Extended Abstract
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Molecular and Histopathological diagnosis of cutaneous leishmaniasis in Urmia, Iran in 2020: Report of Five Cases

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Abstract

Introduction:
Cutaneous leishmaniasis(CL) is the most common form skin disease caused by a single-celled parasite that is transmitted by the bite of a phlebotomine sand fly. bloodshed transmitted from infected animals rodents, dogs or humans to healthy humans can cause contamination. Symptoms usually appear three months to a year after the bite. Leishmaniasis affects almost 12 million people worldwide and it is one of the diseases that special attention should be paid to control and prevent it.

Methods:
In the laboratory, the wounds are prepared from the patients and from the interstitial fluid of the wounds using a smear scalpel blade. Also, for further examination and more definitive diagnosis of the patients' samples, biopsy was performed. In addition, serous fluid from the patient's skin lesion were cultured in Schneider's Drosophila medium (Sigma, Germany), supplemented with 5% heat-inactivated fetal bovine serum (Sigma, Germany). The real time PCR method was performed using clinical samples and it was repeated twice for each batch of samples.

Discussion and conclusion:
Our case report has been done in Iran and in the city of Urmia, and considering that the city of Urmia is located in your region of western Iran, so you are not considered an endemic region. According to the studies on the distribution of Leishmania in Iran, in the eastern regions of Iran such as Mashhad and Neishabour, both forms of skin leishmaniasis are urban and rural.

Introduction
Cutaneous leishmaniasis(CL) is the most common form skin disease caused by a single-celled parasite that is transmitted by the bite of a phlebotomine sand fly. bloodshed transmitted from infected animals rodents, dogs or humans to healthy humans can cause contamination. Symptoms usually appear three months to a year after the bite(1). Leishmaniasis affects almost 12 million people worldwide and it is one of the diseases that special attention should be paid to control and prevent it (2).The disease depends on which of these species causes the infection and depends on the factors associated with the host. The skin, mucosa, and mononuclear phagocytic system may be involved.
The differential diagnosis of this type of lesion is extensive and mainly falls into five categories.
Neoplastic, autoimmune, traumatic, infectious or idiopathic(1, 3). Despite progress in medicine, the diagnosis of such lesions is often incorrect or forgotten. In addition, the diagnosis CL is widely ignored. It is important to know that rapid diagnosis is important for the proper management and treatment of patients Findings that can be the main reason, but many of them overlap, and it can it poses a significant diagnostic problem for the treating physician(3).
Creating ugly-looking sores that stay in place for a long time (usually in open areas of the body and Most in the face (which after healing also scars) leaves a scar of itself and comments on the beauty it causes deep psychological problems in the patient, while the wound is developing in other places(4, 5).
Secondary wound infection causes a bacterial infection that requires antibiotic treatment and may endanger the patient's life, also in some published types require long-term treatment there has been and sometimes there is no improvement(5).
Cutaneous leishmaniasis may be seen in many parts of the world, but is a significant part of the prevalence of leishmaniasis in the Middle East. Approximately 90% of cases of cutaneous leishmaniasis have been observed in Iran, Afghanistan, Pakistan, Brazil, Peru, Syria and Saudi Arabia(6). Iran is the main endemic focus of cutaneous leishmaniasis (CL)(7, 8). In the Middle East and the main causes of CL disease in the country are three species: L. major, L. tropica and less often L. infantum(8-10). Various forms of CL have been reported in Iran, including cutaneous leishmaniasis after kala-azar (PKDL), lymphatic mucosal leishmaniasis and diffuse cutaneous leishmaniasis (DCL). DCL, a rare and unusual form of CL, with no the presence of cellular immunity, the large number of parasites within the host cells, the uncontrolled progression of the

disease and the lack of response to antiepileptic therapy are characterized(11-13).

The most common type of leishmaniasis is the rural type, whose reservoir is rats and about less 22% of cases are infected with urban type leishmaniasis, the reservoir of which is infected patients. After Sand-fly blood-eating from the relevant rodents is contaminated and in re-eating it causes transmission to Becomes human. Disease at the site of a mosquito bite in the form of a small boil after the incubation period (1-2 months). It has become bigger and bigger and turns into a wound, which depending on its type is 12-8 months It lasts and then heals and the scar remains instead. Most cases with topical treatment include glucantime injection Cryotherapy around the lesion and cold therapy. In some cases, only glucantime to It is prescribed intramuscularly, however, the scar remains, so treat it as soon as possible The scar remains smaller(13, 14). Disease prevention depends on personal protection from contact with sand-fly bites. That uses this. From netting, mosquito nets impregnated with insecticides, insecticides as well as proper disposal of waste to reduce. Reproduction of sandflies as well as environmental improvement is done in the rural type of control. Rodents and in the urban type, diagnosis and treatment of patients is important(15, 16).

CASE REPORT

five patients with skin complication refer to Dr. Nemati's clinical laboratory located in Urmia, Iran in eight months ago. Two patients had seen a doctor several months before visiting our center, but after a clinical examination by a doctor, a possible diagnosis for two patients was considered for lupus erythematosus. Therefore, corticosteroids for treatment with a daily dose of 72 mg is prescribed. But the lesions did not heal significantly. Finally, these patients referred to Dr. Nemati's clinical laboratory and complained about the lack of healing of their lesions. The other three patients were initially diagnosed with dermatophytosis and mistakenly received antifungal therapy. But six months after their treatment, they reported to the laboratory that their lesions had grown rather than healed. Some patients had a crusty surface with a raised border and produced a serous exudate when the scab was removed. The dimensions of these lesions ranged from 1 to 6 cm in diameter in all locations.

Figure 1: An example of images taken from the wounds of patients with cutaneous leishmaniasis

Methods:
In the laboratory, the wounds are prepared from the patients and from the interstitial fluid of the wounds using a smear scalpel blade. Then, after fixing with methanol, it is stained with Giemsa staining for 10 minutes. After staining the slides of patients are examined under a microscope with a magnification of 1000. Finally, by observing the amastigote form of Leishmania parasites inside macrophages, leishmaniasis was confirmed**.**

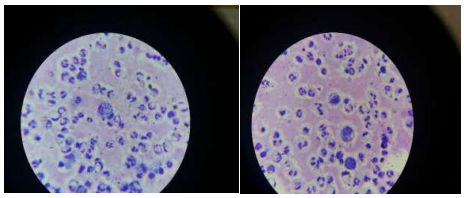

Figure2: Images of Leishmania amastigotes in slides painted with Giemsa coloring.

Also, for further examination and more definitive diagnosis of the patients' samples, biopsy was
performed. After preparing the pathology slide and staining with hematoxylin and eosin and
examining it under a microscope with a magnification of 1000, we were able to see the
leishmaniasis inside the tissue with confidence.

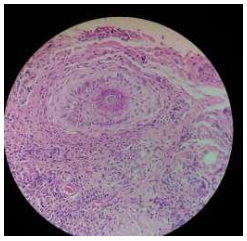

Figure3: The image above is of the body leishmaniasis in the pathology specimen

In addition, serous fluid from the patient's skin lesion were cultured in Schneider's Drosophila medium (Sigma, Germany), supplemented with 5% heat-inactivated fetal bovine serum (Sigma, Germany). Isolated parasites were subcultured and monitored regularly to check promastigotes growth. In this experiment When the amount of parasite in the logarithmic phase reached the desired value used for DNA extraction. Total genomic DNA of Leishmania parasites was extracted from cultured parasites and also from paraffin-embedded blocks of the patient's skin biopsy using the Kia Gene extraction kit, and Leishmania ITS1 was amplified by polymerase chain reaction. The real time PCR method was performed using clinical samples and it was repeated twice for each batch of samples. One positive and two negative controls were included for each PCR reaction. ITS1 real time PCR method was applied using samples containing 20–50 ng of genomic DNA, 400 nM of each primers , 200 nM of each probes, 2 mM of MgCl2, 1 µl LightCycler FastStart DNA Master Hybridisation probe (Roche Applied Science), and 1,5 µl PCR grade water (Roche Applied Science) to a reaction total volume of 10 µl. PCR amplification was performed as follows: one cycle of 10 minutes at 95°C, followed by 45 cycles consisting of denaturation at 95°C for 10 seconds, annealing at 50°C for 10 seconds, extension at 72°C for 20 seconds, and melting at 95°C for 0 second, 50°C for 10 second, 40°C for 10 second, 80°C for 0 second and cooling at 40°C for 30 seconds. Melting curves were analysed(17). All five samples were sent for sequencing to determine the type of leishmaniasis. The sequencing of our samples was performed using South Korean bioneer technology. The sequencing results indicated that the sequences of all five samples belonged to Leishmania major. The patient's ITS1 sequence was similar to the Iranian strain of L. major.

After proving the presence of Leishmania in patients, all patients were treated with glucantime and according to the patients' condition, all patients responded well to glucantime treatment. These cases are among the unusual reports in northwestern Iran. Some parts of Iran are considered endemic to Leishmania, but the northwestern region is not endemic to Leishmania. Of course,
Phlebotomus mosquitoes are present in this region, but the parasite has not the ability to complete its cycle in these areas. Discussion and conclusion Cutaneous leishmaniasis is one of the health problems in the world especially in tropical and subtropical countries. Its distribution range in recent years has exceeded the borders of 88 countries. More than 12 million people in the world are affected by this disease and at least 350 million people are at risk. Disease in Iran in two epidemiological forms, urban or humanitarian type and rural type or zoonosis has been reported.(18) Leishmania skin lesions has several types (localized,)Diffuse, disseminated, lupoid, and It is sporotricoid. In immunocompromised patients Can be seen Cells such as AIDS / HIV due to decreased response Th1, relative increase in Th2 response and no sensitivity the immune system against the parasite energy of this disease with Unusual clinical types, resistance to treatment and lesions Numerous pretends(19) In addition to the two types of urban and rural leishmaniasis, it is sometimes seen in a diffuse form, in which case the wounds can be warts and tumors, and sometimes the wounds can be chronic. This slows down accurate and rapid diagnosis and subsequent treatment of the disease, so rapid diagnosis is important to avoid imposing additional costs on patients as well as earlier treatment (20). Our case report has been done in Iran and in the city of Urmia, and considering that the city of Urmia is located in your region of western Iran, so you are not considered an endemic region. According to the studies on the distribution of Leishmania in Iran, in the eastern regions of Iran such as Mashhad and Neishabour, both forms of skin leishmaniasis are urban and rural(20). In recent years, increasing population and urban density and the availability of climatic conditions for the reproduction of sandflies in the east and south of Iran can cause these areas to become
endemic areas(21). However, the mountainous nature of Urmia region and its location in the Zagros Mountains and
its coldness are not good conditions for the reproduction of sandflies and Leishmania parasites, and therefore the presence of cutaneous leishmaniasis in this region seems a little strange. There are sandflies in Urmia, but the conditions are not such that the parasite can grow in it and complete its cycle. In this study, we tried to use all the facilities available in the medical laboratory to diagnose the parasite. Therefore, direct smear Pathological biopsy was performed and finally
used for final confirmation using Real time PCR and ITS1 gene. With clinical examination, the wounds had a dry appearance and we suspected Leishmania major from the beginning, but to ensure the quality of treatment, the method was used Real time PCR.

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