DETERMINATION OF HEMATOLOGICAL PARAMETERS AND D-DIMER AMONG SUDANESE CHILDREN INFECTED WITH PLASMODIUM FALCIPARUM IN KHARTOUM STATE – SUDAN.

Fatima H. Abdallah¹, Abuzar Elnager², Safa Wdidi³, Alneil M. Hamza⁴ and Abdalla E. Ali*⁵

^{1,2}Alzaiem Alazhari University-faculty of Medical Laboratory Science - Hematology Department

³Omdurman Friendship Hospital-Laboratory – Hematology Department

⁴Clinical Laboratories Sciences Department, College of Applied Medical Sciences, AlJouf University, Saudi Arabia

⁵Alzaiem Alazhari University-Faculty of Medical Laboratory Science- Clinical Biochemistry Department

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Address for Correspondence: Abdalla E. Ali, Alzaiem Alazhari University-faculty of medical laboratory science- Clinical Biochemistry Department

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Abstract

Background: Falciparum malaria is one of the causes of morbidity and mortality in third world, the pathogenesis of the infection results from the sequestration of infected hematological change in vital organs.

Aim: this study aim to measuring hematological changes and D- dimer among children affected with P falciparum.

Methods: The total study group 100 children, 60 as case study infected with Plasmodium Falciparum and 40 as control group healthy children. Hematological parameter measuring by hematological analyzer and D dimer measured by fluorescence Immunoassay used I. Chroma instrument.

Results: Statistical analysis results of sixty children infected with malaria falciparum participated in the study the mean $\pm$ SD had a significantly lower Hb (8.14gm/dl $\pm$ 2.2) and haematocrit (24.4% $\pm$ 6.6) than control children (p <0.001), Thrombocytopenia was found in 59.3% of enrolled patients. Platelet count (98.53% $\pm$ 48.9), D. dimer (7397.58 ng/ml $\pm$ 5867.07) (p <0.001). D. dimer showed correlation with parasite density, Platelet count and Haematocrit correlated (r = 0.4, p <0.0001); (p <0.001).

Conclusion: Malaria infection by plasmodium falciparum had major effects on hematological parameters such as thrombocytopenia anemia and hyper coagulation have been significantly associated with severity of malaria falciparum.

Keywords: Plasmodium, Falciparum, D-dimer, hematological parameters

1. Introduction:

Malaria remains one of the most important causes of morbidity and mortality in the tropical

regions of the world (1). In humans is caused by 5 Plasmodium parasites: Plasmodium falciparum, P. vivax, P. malariae, P. ovale and P. knowlesi. (2). Malaria cases are often under-diagnosed in

hyper endemic countries, where mild symptoms of chronic malaria may possibly lead to misdiagnosis. On the contrary, over-diagnosis may also occur. In fact, not all reported malaria cases are confirmed by microscopy or others assay, such as rapid diagnostic tests (RDTs). Furthermore, in hyper endemic areas febrile illnesses from different causes might be misdiagnosed with malaria (2). *Plasmodium vivax* has unique attributes to support its survival in varying ecologies and climates and very different from *P. falciparum*., and other related simian species have identical biology, *Plasmodium vivax* can be grown in non-human primates (NHP), and in short-term ex vivo cultures.(3) Current estimates suggest that approximately 2.4 billion people are at risk of stable or unstable *Plasmodium falciparum* transmission (2) with 350 to 500 million clinical episodes and 1 million deaths annually (4). Transmission from an infected human host to a susceptible mosquito is mediated through highly specialized sexual-stage parasites, i.e., gametocytes. The gametocytes of *P. falciparum* hold a prominent place in the history of malaria, in that it was the exflagellating male gametocyte that first led Laveran to describe malaria in the late 19th century, exflagellation is a highly active process whereby motile male microgametes free themselves from the red blood cell in order to locate and fertilize a female macrogamete. Also, on routine microscope blood films, the gametocytes of *P. falciparum*, with their unique crescent shape, are prominent. Transitioning from the relatively protected and stable environment within the red blood cell of the human host to being an exposed parasite in the lumen of a mosquito mid-gut obviously requires considerably different characteristics (5-6-7-8). Hematological changes are some of the most common complications in malaria and they play a major role in malaria pathology. (9), these changes involve major cell lines including red blood cells (RBC), leukocytes and thrombocytes. Hematological changes in the course of a malaria infection, such as anemia, thrombocytopenia and leukocytosis or leucopenia are well recognized. These

alterations vary with the level of malarial endemicity, background hemoglobinopathy, nutritional status, demographic factors, and also malaria immunity (10-11-12). In malaria-infected patients, especially non-immunes children, prompt and accurate diagnosis is key to effective disease management for a favorable outcome. Clinical diagnosis is widely used for diagnosis of malaria especially in resource-poor countries (13-14).

2. Material and method

This Study was designed as a case-control study, carried out in the Ahmed Gasum Pediatric Hospital, in Khartoum State, Sudan. Total study one hundred (100) Sudanese children , 60 of them were infected *plasmodium falciparum* as case study and 40 were healthy children used as control group, whole blood samples were withdrawn under hygienic conditions, whole blood samples collected in containers with ethylene diamine tetra acetic acid (EDTA) for complete blood count (CBC), which conducted via hematology analyzer KX21, and tri-sodium citrate container for determination of D-dimer level which conducted via i-chroma device which works with fluorescence Immunoassay techniques . Thin and thick blood films stained by Giemsa for each child performed for confirmation and identification of malaria infection under microscopic examination. Thin smears were used for species identification. All blood slides were read by two independent microscopists with discrepancies resolved by a third microscopist. Parasite density was defined as number of *Plasmodium* parasites per μL of blood, counted against 200 leukocytes assuming a leukocyte count of 8000/ μL of blood. If fewer than 10 asexual parasites were detected in the first 200 leukocytes, counting was continued against 500 leukocytes.

Ethical considerations

This study approved by ethical committee of Alzaeim Al Azhari University. Each participant parent signed consent and written forms before undertaking any study-related activities

Statistical analysis:

Data analysis conducted by the statistical package of social science (SPSS) software version 21. Data are expressed as the mean $\pm$ standard error of the mean (SEM) unless otherwise stated. All data were compared using analysis of variance (ANOVA), P values of ≤ 0.05 were considered statistically significant.

3. Results

60 of children were infected with plasmodium falciparum malaria were enrolled in this study, their mean $\pm$ SD of age and weight were

17.26 $\pm$ 15.41 months and 8.98 $\pm$ 3.89 Kg respectively. And 40 subjects were healthy children, set as control group, their mean $\pm$ SD of age and weight were 18.00 $\pm$ 5.91 months and 9.44 $\pm$ 3.89 Kg respectively.

Parameters measured were haemoglobin (Hb g/dl), Packed cell volume (PCV%), Platelet count /Microliter and D dimer (DD ng/ml). comparing these parameters for patients and control group through independent T test brought significant difference for each one P value < 0.001 as in table 1 which reveal mean $\pm$ SD of each parameter and p value.

Table 1: independent T test for case and control group for hematological parameters and D. dimer (Mean $\pm$ SD).

Parameters	Case (Mean $\pm$ SD)	Control (Mean $\pm$ SD)	P-value
Hb g/dl	8.14 $\pm$ 2.20	13.41 $\pm$ 0.92	< 0.001
PCV %	24.37 $\pm$ 6.59	40.23 $\pm$ 2.77	< 0.001
Platelets count(x10 ⁹ /L)	98.53 $\pm$ 48.99	335.51 $\pm$ 70.09	< 0.001
D.Dimer ng/ml	7397.58 $\pm$ 5867.07	150.22 $\pm$ 79.56	< 0.001

Significant difference p value < 0.05 .

How plasmodium falciparum affect coagulation, Hb, PCV and platelet count as well. Considering formation of D dimer and its effect on Hb concentration PCV and platelet count, a negative correlation was found for each, meaning decline of Hb concentration, PCV % and platelet count paralleled with increasing D dimer formation and significant difference was obtained as p value was < 0.001 for Hb, PCV and platelet count as in figures (1, 2, 3) respectively.

Figure 1: correlation of D dimer and Hb concentration in infected children with malaria.

Figure 2: correlation of D dimer and PCV in infected children with malaria.

Figure 3: correlations of D dimer and platelet count in infected children with malaria.

Figure 4" the relationship between D. Dimer and parasite count among patient with malaria (n = 60). The parasite count means (SD) 3.28 (0.74). Simple linear regression was used the p value = 363 and $R^2 = 0.014$

4. Discussion

Changes in hematological parameters are likely to be influenced by any disease condition which affects the hemopoetic physiology at any level. This is expected to happen with an endemic disease such as malaria that affects the host homeostasis at various fronts resulting in a many of clinical presentation. Malaria is a major cause of morbidity in the tropical countries. Two hundred and forty seven million cases were reported worldwide in 2006 (15). Hematological changes are some of the most common complications in malaria and they play a major role in malaria pathology. These changes involve the major cell lines such as red blood cells, leucocytes and thrombocytes. In malaria-infected patients, especially non-immunes children, early and accurate diagnosis is key to effective disease management for a favorable outcome. Clinical diagnosis is widely used for diagnosis of malaria especially in resource-poor countries. Although fever and other signs and symptoms are known to be fairly sensitive measures of malaria they lack specificity and positive predictive values especially in areas where malaria is less prevalent (10-13). In this study infected children results in ineffective erythropoiesis and hematological parameters change like Hb concentration, PCV, platelet count and D dimer. All parameters gave significant difference when compared with healthy children; this agrees with a study conducted in children living in Western Kenya, severe anemia is the predominant severe malaria syndrome peaking in the first two years of life and is attributed to *Plasmodium falciparum*. (9) Children living in malarial endemic areas repeated attacks of malaria causes of severe anemia due to the red cell destruction and reduced red cell production led to hemolysis and phagocytosis or rupture of infected cells, removal of uninfected cells due to antibody sensitization or other physicochemical membrane changes, and increased reticuloendothelial activity, particularly in organs such as the spleen. Decreased production results from marrow hypoplasia seen in acute infections,

and dyserythropoiesis, a morphological appearance, which in functional terms results in ineffective erythropoiesis.(16) The removal of non-parasitized RBCs (nEs) is thought to be the most important, accounting for approximately 90% of the reduction in hematocrit in acute malaria.(17)

In previous study had similar results of hematological parameters changes among infected group were compared with those of the controls using t-test. Median values for Hemoglobin and Platelet count were significantly lower for the malaria group compared with the controls (18). As well as data obtained from study in Thailand-Myanmar border, it concerned about platelet count which found low among infected children by 31.8 times (19), indicating agreement with our finding as platelet count was low than healthy subjects count with significant difference and also the same finding data in Ethiopian study targeted *Plasmodium falciparum* infected subjects (20).

Previous studies study the Mechanisms for thrombocytopenia is dissimilar in malaria species infection, thrombocytopenia in vivax malaria presence to be immune mediated due to nonappearance of blood coagulation activation, while thrombocytopenia in falciparum malaria is most often accompanied by activation of the coagulation cascade among other mechanisms.(21,22)

Elevated the D-dimer result may specify the presence of an abnormally high level of fibrin degradation product, an elevated D-dimer does not always indicate the presence of a clot because a number of other factors can cause an increased level, infection *P. falciparum* malaria infection influences blood coagulation by various interacting pathobiological mechanisms, most important being the cytokine storm, disseminated intravascular coagulation (DIC) and very elevated D-dimer level. The D-dimer in this study showed significant difference with infection of *plasmodium falciparum*, and increased level of D dimer among case group children, this agreement with a couple of studies

concerned about many parameters included D dimer, both bring suggestion that hyper coagulation can be present even parasite density low. (23-24). Increased fibrinolytic activity and coagulopathy in the malaria patients was reflected by significantly elevated levels of fibrinogen and D-dimer. In non-endemic countries low incidence of *P. falciparum* malaria infection, diagnosis and treatment are often delayed, potentially leading to complications with an increased mortality due to the lack of physicians experience dealing with the malaria disease. (25)

5. Conclusion

Hematological changes are some of the most common complications in malaria and they play a major role in malaria pathology

Increase the rate of severity with plasmodium falciparum associate with a variety of hematological complication such as severe anemia, thrombocytopenia, DIC Disseminated intravascular coagulation .

Thrombocytopenia can be taken as a valid marker of development of hypercoagulation dysfunction, irrespective of the severity of the disease.

Complete Blood Count (CBC) and D dimer test can be utilized as an important tool for early detection the complication and management in malaria patients.

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REFERENCES

1. Carlos A Guerra, Priscilla W Gikandi, Andrew J Tatem, Abdisalan M Noor, Dave L Smith, Simon I Hay , Robert W Snow , the limits and intensity of *Plasmodium falciparum* transmission: implications for malaria control and elimination worldwide. *PLoS Med.* 5:e38 2010;1:175–187.
2. Sullivan D. Uncertainty in Mapping Malaria Epidemiology: Implications for Control. *Epidemiol Rev.* 2010;1:175–187.
3. Galinski, M. R., Meyer, E. V. & Barnwell, J. W. *Plasmodium vivax*: modern strategies to study a persistent parasite's life cycle. *Adv. Parasitol*, 2013; 81, 1–26.
4. Simon I. Hay , Emelda A. Okiro, Peter W. Gething, Anand P. Patil, Andrew J. Tatem, Carlos A. Guerra, Robert W. Snow ,estimating the global clinical burden of *Plasmodium falciparum* malaria in 2007, *PLoS Med.* 2010 Jun; 7(6): e1000290.
5. Laurence Florens, Michael P. Washburn, J. Dale Raine, Robert M. Anthony, Munira Grainger, J. David Haynes, J. Kathleen Moch, Nemone Muster, John B. Sacci, David L. Tabb, Adam A. Witney, Dirk Wolters, Yimin Wu, Malcolm J. Gardner, Anthony A. Holder, Robert E. Sinden, John R. Yates & Daniel J. Carucci , a proteomic view of the *Plasmodium falciparum* life cycle. *Nature* 2002; 419:520–526.
6. Gardner M. J., et al. Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* 2002 ; 419:498–511.
7. Lasonder E., et al , analysis of the *Plasmodium falciparum* proteome by high-accuracy mass spectrometry. *Nature* ,2002; 419:537–542
8. Le Roch K. G., et al, discovery of gene function by expression profiling of the malaria parasite life cycle. *Science* 2002; 301:1503–1508
9. Waitumbi JN, Opollo MO, Muga RO, Misore AO, Stoute JA. Red cell surface changes and erythrophagocytosis in children with severe *Plasmodium falciparum* anemia. *Blood.* 2000;95:1481–1486
10. Erhart LM, Yingyuen K, Chuanak N, Buathong N, Laoboonchai A, Miller RS, et al. Hematologic and clinical indices of malaria in a semi-immune population of western Thailand. *Am J Trop Med Hyg.* 2004;70: 8–14.
11. Price RN, Simpson JA, Nosten F, Luxemburger C, Hkijaroen L, ter Kuile F, et

- al. Factors contributing to anemia after uncomplicated falciparum malaria. *Am J Trop Med Hyg.* 2001;65: 614–622.
12. Wickramasinghe SN, Abdalla SH. Blood and bone marrow changes in malaria. *Baillieres Best Pract Res Clin Haematol.* 2000;13: 277–299.
13. World Health Organization. New perspective, malaria diagnosis. Geneva. 2000
14. Lathia TB, Joshi R. Can hematological parameters discriminate malaria from non malarious acute febrile illness in the tropics? *Indian J Med Sci.* 2004;58:239–244.
15. World Health Organization. World malaria report. Geneva; 2008
16. Phillips RE, Pasvol G, anaemia of Plasmodium falciparum malaria, *Baillieres Clin Haematol.* 1992 Apr;5(2):315-30.
17. Awah NW1, Troye-Blomberg M, Berzins K, Gysin J ,Mechanisms of malarial anaemia: potential involvement of the Plasmodium falciparum low molecular weight rhoptry-associated proteins, *Acta Trop.* 2009 Dec;112(3):295-302.
18. Robert N Maina, Douglas Walsh, Charla Gaddy, Gordon Hongo, John Waitumbi, Lucas Otieno, David Jones, and Bernhards R Ogut. Impact of Plasmodium falciparum infection on hematological parameters in children living in Western Kenya. *Malar J.* 2010; 9(Suppl 3): S4.
19. Manas Kotepui, Bhukdee Phunphuech, Nuoi Phiwklam, Chaowanee Chupeerach, and Suwit Duangmano. Effect of malarial infection on hematological parameters in population near Thailand-Myanmar border. *Malar J.* 2014; 13: 218
20. Sirak S, Fola AA, Worku L, Biadgo B. Malaria parasitemia and its association with lipid and hematological parameters among malaria-infected patients attending at Metema Hospital, Northwest Ethiopia. Dove press, 18 October 2016 Volume 2016:8 Pages 43—50.
21. Ivo M. B. Francischetti1*, Karl B. Seydel 2, and Robson Q. Monteiro 3 , Blood Coagulation, Inflammation and Malaria ,*Microcirculation.* 2008 February ; 15(2): 81–107.
22. Yamaguchi S, Kubota T, Yamagishi T, Okamoto K, Izumi T, Takada M, Kanou S, Suzuki M,Tsuchiya J, Naruse T. Severe thrombocytopenia suggesting immunological mechanisms in two cases of vivax malaria. *Am J Hematol* 1997;56:183–186.
23. Sabine Stauga, Andreas Hahn, Norbert W Brattig, Johanna Fischer-Herr, Stephan Baldus, Gerd D Burchard, and Jakob P Cramer- Clinical relevance of different biomarkers in imported plasmodium falciparum malaria in adults: a case control study. *Malar J.* 2013; 12: 246.
24. Julia Riedl, Benjamin Mordmüller, Silvia Koder, Ingrid Pabinger, Peter G. Kremsner, Stephen L. Hoffman, Michael Ramharter and Cihan Ay. Alterations of blood coagulation in controlled human malaria infection.*Malaria Journal* 2016 ;15:15
25. Sabine Stauga, et al. Clinical relevance of different biomarkers in imported plasmodium falciparum malaria in adults: a case control study , *Malar J.* 2013; 12: 246.