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Serum Cytochrome C and plasma lipids levels as surrogate markers of hepato-cellular toxicity in Sudanese visceral leishmaniasis

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ABSTRACT

Visceral leishmaniasis (VL) is an important cause of morbidity and mortality that is characterized by fever, lymphadenopathy and hepato-splenomegaly. Hepatic toxicity greatly contributes to VL morbidity. This study aimed to evaluate liver damage in Sudanese patients with VL as evidenced by apoptosis and lipid metabolism derangement. In a prospective analytical, hospital-based and case-controlled study and following informed consent, eighty patients with parasitologically confirmed visceral leishmaniasis and eighty apparently healthy age and sex unmatched volunteers [comparators] were enrolled in the study. Serum cytochrome C was measured by ELISA while serum lipids were measured using BioSystems A15 Chemistry Auto-analyzer. Cytochrome C concentrations in VL patients were significantly higher compared to apparently healthy volunteers with no significant difference between pre and post-treatment samples. Patients with VL showed marked hypo-cholestereamia, very low serum levels of LDL and HDL with most patients showing markedly increased triglycerides levels. Deranged lipid metabolism in VL patients could be due to hepatotoxicity or sequestration and/or degradation of lipoproteins in enlarged livers and spleens. In conclusion, hypocholestereamia, low levels of LDL/HDL, high triglycerides levels and increased serum cytochrome C are important features of hepatotoxicity in VL. Increased serum cytochrome C level is probably an important surrogate marker of hepatocytes apoptosis.

Introduction

Visceral leishmaniasis (VL; kala-azar) is caused by an intra-cellular protozoal parasite of the genus *Leishmania* which is transmitted to humans by bites of infected female sand flies. It is the most severe form of all *Leishmania* infections and is fatal in the absence of treatment. The great majority of VL cases occur in India, Bangladesh, Nepal, Sudan and Brazil. In Sudan, VL has been among the most important health problems, particularly in the main endemic area in the eastern and central regions. VL is characterized by fever, weight loss, anorexia, epistaxis, lymphadenopathy and hepato-splenomegaly. Hepatic toxicity greatly contributes to VL morbidity and mortality [1, 2, 3, 4].

Infection with *L. donovani* results in the development of organ-specific immunity where the liver and spleen are the main targets of infection. The liver is the site of an acute resolving infection associated with the development of inflammatory granulomas around infected Kupffer's cells. Efficient immune responses to *L. donovani* in the liver depend on the formation of granulomas, a process that is dependent on chemokine production, subsequent recruitment of monocytes, neutrophils, $CD4^+$ T cells and $CD8^+$ T cells, production of pro-inflammatory cytokines and activation of infected cells [5]. The liver is enlarged and weighs on average 1700 gram; it may show fatty changes indicating a poor prognosis. Microscopically there is hypertrophy and hyperplasia of the Kupffer's cells [4]. Kupffer cells are the major tissue macrophages found in the liver lining the sinusoids, and are a major target for *Leishmania* infection [5]. Parasites are visible in Kupffer's cells and in macrophages in the portal tracts in up to 80% of autopsied cases. There is often disarray of the liver columns with swollen hepatocytes. In some patients with severe liver dysfunction, necrosis of individual hepatocytes is observed with bile retention. Fibrosis of the wall of the central vein is not uncommon and the fibrosis may extend from the central vein to surround the hepatocytes in the vicinity resulting in Roger's fibrosis as a consequence of transformation of Ito cells into fibroblasts [5].

Two major forms of cell death affect hepatocytes in VL: necrosis with a strong inflammatory response surrounding the necrotic tissue. Apoptosis, on the other hand results in shrinking of the cell and formation apoptotic bodies without any inflammatory response. It is proposed that increased serum LDH activity is more indicative of necrotic processes *in vivo*, whereas the release of cytochrome C characterizes apoptotic events [6, 7, 8].

Cytochrome C is a 12.4-kDa electron carrier localized in the inter-membrane space of mitochondria and is the substrate of the last reaction in the electron transport chain [9]. Cytochrome C and the mitochondria play a central role in apoptosis, signalling the cell to begin the process of programmed cell death. The crucial step in the mitochondrial apoptotic pathway is permeabilization of the mitochondrial outer membrane that triggers release of apoptogenic factors, such as cytochrome C with propagation of the apoptotic cascade and execution of cell death [6, 10, 11, 12].

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Cholesterol of eukaryotic cell membrane is required to establish proper membrane permeability and fluidity. Its depletion from macrophages results in a significant reduction in the extent of leishmanial infection and may lead to perturbation of receptor–cholesterol interactions leading to loss of receptor function. LDL and HDL lipoproteins play a central role in extracellular lipid transport in species ranging from insects to mammals and participate in innate immunity [13, 14, 15, 16].

This study aimed to evaluate liver damage in Sudanese patients with VL as evidenced by apoptosis and lipid metabolism derangement.

Materials and Methods:

Scientific and Ethical Considerations:

The study protocol was scientifically reviewed and passed by the Ethics and Scientific Committees of the Institute of Endemic Diseases, University of Khartoum and the Ethics and Committees of the Federal Ministry of Health, Khartoum.

Study design:

This was a prospective analytical, hospital-based and case-controlled study. Following informed consent, eighty patients with parasitologically confirmed VL patients and eighty sex and age unmatched apparently healthy volunteers [comparators] were enrolled.

Clinical Data and samples:

Study VL patients were consented to lymph node or bone marrow aspiration. Pre-treatment and post treatment serum samples were collected for haematological and chemical assessment [AST, ALT enzymes and bilirubin], detection of cytochrome C and assessment of lipid profile.

An enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of human cytochrome C in serum was used as described by the manufacturer (Bender Medical Systems Diagnostics GmbH, Vienna, Austria). Serum cholesterol, LDL, HDL, and triglycerides were carried out using BioSystem A15 Chemistry Auto-analyzer.

Statistical analysis was performed using the data analysis software; Statistical Package Social Sciences (SPSS) version 13. Results were expressed as mean± standard deviation. The Significance of difference between two mean values among cases were determined by the Student independent t test with p <0.05 considered significant. Proportional data were presented as frequencies and percentages**.**

Results:

Eighty patients with a mean age of 13.8 ± 8.1 years and a male: female ratio of 1.3:1 and eighty apparently healthy volunteers [comparator group] had a mean age of 27.1±5.6 years and a male: female of 1 were in the study. Hepatomegaly was seen 63.8% of patients.

The cytochrome C concentration among VL patients ranged from 3 to12 ng/ml with a mean concentration of 4.5 ± 1.2 ng/ml compared to 0.6 ± 0.2 ng/ml in the comparator group (*p*=0.0001*)*. The mean cytochrome C concentration in pre-treatment and post treatment samples was 4.3 ± 0.4 ng/ml and 4.5 ± 0.1 ng/ml respectively with no statistically significant difference (*p*= 0.08).

The mean concentrations of bilirubin, AST and ALT were within normal levels for VL patients and were comparable to the levels of the comparator group.

The mean cholesterol concentration in VL patients was 87.8±42.1 mg/dL compared to 165.6 ± 20.2 mg/dL in the comparator group (*p value is* <0.0001). The mean concentration among female VL patients (77.3±31.1 mg/dL) was significantly lower than that among male patients (96.7±46.5 mg/dL) (*p = 0.03*).

The mean triglycerides concentration was 196.7±66.2 mg/dL among VL patients and 124.3±17.3 mg/dL among the comparator group, with significantly high levels among VL patients (*p*<0.0001).

The mean LDL concentration was 29.7±19.6 mg/dL with most VL patients showing significantly decreased levels of LDL when compared to the mean LDL concentration of the healthy volunteers which was 115.3±11.2 mg/dL (*p*<0.0001).

The mean HDL concentration among VL patients and comparator group was 8.1±5.1 mg/dL and 70.1±20.3 mg/dL respectively with significantly decreased HDL serum levels among VL patients (*p*=0.0001).

Discussion:

Circulating cytochrome C concentrations is usually increased in subjects with a variety of hepatic disorders and the level closely correlates with the apoptotic index in the liver. In this study an increased cytochrome C concentration supports our hypothesis that hepatocytes apoptosis is a feature of VL, while a normal serum creatinine rules out decreased renal clearance which is in agreement with previous reports [18]. Normal serum total bilirubin and enzymes levels in these VL patients indicate that hepatic apoptosis is a limited process and that the increase in serum cytochrome C is not due to failure of hepatic clearance as reported previously [17, 18]. The increase in cytochrome C in VL is not similar to the marked rise in a number of chronic and fulminant liver diseases like primary sclerosing cholangitis and hepatitis B & C virus [17]. The increase in serum cytochrome C did not change with VL treatment, probably indicating that anti-leishmanial drugs did not affect the apoptotic process.

Hypocholesterolemia, hypertriglyceridemia and markedly reduced levels of HDL and LDL have been reported before [19, 20, 21]. Many theories have been put forward to explain the hypocholesterolaemia: cholesterol may be used for lymphocyte activation and proliferation. Furthermore, infection is often associated with cellular injury, and injured areas may need extra cholesterol for new membrane formation. One of the possible mechanisms for the altered lipid profile in patients with VL might be sequestration and/or degradation of lipoproteins in the enlarged spleen and liver [16]. On the other hand decreased serum HDL-cholesterol may be due to decreased LCAT (lecithin/cholesterol acyltransferase) activity that has been reported to be markedly reduced in VL patients [22].

Increased triglyceride can cause an enlargement of the liver and spleen leading to sequestration of lipoproteins in the enlarged organ with reduction in serum HDL-cholesterol [23]. The increased serum triglyceride may be due to decreased hepatic lipase activities, resulting in slow clearance of VLDL ultimately leading to conversion to LDL probably under the effect of TNF-α, which is elevated in chronic parasitic infections [24]. The levels of lipoproteins found in some VL patients is be below the threshold required for the maximal production of TNF-α and of IL-6 [21].

In conclusion, hypocholestereamia, low levels of LDL/HDL, high triglycerides levels and increased serum cytochrome C are important features of hepatotoxicity in VL.

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