced IL-8 than did those of adults (Archibald *et al.*, 2001). Consistent with this, the percentages of both $CD8^-$ and CD8* T-cells producing IL-8 decreased with age (Pettiford *et al.*, 2002). IL-8 is a proinflammatory, chemotactic chemokine for neutrophils and T cells and a neutrophil stimulant that is involved in the severity of infectious diseases (Marie *et al.*, 2000; Burgman *et al.*, 1995; Mukaida *et al.*, 1992). It activates neutrophil to release lysosomal enzymes and induces them to adhere to the vascular endothelim, all extremely useful effects in infants, whose leucocyte function is immature (Mukaida *et al.*, 1992).

In this study, *P. falciparum* infection has been shown to induce the production of IL-8 and so implicate it in the pathogenesis of malaria disease. Our finding suggests that high levels of IL-8 is associated with high risk of *P. falciparum* infection and hence can be used as biomarker of severe malaria infection in our locality. Since there is dearth of information, it is therefore recommended that data in this direction be investigated in order to establish their roles in malaria disease pathogenicity, considering the role of cytokines in immunopathology of diseases and the global public health significance of malaria.

Corresponding author:
O.P.G. Nmorsi
Tropical Disease Research Unit, Department of Zoology,
Ambrose Alli University, Ekpoma, Edo State, Nigeria
E-mail: nmorsiopg@yahoo.com

REFERENCES

Archibald, L. K., McDonald, L. C., Adison, R. M., McKnight, C., Byrne, T., Dobbie, H., Nwanyanwu, O., Kazembe, P., Reller, L. B. and Jarvis, W. R. (2000). Comparison of Bactec Myc/Flytic and WAMPOLE ISOLATOR 10 (lysis-centrifugation) systems for detection of bacteremia, mycobacteremia, and fungemia in a developing country. *J. Clin. Microbiol.* 38: 2994-7

Armah, H. B., Wilson, N. O., Sarfo, B. Y., Powell, M. D., Bond, V. C., Anderson, W., Adjei, A. A., Gyasi, R. K., Tettey, Y., Wiredu, K. E., Tongren, J. E., Udhagajumar, V. and Stiles, J. K. (2007). Cerebrospinal fluid serum biomarkers of cerebral malaria mortality in Ghanian children. *Malaria Journal*, 6: 147.

Brenier-Pinchart, M. P., Pelloux, H., Derouich-Guergour, D. and Ambroise-Thomas, P. (2001). Chemokines in host-protozoan-parasites infections. *Trends Parasitol.* 17: 292-296.

Burgmann, H., Hollenstein, U., Wenisch, C., Thalhammer, F., Looareesuwan, S. and Granninger, W. (1995). Serum concentrations of MIP-1α and interleukin-8 in patients suffering from acute *Plasmodium falciparum* malaria. *Immunology and immunopathology,* 76: 32-36(5).

Cranston, H. A., Boylan, C. W., Carrol, G. L., Sutera, S., Williamson, J. R., Gluzman, I. Y. and Kroystad, D. J. (1984). *Plasmodium falciparum* maturation abolishes physiologic red cell deformability. *Science,* 223: 400-402.

Friedland, S. J., Ho, M., Rennick, G. D., Bunnay, D., White, N. J. and Griffin, G. E. (1993). IL-8 and *Plasmodium falciparum* malaria in Thailand. *Trans. R. Soc. Trop. Med. Hyg.* 87: 54-57.

Ho, N., White, N. J., Looareesuwan, S., Wattanagoon, Y., Lee, S. H., Walporl, M. J., Bunnay, D. and Harinasuta, T. (1990). Splenic Fc receptor function in host defence and anemia in acute *Plasmodium falciparum* malaria. *J. Infect. Dis.* 161: 556-561 *Am. J. Med. Hyg*.

Kremsner, P. G., Winkler, S., Brandts, C., Wildling, E., Jenne, L., Graninger, W., Prada, J., Bienzle, U., Juiliard, P. and Grau, E. G. (1995). Prediction of accelerated cure in *Plasmodium falciparum* malaria by the elevated capacity of tumor necrosis factor production. *Am. J. Trop. Med. Hyg.,* 53(5): 532-538.

Linton, P. J., Thomas, M. L. (2000). T-cell senescence. *Front Biosci.,* 6:248-61.

Looareesuwan, S., Ho, M., Wattanagoon, Y., White, N. J., Warrel, D. A., Bunnay, D., Hannasuta, T. and Wyler, D. J. Dynamic alterations in splenic function in falciparum malaria. *N. Engl. J. Med.* 317: 675-679.

Luse, S. A.and Miller, L. H. (1971). *Plasmodium falciparum* malaria. Ultrastructure of parasitized erythrocytes in cardia vessels. *Am. J. Trop. Med. Hyg*., 20: 655-660.

Luster, A.D. (2000). The role of chemokines in linking innate and adaptive immunity.
Curr. Opin. Immunol., 14: 129-135.

MacPherson, G.G., Warell, M.J., White, N. J., Looareesuwan, S. and Warrel, D.A. (1985). Human cerebral malaria: a quantitative ultrastructural analysis of parasitized erythrocyte sequestration. *Am. J. Pathol.* 119: 385-401.

Marie, C. M., Fitting, C., Muret, J., Pagen, D.and Cavaillon, J. M. (2000). Interleukin 8 production in whole blood assays. Is interleukin 10 responsible for the down regulation observed in sepsis? *Cytokine.* 12: 55-61.

Moormann, A.M., Sullivan, A.D., Rochford,R.A., Chensue, S.W., Bock, P. I., Nyirenda, T. and Meshnick, S. R. (1999). Malaria and pregnancy: placental cytokine expression and its relationship to intrauterine growth retardation. *J. Infect. Dis.* 180: 1987.

Mukaida, N., Harada, A. and Yasumoto, K. (1992). Properties of proinflammatory cell type-specific leukocyte chemotactic cytokine, interleukin-8 (IL-8) and monocyte chemotactic and activating factor (MCAF). *Microbiol. Immunol.* 36: 775-779.

Neote, K., Darbonne, W., Ogez, J., Horuk, R. and Schall, T. J. (1993). Identification of a promiscuous inflammatory peptide receptor on the surface of red blood cells. *J. Biol. Chem.* 268: 12247-12249.

Pawelec, G., Effros, R. D., Caruso, C., Remarque, E., Barnett, Y. and Solana, R. (1999). T-cells and aging. *Front Biosci*., 4: 216-69.

Pettiford, J. N., Jason, J., Nwanyanwu, C. O., Archibald, L. K., kazembe, P. N., Dobbie, and Jarvis, W. R. (2002). Age-related differences cell-specific cytokine production by acutely ill Malawian patients. *Clin. Exp. Immunol*.,128(1): 110-117.

Sturdler, D. (1989). How much malaria is there world wide? *Parasitol. Today*, 5: 39.
Suguitan, A. L., Jr., Leke, R. G. F., Fouda, G., Zhou, A., Thuita, L., Metenou, S.,Fogako, J., Megnekou, R. and Taylor, W. D. (2003). Changes in the levels of chemokines and cytokines in the placentas of women with *Plasmodium falciparum* malaria. The journal of infectious Diseases, 188: 1074-1082.

World Health Organization (2000). Severe falciparum malaria. *Trans. R. Soc. Trop. Med. Hyg.* 94: (Suppl. 1): S1-S90.

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