

Original Research Article

Autoantibodies in visceral leishmaniasis-a comprehensive study

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A B S T R A C T

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Iraq is an endemic area for visceral leishmaniasis (VL) (kala-azar), which is caused by the intracellular parasite *Leishmania donovani*. Patients with VL have markedly elevated immunoglobulin levels and associated with existence of autoantibodies against cellular and humoral components. This study aimed to investigate the occurrence of some autoantibodies {anti-cardiolipin antibody (aCL), rheumatoid factor (RF), anti-neutrophil cytoplasmic antibody (ANCA)} in Iraqi children with VL. A total of 135 children with parasitologically and serologically proven visceral leishmaniasis (71 males and 64 females, aged 2 months to 12 years), with 36 healthy children were included in this study. The estimation of aCL by enzyme-linked immunosorbent assay (ELISA), RF by latex agglutination test (LAT) and ANCA by immunofluorescence (IF) technique was carried out. 18 (29%) of 62 cases of visceral leishmaniasis and 1 (4.2%) of 24 healthy children revealed positive aCL. Positive rheumatoid factor (RF) was detected in 21 (26.9%) of 78 kala-azar patients and in 1 (3.3%) of 30 healthy children. Three (10.7%) of 28 VL cases showed positive (ANCA) of cytoplasmic pattern (c-ANCA), but none of the 18 healthy children showed positive ANCA. There is a positive correlation between VL and aCL or RF, but there was no correlation between age, sex and community of children with VL and aCL or RF.

Introduction

Visceral leishmaniasis is a vector borne disease caused by obligate intra-cellular protozoa of the genus *Leishmania*, and capable of causing spectrum of clinical syndromes affecting millions of people in endemic areas of the tropics and subtropics, in which the amastigotes replicate in macrophages of the

mononuclear phagocyte system and then spread to the entire reticuloendothelial system (Herwaldt, 1999; Kafetzis, 2003). A common feature of autoimmunity is the presence of autoantibodies. Two types of autoantibodies have been described; the pathogenic autoantibodies that is associated with the autoimmune disease,

and the so-called natural autoantibodies. The latter are present in all normal individuals. Both the pathogenic and the natural autoantibodies can be detected at higher frequencies among individuals exposed to viral, bacterial and parasitic infections (Baniel-Ribeiro and Zanini, 2000).

The idea that the infectious agents may represent one of the major environmental factors initiating auto-immune responses is now accepted and the mechanisms of induction are currently being re-examined (Camacho et al., 005). Patients with VL have markedly elevated immunoglobulin levels, though much of this immunoglobulin is not *Leishmania* specific, and the elevated immunoglobulin (specific and non-specific antibodies) in VL may be expressed as hypergammaglobulinaemia (Argov et al., 1989).

Cardiolipin Antibody (aCL): Anti-cardiolipin antibody is one of the antiphospholipid antibody groups that is associated with antiphospholipid syndrome (Wilson et al., 1999). Anti-cardiolipin antibodies are directed against cardiolipin and they are strongly associated with venous and arterial thrombosis, both in patients with systemic lupus erythematosus (SLE) and in patients without any apparent autoimmune disease (Mohammed et al., 2008). Anti-cardiolipin antibody has been reported in a number of infections including parasitic diseases such as African trypanosomiasis, schistosomiasis, filariasis and malaria (Consigny et al., 2002).

Rheumatoid Factor (RF): Rheumatoid factors are antibodies that react with IgG heavy chain epitopes in the interface of $\gamma 2$ - $\gamma 3$ domains. These autoantibodies are

more frequently found in rheumatoid arthritis, but they may be observed in other autoimmune diseases, mainly Sjögren syndrome, SLE, systemic sclerosis, juvenile rheumatoid arthritis and polymyositis, and also in some infections caused by: viruses (as viral hepatitis and infectious mononucleosis), bacteria (as tuberculosis and leprosy) or parasites (as VL) (Westwood et al., 2006; Dorner et al., 2004).

Anti-neutrophil Cytoplasmic Antibody (ANCA): Anti-neutrophil cytoplasmic antibodies are circulating autoantibodies directed mainly toward constituents of neutrophil cytoplasmic granules and monocyte lysosomes, and have been initially reported in patients with primary systemic vasculitis (Kager and Rees, 2003) By indirect immunofluorescent technique, two staining patterns of ANCA are described [cytoplasmic (c-ANCA) and perinuclear (p-ANCA)]. The major c-ANCA target antigen is proteinase 3, a serine protease. The major p-ANCA target antigen is thought to be myeloperoxidase (Kager and Rees, 2003).

Materials and Methods

135 diagnosed patients of kala-azar (71 males and 64 females), aged 2 months to 12 years, by two serological tests (rK39 strip test and IFAT) and/or microscopic examination of bone marrow smear were included in this study. A total of 36 age and gender-matched healthy children, with no history of kala-azar served as controls. Immunological evaluation for Autoantibodies: Anti-cardiolipin Antibody (aCL) by ELISA; Rheumatoid Factor (RF) by LAT and 3- Anti-neutrophil Cytoplasmic Antibody (ANCA) by IFAT was performed.

Results and Discussion

On evaluation of anti-cardiolipin antibody (aCL) seropositivity (total IgG, IgM & IgA) in VL cases and healthy controls by ELISA, 18 (29%) of 62 VL cases showed positive aCL (concentration > 10 U/ml), while only 1 (4.2%) of 24 healthy children showed positive aCL. The frequency of positive aCL, 10/30 (33.3%) in females was higher than in males, 8/32 (25%), while only one healthy female child, 1/24 (4.2%) showed positive aCL. The age of VL patients with positive aCL ranged from 5 months to 6 years, with the highest frequency detected within the first year of age. (Table 1).

Urban kala-azar patients showed the highest frequency of positive aCL cases, 4/10 (40%), followed by semirural-suburban patients, 10/32 (31.2%), while the lowest frequency was seen in rural patients, 4/20 (20%).

21 (26.9%) of 78 kala-azar patients revealed positive RF as compared to 1 (3.3%) of 30 healthy children by latex agglutination test. Males showed higher frequency, 12/41 (29.3%) of positive RF than females, 9/37 (24.3%), while only one healthy male child, 1/15 (6.7%) showed positive RF.

The highest frequency of positive RF cases, 4/13 (30.8%) was detected among urban patients, followed by semirural-suburban patients, 11/40 (27.5%), while the lowest frequency was seen in rural patients. 6/25 (24%).

3 (10.7%) of 28 VL cases showed positive ANCA by IF and all the 3 positive cases showed c-ANCA staining pattern. (Figure 1 and Table 2) No positive ANCA result was detected among the 18 healthy

controls. There was a slightly higher frequency of positive ANCA among male VL cases, 2/18 (11.1%) than female cases, 1/10 (10%). No urban patient was detected with positive ANCA while 2/14 (14.3%) semirural-suburban patients and 1/10 (10%) rural patients showed positive ANCA.

The presence of various autoantibodies observed in the present study could be explained by several mechanisms, such as: release of sequestered antigens by parasite-induced lysis of host cells, cross-reactivity between parasite and host tissue antigens, polyclonal B cell activation, cytokine stimulation and over-expression of major histocompatibility complex molecules (Baniel-Ribeiro and Zanini, 2000; Kager and Rees, 2003; Bohme et al., 1980). Also antigen modification by adsorbing self-antigens of parasite material to surrounding host cells are reported (Kafetzis, 2003; Bohme et al., 1980).

The study revealed a positive correlation (Yates corrected $X^2 = 4.85$, $p = 0.0275$) between the disease and aCL. Our result was discordant with Santiago et al. who reported 6% and 3% frequencies of IgG aCL and IgM aCL respectively (Santiago et al., 2001). The difference in the results of aCL seropositivity in VL patients may be attributed to genetic and environmental factors (Dighiero G and Rose, 1999; Klareskog).

Santiago et al reported the prevalence of IgG and IgM aCLs to be 6% (2/30) and 3% (1/30) respectively after measuring of aCLs by ELISA in patients with kala-azar. Voulgarelis et al,(2003) described three cases of VL with positive ANCA, aCL, VDRL, RF and direct Coomb's test, which subsided after treatment, and they

Figure.1 c-ANCA staining pattern on neutrophil cells (40X).

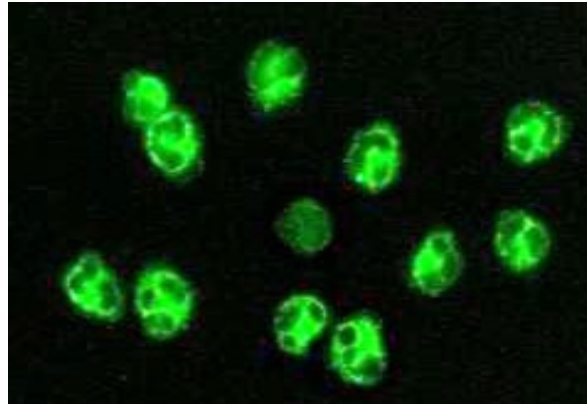


Table.1 Age distribution of aCL seropositivity in vl cases.

Visceral Leishmaniasis		aCL			
Age in years	No of Cases	>10 U/ml Positive	Percentage	≤10 U/ml Negative	Percentage
<1	25	9	36	16	64
1-2	20	6	30	14	70
2-3	10	2	20	8	80
3-4	3	0	0	3	100
4-6	4	1	25	3	75

Table.2 Anca sero-positivity in vl cases and healthy controls according to staining pattern

ANCA PATTERN		VL PATIENTS		HEALTHY CONTROLS	
		No of Cases (28)	Percentage	No of Cases (18)	Percentage
c-ANCA	Positive	3	10.7	-	-
	Negative	25	89.3	18	100
p-ANCA	Positive	-	-	-	-
	Negative	28	100	18	100
Total ANCA	Positive	3	10.7	-	-
	Negative	25	89.3	18	100

suggested that leishmaniasis should be taken into consideration in the differential diagnosis of autoimmune disorders (Voulgarelis et al., 2003). Sakkas et al

reported positive aCL in one of their six patients with VL (Sakkas et al., 2008).

A positive correlation (Yates corrected $X^2 = 6.05, p = 0.0139$) was noted between the

disease and RF in this study, a finding concordant with Atta et al. (2007) and Sakkas et al (2008). Grimaldi and Tesh stated that hypergammaglobulinemia, RF and circulating immune complexes suggesting polyclonal B-cell activation, are characteristic features of VL (Grimaldi G and Tesh, 1993). Atta et al found that VL is associated with important IgM-RF production and anti-cyclic circullinated peptide antibody (CCP-Ab) synthesis. Sakkas et al, reported IgM-RF positivity in 4 out of 6 patients with VL.

The difference of positive ANCA was not statistically significant (Yates corrected $X^2 = 0.68$, $p = 0.4046$) between the patients and healthy controls with no correlation between VL and ANCA in this study, a finding discordant with results obtained by Kager et al. Kager et al reported positive cases of parasitologically proven VL with antibodies against granulocytes but no positive case with antibodies against lymphocytes. In other study, Pollack et al, reported positive membrane-associated anti-platelet, anti-neutrophil and anti-erythrocytic IgG antibodies in patients with VL, by immunofluorescence and antiglobulin (Coomb's) test.²⁰

A positive correlation was noted between Visceral Leishmaniasis and aCL or RF, but no correlation between age, sex and community of children with VL and aCL or RF was seen.

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