

***Plasmodium berghei* induced biochemical alterations in pregnant mice**

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ABSTRACT

Malaria leads to pathophysiological and biochemical alterations in placenta and blood of pregnant mice. A significant decrease in the sugar, protein and lipid levels in the placental homogenate of pregnant-infected mice was observed compared to the pregnant mice. However, serum protein content was not altered much in the pregnant-infected mice as compared to the levels in control mice. The serum lipid level enhanced significantly in both pregnant and non pregnant-infected mice. The enzymatic activities of alkaline phosphatase and acid phosphatase altered significantly in malaria-infected placenta. Our study clearly highlights the possible role of these enzymes in damaging the placenta which in turn may jeopardise the fetal growth together with altered biochemistry of placenta. Therefore biochemical along with pathological alterations occurring during malaria infection in pregnancy may account for compromised maternal fetal relationship.

Keywords: *P. berghei*, placenta, pregnant, biochemical changes.

INTRODUCTION

Malaria affects more than 400 million people in the world. It continues to be a serious public health problem in tropical and subtropical countries, accounting for between 5 and 15.0% deaths of children in endemic areas.¹ Higher frequencies of *Plasmodium falciparum* infections have been observed amongst pregnant women in holoendemic malarious areas. *P. falciparum* malaria is much more frequent and severe in pregnant women particularly primigravidae than multigravidae/non pregnant women residing in endemic area.² Since clinical observations indicate that pregnancy exerts a dampening effect on malarial immunity, pregnant women suffer from recurrent and severe infections as a result of their increased susceptibility and high density of Plasmodium infection.^{3,4} Malaria during pregnancy promotes placental insufficiency, leading to intrauterine growth retardation. In addition, it may also cause prematurity, low birthweight and fetal death.^{5,6} Various scientists have highlighted the association between the density of placental parasitization and reduced birthweight.⁷ Pathological studies on infected placenta have shown that marked placental alteration occur both at surface as well as ultrastructural level. Infected placenta had plugged placental sinusoids with malaria pigment, parasitized erythrocytes and inflammatory cells along with vacuolated and necrosed trophoblastic membrane.⁸ However, the biochemical alterations occurring in the placenta have not yet been fully understood. Therefore, the present study was carried out to examine the biochemical alterations occurring in the placenta of pregnant-infected mice.

MATERIALS AND METHODS

Parasite: *P. berghei* (NK-65) initially procured from National Institute of Communicable diseases, New Delhi was employed in the present study.

Animals: Close bred 5-6 weeks old LACA female mice weighing 17-23g, obtained from Central Animal House of Panjab University, Chandigarh were used. The mice were fed with pellet diet and water ad libitum.

Experimental design: Two female mice and one male mouse were kept for mating. The first gestational day (GD) was assessed by the presence of vaginal plug or cornified squamous epithelial cells in the papanicolaou stained vaginal smears.⁹ The animals were divided into four groups as follows. **Group I (n=6):** Non-pregnant uninfected mice (Control mice). The mice were inoculated with normal saline intraperitoneally (i/p). **Group II (n=6):** Non pregnant-infected mice (Infected). These mice were infected with 10^6 parasitized erythrocytes intraperitoneally (i/p). **Group III (n=8):** Pregnant-uninfected mice (Pregnant, GD-10). These mice were inoculated with normal saline intraperitoneally (i/p) on 10th gestational day. **Group IV (n=8):** Pregnant-infected mice (Pregnant-infected, GD-10). Pregnant mice were inoculated with 10^6 parasitized erythrocytes intraperitoneally on 10th gestational day.

Parasitaemia: The parasitaemia was quantitated on every alternate day by examining Giemsa stained tail vein blood film. Percent parasitaemia was calculated by counting at least 500 cells. Pregnant - infected and infected mice having parasitaemia in the range of 50.0-60.0% were bled by retro-orbital

puncture. The biochemical parameters e.g sugar, lipid, protein as well as acid and alkaline phosphatase were estimated in the serum and placental homogenates.

Preparation of post mitochondrial fraction: Placenta from pregnant mice were collected after sacrificing them by bleeding through retro orbital puncture. Placental homogenates (10.0% w/v) were prepared in cold phosphate buffered saline, pH 7.2 using mechanically driven homogenizer at 4°C, centrifuged at 10000g for 20 min to get the post mitochondrial fractions with which the biochemical estimations were performed.

Biochemical estimation: Total sugar, protein and lipid contents in the serum and postmitochondrial fractions from all the four groups were estimated¹⁰⁻¹². The enzymatic activity of alkaline phosphatase and acid phosphatase was monitored by using para-nitrophenyl phosphate as the substrate.¹³ Enzyme activity was expressed as μM of p-nitrophenol/min/mg of placenta and μm of p-nitrophenol/min/ml in serum.

Statistical Analysis: The data was analyzed by students paired 't' test with equal number of observations.

RESULTS

The level of sugars in the blood of infected and pregnant-infected mice were significantly lower than the level in the control and pregnant mice. However the sugar level further decreased significantly in the placental homogenate of pregnant-infected mice as compared to pregnant mice (Table-1). A significant ($p < 0.01$) decrease in the protein content of placental homogenate was observed in pregnant-infected mice as compared to the pregnant mice, whereas the protein content in the serum of pregnant-infected and infected mice did not altered much as compared to the protein content in the pregnant and control mice (Table-1). Similarly lipid level in the placental homogenate of *P. berghei* infected pregnant mice was also observed to be decreased significantly ($p < 0.01$) as compared to pregnant mice whereas in the serum the lipid level was significantly higher in both pregnant-infected and infected mice than the pregnant and control mice (Table-1).

Alkaline phosphatase activity was significantly ($p < 0.01$) more in the serum of pregnant mice compared to pregnant infected and control mice. (Fig. 1) However, the activity of this enzyme increased significantly in the placental homogenate of pregnant mice (Fig. 2). But, following *P. berghei* infection, the activity of alkaline phosphatase decreased significantly in placental homogenate as well as in the serum of infected and pregnant-infected mice (Fig. 1 and 2). It was also observed that the acid phosphatase activity was significantly ($p < 0.01$) more in the placental homogenate and serum of pregnant-infected mice compared to respective controls (Fig. 1 and 2), indicating enhanced level of acid phosphatase activity during malaria infection.

DISCUSSION

Malaria spares none but affects mostly young children, travelers and pregnant women living in endemic area. Maternal malaria has deleterious effect on developing fetus such as low birth weight, fetal death or abortion as observed by various scientists.^{5,6} However, biochemical changes occurring in the serum as well as in the placenta following malarial infection have not been studied.

The decreased sugar level in placental homogenate of pregnant-infected mice may be due to damaged placental surface or high vascular structure of placenta resulting in to high parasite predilection for placenta resulting in high parasite density, as these obligate anaerobic parasites require more sugar for their growth and multiplication which in turn may cause complete exhaustion of liver glycogen.¹⁴⁻¹⁶ It has also been documented that hypoglycemia in malaria is due to hyperinsulinemia stimulated by the parasite toxins.¹⁷⁻¹⁹ It is clearly indicated that sugar during pregnancy may get diverted from circulation to the developing fetus (needed for its growth) resulting into decreased level of sugars. In malaria infected mice, the decreased sugar levels could be due to the impairment of hepatic gluconeogenesis as liver itself is severely damaged.²⁰ The data, therefore suggests that due to increased demand for sugar by the parasite for its growth, high parasite density (occurring due to immunosuppression), damaged liver and hyperinsulineamia may be responsible for hypoglycemia in pregnant-infected mice.

Decreased placental protein in pregnant-infected mice may be due to damaged placental membrane occurring due to adherence of malarial parasite or it may be due to the fact that during infection, the proteins are the first reserve material to be utilized by the infectious pathogens, thereby suggesting the mal-development of fetus during pregnancy following infection. The decrease in serum protein in pregnant mice compared to control mice could be either due to increase in plasma volume during pregnancy or mobilization of protein for the protein synthesis necessary for the defense mechanism against parasitic infection, as fat utilization is decreased in comparison to protein.²¹

Decreased lipid level in placenta may be due to severely damaged placental membrane leading to loss in its function of selective barrier. This observation is in concordance with the earlier reports.²² Interestingly, lipid level was more in serum which could be due to various hormones having dual functions. It is very well documented that hormones do have influence on high density lipid and low density lipid by enhancing the hepatic lipase activity, which in turn alters the lipid metabolism. Moreover, lipid metabolism takes place in liver, which in turn is affected by malaria more severely than any other organ as it is the first organ where parasite multiplies and results into hepatomegaly. The increase in serum lipid level may be a direct evidence of liver damage which in turn may be associated with decreased uptake of lipoprotein by receptor mediated endocytosis.^{23,24}

As alkaline phosphatase is produced by the liver, bone and placenta and all these organs are severely affected in pregnant infected mice. Therefore, the decreased activity of this enzyme further supports the destruction of defense mechanism present in leucocytes and disturbed phosphate metabolism in placenta during malarial infection. Altered enzyme activity in turn, may also affect the transportation of several metabolic substances that may lead to either fetal death, reduced birth weight, premature delivery and abortion. Earlier, it has been reported that any disturbance in this enzyme level affects the fetal development.²⁵

Red blood cells and prostrate gland are the main source of acid phosphatase enzyme. The increased activity in placenta and serum of infected mice may be attributed to rapid destruction of erythrocytes as a result of heavy malarial infection, its metabolism or could be due to the involvement of lysosomal enzymes that degrade the protein of placenta under parasitic stress. Thus, the altered placental surface as well as the necrosed placenta may be resulting due to enhanced activity of this enzyme, which could be due to either malarial toxins or parasite multiplication.

Thus, it seems that the activity of alkaline and acid phosphatase in the placenta of infected mice are altered due to malaria infection which may play a crucial role in the fetal development indicating an immense role of these enzymes along with other moieties in controlling the physiology of placenta. In other words, malarial parasite may damage the various vascular organs by altering their biochemical physiology. Therefore, it seems that malaria during pregnancy may induce several alterations in the body that makes the host more vulnerable to infection resulting into compromised materno-fetal development.

REFERENCES

1. WHO. International travel and health : situation as on 01 January 2003 (WHO, Geneva) 2004.
2. Steketee RW, Nahlen BL, Parise ME, Menendez C. The burden of malaria in pregnancy in malaria endemic areas. *Amer J Trop Med Hyg* 2001; 64: 28-35.
3. Menendeze. Malaria during pregnancy, a priority area of malaria research and control. *Parasitol Today* 1995; 11: 178-83.
4. Okoko BJ, Enwere G, Ota MO. The epidemiology and consequences of maternal malaria; a review of immunological basis; *Acta Trop* 2003; 87: 193-205.
5. Brabin BJ, Rogerson SJ. The epidemiology and outcomes of maternal malaria. In: Duffy PE, Fried M, eds. *Malaria in Pregnancy: Deadly parasite, susceptible host*. London: Jaylor and Francies 2001; 27-52.
6. Adam I, Khamis AH, Elbashir. Prevalence and risk factors for *Plasmodium falciparum* malaria in pregnant women of Eastern Sudan, *Malaria J* 2005; 4: 18.
7. Ismail MR, Ordi J, Menendez C *et al*. Placental pathology in malaria: histological, immunohistochemical and quantitative study. *Human Pathol* 2000; 31: 85-93.
8. Shukla G, Asnani PJ, Bushnurm SR, Vinayak VK. Prevention of malaria induced fetal abnormalities following immunization of mice with *Plasmodium berghei* merozoite antigen. *Indian J Med Res* 1990; 91: 372-8.
9. Papanicolaou GN. New Procedure for staining vaginal smears. *Science* 1942; 95: 438-43.
10. Dubois M, Gills VA, Hamilton TK, Roberts PA, Smith F. Calorimetric method for determination of sugar and related substance. *Anal Chem* 1956; 28: 350-6.
11. Lowry OH, Rosebrough NS, Farr AL, Randall RJ. Protein measurements with phenol reagent. *J Biol Chem* 1951; 193: 265-275.
12. Frings CS, Dunn RT. A calorimetric method for the determination of total serum lipids based on sulfo-phosphovanillin reaction. *Amer J Clin Path* 1970; 53: 89-93.
13. Bergmyer MVC. In: *Methods of enzymatic analysis*, Academic Press Inc. New York 1963; 783-5.
14. Sherman IW. Biochemistry of *Plasmodium* (malaria parasite). *Microbiol Rev* 1979; 43: 453-495.
15. White NJ, Looareesuwan S, Warrel *et al*. Quinine loading dose in cerebral malaria. *Amer J Trop Med Hyg* 1983; 32: 1-5.
16. Davis TME, Looareesuwan S, Pukrittayakamee S, Lecy JC, Nagachinta B, White NJ. Glucose turnover in severe falciparum malaria. *Metabolism* 1993; 42: 344-50.
17. Elased KM, Playfair JHL. Hypoglycemia and hyperinsulinaemia in rodent models of severe malaria infection. *Infect Immun* 1996; 62: 5157-60.
18. Taylor TE, Molyneus MW, Wirima JJI, Fletcher KA, Morris K. Blood glucose levels in Malawian children before and during the administration of intravenous quinine for server falciparum malaria. *New Eng J Med* 1988; 319: 1040-7.

19. Holloway PAH, Krishna H, White NJ. *Plasmodium berghei* : Lactic Acidosis and Hypoglycemia in a Rodent model of severe malaria; Effects of glucose quinine and dichloroacetate. *Exp Parasitol* 1991; 72: 123-133.
20. Dawn. In: Textbook of obstetrics Dawn. Book Publ. Calcutta (India).
21. Wannemacher RW, Beisel, WR. Metabolic response of the host of infectious disease. In nutritional aspects of care in the critically ill. J.d. Richards and J.M. Kinney (eds) Chrichill, Livisgstone, London 1977; 135-59.
22. Shukla G, Neeraj P. Surface alterations of placenta in *Plasmodium berghei* infected pregnant mice. *Punjab Univ Res J (Science)* 2005; 55: 35-9.
23. Vinayak VK, Pathak G, Jain S, Asnani PJ, Malik AK. Influences of malaria infection of the maternal relationship in pregnant mice. *Aus J Exp Biol Sci* 1986; 64: 223-7.
24. Angus MGN, Fletcher KA, Maegraith BG. Studies on the lipids of *Plasmodium knowlesia*-infected rhesus monkeys (*Macaca multa*). II: Changes in serum non-esterified fatty acid. *Ann Trop Med Parasit* 1971; 65: 155-60.
25. Saxena N, Pandey VC, Saxena PN, Upadhyay S. Hydrolytic enzymes of rhesus placenta during *Plasmodium cynomolgi*. Infection: Ultrastructural and Biochemical studies. *Indian J Exp Biol* 1993; 31: 54-6.

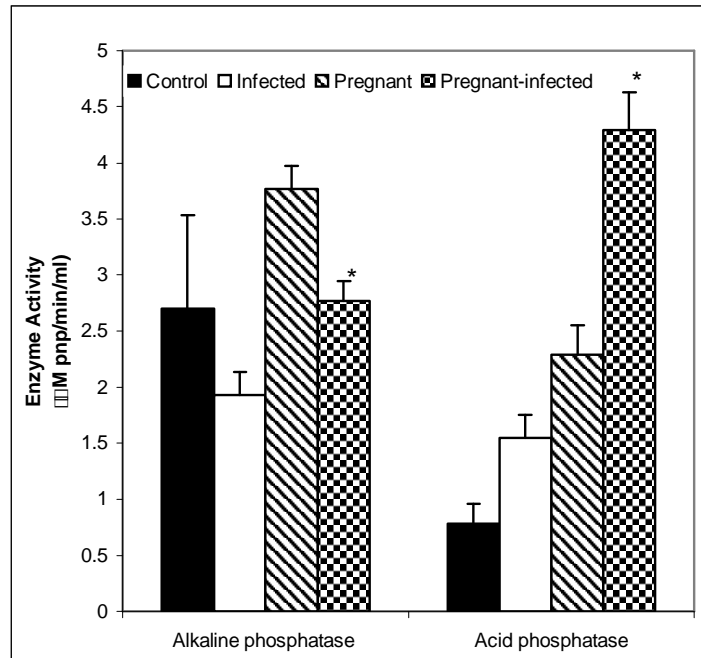


Fig. 1. Alkaline phosphatase activity and acid phosphatase activity in the **SERUM** of pregnant and pregnant-infected mice. Values are expressed as mean \pm standard deviation, *(p<0.01)

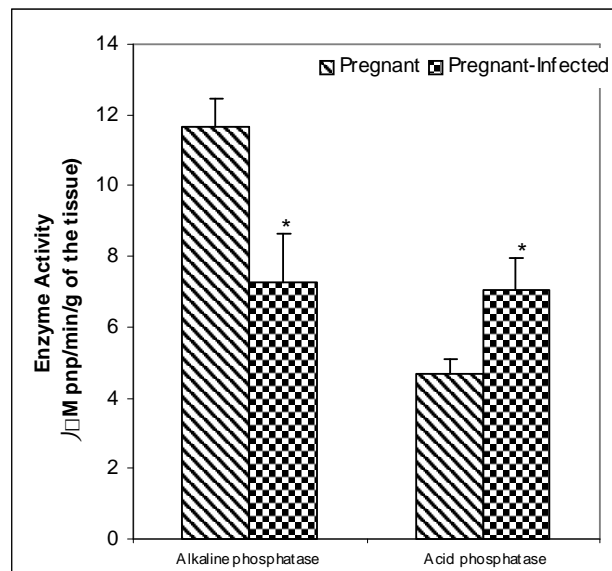


Fig. 2. Alkaline phosphatase activity and acid phosphatase activity in the **PLACENTA** of pregnant and pregnant-infected mice. Values are expressed as mean \pm standard deviation, *(p<0.01)

Table-1: Comparative sugar, protein and lipid levels in the placenta and serum of control, infected, pregnant and pregnant-infected mice.

Biochemical Assays	Groups of Animals	Placenta (mg/g tissue)	Blood (mg/dl)
Sugar level	Control	-	66.70±14.39
	Infected	-	49.67±6.95*
	Pregnant	21.94±0.976	58.04±6.92
	Pregnant infected	10.98±1.65**	39.50±8.70**
Protein level	Control	-	5.37±0.14
	Infected	-	5.02±0.21
	Pregnant	39.27±2.54	4.98±0.09
	Pregnant infected	18.38±11.32*	4.83±0.15
Lipids level	Control	-	84.51±5.56
	Infected	-	98.92±4.02*
	Pregnant	84.58±11.11	100.94±4.02
	Pregnant infected	39.92±3.85*	116.36±5.63*

Values are expressed as mean ± standard deviation, *(p<0.01); **(p<0.001)