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Duration of Immunity in Cutaneous Leishmaniasis

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ABSTRACT

This article presents the results of studies of the state of immunity in blood serum and affected areas of the skin in patients with cutaneous leishmaniasis in comparison with the indicators of healthy individuals.

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Leishmaniasis is one of the main problems of tropical and subtropical countries, ranking second after malaria [1,3,5,7,9,11]. There is evidence that from 12 to 40 million people in the world suffer from leishmaniasis, and up to 350 million are at risk of contracting this disease. It has been established that 1-1.5 million people are infected with cutaneous leishmaniasis every year, and 500 thousand people are infected with visceral leishmaniasis [2,4,6].

Up to 500 species of mosquitoes are known, of which 30 are carriers of leishmaniasis. Leishmaniasis (or leishmania) refers to a group of diseases, the causative agents of which are intracellular protozoa of the genus Leishmania. According to the features of the morphological structure, the causative agents of leishmaniasis are divided into amastigous and promastigous forms. Amastigous pass the cycle of development in humans and other mammals, and promastigous – in the body of mosquitoes of the genus Phlebotomus.

Leishmaniasis is still one of the most neglected diseases in the world, affecting mainly the poorest of the poor, mainly in developing countries; it is estimated that 350 million people are at risk of infection with leishmaniasis, and about 2 million new cases of the disease are detected annually [8,10].

There are two forms of human leishmaniasis: visceral leishmaniosis (leishmaniosis visceralis), characterized by damage to the lymphohistiocytic system, remitting fever, cachexia, progressive anemia, leukopenia, a sharp increase in the spleen, and cutaneous leishmaniosis (leishmaniosis cutanea), in which the skin (mucous membranes) is mainly affected; the process manifests itself by ulceration followed by scarring. Both forms include various geographical and clinical-epidemiological variants [13,15,17,19].

For the first time, the causative agent of leishmaniasis (cutaneous) was discovered in 1898 by P.F. Borovsky in Tashkent and he attributed it to the simplest. Leishman U. [1900] and John Donovan [1903] found parasites morphologically identical to those described by Borovsky P.F. Ross R. in 1903, attributed the causative agents of leishmaniasis to a new genus of protozoa - Leishmania [20,22].

The main endemic foci in Central Asia are located in Uzbekistan and Turkmenistan [1], as well as in certain regions of Kazakhstan [2]. In these territories, the degree of manifestations of the disease is different and the morbidity of the population in each of them has its own characteristic features due to the mutual location of settlements and natural foci, the degree of contact of the population with foci and the level of the immune layer [21,23].

Quite often, despite the treatment, the course of zoonotic cutaneous leishmaniasis takes a chronic course, sometimes for several years, creates a problem not only for the patients themselves, but also for dermatologists.

These data indicate the need for further pathogenesis studies to address the development of cutaneous leishmaniasis, as well as the possibility of the development of complicated forms of cutaneous leishmaniasis, in particular, metalleishmaniasis.

The purpose of the study. To develop a method of molecular genetic diagnosis and pathogenetic therapy of cutaneous leishmaniasis based on the study of immune-biochemical studies.

Material and methods of research. Molecular genetic studies were performed in 112 patients with cutaneous leishmaniasis. Primers and probes for 3 types of leishmania were selected to determine the species specificity of Leishmania.

A real-time PCR amplification reaction was performed using primers and probes for the marker gene (ribosomal RNA small subunit gene (SSU rRNA)) leishmania. In our study, we used a new effective RTQ1 carrier modeled for the SSU rRNA of the Leishmania gene, for the Kinetoplast DNA of the L. Infantum and L. Major genes.

In 112 patients with cutaneous leishmaniasis, biological materials were selected from the skin epithelium and DNA was isolated. During Real-Time PCR, the presence of leishmania was detected in all 112 patients (fig. 1)

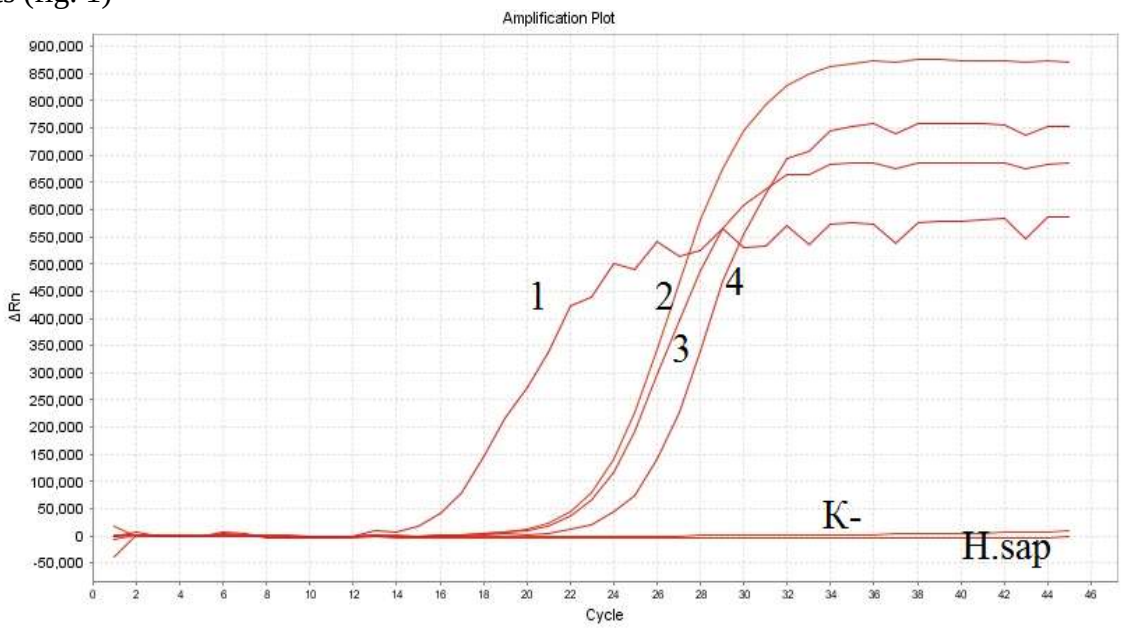

Fig. 1. Real-Time PCR amplification graph for the purpose of determining the presence of leishmania. In patients No. 1-4, the presence of leishmania was determined and the amplification schedule rises from cycle 16. 2- K-H20 (control) and H.sapiens (control) amplification is absent.

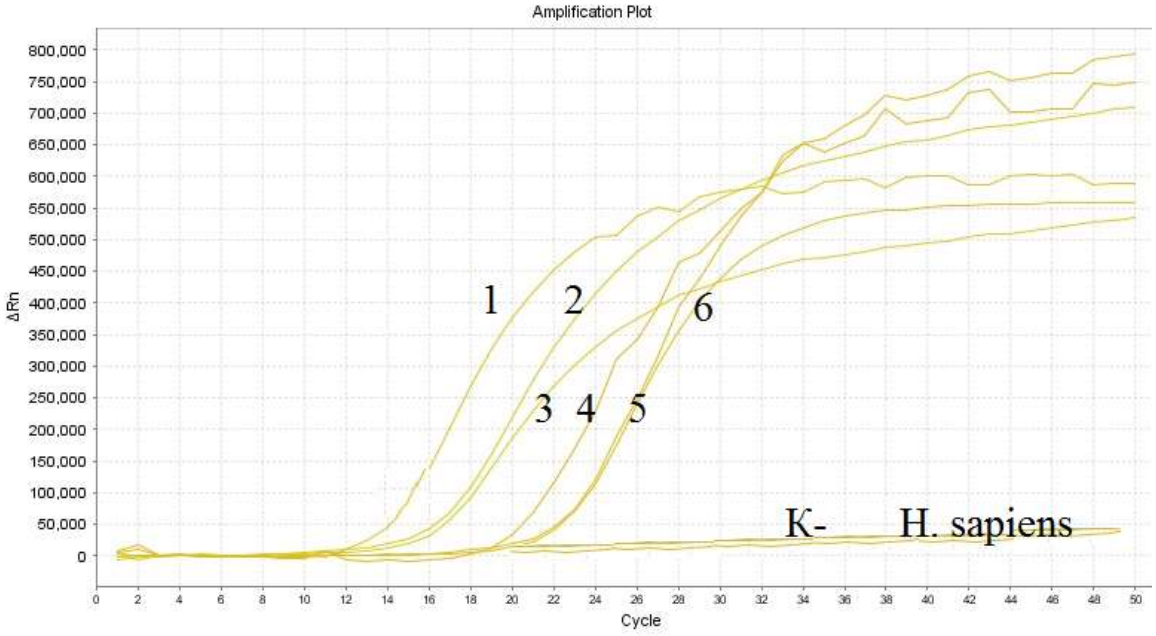

Fig. 2. Graph of PCR Real-Time amplification. In patients 1-6, the presence of L. Infantum was detected, the amplification schedule rises from cycle 13. K-control of H2O and in the Homo sapiens DNA sample (control) amplification of L. Infantum is absent.

To determine the species specificity of leishmania, a Real-Time PCR reaction was performed in 112 patients on L.Infantum, L.Major and L.Tropica. Among the examined 108 (96.4%) patients were found to have L.Infantum (Figs. 1 and 2). And 4 patients with L.Infantum were not identified.

In the study of the species specificity of leishmania in these 4 patients in whom L.Infantum was not detected, the presence of L. Tropica was also not detected on the Real-Time PCR reaction. The amplification schedule did not rise for the selected data groups. In these samples, the amplification graph was not raised as standard for the Real-Time PCR reaction to the presence of L. Major (Fig. 4.8). This indicated that in these samples it is necessary to conduct a study on the species specificity of leishmania for the presence of L. Major with other research methods.

Since the amplification schedule of L. Major in 4 samples was not raised as standard, we used 3% gel electrophoresis, by which we made sure that the amplification process was successful. Primers taken for L. Major (F and R) Major are adjacent to the Glucosephosphate isomerase gene and amplify the PCR product, but the probes are not adjacent to the gene. This contributes to the promotion of two hypotheses. That is, firstly: there may be a mutation specific to our region at the junction of the probe, or the process of splitting the probe may occur.

In the scientific studies conducted earlier to determine the types of leishmania studied in Uzbekistan, primers created on the basis of the design of leishmania isolates found in other geographical regions were used. The data obtained indicate that today, without refuting other methods, the Real-Time PCR method is used for rapid and high-quality diagnosis of leishmania. This method is effective even if the epithelium contains a negligible amount of leishmania (5-10 ng of DNA).

Thus, the data obtained show that in our region, in most cases (96.4%), the causative agent of cutaneous leishmaniasis is L. Infantum. It should be noted that when determining the type of leishmania in molecular diagnostics, it is necessary to use specific genes as markers, such as the SSU rRNA (small subunit ribosomal RNA (SSU-rRNA) gene), mitochondrion kinetoplast (kDNA), Cytochrome B, MSP associated gene, DNA polymerase, glucose-6-phosphate dehydrogenase (G6PD)- glucose-6-phosphate dehydrogenase, Glucosephosphate isomerase, heat shock protein 70 (hsp70), cysteine protease B(cpb), internal transcribed spacer 1 (ITS1) region and other genes.

Thus, for further full study of common Leishmania species on the territory of the republic, it would be advisable to use both the Real-Time PCR method and sequencing of the above marker genes, which will contribute to obtaining specific qualitative information.

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