Total serum lactate dehydrogenase activity in acute *Plasmodium falciparum* malaria infection

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ABSTRACT

Introduction: Lactate dehydrogenase (LDH) activity was assayed in the sera of 76 adult male and 76 adult female patients within the age group of 18 - 40 years presenting with acute, uncomplicated *Plasmodium falciparum* malaria infection and a control group of 80 healthy adults within the same age group.

Methods: Patient selection and pre-qualification were done by simple random sampling of individuals presenting at the Bauchi Specialist Hospital Outpatient Department with a history of fever and malaise within a period of one to eight days, and who were confirmed to be infected with the *P. falciparum* malaria parasite by microscopical examination of Giemsa-stained thin blood slides.

Results: The mean serum LDH activity in male patients was found to be 789.4 ± 35.0 IU. This activity is significantly higher than the control LDH activity of 247.10 ± 19.0 IU (p-value is less than 0.05). The mean serum LDH activity among female patients was 634.0 ± 35.0 IU, which is a relatively higher activity compared to the control LDH activity of 247.10 ± 19.0 IU (p-value is less than 0.05).

Conclusion: 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, uncomplicated *P. falciparum* malaria infection, especially in the absence of other complicating diseases known to be associated with the above normal serum LDH activities.

Keywords: lactate dehydrogenase, malaria, *Plasmodium falciparum*, serum enzymatic marker

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INTRODUCTION

Lactate dehydrogenase (LDH) is an intracellular enzyme, which catalyses the readily reversible reaction involving the oxidation of lactate to pyruvate with nicotinamide adenine dinucleotide (NAD) serving as coenzyme[1]. Five theoretically possible forms of this enzyme are found in human tissues, their concentrations differing in various organs[2]. LDH is an example of the enzyme, which is classified as a true intracellular enzyme[3] because of its high degree of tissue specificity where overall tissue concentrations are some 500-fold greater than serum levels under normal circumstances[4]. Generally, high concentrations of LDH are found in the liver, heart, erythrocytes, skeletal muscles and kidneys[5].

Consequently, diseases affecting those organs, such as renal infarction, myocardial infarction and haemolysis, have been reported to be associated with significant elevations in total serum LDH activity. Such elevations have been widely applied as diagnostic indices for kidney, liver, heart and red blood cell dysfunction[6-8]. Additionally, high serum LDH activity has also been reported in a variety of cancers, e.g. small cell carcinoma of the lung, nephroblastoma, neuroblastoma and metabolic neuroendocrine tumour[9]. Serum LDH is also increased in patients with measles and cervical lymphadenitis[10]. Furthermore, in monitoring the progress of diseases, LDH has been found to be relevant in establishing the survival duration and rate in Hodgkin's disease and non-Hodgkin's lymphoma, and in the follow-up of ovarian dysgerminoma[11]. LDH plays an important role in predicting response to therapy and prospects of remission in leukaemia and colon cancer[12], and as an important clue to the diagnosis of reactive haemophagocytic syndrome (RHPS) in febrile cytopaenic patients with immunodeficiency[13].

Plasmodium falciparum malaria infection is a febrile illness accounting for 300-500 million clinical cases annually[14], with 90% of such cases occurring in Africa. It is the commonest type of malaria in Africa[15], where its hyperendemicity has been estimated to cost about 1.8 billion US dollars in direct costs of prevention and care, and in indirect costs such as lost productivity, time costs and other indirect costs and losses[16]. The life cycle of this parasite in the human host includes the developmental cycle in

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red blood cells, and the cycle taking place in the liver cell parenchyma, includes a series of transformations in the host hepatocytes[17]. Since the pathogenesis of this disease involves both the liver and red blood cells[18,19], this study aimed at assaying the serum level of LDH with the objective of assessing the effect of acute *P. falciparum* malaria infection on the serum activity of this key intracellular enzyme, considering its high concentration in both the liver and red blood cells.

METHODS

The southern and northern limits of Bauchi State where the study was conducted are demarcated by latitudes 9º30' North and 10º30' North, respectively. Its western and eastern limits are bounded by longitudes 8º45' East and 11º0' East, respectively. Two-thirds of the land area is in the south of latitude 11º15'. Mean daily temperature in August, the month in which the study was conducted, is 29.2ºC and a humidity of 68%. August is the month of year where the incidence of *P. falciparum* malaria endemicity is at its highest peak, because the highest average rainfall occurs in that month.

Patient selection and pre-qualification were done by simple random sampling of individuals presenting at the Bauchi Specialist Hospital Outpatient Department with a history of fever and malaise within a period of one to eight days, and who were confirmed to be infected with the *P. falciparum* malaria parasite by microscopical examination of Giemsa-stained thin blood slides. All the patients were found to present moderate parasitaemia, with parasite density in the range of 1,000-10,000 asexual forms/ml of blood. Based on the following selection criteria, only 152 patients were found to be qualified for participation in the study. Among the qualified patients, there were 76 males and 76 females. Both groups of patients fell within the 18-40 year age group.

Patients, whose case history showed a concomitant presentation with the following conditions: acquired immune deficiency syndrome (AIDS), anaemia, liver cirrhosis, hepatitis, alcoholism and kidney disorders, were excluded from this study. Similarly, patients on self-medication with any anti-malarial drug prior to presentation were also excluded from the study. This is because these disease conditions are known to present with significant elevation in serum LDH activity. For comparative purposes, a control group of 80 healthy adults (age group 18-40 years) were also enrolled in the study.

Venous blood (5ml) was obtained from each of the patients by venepuncture of the antecubital vein using a sterile needle and syringe between eight and ten o'clock in the morning. The blood samples were then transferred into clean, sterile centrifuge tubes and allowed to clot. Each clotted sample was centrifuged (Griffith and George Centrifuge, Griffith and George Ltd, England) at 3,000g for ten minutes to obtain the sera. Enzyme assay was carried out within 24 hours of collection. Serum LDH activity was assayed according to the method described in Stroeve and Makarova[20]. This involved incubating the serum sample with nicotinamide adenine dinuceotide (3 µg/ml) and DL-lactic acid (0.45M) with sodium pyrophosphate buffer, pH 8.8. This assay condition eliminates the contribution of parasite LDH, which has a different pH and substrate optima for activity. All the reagents used in the work were of AnalaR grade. LDH activity is reported in International Units (IU).

Data was analysed using the Minitab-10 statistical software (Minitab Inc, Quality Plaza, Pennsylvania, USA). Results are expressed as mean ± standard error of the mean. The difference between the mean serum LDH activity in healthy and infected male and female *P. falciparum* malaria patients was analysed using the one-way analysis of variance test. The Duncan's multiple range test was used to test for significant difference between means of the three groups. P-values of less than 0.05 were considered significant.

This work was conducted in accordance with the following ethical declarations: World Medical Association's Declaration of Helsinki[21], American Psychological Association's (APA) Ethical Principles in the Conduct of Research with Human Participants[22], World Medical Association's Declaration on the Rights of the Patient[23], and Council for International Organisations of Medical Sciences/World Health Organisation (CIOMS/WHO): International Ethical Guidelines for Biomedical Research Involving Human Subjects[24].

RESULTS

Table I shows the serum LDH activity in both categories of patients and the control group. The mean serum LDH activity in male patients was found to be 789.40 ± 35.0 IU. This is over three times above the control LDH activity of 247.10 ± 19.0 IU. Similarly, in female patients, the serum LDH activity of 634.00 ± 35.0 IU is over twice the control serum LDH activity of 247.10 ± 19.0 IU. Among the patients, the males were found to have a significantly higher serum LDH activity relative to their female counterparts, ($p<0.05$).

DISCUSSION

Maegraith[25] postulated that the factors involved in hepatic dysfunction in acute *P. falciparum* malaria infection involve a synergy between local circulatory failure and centrilobular cellular damage. Since LDH is found in clinically-significant amounts in

Table I. Serum LDH activity in adult male and female *P. falciparum* malaria patients and controls.

Subjects	Serum LDH activity (IU)
Male patients	789.40 ± 35.0[a,b]
Female patients	634.00 ± 35.0[a,b]
Controls	247.10 ± 19.0[a]

Values with the same superscript differ at p<0.05.

[a]: One-way ANOVA; [b]:Duncan's multiple range test.

both the liver and red blood cells, the observed increase in serum LDH activity during acute *P. falciparum* malaria infection in this study can be accounted for by a synergy between the two pathophysiological processes usually associated with acute *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[25]. Being rich sources of LDH, the acute liver injury and red blood cell 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 acute *P. falciparum* malaria infection, particularly when all other possible causes of increased serum LDH levels have been eliminated.

Grover et al[26] reported a serum LDH level of 432 IU in hospitalised acquired immune deficiency syndrome (AIDS) patients with *Pneumocystis carinii* pneumonia. Similarly, Cassidy and Reynolds[27] showed that patients with acute viral hepatitis A and B, ischaemic hepatitis and acetaminophen-induced injury are all associated with increases of up to five times the upper limit of normal LDH activity. These variations in the relative magnitudes of serum LDH activities place the *P. falciparum*-induced increase in serum LDH in between the values reported in the studies by Grover et al[26] and Cassidy and Reynolds[27]. This is a reflection of the differences in the aetiology and pathogenesis of these varied conditions.

Ischaemic hepatitis, viral hepatitis and acetaminophen-induced injury are much more severe manifestations of a progressive and irreversible liver damage, sometimes involving other organs like the kidney and brain as seen in viral hepatitis[28], while the picture in AIDS is a consequence of the onset of a multisystem disease whose progression has been slowed down by anti-retroviral drug therapy[26]. In addition, the magnitude of changes in serum LDH activity during acute *P. falciparum* malaria infection and other diseases/conditions like *Pneumocystis carinii* pneumonia, hepatitis and drug-induced liver injury can also potentially be used in distinguishing the aetiology and pathogenic outcomes of these conditions.

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