Bas.J.Vet.Res.Vol.2 No.5,2006.

LEVELS OF SERUM IMMUNOGLOBULINS AND COMPLEMENTS IN PATIENTS WITH VISCERAL LEISHMANIASIS

Wafaa Sadoon Shani

Department of Biology, College of Science, University of Basrah, Basrah ,Iraq.

(Received 11 January 2006,Accepted 26 April 2006)
Key words: IgG,leishmaniasis,Heteroxenous.

ABSTRACT

Forty sera of patients infected with *Leishmania donovani* and (10) sera from apparently healthy subject were tested by single radial immunodiffusion to visualize the concentration of IgG, IgM, IgA, C3 and C4. Recent results showed that there was a highly significant increase in the level of IgG, IgM, C3 and C4 but there were no significant differences in the level of IgA between the two-studied groups.

INTRODUCTION

Leis mania donovani is an obligate intracellular parasite that infects macrophage of the vertebrate host resulting in visceral leishmaniasis in humans, which is responsible for morbidity and mortality in many humans throughout the world (1).

This protozoan has a heteroxenous life cycle, living first as an intracellular amastigote in the vertebrate host, then as a motile flagellated promastigote in the gut lumen of the sand fly vector (2). Infective promastigote entering the blood of vertebrate is covered by two key molecules: the protein gp 63 and a lipophosphoglycan. Both of these molecules mediate the uptake of promastigote by interacting with components of the complement system and with surface molecules on the macrophage (3).

Clinically *L. donovani* infection may range from asymptomatic to progressive, fully developed Kala-azar. The immunology of *L. donovani* has not been studied to quite the extent that *Leishmania major* has, at least in mice (4). Beside this the presence of cases infected with this parasite in Basrah hospitals, so the aim of the present study is to throw a light on the immune state of patients with kala-azar, specially a humoral part of immunity related with the levels of serum Immunoglobulins and complements.

MATERIALS AND METHODS

i- Sample collection

Forty sera from patients with visceral leishmaniasis were collected, the age of those patients ranged from (1-9) year. And those patients diagnosed by testing the bone marrow in order to diagnose the infection, which is done in general Basrah hospital. Ten apparently healthy children were chosen as a control group.

Sera of patients and control group were tested by single radial immunodiffusion (SRID) to determine the concentration of IgG, IgM, IgA, C3 and C4.

ii- Single radial immunodiffusion (SRID)

SRID test was done by using Biomaghreb kit.

The plates were opened and left to stand at room temperature for a few minutes to allow any condensed water in the wells to evaporate. The wells were filled with 5µl of testing sera (patients and controls). Then left the plates to stay at room temperature for about (48) hours in the case of IgG, IgA, C3 and C4 and for (72) hours in the case of IgM.

The diameter of each immunoprecipitating ring formed around each well was measured in mm by using immunoviewer and the concentration of each immunoglobulin class and complement

was calculated from standard curve in the kit test which represents the correlation between the diameter of ring in mm and concentration of antibody in mg / dl.

iii- Statistical analysis

The data analyzed by using analysis of variance (ANOVA) test.

RESULTS

Present results of (SRID) revealed that there were a highly significant differences ($P<0.001$) in mean levels of serum IgG and IgM between patients (1488.5, 168.9) and control group (617.9, 119.83) respectively, tables and figures (1, 2).

Table (1): Mean level of serum IgG in patients and control group.

Group	No.	Range	Mean	SD±	SE
Patients	40	981.3 – 1946.2	1488.5	278.5	44.06
Controls	10	529.0 – 836.7	671.9	128.1	40.53

Where:

No. = Number
SD = Standard deviation
SE = Standard error

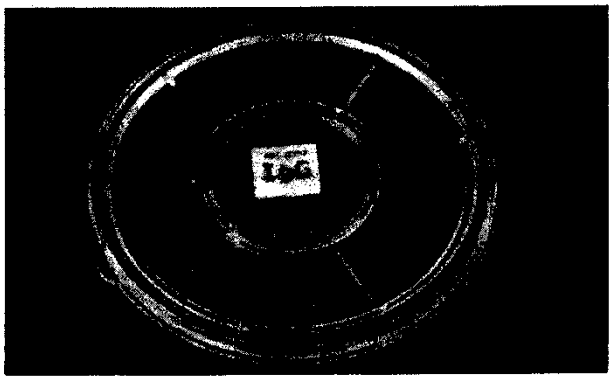

Figure (1): Precipitin ring of serum IgG in tested sera

Table (2): Mean level of serum IgM in patients and control group.

Group	No.	Range	Mean	SD±	SE
Patients	40	81.5 – 227.1	168.9	151.04	14.30
Controls	10	76.3 – 141.4	119.83	141.74	40.51

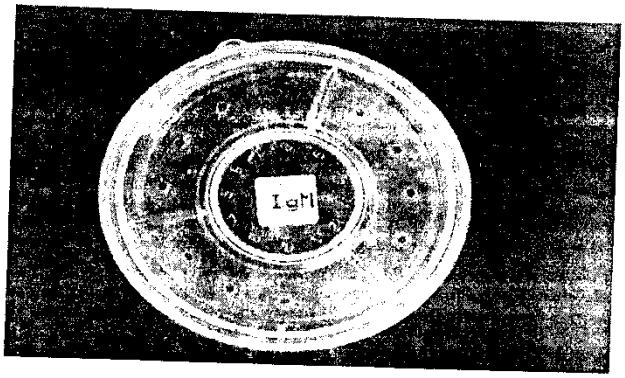

Figure (2): Precipitin ring of serum IgM in tested sera.

Whereas IgA mean level didn't show any significant differences (P> 0.05) between patients (191.73) and control group (225.62), table and figure (3).

Table (3): Mean level of serum IgA in patients and control group.

Group	No.	Range	Mean	SD±	SE
Patients	40	43.7 – 225.4	191.73	90.39	14.30
Controls	10	81.7 – 255.4	225.62	128.02	40.51

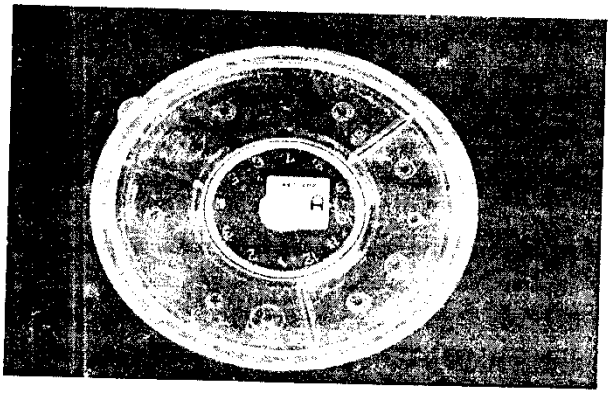

Figure (3): Precipitin ring of serum IgA in tested sera.

In the case of serum complement levels, present data represent a highly significant differences (P<0.001) in mean concentration of C3 and C4 between patients (170.31, 46.09) and control group (141.10, 27.40) respectively, tables and figures (4, 5).

Table (4): Mean level of serum C3 in patients and control group.

Group	No.	Range	Mean	SD±	SE
Patients	40	93.4 – 252.6	170.31	44.63	7.06
Controls	10	93.4 – 132.1	141.10	13.52	4.27

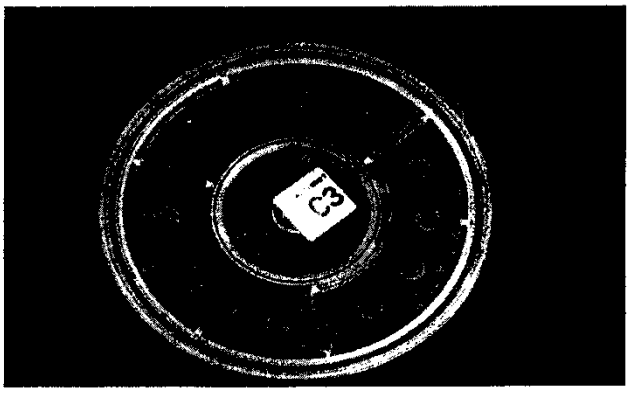

Figure (4): Precipitin ring of serum C3 component in tested sera.

Table (5): Mean level of serum C4 in patients and control group.

Group	No.	Range	Mean	SD±	SE
Patients	40	29.3 – 69.4	46.09	12.04	1.90
Controls	10	24.5 – 43.9	27.40	5.96	1.88

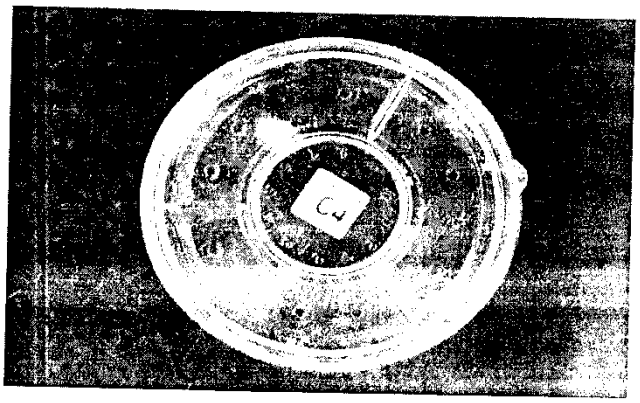

Figure (5): Precipitin ring of serum C4 component in tested sera.

DISCUSSION

Leishmaniasis exist as a complex of diseases that from an immunological point of view, fall into two categories, the self-healing dermal forms such a *Leishmania tropica* and effectively non-healing visceral forms caused by *L. donovani* (5).

The humoral part of immunity which studied here demonstrated that the serum of person infected with visceral leishmaniasis have a high level of IgG and IgM in comparison with control group, and this may be indicated the role of these antibodies during the course of infection, the same results reported also by (6) when showed that the hamster produce IgG which directed against certain parasite surface membrane antigens. (7) also recorded high level of globulin mainly IgG, during visceral leishmaniasis. In addition, recent results were in agreement with the results of (8) and (9) whom showed that the patients with symptomatic kala-azar have a high titer of anti-leishmanial antibodies. (10) showed that the promastigote of *L. donovani* was affected by normal human serum in vitro. Moreover, the importance of IgG, IgM and natural antibodies was also suggested by (1) and (11). Massive hypergammaglobulinemia was also documented by (12).

SRID results was not indicated any differences in serum concentration of IgA between patients and control group and this may be due to that the infection trigger the classical complement pathway which activate initially by IgG and IgM not by IgA (13). So there was an increasing in the concentration of IgG and IgM but not IgA. Or may be due to that the *L. donovani* antigens do not induce the production of IgA.

The increasing in the concentration, of C3 and C4 may be contribute to that the infection appeared to activate the complement cascade through the classical pathway which is needed for expression of the lethal effect (1). (11) also noted the same observations that after inoculation the promastigote interacts with opsonic serum factors and activates the complement system, the third component of complement (C3)-coated parasite adheres to mononuclear phagocytes through CR1 and CR3 complement receptors.

Finally, the considerable humoral immune response was triggered during visceral leishmaniasis infection, which documented during this study revealed the cooperation between IgG, IgM, C3 and C4 in defending mechanism against the infection via the opsonization and then phagocytosis.

مستويات الكلوبيولينات المناعية ومكونات المتمم في مصول المرضى المصابين باللشمانيا الاحشائية

وفاء سعدون شاني
قسم علوم الحياة ، كلية العلوم ، جامعة البصرة ، البصرة ، العراق

الخلاصة

في هذه الدراسة تم فحص أربعين مصل للمرضى المصابين بالـ *L. donovani* وكذلك عشرة مصول للأشخاص غير المصابين باستخدام فحص الانتشار المناعي القطري البسيط لمعرفة تراكيز الكلوبيولينات المناعية IgG ، IgM ، IgA وكذلك مكونات المتمم C3 و C4 . وقد أظهرت النتائج بان هناك فروقات عالية المعنوية في مستوى IgG و IgM و C3 و C4 بينما لم تظهر النتائج أي فروقات معنوية في مستوى IgA بين المجموعتين قيد الدراسة (المرضى والأصحاء).

REFERENCES

1- Pearson, R. D. and Steigbigel, R. T. (1980). Mechanism of lethal effect of human serum upon *Leishmania donovani*. J. Immunol. 125(5): 2195-2201.
2- Riberio, J. M. C. (1995). Blood feeding arthropods: live syringes or invertebrate pharmacologists?. Infect. Agents. Dis. 4: 143-152.
3- Marquardat, W. C.; Demaree, R. S. and Grieve, R. B. (2000). Parasitology and vector biology. 2nd edition. Harcourt academic press. pp. 62.
4- Roberts, L. S. and Janovy, J. Jr.. (1996). Foundations of parasitology. 5th edition. Wm. C. Brown Publications. pp. 73.
5- Cox, F. E. G. (1982). Modern parasitology. Blackwell Scientific Publications. pp. 185.
6- Dwyer, D. M. (1976). Antibody-induced modulation of *Leishmania donovani* surface membrane antigens. J. Immunol. 117(6): 2081-2091.
7- Mcleod, J. (1984). Davidson's principles and practice of medicine. 14th edition. Churchill Livingstone. pp. 777.
8- Carvalho, E. M.; Badaro, R.; Reed, S. G.; Jones, T. C. and Jhonson, W. D. (1985). Absence of gamma interferon and interleukin -2 production during active visceral leishmaniasis . J. Clini. Invest. 76: 2066-2069.
9- Pearson, R. D.; Evans, T.; Whecler, D. A.; Naidu, T. G.; Alencar, J. E. de. and Davis, J. S. (1986). Humoral factors during South American visceral leishmaniasis. Ann. Trop. Med. Parasitol. 80: 465-468.
10- Hoover, D. L.; Berger, M.; Nancy, C. A.; Hockmeyer, W. T. and Meltzer, M. S. (1984). Killing of *Leishmania tropica* amastigotes by factors in normal human serum. J. Immunol. 132(2): 893-897.
11- Dominguez, M. and Torano, A. (1999). Immune adherence-mediated opsonophagocytosis: The mechanism of *Leishmania* infection. J. Exp. Med. 189(1): 25-35.
12- Parslow, T. G.; Stites, D. P.; Terr, A. I. and Imboden, J. B. (2001). Medical immunology. 10th edition. McGraw-Hill. pp. 679.
13- Paul, W .E. (1999) . Fundamental immunology . 4th edition . Vol .2. Lippincott – Ravene publishers .