



## LDH – THE BIOCHEMICAL MARKER OF SEVERITY IN CLINICAL MALARIA

## Medical Science

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## ABSTRACT

**BACKGROUND:** Malaria is the most important parasitic disease of mankind and caused by the protozoa parasite plasmodium. Malaria presents a diagnostic challenge to laboratories in most countries. Liver involvement in severe p.falciparum infection is commonly a significant cause of morbidity and mortality among humans LDH activity as an index in the monitoring of p.falciparum malaria infection, particularly when all other possible causes of increased serum LDH levels have been eliminated.

**OBJECTIVES:** To study the LDH enzyme assay for the detection of p.falciparum severity in patients and to investigate the varying effects that different levels of parasite density have on blood cell count.

**MATERIAL AND METHODS:** The present study was carried out on 104 cases of falciparum malaria in the DTR Hospital Kurnool. A.P. Microscopic examination was used to count parasites. LDH activity was determined in patients with falciparum malaria. All these patients were subjected to blood investigation like Hemoglobin level RBCs count, WBC count and platelets count to know the severity of parasitaemia in children and adults patients.

**RESULTS:** A total 104 patients were enrolled. Out of the total number of cases 46 were children and 58 were adults. Irrespective of age LDH activity was significantly higher in the high level of parasitaemia patients. RBC count was significantly lower in 71(68%) of total patients with p.falciparum. leukocytes, neutrophil was higher in 65(63%) patients where as eosinophil and monocyte count was lower in 59(57%) and 62 (60%) patients respectively. Platelet count was significantly lower in 72(69%) of total patients with p.falciparum infection.

**Conclusion:** Serum LDH activity is potentially valuable enzymatic marker of acute p.falciparum malaria infection. The study finds correlation between levels of parasitemia and the activity of parasite LDH and also focuses on laboratory value in p.falciparum infected malaria with haematological parameters.

## KEYWORDS

Malaria, Parasitaemia, LDH, Thrombocytopenia

## 1. INTRODUCTION

Malaria is one of the most important parasitic diseases of mankind and caused by the protozoan parasite plasmodium. Only four of the known species of plasmodium are able to infect humans. P.falciparum, P.vivax, P.ovale and P.malariae. The WHO estimates 300 to 500 million malaria cases annually. In India about 2 million confirmed malaria cases and 1,000 deaths and reported annually. India's geographical position and climatic conditions are favourable of the transmission of malaria. In this Kurnool area of Andhra Pradesh where the disease is endemic. Malaria presents a diagnostic challenge to laboratories in most of the areas. Liver involvement in severe p.falciparum infection is commonly a significant cause of morbidity and mortality among humans. The most important antigenic markers targeted for detection by RDT's are Histidine Rich proteins -2(HRP-2), parasitic lactate dehydrogenase (pLDH) and plasmodium aldolase (1,2).

Plasmodium lactate dehydrogenase (pLDH) is a soluble glycolytic enzyme produced by the asexual and sexual stages of the liver parasites and it is present in an released from the parasite infected erythrocytes. LDH is an intracellular enzyme, involved in the reaction of oxidation of lactate to pyruvate with nicotinamide adenine dinucleotide (NAD) serving as coenzyme. LDH is an enzyme, which is classified as a true intracellular enzyme because of its high degree of tissue specificity where over all tissue concentrations are some 500 fold greater than serum levels under normal circumstances. LDH have five theoretically possible forms, which are found in human tissues eq: liver, heart, erythrocytes, skeletal muscles and kidneys, so disease affecting these organs such as renal infarction, myocardial infarction and hemolysis have been identified as diagnostic indices for kidney, liver, heart and red blood cell dysfunction. High serum LDH activity has also been reported in small cell carcinoma of the lung, neuroblastoma, neuroblastoma, neuroendocrine tumor measles and cervical lymphadenitis (2,3)

Pathophysiological processes usually associated with severe p.falciparum malaria infections i.e. the hepatic activity of the invading sporozoites leading to centrilobular liver damage and the destruction of the host red blood cells consequent to erythrocytic merogony. Being rich sources of LDH, the liver injury and red blood cells destruction will be followed by the release of LDH into the circulation. This finding has important implications because it highlights the potential of using serum LDH activity as an index in the monitoring of p.falciparum malaria infection, particularly when all

other possible causes of increased serum LDH levels have been eliminated pLDH activity in patients follows the level of parasitaemia. pLDH may be a good marker for active malarial infections (9,10,11). Hematological changes in the course of malaria infection such as anemia, thrombocytopenia and leucocytopenia are well recognized. These parameters vary with the level of malarial endemicity. Patients with the highest parasite densities also have the highest fatality rates. Excessive hemolysis of parasitized RBCs in malaria infection may lead to LDH level increased and causes anemia. Increasing levels of p.falciparum parasite loads results in a decreased count (5,6,7).

## MATERIALS AND METHODS:

The present study was conducted in the DTR Hospital, Kurnool from June 2016 to Dec 2016. In these seasons, when malaria endemicity is at its highest peak in this area. The present study included 104 patients diagnosed as malaria by peripheral smear study admitted to DTR Hospital Kurnool A.P. of the 104 cases 46 were children and 58 were adults. P.falciparum and P.vivax status were diagnosed by rapid diagnostic test. All the subjects were examined clinically in detail and bio chemical evaluation done. The various symptoms exhibited in p.falciparum affected patients are fever, headache rigors chills, sweating abdominal pain, vomiting, pallor splenomegaly and convulsions.

Both thick and thin smears were prepared and stained with Giemsa and examined for malaria parasites by lab technician. Metabolism is related to the activities of different enzymes. In order to investigate the metabolic abnormalities in patients with falciparum malaria LDH enzyme activity in the blood serum of children and adult patients were measured by using automated analyzer. For hematological analysis, like Hb, RBC, WBC count, platelet count using automated hematological analyzer in children and adult cases.

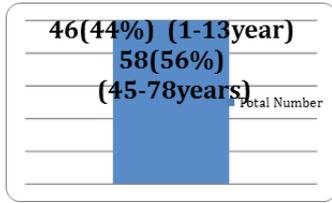
## DATA ANALYSIS:

Data was collected using notebooks and transferred to a computer and kept confidential they were entered into Microsoft word and analysed using statistical package for social sciences SPSS version 17 and Graphed Prism.

**Table :1 Age wise distribution**

Children Number	Adults Number	Total Number
46(44%) (1-13year)	58(56%) (45-78 years)	104

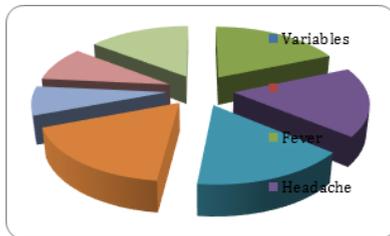
**Fig:1 Age wise distribution**



**Table :2 Clinical Profile of children and Adults**

Variables	Children N=46		Adults N=58		Total N=104%	
	N	%	N	%		
Fever	46	100%	58	100%	104	100%
Headache	43	93%	51	88%	94	90%
Chills	39	85%	46	79%	85	82%
Nausea & vomiting	43	93%	36	62%	79	76%
Convulsions	18	39%	9	15%	27	26%
Pallor	22	48%	35	60%	57	55%
Splenomegaly	36	78%	42	72%	78	75%

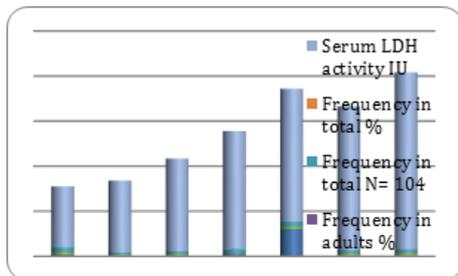
**Fig: Special wise distribution**



**Table : 3 Parallel findings of LDH concentration verses parasitaemia density in children and adults in patients by microscopy.**

Parasitaemia (Micro liter)	Frequency in children		Frequency in adults		Frequency in total		Serum LDH activity IU
	N=46	%	N=58	%	N= 104	%	
>100	7	15%	12	21%	19	18%	272
>500	2	4%	6	10%	8	8%	320
>1000	5	11%	6	10%	11	11%	412
>5000	13	28%	03	5%	16	15%	523
>10000	12	26%	10	17%	22	21%	590
>20000	04	9%	09	16%	13	13%	639
>25000	03	7%	12	21%	15	14%	786

**Fig : 4 : Clinical Profile of Children and Adults**

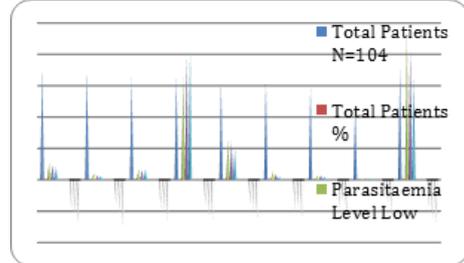


**Table : 4 Haematological Parameters at different Parasitaemia level**

Parameters	Total Patients		Parasitaemia Level		
	N=104	%	Low	Moderate	High
Low Hg(gm/dl)	71	68%	10.9 (19)	9.6 (23)	8.2 (29)
Low RBC (x106/ $\mu$ l)	69	66%	3.9 (16)	3.2 (22)	2.8 (31)
High Leuko cytes (x103/ $\mu$ l)	65	63%	6.82 (18)	6.47 (20)	7.21 (27)
High Neutrophils (x103/ $\mu$ l)	66	63%	67.2 (17)	78.3 (24)	82.1 (25)

Low Lymphocytes (x103/ $\mu$ l)	62	60%	25.6 (16)	24.8 (19)	19.2 (26)
Low Monocytes (x103/ $\mu$ l)	62	60%	5.52 (19)	3.6 (18)	2.0 (25)
Low Eoisionphil (x103/ $\mu$ l)	59	57%	2.83 (18)	2.61 (15)	2.10 (24)
High Basophil (x103/ $\mu$ l)	46	44%	0.81 (11)	0.86 (14)	0.98 (21)
Platelets (x103/ $\mu$ l)	72	69%	96.5 (18)	82.9 (25)	65.4 (29)

**Fig 4 : Haematological Parameters at different parasitaemia level**



**RESULTS**

A total of 104 cases were enrolled. Out of the total number of cases 46 (44%) were children and 58(56%) were adults. The age group of children to adults is 1:1.3. the age group of children to adults is 1:1.3. the age group of children 1-13 years and adults were in the age group of 45-78 years (Table-1)

The present study reveal that irrespective of age LDH activity was significantly higher in the high level parasitaemia patients both in children and adults. (Table:3). The parasitaemia level and changes in blood cell parameters were directly proportional. (Table:4)

In all the patients (100%) fever was the predominant complaint, other main symptoms were Headache, chills, nausea and vomiting in p.falciparum infections. Headachhe was seen in 43 (93%) cases of children and 51(88%) of adults. Chills were seen in 39 cases (85%) of children and 46(79%) cases of adults, nausea and vomiting were seen in 43(93%) and 36(62%) of children and adults respectively. Convulsions were seen in 18(39%) of children and 9(15%) of adults. Pallor was observed in 55% of total patients. Splenomegaly was also seen in 75% of total patients. (Table :2)

This study describes an enzyme LDH assay for the detection of plasmodium falciparum. We found a correlation between levels of parasitemia and the activity of LDH. pLDH activity could be measured in blood serum from patients with cerebral malaria. Results indicated that 66(63%) were from patients with moderate to high parasitaemia (10,000-25,000/ $\mu$ l) where Pf LDH in the serum was high [523 iu-786Iu) had been observed. While 38(37%) were from patients with low parasitemia (100-500/ $\mu$ l) LDH activity was also lower [272Iu) (Table-3). The findings were tabulated in both low and high profiles. The observations were consistent at both ends. That is low parasitaemia was having low LDH while high parasitaemia was having high LDH. This proved that the number of parasites ( $\mu$ l) in the smear is the direct index for organ damage in term of LDH. The results indicated that there is a direct correlation between levels of Pf LDH in serum and blood parasitaemia (Table:3).

Haemetological changes were also observed in this study. Hemoglobin of less than 8.2gm/dl was seen in 29(41%) cases of total with high level parasitaemia in moderate parasitaemia infected patients 23(32%) Hemoglobin was 9.6gms/dl. Where as 10.9gms/dl Hb was seen in low level parasitaemia patients 19(18%) were shown in Table:4 RBC count was significantly lower in 71(68%) patients with p.falciparum infections. Leukocyte count, neutrophil count was higher in 65(63%) patients with malaria where as eosinophil count was lower in 59(57%) patient with infected falciparum parasites monocytes count was lower in 62(6%) where as Basophils count was higher in 46(%) patients with high parasitaemia. Platelet count was significantly lower in 72(69%) of total patients with falciparum infection Table-4.

**RBC, Leukocyte and platelet counts in different parasitaemia level:**

Red Blood Cell count and Hb were significantly reduced in patients

with high parasitaemia (2.8r106/ $\mu$ l) compared to those with low [3.9x106/ $\mu$ l] and moderate parasitaemia groups [3.2x106/ $\mu$ l] (Table-4). Both lymphocytes and monocyte count were significantly lower in both the children and adult patients with high parasitaemia compared to those with low and moderate parasitaemia. Platelet count was notably reduced in patients with high parasitaemia [65.4x103/ $\mu$ l] in 29 cases of children and adults. It could be predicted that the association between malarial parasitaemia and hematological parameters is not influenced by age. Thus, based on the statistical data, age is not a factor that causes any significant differences between falciparum malarial parasitaemia and hematological variables. (Table:4).

Hematological changes in the course of malaria infection, such as anemia, thrombocytopenia and leucopenia were recognized both in children and adults. These alterations vary with the level of malarial endemicity. Hemoglobin level, nutritional status and also malaria immunity.

#### DISCUSSION :

Serum LDH activity is a potentially variable enzymatic marker of acute p.falciparum malaria infection. LDH level was significantly higher in patient infected with Ip.falciparum infection. Among the patients irrespective of the age children and adults were found to have a parasite significantly higher serum LDH. During this infection hepatocellular injury and red cell hemolysis induced by the invading merozoites leading to centrilobular hepatocytes and the destruction or erythrocytes consequent will be followed by the release of LDH in to the circulation. This finding has an important implications because it highlights the potential of using serum LDH activity as an index in the monitoring of acute p.falciparum malaria infection.

This study reported a significant reduction of Hb level below 8.2gm/dl in the high level parasitaemia patients both in children and adults reduced Hb in malaria. It may be attributed to the increase of break down red blood cells by the parasites. It was observed that anemia is more common amongst patients infected by p.falciparum in this endemic area. RBC count in patients with severe parasitaemia of falciparum was lower. Infected RBCs display a reduced deformability and altered surface characteristics which usually would lead to them being filtered and cleared by the spleen, parasites had found a way to counter this protective measure. They modify their host cell membrane, which ultimately results to the cytoadherence of RBCs on to the endothelium. Due to sequestration, rosetting and clog up the capillary and post capillary venules of various organs. This destruction leads too decrease in erythrocyte production all add to malaria related anemia.

This study showed that p.falciparum malaria infections can lead to significant changes of WBC cells. Higher neutrophil and lower eosinophil count were founded. Lymphocytes and monocytes count were significantly lower in children as well as in adults, it may indicate that immunopathogenesis is more important in the disease due to p.falciparum malaria Lymphopenia in infection has been attributed to redistribution, margination and apoptosis of lymphocytes.

In this study low platelet count is a finding of malarial infection and thrombocytopenia is common in children and adults. Thrombocytopenia seems to occur through peripheral destruction, immun-mediated destruction of circulating platelets in malaria infection. Low platelets count were associated with increased parasite density. Consequently increase in platelet sensitivity to adenosine diphosphate (ADA) and higher density granular secretion. These alternations could promote platelet aggregation on the endothelium in cerebral malaria. It has been noted that increasing levels of p.falciparum parasite loads results in a decreased platelet count.

**CONCLUSION :-** The present study indicates that LDH can be a useful tool for the detection of malaria in this endemic area. In the estimation of clinical severity of diseases like typhoid enteritis, Hepatitis -B, Dengue fever antigen and antibodies are utilized in terms of dilution. This is done by automated kits which are readily available in market. But in malaria antigen and anti bodies are not being done as it is a protozoan parasitic infection. Hence the load of the disease is calculated by counting parasites in the peripheral smears. This needs a dedicated lab technician with moderate to high technical skills. In this vogue LDH being the biochemical parallel to parasitaemia gives a hope of easy and definite understanding of malaria in the patients. Thus it can be a good prognostic marker for malaria.

Haematological complications are prominent manifestations of severe malaria, commonly representing indices of prognostic value. Measuring the parameters of RBC, WBC and platelets count is important for follow up the patient, hence these parameters help in both individual and endemic study of malaria patient. They offer a new perfection in estimation of the severity of the disease and help to plan therapeutic and preventive measures for pf malaria infection.

#### Acknowledgement :

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