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Full Length Research Paper

Complement C1, C3 and C4 levels in sera of leishmaniasis cases in Turkey

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Leishmaniasis is still a problem for many countries including developed ones. The subgroups of the serum complements have significant roles on the onset of the infection. The aim of this study was to determine the serum complement (C1, C3, C4) levels of cases with definite diagnosis of leishmaniasis and compare them with healthy controls. The study group included 43 visceral leishmaniasis (VL) and 13 cutaneous leishmaniasis (CL) cases. Two control groups were formed for the study. The first control group included 50 individuals of the same age group having no health complaints, admitted for routine control and found to be serologically negative. The second control group included 28 individuals, consistent with the diagnostic criteria of VL. The serum complement levels of C1, C3 and C4 were measured by the nephelometric method. The average levels of C1 were found to be high in VL cases (78.98%) and low in CL (41.69%) group, which was statistically significant (p=0.011). In addition, the average levels of C3 were high in CL group (96.08%) and low in VL group (62.00%), which was also statistically significant (p=0.010). C4 levels were found to be high in the control group, while similar in VL and CL groups. C1, C3, C4 levels were found to be lower in the first control group of healthy individuals. The levels of the subgroups of complement system show statistically significant in both VL and CL cases, which suggested that they could be useful in verifying the results of the serological examinations.

Key words: Leishmaniasis, complement levels, C1, C3, C4.

INTRODUCTION

The immune system is an organization of cells and molecules with specialized roles in defending against infection. There are two fundamentally different types of responses to invading microbes. Innate (natural) responses occur to the same extent; however, many times the infectious agent is encountered, whereas acquired (adaptive) responses improve on repeated exposure to a given infection. The innate responses use phagocytic cells (neutrophils, monocytes, and macrophages), cells that release inflammatory mediators (basophils, mast cells, and eosinophils), and natural killer cells. The molecular components of innate responses include complement, acute-phase proteins, and cytokines such as the interferons (Delves et al., 2000; Rus et al., 2005).

The complement system contains more than 20 proteins, present in sera of vertebrates, which could cause cytolysis via binding to antigen-antibody complex. The soluble and inactive proteins of the complement system comprise 10% of total protein amount in the serum. The most significant and abundant component of the complement system is C3. The biologically active forms of C3 are its proteolytic cleavage products (Gülmezoğlu et al., 1994; Kiliçturkay, 2003; Delves et al., 2008).

In the early steps of the “classical pathway”, antibody molecules that are complexed with specific antigen

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sequentially bind and proteolytically activate three complement proteins, called C1, C4 and C2 (Abbas et al., 2007).

There is no specific antigen-antibody complex in the alternative pathway. The activation of the alternative pathway is initiated via the degradation of C3, which requires the formation of C3 convertase. The alternative pathway includes five proteins-Factor B, D, H, I and propertin (Abbas et al., 2007).

The ability of fresh serum to lyse *Leishmania* promastigotes was observed as early as 1912. In 1926, Hindle reported that sera of patients with Kala-azar, as well as non-immune individuals, were capable of killing *Leishmania donovani* promastigotes. This activity was shown to be heat-sensitive, suggesting a role for complement in this process. A more thorough examination of the mechanism of complement activation by promastigotes has revealed that *Leishmania* can activate complement via the alternative pathway (Brittingham et al., 1996).

Leishmania are obligate intracellular parasites of mononuclear phagocytes. Infection with *Leishmania* triggers an acute inflammatory response, in which cells and molecules of the immune system move into the affected site. The activation of complement generates C3b, which coats the surface of *Leishmania*. This event enhances phagocytosis of the microbe, because phagocytic cells have cell surface receptors for C3b. *Leishmania* parasites can use this response to attach and enter to the phagocytic cells. Activation of complement also plays an important role in the intracellular survival of the parasite in the macrophage (Delves et al., 2000; Mosser et al., 1987).

MATERIALS AND METHODS

Serum samples

The study group contained 43 patients with visceral leishmaniasis (VL) and 13 patients with cutaneous leishmaniasis (CL). The VL patients were aged between 8 months and 17 years, having one or more clinical symptoms of hepatosplenomegaly, pancytopenia, fever and weight loss, and diagnosed serologically using whole cell ELISA and IFA (cutt-off titer : ≥128) tests. The antigen for these tests was prepared using *Leishmania infantum* (MON-1) strain isolated in Turkey. The diagnosis was confirmed by the detection of amastigotes in the bone marrow aspiration samples of the patients. All serum samples included the present study were collected between 1998 and 2001.

The CL patients were also diagnosed parasitologically by the detection of *Leishmania* amastigotes in lesion samples. In addition, 50 seronegative serum samples from healthy individuals and 28 sera samples from VL suspected individuals were assigned in the control group. The patients in the VL suspected group have one or more appropriate clinical syptoms of VL but they were found as negative using all ELISA and IFA tests. Their bone marrow aspirates were also found as negative.

Measurement and evaluation of complement levels

The C1, C3 and C4 levels were measured twice in the serum samples by the nephelometric method with “N antisera to human C3, Code No.OSAP”, “N antisera to human C4, Code No.OSAO” and “N antisera to human C1” (Dade Behring). The normal levels of the complement proteins accepted as (C1 = 0.22-0.34 g/l, C3 = 0.5 – 0.9 g/l, C4 = 0.1 - 04 g/l), according to the company. The data were assessed by SPSS for Windows 10.0® compatible with Windows 2000®. The differences between the quantitative variables were evaluated by Krushal–Wallis variance analysis and $p < 0.05$ were accepted as statistically significant.

RESULTS

Serological levels of C1 were higher than the normal levels in 58.1% (25/43) of VL cases, 30.8% (4/13) of CL cases, 25% (7/28) of the second control group and 24% (12/50) of the healthy individuals (Figure 1). C3 levels were higher in 58.1% (25/43) of VL cases, 92.3% (12/13) of CL cases, 30% (15/50) of the healthy controls and 53.6% (15/28) of the second control group (Figure 2). C4 levels were higher in 18.6% (8/43) of VL cases, 35%7 (10/28) of the control group and 4% (2/50) of the healthy individuals (Figure 3 and Table 1).

According to these results, the average C1 levels were high in VL cases (78.98) and law in CL cases (41.69) ($p = 0.01$). Average C3 levels were high in CL cases (96.08) and low in VL patients (62.00), which was found statistically significant ($p = 0.01$). C4 levels were found to be higher in the control group and similar levels in VL and CL groups. In the control group of healthy individuals, C1, C3 and C4 levels were found lower (Table 2).

DISCUSSION

There are four main biological functions of the complement system; cytolysis and osmotic lysis, opsonization, chemotaxis and clearence of the immune complexes (Densen, 2000; Gasque, 2004; Delves et al., 2008).

The complement system provides a critical level of defense against bacterial invasion. Various microorganisms have evolved a variety of mechanisms to allow them to avoid complement lytic and opsonic activity. These range from the formation of factors that destroy activity of complement proteins to the evolution of surface structures that fail to bind, facilitate degradation of, or shed, complement proteins. Complement activation constitutes a critical link between the innate and acquired immune responses (Frank et al., 1992; Mor, 2009).

There are three mechanisms which activate the complement cascade; (a) the classical pathway, which is primarily activated by immune complexes; (b) the alternative pathway, which is activated by direct binding

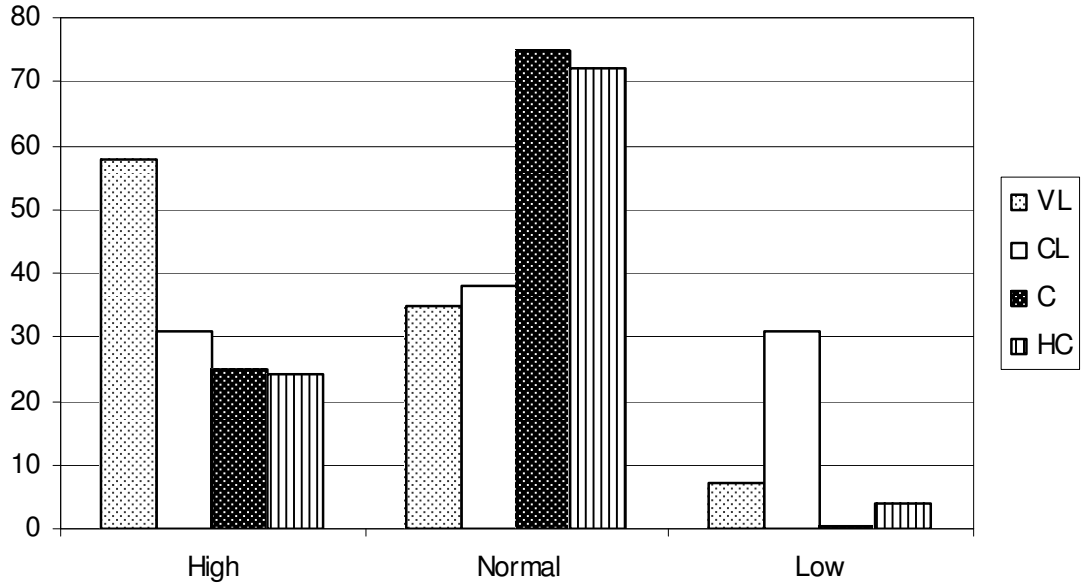

Figure 1. C1 levels in the sera samples of all groups.

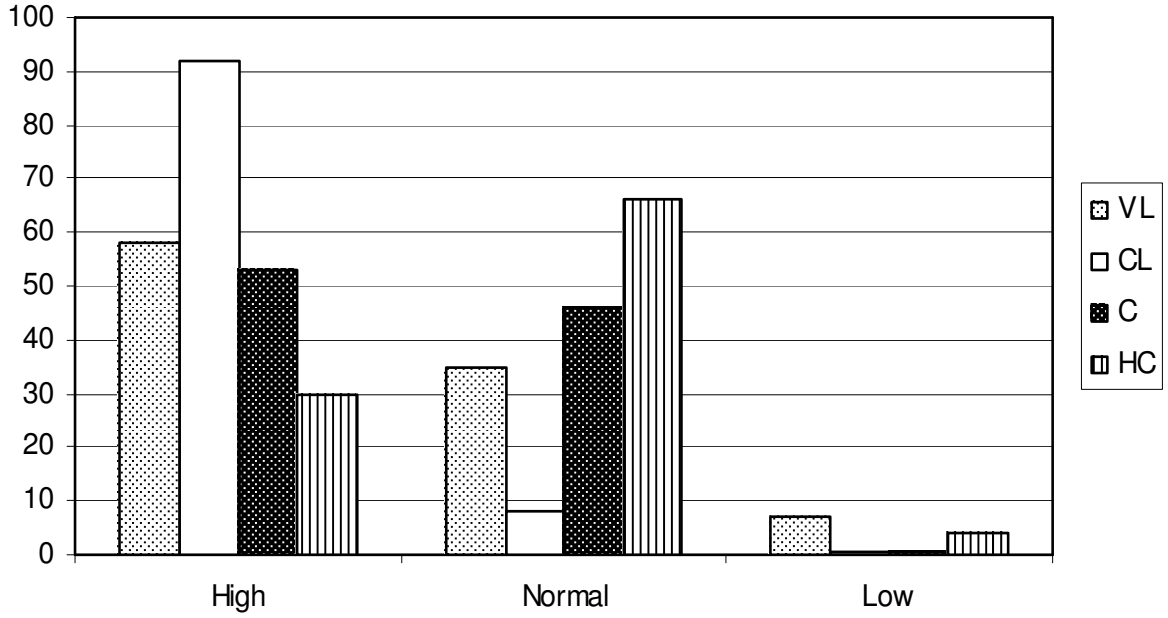

Figure 2. C3 levels in the sera samples of all groups.

of the component C3 to the microbe surface; (c) the lectin pathway, which is initiated by binding of the mannose-binding protein to terminal mannose residues on microbial surfaces (Abbas et al., 2007).

The mechanism of complement activation by *Leishmania* promastigotes has revealed that *Leishmania* can activate complement via the alternative pathway, a process that proceeds in the absence of antibody. Complement activation by *L. donovani* and *L. major* metacyclic promastigotes may also involve components of the classical pathway (Brittingham et al., 1996).

Our findings have shown that, the C3 level in VL patients (58.13%) and CL patients (92.30%) was higher than component C4 level, which can evaluate the indication of usage of alternative pathway by *Leishmania* promastigotes.

The distinct characteristic pattern of complement sensitivity during growth *in vitro* was studied using fresh

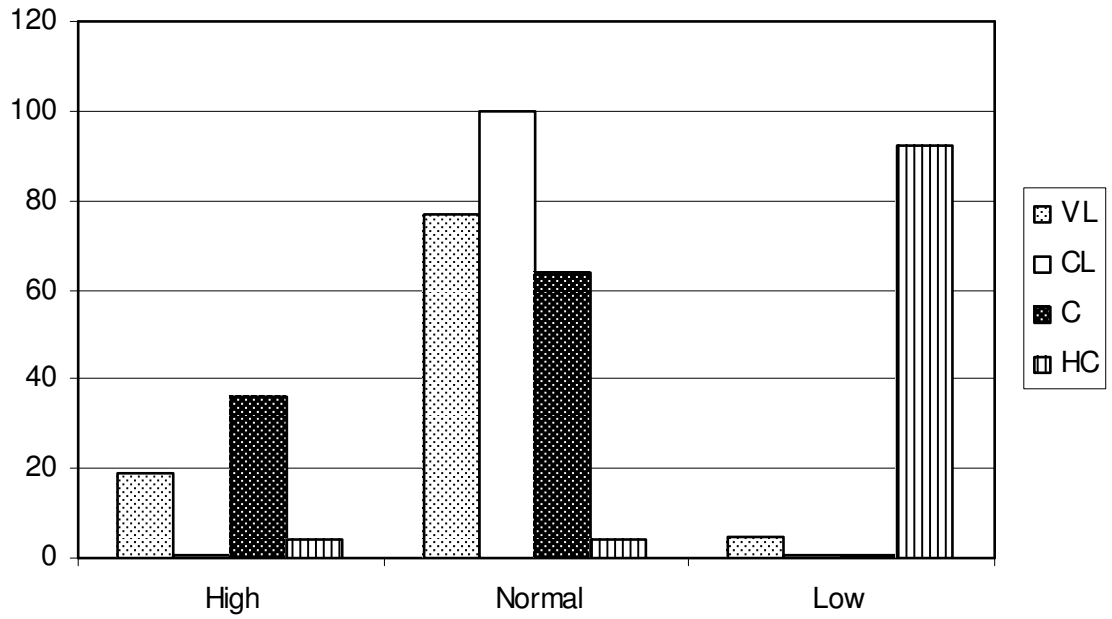

Figure 3. C4 levels in the sera samples of all groups.

Table 1. C1, C3 and C4 levels in the sera samples of all groups.

	C1 (g/l)						C3 (g/l)						C4 (g/l)					
	>0.34		0.22-0.34*		< 0.22		>0.9		0.5-0.9*		< 0.5		>0.4		0.1-0.4*		< 0.1	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
VL (n = 43)	25	58.1	15	34.9	3	7	25	58.1	15	34.9	3	7	8	18.6	33	76.7	2	4.7
CL (n = 13)	4	30.8	5	38,4	4	30.8	12	92.3	1	7,7	0	-	0	-	13	100	0	-
C (n = 28)	7	25	21	75	0	-	15	53.6	13	46.4	0	-	10	35.7	18	64.3	0	-
HC (n = 50)	12	24	36	72	2	4	15	30	33	66	2	4	2	4	2	4	46	92

*: Normal values; VL: Visceral leishmaniasis; CL: Cutaneous leishmaniasis; C: Control group; HC: Healthy control group.

Table 2. Statistical evaluation.

	Visceral leishmaniasis (n=43)	Cutaneous leishmaniasis (n=13)	Control group (n=28)	Healthy control group (n=50)	*p*	*K-W*
C1	78.98	41.69	64.92	61.70	0.011	11.221
C3	62.00	96.08	72.24	58.50	0.010	11.353
C4	76.37	74.65	89.58	43.04	0.000	33.281

K-W: Krushal–Wallis.

normal human serum. Complement resistance of the subgenus *Leishmania* is temporary, reaching its peak at the beginning of the stationary phase of growth, and decreasing thereafter and heat inactivation or the presence of EDTA completely abolished the lytic effect of normal serum against the promastigotes, indicating that parasite killing is dependent on complement activation (Fa´tima SM et al., 1998). This point can explain our results on the levels of component C4 are much higher in healthy control, VL and CL patient's sera than component C3 level.

Various kinds of effectors cells such as macrophages, neutrophils, eosinophils - and even platelets help defend the host against invasion by the parasites and act to

control the multiplication and spread of parasites already in residence. The anatomical location of these effector cells is obviously important. In cutaneous leishmaniasis, it is chiefly the patient's immune response to the infection that determines the form taken by the clinical disease, however, the species of parasite may also determine part of the host response. If the patient mounts an adequate but not excessive cell-mediate immune response to the parasite, healing of the ulcerative lesions and specific protection result. However if cell-mediated immunity to the parasite is inadequate or supressed, the result may be diffuse cutaneous disease. The immune response to visceral leishmaniasis is remarkably different from that of cutaneous leishmaniasis, although the parasites are essentially indistinguishable. Massive polyclonal hypergammaglobulinemia with little or no evidence of cell-mediated immunity is the rule in visceral leishmaniasis. There is no quantitative relationship between the elevated serum immunoglobulin and antiparasite antibodies, which are, moreover, not species-specific (Reed, 1981).

High levels (92%) of C3 in, especially CL patients in the present study have shown that, in cases with low number of amastigotes or in regions where secondary bacterial and fungal infections are common, determination of C3 level could be a parameter in the diagnosis of leishmaniasis.

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