

A PRELIMINARY STUDY OF ASSOCIATION OF ERYTHROCYTE SEDIMENTATION RATE WITH MALARIA-SPECIFIC IMMUNOGLOBULIN G AND MALARIA-INDUCED ANAEMIA

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ABSTRACT

Evidence-based practice in medicine requires clinical signs and symptoms of malaria to be confirmed by microscopic detection of the parasite in blood sample of patients. In some cases, however, such signs and symptoms persist without microscopic detection of the parasite in blood smears, making it difficult for treatment decision to be made by medics. To help solve the above problem and to ensure that antimalarial drugs are given to patients who actually suffer from malaria infection, we designed this cross-sectional study to measure ESR, mIgG, Hb and parasitaemia levels in 78 clinically diagnosed malaria patients attending the Elmina Urban Health Centre, in the Komenda-Edina-Eguafo-Abirem Municipal Assembly in the Central Region of Ghana, with the view to finding alternative biomarkers for malaria infection. Apart from the Hb levels which differed significantly ($P=0.001$) between the sexes, the levels of all the other measured indicators of malaria infection were comparable between the various groups. ESR correlated positively with mIgG ($r = 0.242$, $P = 0.033$) but negatively with Hb ($r = -0.348$, $P = 0.002$) irrespective of age, sex or percentage parasitaemia. Our results appear to suggest that an ESR level of twice the upper reference value of an individual may be diagnostic of malaria infection in the presence of the appropriate recognized signs and symptoms of malaria without microscopically detectable parasitaemia in the blood sample of patients. We propose that ESR and mIgG be considered as complementary markers of malaria infection in cases of unsuccessful microscopic detection of the malaria parasite in blood smears.

INTRODUCTION

Malaria is an important parasitic infection, affecting an estimated 500 million people worldwide and resulting in about one million deaths annually (Milner *et al.*, 2009). In Sub-Saharan Africa, malaria accounts for between 20 and 40 percent of outpatient visits and between 10 and 15 percent of hospital admissions (WHO, 1999) and 40% of public health expenditure (Adams

et al., 2004). In Ghana, significant economic burden of malaria on the economy has been reported (Asante and Asenso-Okyere, 2003). The total treatment cost per episode of malaria has been estimated as US\$ 15.79 per household (Asante and Asenso-Okyere, 2003). This constitutes just 1% and as much as 34% per capita income of the rich and poor households respectively, in Northern Ghana (Akazili, 2002). In-

terestingly, only 29% of the total treatment cost per episode of malaria is a direct cost with the remaining 71% being an indirect cost (Akazili *et al.*, 2007). These revelations point to the need for proper, accurate and reliable diagnostic marker for malaria infection as misdiagnosis may lead to improper drug prescription, drug abuse and possible development of parasite resistance with its consequent increased economic, social and physiological burden. In this era of evidence-based practice in medicine, clinical signs and symptoms of malaria must be confirmed by microscopic detection of the parasite in the blood sample of patients (Reeder *et al.*, 1999; John *et al.*, 2004). In some cases, however, such signs and symptoms persist without the microscopic detection of the malaria parasite in the blood smear. Such cases make the task of clinicians more daunting in prescribing the appropriate medication for the patient. It also has the potential to heighten patients' anxiety and increase the treatment time and cost of the disease. Successful microscopic detection of the *Plasmodium* parasite in blood smears depends on the experience of the technician and time of blood sampling (John *et al.*, 2004) since parasite sequestration (Reeder *et al.*, 1999) could lead to false negative results. To alleviate the above challenges, we measured erythrocyte sedimentation rate (ESR), malaria-specific immunoglobulin G (mIgG), haemoglobin (Hb) and parasitaemia levels in a cross-sectional study of clinically diagnosed malaria patients attending the Elmina Urban Health Centre, in the Komenda-Edna-Eguafo-Abirem Municipal Assembly in the Central Region of Ghana and to investigate the relationship among the measured indicators.

MATERIALS AND METHODS

Study Area

The study was carried out at the Elmina Urban Health Centre, which is located in Elmina, in the Komenda-Edna-Eguafo-Abirem Municipality from October, 2008 to May, 2009. The Komenda-Edna-Eguafo-Abirem Municipality is bounded on the south by the Atlantic Ocean (Gulf of Guinea), the east by the Cape Coast

Metropolis, the north by the Twifo Hemang - Lower Denkyira District and the west by the Mpohor-Wassa East District. It is perched between longitude 1° 20' West and 1° 40' West and latitude 5° 05' North and 5° 15' North. The municipality covers an area of 1,372.45 square kilometers and is generally humid. The coastline about 30 kilometers, form part of the littoral anomalous zone of Ghana, experiencing less rainfall than the interior. Annual rainfall ranges from 1,000 mm along the coast to about 2,000 mm in the interior. The wettest months are May-July and September-October while the drier periods occur in December-February. Mean monthly temperature ranges from 25 °C in the coolest month (August) to about 30 °C in the hottest months (March-April). The Municipality population was estimated to be 112,435 (53,755 males and 58,682 females) (MLGRD, 2006). The Municipality's share of the total population of the Central Region is 7.1%. The age and sex structures of the Municipality reflect that of the region and the nation at large. The inhabitants of this Municipality are mainly into fishing and farming.

Study design and sample collection

This was an out-patient-based cross-sectional study involving 18 male and 60 female patients who were clinically diagnosed of malaria at the Elmina Urban Health Centre by a qualified medical practitioner. Questionnaires were administered to collect demographic, socio-economic and general health information. Respondents with other disease conditions that could influence the ESR and mIgG levels were excluded from the study. In addition, those with viral, bacterial and other infections that have similar symptoms as malaria infection as well as children below five years were also excluded from the study. The children below five years were excluded because their immune system might not have developed to levels that would allow for enough production of mIgG (Riley *et al.*, 2000). Venous blood was collected from respondents who met the inclusion criteria and volunteered to participate in the study after informed consent was sought.

Measurements of haematologic indicators of malaria infection

Parasitaemia was determined by microscopic inspection of thin blood smears as previously described (John *et al.*, 2004). Blood smears were prepared and stained with Giemsa and examined by an experienced licensed laboratory technician.

Haemoglobin level was measured by a HemoCue photometer (Ångelholm, Sweden). ESR was determined by the standard Westergren method described by Bomford *et al.* (1975).

For malaria-specific immunoglobulin G measurement, we used the indirect ELISA method described by Riley *et al.* (1991) with modifications. Briefly, the first two columns of a 96 well microtitre plate were coated with a two-fold serial dilution of 1mg/ml reference IgG standard in coating buffer (0.05% phenol red in PBS buffer) at 50µl/well. A blank (0.05% phenol red in plain PBS buffer) was added to four other wells at 50µl/well to prepare a standard curve for the determination of antibody units (AU). The rest of the wells were coated with 5 x 10⁶ cell/ml of crude antigen extract in a coating buffer (0.005% phenol in phosphate buffer saline) at 50µl/well and the plate incubated overnight at 4°C before being allowed to attain room temperature. The plates were then washed 4 times with PBS containing 0.1% Tween-20 and padded-dried. The wells were blocked with 150µl/well of 3% non-fat milk (skimmed milk) in PBS for 1hr at room temperature on a shaker after which they were washed again. Plasma samples diluted at 1:200 with dilution buffer (1% non-fat milk) were then added in duplicates at 100µl/well to antigen coated portion of the plates. The dilution buffer alone, however, at 100µl/well was added to the reference IgG. The prepared plates were incubated for 2 hours at room temperature on a shaker after which they were washed 4 times and 100µl of enzyme conjugate (Horse Radish Peroxidase Goat anti-human IgG) diluted at 1:3000 in 1% skimmed milk added per well. Following incubation for 1hour on a shaker at

room temperature, the plates were washed 4 times again with PBS/Tween-20 (0.1%). Equal mixture of A and B solutions of ABTS (2,2'-azino-di-(3-ethylbenzthiazoline sulphonic acid) was added into the padded-dried wells of the plate at 100µl/well. The plates were incubated in the dark for 10 minutes for a colour reaction to occur. The reaction was stopped by the addition of 50µl/well of 2M HCl solution. The optical densities (OD) of the colour produced in each well were read at a wavelength of 405nm using a multiscan ascent plate reader. A negative control was prepared alongside the ELISA procedure using antigens of the RBCs that were isolated from the uninfected RBCs during the 'Freeze Thawing' processes. The OD values obtained for the negative control was subtracted from the OD values of the corresponding plates coated with the crude parasite antigen.

Statistical analysis

Data obtained were analysed with the SPSS version 16.0 statistical software. Values were reported as mean ± standard deviation (SD). One way analysis of variance (ANOVA) was used to compare mean values. Pearson correlation was used to assess association among the measured parameters in the sexes. The study sample was divided into parasitaemic and non-parasitaemic groups. Mean values in these groups were compared using one way ANOVA. Pearson correlation was used to test association among the other parameters except percentage parasitaemia. All analyses were carried out at 5 % level of significance

Ethical Approval

The study was approved by the Ethics Committee of the University of Cape Coast, Cape Coast, Ghana. Informed consent was obtained from all the study participants. In the case of children, informed consent was obtained from the parent or guardian. All protocols followed were in line with the ethical standards of the Ministry of Health, Ghana.

RESULTS

Seventy-eight respondents consented and donated blood for the study. They were made up

of 18 (23.1%) males aged 5 years to 64 years and 60 (76.9%) females aged 5 years to 68 years (Table 1). The mean age of males and females did not differ significantly ($P = 0.465$). A similar trend was observed for the parasitaemic and non-parasitaemic groups (Table 2; $P = 0.269$).

The mean Hb for males and females were 12.51 and 10.60 respectively, generally indicating that the respondents were anaemic compared to their respective reference values. Apart from the mean Hb level, which was significantly ($P = 0.001$) higher in males than in females, all the other parameters had comparable ($P > 0.05$) mean values between the sexes (Table 1). A comparison of the parasitaemic and non-parasitaemic groups did not reveal any significant ($P \geq 0.26$) difference between them for the assessed parameters (Table 2).

A significant positive correlation was observed between age and mIgG in males ($r = 0.516$, $P = 0.028$) but not in females ($r = 0.151$, $P = 0.251$). Age, however, correlated negatively with percentage parasitaemia in females ($r = -0.29$, $P = 0.025$). In the parasitaemic group, age correlated negatively with percentage parasitaemia ($r = -0.392$, $P = 0.009$) but positively with Hb ($r = 0.384$, $P = 0.011$). There was no correlation between age and Hb in the non-parasitaemic group. The negative correlation between age and percentage parasitaemia persisted in the entire study sample irrespective of sex ($r = -0.301$, $P = 0.007$).

Hb correlated negatively with ESR in males ($r = -0.572$, $P = 0.013$) and females ($r = -0.321$, $P = 0.012$) and in the parasitaemic ($r = -0.423$, $P = 0.005$) but not the non-parasitaemic group ($r = -0.23$, $P = 0.67$). The negative correlation between Hb and ESR persisted in the entire sample irrespective of age, sex or percentage parasitaemia ($r = -0.348$, $P = 0.002$; Figure 1). However, ESR correlated positively with IgG irrespective of sex, age or percentage parasitaemia ($r = 0.242$, $P = 0.033$; Figure 2).

DISCUSSION

Evidence-based practice in medicine requires

that clinical signs and symptoms of malaria are confirmed by microscopic detection of the parasite in the blood sample of patients. In most cases, such signs and symptoms persist even when laboratory detection of the malaria parasite in blood is unsuccessful. This study therefore sought to find other markers that can be used to complement microscopy in diagnosis of malaria. The ultimate aim is to ensure that, anti-malarial drugs are given to people who are actually suffering from malaria. This may promote compliance and reduce antimalarial drug abuse with its consequent drug resistance.

The mean Hb level for both sexes revealed that in general, our respondents were anaemic. Anaemia as an important public health problem irrespective of the cause has long been recognized (Dallman *et al.* 1978; Perry *et al.*, 1992; Johnson-Spear and Yip, 1994; WHO, 2001; WHO and UNICEF, 2004; Beutler and West, 2005; Sullivan *et al.*, 2008). Malaria-induced anaemia has been observed in children (Premji *et al.*, 1995; Branch *et al.*, 1998; Biemba *et al.*, 2000) and adults (White and Ho, 1992; Branch *et al.*, 1998). Since possible confounding factors were eliminated in our study design, the anaemia observed in our sample can be ascribed to the pathophysiology of the *Plasmodium* parasite as described elsewhere (White and Ho, 1992).

The higher proportion of anaemic females than males could result from the increased exposure of the females to the infective mosquito bites than their male counterparts. Indeed, the females in our study sample stayed much longer outside the room to undertake various household chores, coupled with the nature of their dresses which exposed them more to mosquito bites than the males whose clothing cover almost their entire bodies and therefore derive some level of protection against the bite of female *Anopheles* mosquito.

Interestingly, age correlated negatively with percentage parasitaemia in females but not in males. The absence of correlation between age and percentage parasitaemia in males could be due to their small size ($n = 18$) compared to

females (n =60) in the study sample. On the other hand, the negative correlation between age and percentage parasitaemia could reflect increased parasite clearance ability of females with age as a result of development of the appropriate immune response to increased exposure to the *Plasmodium* parasite (Ofori *et al.*, 2002; John *et al.*, 2005).

To find other markers that can be measured to confirm malaria as the underlying cause of the appropriate clinical sign, we measured ESR and malaria-specific IgG to observe how they are related to malaria. Our results indicated that in general, there was no significant ($P > 0.05$) difference between the sexes. This observation may appear to deviate from the finding that sex and age influence ESR (Bottiger and Svedberg,

1967; Bain, 1993; Lowe, 1994; Kanfer and Nicol, 1997) particularly when no significant ($P > 0.05$) correlation was seen between ESR and sex or age in our study sample. This observation could be due to the relatively small sample size (n =78) in the current study. Indeed, studies (Bottiger and Svedberg, 1967; Bain, 1993; Lowe, 1994; Kanfer and Nicol, 1997) that have documented association between ESR and sex or age worked with a relatively larger sample size (n > 100) than the current study.

ESR, however, correlated negatively with Hb in both sexes and in the parasitaemic group but not the non-parasitaemic group. Irrespective of age, sex or percentage parasitaemia, the negative correlation between ESR and Hb persisted in our sample. This negative association has

Table 1: Age range and indicators of *Plasmodium falciparum* infection by sex of respondents

<i>Age and sex of respondents</i>						
Sex	No. of donors	Mean age \pm S.D	Age range (years)	95 % C.I.	P-value	
Male	18	24.56 \pm 13.58	5 - 64	18.29 - 30.83	0.465	
Female	60	26.87 \pm 11.10	5 - 68	24.06 - 29.68		
<i>Hb level of male and female respondents</i>						
Sex	Mean \pm S.D (g/dL)	Range (g/dL)	95% C.I.	Reference Hb (g/dL)	% within ref. range	P-value
Male	12.51 \pm 1.96	8.20-14.70	11.61 - 13.42	13	55.56 (44.44)*	< 0.001
Female	10.60 \pm 1.37	7.40-13.30	10.25 - 10.95	12	25 (75)*	
<i>ESR in male and female respondents</i>						
Sex	Mean \pm S.D (mm/h)	Range (mm/h)	95% C.I.	Ref. range (mm/h)	% within ref. range	P-value
Male	28.88 \pm 27.33	2-82	16.25 - 41.51	4-14	30 (70)*	0.09
Female	41.87 \pm 31.30	5-125	33.95 - 49.79	3-20	30 (70)*	
<i>mIgG levels in male and female respondents</i>						
Sex	Mean \pm S.D (ng/mL)	Range (ng/mL)	95% C.I.	P-value		
Male	22704.24 \pm 16028.66	2920.58-56014.98	15299.51 - 30108.97	0.335		
Female	19163.59 \pm 12777.16	2977.87 - 64215.16	15930.58 - 22396.6			
<i>Percentage parasitaemia in male and female respondents</i>						
Sex	Mean \pm S.D (%)	Range (%)	95% C.I.	P-value		
Male	0.29 \pm 0.61	0.00 - 2.50	0.01 - 0.57	0.795		
Female	0.34 \pm 0.67	0.00 - 3.50	0.17 - 0.51			

Key: IgG = malaria-specific immunoglobulin G; S.D. = standard deviation; C.I. = confidence interval; (*) = proportion of individuals with values outside the reference range.

Table 2: Age and measured indicators of *Plasmodium falciparum* infection by parasitaemia

<i>Age</i>						
Group	No. of donors	Mean age \pm S.D	Age range (years)	95 % C.I.	P-value	
Parasitaemic	35	24.63 \pm 10.25	10 - 68	21.57 - 27.69	0.269	
Non-parasitaemic	43	28.43 \pm 13.05	5 - 43	24.11 - 32.75		
<i>Hb</i>						
Group	Mean \pm S.D (g/dL)	Range (g/dL)	95% C.I.	Ref. range	% within Ref. range	P-value
Parasitaemic	11.12 \pm 1.85	7.40 - 14.70	7.49 - 14.75	12-13	29 (71)*	0.277
Non-parasitaemic	10.97 \pm 1.56	8.6 - 14.2	10.45 - 11.49	12-13	31 (69.)*	
<i>ESR</i>						
Group	Mean \pm S.D (mm/h)	Range (mm/h)	95% C.I.	Ref. range	% within Ref. range	P-value
Parasitaemic	38.37 \pm 30.45	2 - 106	29.27- 47.47	3 - 20	37.2(62.8)*	0.926
Non-parasitaemic	39 \pm 31.67	2 - 125	28.51 - 49.49	3 - 20	29 (71)*	
<i>mIgG levels</i>						
Group	Mean \pm S.D (ng/mL)	Range (ng/mL)	95% C.I.		P-value	
Parasitaemic	20998.95 \pm 13284.80	3073.56 - 64215.16	14545.30 - 22912.70		0.44	
Non-parasitaemic	18729.63 \pm 13997.38	2920.58 - 56014.98	16597.77 - 25400.13			

Key: mIgG = malaria-specific immunoglobulin G; S.D. = standard deviation; C.I. = confidence interval; (*) = proportion of individuals with values outside the reference range.

been observed by earlier researchers (Morris *et al.*, 1975; Bottiger and Svedberg, 1967; Bain, 1993; Kanfer and Nicol, 1997). It is worthy of note that, although the difference in mean ESR values between the sexes did not reach statistical significance, the values for both males and females were about twice the upper limit of their respective reference values. A similar trend was seen in the parasitaemic and non-parasitaemic groups though the mean values for both groups were closer to the mean ESR of females than that of males. This is not surprising as females constituted over 76% of respondents in the study. In addition, the comparable ESR values between the parasitaemic and non-parasitaemic groups appear to suggest similar underlying pathology in the groups. Thus, an ESR value of at least twice the upper reference value for a given individual of a specific age and sex coupled with the appropriate signs and symptoms of malaria could be indicative of

clinical malaria notably in cases where the gold standard microscopy, fails to detect the *Plasmodium* parasite in the blood sample of such patients. Although Lowe (1994) and Kanfer and Nicol (1997) have proposed the replacement of ESR with plasma viscosity due to the low specificity of the former, our findings do not support this proposition, especially in the presence of appropriate signs and symptoms of malaria infection.

Since high levels of immunoglobulin G has long been associated with the blood stage forms of the *Plasmodium falciparum* parasite (Taylor *et al.*, 1995; Rzepczyk *et al.*, 1997; Taylor *et al.*, 1998; Ofori *et al.*, 2002), we investigated how the levels of malaria-specific IgG in the study subjects were related to the other malariological indicators. The positive correlation observed between ESR and IgG irrespective of age, sex or percentage parasitaemia supports the hypothesis of similar underlying pathology

in our study sample. This view is further enhanced by the comparable levels of mIgG and ESR in the parasitaemic and non-parasitaemic groups and thus reinforces the suitability of both markers to be considered as indicators of *Plasmodium falciparum* infection.

However, the positive correlation between mIgG and age of males requires further investigation on a larger scale to ascertain its relevance for malaria treatment.

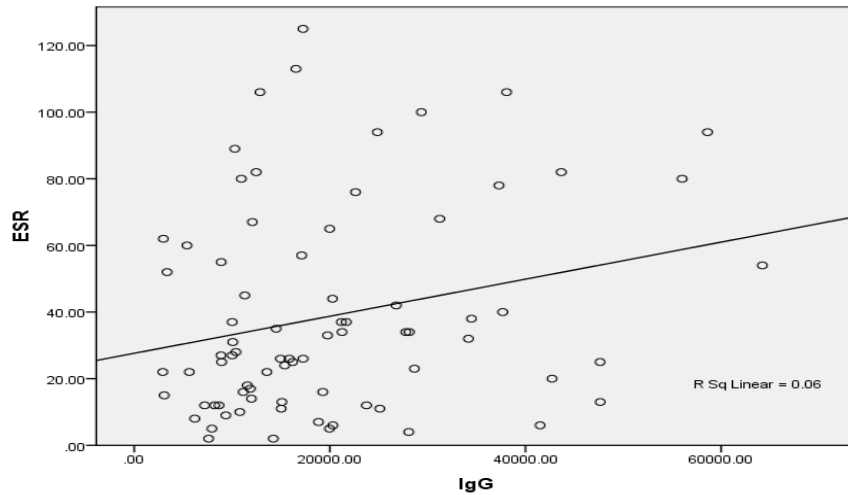


Fig.1: Simple scatter plot of positive correlation between ESR and mIgG levels of respondents irrespective of sex, age or percentage parasitaemia. ($r = 0.242, P = 0.033$)

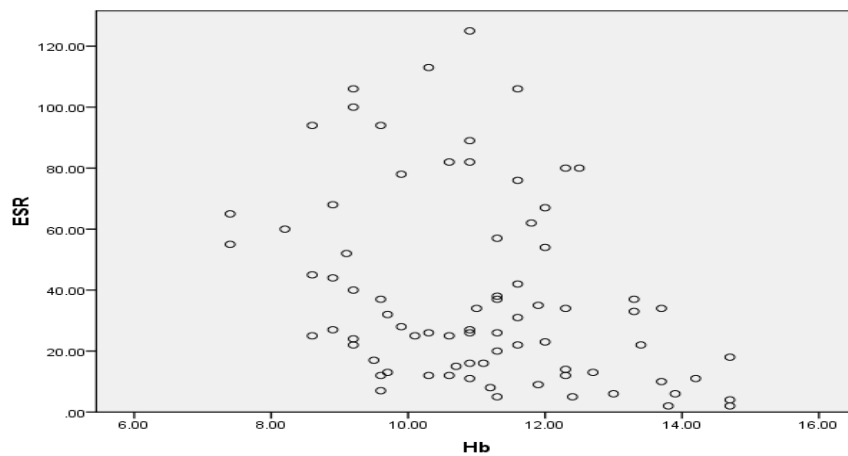


Fig. 2: Simple scatter plot of negative correlation between ESR and Hb levels of respondents irrespective of sex, age or percentage parasitaemia ($r = -0.348, P = 0.002$)

CONCLUSION

ESR and mIgG levels can be used as complementary markers of malaria infection in the presence of appropriate signs and symptoms of malaria but microscopically undetectable levels of the *Plasmodium* parasite in the blood sample of patients. ESR correlated negatively with Hb but positively with malaria-specific IgG in this study. Further research is needed to ascertain the relevance of the positive correlation between age and malaria-specific IgG to malaria treatment

ACKNOWLEDGEMENT

We acknowledge the cooperation and endurance of all respondents in this study. Staff of the Elmina Urban Health Centre who assisted in diverse ways for the successful completion of this work deserves our deep appreciation for their effort. We would like to acknowledge all senior colleagues at the Schools of Biological and Medical Sciences, University of Cape Coast, who read and suggested constructive changes to this work.

REFERENCES

- Adams, I., Darko, D. and Accorsi, S. (2004). Malaria: a burden explored. *Bulletin of Health Information* 1(1): 28-34.
- Akazili J (2002). Costs to households of seeking malaria care in the Kassena-Nankana District of Northern Ghana. *3rd MIM Pan-African Conference on Malaria, Arusha, Tanzania*.
- Akazili J. M. A, Aikins M., and Binka F. N. (2007). Malaria treatment in Northern Ghana: What is the treatment cost per case to households? *Afr J Health Sci* 14:70-79.
- Asante A. and Asenso-Okyere K. (2003). Economic Burden of Malaria in Ghana. A Technical Report Submitted to the World Health Organisation (WHO), African Regional Office (AFRO).
- Bain, B. J. (1993). Some influences on the ESR and the fibrinogen level in healthy subjects. *Clinical Laboratory Haematology* 5:45-54.
- Beutler, E. and West, C. (2005). Hematologic differences between African-Americans and whites: the roles of iron deficiency and alpha-thalassemia on hemoglobin levels and mean corpuscular volume. *Blood* 106: 740-745.
- Biemba, G., Dolmans, D., Thuma, P. E., Weiss, G. and Gordeuk, V. R. (2000). Severe anaemia in Zambian children with *Plasmodium falciparum* malaria. *Tropical Medicine and International Health* 5: 9-16.
- Bomford, R., Mason, S. and Swash, M. (1975). Elementary haematology. *Hutchison's Clinical Methods*. London: Bailliere Tindall 133-160.
- Bottiger, L. E. and Svedberg C. A. (1967). Normal erythrocyte sedimentation rate and age. *British Medical Journal* 2:85-87.
- Branch, O. H., Udhayakumar, V., Hightower, A. W., Oloo, A. J., Hawley, W. A., Nahlen, B. L., Bloland, P. B., Kaslow, D. C., and Lal A. A. (1998). A longitudinal investigation of IgG and IgM antibody responses to the merozoite surface protein-1 19-kiloDalton domain of *Plasmodium falciparum* in pregnant women and infants: associations with febrile illness, parasitemia, and anemia. *American Journal of Tropical Medicine & Hygiene* 58: 211-219.
- Dallman, P. R., Barr, G. D., Allen, C. M. and Shinefield, H. R. (1978). Hemoglobin concentration in white, black, and oriental children: is there a need for separate criteria in screening for anemia? *American Journal of Clinical Nutrition* 31:377-380.
- John, C. C., Moormann, A. M., Pregibon, D. C., Sumba, P. O., Mchugh, M. M., Narum, D. L., Lanar, D. E., Schluchter, M. D. and Kazura, J. W. (2005). Correlation of high levels of antibodies to multiple pre-erythrocytic *Plasmodium falciparum* antigens and protection from infection. *Am. J. Trop. Med. Hyg.*, 73 (1): 222-228
- John, C. C., O'Donnell, R. A., Sumba, P.O., Moormann, A. M., de Koning-Ward, T. F.,

- King, C. L., Kazura, J. W. and Crabb, B. S. (2004). Evidence that invasion-inhibitory antibodies specific for the 19-kDa fragment of merozoite surface protein-1 (MSP-1 19) can play a protective role against blood-stage *Plasmodium falciparum* infection in individuals in a malaria endemic area of Africa. *Journal of Immunology* 173: 666–672.
- Johnson-Spear, M. A. and Yip R. (1994). Hemoglobin difference between black and white women with comparable iron status: justification for race-specific anemia criteria. *American Journal of Clinical Nutrition* 60: 117–121.
- Kanfer, E. J. and Nicol B. A. (1997). Haemoglobin concentration and erythrocyte sedimentation rate in primary care patients. *Journal of Royal Society of Medicine* 90:16–18.
- Lowe, G. D. (1994). Should plasma viscosity replace the ESR? *Br J Haematol* 86:6-11.
- Milner D. A., Montgomery, J., Seydel, K. B. and Rogerson S. J. (2009). Severe malaria in children and pregnancy: an update and perspective *Trends Parasitol* 24 (12): 590-595.
- Ministry of Local Government and Rural Development {MLGRD} (2006). <http://www.ghanadistricts.com/districts/>
- Morris, M. W., Skrodzki, Z. and Nelson, D. A. Zeta sedimentation ratio (ZSR), a replacement for the erythrocyte sedimentation rate (ESR).(1975). *American Journal of Clinical Pathology* 64:254-256.
- Ofori, M. F., Dodoo, D., Staalsoe, T., Kurtzhals, J. A. L., Koram, K., Theander, T. G., Akanmori, B. D. and Hviid L. (2002). Malaria-induced acquisition of antibodies to *Plasmodium falciparum* variant surface antigens. *Infection and Immunity* 70 (6): 2982–2988.
- Perry, G. S., Byers, T., Yip, R. and Margen, S. (1992). Iron nutrition does not account for the hemoglobin differences between blacks and whites. *Journal of Nutrition* 122: 1417–1424.
- Premji, Z., Hamisi, Y., Shiff, C., Minjas, J., Lubega, P. and Makwaya, C. (1995). Anaemia and *Plasmodium falciparum* infections among young children in an holoendemic area, Bagamoyo, Tanzania. *Acta Trop* 59: 55–64.
- Reeder J. C., Cowman A. F., Davern K. M., Beeson J. G., Thompson J. K., Rogerson S. J., And Brown G. V. (1999). The adhesion of *Plasmodium falciparum*-infected erythrocytes to chondroitin sulfate A is mediated by *P. falciparum* erythrocyte membrane protein 1. *Proc. Natl. Acad. Sci. USA* 96: 5198–5202.
- Riley, E. M., Allen, S. J., Wheeler, J.G., Blackman, M. J., Bennett, T. B., Schonfeld, H. J. and Greenwood, B. M. (1991). Naturally acquired cellular and humoral immune responses to major Merozoite Surface Antigen (PfMSP1) of *Plasmodium falciparum* are associated with reduced malaria morbidity. *Parasite Immunology* 14: 321-337.
- Riley, E. M., Wagner G. E., Ofori M. F., Wheeler, J. G., Akanmori, B. D., Tetteh, K., McGuinness, D., Bennet, S., Nkrumah, F. G., Anders, R. F. and Koram, K. A. (2000). Lack of association between maternal antibody and protection of African infants from malaria infections. *Infect Immun* 68:5856-5863.
- Rzeczyk, C. M., Hale, K., Woodroffe, N., Bobogare, A., Csurhes, P., Ishii, A. and Ferrante, A. (1997). Humoral immune responses of Solomon Islanders to the merozoite surface antigen 2 of *Plasmodium falciparum* show pronounced skewing towards antibodies of the immunoglobulin G3 subclass. *Infect Immun* 65: 1098–1100.
- Sullivan, K. M., Mei, Z., Grummer-Strawn, L. and Parvanta, I. (2008). Haemoglobin adjustments to define anaemia. *Tropical Medicine & International Health* 13 (10): 1267–1271.

- Taylor R. R., Allen, S. J. Greenwood, B. M. and Riley E. M. (1998). IgG3 antibodies to *Plasmodium falciparum* Merozoite Surface Protein 2 (MSP2): increasing prevalence with age and association with clinical immunity to malaria. *Am. J. Trop. Med. Hyg* 58 (4) : 406–413.
- Taylor, R. R., Smith, D. B., Robinson, V. J., McBride, J. S. and Riley, E. M. (1995). Human antibody response to *Plasmodium falciparum* merozoite surface protein 2 is serogroup specific and predominantly of the IgG3 subclass. *Infect Immun* 63: 4382–4388.
- White, N. J. and Ho, M. (1992). The pathophysiology of malaria. *Adv. Parasitol.* 31:83–173.
- World Health Organization (1999). World Health Report, 1999; *making a Difference*. <http://www.who.int/whr/1999/>
- WHO (2001). Iron deficiency anaemia: assessment, prevention, and control: a guide for