



Detection of Serum Highly Sensitive C-Reactive Protein and NT pro Brain Natriuretic Peptides Levels in Visceral Leishmaniasis Patients

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Abstract

Background: Visceral leishmaniasis is an endemic and potentially life-threatening disease in different area caused by *Leishmania donovani*, which can involve different organs and the disease presents with many different features, even heart involvement.

Objective: To detect high sensitivity C reactive protein (HS CRP) and N terminal pro Brain natriuretic peptide (NT-proBNP) in children with visceral leishmaniasis in Baqubah city.

Patients and Methods: This prospective study was conducted in the Al-Batool Teaching Hospital in Baqubah city, from January to May 2016. A total of 56 cases of infected child with visceral leishmaniasis, these patients have complained of clinical and laboratory diagnosis of Leishmania were included in the present study. Venous blood specimens were collected from each patients use for estimation of serum HS CRP and NT-proBNP by immune assay.

Results: HS CRP levels recorded high risk (4.46+0.66) was recorded at 8.9% of patients. While 53.6% of patients showed low risk (0.38+0.47). 5.5% patients had high level (2244.00+164.94) of NT-proBNP, whilst 30.9% of patients recorded mild increase of NT-proBNP (439.29+ 17.84).

Conclusion: The results of the present study proved that the HS CRP and NT proBNP levels were increased in association with visceral leishmaniasis and can be used for monitoring of clinical presentation of visceral leishmaniasis patients.

Key words: Leishmaniasis, heart failure, C - reactive protein, NT pro Brain Natriuretic peptides.

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Received: 5th September 2016

Accepted: 28th November 2016

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Introduction

Visceral leishmaniasis (VL) or Kalazar is one of the World's most careless and poorness-related diseases, affecting 500,000 cases each year, more than 90% of the VL cases reported from 5 countries: India, Nepal, Bangladesh, Brazil and Sudan and then Iraq with 5,000 annual cases will be added to other country [1]. Visceral

leishmaniasis is a vector-borne disease transmitted occur where the sand fly vector is present, which inject the protozoan parasite *Leishmania donovani*, *L. infantum*, or *L. amazonensis* into the skin of human. The protozoan can overcome the immunity, diffusion in organs, and multiply in macrophages mainly in the liver, spleen, and bone marrow and lymph nodes. The disease was characterized by a broad range



of clinical findings, such as fever, pancytopenia, cachexia, hepatosplenomegaly, hypergammaglobulinemia and hypoalbuminemia that may develop with severe complications such as sepsis, hemorrhage, pericardial effusion and ultimately death [2][3].

C-reactive protein (CRP) is a protein in acute phase, which synthesized in the liver in response to pathogens, tissue damage and autoimmune disease [4][5]. CRP has been demonstrated to have many calcium dependent binding properties including phosphoryl choline. This ligand accounts for binding to bacteria also to various cells including neutrophils and monocytes, and when bound may act as a galactose specific receptor. *Leishmania donovani* when entering the mammalian host express CRP ligand and CRP has been shown to change macrophage role [6].

The proposition to achieve this, is partly may be through a biomarker called NterminalproBrain natriuretic peptide (NTproBNP). NTproBNP is the biologically passive 76 amino acid peptide product of the cleavage of a 108 amino acid peptide called proBNP [7]. The other output of the process is the biologically active BNP. This process was happened primarily in the left ventricle and raise level of NTproBNP is equivalent with cases causing raised tension on the ventricular walls and heart failure [8].

On other hand data on relationship between visceral leishmaniasis and heart failure in patients, which are considered one possible cause of death especially in children, are available only from limited study. More data about this relationship was needed in our country especially in children, so this study aims to detect high sensitivity C reactive protein (HS CRP) and N terminal pro Brain natriuretic peptide (NT-proBNP) in children with visceral leishmaniasis.

Patients and Methods

This prospective study was conducted in the Al Batool Teaching Hospital in Baqubah city, from January to May 2016. A total of 56 child infected with visceral leishmaniasis, these patients have complained of clinical and laboratory diagnosis of Leishmania were detected in this study.

Visceral leishmaniasis was defined as detection of the amastigote stage of Leishmania in bone marrow smear and positive serological test (immunofluorescence antibody test), or both in a patient with symptoms compatible with VL. Name, age, sex, address, duration of disease, all enrolled patients were recorded. Venous blood samples were collected from each patient and separated by blood centrifugation at 3000 rpm for 5 minutes and stored at -20 °C until use for:

Estimation of serum HS CRP

The patient serum had been tested for CRP by high sensitive ELISA (Human CRP ELISA kit, BioCheck, USA) and according to manufacturer instructions as follows: After incubation with serum, specific goat anti-CRP antibodies are bound to the antigen on the inner walls of the microtiter plate. Extracted of excess serum by washing step, and (anti-IgG-HRP) is added. After further washing, the substrate (TMB) is pipetted. In positive reaction change the colorless of substrate to blue. The action of the enzyme on the substrate is stopped by the addition of 1 M H₂SO₄, and the stop reagent change the blue color to yellow. A photometric measurement by ELISA reader was carried out at the extinction 450 nm, then the mean absorbance values was converted to proper concentration in mg/l using a special table provided with each kit.

Estimation of NT-proBNP

The serum level of proBNP from Biomerieux Company France (a biomarker of heart failure) was determined by Enzyme Linked Fluorescent Assay (VIDAS NT-



proBNP automated test for use on the VIDAS instrument). The method of the kite is a one-stage immunoassay sandwich method with a final fluorescent revelation (ELFA) and the range of measurement is (20-25000 pg/ml). Levels less than 100 pg/ml suggest no failure in heart, 100-300 pg/ml indicate failure in heart is present, >300 suggest mild failure in heart, > 600 ml suggest moderate failure in heart and >900 pg/ml suggest severe failure in heart.

Statistical analysis

The data were analyzed by using SPSS version 20 (SAS, 2010). in the present study

compare between means by used Least Significant Difference-LSD test [9].

Results

Fifty-six visceral leishmaniasis patients were included, the data of Serum Highly Sensitive C Reactive Protein levels recorded significant differences ($p < 0.05$) among VL patients at ages 1 up to 3 years. High risk (4.46+0.66) was presented at 8.9% of patients. While 37.5% of patients showed average risk (2.15+ 0.10). While 53.6% of patients showed low risk (0.38+0.47) as shown in Table (1).

Table (1): Serum Highly Sensitive C Reactive Protein levels in visceral leishmaniasis patients.

Tests	LOWRISK	AVERAGERISK	HIGHRISK	**LSD
HSCRCP	0.38+0.47	2.15+ 0.10	4.46+0.66	0.7
VL patients %	53.6%	37.5%	8.9%	
*($p < 0.05$)				

*=Significant ($p < 0.05$), **LSD : Least Significant Difference

In Table (2), 5.5% patients had high level[2244.00+164.94] of NT-proBNP, while 12.7% showed moderate increase in NT-proBNP level[702.14+36.07].30.9% of patients recorded mild increase of NT-

proBNP(439.29+ 17.84).However, 50.9% of VL patients showed normal value [154.21+13.89]. The result detected that there were significant differences [$p < 0.05$] in level of NT-proBNP among VL patients.

Table (2): N-terminal prohormone of brain natriuretic peptide Levels in visceral leishmaniasis patients.

TESTS	Normal heart failure	Mild heart failure	Moderate heart failure	Sever heart failure	**LSD
NT-proBNP	154.21+13.89	439.29+ 17.84	702.14+36.07	2244.00+164.94	167.4
VL patients %	50.9%	30.9%	12.7%	5.5%	
*($p < 0.05$)					

*=Significant ($p < 0.05$), **LSD : Least Significant Difference

Discussion

Visceral leishmaniasis is an endemic and potentially life-threatening disease in the tropical and subtropical regions [10]. Leishmania Protozoa can involve different organs, and the disease presents with many different features, even heart involvement. Although clinically pericardial effusion was not significant, it is not unbelievable that the pericardial included may related to sudden heart deaths in untreated VL patient [3]. However, the present study indicated CRP level was increased in VL patients, this result

was accepted with prior results that presented CRP level increased in VL patients like [11][12]. CRP is the prototypic acute phase protein in man, in severe inflammation cases CRP increasing in concentration by up to 1000-fold within 24 h, also was increased following election of hepatocytes by inflammatory cytokines. Here, the specificity of CRP binding to the surface of *Leishmania donovani*, an obligate intracellular parasite of mononuclear phagocytes, is presentation to link to promastigotes at the infectious metacyclic phase of evolution [13]. When the surface of promastigotes presence CRP



substantially increases uptake into human monocyte-derived macrophages, that may influence the pathology and course of VL infection [14].

In the heart, macrophages accumulation between areas of cardiac muscle atrophy and the muscle fibers appeared in the myocardium, degeneration and loss of myocardiocytes. Sever inflammatory reaction was showed was practically in the heart, where wide parts of the myocardium showed infiltrated with large numbers of mononuclear immune cells, causing cardiac muscle atrophy and degeneration [15].

NT-pro-BNP could be increased in patients with inflammatory heart sickness, as well as in patients with left ventricular impairment in the function and myocardial ischemia [16]. However, NT-proBNP levels in the blood are used for screening, diagnosis of acute congestive failure in heart and may be helpful to confirm prognosis in heart failure, as indicator is higher in patients with worse consequence [17].The NT-proBNP levels in plasma are also increased in patients with a symptomatic or symptomatic left ventricular impairment in the function and is link with coronary artery sickness and myocardial ischemia [18][19].

NT pro Brain Natriuretic peptides levels showed different trends in VL patients at ages 1 up to 3 years, this may be due to differences in development and the severity of VL infection. This rare picture of VL may remain unrevealed due to deficiency of awareness. According to that, thorough work up should be considered in every patient with suspected VL.

In conclusion, the results of the present study proved that the HS CRP and NTproBNP levels were increased in association with visceral leishmaniasis and can be used for monitoring of clinical presentation of VL patients.

Further investigation for those who infected with VL should be done by

physician like Echo study and ECG to establish presence of heart failure.

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