

Original Article

Comparison of various cytodagnostic tests in the rapid diagnosis of cutaneous leishmaniasis

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Abstract *Background* In poor endemic areas of cutaneous leishmaniasis (CL), lacking sufficient laboratory infrastructure, cheap and rapid diagnostic methods are critical in management.

Objective To compare the diagnostic accuracy of newer smear techniques with slit skin smear technique in the rapid diagnosis of CL.

Patients and methods: This interventional and comparative study was carried out at Combined Military Hospital Muzaffarabad, Azad Kashmir, from January 2008 to June 2008. Patients with suspected CL lesions, fulfilling the inclusion criteria were enrolled in the study. In every patient, dental broach smear (DBS), dental broach saline smear (DBSS), slit-skin smear (SSS) and fine needle aspiration biopsy smear (FNABS) were made from the active edge of the lesions and then stained with Leishman's stain, except FNABS (stained with H&E stain). At least 100 fields were examined under oil immersion lens to identify Leishman-Donovan (LD) bodies based on their morphology before declaring the specimen as negative. Newer techniques i.e. DBS, DBSS, and FNABS were compared with SSS. Sensitivity, specificity, positive and negative predictive values, accuracy, and likelihood ratio were calculated using statistical program SPSS version 12.0.

Results Sixty patients were enrolled which included 31 (51.7%) males and 29 (48.3%) females, ranging in age from 3 to 68 years (mean 25.4±21.9 years). FNABS showed highest positive rate (55%) followed by DBS (46.6%), SSS (36.6%) and DBSS (25%). DBS showed highest sensitivity and specificity, followed by FNABS.

Conclusion DBS and FNABS showed a better yield than the traditional SSS method of direct microscopy in CL.

Key words

Cutaneous leishmaniasis, smear techniques, fine needle aspiration biopsy smear, dental broach smear, dental broach saline smear, slit skin smear.

Introduction

In recent years, cutaneous leishmaniasis (CL) has grown into a major public health problem in

Pakistan. Once endemic in Balochistan province, CL has now become ubiquitous in all provinces including Azad Kashmir.¹ It is a disease associated with rural areas and poverty, but has adapted to the urban environment as well.²

Morphology of the disease may be quite variable depending on the site of bite, number of bites, type of sandfly, genus of parasite and above all

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the host immune responses.^{3,4,5} In view of large number of clinical presentation of CL, a correct diagnosis of the disease becomes important. Clinical appearance along with history of travel to an endemic area may not be sufficient for diagnosis and need to be supported and confirmed by parasite identification. Cytodiagnosis is a rapid and cheap method of identifying parasites in lesional tissue. Cutaneous scraping is the simplest and most common test. Smears obtained from the lesions and examined under the microscope, after staining with Giemsa or Leishman's stain, are a rapid means of diagnosing CL. The traditional method of making smears is slit skin smear while other less used methods include dental broach smear, dental broach saline smear, and fine needle aspiration cytology.⁵⁻⁷ It is recommended that the sample should be taken from the edge of the active lesion, as far away from crusting, ulceration and secondary infection as possible. The cytodiagnosis if performed correctly can give a positive yield in over 70% cases.^{8,9}

In developing countries like Pakistan, where disease is highly endemic and sufficient laboratory infrastructure is not available in remote areas, the need for an easy to perform, cost-effective and rapid diagnostic method becomes imperative. Traditional slit skin smear technique is established and can be considered as a gold standard among the rapid diagnostic techniques of CL. The purpose of this study was to compare the diagnostic accuracy of the traditional slit skin smear with the newer techniques of dental broach smear, dental broach saline smear, and fine needle aspiration cytology in diagnosing CL and to recommend the most appropriate test to diagnose CL in primary / secondary care setting.

Patients and methods

During the study period, patients of both sexes and all ages reporting from Muzaffarabad and its surrounding areas, having clinically suggestive lesions of CL were included in the study. Patients with a doubtful clinical lesion, or who did not agree to be included in the trial or who had received some definitive treatment for CL were excluded from the trial. Written informed consent was obtained from all the participating patients. The study was approved by the research and ethics committee of the concerned hospital.

Clinical diagnosis was made by the agreement of two investigators. Name, age, sex, address, duration of disease, number of lesions, and site of lesions of all enrolled patients were recorded. All patients were subjected to four cytodiagnostic tests namely slit skin smear (SSS), dental broach smear (DBS), dental broach saline smear (DBSS), and fine needle aspiration cytology (FNAC).

In the DBS technique sterile corrugated dental needle (**Figure 1**) was inserted by rotating and gently pushing it into the skin at the margin of the ulcer, pointing towards the floor of the ulcer. The needle was then withdrawn without rotating; smear was made directly from the needle on a clean glass slide and stained with Leishman's stain. In the DBSS technique a fine drop of normal saline was placed on the glass slide with the help of micropipette tip, smear was then made by spreading this drop with the help of dental broach after being inserted into the skin afresh in the same manner as quoted above with the aim to dislodge most of the tissue material from the corrugated needle that might have been trapped in between and difficult to smear with the direct technique; smear was then stained with Leishman's stain. In the SSS



Figure 1 Dental broche needles of different sizes and close up of a needle showing spiral corrugations.

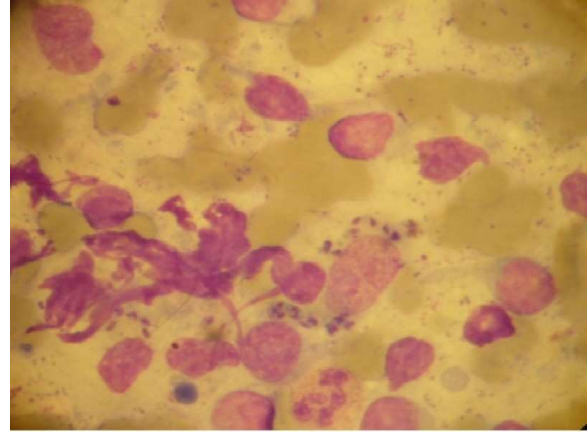


Figure 4 Skin slit smear showing intracellular and extracellular *Leishmania* amastigotes (LD bodies).

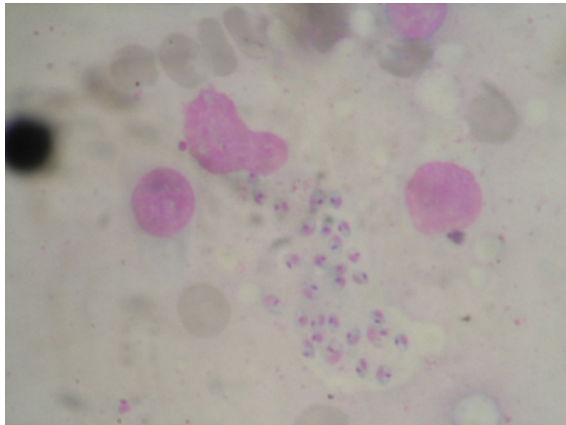


Figure 2 Dental broche smear preparation showing multiple well-demarcated LD bodies having distinct nucleus and kinetoplast.

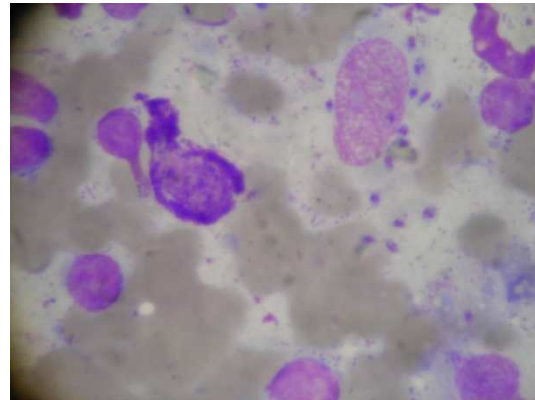


Figure 5 Fine needle aspiration cytology smear revealing multiple scattered LD bodies.

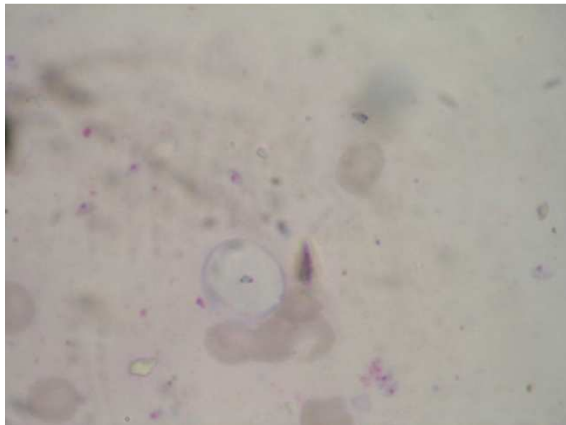


Figure 3 Dental broche saline smear preparation exhibiting discrete and scattered extracellular *Leishmania* amastigotes (LD bodies).

technique, while applying little pressure by squeezing the lesion between thumb and index finger; a 5 mm long and 2 mm deep incision was made by number 15 Bard Parker blade, the blade was then rotated at right angle and incision was scraped several times. Total slitting of the skin with surgical blade was not done to avoid excessive bleeding which obscures the morphology; smears were stained with Leishman's stain. 10cc disposable syringe with 21 G needle was used for FNAC. Needle was introduced into the skin one cm away from the ulcer margin with aseptic technique, penetrating the subcutaneous tissue in the direction of the ulcer. Suction was applied when the needle reached below the ulcer margin and released before withdrawing the needle. The needle was

detached, syringe was filled with air, needle was reattached and contents were blown out onto a clean glass slide, smear was made and stained with hematoxylin and eosin (H&E) stain.

Leishman-stained slides were first examined under light power objective to look either for large macrophages containing the parasite, or areas with predominance of mononuclear inflammatory cells. The slides were then examined under oil immersion lens to identify Leishman-Donovan (LD) bodies based on their morphology (intracellular or extracellular, ovoid in shape, containing nucleus and/or kinetoplast) [Figures 2-4]. H&E stained slides after mounting and application of cover slip were initially examined under low (10x) and then high power (40x) to localize the fields with maximum cellular yield and finally moved to oil immersion lens for the morphological diagnosis of parasite (Figure 5). In both Leishman- and H&E-stained slides, at least 100 different well stained and preserved fields mainly in and around the vicinity of mononuclear inflammatory cells were examined under oil immersion lens before giving a negative diagnosis for LD bodies.

Data were recorded on a separate pro forma for every patient and later transferred to statistical program SPSS version 12.0. Descriptive analyses were calculated for the variables. Sensitivity, specificity, positive and negative predictive values, accuracy, and likelihood ratio were calculated and compared with SSS.

Results

Sixty consecutive patients of both sexes and all ages were included in the study. The study group included 31 (51.7%) males and 29 (48.3%) females, ranging in age from 3 to 68 with a mean age of 25.4 years. Most of the patients were young adults belonging to rural areas. In

80% of cases, the lesions were localized to the face. Out of the face lesions, cheeks were the commonest site followed by nose. Ear was the least common site on the face region to have a sandfly bite. Other than the face, hands were second commonest site seen in 15% of cases. Only one case on trunk was seen. Duration of lesion of three months was seen in 38% cases, whereas one month and six month duration was seen in 25% cases each. More than one year duration was seen in only 11% of cases. Clinical morphology of lesions was segregated into various types seen. Dry scaly type was the commonest form seen in 48% cases, followed by indurated plaque which was seen in 28% cases. Dry crusted (15%), papular erythematous (5%), and macular erythematous (3%) were the least common clinical patterns.

DBS showed a sensitivity of 65% and specificity of 67%. Fine needle aspiration biopsy smear (FNABS) showed a sensitivity of 60% and specificity of 57%. Both were better than SSS in this regard. Maximum positive cases were picked by FNABS (48.3%), followed by DBS (43.3%), SSS (33.3%) and DBSS (21.6%). Detailed demographic features are shown in Table 1. Statistical comparison is shown in Table 2.

Discussion

Cytodiagnosis is a relatively new science. Various cytodagnostic methods used in dermatology include aspiration cytology, imprint smear, exudate smear, skin scraping smear, and Tzanck smear. There are certain cytodagnostic techniques that can be employed to confirm CL even in an under-equipped field laboratory with minimum resources, which include, skin slit smear, saline skin smear, impression or touch smear, scalpel skin scraping, dental broach smear and fine needle aspiration biopsy smear.

Table 1 Demographic features of patients of cutaneous leishmaniasis

Parameter	N (%)
Age groups (years)	
0-12	10 (16.7)
12-40	40 (66.6)
>40	10 (16.7)
Gender	
Males	31 (51.7)
Females	29 (48.3)
Residence	
Urban/Suburban	8 (13.3)
Rural	52 (86.7)
No. of lesions	
1	41 (68.6)
2	14 (23.2)
>2	5 (8.2)
Site of Lesions	
Face	48 (80)
Limbs	8 (13.3)
Trunk	2 (6.7)

Skin slit smears and impression smears are frequently used conventional techniques for detecting the parasite in Pakistan, while FNABS, saline skin smears and dental broach smears are used rarely.¹⁰ Positive yields of these cytological tests have been variable in various studies (ranging from 27.5% to 80%).^{8,10-14}

Although cases of *Leishmania* lymphadenitis are reliably diagnosed with FNABS, it has not attained popularity in the diagnosis of cutaneous leishmaniasis, and only a few reports are available.¹⁵ The present study has shown a positive percentage of 55% with FNABS, whereas the sensitivity and specificity were 60% and 57%, respectively. Other investigators from Pakistan have shown a positive yield of 35% and 100%.^{10,12} Slit skin smears, on the other hand have been shown to have a positive percentage of 32% in a comparative trial.¹⁶

Dental broach smear has not been employed or promoted as an easy to use technique in the rapid diagnosis of CL. Tissue sampling using dental broach appeared to be more efficient and easier than other sampling techniques used in

this study. The present study has shown the best results with this technique. It was found positive in 43% of cases. This technique can be learnt very easily and can be performed in a primary care set up with minimum facilities. Though rarely used, other investigators have shown even better results with dental broach smears and found it to be the best among smear techniques.⁸

Dental broach saline smear was thought to be an improvement over the dental broach smear technique. Theoretically, it should have improved the yield of smears by dissolving the tissue in between the grooves of the broach, but our results do not show an improvement over the dental broach smear technique. Further research in this direction may be able to improve upon the technique so as to improve the yield of smears.

Staining technique is very important in improving the yield of smears. It has been suggested that visualizing the parasites is easier using the Giemsa staining.¹⁷ Indeed an interesting observation, which might appear as a limitation of our study as we used Leishman's stain in our cases. However, we could not find any substantial study addressing the issue. This may be a promising area for further research to enhance the diagnostic potential of direct microscopy in CL.

Traditionally, smear samples are taken from the active edge of the leishmanial lesion, presumably due to the maximum number of parasites in the active edge. Robinson *et al.*¹⁸ demonstrated maximum yield of parasites from the center of the lesion. We used the traditional method of obtaining tissue from the active margin. We believe that collecting material from the center of the lesion may increase the chances of positive samples. Further research in this direction can help to improve the quality of

Table 2 Statistical analysis of cytodiagnostic tests in comparison with slit skin smear.

	Dental broach smear	Dental broach saline smear	Fine needle aspiration cytology
Sensitivity (%)	65	45	60
Specificity (%)	67	90	57
Positive predictive value (%)	50	69	41
Negative predictive value (%)	79	76	74
Accuracy	66.6	75	58
Likelihood ratio	5.7	9.1	1.6
p value	0.01	0.002	0.20

smear techniques.

CL is largely a disease of the poor. Endemic areas lack basic laboratory and diagnostic facilities. Referral to larger set up may not be feasible economically for the patients. Therefore, bedside rapid diagnostic techniques are imperative in improving patient care in this particular disease. We have shown improvement over the traditional slit skin smear technique in the form of dental broach and FNABS. Both have their advantages. Dental broach gives somewhat better yield whereas FNABS is easier to perform, requiring minimum apparatus. Keeping in mind the staining and sampling techniques we believe the positive yield of tissue smear can be much improved. In the end we think it is the clinician's preference of which technique he is really comfortable with.

Conclusion

Dental broach smear and FNABS techniques appear to be an improvement over the standard slit skin smear technique. With an improvement in staining and sampling methods, the yield of smear techniques can be improved.

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Authors Declaration

Authors are requested to send a letter of undertaking signed by all authors along with the submitted manuscript that:

The material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the *Journal of Pakistan Association of Dermatologists*.