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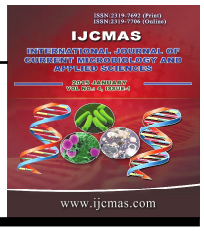

Original Research Article

Serological detection and liver functions of pediatric visceral leishmaniasis in Baghdad hospitals

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A B S T R A C T

A cross- sectional study was carried out for serological detection and liver functions of pediatric visceral leishmaniasis (VL) in Baghdad hospitals during the period from July 2013 to September 2014. Results were showed that the distribution of leishmaniasis according to sex which diagnosed with IFAT and rk39 techniques revealed there was no significant difference (p□0.05). From total of one hundred and seventy eight clinical suspected cases of Kala-azar who had studied the majority of cases 23 patients (46.0%) below 4 years old, so it is proved that the majority of visceral leishmaniasis among children. Analysis of total serum protein level of patients mean were 7.475 g/L (mean ± SD0.626) as compared to controls mean (7.292 g/L) (mean ± SD 0.705), and there were significantly increase of mean serum albumin level, while globulin was observed in the patients 3.225 g/L (mean ± SD 0.639) and 4.252 g/L (mean ± SD0.738) in compared to healthy control (4.384 g/L mean ± SD0.549 and 2.902 g/Lmean ± SD0.493), respectively. Albumin/globulin ratio was highly significant (P<0.01) in VL patients as compared to healthy control groups, so its mean value was in apparently healthy control 1.561 (mean ± SD0.379), while in leishmaniasis patients 0.793 (mean ± SD0.251). Determination of mean total serum bilirubin, mean serum direct bilirubin and mean serum indirect bilirubin of the study were showed there were no significant effects through leishmaniasis cases (P□0.05), which was 0.979 mg/dl, 0.533 mg/dl and 0.624 mg/dl, respectively. The biochemical tests includes assays of alanine 2-oxoglutarate aminotransferase (ALT), aspartate: 2-oxoglutarate aminotransferase (AST) and alkaline phosphatase were estimated that revealed higher significant P<0.01 value in leishmaniasis patients than healthy controls, with mean value of 58.43 (IU/L), 87.374 (IU/L) and 238.14 (IU/L), respectively. The study indicated that serological tests and detection of liver enzymatic functions results having better estimation in diagnosis of VL patients.

Keywords

Visceral leishmaniasis, Serological detection and liver functions.

Introduction

Visceral leishmaniasis (VL) is a vector-borne disease that is caused by obligate intra-macrophage protozoa, is endemicin large areas of the tropics, subtropics and the Mediterranean basin. This disease is characterized by both diversity and complexity (Chappuis *et al.*, 2007), so it is caused by more than 20 leishmanial

species and is transmitted to humans by about 30 different species of phlebotomines and flies, also (VL) or kala-azar, is a disease cause by intracellular protozoan parasites of the *Leishmania donovani* complex that consists mainly of *L. infantum*, *L. donovani* and *L.chagasi* (Singh, 2006). Leishmaniasis is endemic in 98 countries and 3 territories on five continents; it is estimated that there are 2 million new cases annually worldwide and up to 350 million people at risk of the disease (WHO, 2010; Alvar *et al.,* 2012). Kala-azar disease characterized in children by irregular fever, loos of weight, anaemia, splenomegaly, thrompocytopaenia, hypergamma globulinemia, leucopaenia and hepatomegaly (Kafetzis, 2003).

Technological advancements have led to significant improvements in the development of new diagnostic tools that are useful in rapid assessment of the disease burden to allow the rational design of control strategies (Boelaert *et al.,* 2008).

The indirect fluorescent antibody (IFA) test is one of the commonly tests used for anti-leishmanial antibody detection by using fixe promastigotes. This test is based on detecting IgG antibodies against *Leishmania spp.*, which are demonstrated in the serum during early and different stages of infection but are undetectable up to nine months after cure (Pedras *et al.,* 2008).

A recombinant antigen, rK39 has been shown to be specific for antibodies arising during VL caused by member of the *L. donovani* complex. It is highly sensitive and predictive for onset of acute disease and evokes high antibody titers of VL patients (El-Moamly *et al.,* 2012).

The presence of the parasites inside the hepatocytes has been documented by laboratory diagnosis which revealed elevated liver function through different tests and hypergammaglobulinemia (Christoph *et al.,* 1999). Functional derangement of liver in visceral leishmaniasis is reported in many cases and wrongly diagnosed and treated as hepatitis (Mathur *et al.,* 2008).

Methods

Study groups

Cross- sectional study was carried out during the period from July 2013 to September 2014. The age of patients ranged from 8 months to 13 years. Two study groups were involved:

A. Blood serum obtained from a total of one hundred and seventy eight clinical suspected cases with Kala-azar who had examined and defined as suspected cases by specialized physician, and fifty confirmed children pick up with visceral leishmaniasis were included in this study from five hospitals; Baghdad teaching children hospital, Ibin-Albalady children hospital, Al-Elwyia children hospital, Central child hospital, Al-Kademia children hospital.

B. Blood serum obtained from a total of fifty healthy control group were involved in this study from Baghdad province including health centers and Ibin-Albalady children hospital, they were apparently healthy by specialist physical examination without history of kala-azar. All sera samples were stored at -20ºC until use.

Samples collection

About 5 ml of venous blood was collected from each child, the blood was collected into a sterile screw plastic tube, left for 30 minutes at room temperature, then centrifuged at 3000 rpm for 5minutes, and then the serumwas collected in another sterile tubes which was stored in freeze at -20ºC.

Serological examination

rk39

Antibody response by the Recombinant K39 Immunochromato graphic strip Test (In Bios International, USA) was determined by the manufactures' instruction. 30 µL of serum was added to the dipstick and then placed vertically in a test tube. Two drops of the chase buffer solution provided with the dipstick kit were added to the test tube. The results were read after five minutes. A band in the test region considered as positive result, while the control line should be positive.

IFAT

Indirect fluorescence antibody test was carried out according to manufacture procedure (Leishmania IFA IgG vircell microbiologists, SPAIN) briefly serum of patient was diluted by phosphate buffer saline (PBS) (1/40 dilution) and20 µl of diluted serum was applied in the slide wells. Slide was incubated in a humid chamber for 30 minutes at 37ºC.The slide rinsed briefly with a gentle stream of PBS and then immersed for ten minutes in PBS and dip washed briefly in distilled water. 20µl of anti-human IgG FITC conjugate solution was added to each well. One drop of mounting medium was added to each well and carefully covered with a coverslip. Fluorescence microscope was used to read the slide with magnification power at 400x.

Biochemical tests

Alanine: 2-oxoglutarate amino-transferase (ALT or SGPT), Aspartate: 2-oxoglutarate amino-transferase (AST or SGOT), alkaline phosphatase, total serum bilirubin, serum direct bilirubin and serum indirect bilirubin were estimated following the instructions of commercial kits provided by Syrbio. Total serum protein, serumalbumin and globulin were measured according to (Biomaghreb) kit. All these analysis performed in Baghdad technical institute laboratories.

Statistical analysis

Patients and controls data were comparison by using ANOVA test. All statistical analysis was conducted with the statistical package for the social sciences (SPSS) software at significant levels of 0.05.

Result and Discussion

Visceral leishmaniasis is known as an endemic disease in some parts of the world and the most common clinical features of the disease are its prevalence among gender variants (Sayyahfar *et al.*, 2014).

This study included one hundred seventy eight children suspected of having visceral leishmaniasis and fifty healthy children as a control group. Which tested by using two immunochromatographic dipstick assays to detect antibodies to *Leishmania donovani* complex.

Table.1 Mean distribution of leishmaniasis according to sex

Gender		Studied groups		Total	Chi-Square (P-value)
		Apparently Healthy control	**Patients**		
Male	**N**	**25**	**26**	**51**	**P= 0.841 NS (P>0.05)**
	%	**50.0%**	**52.0%**	**51.0%**	
Female	**N**	**25**	**24**	**49**	
	%	**50.0%**	**48.0%**	**49.0%**	
Total	**N**	**50**	**50**	**100**	
	%	**100.0%**	**100.0%**	**100.0%**	

Table.2 Mean distribution of leishmaniasis among age groups

Age groups / Year		Studied groups		Total	Chi-Square (P-value)
		Apparently Healthy control	**Patients**		
>4	**N**	**21**	**23**	**44**	**P= 0.690 NS (P>0.05)**
	%	**42.0%**	**46.0%**	**44.0%**	
4.1 –8	**N**	**22**	**18**	**40**	
	%	**44.0%**	**36.0%**	**40.0%**	
8.1 –12	**N**	**7**	**9**	**16**	
	%	**14.0%**	**18.0%**	**16.0%**	
Total	**N**	**50**	**50**	**100**	
	%	**100.0%**	**100.0%**	**100.0%**	

Table.3 Effects of leishmaniasis on mean total serum protein, serum albumin and serum globulin

Tests	Studied groups	N	Mean	Std. Deviation	Std. Error	t-test (P-value)
Total Serum Protein (gm/dl)	Healthy control	50	7.292	0.705	0.099	P=0.205 NS (P>0.05)
	Patients	50	7.475	0.726	0.103	
	Total	100				
Serum Albumin (gm/dl)	Healthy control	50	4.384	0.549	0.078	P=**0**.00 HS (P<0.01)
	Patients	50	3.225	0.639	0.090	
	Total	100				
Serum Globulin (gm/dl)	Healthy control	50	2.902	0.493	0.069	P=**0**.00 HS (P<0.01)
	Patients	50	4.252	0.738	0.104	
	Total	100				
Albumin/ Globulin Ratio	Healthy control	50	1.561	0.379	0.054	P=**0**.00 HS (P<0.01)
	Patients	50	0.793	0.251	0.036	
	Total	100				

Table.4 Effects of leishmaniasis on mean total serum bilirubin, serum directbilirubin, andserum indirect bilirubin)

Tests	Studied groups	N	Mean	Std. Deviation	Std. Error	t-test (P-value)
Total Serum Bilirubin (mg/dl)	Healthy control	50	0.851	0.407	0.057	P=0.395 NS (P>0.05)
	Patients	50	0.979	0.974	0.138	
	Total	100				
Serum direct Bilirubin (mg/dl)	Healthy control	50	0.448	0.309	0.044	P=0.438 NS (P>0.05)
	Patients	50	0.533	0.702	0.099	
	Total	100				
Serum indirect Bilirubin (mg/dl)	Healthy control	50	0.403	0.226	0.032	P=0.091 NS (P>0.05)
	Patients	50	0.624	0.887	0.125	
	Total	100				

Table.5 Effects of leishmaniasis on mean serum ALT, serum AST, and serum alkaline phosphatase

Tests	Studied groups	N	Mean	Std. Deviation	Std. Error	t-test (P-value)
SerumALT (IU/L)	Healthy control	50	20.02	7.055	0.998	P=**0**.00 HS (P<0/01)
	Patients	50	58.43	50.303	7.113	
	Total	100				
Serum AST (IU/L)	Healthy control	50	32.304	8.131	1.149	P=**0**.00 HS (P<0/01)
	Patients	50	87.374	53.993	7.636	
	Total	100				
Serum Alkaline Phosphatase (IU/L)	Healthy control	50	132.94	48.433	6.849	P=**0**.00 HS (P<0/01)
	Patients	50	238.14	157.778	22.313	
	Total	100				

Results listed in Table 1 were showed that the mean distribution of leishmaniasis according to sex through IFAT and rk39 techniques revealed there was no significant difference (P□0.05) (male: 26 and female: 24), so this may lead that males and females exposed to the parasite equally through environments. Khlabus (2007) was explained also no differences in the morbidity of leishmaniasis cases between males and females. While AL-

Khayyt and AL-Qadhi, (2014) was recorded that from 52 studied children proved having visceral leishmaniasis, there were 21 of them were males and 31 females, the percentage of females was significantly higher P<0.01 than males (59.61%),(40.38%) respectively.

Results of present study was showed that themean distribution of leishmaniasis among age groups, so that the majority of cases 23 patients (46.0%) below (4) years old, 18 patients (36.0%) within (4.1-8) years old and 9patients (18.0%) within (8.1-12) years. Incidence of leishmaniasis reported at Table 2 detect the worldwide distribution and about 50%of the patients are children (Palumbo, 2010). Newly studies observe that the occurrence of death from VL is associated with several factors, including young age and the presence of comorbidities such as infections, malnutrition, and AIDS. The following features have been cited as markers of poor prognosis: fever lasting for more than 60 days, bacterial infection, jaundice, severe anemia, severe neutropenia, and thrombocytopenia (Werneck *et al.,* 2003; Costa *et al.,* 2010; Sampaio *et al.,* 2010).

Investigation of the serum proteins in diseases is handicapped by diffuses of clinically applicable methods for resolving the complex system of proteins present in blood serum into homogeneous components. Table 3 was showed no significant effects (P□0.05) of leishmaniasis on the mean total serum protein, and it was (7.475 g/L, mean ± SD0.726) as compared with mean controls (7.292g/L, mean ± SD0.705). Hypo-albuminaemia was accrued because of hepatic damage. Also the results were revealed significant increased level of the mean serum albumin and globulin was observed in the patients 3.225 g/L (mean ± SD0.639) and4.252 g/L (mean ± SD0.738)in compared to healthy control (4.384 g/L, mean ± SD0.584 and 2.902 g/L, mean ± SD0.493) respectively. The albumin/globulin ratio was significantly higher(P<0.01) in VL patients as compared to healthy control groups (Mishra *et al.,* 2010), so its mean value was in apparently healthy control 1.561 Std. Dev. 0.379, while in leishmaniasis patients 0.793 Std. Dev. 0.251 (Gani *et al.,* 2010).

Table 4 summarizes the study results by shows there were no significant effects of leishmaniasis cases (P□0.05) on mean total serum bilirubin, serum direct bilirubin and serum indirect bilirubin, which was (0.979 mg/dl, mean ± SD 0.974), (0.533 mg/dl, mean ± SD 0.602) and (0.624 mg/dl, mean ± SD 0.887) respectively. Kala-azar mimicking chronic liver disease it has been suggested that screening of the disease in children presenting with chronic liver disease is important in endemic areas, so serum bilirubin and liver enzymes level significantly reduced and there was increase in serum proteins (Mona and Dhakal, 2013).

Liver enzymes – alanine, 2-oxoglutarate aminotransferase (ALT), aspartate, 2-oxoglutarate aminotransferase (AST) and serum alkaline phosphatase were significantly higher in leishmaniasis patients (P<0.01) than in healthy controls, which its means was (58.43IU/L, mean ± SD 50.303), (87.374IU/L, mean ± SD 53.993) and (238.14IU/L, mean ± SD 157.778) respectively. Salwa *et al.* (2014) was discussed the results of his study (in Yemen) as the lack of significant increasing of AST, ALT and bilirubin in

reflect of the normal liver function and absence of VL among patients. While Kashani *et al.* (2007) reported no significant change in the levels of bilirubin, AST and ALT in leishmaniasis patients. Table 5 shows the effects of leishmaniasis on mean serum ALT, serum AST, and serum alkaline phosphatase.

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