International Journal of Natural and Engineering Sciences 3 (2): 49-51, 2009
ISSN: 1307-1149 www.nobel.gen.tr

The Investigation of Antibody Existence Against *Leishmania* Parasites in Patient Sera Infected by Different Antibodies

M.S. NOVRUZOVA[1] M.İ. BAĞIROVA[2] S. CANIM[2*] A.И. QURBANOV[1] A.M. ALLAHVERDIYEV[2]
[1]*Department of Medical Microbiology and Immunology, Azerbaijan Medicine University, Baku, AZERBAIJAN*
[2] *Department of Bioengineering, Yildiz Technical University, Istanbul, TURKEY*

*Corresponding Author
e-mail: scanim@gmail.com

Received: November 28, 2008
Accepted: January 07, 2009

Abstract:

Leishmania parasites are the causal agents of leishmaniasis; a group of protozoan diseases transmitted to mammals, including human beings, by phlebotomizes sandflies. It is known that 12 million people are infected with *Leishmania* in the world and approximately 350 million people are at a risk of infection and disease. Also in Turkey approximately 20 million people have the risk of leishmaniasis. It is estimated that each year globally 1 to 1.5 million cases of cutaneous leishmaniasis (CL) and 500.000 cases of visceral leishmaniasis (VL) occur. For diagnosis of this disease there are various methods, especially serological methods. But the sensitivity and specificity of these methods are not stable and can cause cross reactions in various infections.

The aim of this study is to investigate antibody existence against *Leishmania* in different infection antibodies having patient sera. 37 healthy people's and 113 different patient's sera were investigated for antibody existence against *Leishmania* and also *Cytomegalovirus, Toxoplasmosis gondii, Chlamydia pneumonia, Rubivirus, Herpes simplex II virus* and *Hepatitis B virus* in the experiments. IFAT and ELISA methods were used for this aim.

It is determined that 52 of investigated patient's blood serums have *Cytomegalovirus* antibody, 14 have *Toxoplasmosis gondii* antibody, 9 have *Chlamydia pneumonia* antibody, 13 have *Rubivirus* antibody, 7 have *Herpes simplex II* antibody and 13 have antibody against HBs antigens of *B hepatitis virus*. Antigens that we prepared from *Leishmania*'s promastigot forms were used within the IFAT method. IgG and IgM against *Leishmania* were detected in serum samples. 58 (51, 3%) of 113 patient's serum gave positive result against *Leishmania* antigens and 40 (35, 4%) of them against *Leishmania* IgG, although 18 (15, 9%) of IgM was determined.

The obtained results show that IFAT and ELISA methods are not specific for the diagnosis of Leishmaniasis and give cross reactions with other infections. This results show that, these mentioned methods are useful for scanning more than diagnosis.

Key words: IFAT, ELISA, Leishmania, diagnosis

INTRODUCTION

Leishmania parasites are the causal agents of leishmaniasis, a group of protozoan diseases transmitted to mammals, including human beings, by phlebotomine sandflies. It is known that 12 million people are infected with *Leishmania* in the world and approximately 350 million people are at risk of infection and disease [1]. It is estimated that, there are 1 to 1.5 million cases of cutaneous leishmaniasis (CL) and 500.000 cases of visceral leishmaniasis (VL) occurring each year globally [2]. Leishmaniasis is associated with about 2.4 million disability-adjusted life years and around 70 000 deaths each year. Also in Turkey approximately 20 million people have the risk of leishmaniasis [3, 4].

Leishmaniasis is divided into four main clinical forms, namely Cutaneous leishmaniasis (CL), Mucocutaneous leishmaniasis (MCL), Diffuse cutaneous leishmaniasis (DCL) and Visceral leishmaniasis (VL) [5,6]. Different methods are used for the diagnosis of Leishmaniasis. Leishmaniasis has an important role for the regional pathology in tropical and subtropical countries. Parasitological, immunological and molecular methods are being used in this field [6]. Some of the most commonly used serological tests are the enzyme-linked immuno sorbent assay (ELISA), with several modifications, the indirect fluorescent antibody test (IFAT) counterimmunoelectrophoresis, the direct agglutination test and others [7]. But there are concerns about the sensitivity and specificity of serological methods. The most used method for visceral leishmaniasis is IFAT. The accuracy of such a method varies from 80-100% in accordance with the laboratory.

Applying kits for serological diagnosis of leishmaniasis is resulting in cross reactions with antibodies that were composed against other microorganisms in patient's blood serum. Several studies have shown that the *Chagas* disease serum perform positive results by ELISA when the *Leishmania braziliensis* and *Leishmania chagasi* antigens were used. Kala-azar serums are also positive against *Trypanosoma cruzi* or *Leishmania braziliensis* antigens with IFAT and similarly mucocutaneous leishmaniasis patients' serum antibodies are positively react with *Trypanosoma cruzi* or *Leishmania braziliensis* antigens with ELISA [8]. However, there is no sufficient research regarding cross antibody reactions against leishmaniasis factors in blood sera of patients infected by Cytomegalovirus, Chlamydia pneumoniae, Rubivirus, Herpes simplex II or hepatitis B.

Therefore, the aim of this study is to investigate the antibodies against *Leishmania* parasites in patient sera infected by different antigens (Cytomegalovirus, Chlamydia pneumoniae, Rubivirus, Herpes simplex II or hepatitis B).

MATERIALS AND METHODS

In this research 113 different patients and 37 healthy people's sera were investigated. Patient samples were obtained from Azerbaijan Medicine University, Medical Microbiology and Immunology Department. In this study ELISA and IFAT methods were used. The investigation of *Leishmania* antibody by using IFAT method was performed at the Cell Culture Laboratory of Yildiz Technical University, Bioengineering Department.

The antigens were prepared from the continuos culture of *Leishmania tropica* (MHOM/TR/999/Ep-39). In the cultivation of *Leishmania tropica* PMI – 1640 + 10% fetal calf serum (FCS) were used.

IgG and IgM antibodies against *Leishmania* parasites were investigated in patients' sera which have antibodies against different infection. 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128 and higher dilutions of blood serums were prepared to determine the titre of the reaction (Figure 1, 2).

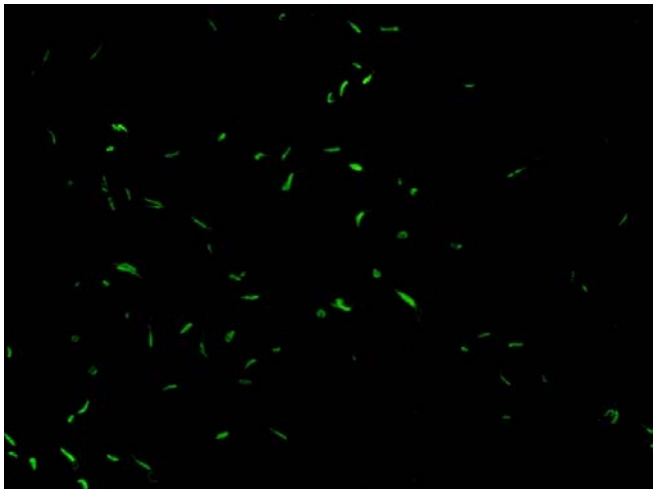

Figure1: Positive *Leishmania tropica* IFAT result at dilution 1/32

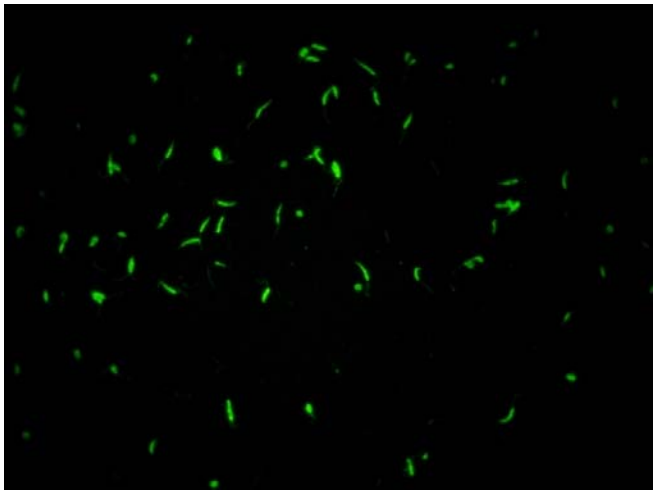

Figure 2: Positive *Leishmania tropica* IFAT result at dilution 1/64

RESULTS

It is determined that 52 of investigated patient's blood sera have Cytomegalovirus antibody, 14 have *Toxoplasmosis gondii* antibody, 9 have Chlamydia pneumonia antibody, 13 have Rubivirus antibody, 7 have Herpes simplex II antibody and 13 have antibody against HBs antigens of B hepatitis virus. 58 (51,3%) of 113 patient's serum gave positive result against *Leishmania* antigens and 40 (35,4%) of them against *Leishmania* IgG, 18 (15,9%) of IgM were determined.

From 14 blood sera which have antibodies against *Toxoplasmosis gondii*, 8 (57,1%) of them gave positive result with *Leishmania* antigens. Among these in 7 (50%) of them IgG and in 1 (7,1%) IgM against *Leishmania* were determined.

From 9 blood sera which have antibodies against *Chlamydia pneumonia*, 4 (44.5%) of them which were IgG class gave positive result with *Leishmania* antigens and in all of them (44.5%) IgG was determined. In these serums against *Leishmania* IgM was not determined.

From 52 blood sera which have antibodies against *Cytomegalovirus*, 28 (53,9%) of them gave positive result with *Leishmania* antigens. Among these in 18 (34,6%) of them IgG and in 10 (19,3%) IgM against *Leishmania* was determined.

From 7 blood sera which have antibodies against Herpes simplex II virus, 4 (57,1%) of them gave positive result with *Leis mania* antigens and in all of them (57,1%) IgG was determined. In the serums against *Leishmania* IgM was not determined.

From 18 blood sera which have antibodies against Rubivirus, 10 (55,6%) of them gave positive result with *Leishmania* antigens. Among these in 7 (38,9%) of them IgG and in 3 (16,7%) IgM against *Leishmania* was determined.

From 13 blood sera which have antibodies against HBs antigens of Hepatitis B virus, 4 (30,8%) of them gave positive result with *Leishmania* antigens and in all of them IgM was determined. In these sera against *Leishmania* IgG was not determined.

None of the 37 healthy people's blood serum samples gave positive result with *Leishmania* antigens. In other words in these sera no antibodies were found which gave cross reacts with *Leishmania* antigens.

DISCUSSION

As it is seen from the results obtained, antibodies against *Leishmania* were found in the blood serum of 57, 1% of the patients who were exposed to various inflectious diseases. Therefore it is difficult to explain this fact from only one point of view. At first sight this can be explained by the other microorganisms having general antigens of *Leishmania.* As a result of this it is natural to see that blood serum which has antibodies against *Toxoplasmosis gondii* also has antibodies against *Leishmania. Toxoplasmosis gondii* and *L.tropica* are microorganisms which are similar on philogenetic bases (Protozoa type). For example, *Leishmania infantum* and *Crithidia luciliae* antigens gave the same results with IFA and ELISA [9]. The blood sera of the patients of Trypanosomes gives positive reactions with *Leishmania braziliensis* and *Leishmania chagasi* antigens with ELISA, the blood serum of Kala-azar patients gives positive reaction with *Trypanosoma cruzi* and *Leishmania braziliensis* antigens with immunofluorescence assays and mucocutaneous patients' blood serum with *Trypanosoma cruzi* and *Leishmania chagasi* with ELISA [10]. Recently the existence of the cross antigens between *Leishmania* and

pathogen fungi were examined. Antibodies against *Paracoccidioides brasiliensis* were examined with ELISA in the dogs which were seropozitive or seronegative for the *Leishmania* [11]. Antibodies against *Paracoccidioides brasiliensis* were found in 67.8 % of seropozitive dogs and 7.3% of seronegative dogs. Most of the seropozitive dog (79.9%) blood sera reacted with gp43 antigens of *Paracoccidioides brasiliensis*. Researchers stated that cross reaction or co-infection between *Leishmania* and *Paracoccidioides brasiliensis* was possible.

However it is difficult to explain virus infections and *Leishmania* antibodies only with cross reactions. Antibodies which cross react as a result of the increasing antibody concentration in the blood sera of the patients who were exposed to various infectious disease. Antibodies can be seen which gives cross reaction as a result of the increased concentration of the antibodies in the blood serums which were exposed to various inflectional diseases. For example, formal gel or aldehit method which is used in the diagnosis of leishmaniasis depends on the increase of IgG and IgM immunoglobulin in the examined sample [10].

The results of this study show that, the antibodies against *Leishmania* in the blood serum of the patients who were exposed to various inflectious diseases can be seen not only in protozoa but also with virus based infections. This may be the main reason of the weakness of the specificity of the serological method which is used in the diagnosis of leishmaniasis. This fact should be considered in the diagnosis of leishmaniasis. In other words, these results show that above mentioned methods are useful for scanning more than diagnosis.

ACKNOWLEDGEMENTS

This study has been supported by DPT project (Project no: 25-DPT-07-04-01).

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