Molecular detection of Leishmania in dermal tissue: Case control study

Moayad Aziz Abdulqadir[1*]

[1]Department of Dentistry, AlNoor University College, Nineveh, Iraq

Abstract Background: An endemic disease cutaneous leishmaniasis CL was spread in Iraq. In 2020, there were more CL cases than in previous years, with most occurring in rural areas. In this scenario, the current diagnostic technique must be replaced with a more sensitive one. This research aimed to examine if polymerase chain reaction (PCR) is an efficient approach for identifying leishmanial parasites in skin biopsies from Iraqi patients.
Methodology: PCR was used to evaluate samples collected from one hundred people who had cutaneous ulcers that were compatible with leishmaniasis. The primers used in the analysis were produced from the gene for the small subunit of the ribosomal complex. In order to determine which method was most sensitive to the presence of Leishmania, PCR, smear staining, and in vitro culture were put through their paces.
Results: In vitro culture test had a sensitivity of 72 percent, while the direct microscopy smear had a sensitivity of 88 percent. The sensitivity of the PCR test was much greater (88 percent) (71 percent). In addition to this, the specificity of the PCR test that we used was rather good (99 percent).
Conclusions: When it comes to making a diagnosis of cutaneous leishmaniasis, the polymerase chain reaction, commonly known as PCR, has to be regarded as a method that is not only advantageous but also sensitive and more time and cost effective. It is especially important to keep this in mind for patients whose parasitological testing came back negative.
Keywords: PCR, leishmania, skin, dermal tissue, molecular detection, parasite.

1. Introduction

The illness known as leishmaniasis can be found in every region of the world, and it is estimated that it endangers the lives of around 350 million people worldwide. Only 500,000 people are affected with visceral leishmaniasis, but between one and one and a half million people are affected by cutaneous leishmaniasis[1]. It is estimated that there are 12 million occurrences all over the world, with between 1.5 and 2 million new instances emerging year[2]. This number is based on the belief that there are 12 million occurrences. The number of new cases of cutaneous leishmaniasis that were reported in Iraq in 2020 was rather high overall. This indicates a significant increase when compared to the elevated instances that

* Corresponding Author: researcherstaff08@alnoor.edu.iq

© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

were recorded in 2021, which demonstrates that there has been a discernible trend toward an increase over the course of the last decade[3]. The number of cases of cutaneous leishmaniasis that were documented in Iraq in the year 2020 was much greater than in the years that came before. When the parasite forms one or more ulcers on the skin that heal extremely slowly, this is known as the clinical manifestation of cutaneous leishmaniasis, which is also known as CL and is usually abbreviated as CL. One of the distinguishing characteristics of this illness is how long it takes to get better after an episode of it. The species of Leishmania that are found in the ancient world are the ones that are responsible for causing the benign and self-limiting ulcers[4]. These sores are brought on by a form of Leishmania that is found in the old world. Despite the fact that the mucocutaneous form of leishmaniasis is the most severe kind of the disease, the New World species is to blame for a wider variety of clinical signs. The vast majority of the diagnostic procedures that are currently being used place a primary focus on the collection of clinical and epidemiologic data in addition to parasite specimens as their primary research objectives[5]. This is the case for the majority of the diagnostic procedures that are currently being used. This is true for the vast majority of the different diagnostic techniques. There is not a single laboratory test that has attained the level of general acceptance required to perform the role of the diagnostic "gold standard" for CL at this point in time. In patients who have been given a clinical diagnosis of cutaneous leishmaniasis, it is not always possible to draw a conclusive conclusion from the findings of parasitologic testing carried out on tissue retrieved from a skin biopsy[6]. This is because the results of these tests are dependent on the presence of the parasite that causes cutaneous leishmaniasis. This holds true even in circumstances in which doing so would be feasible. There are a few distinct PCR tests that have been developed specifically for the goal of determining whether or not the Leishmania parasite is present in the body. These tests can be found in a number of various places online. Surprisingly high levels of sensitivity may be achieved by technologies that are founded on PCR in a broad variety of different settings and conditions[7]. Primers for PCR that are specific to Leishmania have the ability to amplify either repeated nuclear sequences, such as those found in ribosomal, miniexon, and repetitive nuclear DNA; or minicercle kDNA, which is present at a rate of approximately 10,000 copies per parasite. Both of these types of sequences can be found in the parasite's genome. Both of these kinds of sequences may be discovered in nuclear DNA. Nuclear DNA is composed of nucleotides. Examples of both of these types of sequences may be found in the genome of the leishmaniasis parasite[8]. The kDNA primers have the ability to either amplify the full of the minicircle or certain parts of the variable and conserved regions depending on the situation. The results will be closer to what was intended if the minicircle is amplified in its full. In any event, this is one of the outcomes that may occur. One of the sequences that is a part of the multicopy group and is one of the sequences that has been analyzed the most completely is the gene sequence for the small subunit of ribosomal RNA, which is also known as the 18S rRNA gene sequence[9]. Both of these names refer to the same sequence. It is also one of the sequences that is popularly known as the 18S rRNA gene sequence. rRNA stands for ribosomal RNA. Each and every one of a host's parasites carries with it a sizeable collection of 18S rRNA gene copies that are identical to one another (about 160). In order to develop a sensitive detection approach for Leishmania DNA that can be used as a target in a PCR experiment, van Eys et al. decided to focus their efforts on the core part of the SSU rRNA gene[10]. This decision allowed them to design a method that can be used as a target in the experiment. Because of this, they were able to accomplish their objective of establishing the approach. As a direct consequence of this, they will be able to devise a technique of detection that is very sensitive. The findings that may be obtained by applying this way of detection will have a better degree of accuracy than those that may be obtained using other methods[11]. The numerous applications of PCR primers that have been created as a direct result of this study are outlined in the following list. We

tested the ability of polymerase chain reaction, also known as PCR, to identify Leishmania DNA in skin biopsy specimens taken from one hundred people living in various regions of Iraq who were clinically suspected of having cutaneous leishmaniasis. These individuals had clinical symptoms consistent with having the disease. These people displayed clinical symptoms that were compatible with having the condition. These individuals exhibited clinical symptoms that were consistent with having the illness [12]. The aim of this study is to investigate Molecular detection of Leishmania in dermal tissue in a Case control study research.

2. Materials and Methods

Sampling

Twenty-six people in all who were suspected to have cutaneous leishmaniasis each gave a clinical specimen for examination. Twenty-three of the patients came from rural areas in the south of Iraq, and three of the patients came from rural areas in the south of Iraq near the cities of Wasit and Maysān. Patients were selected for this investigation based not only on the clinical signs of the disease but also on the epidemiological risk factors associated with cutaneous leishmaniasis. All of the patients who took part in this research were from endemic areas. After doing a thorough dermatological examination on each patient, a dermatologist discovered that each patient had cutaneous lesions that were typical of leishmaniasis. This conclusion was reached after the dermatologist examined all of the patients.
The disinfectant was used to clean and sterilize not just the skin lesions, but also the skin close to them that seemed to be normal. Biopsies of the skin were taken in a sterile environment from the margin of the ulcer using a disposable scalpel blade. The size of the biopsies ranged from 2 to 4 millimeters in diameter. After the lesions had been cleaned, the tip of the blade was inserted into the margin of each one to create a minute cut. After making a 90-degree rotation with the blade, it was scraped along the cut edge of the incision to remove and collect the skin tissue that had been sliced into three portions. This was done while the blade was still turned. One piece was used for the smear, another for the culture, and the other component was frozen at -80 degrees Celsius until it could be used for the PCR analysis. Both of these procedures were carried out on separate portions of the sample. The biopsies of the patients' skin that were collected acted as negative controls for the experiment. There were five patients total.

Rapid Testing

The smears were produced by bringing the tissue sample from the biopsy into contact with a glass microscope slide. After the drying step was finished, the smears were fixed with methanol at a concentration of one hundred percent, allowed to dry a second time, and then stained with Giemsa. The slide inspection was completed with a 100X immersion objective, which was used for the whole of the process. Each of the slides was looked at not one but twice before it was decided that the result was negative and could be confirmed.

Isolation of parasite

Before continuing on to the subsequent step of each biopsy, the specimen was cultured once the first stage was finished successfully. By mashing the chunks of tissue using a plastic pestle and a sterile mortar, the pieces of tissue were able to become more uniform in size while also being decreased in size. This chemical was used in the process of inoculating four tubes, each of which contained a biphasic culture media. The tubes were inoculated in a biphasic culture medium. The process was referred to as inoculation. The temperature of the

medium that was used for inoculation was maintained at a steady 25 degrees Celsius for the entirety of the procedure. In order to determine whether or not promastigotes were present, it was possible to conduct thorough testing by making use of microscopy on a weekly basis. It was found that a culture was positive for the presence of the parasite by studying it under a microscope and looking for indicators of the existence of at least one promastigote in the culture. This allowed for the determination of whether or not the culture contained the parasite. On the other hand, a culture was considered to have yielded unfavorable results if, after a period of one month, it was determined that no parasites had been cultivating there. This was the criteria used to determine whether or not the culture had generated negative outcomes **(figure 1).**

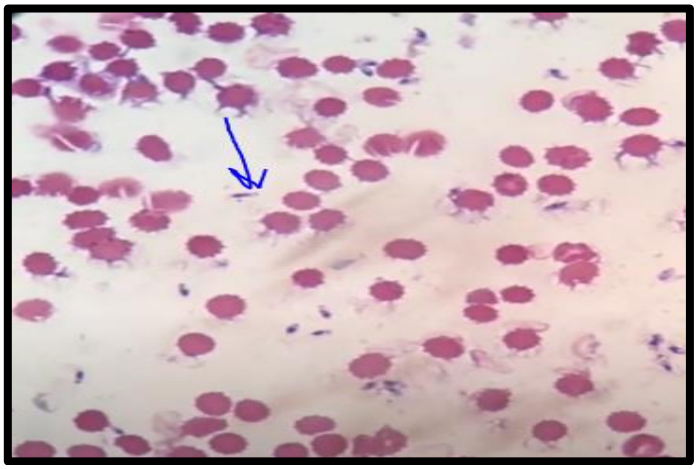

Figure 1: Isolated leishmania from skin of patients.

DNA extraction and PCR processing

Frozen biopsy samples, after thawed, were put through an incubation procedure that lasted for four hours at a temperature of 45 degrees Celsius. This process was carried out after the samples had been thawed. In the incubation solution, there was 1.5 percent SDS and 100 ug/ml of proteinase K. Additionally, the solution contained 10 mM Tris HCl with a pH of 8.0, 10 mM EDTA, and 10 mM NaCl with a NET concentration of 10. Following that, the samples were put into the solution. Following this step, the required chemical was obtained by the use of two separate phenol extractions as well as ethanol precipitation. After going through the distillation process, the precipitate was dissolved in the water that was left behind.

Primer's reaction

The following is a description of the small subunit of the ribosomal gene, which is where the primers that are used for the detection and identification of the Leishmania parasite are located: 5'- AAGATGCCGCCGTACTTTGA-3', 5'- AAGACGTAGCGACCACCAAG- 3'. The reaction mixture had a total volume of 50 l and had between 150 and 180 pM of each primer in addition to a buffer that contained 1x DNA polymerase, 100 M dNTP, and the buffer. The reaction mixture also had a total volume of 50ml. In addition, the mixture had a total of 2 l of sample DNA and 0.5 U of Taq polymerase. All of these components were combined together to make up the mixture. After each of the reactions had been carried out to completion, a layer of mineral oil totaling fifty liters was poured. A grand total of thirty-two cycles were completed in a device called a thermocycler. There was a denature phase that lasted seventy-five seconds at a temperature of 94 degrees Celsius, an anneal step that

lasted the same length of time at a temperature of sixty degrees Celsius, and an extension step that lasted seventy-two degrees Celsius. These three phases were repeated in every cycle (2 m). In every single experiment, we made it a point to carry out the procedure using both positive and negative controls. DNA from Leishmania infantum, Leishmania tropica, and Leishmania major were used as the positive controls. The samples used as negative controls did not have any trace of DNA. Electrophoresis was carried out on an agarose gel with a concentration of two percent in order to visualize ten liters of the reaction mixture. The temperature of the gel bath was kept at room temperature. Following the amplification step, the sample that had been created was digested with 10 units of. The samples were analyzed by utilizing reference strains of Leishmania infantum, Leishmania tropica, and Leishmania major on an agarose gel with a concentration of 2 percent.

Diagnostic criteria for determining cutaneous leishmaniasis (CL)

When cultures or stained tissue smears yielded positive results for the presence of parasites, it was concluded that the findings of the specimens were positive for the presence of parasites. This was done in order to identify whether or not the specimens' included parasites. In order to investigate the level of sensitivity offered by the PCR test, samples were employed that were collected from people who had previously been determined to have cutaneous leishmaniasis. On the other hand, the specificity of the test was examined by using the test results of patients who did not have leishmaniasis and who resided in places that did not have leishmaniasis. These patients were tested in regions where leishmaniasis was not present. The patients in question were examined in settings free of the leishmaniasis parasite (Table 1,2).

Table 1: Smear diagnosis.

Smear					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Negative	77	73.3	77.0	77.0
	Positive	23	21.9	23.0	100.0
	Total	100	95.2	100.0	
Missing	System	5	4.8		
Total		105	100.0		

Detection of parasites

One hundred Iraqi patients who were thought to have cutaneous leishmaniasis each had a specimen obtained from them, and those specimens were evaluated using three different diagnostic strategies. The total number of specimens taken from Iraqi patients was one hundred. On the same day, a microscopical examination of a third of each biopsy specimen was performed in order to search for evidence of the existence of amastigote. This was done in order to determine whether or not the amastigote was present. The middle third was utilized for culture in vitro, and the remaining third was frozen at -80 degrees Celsius in order to be ready for PCR processing the following day. In addition to this, five samples were included from patients who suffered from a range of skin conditions that were clinically comparable to CL. These patients' samples were also included. These patients were selected at random from the general public to participate in the study. During the PCR, we made use of the SSU rRNA gene, which may be found in the nucleus DNA in a total of 160 different copies. As a direct consequence of this, the final product had a length of 650 base pairs across its whole. In the vast majority of cases, the results of the PCR test may be collected within a period of twenty-four hours (Table 1,2).

Molecular detection of cutaneous leishmaniasis

There was some comparing and contrasting done between the results of the various tests. Both parasite cultures and microscopic analysis of smears were highly specific for the diagnosis of CL, as defined by the consensus criteria, and when studied together, they correctly identified fifty out of one hundred of the suspected specimens as having CL. The consensus criteria were developed by a group of experts in the field. When they were applied, the standards showed this to be the case. However, eight of the positive specimens were recognized by one strategy but not by the other, suggesting that it is desirable to use both approaches concurrently for the highest possible level of effectiveness. The sensitivity of both of these tests was shown to be seventy percent. 45 out of the 50 people who were determined to have cutaneous leishmaniasis by culture and/or microscopy also had positive PCR findings. This indicates that the disease is spread through the skin. It is abundantly clear that the polymerase chain reaction (PCR) had the highest sensitivity of any individual test because it correctly diagnosed 85.2 percent of patients with confirmed cases of cutaneous leishmaniasis while failing to identify two specimens (Table 2).

Table 2: PCR diagnosis

PCR					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Negative	40	38.1	40.0	40.0
	Positive	59	56.2	59.0	99.0
	22.00	1	1.0	1.0	100.0
	Total	100	95.2	100.0	
Missing	System	5	4.8		
Total		105	100.0		

Our findings make this conclusion abundantly clear. These two erroneous negative results might have been caused by PCR inhibition, as it is a possibility. The outcomes of biopsies performed on individuals whose skin problems were not caused by cutaneous leishmaniasis were consistently unfavorable. During the course of this inquiry, a PCR was utilized that possessed a specificity that was accurate each and every time it was put to use. PCR amplification of leishmania DNA from a patient who was diagnosed as having chronic leishmaniasis but for whom a diagnosis could not be made. Six out of thirteen patients with cutaneous leishmaniasis who tested negative for smear and culture were able to have their Leishmania DNA discovered by PCR. This percentage represents a significant improvement over previous diagnostic method. This percentage reflects a substantial improvement over the diagnostic approaches that were used in the past. This reveals that the smear and culture procedures, particularly when performed simultaneously, are not helpful in diagnosing cutaneous leishmaniasis. This is especially true when many actions are combined into one. The nine negative PCR samples were purified by diluting them two, four, and even 10 times; nonetheless, the results did not alter at any point throughout the process of purification or dilution. This was true regardless of how many times the samples were diluted. This was done in an effort to rule out the likelihood of PCR inhibition, which was the primary aim at the beginning of the investigation(figure 2).

Table 3: diagnosis of current methods

Diagnosis by Current Methods					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Non	28	26.7	28.0	28.0
	+CL	72	68.6	72.0	100.0
	Total	100	95.2	100.0	
Missing	System	5	4.8		
Total		105	100.0		

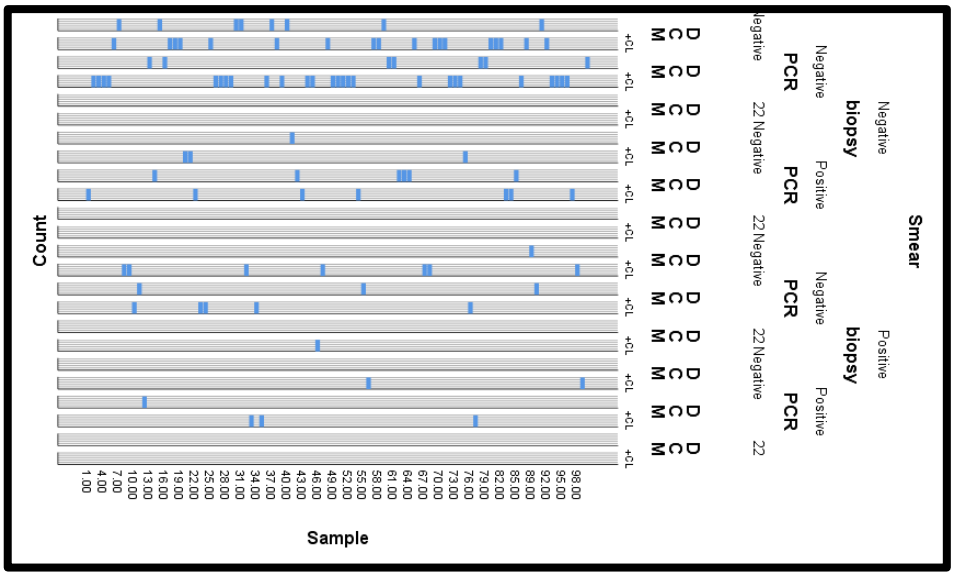

Figure 2: comparing diagnosis (DCM; Diagnosis of current methods)

3. Discussion

In many instances, it may be difficult to diagnose chronic lymphocytic leukemia (CL). Traditional diagnostic approaches, such as in vitro culture, smear, and direct examination, are easy to use; nevertheless, in order for them to be accurate, a relatively high number of living or morphologically intact microorganisms need to be present [1]. Traditional diagnostic procedures include: The findings that were provided in this study for the diagnosis of CL through the use of microscopy (72 percent) or through the use of parasite culture (71 percent) are comparable to the findings that were published by other organizations[2]. It has been claimed that the sensitivity of microscopic procedures for CL can range anywhere from 20 percent to 88 percent. Some examples of these tests are histopathology and tissue smears, touch preparations, and exudates. The clinical presentation, the type of the parasite, the amount of technical skill, and a variety of other factors all play a role in determining this. It has also been shown that the sensitivity of cultivated parasites can range anywhere from twenty to eighty-seven percent of the time[3]. Additionally, depending on the kind and number of parasites that were implanted during the time of the biopsy, it may take anywhere from a few days to a few weeks before the parasites are discovered. In addition, cultures may be contaminated, and the proportion of infected samples may reach as high as 33 percent in some unusual circumstances[4]. Numerous studies have been conducted to compare and

contrast the PCR diagnosis with other, more conventional diagnostic approaches. With the exception of a few particular cases, it has been demonstrated that PCR-based diagnostic assays are significantly more sensitive than the conventional parasitologic methods of diagnosis[5]. They were able to show via their investigation that the sensitivity of the PCR as a diagnostic tool for cutaneous leishmaniasis is 96 percent. [Further citation is required] On the other hand, smears acquired through the use of direct microscopy demonstrated a sensitivity of 67%. Conventional diagnostic procedures for cutaneous leishmaniasis, such as culture and histopathologic evaluation of biopsies, were reported to have a lower sensitivity than PCR. More specifically, conventional diagnostic procedures had a sensitivity of 67.5 percent, while PCR had a sensitivity of 64.3 percent (90.4 percent) [6]. These findings have been verified by the outcomes of our research, which shown that PCR is more effective than traditional methods in the diagnosis of CL (84.6 percent). In order to accomplish this goal, more patients who had been missed by either microscopic examination or culture were located and identified. It is likely that additional factors, such as the sample location, play a role in altering the outcome of any diagnostic test for cutaneous leishmaniasis. This is because cutaneous leishmaniasis is a disease that affects the skin (CL) [7]. This is demonstrated by the fact that two people whose PCR results were false negatives tested positive for cutaneous leishmaniasis when they were cultured for the condition. When it comes to CL, it has been established that the levels of parasites and, as a consequence, the diagnostic sensitivities for both PCR and other traditional diagnostic procedures can alter spatially inside a lesion. This is true for both types of diagnostic tests. Using the sequences from the small subunit ribosomal gene as a template for PCR amplification gives a substantial benefit. This is due to the fact that ribosomal genes are highly repetitive in the Leishmania genome (about 160 copies apiece) [8]. It has been shown via the use of clinical samples obtained from human patients that this target is quite useful in the diagnostic process for leishmaniasis. Using ribosomal primers in a PCR experiment was shown to successfully amplify DNA at a concentration corresponding to fewer than 10 promastigotes, according to a publication. By employing these PCR primers, we were able to show that the PCR method is a useful tool for identifying Leishmania DNA in skin samples acquired from Moroccan patients as part of the current experiment. Even though both the direct microscopy smear and the culture came back negative, the polymerase chain reaction (PCR) came back positive in six of the thirteen patients (46 percent) who were suspected of having CL. This was despite the fact that both the direct microscopy smear and the culture came back negative[9]. This reveals that the direct microscopy smear and culture are useless in diagnosing CL, even when used jointly. This was the case even when they were employed simultaneously. The high sensitivity and specificity of PCR, the case history (i.e., whether the individuals were exposed to risk of acquiring the disease), and the clinical examination of lesions confirmed that these samples are not false positives, but rather true positives that contain very few parasites. Additionally, the case history confirmed that the individuals were exposed to risk of acquiring the disease. In addition, the case history provided evidence that the patients were put in situations where they were at danger of contracting the disease. Because of the presence of contamination, the negative control samples that were utilized in each and every PCR experiment did not produce any bands. In comparison to more conventional methods, the polymerase chain reaction (PCR) method proved to be the diagnostic test with the highest levels of sensitivity and specificity when applied to tissue samples[10]. In addition to this, it was demonstrated to be a reliable instrument for making a differential diagnosis of cutaneous lesions that were brought on by other reasons. It is vital to establish a precise diagnosis and begin curative treatment of the illness as soon as possible in order to halt the course of the condition while it is still in its early stages, therefore preventing the production of disfiguring scars as well as long-term chronic disorders. In addition to this, its relevance for the reduction of the human reservoir is of the highest importance. The parasite will continue to spread

throughout the community if not all cases are correctly recognized and treated in a timely manner. In accordance with the restriction for amplified products of 17 biopsies, we were only able to get one profile of Leishmania infantum[11]. This particular case occurred from a region in the province of Taounate, which is a center for cutaneous leishmaniasis caused by L. infantum. The remaining 16 biopsies were able to offer information on the profiles of L. major and L. tropica. Two of these 16 biopsies were from the Northern Slope of the High Atlas, which is an endemic zone for cutaneous leishmaniasis caused by L. tropica. The Northern Slope of the High Atlas is located in Morocco[12]. Tanant and Smimou were present. The culture-positive strains TN2 and TN5, which were later proven to be L. tropica by the utilization of enzyme electrophoresis on a panel of 15 enzymes, are referred to as "culture-positive strains" (data not shown). According to the findings of five separate biopsies, L. major is most likely to blame for cutaneous leishmaniasis[13]. Using a method called isoenzyme electrophoresis, it was found that the isolated bacteria belonged to L. major (data not shown). The PCR-RFLP method that was utilized for this research produced identical profiles for both L. tropica and L. major. However, if we take into account the patient's place of origin, we will be able to establish whether the patient is infected with L. tropica or L. major. Both MR1 and KH1 originated in the Fes province, which is becoming an increasingly important location for cases of cutaneous leishmaniasis caused by Leishmania tropica. The L. major population is almost entirely restricted to the dry Saharan area, whereas the L. tropica population has the broadest geographic range and has even been observed in central Iraq[10]. In point of fact, these two species have quite distinct geographical distributions in Morocco: the L. major population is almost entirely limited to the dry Saharan area; the L. tropica population has the broadest geographic range. The ability to identify species is very important for identifying not only the prognosis of the ailment but also the therapy that should be given to the patient[13].

4. Conclusion

In conclusion, the outcomes of this study show that the polymerase chain reaction (PCR) is a viable alternative laboratory technique for diagnosing CL. This is especially true in cases when the sickness was not identified using procedures that are more often used. The polymerase chain reaction, often known as PCR, has a lot of benefits in terms of its therapeutic relevance. These benefits include the following: it is exceedingly sensitive and specific, and most importantly, it is speedier than the existing traditional treatments. However, in order for this plan to be implemented in locations where the illness is endemic, it will first be essential to overcome considerable challenges such as the demand of laboratory facilities and the expense involved.

5. Highlights

PCR is a highly effective and sensitive diagnostic method for cutaneous leishmaniasis, with 88% sensitivity. It is a cost-effective alternative to traditional diagnostic methods, especially for negative parasitological testing. However, implementation of this technique in endemic areas may face challenges due to the need for laboratory facilities and associated costs.

6. References

1. World Health Organization. (2020). Leishmaniasis. Retrieved from https://www.who.int/news-room/fact-sheets/detail/leishmaniasis
2. Alvar, J., Vélez, I. D., Bern, C., Herrero, M., Desjeux, P., Cano, J., Jannin, J., & Boer, M. D. (2012). Leishmaniasis worldwide and global estimates of its incidence. PloS one, 7(5), e35671. https://doi.org/10.1371/journal.pone.0035671
3. Sahoo, Pallavi, Nikhil Kothari, Shilpa Goyal, Ankur Sharma, and Pradeep K. Bhatia. "Comparison of norepinephrine and terlipressin vs norepinephrine alone for management of septic shock: a randomized control study." Indian J Crit Care Med 26, no. 6 (2022): 669-675.
4. Kumar, Pradeep HG, Madhusudan A. Kalluraya, C. Jithendra, Ashwin Kumar, Sudhindra P. Kanavehalli, Arul D. Furtado, and Ravindra Mehta. "Venoarterial Extracorporeal Membrane Oxygenation is Effective in Severe Aluminum Phosphide Overdose Despite Delayed Presentation." Indian Journal of Critical Care Medicine: Peer-reviewed, Official Publication of Indian Society of Critical Care Medicine 25, no. 12 (2021): 1459.
5. de Vries, H. J., Reedijk, S. H., & Schallig, H. D. (2015). Cutaneous leishmaniasis: recent developments in diagnosis and management. American Journal of Clinical Dermatology, 16(2), 99-109. https://doi.org/10.1007/s40257-015-0114-z
6. Salam, N., Al-Shaqha, W. M., & Azzi, A. (2014). Leishmaniasis in the Middle East: incidence and epidemiology. PLoS Neglected Tropical Diseases, 8(10), e3208. https://doi.org/10.1371/journal.pntd.0003208
7. Mary, C., Faraut, F., Lascombe, L., & Dumon, H. (2004). Quantification of Leishmania infantum DNA by a real-time PCR assay with high sensitivity. Journal of Clinical Microbiology, 42(11), 5249-5255. https://doi.org/10.1128/JCM.42.11.5249-5255.2004
8. Schönian, G., Nasereddin, A., Dinse, N., Schweynoch, C., Schallig, H. D., Presber, W., & Jaffe, C. L. (2003). PCR diagnosis and characterization of Leishmania in local and imported clinical samples. Diagnostic Microbiology and Infectious Disease, 47(1), 349-358. https://doi.org/10.1016/S0732-8893(03)00093-2
9. Hallek, M., Cheson, B. D., Catovsky, D., Caligaris-Cappio, F., Dighiero, G., Döhner, H., ... & Kipps, T. J. (2018). iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Blood, The Journal of the American Society of Hematology, 131(25), 2745-2760.
10. de Vries, H. J., Reedijk, S. H., & Schallig, H. D. (2015). Cutaneous leishmaniasis: recent developments in diagnosis and management. American journal of clinical dermatology, 16(2), 99-109.
11. Reithinger, R., & Dujardin, J. C. (2007). Molecular diagnosis of leishmaniasis: current status and future applications. Journal of clinical microbiology, 45(1), 21-25.
12. Alvar, J., Vélez, I. D., Bern, C., Herrero, M., Desjeux, P., Cano, J., ... & WHO Leishmaniasis Control Team. (2012). Leishmaniasis worldwide and global estimates of its incidence. PLoS One, 7(5), e35671.
13. Schönian, G., Nasereddin, A., Dinse, N., Schweynoch, C., Schallig, H. D., Presber, W., & Jaffe, C. L. (2003). PCR diagnosis and characterization of Leishmania in local and imported clinical samples. Diagnostic microbiology and infectious disease, 47(1), 349-358.