PITFALLS IN THE INTERPRETATION OF LIVER PARENCHYMAL AND MEMBRANOUS ENZYME RESULTS IN PRE-CLINICAL PLASMODIUM FALCIPARUM MALARIA IN THE NIGERIAN ENVIRONMENT

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SUMMARY

Objective: Both organ congestion and cellular destruction of the liver occur in malaria. While cellular affection leads to loss of parenchymal enzymes such as aspartate and alanine transaminases (AST and ALT), bile stasis causes loss of membranous enzymes such as alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) to the extracellular fluid (ECF). However, in malaise, early elimination of viral cause often casts aspersion on laboratories that report increased plasma enzyme activity of liver enzymes. Is the laboratory really always to blame? This study investigated the role of the ubiquitous malaria parasite in the "errors" of the clinical chemistry laboratory in our environment.

Subjects/Materials/Methods: Fifty-eight adult Nigerians, 24 males and 34 females, with positive Plasmodium falciparum results were enrolled in this self-controlled study. The inclusion criteria was malaria, fever, rigors, jaundice and positive HBsAg were exclusion criteria. Pre-treatment, about 5 ml of venous blood was drawn into heparinised bottles. The subjects were then treated with chloroquine, and repeat samples taken 2-3 weeks after both malaise and malaria parasitaemia (MP) had disappeared. The chemical assays on clear, non-haemolysed plasma were done on the Synchron CX5 (Beckman, USA) by previously established methods for transaminases, membranous enzymes and bilirubin (total, TBIL, and direct, DBIL). Statistical analysis for means and standard error of the mean (sem) was by Epi Info version 5.

Results: The liver parenchymal enzymes, AST (40.5±1.6 vs. 28.8±1.3 iu/l; p<0.001) and ALT (52.7±1.8 vs. 38.5±1.6 mmol/L; p<0.001) were significantly raised; with overall respective increases of 40.6% and 36.9%. The observed values for AST among pre-treatment subjects ranged from 18 to 100 iu/L, while similar values for ALT were 21 to 150 iu/L; meaning parenchymal enzyme activities were raised to about 3xULN (upper limit of normal) in some cases. The changes in the activities of membranous enzymes, ALP (86.6±2.1 vs. 75.8±1.6 iu/L; p<0.05) and GGT (56.4±2.2 vs. 52.5±2.0 iu/L; p>0.05) were minimal, at about 15% of pre-treatment levels. Both TBIL (25.8±1.1 vs. 14.7±0.8 mmol/L; p<0.001) and DBIL (20.4±1.0 vs. 12.0±0.7 mmol/L; p<0.001) had significant increases at about 70% of pre-treatment levels.

Conclusion: The study found increase in plasma activity of liver enzymes, in P. falciparum-induced malaise, which dropped with treatment and clearance of MP, with a conjugating liver. With negative HBsAg and positive P. falciparum parasitaemia, malaria would largely account for these findings. These findings recommend that MP test be included in the panel of investigation of moderately raised plasma activities of transaminases.

KEYWORDS: Malaria, Liver enzymes, Nigerians

INTRODUCTION

The quality of laboratory results is known to be influenced by numerous factors. The environment, however, introduces its own interference and, thus, difficulties to result interpretation on a scale yet to be ascertained and recognized by many laboratory users in this locality. Unfortunately, very few reports in Nigeria have paid attention to the influence of infections and parasitism on clinical chemistry laboratory results in this environment holoendemic for malaria.

Malaria parasites are known to predominantly interfere with three organs in the body, namely the brain, the kidney and the liver. In the liver, the summary of invasion by these parasites includes organ congestion, sinusoidal blockage, cellular inflammation and occasional centrilobular necrosis. That is, features of both congestion and necrosis are present. In addition, hypoxia occurs, arising from both anaemia and interference with microcirculation within the organ. Indeed, the liver is a breeding ground for the maturation of the parasites.

It is known that the plasma activities of aspartate transaminase, AST, and alanine transaminase, ALT (both parenchymal enzymes), rise when there is affectation of hepatocytes. Hypoxia compromises active transport and the cell contents ooze into the
ECF through widened pores. Congestive disease, especially of the outflow channels, is trigger for elevation of plasma activities of alkaline phosphatase, ALP, and gamma-glutamyl transferase, GGT, both of them membranous enzymes. Bilirubin is affected by both hepatocytic and channellary events. The malaria parasite invasion of the organ is, thus, a harbinger of a plethora of changes to constituents of the ECF.

The need to have baseline information on this subject as well as the imperative of LFT results interpretation in a malaria-ridden locality has informed the study, one in a series on the biochemical changes accompanying malaria. It is hoped that the outcome would contribute to the management of the disease.

SUBJECTS/MATERIALS/METHODS

Fifty-eight adult Nigerians, 24 males and 34 females, were enrolled in this study. The average age was 28.4±7.8 years. All patients were enrolled from hospitals within Lagos metropolis. Positive blood film for malaria parasites (P.falciparum) was sine qua non for all patients. The study was self-controlled, with seven patients (3 males and 4 females) defaulting. To be enrolled, a subject must have malaise without fever, rigors, jaundice and a negative HBsAg. Test for human immunodeficiency virus was deemed unnecessary and was not done.

About 4 ml of venous blood was drawn at the ante-cubital fossa into heparinised bottles. The subjects were treated with i.m. or oral chloroquine, and repeat samples taken 2.8 ± 0.5 weeks after malaise and MP (malaria parasites) had disappeared. Plasma was obtained after spinning blood at 3000 rpm for 10 min and stored in plain plastic bottles overnight at 4 °C, if chemical analysis was not done the same day. Parenchymal2 and membranous enzymes3 as well as bilirubin4 were assayed by previously established methods on the Synchron CXS (Beckman, USA).

Statistical analysis for means and standard error of the mean (sem) was by Epi info version 5. And test of difference between means was by the student's t-test, with minimum level of significance at p<0.05. All averages reported here are mean±sem.

RESULTS

The liver parenchymal enzymes, AST and ALT, were significantly raised(p<0.001); with overall respective increases of 40.6% and 36.9%. The observed values for AST among pre-
treatment subjects ranged from 18 to 100 iu/L, while similar values for ALT were 21 to 150 iu/L; meaning parenchymal enzyme activities were raised to about 3XULN in some cases(9).

The liver membranous enzymes, that is, ALP (p<0.05) and GGT(p<0.05) were also elevated in pre-clinical malaria. However, the increase in activities was minimal, about 15% of pre-treatment levels. Both TBIL and DBIL were significantly increased(p<0.001), with increases of about 70%

DISCUSSION

The study found that in preclinical malaria there is increase in the plasma activities of liver enzymes. The membrane species were, however, only marginally raised. The study also found evidence of a liver actively conjugating bilirubin. These observations are in keeping with previous reports which had recognized the malaria parasite as a harbinger of derangements in biochemistry of sufferers2,10.

Researchers have documented that malaria parasites damage the liver; necrosis and hypoxia cause increased loss of transaminases to the ECF, just as congestion and biliary stasis lead to loss of membranous enzymes. This factor would partly explain the increased plasma enzyme activity found in this study.

The laboratory is depended upon to turn out results which must be true reflection of the internal condition of the patient. Plasma transaminases activities of about x3 ULN were obtained, without jaundice. With negative HBsAg, interpretation of such results in malaise would not only task the physician but also question the quality control programme in such a laboratory. This study has found that pre-clinical malaria could complicate enzyme diagnosis of hepatic conditions.

Wilairatana et al. studied Thais with unconjugated jaundice due to falciparum malaria and reported significant increase in the activities of transaminases1. They believed, without isoenzyme studies, that haemolysis was predominantly responsible for this finding. Jaundice usually appears at plasma bilirubin levels of 34 mmol/L and above, but the mean value found for total bilirubin in the present study was 28.8 mmol/L. Thus, while the findings in this Nigerian study largely agreed with the Thai findings of increased transaminases, a marginal conjugated hyperbilirubinaemia of 28.8 mmol/L would hardly support haemolysis as the predominant cause of increased enzyme activity in our study. Indeed, frank hepatitis has been reported in the malaria condition10, supporting the view that liver parenchymal injury may largely be responsible for these enzyme changes in our study.

In summary, the study has shown that the plasma activity of liver enzymes increased in pre-clinical P.falciparum parasitaemia occasioning malaise. These findings convince us that MP study is needful where plasma hyperactivity of liver enzymes is found associated with negative HBsAg results. Thus, the laboratory must be seen sometime, to reflect the environment in which it is situated.

ACKNOWLEDGEMENTS

The authors would like to thank the medical and nursing staff of the Staff Medical Services Department of the Lagos University Teaching Hospital, Ii-Ira-Araba, for their assistance in this study. Also, we thank the staff of Salvation Medical Centre of
the Christian Medical Helps Programme, among other private health institutions in Lagos metropolis, for assisting with the study. We would like also to thank the technologists in the departments of Medical Microbiology and Parasitology, Haematology and Blood Transfusion and those in Clinical Pathology for assisting with smear studies, chemical analyses and other technical aspects of the study. For making the study possible, we thank the patients.

REFERENCES


