MOLECULAR-GENETIC ANALYSIS OF THE CAUSES OF TERMINAL CUTANEOUS LEISHMANIOSIS IN THE SAMARKAND DISTRICT OF SAMARKAND REGION

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Annotatsiya: O‘zbekistoning ko‘plab hududlarida, xususan, Samarqand viloyatining ko‘plab tumanlarida teri leyshmaniozi bilan kasallanish kuzatiladi. Teri leyshmaniozi qo‘zg‘atuvchilari odam organizmiga tashuvchilar orqali tushgach, uzoq vaqt davomida tuzalmaydigan yaralarni hosil qiladi. Kasallik qo‘zg‘atuvchining turiga qarab antroponoz teri leyshmaniozi (ATL) va zoonoz teri leyshmaniozi (ZTL) turlariga bo‘linadi. Teri leyshmaniozi yaralarini davolashda, kasallikning epidemiologik xaritasini tuzishda, tashxislash usullarini takomillashtirishda leyshmaniya turlarini (L.tropica, L.major) aniqlash muhumdir. O‘zbekistonda leyshmanioz yaralaridagi leishmaniya turlarini aniqlashga oid tadqiqotlar yetarlicha emas. Kasallik umumiy tashxislash usulida amalga oshiriladi va uni davolashda alohida usullardan foydalanilmaydi. Teri leyshmaniozi yaralarini molekulyar-genetik jihatdan tahlil qilish, davolash va epidemiologiyaga oid muammolarni hal qilish bilan birga, ilmiy yangilik bo‘lib hisoblanadi. Ushbu tadqiqotlarda eng samarali usul polimeraza zanjiri reaktsiyasidir (PZR).

Kalit so‘zlar: Teri leyshmaniozi, antroponoz, zoonoz, epidemiologik, parazit, polimeraza zanjiri reaktsiyasi (PZR).

Аннотация: Кожный лейшманиоз наблюдается во многих регионах Узбекистана, в частности, во многих районах Самаркандской области. При попадании возбудителей кожного лейшманиоза в организм человека через переносчиков образуются раны, которые долго не заживают. В зависимости от вида возбудителя заболевание подразделяют на антропонозный кожный лейшманиоз (АКЛ) и зоонозный кожный лейшманиоз (ЗКЛ). Выявление видов Leishmania (L.tropica, L.major) имеет важное значение для лечения ран, вызванных кожным лейшманиозом, составления эпидемиологической карты заболевания и совершенствования методов диагностики. В Узбекистане отсутствуют исследования по выявлению видов Leishmania в ранах, вызванных лейшманиозом. Заболевание диагностируется с помощью общедиагностического метода, специфических методов лечения не применяется. Молекулярно-генетический анализ поражений кожного лейшманиоза является научной инновацией, решающей вопросы, связанные с лечением и

эпидемиологией. Наиболее эффективным методом в этих исследованиях является полимеразная цепная реакция (ПЦР).

Ключевые слова: Кожный лейшманиоз, Самаркандская область, эпидемиологический, паразит, полимеразная цепная реакция (ПЦР).

Abstract: Cutaneous leishmaniasis is observed in many regions of Uzbekistan, in particular, in many districts of the Samarkand region. When cutaneous leishmaniasis pathogens enter the human body through vectors, they form wounds that do not heal for a long time. Depending on the type of pathogen, the disease is divided into anthroponotic cutaneous leishmaniasis (ATL) and zoonotic cutaneous leishmaniasis (ZTL). Identification of Leishmania species (L.tropica, L.major) is important in the treatment of cutaneous leishmaniasis wounds, in compiling an epidemiological map of the disease, and in improving diagnostic methods. In Uzbekistan, there are not enough studies on the identification of Leishmania species in leishmaniasis wounds. The disease is carried out by a general diagnostic method and no special methods are used in its treatment. Molecular-genetic analysis of cutaneous leishmaniasis wounds, along with solving problems related to treatment and epidemiology, is considered a scientific innovation. The most effective method in these studies is polymerase chain reaction (PCR).

Keywords: Cutaneous leishmaniasis, Samarkand region, Epidemiological, parasite, polymerase chain reaction (PCR).

Introduction: Leishmaniasis is a very common parasitic disease, which is transmissible in nature. According to the World Health Organization, 14 million people are infected every year. In addition, 50 thousand deaths are recorded from visceral leishmaniasis every year. The disease is more common in countries with a hot climate.

According to the World Health Organization (WHO), leishmaniasis is one of the six most important infectious diseases in the world. Population growth, migration, and immigration can lead to the formation of immunity to endemic areas, or we can often observe people without immunity contracting this disease.

In particular, in some regions of Surkhandarya, Kashkadarya, Bukhara, Samarkand and Jizzakh regions of Uzbekistan, as well as in the Republic of Karakalpakstan, there are endemic foci of the disease. In our country, 3 types of this disease are widespread: visceral leishmaniasis, anthroponosis and zoonotic cutaneous leishmaniasis. The causative agents of leishmaniasis (L.major, L.tropica, L.infantum) also differ from each other.

Studies on the molecular mechanisms of Leishmania pathogenicity conducted in Iran in 2019-2021 showed that some pathogenic factors can be observed between species in the evolution of Leishmania, with the same type of nucleotide sequences.

According to molecular genetic studies conducted in France in 2020, it is possible to be infected with several species of Leishmania, even with several Leishmania in one wound. At least two species of Leishmania (L. major and L. tropica) can also coexist in mosquitoes. In this regard, it is very important to study the genomics of Leishmania in natural conditions, where conditions exist for interspecific hybridization and the transfer of pathogenic factors between different species of Leishmania.

Objective: To conduct molecular genetic studies on cutaneous leishmaniasis lesions, analyze the types of Leishmania pathogens in cutaneous leishmaniasis lesions, develop recommendations for choosing a treatment method, and create an epidemiological map of the spread of cutaneous leishmaniasis in Uzbekistan.Materiallar va metodlar:

In the research conducted at the L.M. Isayev MVYUPKITI under SamDTU on the molecular genetic analysis of smears from cutaneous leishmaniasis lesions, samples from a total of 15 patients were used. DNA extracts from amastigotes were performed in special kits for use in molecular diagnostics of Leishmania species. DNA was extracted from all samples using a DNA isolation kit (Biosan, Russia), and genomic DNA for each case was extracted using proteinase k.

DNA samples were amplified using PCR kit (Invitrogen, Waltham, USA). DNA sequencing: For sequencing, the nucleotide sequence data of L.tropica and L.major samples in DNA PCR were reviewed based on the information submitted to the GenBank database, with accession number EU482829 for L. tropica and accession number EU482830 for L. major.

PCR-amplified DNA samples were analyzed by gel electrophoresis using 2% agarose. Gel staining was performed using ethidium bromide. The 620 bp fragment showed the presence of Leishmania major and the 800 bp fragment showed L. tropica.

Results: In the laboratory of the L.M. Isayev MVYUPKITI under SamDTU, samples were taken from 44 patients suspected of having cutaneous leishmaniasis (TL). The patients from whom the samples were taken came from different regions of Uzbekistan. In the laboratory of the institute, smears were prepared from the samples taken from the patients on a glass slide, stained using the Giemsa method, and Leishmania amistigotes were found under a microscope. Of these samples, 18 were women and 26 were men. More than 90% of the lesions of cutaneous leishmaniasis were dry lesions (simple or lupoid lesions) (Table 1).

Table 1: Analysis of the clinical presentation of lesions in 44 patients with cutaneous leishmaniasis.

Clinical appearance of smears	male	famele	general
Dry (normal form)	*26*	*16*	*42*
Dry (bulging form)	*0*	*1*	*1*
Mucus form	*0*	*1*	*1*
Total	*26*	*18*	*44*

In the table above, we can see that among the samples obtained, 85.7% were normal-shaped, 4.8% were lupoid-shaped, and 9.5% were mucous-shaped.

PCR was performed on 15 smear samples from cutaneous leishmaniasis specimens. The results showed that thirteen isolates (90.5%) were L.tropica and two were confirmed to be L.major (9.5%).

DNA from the smears was extracted using proteinase K and amplified with cDNA-specific primers. The PCR product was analyzed by gel electrophoresis using 2% agarose. The gel was stained with ethidium bromide. The presence of a 620 bp fragment indicated Leishmania major and an 800 bp fragment indicated L. tropica.

Conclusion: In conclusion, both L. tropica and L. major are causative agents of cutaneous leishmaniasis. Based on the above results, we can see that L. tropica is dominant in the Urgut district of Samarkand region. One of the most reliable methods for diagnosing leishmaniasis is the PCR technique.

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