Autoantibodies Status of Leishmanial Patients in Iraq

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Abstract

A total of 145 leishmaniasis Iraqi patients were collected from suspected cases and 30 healthy control. Based on skin smear examination and serum samples analysis, the patients were distributed into two clinical groups: 30 cutaneous leishmaniasis and 30 visceral leishmaniasis patients. The study was conducted for the detection of 17 types of antinuclear autoantibodies (ANA) in studied groups, 10 types including (anti-ds-DNA, anti-Nucleosome, anti-SmD1, anti-PCNA, anti-SS-A/Ro60kD, anti-SS-A/Ro52kD, anti-SS-B/La, anti-CENP-B, anti-Mi-2 and anti-Ku antibodies), showed significant differences ($P \leq 0.05$), ($P \leq 0.01$) in the two groups, while 7 types which were non-significant included (anti-histones, anti-ribosomal P0, anti-Scl70, anti-U1-snRNP, anti-AMA M2, anti-Jo1 and anti-PM-Scl antibodies). With respect to the Anti-cardiolipin IgG antibodies (aCL), it showed a significant increased level in the serum of VL patients ($P \leq 0.01$) as compared with control group (8.123 *vs.* 1.959), also as compared with CL patients (8.123 *vs.* 2.402). As for CL patients, also there was a significant difference ($P \leq 0.01$) (2.402 *vs.* 8.123) when compared with VL patients, but no significant variations (2.402 *vs.* 1.959) were observed in compare with control group. We conclude that Leishmaniasis in iraqi patients may be considered as a cause autoimmune diseases as in case of inflammatory myopathies (IM), and the pathgenesis of human myositis, especially in VL which contributes in the immunological alterations associated with muscle damage.

Keywords: Leishmania species, autoantibodies, antinuclear antibodies, anticardiolipin.

Introduction

Leishmaniasis is a group of vector-borne disease caused by obligate, intracellular protozoa belonging to the genus *Leishmania*, it is characterized by diversity and complexity, *Leishmania* infection is transmitted to susceptible mammalian hosts by about 30 species of female phlebotomine sand flies (1,2).

Leishmania spp. cause awide variety of diseases that range in severity from self-healing cutaneous leishmaniasis to fatal disseminated visceral leishmaniasis (3,4). The parasites have a digenetic life cycle and exist in two distinct morphologies, the promastigote in sand fly vector, and the amastigote in mammalian host (5). In order to develop a successful parasitic relationship with its host, the *Leishmania* must evade both the innate and adaptive immune responses.When *Leishmania* first enter the human body, it is in a promastigote from, which was engulf by macrophages of the reticuloendothelial system, but it is resistant to proteolysis degradation in the phagosome. So, within the mammalian host, *Leishmania* resides as amastigotes in phagocytic cell (6). In visceral leishmaniasis (VL), the intracellular parasite causes intense parasitism in the reticuloendothelial system, affecting the liver, spleen, bone marrow, and lymph nodes. It causes expressive changes in cellular and humoral response, with deficiency of Interferongamma (IFN-γ), increased production of Tumour necrosis factor-α (TNF-α) and other interleukins, besides polyclonal hypergammaglobulinemia (7).

The clinical presentation of VL is characterized by hepatosplenomegaly, fever, paleness, asthenia, weight loss, tachycardia, coughing, epistasis, bleeding of the gums, myalgia, arthralgia, and adenopathy, among others; those symptoms can also be seen in Systemic lupus erythematosus (SLE) (8). And the main signs and symptoms observed in cutaneous leishmaniasis (CL) include: ulcerated, painless lesion with elevated borders, central granulation tissue, with or without exudates, the lesion can be single, multiple, disseminated, or diffuse (6).

Leishmanial infection in human is characterized by the appearance of anti-leishmanial antibodies in the sera of the patient. In CL, usually they are present at low levels during the active phase of disease (9). In contrast strong anti-leishmanial antibodies titer well documented in VL. The elevated antibody titers against promastigote or amastigote antigens, have been extensively exploited for specific serodiagnosis in last two decades (10). and the analysis of sera of with *Leishmania* infection demonstrates the presence of autoantibodies against cellular and humoral components, besides circulating immune complexes and anti-IgG antibodies (11). According to American College of Rheumatology (12). The antibodies make a mistake, identifying normal, naturally-occurring proteins in our bodies as being "foreign" and dangerous. The antibodies that target "normal" proteins within the nucleus of a cell are called Anti-nuclear Antibodies (ANA). ANAs could signal the body to begin attacking itself which can lead to autoimmune diseases, including: SLE, scleroderma, Sjögren's syndrome, polymyositis, dermatomyositis, mixed connective tissue disease (MCTD), drug-induced lupus, and Primary biliary cirrhosis (PBC).

SLE is a multifactorial autoimmune, chronic inflammatory disease with a polymorphic clinical presentation. It is associated with immunologic dysfunction with polyclonal activation of B lymphocytes and autoantibodies against nuclear antigens, some of which participate in immune-mediated tissue lesions. Due to its polymorphic presentation, it can be mistaken with several infectious diseases, including VL (13).

The production of autoantibodies, such as anti-Sm, anti-RNP, anti-SSA, anti-SSB, and antiphospholipid, is found in patients with *Leishmania* infection. The induction of autoantibodies in leishmaniasis can be attributed to the release and exposure of antigens, previously hidden, during tissue damage and breakage of host cells (14).

Accordingly, the present study was designed to inspect the association between the autoantibodies and the two types of *Leishmania*; CL and VL, and to define the antinuclear antibodies recognized by sera from Iraqi patients with leishmaniasis and determine whether these leishmanial infections may correlate with autoimmune manifestations, through the following parameters,through investigating the association of IgG autoantibodies against some antigens, especially; nuclear antigens, like: dsDNA, Nucleosome, histones, SmD1, PCNA, ribosomal P0, SS-A/Ro60kD, SS-A/Ro52kD, SS-B/La, CENP-B, Scl70, U1-snRNP, AMA M2, Jo1, PM-Scl, Mi-2, Ku and against cardiolipin.

Subjects, Materials and Methods:

Subjects:

A total of 145 leishmaniasis Iraqi patients were collected from suspected cases including, 75 uncertainly diagnosed with CL. Their age range was (3 to 66 years), 70 uncertainly diagnosed with VL. Their age range was (3 month to 10 years) attending AL-Karama Teaching Hospital and Central Public Health Laboratory, during the period October 2013 to February 2014 were enrolled in this study. The clinically cases examined and evaluated at these medical centers under the supervision of staff. In addition, 30 healthy (controls), their age range was (3 month to 59 years), were also included in this study. Based on skin smear examination and serum samples analysis, the patients were distributed into two clinical groups: 30 patients of cutaneous leishmaniasis (CL), were diagnosed depending on the clinical picture and laboratory diagnosis of cutaneous ulcer.and their age range was (3 to 59 years), 30 patients of visceral leishmaniasis (VL, were diagnosed by Indirect Immune Fluorescent Antibody Assay Test (IFAT) and their age range was (4 month to 9 years) .

Blood samples collection:

Five milliliter (ml) of venous blood was collected in sterile container from each patient; sera were separated after leaving samples at room temperature for about 15 to 30 minutes, and then centrifuged at 1500 rpm for 5 minutes and the sera dispensed into 4 to 5 Eppendorf tubes, stored at -20°C until used for laboratory diagnosis and serological tests.

Detection of total antinuclear antibodies profile among studied groups:

IMTEC-ANA-LIA MAXX is an indirect membrane based enzyme immunoassay for the qualitative measurement of IgG class antibodies against 17 variables autoantibodies includes:dsDNA, nucleosomes, histones, SmD1, PCNA, ribosomal P0, SS-A/Ro60kD, SS-A/Ro52kD, SS-B/La, CENP-B, Scl70, U1-snRNP, AMA M2, Jo1, PM-Scl, Mi-2 and Ku in human serum or plasma.This test based on the principle the line immune assay (LIA). Nuclear and associated cytosolic antigens are applied as lines on a nitrocellulose membrane. The nitrocellulose membrane is blocked to prevent unspecific reaction. During strip incubation with diluted patient samples autoantibodies bound antibodies a secondary horseradish peroxidase (HRP)- labeled anti-human IgG antibody is used. After addition of the substrate and stop solution the appearance of brown lines indicate the existence of (auto) antibodies against the respective antigen (15).

Test validation

For each strip: function control is visible, cut-off control is visible, intensity function control > intensity cut-off control. The strip were fixed onto scoring sheet and the reference line of the strip was aligned with the reference line on the scoring sheet. The dotted reference line of the evaluation template was aligned with the reference line. The interpretation of the test result take place exclusively on basis of the respected cut-off control regarded for each strip; the test result was negative, if no band was too recognized or if the band exhibits a smaller intensity in comparison to the cut-off. The test was equivocal, if the intensity of cut-off control did not significantly differ, the test result was positive if a band exhibits a stronger staining in comparison to the cut-off control (15).

Detection of total Anticardiolipin (aCL)-IgG Antibody among studied groups:

This method was carried out according to (AESKULISA Company). Cardiolipin-IgG-IgM is a solid phase enzyme immunoassay employing highly Serum purified cardiolipin plus native human β2-glycoprotein1 for the quantitative and qualitative detection of IgG and / or IgM antibodies against cardiolipin in human serum. Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. After wards anti-human immunoglobins conjugate to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of Tetramethylbenzidine (TMB) substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color change to yellow). The rate of color formation for the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample. A standard curve is prepared from standard dilutions and human anti Cardiolipin-IgG antibody concentration is determined from a curve fitting equation (kit leaflet).

Calculation of Sample Results:

For quantitative interpretation establish the standard curve by plotting the optical density (OD) of each calibrator (y-axis) with respect to the correspond concentration values in GPL\ml or MPL\ ml (x-axis).

Statistical Analysis:

The Statistical Analysis System- SAS (2010) was used to effect of different factors in study parameters. Chi-square test was used to significant compare between percentage and Duncan multiple range test was used to significant compare between means in this study.

Results

The study was conducted for the detection of 17 types of antinuclear autoantibodies in studied groups, 10 types including (anti-ds-DNA, anti-Nucleosome, anti-SmD1, anti-PCNA, anti-SS-A/Ro60kD, anti-SS-A/Ro52kD, anti-SS-B/La, anti-CENP-B, anti-Mi-2 and anti-Ku antibodies), showed significant differences (P≤0.05), (P≤0.01) as in table 1, while 7 types which were non-significant included (anti-histones, anti-ribosomal P0, anti-Scl70, anti-U1-snRNP, anti-AMA M2, anti-Jo1 and anti-PM-Scl antibodies) as in table 2.

Anti-cardiolipin IgG antibodies (aCL) in patients with Leishmaniasis :

Anti-cardiolipin IgG antibodies showed a significant increased level in the serum of VL patients (P≤0.01) as compared with control group (8.123 *vs.* 1.959), also as compared with CL patients (8.123 *vs.* 2.402) table 3. As for CL patients, also there was a significant difference (P≤0.01) (2.402 *vs.* 8.123) when compared with VL patients, but no significant variations (2.402 *vs.* 1.959) were observed in compare with control group.

Discussion

All 60 consecutive patients with CL and VL who were tested, were found to have a variety of autoantibodies present without any clinical autoimmune manifestation.

For anti-ds-DNA, among patients with CL (n=30), 1 (3.3%) was positive and 29 (96.7%) were negative, while in patients with VL (n=30), 9 (30.0%) were positive and the results were negative in 21 (70.0%). The anti-ds-DAN autoantibodies in leishmaniasis patients was significantly different at the level of (P≤ 0.01), when compared between the groups.

In a study with 45 visceral leishmaniasis patients and 45 with cutaneous leishmaniasis (11) did not note the presence of anti-ds-DNA in CL patients, while they pointed to its presence in (4.4%) of VL patients, so the present results shared these findings, and the anti-ds-DNA antibodies are more present in VL than in CL patients.

(14) mentioned the case of a patient with visceral leishmaniasis who was positive for anti-DNA antibodies, which disappeared after treatment with corticosteroids, antimonials, and posterior use of liposomal amphotericin B. In a study of 23 patients with visceral leishmaniasis and 14 with cutaneous leishmaniasis, (16) did not observe the presence of anti-ds-DNA despite the production of other autoantibodies in those patients. Also (17) did not detect anti-ds DNA autoantibodies in any VL patients.

For anti-Nucleosomes antibodies in CL (n=30), these antibodies were positive in 5 patients (16.7%) and were negative in 25 (83.3%), whereas in VL (n=30), 16 patients (53.3%) were registered positive and 14 (46.7%) were negative. The anti-Nucleosomes autoantibodies in leishmaniasis patients was significantly different at the level of (P≤0.01), when compared with studied groups.

For anti-SmD1in our CL patients (n=30), no one scored positive 0 (0.0%) and 30 patients (100.0%) gave a negative result, while in patients with VL (n=30), the results of 4 patients (13.3%) tested positive and 26 patients (86.7%) were negative. The anti-SmD1 antibodies in leishmaniasis patients was significantly different at the level of (P≤0.05), when compared between studied groups. (11) did not observe the presence of anti-SmD1, regardless the type of leishmaniasis (visceral or cutaneous), while (16) observed the presence of anti-SmD1 antibodies in 7% of patients with cutaneous leishmaniasis and in 83% of the patients with visceral leishmaniasis. Also (17) revealed about anti-Sm antibodies in 6% of patients with visceral leishmaniasis in their study on 16 patients.

anti-PCNA, among patients with CL (n=30), 1 (3.3%) was positive and 29 (96.7%) were negative, while in patients with VL (n=30), 9 (30.0%) were positive and the results were negative in 21 (70.0%). The anti-PCNA autoantibodies in leishmaniasis patients was significantly different at the level of (P≤ 0.01), when compared between the groups.

Autoantibodies targeting the proliferating cell nuclear antigen have been considered as a specific biomarker for systemic lupus erythematosus. The proliferating cell nuclear antigen-like staining pattern was rarely found (0.07%) in sequential, unselected sera. Further, indirect immunofluorescence is not an accurate screening method to identify anti-PCNA antibodies as their presence may be masked by other autoantibodies (18).

For both anti-SSA/ Ro60 and Ro52, in patients with CL (n=30), no one scored positive (0.00%) and 30 of the patients (100.0%) were negative for anti-SS-A/Ro60kD, whereas one patient (3.3%) gave a positive result and 29 of the patients (96.7%) were negative for anti-SS-A/Ro52kD. While in VL patients (n=30), 9 patients (63.3%) scored positive results and 21 of the patients (36.7%) had tested negative for anti-SS-A/Ro60kD, but 7 patients (23.3%) were positive and 23 of the patients (76.7%) had tested negative for anti-SS-A/Ro52kD.

With respect to the anti-SS-B/La, we found that 4 patients (13.3%) have those antibodies and 26 patients (86.7%) haven't these antibodies in CL patients and 19 of the patients (63.3%) were scored positive result for this antibody in addition to 11 patients (36.7%) who were negative in VL patients respectively. The anti-SS-A/Ro60kD, anti-SS-A/Ro52kD and anti-SS-B/La in leishmaniasis patients were significantly different at the level of (P≤0.05), when compared between studied groups. (11) did not shown the presence of anti- SSA/Ro antibodies regardless the type of leishmaniasis (visceral or cutaneous), the results were negative. As for anti-SSB/La antibodies, only one patient (1.1%) with visceral leishmaniasis had a positive result and all others were negative, while (16) revealed that in sera of patients with VL, the antibodies against Sm, RNP,and SS-B antigens were detected in 83, 86 and 73% of the patients respectively, and only 36% of the VL patients were considered positive to SS-A, while <25% of CL patients exhibit such antibodies. And on the other study, (17) observed the presence of anti-Ro antibodies in sera of 13% of patients with visceral leishmaniasis.

Molecular mimicry between *Leishmania sp.* Antigens and ribonucleoproteins is one of the hypotheses raised by (14) to explain the presence of those autoantibodies (anti-Sm, anti-RNP, anti-SSA, and anti-SSB) in infected patients. The possibility that Sm, RNP, SSB, and *Leishmania* share similar antigenic determinants is raised, due to the inhibition of autoantibodies against those nuclear antigens by intact *Leishmania* promastigotes (16).

In the current study, patients with Visceral Leishmaniasis (VL) demonstrated a significantly different distribution of (ANA) as compared to Cutaneous Leishmaniasis patients (CL), and showed the highest level of autoantibodies especially; Anti-Ku antibodies 24 (80%), anti-Mi-2 antibodies 21 (70%) and anti-SS-B/La antibodies 19 (63.3%) in descending order (Table 4-9). But there was some increase in the level of anti-Ku antibodies 12(40.0) in CL patients.

It is not found that any one gave a positive examination for those antibodies in the group of healthy control.

In a study conducted to analyze the prevalence of myositis-specific and myositis-associated autoantibodies and their clinical correlations in a large series of patients with dermatomyositis/polymyositis, the most frequently found autoantibody was anti-Ro-52 (36.9%), followed by anti-Jo-1 (18.9%), anti-Mi-2 (8.1%), anti-Ku (4.1%), anti-SRP (3.2%), anti-PL-7 (3.2%), anti-PL-12 (2.7%), anti-PM/Scl75 (2.7%), and anti-PM/Scl100 (2.7%). The distributions of these autoantibodies were comparable between polymyositis and dermatomyositis, except for a higher prevalence of anti-Jo-1 in polymyositis. Anti-Mi-2 was more prevalent in dermatomyositis (19). These observations may lead to correlate between leishmaniasis infection and autoimmune diseases in terms of the contribution of leishmaniasis in the formation of autoantibodies and may support the perception that leishmanial infection may lead to autoimmune diseases through the formation of these autoantibodies in patients with leishmaniasis ,especially in (VL) patients.

Leishmaniasis is a spectrum of diseases and the humoral response of patients varies markedly depending on the type of disease present. Patients with VL have markedly elevated immunoglobulins levels and anti-leishmanial specific antibodies, though much of these immunoglobulins is not leishmania specific (20, 21). On the other hand, in the cutaneous forms of leishmaniasis, antibodies specific to the parasite are demonstrable, where the serum immunoglobulin levels are normal (21). Analysis of infected patients by several group of investigators, revealed the existence of autoantibodies against cellular and humoral components (22, 23).

In the present study, we examined the response to nuclear antigens of patients with leishmaniasis. Several possibilities can be taken into account to explain the manifestation of autoantibodies in leishmaniasis. The first is might be due to release of high amounts of self antigens as a result of tissue destruction caused by the parasite. Release of tissue antigens may stimulate the autoreactivity and autoantibody production. Another possible mechanism is altered host immune responses caused by Leishmania parasites. Both T and B cells are affected by VL (24). VL causes polyclonal B cell activation independent of T cells through mitogen activation or stimulation of cytokines by other immune cells (25, 16). B cell hyper activation results in severe hypergammaglobulinaemia observed in VL. Finally, there are some molecular mimicry between leishmanial antigens and host antigens such as ribosomal phosphoproteins expressed on parasites, causing cross-reactivity (26, 24).

Moreover, even in cutaneous leishmaniasis, which is not characterized by hypergammaglobulinaemia, leishmanial antigens can induce blastogenesis and proliferation responses (22, 27). Specific antibodies against the parasite can be induced by antigens released by promastigotes and amastigotes (28).

Another study on *Leishmania donovani* revealed the induction of a non-specific as well as a specific antibody production, much of which is probably due to the parasite-released substances, which act as B cell mitogens (29, 30). As a consequences of The B cell hyperactivity, *leishmania donovani* may cause hypergammaglobulinaemia and it is common in all patients with Kala-azar due to the large production of IgG, IgM and IgA antibodies, after polyclonal activation of B lymphocytes, generating specific and non-specific antibodies, besides the production of autoantibodies such as ANA and others. On the other hand, the prolonged saturation of the reticuloendothelial system infected by parasites contributes to organomegaly and mainly to splenomegaly, causing cytopenias. And the study conclude that: all patients with positive ANA do not have SLE, splenomegaly is not a common sign in patients with SLE and the VL may present with cytopenias and the production of autoantibodies mimicking SLE (31, 11).

The immune response to the infection caused by Leishmania spp. includes both T-cell and B-cell activation. However the pathogenesis of the disease appears to be related T-cell cytotoxicity, and control of VL depends on the magnitude of T helper 1 (Th1) and multicytokine responses early in the course of infection (32). Indeed, it has been shown during progressive infection in mice that Th2 CD_{4+} yT cells expand and secrete interleukin-4, resulting in polyclonal B cell activation. Later on, in fully established VL, a cellular anergy is established. Inappropriate antigen presentation and communication between the antigen-presenting cells and Tcells, as well as the induction of interleukin-10 and interleukin-4, might be the reason for this anergy (32, 33).

With respect to the aCL antibodies, our findings together with the results obtained by (Harimoto and da Costa, 2009) that out of 45 patients with VL, the anticardiolipin IgG antibody was positive in 17.8 % (n=8) and undetermined in 8.9%(n=4), while among patients with CL, only one (2.2%) tested positive for aCL IgG.

In another study conducted by (34), an Iraqi comparative study on autoantibodies in VL, they showed that 18 (29%) of 62 cases of VL revealed a positive aCL.

We did not found in the literature any studies on anticardiolipin antibodies for comparison. The literature reports the presence of anticardiolipin antibodies in other infectious diseases (35, 36), Repka et al.38 observed those antibodies in 8.34% of patients with paucibacillary leprosy and 80.77% of non-treated patients with multibacillary leprosy. The origin of anticardiolipin antibodies in patients with infections has been widely debated and several hypotheses have been suggested. Among them we should mention: non-specific polyclonal activation of B cells; ability of the infectious agent to bind to endogenous phospholipids making them immunogenic; damage of the endothelium by the infectious agent, exposing phospholipid epitopes that trigger the immunologic response; and cross reaction of anti-DNA antibodies giving rise to anticardiolipin antibodies.

The presence of anticardiolipin antibodies in patients with leishmaniasis is important since those antibodies are related with the etiopathogenic mechanisms of venous and arterial thrombosis (35). In 2006, (37) studied 33 dogs naturally infected by Leishmania infantum and observed that 63.3% of them had antiplatelet antibodies and half of those animals had thrombocytopenia and clinical signs of moderate to severe disease.

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Table 1: Distribution of significantly different ANA autoantibody among studied groups.

ANA autoantibody		Cutaneuos n = 30		Visceral n=30		Controls n=30		Chi-squaree	P. value
		No.	%	No.	%	No.	%		
Anti-dsDNA	Positive	1	3.3	9	30.0	0	0.0	9.43 **	0.015
	Negative	29	96.7	21	70.0	30	100		
Anti-Nucleosome	Positive	5	16.7	16	53.3	0	0.0	11.8 **	0.006
	Negative	25	83.3	14	46.7	30	100		
Anti-SmD1	Positive	0	0.0	4	13.3	0	0.0	4.26 *	0.04
	Negative	30	100.0	26	86.7	30	100		
Anti-PCNA	Positive	1	3.3	9	30.0	0	0.0	9.43 **	0.015
	Negative	29	96.7	21	70.0	30	100		
Anti-SS-A/Ro 60	Positive	0	0.0	9	63.3	0	0.0	12.6 **	0.001
	Negative	30	100.0	21	36.7	30	100		
Anti-SS-A/Ro 52	Positive	1	3.3	7	23.3	0	0.0	3.29 **	0.022
	Negative	29	96.7	23	76.7	30	100		
Anti-SS-B/La	Positive	4	13.3	19	63.3	0	0.0	10.9 **	0.002
	Negative	26	86.7	11	36.7	30	100		
Anti-CENP-B	Positive	0	0.0	5	16.7	0	0.0	5.96 *	0.019
	Negative	30	100.0	25	83.3	30	100		
Anti-Mi-2	Positive	4	13.3	21	70.0	0	0.0	12.4 **	0.001
	Negative	26	86.7	9	30.0	30	100		
Anti-Ku	Positive	12	40.0	24	80.0	0	0.0	10.2 **	0.003
	Negative	18	60.0	6	20.0	30	100		
* (P≤0.05), ** (P≤0.01).									

Table 2 : Distribution of non-significantly different ANA autoantibody   among studied groups.

ANA autoantibody		Cutaneous n=30		Visceral n=30		Controls n=30		Chi-square	P.value
		No.	%	No.	%	No.	%		
Anti-Histone	Positive	7	23.3	10	33.3	0	0.0	2.08 NS	0.56
	Negative	23	76.7	20	66.7	30	100		
Anti-P0	Positive	0	0.0	0	0.0	0	0.0	0.00 NS	-
	Negative	30	100.0	30	100.0	30	100		
Anti-Scl70	Positive	0	0.0	0	0.0	0	0.0	0.00 NS	-
	Negative	30	100.0	30	100.0	30	100		
Anti-U1-snRNP	Positive	3	10.0	7	23.3	0	0.0	2.71 NS	0.29
	Negative	27	90.0	23	76.7	30	100		
Anti-AMA M2	Positive	1	3.3	1	3.3	0	0.0	1.42 NS	0.47
	Negative	29	96.7	29	96.7	30	100		
Anti-Jo-1	Positive	0	0.0	0	0.0	0	0.0	0.00 NS	-
	Negative	30	100.0	30	100.0	30	100		
Anti-PM-Scl	Positive	4	13.3	7	23.3	0	0.0	2.65 NS	0.31
	Negative	26	86.7	23	76.7	30	100		
NS: Non- significant.									

Table 3: Mean value of Anti-Cardiolipin IgG antibodies in studied groups.

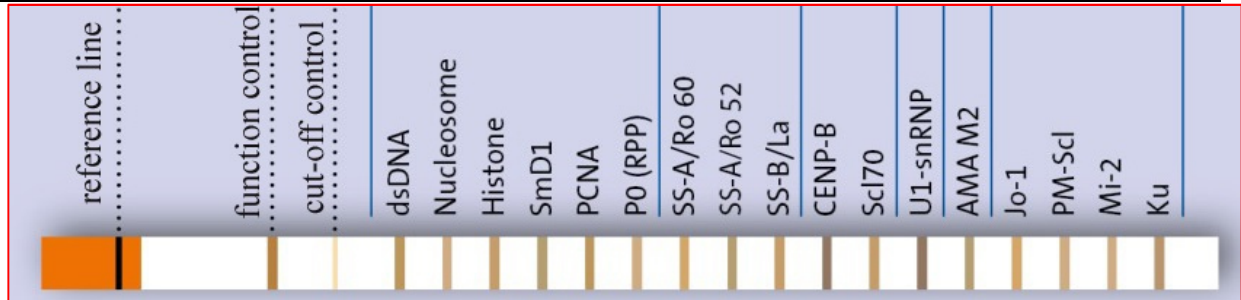| Groups	No.	Mean ± S.E*.	Minimum	Maximum
Cutaneous Leishmaniasis	30	2.402 ± 1.455 [B]	1.081	12.900
Visceral Leishmaniasis	30	8.123 ± 3.828 [A]	1.093	85.108
Control	30	1.959± 0.156 [B]	1.131	4.095
Total	90	4.161 ± 1.306	1.081	85.108
Different letter: Significant difference between means (Duncan test). ** (P≤0.01).				

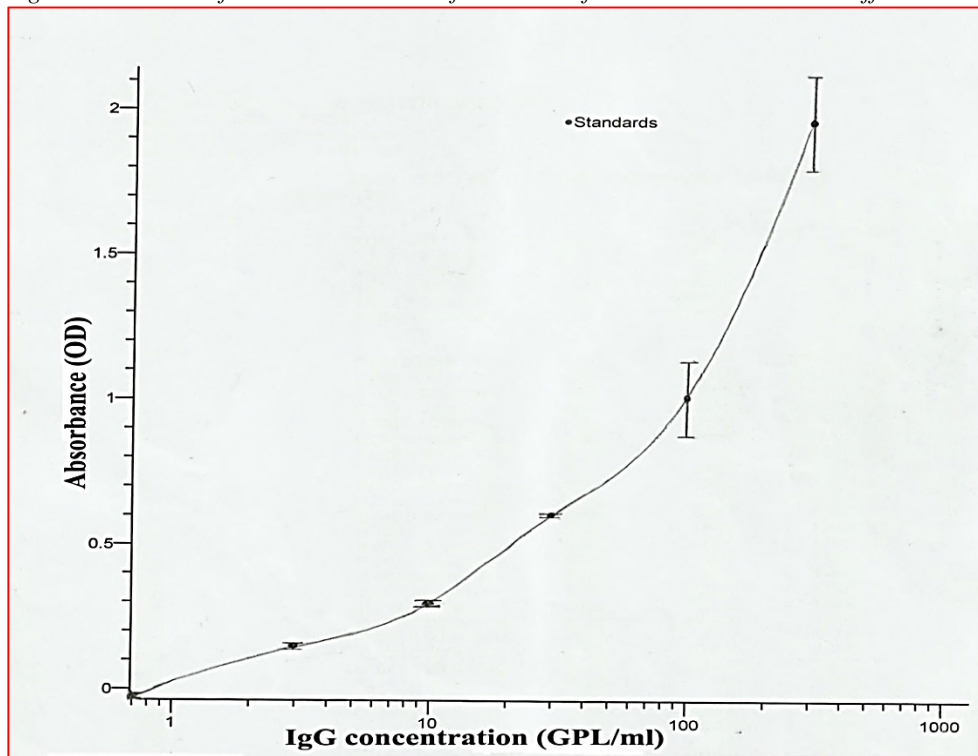*Figure1: Location of the ANA line with reference line function control and cut-off control line.*

Figure 2 : ELISA standard curve of anticardiolipin IgG.