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Research Article

COMPARATIVE STUDY OF SERUM LDH AND CPK IN *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* IN RELATION TO HEALTHY CONTROL GROUP IN WEST BENGAL.

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ABSTRACT

LDH and CPK are both intracellular enzymes. LDH present in large amount in liver and red blood cells, whereas, CPK is present in skeletal muscles in large extent. Our aim was to compare serum LDH and CPK in patients affected by plasmodium vivax and falciparum as compared to control group. In 238 affected patients, blood was collected for estimation of LDH and CPK. After collection of data of p. vivax (n=120), p. falciparum (n=118), and control group (n=100), mean value with standard deviation were calculated and compared to extract 'p' value at 95% confidence interval. In case of falciparum and vivax group, CPK and LDH levels in males were more than females. LDH levels in both affected groups were significantly raised (p=0.00) as compared to control group. Whereas, CPK levels in both affected groups were low as compared to control group, with significant lowering in case of females in falciparum group (p=0.00). CPK and LDH levels were decreased and increased as compared to control group respectively, which may influence the prognosis in malaria. It may open the door of the therapeutic options in malaria in future, so that, we can cure malaria efficiently.

Key-words: Lactate dehydrogenase, Creatinine phosphokinase, *Plasmodium falciparum*, *Plasmodium vivax*, West Bengal

INTRODUCTION

Lactate dehydrogenase (LDH), an intracellular enzyme [1], are the homo and hetero tetramer, composed of M and H protein subunits. M and proteins are encoded LDHA and LDHB genes respectively. LDH has five isoenzymes [2], these are: LDH-1 (present in heart and red blood cells), LDH-2 (in reticulo-endothelial system), LDH-3 (in the lungs), LDH-4 (in kidneys, placenta and pancreas), LDH-5 (in liver and striated muscles). High concentration of LDH is formed in liver, heart, erythrocytes, skeletal muscles and kidney [3]. LDH catalyses the reversible reaction, it includes oxygenation of lactate

to pyruvate in presence of co-enzyme, nicotinamide adenine dinucleotide (NAD) [4]. Intracellular concentration of LDH is five hundred times higher than in the serum [5]. Diseases of the heart (myocardial infarction), red blood cell (hemolysis), kidney (renal infarction), different type cancers (small cell carcinoma of lung, neuroblastoma, nephroblastoma, neuroendocrine tumor, and Hodgkin's disease and non-Hodgkin lymphoma) are associated with elevation of serum level of LDH again [6-9]. Again, LDH also predicts the response to therapy in leukemic and colonic cancers [10]. In febrile cytopenic patients with immunodeficiency, elevation of serum LDH is important clue to the diagnosis of hemophagocytic syndrome [11]. In India, plasmodium falciparum and vivax are responsible for all the malaria cases. It involves three fourth of the cases occurring in South-East Asia [12]. Again, malaria itself is responsible for 1.5 to 2 million of deaths in the world [13]. Life cycle of plasmodium parasite in human being involves hepatic phase; where sporozoites are transformed into schizonts, then to merozoites, which in turn, are released in to circulation; and eryrhrocytic phase, where after invasion, each merozoite produces 24 to 32 merozoites in the process of asexual replication. As RBC ruptures, all the merozoites are at once released into circulation. So, in malaria, liver and RBC are mostly involved and damaged, releasing large amount of LDH in to circulation, and its level will vary according to the amount of damage of liver cells and RBCs.

Creatinine phosphokinase (CPK), another intra-cellular enzyme, is expressed in the tissues, like, skeletal muscles (CPK-MM), brain (CPK-BB) and cardiac muscles (CPK-MB). CPK acts as a catalyst in the reversible conversions of creatine to phosphocreatine by consuming adenosine diphosphate (ATP) and generate adenosine diphosphate. CPK acts as major reservoir for rapid buffering and generation of ATP, and major source of energy in different biochemical reactions in the skeletal muscles and brain [14]. Serum level of CPK will be increased in myocardial infarction, muscle injury, acute renal failure etc. Again, in malaria, muscle fiber damage is very common in malaria. So, our aim in this study was to compare the serum LDH and CPK level between plasmodium falciparum (p. falciparum) and plasmodium vivax (p. vivax) as compared to healthy control group.

MATERIALS AND METHODS:

We started our study only after getting permission from our ethical committee. Total 238 patients were admitted in our hospital in the course of last three years. After getting written consent from the patients' parties, we took proper history along with the required physical examinations in the form of written questionnaire. Their blood was drawn for thick blood film stained by Giemsa stain and rapid antigen tests to diagnose them as malaria cases. After diagnosis, 5 ml of blood was drawn from each patient aseptically between eight to ten o'clock in the morning and was transferred to sterile centrifuge tube to clot. Then each clotted sample was centrifuged in Griffith and George Centrifuge machine (Griffith and George Ltd. England) at 3000 r.p.m. for ten minutes to get sera. Then within twenty four hours of collection of blood, LDH activity was assayed according to the method as described by Stroeve and Makarova [15]. In this method, the serum had to be incubated at ph 8.8 with nicotinamide adenine dinucleotide (3 ug/ml), and DL-lactic acid (0.45 M) along with sodium pyrophosphate buffer. This environment will eliminate the contribution of possible LDH, since it has different pH and substrate for activity. LDH activity was expressed in international unit

(IU). Data were collected and analyzed statistically. Another 5 ml. of blood sample was transferred to centrifuged tube and kept for clotting. Then the blood was centrifuged in the centrifuge machine at 1500 r.p.m. for twenty minutes to separate the serum.. Then the serum was used immediately for estimation of CPK by Kit-method on Micro lab 300, a software controlled system in clinical chemistry (Microlab 300 user manual).

Statistical methods:

Results were expressed in the form of mean with standard deviation (SD) in case of p. vivax, p. falciparum and healthy control group. Then the mean with standard deviation were compared between groups to get the 'p' value at 95% confidence interval. 'p' value of <0.05 was considered statistically significant

RESULTS:

In case of p. falciparum and p. vivax group, CPK and LDH in males were more than females, but in case of p. falciparum, LDH level showed significant difference (563.06±417.9 IU/L vs. 509.74±157 IU/L, p=0.00).[Table 1].

LDH level in both males and females of p. vivax group were significantly raised as compared to control group (684.49±456.05 IU/L vs. 342.48±142.12 IU/L, p=0.00, in case of males and control group, 556.78±189.36 IU/L vs. 278.47±127.43 IU/L, p=0.00, in case of females and control group), as well as, p. falciparum group (563.06±417.9 IU/L vs. 342.48±142.12 IU/L, p=0.00, in case of males and control group, 509.74±157 IU/L vs. 278.47±127.43 IU/L, p=0.00, in case of females and control group) [Table 2].

CPK levels in both males and females of p.vivax and p. falciparum groups were lower as compared to control group, but, females in p. falciparum group demonstrated significant lowering (206.17±114.63 IU/L vs. 239.12±110.11 IU/L, p=0.00) [Table 3].

DISCUSSION:

LDH is found in significant amount in centrilobular region in liver and in RBC. Again, in acute p. falciparum and p. vivax malaria, the pathophysiological process starts in the liver followed by RBC. So, the hepatic activity of sporozoites is associated with centrilobular hepatic damage and eryrhrocytic phase is associated with the destruction of RBCs. Both the phases are associated with release of large amount of LDH in the serum [16]. Again, according to Macgraith et al [16], two factors are responsible for hepatic dysfunction in acute p. falciparum malaria, like, local circulatory failure and centrilobular cellular damage. Our study demonstrated serum level of LDH in both males and females of both p. falciparum and p. vivax group were significantly high as compared to healthy control group, which was similar to study done by Garba IH et al. [14], but the values were little higher than that in our study.

Again, LDH level in p. vivax group was higher than the level in p. falciparum group both in males and females (684.49±456.05 IU/L vs. 563.06±417.9 IU/L in males, 556.78±189.36 IU/L vs. 509.74±157 IU/L in females). The cause may be more RBCs damages and/or centrilobular hepatic damage in the patients affected by p. vivax. This is contradictory to the study done by Pir MA et al [17], where LDH level in p. falciparum was much higher than that in p. vivax (1276.41±147.95 IU/L vs. 508.90±36.34 IU/L).

In the study done by Grover et al [18], serum LDH level was 432 IU/L in hospitalized acquired immunodeficiency syndrome (AIDS) patients with concomitant

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pneumocystis carinii infection, whereas, in viral hepatitis or drug induced hepatitis, serum level of LDH is nearly five times higher than the normal level, as shown by Cassidy et al [19]. The value in our study was nearly midway between the two above observations.

LDH level in males were higher than in females in our study, which is similar to the study done by Garba et al (789.40±35 IU/L vs, 634±35 IU/L) [14]. This may be due to increased muscle bulk in males than females.

CPK level in p. vivax and p. falciparum were low as compared to control group which was similar to the study done by Pir MA et al [17] and Baloch S et al [20]. This may be due to muscle fiber alteration as shown in the study of Carmona et al [21]. Again, the low level of CPK may be due to skeletal muscle problems, like, fatigue, weakness or aches as described by Macro et al [22].

Table 1: Comparison of LDH and CPK between Plasmodium falciparum and Plasmodium vivax group:

Item	Plasmodium falciparum (118)		95% confidence interval	P value	Plasmodium Vivax (120)		95% confidence interval	P value
	Male (68)	Female (50)			Male (90)	Female (30)		
CPK	218.36±247	206.17±114.63	-62.33 – 86.71	0.74	202.69±355	181.5±172.70	- 112.39 – 154.77	0.75
LDH	563.06±417..9	509.74±157	13.88 – 92.75	0.00	684.49±456.05	556.78±189.36	- 42.22 – 294.63	0.14

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Table 2: Comparison of LDH between Plasmodium vivax group and control group.

P. Vivax	**LDH**	**Control**	**LDH**	**95% confidence interval**	**P value**
Male (90)	684.49±456.05	Male (50)	342.48±142.12	308.56 – 375.45	0.00
Female (30)	556.78±189.36	Female (50)	278.47±127.43	207.78 – 348.83	0.00
P. Falciparum					
Male (68)	563.06±417.9	Male (50)	342.48±142.12	- 342.62 -- - 98.53	0.00
Female (50)	509.74±157	Female (50)	278.47±127.43	- 222.93 -- - 111.58	0.00

Table 3: Comparison of LDH between Plasmodium falciparum group and control group.

P. falciparum	**CPK**	**Control**	**CPK**	**95% confidence interval**	**P value**
Male (68)	218.36±247	Male (50)	249.78±117.28	- 105.54 – 42.70	0.40
Female (50)	206.17±114.63	Female (50)	239.12±110.11	14.50 – 94.27	0.00
P. vivax					
Male (90)	202.69±355	Male (50)	249.78±117.28	- 149.11 – 54.94	0.36
Female (30)	181.5±172.70	Female (50)	239.12±110.11	- 120.5 – 5.26	0.07

CONCLUSION:

Present study showed diminished level of CPK and elevated levels of LDH in plasmodium vivax and plasmodium falciparum as compared to control healthy group. This imbalanced enzyme levels may influence the progress of malaria. This study may open the new door in respect of therapeutic advances for the malaria treatment along with the improvement the abnormal enzyme levels.

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