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REVIEW ARTICLE

Estimation of serum electrolytes and renal function in patients with kidney failure in Mosul city

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Abstract:

The Background: Renal failure, additionally referred to as kidney failure, is an illness in which the kidneys are unable to filter bodily waste from circulation. This can cause an accumulation of harmful substances and electrolytes in the human body, which can result in a variety of unpleasant side effects. Electrolytes are minerals that are essential for proper body function, including maintaining fluid balance, regulating nerve and muscle function, and supporting heart health. Imbalances in electrolyte levels can occur in individuals with kidney failure due to the kidneys' inability to regulate electrolyte levels properly. Objectives: The purpose of this study is to determine the approximate levels of chloride, sodium, potassium, calcium, and magnesium in individuals with kidney failure. Materials and methods: 50 dialysis patients from Ibn Sina Teaching Hospital/Dialysis Unit in Al-Mosul, Iraq, aged 18–77, were studied from October 2021 to January 2022. Controls were 50 physically healthy people. They were 19–75. Blood sampling and biochemical analysis were performed. Results: This study examined the differences in age and gender groups and electrolyte levels between individuals with kidney failure and healthy controls. The findings revealed no statistically significant differences in age and gender distribution between cases and controls. However, observable differences were significant in the levels of electrolytes between cases and controls, with increased levels of potassium, magnesium, and phosphate and decreased levels of sodium, calcium, and chloride in individuals with kidney failure. Conclusion: This study suggests that regular monitoring of electrolyte levels and implementing interventions to correct electrolyte imbalances, as well as early detection and management of kidney disease, may help prevent the progression of kidney failure. Further study is required to investigate the relationship between electrolyte imbalances and kidney disease in further explanation.

Zahr**Keywords:** Cutaneous Leishmaniasis; Leishmania major; kDNA

Introduction

The parasites known as Leishmania are responsible for the transmission of the neglected tropical disease known as cutaneous leishmaniasis (CL). This disease is transmitted by sand flies known as phlebotomine. Because there are only a limited number of treatments available and there is not yet either a vaccine to prevent the disease or a treatment for it, CL continues to be a significant threat to public health all over the world (1). The disease is transmitted by the Sand-fly Phlebotomus, where the dogs considered to be host families in the Middle East and the Eastern Mediterranean Region. In Asia and Africa, the rodent mice are their host, is a disease that is found to be endemic in Middle Eastern countries such as Syria, Iraq, the Kingdom of Saudi Arabia, and Jordan, and it is still seen as an important health concern that calls for international notice (2,3). Overall, the infection begins as a small erythema around the site of the sandfly bite, gradually converts to an inflammatory papule then increases in size, after that becomes a nodule not painful. Finally, it appears discoloration scar (4). Several techniques have been described for the identification of Leishmania at the molecular level. These techniques include sequence analysis of multicopy genes, restriction fragment length polymorphism, inter genic spacer regions, DNA fingerprinting, polymerase chain reaction (PCR), and randomly amplified polymorphic DNA (5,6). However, Leishmania parasites have a single branched mitochondrion (kinetoplast), that contains a large network of kDNA, a gene has highly shown sensitivity and specificity in detecting Leishmania (7). In short, the kDNA minicircle of Leishmania parasite is provide an ideal target in order to the genotyping, because the sequence differences are allowing for correct discrimination between species (8). Considering to importance of Wasit province as a one of the mixed foci of cutaneous and visceral leishmaniasis. One of the primaries aims of this study is to identify the epidemiology of CL in spatially and timetable epidemic areas. Also, to determine the rate of L. tropica prevalence in the local area by amplifying kinetoplast minicircle DNA with nested-PCR and characterized by kDNA sequencing and phylogenetic tree to examine the intra-species relationships within L. major.

Materials and methods

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Samples collection:

This investigation was carried out at the dermatology clinic of Zahra Teaching hospital in Kut city during the months of December 2021 and at the end of March 2022. This study included a total of twenty people who had a possible case of cutaneous leishmaniasis. Cutaneous leishmaniasis was the clinical diagnosis made by a particular dermatologist after the patients complained of skin lesions in exposed portions of the body, particularly in the face, leg, and arm. Based on their clinical symptoms, it was determined that these people had CL.

After a detailed clinical and epidemiologic history had been obtained, the primary isolation was made from patients with cutaneous lesions. The puncture site (lesion) was cleaned with 70% ethanol before sample aspiration. 1 ml syringe containing 0.1-0.2 ml of sterile saline was inserted intradermal into outer border of the lesion. The syringe was rotated, and the tissue fluids were gently aspirated into the needle while its withdrawal. The aspirated material was inoculated in culture tubes containing five ml of NNN medium supplemented with 10% human blood and antibiotic. All inoculated tubes were incubated at 25º C. All cultures were incubated and examined for 7-28 days before being considered negatives. Patients were positively diagnosed for CL when actively motile promastigotes were seen in culture (9).

Microscopic examination:

A small amount of aspirated fluid was taken and smeared on a clean glass microscope slide. After the smears dried completely, they were fixed with 100% methanol, allowed to dry again, and stained with Giemsa stain for microscopic examination for presence of amastigotes (10). And then, they were examined under a light microscope with magnification at 100×.All the smear of samples where amastigote was observed were accepted to be positive, and those preparations where amastigote was not observed were negative.

Genomic DNA extraction:

The gSYAN DNA kit (Geneaid) was used for DNA extraction from positive culture media (Nove-MacNeal-Nicolle) samples, according to protocol of producing company. Extracted DNA was examined using Nanodrop spectrophotometer, then stored at -20°C until used in PCR amplification.

Nested PCR:

The Nested PCR technique was performed for detection of cutaneous leishmania based on the kinetoplast DNA (kDNA) for detection L. major and L. tropica. This method was carried out according to method described by (11). Amplification of the kDNA gene of Leishmania species using the primers was provided from Macrogen company, Korea as following table (1).

Nested PCR master mix preparation: the mix was prepared using master mix reagent and done depend on company instructions as following, First run table (2)

The PCR master mix placed in PCR premix tubes provided by kit that contain other PCR components Then, all the PCR tubes transferred into vortex for 2minutes. Then transferred into PCR thermocycler (Biorad, Bioneer. Korea). PCR thermocycler conditions table (3).

Second Run Nested PCR table (4):

PCR thermocycler conditions table (5):

PCR product analysis: The Nested PCR products were checked in electrophoresis in a 1% agarose gel and 1X TBE buffer, then stained by ethidium bromide, and investigation under UV-transilluminator.

DNA sequence method:

Gene sequencing (phylogenetic tree analysis) has been performed using the Molecular Evolutionary Genetics Analysis (VEGA) 6.0 version. (Mega 6.0) and ClustalW alignment analysis of multiple sequence alignments based on the Maximum Composite, Likelihood technique was used to calculate the evolutionary space by the phylogenetic tree UPGMA method. Finally found L. major separates was submitted to NCBI-GenBank to acquire a Genbank accession number.

Results and Discussion

Twenty skin samples of suspected patients were enrolled in our study, as shown in table (6) representing microscopic (Giemsa staining) detection results, 13 (65%) were found to be positive for amastigote of cutaneous Leishmania, and 7 (35%) were negative. The Giemsa stain provides a better stain intensity, and show some details that may be unclear otherwise, especially in cells, but some smears of Giemsa stain gave negative results, and the parasite doesn't see or disappeared (12), that attributed to many reasons, patient take treatment, mistake in time staining, smear thickness and sometimes distortion of the cell wall may occur (13).

Molecular identification of Leishmania spp. using Nested-PCR technique

In the present study, DNA extraction of three positive culture isolates for detection of Leishmania spp. Specific primers were used to detect of mitochondrial kinetoplast minicircle fragment DNA (kDNA) (non-protein coding region) of L. tropica, and L. major. Agarose gel electrophoresis results for three DNA samples were detected L. major. Parasite.There are three positive samples appear to be L. major in which the DNA bands of L. major was 650 bp molecular weight, as shown in the figure (1). Nested-PCR technique is considering a suitable method, has high sensitivity and very resolution in identification of Leishmania parasites in clinical samples (14,15). Moreover, kDNA gene has highly shown sensitivity and specificity in detecting Leishmania (16). Nested-PCR of kDNA was demonstrated to be an efficient method with good sensitivity and specificity to identify human Leishmania spp. by (17). Additionally, (18) in 2016 suggested exploiting the high sensitivity and specificity of the kDNA gene to diagnose Leishmania species. In short, high abundance of kDNA minicircles in each kinetoplast make them an ideal target for diagnosis of Leishmania parasites (19). Nested PCR was used by (20) to identify the species of Leishmania based on kDNA minicircles (big number), and they noted that this method can be a useful diagnostic and identification tool for Leishmania species. According (21) in 2016, kDNA fragments are more sensitive than other molecular diagnostic methods for leishmaniasis.

Analysis of the DNA sequence and a phylogenetic tree

The DNA sequencing method was utilized in order to identify genetic relationships and conduct genetic variation (Mutations) analysis in mitochondrion kinetoplast DNA sequence in the locally isolated Leishmania major IQ-Kut strain as well as NCBI-Blast related Leishmania major isolates.

According to the results of the phylogenetic tree genetic relationship analysis, the local Leishmania major IQ-Kut isolate is

closely related to the NCBI-BLAST Leishmania major isolate IQ LM.No.2 isolate at a total genetic change level of 0.0020 %, as shown in the figure (2).

The genetic homology sequence identity between the local Leishmania major IQ-Kut isolate and the NCBI BLAST related Leishmania major isolate IQ LM.No.2 isolate was shown to range from (99.19 %). The table (7) illustrates this point.

The genetic variation (substitution Mutations) analysis in mitochondrion kinetoplast DNA sequence between local Leishmania major IQ-Kut isolate and NCBI-Blast related NCBI-BLAST Leishmania major isolate IQ_LM.No.2 isolate were found three substitution mutations at total genetic variation percentage ranged (0.81%). As showed in table (8). And figure (3).

Finally, the Leishmania major IQ-Kut isolate was submitted into NCBI Genbank and identified by accession numbers (OP046364.1).

The kDNA minicircle sequence of the L. major isolate had minimal variability, according to the findings. A phylogenetic tree revealed that the local L. major isolate had a tight genetic relationship to the Iraqi MF166792.1 strain and was genetically more like its sequences. The result is matching with Al-Tamemy and Al-Qurashi ,2017 (22) were mentioned that phylogenetic analysis of Leishmania strains by utilizing the cytochrome b gene sequences in the Iraqi border region, it was discovered that the Iraqi MF166792.1strain strain was closely related.

Conclusion

Nested PCR is appropriate for Leishmania identification at the species level. Wasit province is considered one of the foci of leishmaniasis, especially, L. major. There is still much to learn about molecular epidemiology from the phylogenetic data of L. major in Wasit. The degree of genetic convergence between local isolates may be estimated using the phylogenetic tree. Different clinical symptoms of the cutaneous lesion might result from genetic diversity of kDNA minicircle sequences among L. major isolates. Therefore, when the characteristics of L. major are clinically significant, genetic heterogeneity diagnosis is crucial for the detection of therapy, control, and epidemiological investigations.

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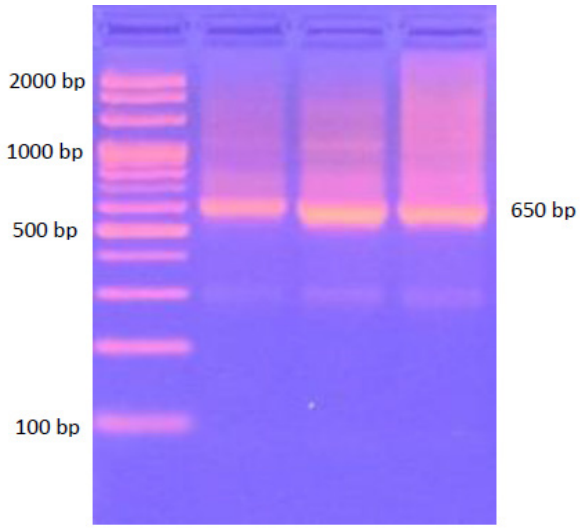

Figure (1): Agarose gel electrophoresis image that shows the Nested PCR product analysis of mitochondrial kDNA in Cutaneous Leishmania positive isolates from positive culture samples. Where M: DNA Ladder marker (100-2000 bp), lane (1-3) positive L. major at (650bp) product size.

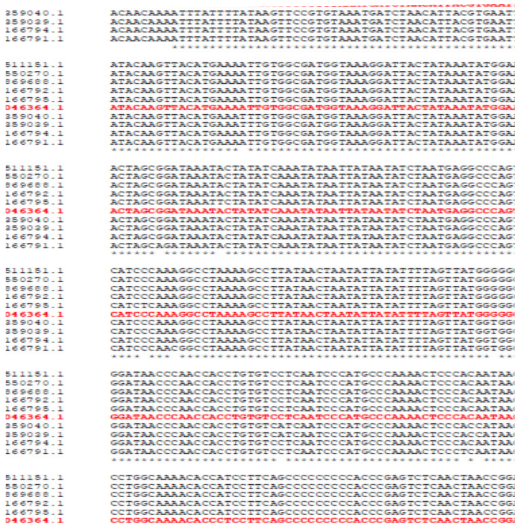

Figure (2): Multiple sequence alignment analysis of mitochondrial kinetoplast DNA in the IQ-Kut isolate of Leishmania major and NCBI-BLAST Leishmania major isolates. ClustalW alignment tool was used to create the multiple alignment analysis (MEGA 11 version). This demonstrated the alignment similarity of nucleotides as (*) and substitution mutations in mitochondrial kinetoplast DNA between different Leishmania major isolates.

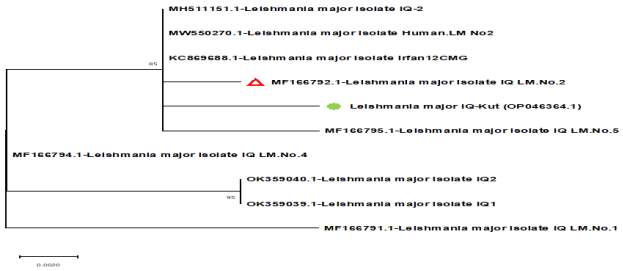

Figure (3): Phylogenetic tree analysis based on mitochondrion kinetoplast DNA partial sequence in local Leishmania major IQ-Kut isolate that used for genetic confirmative detection and genetic relationship identification. The phylogenetic tree was constructed using the Unweighted Pair Group method with the Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local Leishmania major IQ-Kut was showed closed related to NCBI-BLAST Leishmania major isolate IQ_LM.No.2 at total genetic changes (0.0020%).

Table (1) Primer sequences that used in Nested PCR for Leishmania species typing.

Gene	Primer	Sequence (5'-3')	Product size
First PCR kDNA primer	LINR4 F	GGGGTTGGTGTAAAATAGGG	720bp
	LIN17 R	TTTGAACGGGATTTCTG	
Second Nested PCR kDNA primer	LINR4 F	GGGGTTGGTGTAAAATAGGG	650bp
	LINR19 R	CAGAACGCCCCTACCCG	

Table (2) External PCR Master Mix Components.

Master mix components	Volume
DNA template 5-50ng	5µL
(First PCR kDNA primer Forward primer (10pmol	2µL
First PCR kDNA primer Reveres primer (10pmol)	2µL
GoTaq ®Green PCR master	12.5µL
PCR water	3.5µL
Total volume	µL 25

Table (3) External PCR cycle conditions.

PCR cycle	Repeat	Temp.	Time
Initial denaturation	1	95C	5min
Denaturation	30	95C	30sec.
Annealing		55C	30sec
Extension		72C	1min
Final extension	1	72C	5min
Hold	-	4C	Forever

Table (4) Internal PCR Master Mix Components.

Master mix components	Volume
PCR product	2.5µL
(Second PCR kDNA primer Forward primer (10pmol	2µL
Second PCR kDNA primer Reveres primer (10pmol)	2µL
GoTaq ®Green PCR master	12.5µL
PCR water	5.5µL
Total volume	25 µL

Table (5) Internal PCR cycle conditions.

PCR cycle	Repeat	Temp.	Time
Initial denaturation	1	95C	5min
Denaturation	30	95C	30sec.
Annealing		55C	30sec
Extension		72C	1min
Final extension	1	72C	5min
Hold	-	4C	Forever

Table (6): Microscopic investigation of the Leishmania parasite in skin lesions using Giemsa stain.

Detection method	Total number of samples	Positive		Negative	
		n	%	n	%
Microscopic examination	20	13	65%	7	35%

Table (7): The NCBI-BLAST Homology Sequence identity (%) between local Leishmania major IQ-Kut and NCBI-BLAST submitted related isolates:

Leishmania major isolate	Accession number	NCBI-BLAST Homology Sequence identity (%)			
		Identical isolate	GenBank Accession number	Country	Identity (%)
Leishmania major IQ-Kut	OP046364.1	Leishmania major isolate IQ_LM.No.2	MF166792.1	Iraq	99.19%

Table (8): The NCBI-BLAST genetic variation analysis between local Leishmania major IQ-Kut and NCBI-BLAST submitted related isolates.

Leishmania major isolate	Accession number	Homology sequence identity (%)		
		Number Mutations	Type of Mutation	Mutation %
Leishmania major IQ-Kut	OP046364.1	3	A/T, A/C, C/T	0.81%