DOI: https://doi.org/10.53350/pjmhs22167112

ORIGINAL ARTICLE

Diagnostic Accuracy of Slit Skin Smear and Fine-Needle Aspiration Cytology (FNAC) in Diagnosis of Cutaneous Leishmaniasis Taking Histopathology as Gold Standard

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ABSTRACT

Aim: To assess the efficacy of slit skin smear and fine-needle aspiration cytology (FNAC) for diagnosing cutaneous leishmaniasis while keeping histopathological analysis as a gold standard.
Place and duration of study: From 6th Jan 2020 to 6th Jan 2021 at Bakhtawar Amin Medical & Dental College, Multan.
Study design: A Cross-Sectional Study
Methodology: In this study, a total of 180 patients were observed. In the slit skin smear technique, the Smear was fixed and stained with Leishman or Giemsa stain. For the FNAC technique, hematoxylin and eosin (H & E) stained smear slides were prepared which were examined under light power. For FNAC, the stained slides were examined under low (10x) and high power (40x) to examine cell mass and product formation and then under oil immersion lens (100x) for identifying the morphology of the parasite.
Results: The diagnostic accuracy of slit skin smear was 44.44% and that of fine-needle aspiration cytology was 88.33%.
Conclusion: Fine needle aspiration cytology has better diagnostic accuracy as compared to slit skin smear in diagnosing cutaneous leishmaniasis.
Keywords: slit skin smear, fine needle aspiration cytology, cutaneous leishmaniasis, histopathology.

INTRODUCTION

Cutaneous Leishmaniasis (CL) is a condition that is prevalent globally with 89 countries currently affected by it[1]. All the major continents including America, Asia, Africa and the Mediterranean region are equally suffering from this endemic, reporting 1.5 million affected individuals each year. In Pakistan too, this endemic has affected all the major cities in the five provinces especially in Balochistan, Interior Sindh and Multan[2,3]. Although this disease presents in its classic form, some rare forms including annular, erysipeloid, acute paronychia, zosteriform and chancriform have also been observed in some cases[4]. Literature indicates that this disease is mostly found in people living in hilly areas[5]. WHO has taken steps to control this outbreak in the NWFP province reporting a total of over 2000 cases which included children mostly who were younger than 15 years[6]. Among these infected people, more than 70% of the individuals were unable to seek medical care due to lack of facilities, poverty and illiteracy.

Generally, CL can be diagnosed by using various techniques[7]. The most commonly used methods are slit skin smears and histopathological analysis of skin biopsy. This histopathological examination detects the Leishmania parasites in the host. Similarly, slit smears can identify the amastigotes in macrophages. These parasitic organisms appear round with well-developed nuclei and kinetoplasts.

The efficiency of fine-needle aspiration cytology has also been reported by Kassi M et al[8]. According to the results of this study, this procedure had a sensitivity and specificity of 89% and 100% respectively. This study used histopathological analysis as a gold standard and observed a 100% positive predictive value in diagnosing CL. In comparison to this, Slit Smears had a relatively low diagnostic efficiency as reported by Mashood AA[9]. The smear test had a 41.94% sensitivity a d 73.68% specificity. This study also used histopathological analysis as a gold standard and reported a 72.22% positive predictive value of the slit smear test in diagnosing CL. PCR is also an effective method of CL diagnosis, however, it is costly. Therefore, there is a simple, inexpensive and sensitive method for diagnosis of Cl.

Received on 15-01-2022
Accepted on 27-06-2022

This study was conducted to efficacy of slit skin smear test and fine-needle aspiration cytology for diagnosing cutaneous leishmaniasis while keeping histopathological analysis as a gold standard.

METHODOLOGY

A cross-sectional study was conducted after permission from Ethical Committee from 6th Jan 2020 to 6th Jan 2021 at Bakhtawar Amin Medical & Dental College & Hospital Multan. The minimum sample size was calculated by using the Non-probability consecutive sampling technique and taking statistics for 41.94%[8] sensitivity, 73.68%[8] specificity, 5.17%[9] prevalence, 95% Confidence interval, 10% error margin and 50% precision. A total of 180 patients were included in the study aged from 15-60 years, both male and female who were clinically diagnosed with Cutaneous Leishmaniasis. All the patients provided their informed consent to become a part of the study. The patients who had received any treatment in the last 30 days and who refused to provide their informed consent were excluded from the study. All the patients coming from an endemic area to the skin department , having lesions clinically suggestive of CL and fulfilling the inclusion criteria were subjected simultaneously to a skin biopsy, and two slit skin smears (SSS) and two FNAC smears, from two different sites. Slit skin smear and FNAC were examined by two observers.
For performing the slit smear procedure, number 15 bard parker blade was used to make a 5mm long and 3mm deep incision after squeezing the lesion between the thumb and index finger. After making the incision, the blade was rotated at a 90-degree angle and the lesion was scraped multiple times. The culture obtained on the blade was placed on the glass slide, the smear was fixed and then stained with Leishman stain.

These stained slides were then examined for detecting the abundance of mononuclear inflammatory cells or large macrophages with Leishmania parasites. To examine the morphology of these parasites, slides were examined under an oil immersion lens. For performing the fine needle aspiration cytology, a 10cc/mL sterile syringe with a 21 G needle was used. The patient was administered anaesthesia and after the aseptic measures, the needle was inserted into the lesion in the direction of the ulcer through the subcutaneous tissue. The pressure was

applied once the needle was below the lesion margin and was released before the needle was removed. The material obtained was blown on the glass slide, the smear was fixed and was stained with hematoxylin and eosin stain.

The stained slides were examined under low (10x) and high power (40x) to examine Cell mass and product formation and then under oil immersion lens (100x) for identifying the morphology of the parasite. For every patient, data were recorded for diagnosis of cutaneous leishmaniasis as per operational definition by all three methods on a separate proforma.

All the data were analyzed by SPSS version 23. Quantitative quantities such as age were assessed by standard deviation. Qualitative quantities were evaluated by percentage. Sensitivity, specificity, positive and negative predictive value and diagnostic accuracy for Slit skin smear/fine needle aspiration cytology against histopathology were calculated by using the 2X2 model for both the diagnostic tools. A p-value of less than or equal to 0.05 was regarded as statistically significant.

RESULTS

A total of 180 patients were included in the study with an average age of 33.1±0.62. 72% of patients were older than 18 years and younger than 40 years, while 28% of patients were older than 40 years but not exceeding 60 years (Table I). 68 patients out of 180 were men and 112 were women.

Histopathological analysis showed a positive in 175 (97%) patients and negative in 5 (3%) patients (Table II). The slit smear test showed positive in 81 (45%) patients and negative in 99 (55%) patients (Table III). FNAC results showed positive in 158 (88%) patients and negative in 22 (12%) patients (Table IV).

Tables V and VI show that FNAC had a more diagnostic accuracy (88.33%) than the slit smear test (44.44%) with a sensitivity of 89.14% and 44.57% respectively. The positive and negative predictive values of both procedures are presented in Table V and VI.

Table I: Age distribution of study patients (n=180)

Age	Frequency	Percentages
20-40 years	130	72%
41-60 years	50	28%
Total	180	100%

Mean age was 33.1±0.62

Table II: Histopathology Findings (n=180)

Histopathology	Frequency	Percentage
Positive	175	97%
Negative	5	3%
Total	180	100%

Table III: Slit Skin Smear (n=180)

Slit Skin Smear	Frequency	Percentage
Positive	81	45%
Negative	99	55%
Total	180	100%

Table IV: Fine Needle Aspiration Cytology (n=180)

FNAC	Frequency	Percentage
Positive	158	88%
Negative	22	12%
Total	**180**	**100%**

Table V: Slit Skin Smear Vs Histopathology Findings (n=180)

Slit Skin Smear Findings	Histopathology findings		
	Positive	Negative	Total
Positive	78	3	81
Negative	97	2	99
Total	175	5	180

Sensitivity=44.57% Specificity = 40%
Positive predictive value = 96.29%
Negative predictive value = 2.02%
Diagnostic accuracy = 44.44%

Table VI: Fine Needle Aspiration Cytology Vs Histopathology Findings (n=180)

FNAC Findings	Histopathology findings		Total
	Positive	Negative	
Positive	156	2	158
Negative	19	3	22
Total	175	5	180

Sensitivity=89.14% Specificity = 60%
Positive predictive value = 98.73%
Negative predictive value = 13.63%
Diagnostic accuracy = 88.33%

DISCUSSION

This study was conducted to determine the efficacy of the slit smear test and fine-needle aspiration cytology in diagnosing Cutaneous Leishmaniasis while taking histopathological analysis as standard. The average age of the study patients was 33± 10.62 years and most of the patients (62%) were women. 97% were positive after the histopathological analysis. As indicated by the results, the slit smear test had a specificity and sensitivity of 40% and 44.57% respectively, showing a diagnostic accuracy of 44.44%. FNAC had a way better diagnostic accuracy of 88.33% with a sensitivity and specificity of 89.14% and 60% respectively.

These results comply with Kassi M et al[8] where the study patients had an average age of 28.4 years. 86% of patients were positive for CL after histopathological examination. Similar to our study, FNAC had 89% sensitivity, 100% positive and 60% negative predictive values for diagnosing CL. The results of our study can also be proved by comparing with Mashhood AA et al[9]. In which the slit smear test showed a 41.94% sensitivity as in our study i.e., 44.57% in diagnosing CL.

Our study suggests that FNAC is a more suitable method for diagnosing CL. Similar results were shown in Rajabi P et al[10], FNAC showed a high specificity (89%), positive predictive value (100%) and negative (60%).

The results of our study are also comparative with the Markle et al[11] study with a sensitivity of 74.1% and specificity of 70-75%. However, Mengista et al[12] showed a much lower value of specificity i.e., 37.5%. Also, this study showed that FNAC had low sensitivity (51.9%) than the slit smear test (74.1%).

The diagnostic accuracy of FNAC has also been proved by Ikramullah K et al[13]. The study showed that FNAC had an 80% diagnostic rate. Massood H[14] also concluded that FNAC was a reliable method to diagnose cutaneous leishmaniasis. Mallik MK[15] and Pezeshkpoor F et al[16] suggested that FNAC was more effective than scrape smears.

CONCLUSION

Fine needle aspiration cytology has better diagnostic accuracy as compared to slit skin smear in diagnosing cutaneous leishmaniasis.

Authors Contribution: Yasin, Seemab,Shagufta,conceived, designed and did statistical analysis & editing of manuscript, Nighat, Yasin, Shagufta,did data collection and manuscript writing, Farooq, Nighat, did review and final approval of manuscript
Grant Support & Financial Disclosures: None

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