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Research Article

Role of fine needle aspiration cytology in diagnosing leprosy: in a tertiary care hospital

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ABSTRACT

Background: Leprosy, a chronic inflammatory granulomatous disease chiefly involving skin and peripheral nerves and occasionally other organ systems, caused by Mycobacterium leprae. It has tormented the human civilization through time immemorial. Leprosy remains a significant public health problem worldwide, especially in developing countries like India. The diagnosis of leprosy is not always easy because of long incubation period, over dependence of clinical expertise and a lack of rapid and simple diagnostic tool, patients remain undiagnosed for longer time. Fine needle aspiration (FNAC) technique is an inexpensive, rapid and accurate procedure for diagnosis of leprosy. We conducted a prospective study evaluating the ability of fine needle aspiration cytology in diagnosing and classifying leprosy lesions on Ridley-Jopling scale (R-J scale). The aim of this prospective study was to assess the usefulness of fine needle aspiration cytology in early diagnosis of leprosy, to identify specific cytological characteristics of diagnosis and to correlate the cytological smear findings with histopathology and to evaluate merits of relatively non-invasive procedure of FNAC over more invasive procedure - biopsy.

Methods: The study is a hospital based prospective study carried out in the Department of Pathology and Department of Skin, Venereal Diseases, Leprosy, N.S.C.B. Medical College & Hospital, Jabalpur (M.P.) September 2010 to September 2013. Patients with new skin lesions were selected for the study. FNAC was performed and aspirates were evaluated for cytology using Hematoxylin and Eosin staining (H&E staining), Ziehl-Neelsen staining (ZN staining) and punch biopsy was collected.

Results: Out of 50 cases, clinical and cytological correlation was seen in 88% tuberculoid leprosy, 93.7% of borderline tuberculoid, 33% of borderline lepromatous leprosy and 66% of lepromatous leprosy. While clinical with histopathological correlation revealed 100% specificity in tuberculoid leprosy, 100% in borderline tuberculoid, 66.6% in borderline lepromatous, 83.3% in lepromatous leprosy and 80% in indeterminate leprosy and 100% in histoid leprosy in our study. The overall cytodiagnostic accuracy has been 92% in present study.

Conclusion: Our study demonstrates that the combination of FNAC and ZN staining for Acid Fast Bacilli (AFB) can provide a rapid diagnosis in majority of leprosy suspected cases. FNAC is a safe, simple, rapid, less-invasive, OPD procedure for early diagnosis and classification of leprosy cases.

Keywords: Mycobacterium leprae, Fine needle aspiration cytology, Acid fast bacilli, Ziehl-Neelsen staining, Hematoxylin and eosin staining

INTRODUCTION

Leprosy, a chronic inflammatory mycobacterial disease chiefly involving skin and peripheral nerves and occasionally other organ systems, has tormented the human civilization through time immemorial. Leprosy as a disease is indeed a problem which requires a multi-disciplinary approach. It still afflicts the humanity, brings challenges and still arouses our curiosity.

In spite of our knowledge about leprosy, it still remains a major public health problem in many developing countries for centuries. According to WHO the estimated global new cases detection in 2011 was 2, 19, 075[1] and India accounts for 1, 27, 295 (58%) of new cases of leprosy.[2]

The clinical manifestations of leprosy are so varied and diverse and can mimic a variety of unrelated diseases. Presentation may vary from an insignificant skin lesion to extensive disease causing profound disability / deformities.[3] The histopathological findings in leprosy are related to the immunological status of the patient.[4] Ridley and Jopling (1966) proposed a five group histological classification reflecting the immunological spectrum: Tuberculoid (TT), Borderline Tuberculoid (BT), mid-borderline (BB), Borderline Lepromatous (BL) and Lepromatous (LL).[5] Later they developed clinical and bacteriological finding in each group with respective immunological and histopathological findings.[6] Clinicians have also adopted the same nomenclature for classifying leprosy on clinical grounds. WHO in its 6 technical report of 1988, classified leprosy into multibacillary and paucibacillary forms to facilitate the institution of accurate mode of therapy and regular follow-up of patients to prevent undesirable complications.[7]

METHODS

Diagnosing leprosy is an uphill task, because of its long incubation period, patients remain undiagnosed for longer time. The diagnosis of leprosy has been based on clinical criteria of anesthetic or hypoesthetic skin lesions, enlarged or thickened peripheral nerves and the presence of Acid Fast Bacilli (AFB) in slit skin smears or tissue biopsy. The gold standard for leprosy diagnosis is histopathology examination, and it also gives information on nature of host response. Biopsy is an invasive procedure and possible only in specialized centers .Thus, early diagnosis is of utmost importance for all purposes including epidemiology, case management, and the prevention of deformity and disability. To overcome this issue Fine Needle Aspiration Cytology (FNAC) of leprosy lesions has emerged as a safe, simple, rapid and less traumatic technique with good diagnostic accuracy.[8]

With this background, this study is aimed to evaluate and compare the effectiveness of cytology in early diagnosis of leprosy, to identify specific cytological characteristics of diagnosis and to correlate the cytological smear patterns with histological spectrum of leprosy. To evaluate merits of relatively non-invasive procedure of FNAC over more invasive procedure - biopsy in different entities of leprosy for rapid, early, simpler and possible accurate diagnosis.

Techniques

This hospital based prospective and descriptive study was carried out in setting of Department of Pathology and Department of Skin, Venereal Diseases, Leprosy, N.S.C.B. Medical College & Hospital, Jabalpur, M.P. between September 2011 to September 2013. After duly signed informed consent forms and institutional ethical clearance a total of fifty clinically suspected untreated patients regardless of their age, sex, socioeconomic status and occupation were included. Previously diagnosed cases and cases already getting treatment were excluded. The clinical diagnosis was done by experienced dermatology consultant and the investigator.

History of patients was recorded. Clinical examination of skin lesions included the type, number, size, site, margins, erythema, dryness, loss of hair and sensation and presence of neural involvement. All the cutaneous and peripheral nerves were palpated and findings like number, size, nodularity, abscess formation, tenderness and sensory or motor complaints were noted. The leprosy patients were classified according to Ridley-Jopling (RJ scale) into tuberculoid (TT), Borderline Tuberculoid (BT), mid-borderline (BB), Borderline Lepromatous (BL), and lepromatous (LL) types. Apart from RJ scale Indeterminate Leprosy (IL) category was also included for classification of cases.

Collection of samples

Samples from untreated patients were collected after duly signed informed consent forms and institutional ethical clearance.

Fine needle aspiration

Fine needle aspiration was done as described by Prasad PVS et al.[8] The site was cleaned with alcohol and an assistant pinched the skin for 30 seconds to blanch the skin. A 20 mL syringe was fitted with a 21-gauge needle and the assistant created negative pressure by holding back the piston with the forefinger and index finger of the right hand. The aspirated material was transferred onto glass slides. The flat surface of another slide was used to smear the material. The aspirates were subjected to cytological examination with hematoxylin and eosin and Ziehl Nelson's staining. Cytological smears were assessed by two cytopathologists who were not informed about the clinical and histopathological findings. We used the cytological criteria laid down by Singh et al.[9] and modified by Prasad PVS et al.[8] for reporting of the FNAC smear. Diagnostic criteria: Table 1.

Table 1: Diagnostic cytology criteria used to classify leprosy cases by Singh et al.[9]

Type	Cytological features	AFB stain ability
Tuberculoid leprosy (Including TT and BT)	Cellular smears Cohesive epithelioid cell granulomas Numerous lymphocytes not infiltrating granuloma	No stainable AFB (BI = 0)
Borderline tuberculoid (BT) (Modified according to Prasad PVS et al.)[8]	Cellular material with lymphocytes, histiocytes and epithelioid cells Foamy macrophages are a not features	No stainable AFB
BB (Mid-borderline leprosy)	Fair cellular yield Poorly cohesive granulomas composed of an admixture of epitheloid cells and macrophages Few lymphocytes infiltrating the granulomas	BI = 1+ to 2+
Borderline lepromatous leprosy (BL)	Moderate cellularity Singly dispersed macrophages with "negative images", no epitheloid cells Numerous lymphocytes (Predominant cell type) diffusely admixed with macrophages	BI = 3+ to 4+
Lepromatous leprosy (LL)	Heavy cellularity Numerous foamy macrophages (Predominant cell type) in a fatty background with intracellular and extracellular negative images Few lymphocytes	BI= 5+ to 6+ (Globi)
Erythema nodosum leprosum (Type II reaction in LL)	Numerous fragmented AFB (MI <1) and neutrophils	
Histoid leprosy (Modified according to Prasad PVS et al.)[8]	Cellular yields, elongated spindle cells, scattered lymphocytes	BI 6+

Punch biopsies

Samples for punch biopsy of approximately 4 mm size were collected according to standard procedures.[10] A portion of biopsy samples from each patient was fixed in 4% buffered neutral formalin and then dehydrated in a graded series of ethanol and embedded in paraffin. Sections were stained with H and E staining for histopathological examination. The Ziehl Neelsen (ZN) and Fite Faraco (FF) stain for demonstration of AFB were used whenever required.

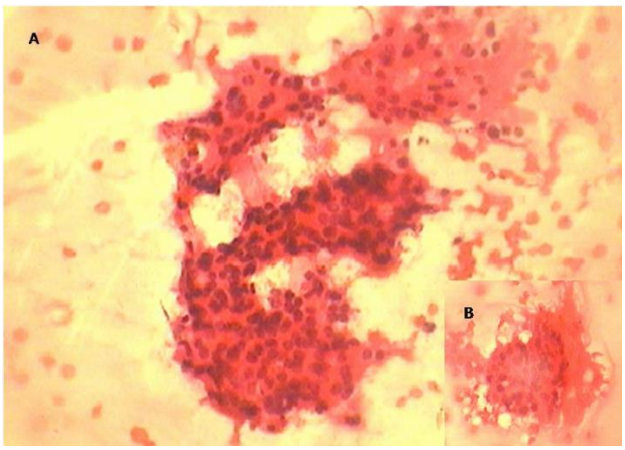

Figure 1: Cellular smear tuberculoid leprosy B-Giant cell.

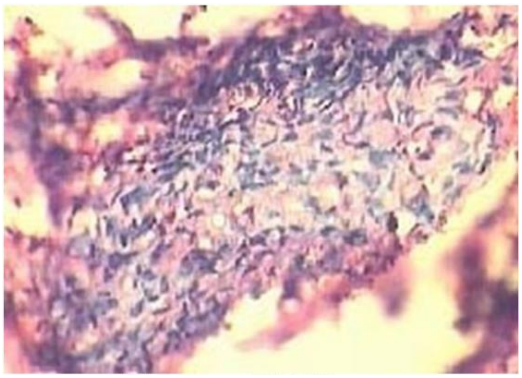

Figure 1: Cellular smear of histoid leprosy.

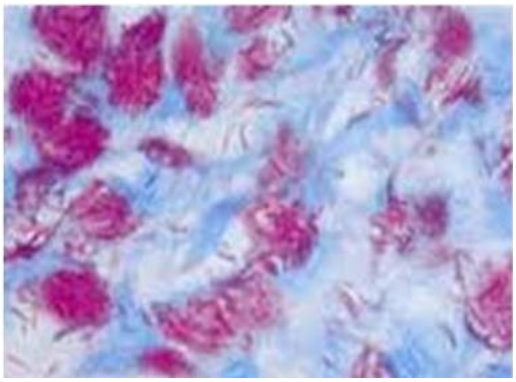

Figure 3: Acid fast bacilli in lepromatous leprosy.

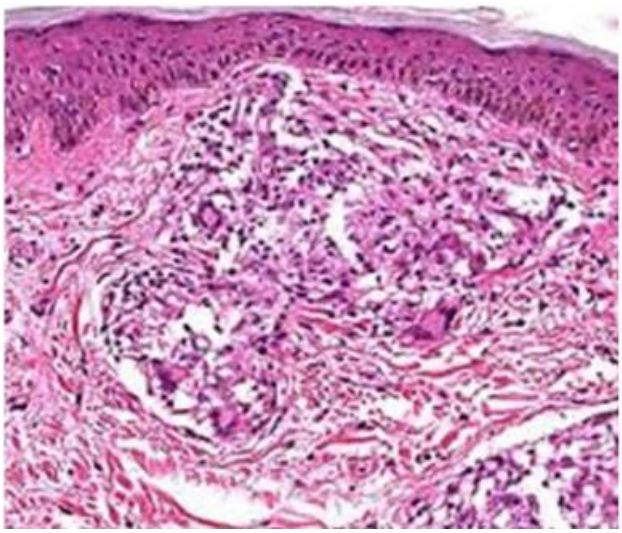

Figure 4: Histopathology of tuberculoid leprosy.

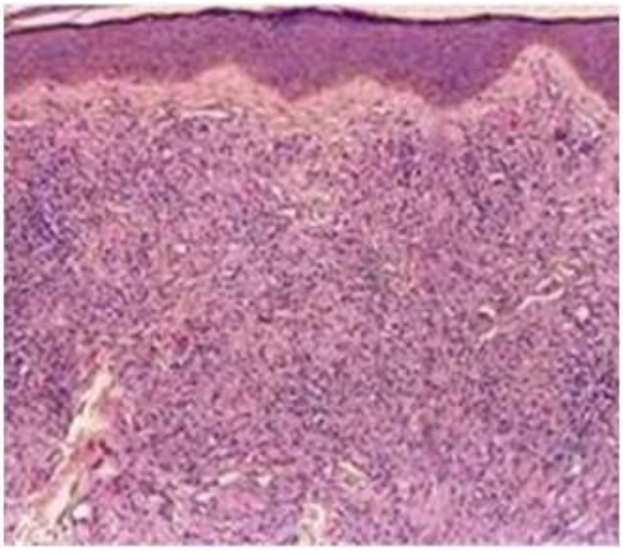

Figure 5: Histopathology of lepromatous leprosy.

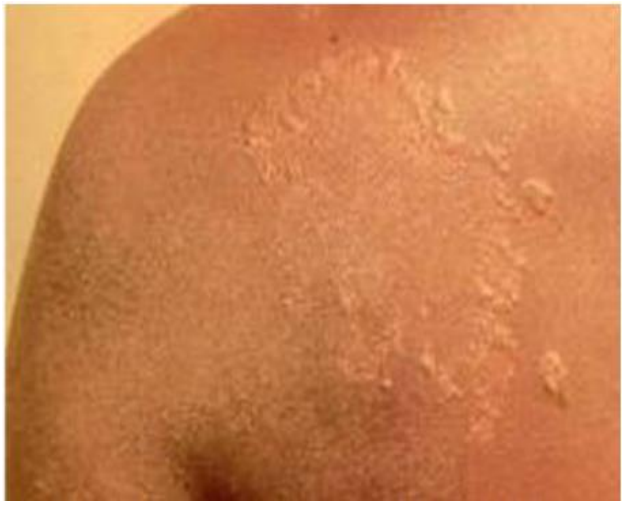

Figure 6: Showing clinical case of tuberculoid leprosy.

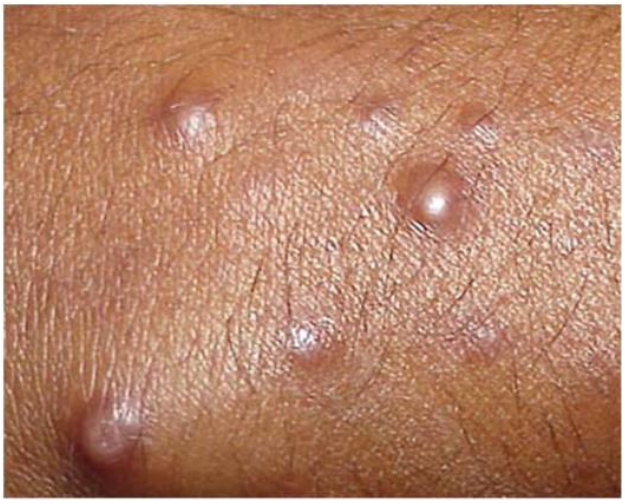

Figure 7: Showing clinical case of lepromatous leprosy.

Data analysis

Data was analyzed by SPSS version 10. Quantitative variables are expressed as mean ± standard deviation and qualitative variables as percentages. Agreement between the clinical, cytological and histopathological classification was calculated using percentage of parity. Statistical tests applied included diagnostic tests for sensitivity and specificity.

RESULTS

The study included fifty (n=50) patients clinically newly diagnosed as leprosy cases were enrolled in this study. The youngest patient studied was 8 years old and the oldest was 70 years old. The mean age and standard deviation 38.06 and 16.92 years respectively (Table 2). Majority of cases belongs to age group of 21 to 40 years. There were 35 (70%) male and 15 (30%) females. Male to female ratio was 7: 3 (Table 3).

According to the RJ classification, 46% (n=23) patients were in the borderline spectrum. Borderline Tuberculoid (BT) was the most frequent morphologic type, seen in 32% (n=16) followed by tuberculoid leprosy seen in 18% (n=9), borderline (BB) in 2% (n=1), Borderline Lepromatous (BL) in 12% (n=6) and lepromatous (LL) in 12% (n=6) patients. Additionally, special types of leprosy were recorded in 4% patients (n=2) with Histoid, and indeterminate forms in 10 (20%).

On cytological aspiration taken from these patients suggested 11 (22%) tuberculoid (TT), 6 (12%) lepromatous, 20 (40%) borderline including [BT 15 (30%), BB 3 (6%) and BL 2 (4%)], 2 (4%) cases of histoid leprosy on FNAC. Out of 50 cases, 11 (22%) cases were reported as unsatisfactory for evaluation on cytology due to insufficient material (Table 4). Considering Clinical diagnosis and FNAC the concordance was as follows high correlation was seen in

all types of leprosy except in Borderline Lepromatus leprosy and complete concordance for FNAC was 64% (Table 5).

Biopsy records were available for all cases. BT leprosy was the most common histological diagnosis. A clinico-histopathological correlation was observed in 45 out of 50 cases (90%) with 4 cases (8%) demonstrating non-specific dermatitis features. 100% correlation between clinical diagnosis and HPR was seen in tuberculoid leprosy, borderline tuberculoid, mid borderline and histoid leprosy (Table 6).

Table 2: Distribution of cases according to age and HPR findings.

Age (years)	Tuberculoid leprosy	Borderline tuberculoid	Mid-border-line	Borderline lepromatus	Lepromatus leprosy	Histoid leprosy	Indeterminate leprosy	Chronic dermatitis	Total
<20	2	1	0	0	2	0	0	1	6
20-29	1	4	1	1	1	0	1	2	11
30-39	4	4	1	1	1	1	1	1	14
40-49	0	4	0	0	1	0	1	0	6
50-59	2	0	0	1	0	1	1	0	5
60-69	0	3	0	0	0	0	3	0	6
70+	0	0	0	1	0	0	1	0	2
Total	9	16	2	4	5	2	8	4	50

Above table shows the age wise distribution of cases. Their ages ranged from were 8-70 years with mean age 38.06 ± 16.92 years. Most patients belong to 20-40 years of age.

Table 3: Distribution of cases according to sex.

HPR (No. of cases)	Male	Female	Total
Tuberculoid leprosy	7	2	9
Borderline tuberculoid	12	4	16
Mid-borderline	1	1	2
Borderline lepromatus	2	2	4
Lepromatus leprosy	5	0	5
Histoid leprosy	1	1	2
Indeterminate leprosy	4	4	8
Chronic dermatitis	3	1	4
Total	35 (70%)	15 (30%)	50

There were 35 (70%) male and 15 (30%) females, the male female ratio of 7:3 and the mean age of male was 37.34 ± 18.31 years and female cases was 39.73 ± 13.55 years.

Table 4: Distribution of cases according to cytology diagnosis.

Type of leprosy	No. of cases	Percentage (%)
Tuberculoid leprosy	11	22
Borderline tuberculoid	15	30
Mid-borderline	3	6
Borderline lepromatus	2	4
Lepromatus leprosy	6	12
Histoid leprosy	2	4
Inadequate	11	22
Total	50	100

Table 5: Correlation between clinical and fine needle aspiration cytology diagnosis.

Clinical spectrum	Clinical diagnosis	FNAC diagnosis	Complete concordance	Percentage correlation
Tuberculoid leprosy	9	11	8	88%
Borderline tuberculoid	16	15	15	93.7%
Mid-borderline	1	3	1	100%
Borderline lepromatus	6	2	2	33%
Lepromatus leprosy	6	6	4	66.6%
Histoid leprosy	2	2	2	100%
Indeterminate leprosy	10	-	-	-

Correlation of clinical diagnosis with FNAC examination revealed high correlation in all types of leprosy except in lepromatus and borderline lepromatus leprosy. Indeterminate cases were diagnosed histologically in 10 cases, out of which 5 cases had inadequate cellularity while three cases showed tuberculoid features in aspiration and two cases showed a lepromatus spectrum by FNAC.

Table 6: Correlation between clinical and histopathological diagnosis.

Type of leprosy	Clinical diagnosis	HPR diagnosis	Complete concordance	Percentage Correlation
Tuberculoid leprosy	9	9	9	100
Borderline tuberculoid	16	16	16	100
Mid-borderline	1	2	1	100
Borderline lepromatus	6	4	4	66.6
Lepromatus leprosy	6	5	5	83.3
Histoid leprosy	2	2	2	100
Indeterminate leprosy	10	8	8	80
Non-specific dermatitis	-	4		
Total	50	50		

Above table reveals 100% correlation between clinical diagnosis and HPR in tuberculoid leprosy, borderline tuberculoid, mid-borderline and histoid leprosy.

In our study we observed maximum parity of both cytology and histopathology against the clinical diagnosis in polar groups, tuberculoid and lepromatous. FNAC shows concordance in 8 (88%) for tuberculoid and 4 (66%) for lepromatous leprosy and histopathology shows concordance in 9 (100%) for tuberculoid and 5 (83.3%) for lepromatous leprosy. While in borderline groups (BT, BB, BL) concordance of FNAC was 18 (36%) and histopathology was 21(42%) respectively. This clearly indicates that the FNAC is useful usually for polar groups than the borderline cases because of overlapping in the smear findings. Therefore histopathology is better choice over the FNAC in borderline or unstable group of leprosy cases (Table 7).

Table 7: Correlation between clinical and fine needle aspiration cytology diagnosis.

Type of leprosy	Clinical diagnosis n	FNAC diagnosis	Percentage correlation %	HPR diagnosis	Percentage correlation %
Tuberculoid leprosy	9	11	88	9	100
Borderline tuberculoid	16	15	93.7	16	100
Mid-borderline	1	3	100	2	100
Borderline lepromatus	6	2	33	4	66.6
Lepromatus leprosy	6	6	66.6	4	83.3
Histoid leprosy	2	2	100	2	100
Indeterminate leprosy	10	-	-	9	80
Inadequate		11			
Non-specific dermatitis				4	
Total	50	50			

Histoid leprosy showed complete concordance 100% both in FNAC as well as histopathology accounting to its unique cytological features of elongated spindle shaped cells along with scattered lymphocytes and bacillary index of 6+. Indeterminate leprosy was diagnosed histologically in 10 cases, out of which 3 showed features of tuberculoid spectrum, 2 showed features of lepromatus spectrum while five cases had insufficient cellularity for evaluation.

On correlating cytological and histopathological results of the cases, showed that out of 50 FNAC which were performed diagnosis were possible in 39 cases (78%) and 11 (22%) were negative. HPR results of these 50 cases showed definite results were seen in 46 (92%) and 4 (8%) were negative. Out of these 46 positive cases of HPR, FNAC confirmed positive results in 39 (84.8%) cases and 7 (15.2%) cases were negative. While out of the 4 negative HPR reports, FNAC confirmed negative results for all 4 (100%) cases. Thus the sensitivity of the FNAC over HPR was found 84.8%. Specificity 100%, positive predictive value was observed at 100% and negative predictive value at 36%. The overall diagnostic accuracy was found at 92% (Table 8).

Table 8: Showing the measurements of sensitivity and specificity of FNAC and HPR.

FNAC	HPR		Total
	Positive	Negative	
Positive (n=39)	39 (78 %)	0 (0%)	39 (78%)
Negative (n=11)	7 (15.2%)	4 (100%)	11 (22%)
Total	46 (92.0%)	4 (8.0%)	50

Sensitivity = 84.8%, Specificity = 100%, PPV = 100%, NPV = 36.36%, DA = 92%

DISCUSSION

Present study comprises 50 clinically newly diagnosed cases of leprosy. All the patients were investigated by fine needle aspiration cytology and biopsy for histological assessment.

Age incidence

In the present study of 50 cases, age ranged from 8 years to 70 years with the mean age 38.06 years. Most patients belonged to 20-40 years of age.

Similar age incidence was reported by Prasad P, George RV et al.[8] in year 2008. Age incidence of present series favorably simulates with the age incidence reported by Jindal et al.,[11] also observed maximum number of cases 47.8% in 21-40 years .

Sex incidence

In our study, 50 cases of both the sexes were included there were 35 (70%) males and 15 (30%) females and the ratio of male and female was 7:3, showing a male preponderance. Male predominance may be because of many factors such as industrialization, urbanization and more opportunities for contact in males, social customs and taboos may account for the smaller number of females reporting for treatment to the hospital.[9]

Sex incidence of our study favorably simulates study of Prasad P, George RV et al.[8] male female ratio of 2.4:1 and Rao et al 5:1.[12]

Clinical spectrum

In present study, clinically 46% (n=23) patients were in the borderline spectrum. Borderline Tuberculoid (BT) was the most frequent morphologic type, seen in 32% (n=16). Observations were similar to observations made by Prasad PVS et al.[8] Sheoni et al.,[13] Moorthy et al.[14]

Cytological examination

Correlation of clinical diagnoses with cytological examination (FNAC) revealed varying results. A correlation of clinical diagnoses with FNAC is noticed in 88% of tuberculoid leprosy, 93.7% of borderline tuberculoid, 33% of borderline lepromatous and 66.6% of lepromatous leprosy cases in our study. In our study we have found maximum clinico-cytological correlation (100%) in histoid leprosy and mid-borderline type. Prasad PVS et al.[8] observed maximum clinico-cytological parity in TT (87.5%), BT (92.1%), and BL (80.9%), which are similar to our findings.

Histopathological examination

Literature reveals that the histopathological conformation rate in several studies varies from 29% to 58% in early stages of leprosy and clinically.[15] In the present study the histopathological diagnoses were consistent with the clinical diagnoses in 45 out of 50 (90%) cases as shown in table. The percentage of parity between the clinical and histopathological classification was highest at the polar ends of the spectrum .The percentage of concordance was less for the borderline group with least correlation in borderline lepromatous cases in the present study which is comparable to the results of Moorthy et al.[14] (2001), Bijjaragi S et al.[16] (2012). Histopathology continues to be regarded as the gold standard for the diagnosis, particularly in early stage of leprosy. The histopathological features in leprosy indicate the accurate tissue response while the clinical features indicate only the gross morphology of the lesions caused by the underlying pathology. It also provides information on the nature of host response by which one can predict the likely course of the disease and the likely response to therapy.

The sensitivity of FNAC over HPR was found 84.8% and specificity 100% in our study. Thus the overall cytodiagnostic accuracy has been 92% in present study which is similar to study carried by Mehdi G, et al.[17] in 2010 who noted 92% accuracy in the diagnosis of leprosy and it is higher than 81.8% reported by T. S. Jaswal, et al.[18] in 2001. This could be due to proper selection of cases with full blown picture.

The cytological study (FNAC) in leprosy skin lesions provides an accurate provisional diagnosis in the majority of cases; especially in polar/stable type leprosy. It is a minimally invasive, requires very little expertise and can be performed as an outpatient department procedure.

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Ethical approval: The study was approved by the institutional ethics committee

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