Original Article

Comparison of Hepatic Biochemical Derangements Induced By Falciparum and Vivax Malaria

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Objective: To compare the hepatic biochemical derangements induced by falciparum and vivax malaria

Study Design: A descriptive study

Place and duration of study: Department of Biochemistry, Basic Medical Sciences Institute, JP M C, Karachi from August 2005 to July 2006.

Materials and Methods: Total eighty-one patients of different ages and both sexes suffering from acute malaria, confirmed by peripheral blood smear were selected by convenient sampling. Nine out of eighty-one patients were infected by Hepatitis B and C infections and were excluded from the study. Out of seventy-two patients 48(70%) were suffering from malaria by Plasmodium falciparum and 24(30%) from Plasmodium vivax infection. The falciparum infected patients were divided into two groups on the basis of duration of illness. Group 1 comprised of 24 patients complaining of fever with or without rigors ranging from 1 – 7 days. Group 2 also consisted of 24 patients with duration of illness lasting from 8-20 days. Group 3 comprised of 24 Plasmodium vivax infected patients having illness of 1 – 20 days. Samples were analyzed in the Biochemistry Department at Basic Medical Sciences Institute, JPMC, Karachi.

Results: Liver was enlarged in 26 (54%) patients suffering from falciparum and 2 (8.3%) from vivax malaria. Spleen was also palpable in 23 (47.9%) patients from falciparum group and 4 (16.7%) from vivax group. This difference was statistically highly significant (P<0.001). Mean value of blood hemoglobin in Plasmodium falciparum group was 9.3±0.27, while in Plasmodium vivax group was 10.6±0.04 and the difference in haematocrit percentage was statistically highly significant (P<0.001). Hyperbilirubinemia was present in 52.7% of patients. The mean value of indirect Bilirubin in case of Plasmodium falciparum group is 2.4±0.32 comparatively higher than plasmodium vivax group showing a statistically significant P value (<0.01). Difference in the mean value of SGPT and SGOT are statistically highly significant (P<0.001) when results were compared with group I. The results of difference in mean value of alkaline phosphatase was statistically highly significant when group I and group II of Plasmodium falciparum infected were compared, which confirms that, as the duration of illness of Plasmodium falciparum progresses the level of alkaline phosphatase rises.

Conclusion: Although significant biochemical derangements were observed in both falciparum and vivax malaria, they were significantly more pronounced in Plasmodium falciparum infection, particularly in the later phase of illness. Therefore, liver function tests should be performed early in the course of Plasmodium falciparum malarial infections in order to prevent complications and to reduce mortality.

Key words: Plasmodium falciparum, Plasmodium vivax, Hyperbilirubinemia, hepatic biochemical derangements

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Introduction

Malaria is responsible for 1-3 million deaths annually and 300-500 million infections world wide.[1] Most of deaths are due to severe malaria, with one or more complications in a patient showing asexual parasitaemia of falciparum malaria.[2] Malarial transmission occur

through sporozoites injected in blood by female Anopheles mosquito, which attach to hepatocytes through receptor for thrombospondin and properdin.[3] Tissue schizonts then produces large number of merozoites. Each merozoite is capable of invading red blood cells and establishing the asexual cycle of replication with the release of 24 to 32 merozoites.[4] The clinical manifestations are consequent upon the release of cellular products and debris from ruptured erythrocytes and their phagocytosis by reticuloendothelial cells.[5] Hyperplasia of the these cells in the liver occur as part of the response to malaria and is responsible for its enlargement.[6] Plasmodium falciparum differs from other species in that its merozoites are capable of invading both older and younger red cells[7] and is responsible for multiorgan dysfunctions.[8] Liver involvement is common.[5] Unconjugated hyperbilirubinaemia is attributed to hemolysis of both parasitized and non-parasitized erythrocytes and partly due to liver damage.[9] Hyperbilirubinemia is often seen with acute renal failure, cerebral malaria and death.[10] The coagulation system is adversely affected during severe malaria and is proportional to the severity of the illness.[8] The elevated alkaline phosphatase activity indicates hepatic stage of parasites and significant perturbation in the hepatocytic membrane leading to its leakage.[11] Significantly decreased protein synthesis will not become apparent except in severe long standing hepatic diseases[12] or might be due to increased capillary leakage.[13] Hypoglycemia may have four reasons; (1) depletion of liver glycogen (2) glucose consumption by the parasites (3) hypoglycemic effects of elevated level of interleukin-1 and TNF-B and (4) release of insulin during treatment with quinine or quinidine.[4]

Present study was carried out to evaluate the acute hepatic damage induced by Plasmodium falciparum malaria, while Plasmodium vivax infected cases were included for comparative analysis.

Materials and Methods

This study was conducted from August 2005 to July 2006 in the Department of Biochemistry, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre Karachi. Eighty-one patients complaining of fever with or without rigors were selected through convenient sampling technique from Malaria Control Program counter at Accident and Emergency Department, Medical Units I, II and III, Jinnah Postgraduate Medical Centre and Pediatric Units in National Institute of Child Health, Karachi.

Malarial parasites were confirmed by blood peripheral smears. Two drops of blood were obtained by hand lancet from the pulp of the ring finger of the patients after cleaning with spirit swab. One drop was used to make a thin blood smear and the second drop for thick blood smear. Both slides were air dried and Giemsa stained for half an hour, washed and then examined for malarial parasite, under the microscope at 100x. A Performa was filled while taking history. There was no history of blood transfusion in the past four months. Nine patients were excluded because of their association with hepatitis B and hepatitis C. Remaining seventy-two patients were acutely symptomatic and uncomplicated, which was evident from the history, duration of illness and level of consciousness. Liver and spleen sizes were measured subcostally in the midclavicular line using a measuring tape (cm) while patient was lying supine. Patients positive for malarial parasite were selected for study and their venous blood (about 5 ml) was drawn from an antecubital vein through disposable syringe and fractionated as: One ml blood was transferred into a citrated tube for prothrombin time, while another 1 ml blood was transferred into EDTA containing tube for hemoglobin and hematocrit estimation. The rest of blood was left in syringe, to get serum for biochemical parameters. A small drop of blood was also placed on the strip of glucometer (Optium, Abbott) to check the random blood glucose level. The blood was allowed to clot and serum was pipetted out after centrifugation, labeled and stored at -20°C in freezer for later analysis. The selected patients were grouped on the basis of type of species and their duration of illness.

Group I: Plasmodium falciparum positive and having illness of 1 to 7 days.

Group II: Plasmodium falciparum positive and having illness of 8 to 20 days.

Group III: Plasmodium vivax positive and having illness of 1 to 20 days.

EXCLUSION CRITERIA

1. Malarial parasite negative patients
2. Those having jaundice of causes other than malaria
3. Those who were taking hepatotoxic drugs
4. Those having mixed malarial infection
5. Pregnant women
6. Patients found serologically positive for hepatitis.

Blood hemoglobin was estimated by Cyanmathemoglobin method, haematocrit values were estimated by microhaematocrit method on Microhaematocrit Machine, one-stage prothrombin time, serum bilirubin (Total, Direct and Indirect) by Jendrassik Grof Method. Blood Glucose, serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase and alkaline phosphatase by Enzymatic Method. Total protein estimated by Biuret Method. Serum albumin estimated by Bromocresol Method using kit (AB 362) Randox. The biochemical parameters were compared between all the three groups. The statistics were applied by using SPSS version 10.0. The p-value

was calculated by Student't' test (Level of significance: $p < 0.05$). The mean and standard error of the mean (SEM) were found out and further statistical analysis was performed.

Results

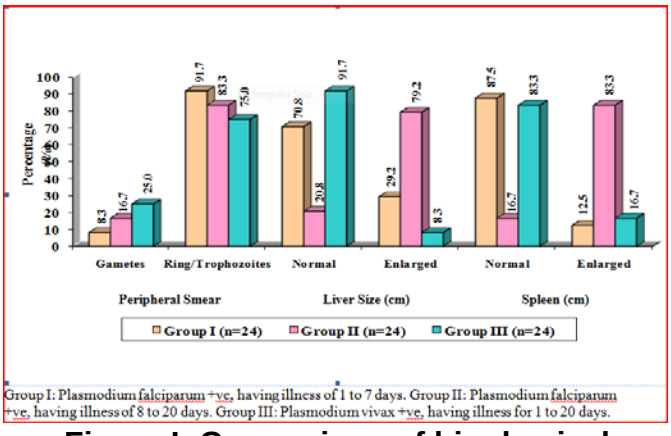
Out of eighty-one malaria infected cases seventy-two confirmed cases of two most common species were selected by convenient sampling. Table 1 shows the distribution of both Plasmodium falciparum malaria infected cases and Plasmodium vivax malaria infected patients in different groups according to their duration of illness.

Table I: Distribution of age and sex in patients with malaria (n=72)

Groups	No. of Subjects	Age	Sex	
			Male No. (%)	Female No. (%)
Group I	24	25.2±3.33	14 (58.3)	10 (41.7)
Group II	24	24.7±2.71	16 (66.7)	8 (33.3)
Group III	24	23.3±2.75	15 (62.5)	9 (37.5)

Values are expressed as Mean ± SEM

Group I: Plasmodium falciparum +ve, having illness of 1 to 7 days.
Group II: Plasmodium falciparum +ve, having illness of 8 to 20 days.
Group III: Plasmodium vivax +ve, having illness of 1 to 20 days.



Group I: Plasmodium falciparum +ve, having illness of 1 to 7 days. Group II: Plasmodium falciparum +ve, having illness of 8 to 20 days. Group III: Plasmodium vivax +ve, having illness for 1 to 20 days.

Figure I: Comparison of biophysical parameters between groups with Plasmodium falciparum and Plasmodium vivax malaria

Table II showing a comparison of biochemical parameters between groups of Plasmodium falciparum infected patients having equal number of patients in both groups.

Table II: Comparison OF BioChemical Parameters Between Group I and Group II

Biochemical Parameters	Plasmodium falciparum +ve		P value
	Group I (n=24) Mean ± SEM	Group II (n=24) Mean ± SEM	
Haemoglobin (g/dl)	9.2 ± 0.31	9.5 ± 0.44	0.578^{NS}
Haematocrit (%)	27.7 ± 0.97	28.6 ± 1.31	0.598^{NS}
Prothrombin time (Control: 11 to 16 sec)	14.4 ± 0.23	15.3 ± 0.31	0.016*
Random Blood Glucose (mg/dl)	93 ± 2.89	95 ± 3.11	0.619^{NS}
Bilirubin – Total (mg/dl)	1.4 ± 0.13	7.1 ± 0.83	0.001***
Direct (mg/dl)	0.6 ± 0.07	3.1 ± 0.49	0.001***
Indirect (mg/dl)	0.8 ± 0.08	4.0 ± 0.43	0.001***
SGPT (U/L)	27.5 ± 1.59	67.9 ± 7.72	0.001***
SGOT (U/L)	27.2 ± 1.19	52.1 ± 4.21	0.001***
Alkaline Phosphatase (U/L)	248 ± 11.6	352 ± 10.7	0.001***
Total protein (g/dl)	7.2 ± 0.14	6.1 ± 0.06	0.001***
Albumin (g/dl)	4.3 ± 0.08	3.4 ± 0.08	0.001***
Globulin (g/dl)	2.9 ± 0.10	2.7 ± 0.10	0.072*
A/G ratio	1.5 ± 0.06	1.4 ± 0.09	0.280^{NS}

Table III is showing a comparison of biochemical parameters between Plasmodium

falciparum and Plasmodium vivax. Both groups (i.e. I and II) of Plasmodium Falciparum were merged and compared with group III representing Plasmodium vivax infected patients. Their P value was calculated by Student't' test.

Table III: Comparison of biochemical parameters between Plasmodium Falciparum And Plasmodium Vivax groups

Biochemical Parameters	P. Falciparum (n=48)	P. Vivax (n=24)	P-value
Haemoglobin (g/dl)	9.3 ± 0.27	10.6 ± 0.34	0.004**
Haematocrit (%)	28.1 ± 0.81	33.0 ± 1.04	0.001***
Prothrombin time (Control: 11 to 16 sec)	14.8 ± 0.20	14.1 ± 0.29	0.042*
Random Blood Glucose (mg/dl)	94 ± 2.11	101 ± 3.33	0.083*
Bilirubin – Total (mg/dl)	4.2 ± 0.58	1.6 ± 0.17	0.003**
Direct (mg/dl)	1.8 ± 0.30	0.7 ± 0.11	0.010**
Indirect (mg/dl)	2.4 ± 0.32	0.9 ± 0.10	0.002**
SGPT (U/L)	47.7 ± 4.89	35.4 ± 1.10	0.083*
SGOT (U/L)	39.6 ± 2.82	32.9 ± 0.95	0.100*
Alkaline Phosphatase (U/L)	300 ± 10.9	273 ± 27.1	0.265
Total protein (g/dl)	6.7 ± 0.11	6.8 ± 0.14	0.297
Albumin (g/dl)	3.8 ± 0.08	4.4 ± 0.15	0.001***
Globulin (g/dl)	2.8 ± 0.07	2.4 ± 0.14	0.006**
A/G ratio	1.4 ± 0.05	2.0 ± 0.18	0.001***

* P<0.05, ** P<0.01, *** P<0.001 (Student 't' test).
Plasmodium falciparum +ve (n=48), having illness of 1 to 20 days. Plasmodium vivax +ve (n=24), having illness of 1 to 20 days.

Discussion

Malaria is a disease of poverty and underdeveloped countries like Pakistan. Among four species Plasmodium falciparum is the most prevalent, can be life threatening because of its severe complications. Malaria has been known to involve liver since the disease was first described.[14] Clinically, pallor reflects the blood hemoglobin levels. Anemia may arise from many mechanisms in falciparum infection including acute haemolysis , destruction of both infected and uninfected red blood cells, dyserythropoiesis and nutritional deficiencies.[15] In our study the level of Hemoglobin with the mean value of 9.2 g/dl and 9.5 g/dl in group I and group II (table 2) respectively, shows an anemic picture (<10 g/dl). These findings are comparable to those reported earlier[16]; however they differ from those of Nadeem et al who reported a hemoglobin level of 13.7 g/dl.[3] In this study hyperbilirubinemia is present in 52.7% of patients while a similar study conducted by Mishra et al reported hyperbilirubinemia in 64.3% of cases.[17] The mean value of indirect bilirubin in case of Plasmodium falciparum group is 2.4±0.32 (table 3) comparatively higher than the plasmodium vivax group showing a statistically significant P value (<0.01). Similarly, the mean value of indirect bilirubin was also higher in group II (table 2); its level correlates with the duration of illness. Similar findings have been reported by Irfan.[18]

Coagulation abnormalities are not uncommon in falciparum malaria. In this study, mean values of the prothrombin time 14.4 seconds in group I and 15.3 seconds in group II, relatative to control value of 11-16 seconds were observed. Only 4 out of 48 patients of Plasmodium falciparum infected group had prolonged prothrombin time without any bleeding disorders. These results are significant and are in accordance with Hemmer et al.[19]

Hypoglycemia is an important complication of severe malaria associated with high morbidity and mortality.[20] The results of random blood glucose were normoglycemic. The mean glucose level in total Plasmodium falciparum group (table III) was 94 mg/dl when compared with the Plasmodium vivax group (101 mg/dl), showing that falciparum malaria causes more hypoglycemia and this value matches with the Grau et al showing P<0.01.[21]

The increased serum level of hepatic enzymes, transaminases (SGOT and SGPT) and alkaline phosphatase are the markers of liver disorders. SGPT (ALT) is a specific enzyme of liver. The difference in the mean value of SGPT and SGOT are statistically highly significant (P<0.001) when results were compared with group I (table 2). Similar finding have been reported by Premaratna R et al.[22] In the present study the P value of alkaline phosphatase was insignificant in table 3, but the results of difference in mean value are statistically highly significant when group I and group II of Plasmodium falciparum infected were compared in table 2 which confirms that, as the duration of illness of Plasmodium falciparum progresses the level of alkaline phosphatase rises. These findings correlate with the results of Garba and Ubom.[11]

The liver has extensive synthetic capacity and plays a role in protein synthesis. The mean value of total protein was within normal range in all groups, but statistically significant difference (P<0.001) was observed when group I and group II were compared (table 2). A non-significant hypoalbuminemia in group II was a regular feature highlighting the fact that patients

were in good health previously. Overall assessment shows that there is highly significant changes of liver function tests in group II as compared to group I and group III, showing that the liver involvement in this group resemble an acute liver damage rather than the chronic one, while the results of total protein and particularly A/G ratio are non-significant.

In this study, the Plasmodium vivax positive cases were selected for comparison with Plasmodium falciparum malaria. There was no difference in the mean age and duration of illness. Liver was palpable in 8.3% of cases showing a marked difference of 29.2% and 79.2% in group I and group II respectively. There was a statistically significant ($P<0.01$) difference in the mean value of hemoglobin, bilirubin total, direct and indirect. The difference in the result of mean values of prothrombin time, random blood glucose, SGPT and SGOT were statistically less significant ($P<0.05$) shown in table 3. These results reflect that the hepatic involvement was present in both species but the severity was more pronounced in Plasmodium falciparum malaria.

Conclusion

Both Plasmodium falciparum and Plasmodium vivax malaria induces hepatic biochemical derangement. Liver was found enlarged in 54% of falciparum positive patients as compared to 8.3% Plasmodium vivax positive patients.

Red blood cells are destroyed reflecting a fall in hemoglobin and hematocrit percentage, The results of prothrombin time and random blood sugar were statistically less significant, while serum bilirubin total, direct and indirect showing a statistically significant results ($P<0.01$). Aminotransferases showed less significant results in both groups with P value of <0.05. On the other hand, the alkaline phosphatase and total protein results were statistically non-significant. Regarding serum albumin, globulin and A/G ratio in Plasmodium falciparum group observed highly significant ($P<0.001$) when compared to the mean value of Plasmodium vivax group. So it is suggested that the liver function tests should be performed along with early detection of Plasmodium falciparum malarial parasites in order to prevent complications and to differentiate with viral hepatitis. We also conclude that the derangement in liver function test bears a broad relationship to the duration of illness. In our study derangement of some parameters were significantly more pronounced during the later phase of Plasmodium falciparum infection.

References

1. Casals- Pascual C and Roberts DJ. Malaria and the red cell. Vox Songuinis ; 87(Suppl. 2): 115-119; 2004.
2. World Health Organization . Severe falciparum malaria (Severe and complicated malaria). 3rd Edition. Trans Royal Soc Trop Med Hyg; 94(Suppl 1):20-34; 2000.
3. Nadeem M, Ali N and Qamar MA. Hematological findings in acute malarial infection list of authors along with highest qualification and institute. Biomedica; 18:62-65; 2002.
4. Donald and Krogstad. Plasmodium species (Malaria). Mandell Doughlas and Bennetts. 5th Edition.; pp 2817-2830; 1995.
5. Baheti R, Laddha P and Gehlot RS. Liver Involvement in falciparum malaria – A histo-pathological analysis. JIACM; 4(1):34-8; 2003.
6. Sowunmi A. Renal function in acute falciparum malaria. Arch Dis Childhood; 74:293-298; 1996.
7. Davis TME. Recognition and management of falciparum malaria. Emergency Medicine; 12:276-284; 2000.
8. Vogetseder A, Ospelt C, Reindl M, Schober M, Schmutzhard E. Time course of coagulation parameters, cytokines and adhesion molecules in plasmodium falciparum malaria. Trop Med Intern Health; 9(7):767-773; 2004.
9. Harinasuta T and Bunnag D. The clinical features of malaria In: Malaria: Principles and Practice of Malariology. Vol. 1, Wernsdorfer WH and McGreogor IA (eds). Edinburgh Churchill Livingstone; p 721 1988.
10. Kochar DK, Agarwal P, Kochar SK, Jain R, Rawat N, et al. Hepatocyte dysfunction and hepatic encephalopathy in plasmodium falciparum malaria. Q J Med; 96:505-512; 2003.
11. Garba IH, and Ubom G. Serum Alakaline Phosphatase activity as a potential biomarker for the integrity of the hepatic draining system in acute falciparum malaria infection. *Inter J Infect Dis;* 4(2): 2005.
12. Fletcher KA and Gilles HM. Chemical pathology of malaria In: Malaria: Principles and Practice of Malariology. Wernsdorfer WH,McGregor IA (eds). Vol. 1, Edinburgh Churchill Livingstone 1988; pp 647-72.
13. Areekul S. Transcapillary escape rate and capillary permeability to albumin in patients with Plasmodium falciparum. *Ann Trop Med Parasitol*; 82:135-40; 1988.
14. Molyneux ME, Looareesuwan S, Menziee IS, Grainger SL, et al. Reduced hepatic blood flow and intestinal malabsorption in severe falciparum malaria. *Am J Trop Med Hyg;* 1989; 4-(5):470-476.
15. Miller LH, Baruch DI, Marsh K, Doumbo KO. The Pathogenic basis of malaria. *Nature*; 415:7; 2002.
16. Bhalli MA and Samiullah. Falciparum Malaria – A review of 120 cases. *JCPSP*; 11(5):300-303; 2000.
17. Mishra SK, Pati SS, Satpathy SK, Mohanty S, Mohapatra DN. The influence of hyperbilirubinaemia on malaria-related mortality" an analysis of 1103 patients. *Ann Trop Med Parasitol*; 98(6):555-558; 2004.
18. Irfan M. Liver dysfunction in falciparum malaraia. Thesis 2000.
19. Hemmer CJ, Kern P, Holst FGE, Radtke KP, et al. Activation of the host response in human plasmodium falciparum malaria: Relation of parasitemia to tumor necrosis factor/cachectin, thrombin-antithrombin III, and protein C levels. *Am J Med*; 91:37-44; 1991.
20. Dekker E, Hellerstein MK, Romijn JA, Neese RA, Peshu N, Endert E, Marsh K, Sauerwein HP. Glucose homeostasis in children with falciparum malaria: Precursor supply limits gluconeogenesis and glucose production. *J Clin Endocrin Metabol*; 82(8):2514-21; 1997.
21. Grau GE, Taylor TE, Trop M, Molyneux ME, *et al*. Tumor necrosis factor and disease severity in children with falciparum malaria. *N Engl J Med;* 320(24):1586-1591; 1989.
22. Premaratna R, Gunatilake AKE, de Silva NR, Tilakaratne Y, Fonseka MMD, de Silva HJ. Case Report – Severe hepatic dysfunction associated with falciparum malaria. Southeast Asia J Trop Med Pub Health 2001; 32(1):70-72