Case Report:

Cutaneous Leishmaniasis in El-Madinna Manowra region, Saudi Arabia in 2012

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Abstract:

Objective*:** Leishmaniasis is a parasitic disease causing major public health problem in form of visceral and cutaneous types. The cutanoue leishmaniasis is caused by *L. tropica*, in low-land areas without reservoir; Arthroponatic leishmaniasis (ACL), Zoonotic Cutaneous Leishmaniasis (ZCL), in high-land. This case report involved; 25 years old Egyptian active young single male adult, stayed in Utama (75 Km far from El-Madina Manowra on the road to Makkah). He presented with three skin lesions on his arms occurred within the last 1-3 months. on examination revealed; volcano- like indurated ulcers which clinically suspected as leishmania lesions. ***Materials and Methods: Laboratory investigations were involved; skin smear using Giemsa stain, Leishmanin test (LST), polymerase chain reaction (PCR), sequencing and phylogenitic analysis BLAST (NCBI). ***Results*:** Microscopy positive LDB (leishmanin donovani bodies), Leishmanin test (LST) was negative. PCR positive *L. major*. Sequence alignment were 100% with nine Iranian isolates and one Tunisian isolate. After one month of treatment with Pentostam (Sodium stibogluconate) local injections at the site of lesions the lesion progressed from ulcer to scar. ***Conclusion*:** L. major is a major species causing cutaneous leishmaniasis in Al-Medina Manowra region in Saudi Arabia. The usage of the polymerase chain reaction (PCR) is a useful diagnostic tool and help to identify the causative species.

Key words: Cutaneous Leishmaniasis*, L. tropica, L. (L) major. Skin lesion, Polymerase chain reactions (PCR)* .

Introduction:

Leishmaniasis is a parasitic disease existed in many countries . Presented in variable clinical patterns mainly visceral and cutaneous leishmaniasis which cause major health problem to communities. The cutaneous leishmaniasis is divided into two types; one type transmitted by arthropods (ACL) the other is transmitted by reservoir animals (ZCL) such as rodents. The disease is transmitted by a vector, sandfly such as Papattasi, Orientalis[1].

Leishmaniasis are prevalent on four continents and considered endemic in 88 countries, 72 of which are developing countries. The worldwide prevalence of the disease is estimated at 12 million cases, with 400,000 to 600,000 new cases per year for visceral forms and of 1–1.5 million for the cutaneous forms _ population information.

There are about 1.5 million cases of Cutaneous Leishmaniasis each year worldwide. According to the World Health Organization leishmaniasis is endemic in 88 countries, with a total of 350 million people at risk. It is believed that worldwide 12 million people are affected by leishmaniasis so one person infected by cutaneous leishmaniasis every 20 seconds, and over 90% of cases occur in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, Syria, Brazil and Peru[2].

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The Leishmania species present with a similar clinical appearance, but with different prognosis during the course of the infection. The ulcers caused by parasites of the subgenus *Viannia* are more aggressive and can recur after treatment. The ulcers caused by parasites of subgenus Leishmania are less severe and more likely to cure spontaneously or after treatment. Post Kala-azar Dermal Leishmaniasis (PKDL) is a form of cutaneous leishmaniasis occur after remission of treated visceral leishmaniasis. Molecular characterization has been used in several studies, it was applied in Giemsa stained smears that found positive for cutaneous leishmaniasis revealed the causative species were *L. tropica*. The causative agent for CL was previously reported in Tihama coastal plain, Yemen in 1989 was *L. tropica*. Almost two decades later, Khatri and his colleagues detected Leishmania amastigotes in 128 cases of CL from northern Yemen. In study done, four CL cases were characterized using the isoenzyme electrophoresis technique and *L. major* was identified in all of these cases. Other study detected *L. tropica* in 133 cases (85.80%), *L. infantum* in 17 cases (10.97%) and *L. donovani* in 5 cases (3.23%) [3], similarly CL cases were characterized using the isoenzyme electrophoresis technique in Al-Madina Almonwra (unpublished data). This study aimed at detection of leishmania parasite species that causing cutaneous leishmaniasis in Al-Madinah region. It was conducted in Al-Utamah around seventy five kilometer north to Al-Madinah. It is a semi-cultivated desert area with houses and many farms containing sheep and poultry breeding houses, irrigated by underground water, which has created a rich habitat supporting endemic animal including desert rodents and sand flies populations. All the inhabitants are relatives with non indigenous workers, their main income depend on agriculture and live stock, i.e., working as farmers and shepherds.

Case report:

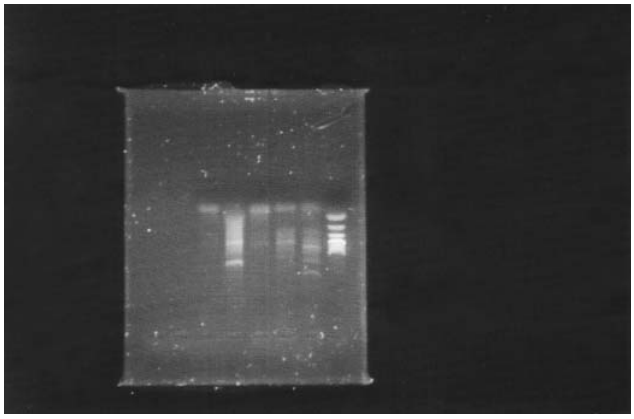
25 years old Egyptian active young single male adult, stayed in Utama (50 Km far from El-Madina Manowra on the road to Makkah). His occupation is farmer and lived with two partners in small room constructed from bricks which located in a valley surrounded with trees like Acasia. There have domestic animals such as goat beside presence of rodents; rats and mices. The house is supplied with electricity. Their sleep habit is indoor and usually sleep with long sheaves and covering clothes and uses of insect repellents but no uses of bed nets. He presented with skin lesions. The patient encountered this illness during the summer season. The skin lesions occurred within the last 1-3 months. Number of lesions were 3 lesions localized to the arms. On examination; his weight was 62 Kg, height was 165 cm and normal BMI (body mass index) 22.8. Clinical examination revealed; volcano- like indurated ulcers which clinically suspected as leishmania lesions. Laboratory investigations were performed which involved; skin smear using Giemsa stain, Leishmanin test (LST), polymerase chain reaction (PCR), sequencing and phylogenitic analysis BLAST (NCBI). The results were; Microscopy positive LDB (leishmanin donovani bodies), Leishmanin test (LST) was negative in day one with diameter less than 5mm and remained negative after repeated one month later. PCR positive *L. major* . Sequence alignment were 100% with nine Iranian isolates and one Tunisian isolate. After one month of treatment with Pentostam (Sodium stibogluconate) local injections at the site of lesions the lesion progressed from ulcer to scar. The patient compliance towards treatment and follow up was good and no side effects. The investigation procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (*Research Center For Medical Colleges*, King Khalid University) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from the patient after clear explanation of this study, after approval of the experimental protocol by the local human ethic committee (*Research Center For Medical Colleges*, King Khalid University). The clinical manifestation of CL can be misdiagnosed and confused with dermal diseases, however the disease in endemic areas can be easily diagnosed by the ulcers in the naked areas and the feature of the ulcers is very characteristic with prominent edged, volcanic appearance, started with papules and takes long term mostly one to six months without pain. The sample was taken from the edges of the lesion using insulin injection aspiration put in two clean microscopic slides with drop of normal saline, then the slides left to dry completely. Then Giemsa stain overlaid on the slides and fixed with 100% methanol, left to dry then covered with 10% Giemsa stain and left for 15 minutes then rinsed with distilled water by pouring water on the edge of the slide, then examined carefully for

Leishmania amastigote microscopically at X100 power using the immersion oil lens. Neither culture nor serology was performed. Leishmanin Skin Test (LTS) was done**.** The Leishmanin antigen obtained from the Pasteur Institute of Iran *L. major* (reference strain MRHO/IR/75/ER) used for preparation of the leishmanin. The preparation contained a final concentration of *L. major* promastigotes of 6×10^6 in 1mL of phosphate-buffered saline (PBS). The LST was performed by intradermal injection of 0.1 mL skin test antigen on the volar surface of the left forearm using a 1.0 –mL sterile syringe and disposable needle. The result was read after 48-72 hours using the ball-point pen technique. Induration size 5 mm was taken as positive reading. Molecular diagnosis was conducted using the filter paper to collect sample from secondarily infected ulcers The patients referred to the Dermatology Department of the AlMiqat Hospital, Al-Madinah Almonawarah, Saudi Arabia, with microscopic confirmed. Lesions and the adjacent normal-looking skin around them were cleaned and sterilized with disinfectant. Sterile saline (0.1 to 0.2 ml) was drawn into a syringe (1-ml, 25-gauge needle), and the needle was inserted into the nodule or ulcer's margin and rotated gently several times. A small amount of saline was expressed into the tissue and some tissue aspirate and freed tissue were withdrawn each was plotted in a sterile Whatman 3 MM filter paper[4]. Filter papers were stored with silica gel at 4°C until DNA extraction**.** DNA extracted from filter papers using; dried Blood Spot or ulcer aspirate Protocol (QIAGEN)**:** placed 3 punched out circles from a dried blood spot into a 1.5ml Eppindorf tube and add 180ml of buffer ATL (cut 3mm by paper puncher), incubated at 85 ºC for 10mint, briefly centrifuge to remove drops from inside lid. 20ml protenase K (stock solution) was added and mixed by vortex and incubated at 56 ºC for 1 hour, briefly centrifuge to remove drops from inside lid. PCR was used by QIAGEN PCR Kit: dNTP mix (2.5 mM) containing all four dNTPs, 10x amplification buffer, Taq polymerase, Sterile distilled water, *L. major* specific set of primers:

Primers**:** L5.8S**:** 5` TGATACCACTTATCGCACTT 3`**.** LITSR: 5` CTGGATCATTTTCCGATG 3`(320 pb).The PCR-Master Mix (MM) was prepared as indicated in **Table 1**. Vortex and centrifuge the MM shortly and dispense the MM in pre-chilled labeled PCR –tubes. To which 5 μl of template DNA was added. The total reaction volume will be 25 μl, vortex and centrifuge the mixture briefly. Overlay (if necessary) the reaction mixtures with 1 drop (50 μl) mineral oil to prevent evaporation.

The PCR program was composed of; Hot start; 5 min 94 ºC**.** Denaturation: 30 sec94 ºC, annealing: 30 sec 65 ºC, extraction: 30 sec 72°C for 35 cycles then extraction: 6 min 72°C, finally stored at 4 ºC. PCR products was checked on agarose gels 1%. Examined by UV light and photographed. The product size 350 bp. For *L. major* isolate (Photo. 1).

PCR product (from left to right: lane 1-4 negative samples, lane 5 positive sample 26 (320bp), lane 6 DNA Leishmania ladder- 1kb).



Left L1 L2 L3 L4 L5 1Kb Right

L: lane

Fig 1. The total genome sequence of the positive sample (26).

1 :
* 20 * 40
tttactgttattgctagtgttagctgttgtattggactgtaccgacc
TTTACTGTTATTGCTAGTGTTAGCTGTTGTATTGGACTGTACCGACC: 47
1 :
* 60 * 80 *
cgccccctttgacgcccttgatgttctccttctttagtttagcatcg
CGCCCCCTTTGACGCCCTTGATGTTCTCCTTCTTTAGTTTAGCATCG: 94
1 :
100 * 120 * 140
agcccgctttacgcccagccagcaaggcccatgtttagccctgccac

AGCCCGCTTTACGCCCAGCCAGCAAGGCC-
CATGTTTAGCCCTGCCAC: 141
1 :
* 160 * 180
tttactgttgtcaccaagttcagggaccagatgtactggccgctaag
TTTACTGTTGTCACCAAGTTCAGGGACCA-
GATGTACTGGCCGCTAAG: 188
1 :
* 200 * 220 *
gggcgcataagccccgttcctactctgggccttgttcgtggacaggc
G G G C G C A T A A G C C C C G T T C C -
TACTCTGGGCCTTGTTCGTGGACAGGC: 235
1 :
240 * 260 * 280
ggttgatgcctaacctcaacaccgttcataattcgtatgggggggaac
G G T T G A T G C C T A A C C T C A A C A C -
CGTTCATAATTCGTATGGGGGGAAC: 282
1 :
* 300 * 320
gcaactatagaatttaaaattaggggaccaaatgttcccctgatcg
GCAACTATAGAATTTAAAATTAGGGGGAC-
CAAATGTTCCCCTGATCG: 329
1 :
* 340 * 360 *
gacagaccgcacggcgcaaacttatcctacccggcatttaggggcg
GACAGACCGCACGGCGCAAACTTATCC-
TACCCGGCATTTAGGGGGCG: 376
1 :
380 * 400 * 420
gtgggggcatcaattggtcagcggaatgctgaaaaggactgcgggca
GTGGGGGCATCAATTGGTCAGCGGAAT-
GCTGAAAAGGACTGCGGGCA: 423
1 :
* 440 * 460 *
cttgcataacaggtagaggtcagatgtgtgggaggaggaatttagtg
CTTGCATAACAGGTAGAGGTCAGATGT-
GTGGGAGGAGGAATTTAGTG: 470
1 :
480
taggaa----------
TAGGAA: 476
1
The collected data were statistically analyzed using the SPSS version.

All these techniques were performed in the laboratory of Al-miquat Hospital in Al-Madinah Almonawarah and molecular analysis department of microbiology, college of medicine, King Khalid University, Abha. Saudi Arabia. The sequencing was performed abroad the sample was send to S. Korea. The total sequence is illustrated in (Fig. 1). The phylogenitic analysis by BLAST (Basic Local Alignment Sequence Tool) using NCBI (National Centre for Biological Information). The outcome showed 100% similar sequence with ten isolates of L. major from middle east region, nine from Iran which (unpublished data) and one isolate from Tunisia (published data).

Table1. Master mix direct PCR

MM (with reagent Roch GMP quality)		Final concentration
10 x PCR buffer (incl.15mM MgCl2)	2.5 µl	1x (incl.15mM MgCl2)
dNTP mix (2.5 mM)	2 µl	200 µM
Primer R223 (15 µM)	0.5 µl	7.5 pmol
Primer R333(15 µM)	0.25 µl	3.75 pmol
Taq (5U/ µl)	0.25 µl	1.25 U
H2o	14.5 µl	--- --
Total volume	20 µl	----- ---

Discussion:

Cutaneous leishmaniasis is an important health problem caused by the flagellated protozoa, *Leishmania major* and *L. tropica*. *Leishmania* is transmitted by female sandflies, *Phlebotomus* species. Cutaneous leishmaniasis occurs 1–12 weeks after exposure with a small inconspicuous papule on the exposed site which enlarges and finally ulcerates.

Direct detection of parasites is done by microscopic examination of clinical specimens or by cultivation. However, all Leishmania are morphologically similar and species identi?cation is not possible using either of these techniques. Therefore, the ability to distinguish between *Leishmania* species is crucial in determining disease prognoses, as well as prescribing appropriate therapeutic regimens since some species are more refractory to treatment[5].

As there is no vaccine, drug treatment is the only way to tackle leishmaniasis. The drugs of choices are sodium stibogluconate and meglumine anti-

monite (both pentavallent antimony derivatives). Pentavalent antimony compounds have been in use for more than half a century, and they have shown response rates between 72% and 100% after intra lesion application. Their mechanism of action is not completely known. Development of resistance is of increasing concern; they require weeks of inttravenous administration and are frequently associated with malaise, anorexia, myalgia and arthralgia, electrocardiographic abnormalities, elevated aminotransferase levels, and chemical pancreatitis. Amphotericin B is recommended as second-line treatment. The most dangerous side-effect of amphotericin B is kidney damage. Other alternative treatments exist, including cryotherapy, excision, currettage and electrodesiccation, but they bear a higher risk of recurrence and are cosmetically unsatisfactory. Unfortunately, no ideal therapy for the cutaneous leishmaniasis has yet been identified. Photodynamic therapy is a developing technique which uses light to induce reactions in the body which are of benefit to patients. Photodynamic therapy uses a drug (photosensitizing agent) and a partticular type of light to destroy diseased tissue/cells whilst sparing normal tissue. Sodium stipogluconate (Pentostam) and liposome are the drugs of choice used in treatment of cutaneous leishmaniasis in Saudi Arabia[6].

A recent study carried out using PCR RFLP involving 155 CL cases originated from 10 governorates of northern Yemen highlighted the possibility of CL being caused by more than one species of *Leishmania*. The predominance of *L. tropica* as a causative agent of CL has been reported from Saudi Arabia. Although *L. tropica* most commonly causes CL, it has been isolated from VL cases. The Cutaneous Leishmaniasis, ' Oriental sore' was reported by Manson as long as 1898 to occur in Saudi Arabia,

Based on findings from several studies in Yemen, *L. tropica* is still the predominant species responsible for CL. The predominance of *L. tropica* as a causative agent of CL has been reported from Saudi Arabi,[7]. L. major is a major species causing cutaneous leishmaniasis in Sudan[8].

The sensitivity of the polymerase chain reaction (PCR) in 35 consecutive outpatients with cutaneous leishmaniasis caused by *Leishmania* (*Viannia*) *guyanensis* in the Brazilian Amazon, was evaluated using, as gold standard, the in vitro isolation of the parasite through culture of aspirates of the cutaneous ulcers. PCR showed 100% sensitivity; 95% CI from 90.0 to 100[9].

The leishmaniasis were either rare or rarely recognized in Saudi Arabia prior to about 1960. LD bodies have been detected in the spleen of two men one who had lived for 15 years in Mecca and the other in Yemen.

Cutaneous leishmaniasis is endemic in the Eastern Province, mainly in Al-Hasa Oasis. ARAMCO health workers, in 1948, began to maintain records of diagnosis in hospital in-patient. In their records for the 1960s are seven cases of Cutaneous Leishmaniasis Subsequently a high prevalence rate of CL was reported from Bisha and Khamis Mushayt in the Asir region and western region of Saudi Arabia[10]. Newer techniques of detection may help in early diagnosis[11].

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Authors' Contribution

Conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, and provision of study materials or patients.

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