Determination of Immunoglobulin's levels in cutaneous Leishmainasis patients

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Abstract :

Human leishmaniasis is distributed worldwide, butmainly in the tropics and subtropics, with a prevalenceof 12 million cases and an approximate incidence of 0.5million cases of VL (visceral leishmaniasis) and 1.5 million cases of cutaneousleishmaniasis (CL) *Leishmania*species are intra-cellularparasites invading monocytes, macrophages, and langerhans celland begin the differentiation process into amastigotes, the parasite form which persists in the host. Theirinfection in man induces both humoral andcellular immune responses, but the balanceof their expression varies with the type of the disease**.** To identify the profile of autoantibodies levels of patients with cutaneous leishmaniasis, this study showed that a significance changes of immunoglobulin levels as high level, especially in IgG and no significance value for elevation of IgM and IgA.

Key words: cutaneous leishmaniasis, immunoglobulins.

Introduction:

Leishmaniasis is a group of vector-borne diseases caused by obligate intracellular protozoan parasites of the genus *Leishmania* ,which transmission primarily occurs via the bite of infected female sandflies, which inoculate *Leishmania* promastigotes into mammalian hosts. *Leishmania* species are intra-cellular parasites invading monocytes, macrophages, and langerhans cell and begin the differentiation process into amastigotes, the parasite form which persists in the host . The infection in man induces both humoral and cellular immune responses, but the balance of their expression varies with the type of the infection. Disease outcome is dependent onboth the host's immune status and the species of parasite. Clinical manifestation can rangefrom self-healing cutaneous lesions to fatal disseminated visceral disease [1,2].Specific antibodies are proteins which, in view of their importance for immunity, are called immunoglobulins; they are localized in the fi- and y-globulin fractions of the serum. On the basis of differences in physico-chemical properties and antigenic structure, five different immunoglobulin classes can be distinguished today, viz. IgM, IgG, IgA,IgD and IgE these immunoglobulin classes differ also inbiological activity[3]. The immunecellular response for the control of the *Leishmania* infection is related to the generation of T- helper cells by the host, which is able to deliver macrophageactivating cytokines, particularly gamma interfere on and interleukin-2. In addition, the humoral immune response occurs during the active phase of the infection with the appearance of low titers of antibodies (Ab), which may disappear later .Serological methods have been used over theyears in the diagnosis of ACL (Anthrobonotic cutaneous leishmanaisis). However, the diagnostic value of the assay using crude antigens is considered to be limited because of their low reproducibility and specificity . Despite this, there are serological tests still used in many diagnostic centers for detecting the prevalence of *Leishmania*s is in endemic areas . Amongthese the direct agglutination test (DAT), the indirect immunofluorescence antibody test (IFAT) and ELISAare some of the most commonly used tests[4,5]. Human visceral leishmaniasis(VL) is marked by high levels of *Leishmania*-specific antibodies which appear soon after infection and before the development of cellular immunologic abnormalities.While the antibody titers in kala-azar have been exploited for specific diagnosis, their role in resolution of disease and protective immunity is largely unknown. It is, however, evident that resistance in a large population of individuals residing in areas of endemicity is detectable only by the development ofspecific antibodies and/or T-cell response to leishmanial antigens[6] .VL patients showed significantly higher IgG responses (93-94%sensitivity, 93-97% specificity), and higher (but not significantly) IgM responses and low IgA levels[7]. Serological tests donot show a good performance in cutaneous leishmaniasis because sensitivity can be variable and because the number of circulatingantibodies tends to be low and the specificity is the most critical point in leishmaniasis diagnosis[8].

The aim of the study was to identify the profile of autoantibodies levels of patients with cutaneous leishmaniasis.

Material and Methods :

This study was conducted at Tikrit Teaching Hospital in Tikrit,during the period from November 2013 to April 2014. sixty peoples with CL lesion patients , were included.Their age ranged from 1-63 years.The clinical diagnosis was confirmed by laboratory demonstration of the parasite in the lesions by direct smears, the edge of the lesion were cleaned and surrounding skin with sterile gauze or cotton wool soaked in 70% ethanol or isopropanol, the alcohol must not be allowed to enter the open part of the lesion ,allowed to dry before proceeding further, then 0.1-0.2 ml of sterile saline or PBSS was injected into the edge of the lesion from 1 ml syringe fitted with a

short needle (20 gauge). then the needle was rotated 2-3 times whilst it is in the skin.(this cut small pieces of tissue from the edge of the needle wound) and applying gentle suction until pink-tinged tissue juice was noted in the hub of the syringe. Exudate materials was smeared, dried in air and fixed by methanol. The smears were stained with Giemsa's stain and examined by light microscope. Microscopic diagnosis was made when amastigotes were identified in the smears[9,10]. In order to confirm the diagnosis, the material was also cultured on RPMI 1640 medium for up to three weeks to detect the leishmanial promastigotes. Blood samples were collected from each patients ,after collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 15-30 minutes. the clot was removed by centrifuging at 1,000-2,000 x g for 10 minutes in a centrifuge. The resulting supernatant is designated serum. Following centrifugation, it is important to immediately transfer the liquid component (serum) into a clean polypropylene tube using a Pasteur pipette. The samples stored at –20°C or lower[11].

Determination of the lgG, IgM, IgA proteins by radial immunodiffusion plate:

-Principle of test:

The examined protein,diffusing in agarose gel containing a specific antibody will form animmuno-complex ,visible as a ring around the well. The ring diameteris direct proportional the concentration of the analyzed protein. The proportion corresponds to the diffusion time. Infact, at the end (72h), the square of diameter will be in linear proportion to the concentration of the sample. With the plate is supplied are ferencetable in which each diameter of the halo is associated a concentration .

-Procedure:

The plates were removed from its envelope and leaved to stand at room temperature for few minutes so that any condensed water in the wells can evaporated. the wells were filled with 5 µl of sample and/or controls and waited, it has been completely adsorbing before handling the plate. The plate were closed and placed in a moist chamber for 72 hours[12].

Results:

The precipitating ring were measured with an appropriate ruler or measuring lens however a system which provides a maximum error of 0.'1mm. the reading were on enclosed reference table the concentration value corresponding to the precipitating ring diameter. The control serum, to be used always, give a ring which differs by a maximum of 0.2 mm from the value reported in the table. As shown in table (1): the immunoglobulin levels of samples according to age, the highest percentage were included as normal levels in different types of immunoglobulin and different ages ,followed by low levels of IgA in 1-4,10-14 age groups.Figures (1,2,3): shows the different levels of IgG,IgM, IgA immunoglobulin according to age as a precipitating rings of immunoglobulin which were measured by an appropriate ruler and the reading compared with the standard measuring in a combined tables of test [13].

Table (1): levels of IgG, IgM, IgA immunoglobulin in patients with cutaneous leishmaniaisis according to age groups.

Age Groups	Type of Immunoglobulin's								
	IgG No.(%)			IgM No.(%)			IgA No.(%)		
	High	Normal	Low	High	Normal	Low	High	Normal	Low
1-4	1(1.66)	13(21.66)	1(1.66)	2(3.33)	12(20)	1(1.66)	3(5)	8(13.33)	4(6.66)
5-9	2(3.33)	10(16.66)	3(5)	1(1.66)	11(18.33)	3(5)	3(5)	9(15)	3(5)
10-14	1(1.66)	10(16.66)	4(6.66)	1(1.66)	14(23.33)	0	3(5)	8(13.33)	4(6.66)
Adults	1(1.66)	14(23.33)	0	2(3.33)	12(20)	1(1.66)	1(1.66)	13(21.66)	1(1.66)
Total	5(8.33)	47(78.33)	8(13.33)	6(10)	49(81.66)	5(8.33)	10(16.66)	38(63.33)	12(20)
Chi^2	6.685			4.854			4.989		
P-value	0.051			0.562			0.545		
Significance	significant			Non			Non		

Figure (1): shows the precipitating rings of IgG immunoglobulin

Figure (2): shows the precipitating rings of IgM immunoglobulin

Figure (3): shows the precipitating rings of IgA immunoglobulin

Discussion:

Human leishmaniasis is distributed worldwide, butmainly in the tropics and subtropics, with a prevalenceof 12 million cases and an approximate incidence of 0.5million cases of VL and 1.5 million cases of cutaneous leishmaniasis (CL)[14]. Cutaneous leishmaniasis (CL) is caused by parasitic infectionof dermal macrophages resulting in intense immune mediated tissue inflammation and skin ulceration. The severity of the disease is dependent on parasite species as well as the immune responses evoked by the host. Most cases of CL heal spontaneously. In rare cases, the ulcer/sbecome chronic, and some Leishmania species may induce mucosal leishmaniasis (MCL) leading to severe tissue damage.Due to difficulties in obtaining skin tissue, most human studies of CL have been limited to the analysis of peripheral blood. While systemic responses may be good correlates ofimmunity, tissue damage and local immune responses at thesite of infection is seldom reflected in alterations in the peripheral blood [15].as seen in table (1),there were no significance change of immunoglobulin levels and 78.333% of IgG included as normal levels and 81.666% of normal IgM level, followed by 63.333% of IgA. While the elevated level of IgG, IgM and IgA were 8.333%,10%,and 16.666% respectively this may be due to Patients with cutaneous leishmaniasis (CL) often have low or no Leishmania antibodies, because of the localized character of thedisease, and thus serological tests are mostly negative[16], and serological tests are rarely used in CL diagnosis because sensitivity can be variable and because the number of circulating antibodies against CL-causing parasites tends to be low . The specificity can also be variable, especially in areas where cross-reacting parasites are prevalent[17].

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تحديد مستويات الكلوبيولينات المناعية في المرضى المصابين اللشمانيا الجلدية

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الملخص

تتوزع إصابات اللشمانيات الجلدية في الإنسان على مستوى العالم ، وبالأخص المناطق الاستوائية وشبه الاستوائية وبنسبه انتشار 12 مليون خمج مع نسبة حدوث الخمج ب 0.5 مليون لحالات اللشمانيا الحشوية و 1.5 مليون خمج للشمانيا الجلدية. يعتبر طفيلي اللشمانيا طفيلي داخل خلوي حيث يهاجم خلايا وحيده النواة والخلايا البلعمية وخلايا لانكرهانز الموجودة في الجلد وتتميز إلى طور العديم السوط وهو الطور الذي يبقى ساكنا داخل خلايا المضيف .وتسبب الإصابة بهذا الطفيلي تحفيز كلا النوعين من المناعة الخلطية والخلوية وتختلف نسبة الاستجابة حسب نوع الخمج . ولغرض تحديد مستوى الأضداد الخلطية في مرضى اللشمانيا الجلدية ,في هذه الدراسة وجد إن هناك ارتفاع واختلاف معنوي في مستوى الأضداد من النوع IgG وعدم وجود اختلافات معنوية في نسب الأنواع الأخرى .

الكلمات المفتاحية : اللشمانيا الجلدية , الكلوبيولينات المناعية