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Research Article

MALARIAL INFECTION ELEVATE ERYTHROCYTE GLUCOSE UTILIZATION: A SYSTEMATIC ANALYTICAL STUDY

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A B S T R A C T

Background: Malaria is one of the most prevalent parasitic disease of global importance. Malaria imposes great socioeconomic burden on humanity and it accounts for 85% of Global infectious disease burden. Plasmodium also requires glucose as a source of energy. Parasitized erythrocytes increase their utilization of glucose as much as 100 times than that of uninfected normal erythrocytes. **Material and methods: B**lood samples of healthy individuals and Malaria infected patients were collected into 2 vials, 2 ml in EDTA and 3 ml vial without any anticoagulant. Plasma and serum glucose concentrations were analyzed from collected blood by GOD-POD method at the interval of 0, 1 and 2 hours. **Results:** Higher rate of glucose utilization, approximately double, was observed in patients suffering with malaria in serum and plasma both. In whole blood, concentration of blood glucose is decreased from 86.3 ± 18 mg/dl to 66 ± 15.8 mg/dl at the end of 2nd hours. Similarly significant decrease observed in glucose concentrations in separated serum also. At 0 hour, observed mean was 86.3 ± 18 mg/dl which was decreased to 69.2 ± 17.3 mg/dl at the end of 2 hours. The concentrations of blood glucose in control and study group were compared statistically, showed significant difference with P value was <0.001. **Conclusion:** It was concluded that utilization of glucose in malaria infection is much higher than normal individual. Increased consumption of blood glucose is due to glycolysis of RBC and added effect of parasite consuming glucose for their growth and development.

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INTRODUCTION

Malaria is one of the most prevalent parasitic disease of global importance and also a major cause of morbidity and mortality in the tropics.[1] Malaria imposes great socio-economic burden on humanity and it accounts for 85% of Global infectious disease burden. [2] According to survey of National Institute of Malaria research about 36% of the world population, i.e. 2020 million is exposed to the risk of contracting malaria in approximately 90 countries. [2] World Health Organization estimates are around 300-500 million malaria cases annually. [3]

Malaria is caused by five species of parasite that affect humans and all of these species belong to the genus *Plasmodium*: *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*.[4] Plasmodium spend important phase of growth and development in RBCs (intraerythrocytic schizogony). Finally RBC ruptures and releases merozoites which further invade fresh RBC. Some merozoites within red cell form gametocytes, host carrying gametocytes called as carrier. These gametocytes taken up by mosquito during its blood meal and the cycle continues. [4] Thus the red cell plays a central role in growth and propagation of malarial parasite. [5]

Inside RBC, along with hemoglobin, Plasmodium also requires glucose as a source of energy. Parasitized erythrocytes increase their utilization of glucose as much as 100 times than that of uninfected RBCs. [4,6] The increased utilization reflects the need of parasite to grow and reproduce. [7] The plasmodium lacks functional Embeden Mayorhof pathway due to absence mitochondria. Therefore, during intra-erythrocytic growth phase, malarial parasites totally rely on glycolysis for source of energy. [8] Entire glucose is metabolized through Embeden Mayorhof pathway to lactic acid. Hypoglycemia and lactic acidosis often associated with severe malaria. [7,8,9] Also the rate of glucose utilization is directly proportional to parasitemia and was highest in trophozoites stage. [8] However, the biochemical basis for increased rate of glycolysis has not been completely understood. [6] Eugene *et al* (1988) had studied enzymes of the glycolytic pathway as well as some ancillary enzymes in

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normal red cell and red cells parasitized with Plasmodium. They found increased concentrations of all enzymes like hexokinase, aldoses, pyruvate kinase and adenosine deaminase.[7] Hence these enzymes might play lead role to decrease glucose concentrations in malaria. The difference in glucose utilization if present it may be either due to enzymes released by destruction of parasitic or may be due to parasite itself utilizing glucose for survival, was ultimate aim of this study.

MATERIAL AND METHODS

The study was conducted in the Department of Biochemistry, MGM Medical College, Navi Mumbai. The aim of our study was to evaluate rate of glucose utilization in patients of malaria. Ethical clearance was taken from the scientific and ethical committee of the institution. The study was conducted as per ICH-GCP guidelines for human research.Written informed consent was obtained from the patients before enrollment of subjects in this study.

Out of total enrollment 50 malaria confirmed patients by thin smear between ages of 25-40 years and 30 healthy individuals after age and gender match were enrolled in this study. Patient beyond study age group, liver disease, renal dysfunction, chronic infections, alcoholics and diabetes mellitus subjects were excluded. [10]

5 ml of blood was collected from each subject by venipuncture with standard blood collection technique. Collected blood was divided in 2 vials, 3 ml in plane vaccutainer for serum and 2 ml in EDTA vaccutainer for plasma. The plane vaccutainer was centrifuged for 15 minutes at 2500 rpm. Serum was separated for glucose estimation under aseptic precautions. Plasma and serum glucose concentrations were measured by GOD-POD method (Teitz) at 0, 1 and 2 hours. The plasma and serum glucose concentrations were compared and co-related statistically.

RESULTS

Data reported were statistically analyzed by R-software which is freely available online.

Table 1 Mean and SD of Blood plasma Glucose at 0, 1 and 2 Hours

Groups	Blood glucose(mg/dl)			P-Value
	0 hour	1hour	2 hours	
Control group	105.5 ± 12.5	97.3 ± 11.6	89.8 ± 11.1	<0.001**
Study group	86.3 ± 18	74.8 ± 16.6	66 ± 15.8	<0.001**

Table 2 Mean and SD of separated serum glucose at 0, 1 and 2 hours

Groups	Serum Glucose(mg/dl)			P value
	0 hour	1hour	2 hours	
Control group	105.5 ± 12.5	99.3 ± 11.5	93.9 ± 11.2	<0.001**
Study group	86.3 ± 18	77 ± 17.7	69.2 ± 17.3	<0.001**

**: Significant at 1% concentration of significance

Table 3 Percent utilization of glucose at the end of 1st and 2nd hour

Groups	Blood Glucose		Serum Glucose	
	At 1 hour	At 2 hours	At 1 hour	At 2 hours
Control group	7.80%	7.60%	5.90%	5.40%
Study Group	13.20%	11.70%	10.70%	10.10%

DISCUSSION

Malaria remains one of the most widespread disease affecting human race in tropical and subtropical regions of the world. [5,11] According to the World Health Organization (WHO) report, of all malaria cases in the world, 90% were occurring in Africa with 75% of global P. *falciparum* malaria and 80% mortality was documented. [12] Drug and insecticide resistance have aggravated the complexity of malaria infection control. [5]

Glucose is a ubiquitous fuel for survival human body cells, hence it's worthy to investigate during malarial infection. [13] Glucose acts as a source of nutrition for human erythrocyte and also for Plasmodium. [7] Since human erythrocyte lacks mitochondria, there is absence of Kreb's cycle. Similarly even Plasmodium also lacks functional of Kreb's cycle. Ultimately anaerobic glycolysis is the main pathway to derive energy for erythrocytes as well as for parasites. However, it is relatively inefficient process as pyruvate, an end product of aerobic glycolysis is unable to enter kreb's cycle due to deficiency of pyruvate dehydronase in erythrocyte. Therefore under anaerobic condition pyruvate is converted to lactate. Lactate can arise also from glucose by the hexose monophosphate shunt or the pentose cycle, alternative oxidative pathway for glucose oxidation through glycolysis. Hence, hypoglycemia and lactic acidosis are often associated in higher parasitic index patients. One of the reasons for hyperlactatemia or acidosis is assumed to be the increased anaerobic glycolysis by the infected erythrocytes. [14]

In this study rate of glucose utilization was assessed by determining glucose concentration in whole blood and also in serum at 0, 1 and 2 hours after blood collection. It was observed that Blood glucose concentrations were on lower side in patients of malaria with mean 86.3 ± 18 mg/dl compared to control group (105.5 ± 12.5 mg/dl) at baseline. This observation is consistent with symptom of hypoglycaemia in patients of malaria. [8]

Decreased blood glucose concentrations (table1) at 0, 1 and 2 hours. At end of 1 hour, mean of blood glucose concentrations fall from 105.5 ± 12.5 mg/dl to 97.3 ± 11.6 mg/dl, and at end of 2 hour, it decreased to mean of 89.8 ± 11.1 mg/dl in control group. Glucose is lost due to continuation of glycolysis at a rate of 5%–7% / hr at concentrations near the reference interval. The results of our study are consistent with findings of study of David E. Bruns. [15]

When studied rate of glucose utilization in cases of malaria patients, it was observed that utilization of glucose was approximately doubled after 1 hour. As mentioned in table 1, at end of 1 hour, concentration of blood glucose is decreased from 86.3 ± 18 mg/dl to 74.8 ± 16.6 mg/dl. Blood glucose concentration at end of 2nd hour is 66 ± 15.8 mg/dl. The concentrations of blood glucose in control and study group were compared statistically, showed significance with P value <0.001.

In a malaria patient the percent of parasite infected erythrocytes rarely exceeds 3-4%, and is generally around 0.1–1% (4,000-40,000/µl). Mehta *et al* (2008), stated that solid [2-^{13}C] glucose was added in control medium consisting of malarial parasite infected erythrocytes mainly consist of trophozoites. The quantitative estimates of metabolites and flux was determined by NMR spectroscopy. Result showed

increase in rate of glucose utilization of infected erythrocytes as compared to that of uninfected erythrocytes. [8]

Study of Onyesom Innocent *et al*, (2013) showed significant decrease in the concentrations of blood and brain glucose in malaria infected mice when compared with the control when they studied changes in the concentrations of blood and brain glucose in *Plasmodium berghei* infected mice with the intention of correlating changes in blood with that of the brain and using observation in mice to predict possible human effect. [13] A research work carried out by Olayemi *et al.* (2012) on the "effect of malarial treatment on some biochemical parameter and plasma pH of mice infected with *Plasmodium berghei*", concluded that blood glucose concentration was significantly lower (P<0.05) in the parasitized untreated mice (22.57±0.3) group compared with the control (33.74±0.1) group. [16]

Glucose concentrations (table 2) at 0, 1 and 2 hours in separated serum of control and study groups. It was observed that there was decrease in glucose concentrations at hourly interval. In control group, glucose concentration at 0 hour was 105.5 ± 12.5mg/dl which was further decreased to 99.3 ± 11.5 mg/dl at end of 1 hour and at end of 2nd hour to 93.9 ± 11.2 mg/dl. Decrease in glucose concentrations in this exercise may be because of presence glycolytic enzymes in serum, it need further scientific validations to support assumption.

However in malaria patients significant decrease in glucose concentrations observed was in serum (Table 2). At 0 hour, observed mean was 86.3 ± 18 mg/dl which was further decreased to 77 ± 17.7 mg/dl at end of 1st hour and 69.2 ± 17.3 mg/dl at 2nd hour. Glucose concentrations in both group showed P value <0.001 which was statistical significant.

The calculated percent rate of glucose utilization found that, in control group, rate of blood glucose utilization is approximately 7.8 % per hour. Whereas it was almost doubled in malaria infected patients with rate of 13.2% as mentioned in Table 3. Similarly, rate of glucose utilization in serum showed more all less same pattern as that of plasma glucose. Table3 shows percent utilization of serum glucose, in control group it is 5.9% and in study group it is 10.7 %.

Parasitized erythrocytes utilize glucose as much as 100 times than that of uninfected erythrocytes. This increase reflects the need of parasite to grow, synthesize RNA, DNA and to reproduce themselves. Eugene Roth *et al*. [1988] and Monika Mehta *et al.* [2006] had investigated the reason behind this increase utilization. [6,8]

Eugene Roth *et al* [1988] studied enzymes of glycolytic pathway in normal and parasitized red cell culture. The concentrations of all enzymes except diphosphoglycerate mutase, glucose-6-phosphate dehydrogenase and adenylate kinase were elevated. Extreme elevations of hexokinase, aldolase, enolase, pyruvate kinase and adenosine deaminase concentration were noted.[6] Certa *et al* (1988) have reported that a 41 kD protein associated with membrane of plasmodium falciparum infected red cell in monkey has aldolase activity. Antibodies to 41 kD provide immunity in monkey. It is not clear whether established immunity is because of inhibition of glycolysis, the main path of energy or because of antibodies of specific class. [17]

Monika Mehta *et al* [2006] suggested that there is down regulation of the glucose utilization rate in the majority (>96%) of uninfected erythrocytes in patients of malaria. Using [2-^{13}C] Glucose and nuclear magnetic resonance (NMR), the glucose utilization rate and 2, 3-diphosphoglycerate (2,3-DPG) concentration produced in normal erythrocytes and plasmodium falciparum infected red blood cell populations (IRBCs, with <4% parasite infected red cells), were measured. The proposed mechanism of down regulation of glucose utilization in uninfected erythrocytes by parasitized RBCs is likely to be due to the selective inhibition of uninfected erythrocytes -PFK due to a decrease in pH.[18]

CONCLUSION

It was concluded that there is approximate double utilization of glucose in malaria infected subject over healthy volunteers. Increased consumption of blood glucose is probably due to increase energy demand through glycolysis of RBC and added effect of parasite glucose utilization for their growth and development. Whereas decreased concentrations of serum glucose is combine effect of release of glycolytic enzymes and plasmodium existence after intravascular hemolysis, which is established mechanism in malarial infection.

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