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RESEARCH ARTICLE

Auto-antibodies Profile in Children Infected with Visceral Leishmaniasis

Ali Hafedh Abbas[1], Entsar Jabbar Saheb[2*], Israa Salim Mussa[2], Dunya Fareed Salloom[2], Dalia Muafaq[3], Raghad Ibrahim[3]

1. *University of Baghdad, College of Science, Tropical, Biological Research Unit /Iraq.*
2. *University of Baghdad, College of Science, Biology Department/Iraq.*
3. *Central Public Health Laboratories, Serology Department/Iraq.*

***Corresponding Author Email:** *ejsaheb@ualr.edu*

Abstract

Visceral leishmaniasis (VL) is a parasitic infection caused by an intracellular growth of *Leishmania* spp. in macrophage cells. The autoimmune disorder is a condition takes place when the immune system produces antibodies which incorrectly attacked its own body tissues. VL has been involved as an effect or on the autoimmune aspect. This study was conducted to identify the auto antibodies profile in patients infected with VL. The presences of auto antibodies in 21 Iraqi children infected with VL were tested for laboratory autoimmune aspect. The highest percentage of seropositive in *Leishmania* patients was observed for anti-ds DNA, anti-Mi-2, anti-Ku and anti-PCNA antibodies (90.5%, 90.5% , 90.5% and 61.9%) respectively, while the lowest percentage was recorded for anti-Histone, anti-R0(RPP), anti-SS-A/Ro 52, anti-Scl 70, anti-Jo-1 antibodies (4.8%) while the auto antibodies (anti-SmD1, anti-SS-A/Ro-60, anti-SS-B/La, anti-CENP, anti-U1snRNP, anti-AMA M2, anti-PM/Scl) showed different profiles in *Leishmania* patients. These results provide evidence that VL can have an effective role in the production of the auto antibodies.

Keywords: *Children, Leishmania spp, Visceral leishmaniasis, Auto antibodies.*

Introduction

Visceral leishmaniasis (VL) is a parasitic infection caused by an intracellular growth of *Leishmania* species in macrophages [1]. It is categorized among the greater importance of parasitic diseases listed by the WHO [2]. Leishmaniasis has a diversity of pathological symptoms with three clinical forms, cutaneous, mucocutaneous and visceral leishmaniasis [3]. *Leishmania spp*. infection is transmitted to their host (mammalian) through the bite of the sand fly female [4].

The life cycle of *Leishmania* spp. includes a series of development processes; the promastigotes and the metacyclic promastigotes (the infectivestage) in the sand fly vector, which then develop into amastigote stage in the mammalian host [5, 6].Following biting by the sand fly into their mammalian host, *Leishmania* parasites face several immune cells in the location of infection [7], these cells release chemokines, which in turn cause the stimulation of inflammatory cells [8].These consequences cause dramatic changes in cellular and humoralimmune response including deficiency of Interferon gamma (IFN-γ), increased production of Tumor necrosis factor-α (TNF-α) and other interleukins, besides polyclonal hypergammaglobulinemia [9, 10]. The analysis of sera with *Leishmania* infection stimulates the production of auto antibodies against cellular and humeral components, besides circulating immune complexes and anti-IgG antibodies [11].

The main signs observed in VL infection includes plenomegaly and hepatomegaly, fever, weight loss, tachycardia, coughing, bleeding of the gums, myalgia, arthralgia, and adenopathy[12, 13].However, VL may combine with clinical autoimmune indicators including increased the levels of rheumatoid factor (RF), antinuclear antibodies (ANAs), rate of cryoglobulins, and low serum complement levels[14,15].

The production of auto antibodies, such as anti-Sm, anti-RNP, anti-SSA, anti-SSB, and antiphospholipid, has found in patients infected with *Leishmania*. The induction of auto antibodies in leishmaniasis can be attributed to the exposure to antigens that previously hidden during tissue damage and breakage of host cells [16].

In this study, the association between the auto antibodies and VL was tested to reveal whether these leishmanial infections correlate with autoimmune manifestations via investigating the association of IgG auto antibodies against 17 antigens including anti-ds DNA, anti-nucleosome, anti-histones, anti-SmD1, anti-PCNA, anti-ribosomal P0, anti-SS-A/Ro 60, anti-SS-A/Ro 52, anti-SS-B/La, anti-CENP-B, anti-Scl70, anti-U1-snRNP, anti-AMA M2, anti-Jo1, anti-PM/Scl, anti-Mi and anti-Ku antibodies.

Subjects, Materials, and Methods

The study was permitted by the ethical committee of Iraqi Ministry of Health, in which 21 leishmanial patients were enrolled. Their age range was 3 – 12 years (7.2 ± 1.6 years). They were checked at the Consultant Clinic in Department of Central Child Hospital (Baghdad, IRAQ) from January 2017 to July 2017 for diagnosis and treatment. The diagnosis was made by the consultant medical staff, which was based on clinical evaluation and laboratory examination.

In addition to patients, 20 apparently healthy persons were also involved in this study as a control group, which they matched patients for age. Sera of patients were first assessed for anti-leishmanial IgG antibody by using a commercially available kit (*Leishmania* ELISA IgG). The principle of this kit was based on the sample antibodies reaction with antigen adsorbed on a polystyrene surface.

Then, were surveyed for 17 autoantibodies which were detected by ANA-LIA MAXX kit (Human Company, Germany).The principle of this kit was based online immune assay on a nitrocellulose membrane. These autoantibodies included: anti-dsDNA, anti-nucleosome, anti-histones, anti-SmD1, anti-PCNA, anti-ribosomal P0, anti-SS-A/Ro 60, anti-SS-A/Ro 52, anti-SS-B/La, anti-CENP-B, anti-Scl70, anti-U1-snRNP, anti-AMA M2, anti-Jo1, anti-PM/Scl, anti-Mi and anti-Ku antibodies. The Win Pepi computer program version 11.65 was used to study the significant differences. Chi-square, Fischer exact probability, Odd ratio and 95% CI were used to a significantcomparison between the studied groups.

Results and Discussion

The sera of leishmanial patients were positive for anti-leishmanial antibody test (100.0%), while none of the control sera was positive for this antibody; therefore, the diagnosis of *Leishmania* was determined. Most researchers confirm that sera of leishmanial patients display positive reaction for anti-leishmanial antibody [14-16].

The sera of leishmanial patients and controls then were tested for 17 auto antibodies. They were anti-dsDNA, anti-nucleosome, anti-histone, anti-smD1, anti-PCNA, anti-P0, anti-SS-A/Ro-60, anti-SS-A/Ro-52, anti-SS-B/La, anti-CENP, anti-SCI-70, anti-U1snRNP, anti-AMA-M2, anti-Jo-1, anti-PM-SCI, anti-Mi2 and anti-Ku antibodies. The sera of control subjects were negative for these antibodies.

The highest percentage of seropositive of leishmanial patients was observed for anti-dsDNA, anti-PCNA, anti-Mi-2 and anti-Ku antibodies (90.5%, 61.9%, 90.5% and 90.5%) respectively, while a lowest percentage was recorded for anti-Histone, anti-R0(RPP), anti-SS-A/Ro 52, anti-Scl 70, anti-Jo-1 antibodies (4.8%, for these auto antibodies).

However, auto antibodies (anti-SmD1, anti-SS-A/Ro-60, anti-SS-B/La, anti-CENP, anti-U1snRNP, anti-AMA M2 and anti-PM/Scl) showed different profiles in leishmanial patients. The results revealed a significant increase frequency percentage in leishmanial patients compared to controls with anti-dsDNA (90.5 *vs*. 0.0%, $p = 9.4x10^{-10}$), anti-PCNA (61.9 *vs*. 0.0%, $p = 1.6x10^{-5}$), anti-Mi-2 (90.5 *vs*. 0.0%, $p = 9.4x10^{-10}$), anti-Ku (90.5 *vs*. 0.0%, $p = 9.4x10^{-10}$) and anti-PM/Scl(23.8 *vs*0.0%, $p = 0.048$) (Table 1).

Table 1: Observed numbers and percentage frequencies of positive sera for autoantibodies in leishmanial patients and controls

Autoantibodies	Leishmanial Patients (No. = 21)		Controls (No. = 20)		Odds Ratio	Etiological Fraction	*p*	95% Confidence Interval
	No.	%	No.	%				
dsDNA	**19**	90.5	0	0.0	**319.80**	0.88	**$9.4x10^{-10}$**	15.53-6585.62
Nucleosome	3	14.3	0	0.0	7.76	0.14	0.232	0.40-149.18
Histone	1	4.8	0	0.0	3.0	0.05	1.5	0.12-72.21
SmD1	6	28.6	0	0.0	17.19	0.28	0.21	0.96-306.39
PCNA	**13**	61.9	0	0.0	**65.12**	0.60	**$1.6x10^{-5}$**	3.72-1140.82
P0 (RPP)	1	4.8	0	0.0	3.0	0.05	1.0	0.12-72.21
SS-A/Ro 60	2	9.5	0	0.0	5.26	0.09	0.488	0.26-108.24
SS-A/Ro 52	1	4.8	0	0.0	3.0	0.05	1.0	0.12-72.21
SS-B/La	2	9.5	0	0.0	5.26	0.09	0.488	0.26-108.24
CENP-B	6	28.6	0	0.0	17.19	0.28	0.21	0.96-306.39
Scl 70	1	4.8	0	0.0	3.0	0.05	1.0	0.12-72.21
U1-snRNP	6	28.6	0	0.0	17.19	0.28	0.21	0.96-306.39
AMA M2	2	9.5	0	0.0	5.26	0.09	0.488	0.26-108.24
Jo-1	1	4.8	0	0.0	3.0	0.05	1.0	0.12-72.21
PM-Scl	**5**	23.8	0	0.0	**13.67**	0.23	**0.048**	0.76-247.29
Mi-2	**19**	90.5	0	0.0	**319.80**	0.88	**$9.4x10^{-10}$**	15.53-6585.62
Ku	**19**	90.5	0	0.0	**319.80**	0.88	**$9.4x10^{-10}$**	15.53-6585.62

p: Two-tailed Fisher's exact probability

The autoimmunedisorder is a condition that occurs when the immune system produces antibodies which incorrectly attacked its own body tissues. The starting invasion of the body's self-molecules in autoimmune diseases is caused due to many factors including genetics, infections and environment [17].

The auto antibodies production mechanisms involve two possibilities, when any viral, bacterial or parasitic infection occur; either by monoclonal lymphocyte secretion specific autoantibodies, or by stimulation of B lymphocytes that secret natural auto antibodies [18]. Several possibilities can explain the development of auto antibodies in VL. Hypergammaglobulinemia is present in all patients with VL due to the increased production of IgG, IgM, and IgA antibodies, after polyclonal activation of B lymphocytes, generating specific and non-specific antibodies.

However, under normal conditions auto antibodies is expressed at low levels. Molecular mechanism between *Leishmania* antigens and ribonucleoproteins is another hypothesis which including the production of autoantibodies such as anti-Sm, anti-RNP, anti-SSA, anti-SSB, and antiphospholipid, in patients with *Leishmania* infection. A third mechanism for the stimulation of autoantibodies in leishmaniasis can be due to the release and exposure of antigens that previously hidden during tissue destruction and breakage of the host cells [19].In contrast, *Leishmania* parasites themselves cause tissue damage and release self-antigens, which in turn may stimulate autoreactivity [20]. The current results are agreed with findings of Liberopoulos *et al*. [20], Nozzi *et al*. [21], Al-Assaf and Al-Ameri [22] and Pouladfar *et al*. [23]. They referred to the highest frequency percentage presence of autoantibodies such as anti-dsDNA, anti-PCNA, anti-Mi-2 and anti-Ku antibodies in VL patients.

The molecular mimicry of self-ribonucleoprotein and leishmanial antigens is one possible mechanism that clarifies the presence of these autoantibodies in the sera of leishmanial infection patients, which play avital role in the production of autoantibodies against leishmanial antigens and some autoimmune diseases such as SLE [24, 25].Moreover, [21, 26] confirmed the relationship between the VL infection and autoimmune diseases progress such as SLE. Patients with VL can be primarily misdiagnosed as having an autoimmune disease (especially SLE), therefore possibly being treated with immunosuppressive drugs.

In conclusion, the results of this study agree with [20] that VL has to be diagnosed in patients with the autoimmune disorder, mainly in endemic areas, before treatment with the immunosuppressive medications because VL can simulate the pre-existing autoimmune disease.

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