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Enzyme activity of AKP, CPK, LDH and S-GOT in Blood Serum of Malarial Patients

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Abstract: Malaria is one of the most serious tropical diseases in the world, such as in North America, South America, Asia and Africa. It has been a severe public health problem in Pakistan due to poor hygienic conditions; malnutrition borne non-defensive immunity system. Enzyme activity plays vital roles in metabolic pathways are expected to be severely disturbed in malarial patients. In present study blood serum Creatine phosphokinase (CPK), Alkaline phosphatase (AKP), Lactate dehydrogenase (LDH), and Glutamine oxalotransaminase (S-GOT) activity was evaluated in twelve malarial patients and in the same number of normal subjects using kit method on Microlab 300. The enzyme activity of AKP, LDH and S-GOT increases significantly, whereas, the activity of CPK decreases in malarial patients as compared to the control subjects. The blood serum enzyme activity determined for AKP was 160.42 U/L, for LDH 549.08 U/L, for S-GOT 43.830 U/L and for CPK it was 77 U/L and those for the normal subjects the activity was determined to be 123.75U/L, 291.17 U/L, 18U/L and 86.33U/L for AKP, LDH, S-GOT and CPK respectively.

Keywords: - Malaria, Serum, Enzyme activity.

INTRODUCTION

Malaria is a communicable disease caused by *"Plasmodium vivax"*, transmitted into humans by the bite of infected female *Anopheles* mosquitoes. The pathogen, which causes the disease, is a parasite protozoon of the genus *Plasmodium*. (Phillips1983). It is a major health problem in about 90 countries of the world, where, 40 % of the world population estimated recently lives in these areas are affected with malaria (WHO 1997, 1998). Almost 300,000 suspected malarial cases have been recorded in Pakistan (WHO 2011).

Enzymes biocatalysts are involved in all chemical transformation reactions in the body. It may even decrease the amount of free energy needed to activate a specific reaction for the body function. An enzyme may favor the production of only one of the products, where, more than one product is possible in a reaction. Enzyme activity can be affected by molecules produced by the attack of any disease such as malaria, as well as by any dysfunction in metabolic pathways. Enzyme Activity is also affected by temperature, chemical environment (e.g. pH), and the concentration of substrate. (http://en.wikipedia.org/wiki/Enzyme).

Effect of Malaria on the Activity of AKP

In malarial patients significantly higher serum alkaline phosphatase activity determined can be used as a potential biomarker in assessing the integrity of the hepatic drainage system during acute malaria (Garba, *et al.*, 2005).

Effect of Malaria on the Activity of LDH

The combination of acute hepatocellular injury and red cell haemolysis induced by the invading merozoites may account for the increase in serum LDH activity during this infection. Therefore serum LDH activity is a potentially valuable enzymatic marker of acute malarial infection, especially in the absence of other complicating diseases known to be associated with the above abnormal serum LDH activities (Garba, *et al.*, 2005).

Effect of Malaria on the activity of S-GOT/ ASAT

The liver is badly affected in malaria. The enzyme S-GOT is associated with liver parenchymal cells and is also present in red blood cells and cardiac muscles. It is raised in acute liver damage which is true in malaria. Aspartate aminotransferase (ASAT) is an enzyme that catalyzes a reversible reaction, where, an amine group is transferred from dicarboxylic alpha-amino and alpha-keto acids by way of a ping-pong bi-bi reaction (Catalytic Site and Anfinsen 1973).

Effect of Malaria on the activity of CPK

There is no clear role of creatine phospho kinase in malaria but due to the decrease in the ATP content of the body its level is also decreased. As we have shown in our previous study. (Baloch, *et al.,* 2010). The variation in serum enzyme activity of patients with malaria shows the severity of disease.

EXPERIMENTAL
MATERIALS AND METHOD

Twelve venous blood samples (10ml) of both the groups each from malarial patients and normal subjects were collected into sample tubes without the addition of anticoagulant. The blood samples were centrifuged at 1500 rpm for 20 minutes; the serum was separated and immediately used for the determination of the enzyme activity by kit method using software controlled system on MicroLab300. (Microlab 300).

All the chemicals and reagents obtain were of Analytical grade obtained from Merck.

Methods and Reactions for tests with microlab 300
Determination of LDH

Reaction

$$\text{Pyruvate} + \text{NADH} + \text{H} \xrightarrow{\text{LACTATE DEHYDROGENASE}} \text{Lactate} + \text{NAD}$$

The rate of NADH decreases then concentration is determined photometrically and is directly proportional to the LDH activity in the sample material.

Method

Take 400 µL of R1 and transfer in a sample tube containing 100 µL of R 2 followed by 20 µL of serum. The color reaction follows beer-lambered law and was measured on Microlab 300.
R 1 = 400 µL + R 2 = 100 µL + Serum = 20 µL

Determination of ASAT /SGOT
Reaction

$$2-\text{oxoglutarate} + \text{L}-\text{aspartate} \xrightleftharpoons{\text{ASAT}} \text{Glutamate} + \text{Oxalacetate}$$

$$\text{Oxalacetate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{MDH}} \text{Malate} + \text{NAD}^+$$

Method

Take 400 µL of R1 and transfer in a sample tube containing 100 µL of R 2 followed by 50 µL of serum.
R 1 = 400 µL + R 2 = 100 µL + Serum = 50 µL

Determination of AKP

Reaction

$$p-\text{Nitrophenylphosphate} + \text{H}_2\text{O} \xrightleftharpoons{\text{AKP}} \text{Phosphate} = p-\text{Nitrophenol}$$

Method

Take 400 µL of R1 and transfer in a sample tube containing 100 µL of R 2 followed by 10 µL of serum.
R 1 = 400 µL + R 2 = 100 µL + Serum = 10 µL

Determination of CPK

Reaction

$$\text{Phosphocreatine} + \text{ADP} \xrightleftharpoons{\text{CPK}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{D}-\text{Glucose} \xrightleftharpoons{\text{HK}} \text{ADP} + \text{D}-\text{Glucose}-6-\text{phosphate}$$

$$\text{D}-\text{Glucose}-6-\text{phosphate} + \text{NADH}^+ \xrightleftharpoons{\text{G}-6-\text{P DH}} \text{D}-\text{Gluconate}-6-\text{phosphate} + \text{NADP} + \text{H}^+$$

The rate of increase in NADPH is determined photometrically, and is directly proportional to the CK activity in the sample material.

G – 6 – PDH = Glucose – 6 – phosphate Dehydrogenase
ATP = Adenosin triphosphate
ADP = Adenosin diphosphate
CPK = ATP: Creatine – N – Phosphotransferase
HK = ATP: D – Hexose – 6 – phosphotrasferase
NADP = Nicotinamide – adenine dinucleotide phosphate
$NADP^+$ = Nicotinamide – adenine dinucleotide phosphate, reduced form

Method
Take 400 µL of R1 and transfer in a sample tube containing 100 µL of R 2 followed by 20 µL of serum.
R 1 = 400 µL + R 2 = 100 µL + Serum = 20 µL. **(Microlab 300).**

RESULTS AND DISCUSSION

Statistical data of enzyme activity

Table 1. Shows the blood serum levels of CPK, AKP, S-GOT and LDH activity in malarial patients including in the normal subjects.

All values are expressed as Mean ± S.D.

Enzyme Activity	No. of Patients	Normal Subjects (Mean ± S.D)	Malarial Patients (Mean ± S.D)
Age	12	45.92±2.05 U/L	34.67±4.86 * U/L
S-GOT	12	18.0±.1.36U/L	43.830±8.02* U/L
LDH	12	291.17±11.85 U/L	549.08±62.45 *** U/L
CPK	12	86.33±9.76 U/L	77±11.203* U/L
AKP	12	123.75±17.05 U/L	160.42±28.98* U/L

* P < 0.05 *** P < 0.001

From the above data it is observed that the enzyme AKP, LDH and S-GOT activity increases significantly whereas serum levels of CPK decreases in malarial patients as compared to that in the normal subjects

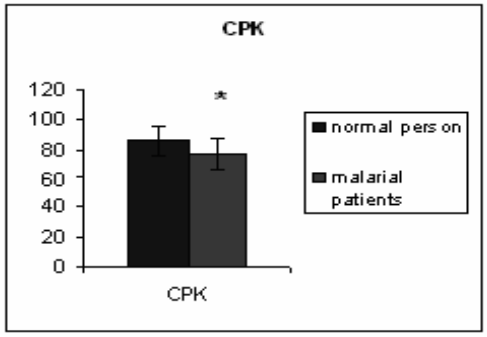

Fig. 1 (a) shows the decreased level of Serum CPK in malarial patients as compared to the normal subjects,

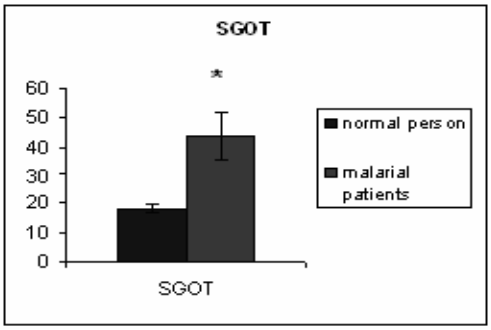

Fig. 1 (b) shows the increased level of Serum S-GOT in malarial patients as compared to the normal subjects.

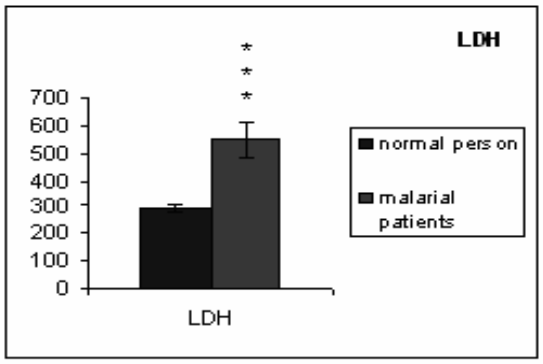

Fig. 2 (a) shows the increased level of serum LDH activity in malarial patients as compared to the normal subjects.

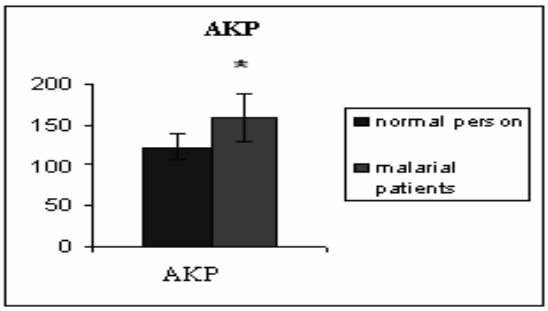

Fig. 2 (b) shows increased level of serum AKP activity in malarial patients as compared to the normal subjects.

In the present work enzyme activity of alkaline phosphatase (ALP), Creatine Phosphokinase (CPK), Serum Glutamic-Oxaloacetic Transaminase (SGOT) and Lactate dehydrogenase (LDH) were determined in the blood serum levels of malarial patients and normal subjects.

It is observed that, enzyme ALP, LDH and GOT activity increases significantly whereas, the activity of CPK decreases in malarial patients as compared to the normal subjects. The blood serum enzyme activity determined for AKP was 160.42 U/L, for LDH was 549.08 U/L, for S-GOT was 43.83 U/L and for CPK was77 U/L and those for the normal subjects determined to be 123.75U/L, 291.17 U/L, 18.0U/L and 86.33U/L respectively.

SGOT is heart related enzyme and an increased in level of S-GOT activity could lead to heart attacks or kidney failure, the enzyme CPK is used for the rapid buffering and regeneration of ATP from ADP and serves as an energy reservoir as well as used for intracellular energy transportation, this enzyme is also related to skeletal muscles, brain, photoreceptors etc, the decreased levels of this enzyme could lead to low energy and immunity in the body, the decreased levels of this enzyme could cause a disaster in human body *(http://en.wikipedia.org/wiki/Creatine_kinase).*

The enzyme AKP is known to be phosphate removing agent, found in liver, bile duct, kidneys, bones and placenta. Its decreased levels could increase the phosphate levels in the cell which could harsh the entire body with toxicity, leading to other complications and fatal for the patient with low immunity *(http://en.wikipedia.org/wiki/Alkaline_phosphatase).* The reason of elevation in LDH in malarial patients could be caused by abnormal hemolysis, which could leads to abnormal growth of tissues and could cause cancer. A normal LDH level is a marker of normal hemolysis**.**

CONCLUSION

In this study, the enzyme activity of AKP, LDH, CPK and S-GOT in malarial patients is compared to the normal subjects, the serum level of CPK decreased whereas, enzyme activity of AKP, LDH and S-GOT shows increased levels in blood serum of malarial patients as compared to the normal subjects from the results of our study, it is concluded that theses enzymes can be utilized as markers in malarial patients.

REFERENCES

Anfinsen C. B. (1973) "Principles that Govern the Folding of Protein Chains". *Science*: 20: 181 (**96**): 223-230.

Baloch, S., G. S. Gachal and S. A. Memon, (2010) Investigation of creatine phosphokinase (CPK) level in blood serum of malarial patients. Sindh Univ. Res. Jour. (Sci. Ser.) Vol.42 (**2**): 71-72.

Garba, I. H. and U. Gregory (2005) “Serum Alkaline Phosphatase Activity as a Potential Biomarker for the Intergrity of the Hepatic Drainage System in Acute Falciparum Malaria Infection”. Journal of Infectious Diseases.Vol. (**4**): N0. 2.

Garba, I. H., and G. A. Ubom (2005) Total serum lactate dehydrogenase activity in acute Plasmodium falciparum malaria infection. Singapore Med Jour. Vol. 46 (**11**): 632-634.

Microlab 300 User Manual. Merck Publishers.

Phillips, R. S. (1983) Studies in Biology no. 152, Malaria Edward Arnold Publisher, Bedford Square, London, United Kingdom.

The Catalytic Site Atlas http://www.ebi.ac.uk/thornton-srv/databases/CSA/ at the European Bioinformatics Institute.

World Health Organization (2010) Health action in crises, Supports malaria epidemic prevention and control in Pakistan.

World Health Organization (1997) World malaria situation in 1994, Weekly. Epidemiological Record, (**72**): 269-276.

World Health Organization (1998) World Health Report: Fifty Facts of Health. WHO, Geneva.