Correlation of FNAC to Histopathology in Skin Lesions of Leprosy as Per Ridley-Jopling Spectrum

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Abstract

Leprosy is a chronic infectious disease of humans caused by Mycobacterium leprae. Leprosy is present in practically every corner of the globe, but in tropical countries like lndia, it is a major problem of public health importance. This problem can be tackled by correct diagnosis and timely treatment.

The clinical diagnosis of leprosy has to be confirmed by various diagnostic procedures e.g., histopathology, slit skin smears for Bacteriological Index (BI) and Morphological Index (Ml). These procedures are well established and accepted all over the world.

With the increasing role of cytology in the diagnosis of many diseases, few workers thought of employing the technique of Fine Needle Aspiration Cytology (FNAC) in leprosy cases and tried to find its correlation with histopathology.

Thus, the present study was undertaken to know the possible role of cytology in the diagnosis of leprosy, as it was felt that further documentation is required, due to paucity of similar studies.

Introduction

Leprosy, also called Hansen's disease, is a chronic infectious disease that affects the peripheral nerves, skin, upper respiratory tract, eyes and nasal mucosa. It causes skin sores that are disfiguring, nerve damage and muscle weakness that gets worse over time. It was discovered by G.A. Hansen in Norway in 1873. There are 2 types of leprosy: tuberculoid leprosy and lepromatous leprosy. Tuberculoid leprosy is the less severe and less contagious. Lepromatous leprosy is the more severe and is more contagious. This type affects the organs such as kidneys, testicles, eyes and nose. Leprosy is difficult to study. Mycobacterium Leprae multiplies slowly and symptoms can take as long as 20 years to appear. Armadillos are the only animal other than humans that have been found to become naturally infected by this disease.[1]

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Aims and Objectives

- To study the cytopathology of leprosy obtained by FNAC and slit skin smears.
- To study the histopathology of leprosy.
- To study and correlate the cytopathology and histopathology of leprosy.
- To evaluate the possible role of cytopathology in classifying leprosy lesions according to Ridley-Jopling classification.
- To find out the incidence of leprosy in patients attending this hospital.

Material and Methods

The present prospective study was carried out during the period 1st April 1999 to 31st December 2000. 40 patients who were clinically diagnosed or suspected of having leprosy and whose histopathology and/or cytology were available were included for this study. Initially, detailed history and complete clinical examination was done.

The patients were subjected to the following procedures

1. FNAC of skin lesions: This was done using 22-24 gauze needle attached to 10 ml disposable plastic syringe. The aspirated material was smeared on a glass slide.[16]

   Smears were fixed in methanol and ethanol. The smears fixed in methanol were air dried and stained with May-Grunwald Giemsa stain (MGG). The smears fixed in ethanol were wet fixed and stained by Modified Ziehl Neilsen stain (Z-N stain). The slides were studied and classified as per Ridley-Jopling classifications.[6,11,13]

2. Slit skin smears: These were obtained from the following sites;

   (I) Right and left ear lobes.

   (ll) Active skin lesions

   Under all sterile and aseptic precautions, sufficient material was obtained for the smear. The material collected was smeared on a glass slide and was air dried. It was than stained by Modified Z-N stain.[10]

3. Skin Biopsy: Biopsies were performed using a 4 mm punch. The lesion to be biopsied was infiltrated with local anaesthetic and the specimens obtained were fixed in 10% formalin. Paraffin blocks were made which were cut at 4-5 microns' thickness and the slides were stained by routine H&E Stain.

Punch Biopsy

The stains used for the study were

1. May-Grunwald-Giemsa(MGG)[4]

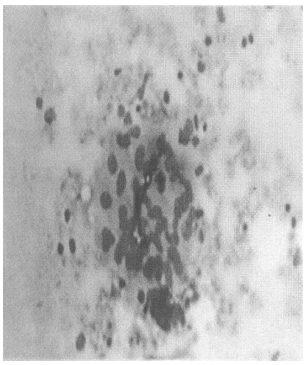
   Results-Nuclei-blue.

   Background-Pink to pale blue.

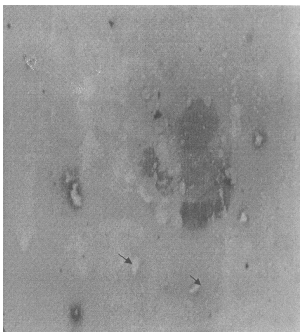
The smears were observed under low power and high power magnification for evaluation of type of cells; forming the granulomas i.e. epitheloid cell or macrophage, lymphocyte infiltration both number and pattern wise and negative images.

Cytologic criteria[16] used to classify the cases were as follows;

Type	Cellularity	Cohesive Epitheloid Cells	Other Cells	Bacterial Index
TL (including TT & BT)	Cellular	Cohesive epitheloid cell granuloma	Numerous lymphocytes not infiltrating granuloma	BI=0
BB	Fair Cellularity	Poorly Cohesive granulomas, admixture of epitheloid and macrophages.	Few lymphocytes infiltrating granuloma	BI=1+ to 2+
BL	Moderate	Singly, dispersed Macrophages with 'negative images', no epitheloid cells	Numerous lymphocytes (predominant cell type) diffusely admixed with macrophages	BI=3+ to 4+
LL	Heavy Cellularity	Numerous foamy macrophages with intracellular and extracellular negative images	Few lymphocytes	BI=5+ to 6+ (globi)



FNAC of BB showing poorly cohesive FNAC of epitheloid cell granuloma



LL showing negative images

2. Modified Z-N stain.[2,3]

   Results-M lepra bacilli-red.

   Background-pale blue.

The Z-N stained smears were examined under oil-immersion lens and the acid fast bacilli counted as per the Bacteriological Index (BI)[10] as follows;

- 0-No bacilli seen after examining 100 oil immersion fields.
- 1+ 1-10 bacilli/100 oil immersion fields.
- 2+ 1-10 bacilli/10 oil immersion fields.
- 3+ 1-10 bacilli/oil immersion fields.
- 4+ 10-100 bacilli/oil immersion fields.
- 5+ 100-1000 bacilli/oil-immersion field.
- 6+ More than 1000 bacilli/oil immersion field.

Bacteriological index was found out by the arithmetic mean of positivity of all smears taken.

3. Standard Haematoxylin and Eosin for paraffin sections:[2,3]

   Results- Nucleus-blue, black.

   Cytoplasm-varying shades of pink.

   Muscle fibres-deep pink red.

   Red blood cells-orange, red.

   Fibrin-deep pink.

Histopathologic criteria[11,12,13] used for classification of leprosy were as follows:

A. Tuberculoid leprosy(TT):

   Epidermis is normal.

   Clear zone is not seen beneath the epidermis.

   Granulomas formed by epitheloid cells and Langhan's giant cells along with neurovascular bundles.

   Many lymphocytes peripheral to the granulomas. They do not penetrate the granuloma.

   Or a large granulomatous nerve with intact perineurium or caseation in a nerve centre.

   Granulomas may extend up to the epidermis.

   BI=0

B. Borderline Tuberculoid (BT).

   Epidermis normal.

   Granulomas are compact with peripheral mantle of lymphocytes. They follow neurovascular bundles and infiltrate sweat glands and errector pili muscles.

   Granulomas do not infiltrate into the epidermis.

   BI=0,1+ or 2+

C. Mid borderline (BB).

   Epidermis is normal or flattening of

rete pegs.

Clear zone is well demarcated.

Granulomas are indistinct and lymphocytes are scanty.

Langhan's giant cells are absent.

Dermal oedema is prominent between inflammatory cells.

BI = 3+ to 4.5+

D. Borderline Lepromatous (BL)

Epidermis is usually flattened or atrophic.

Clear subepidermal zone is present.

Granulomas are composed of macrophages with numerous lymphocytes densely packed over the whole of at least one segment of granuloma but not all segments or solitary clumps of epitheloid cells amongst the macrophages with or without lymphocytes or a combination of moderate number of lymphocytes and undifferentiated granuloma cells.

Nerve commonly shows an onion skin perineurium with cellular infiltrate.

Foamy change can be seen sometimes, usually absent.

BI = 5+

E. Lepromatous Leprosy(LL).

Epidermis is atrophic and thinned out.

Sub-epidermal clear-zone, called as grenz-zone is present.

Foamy cells seen with vacuolated giant cells in regression lesions. Nerves are damaged.

BI= 5+ to 6+

Results

Over the period of 1.5 year study 40 patients presented with hypopigmented patches, erythematous plaques, and nodules. These cases were selected for further study.

Taking histopathology as final diagnosis, FNAC/slit skin smears were compared in 40 cases and results were noted.

Cytology

1. Age and sex incidence in lesions of leprosy.

Results

	No of cases		
Age in years	Male	Female	Total
0-10	0	0	0
11-20	5	2	7
21-30	8	4	12
31-40	4	4	8
41-50	4	1	5
51-60	2	3	5
61 and above	3	0	3
Total	26(65%)	14(35%)	40(100%)

2. Adequacy

It was assigned as mentioned by Singh et al.[16]

According to them cytologic specimen were considered adequate if the cellular yield of inflammatory cells was heavy or when eccrine sweat glands were present even in the presence of scanty inflammatory cells.

Results

Cytology	No of cases
TL	17
BB	04
BL	04
LL	04
Inadequate	11
Total	40

3. Cellularity - it was divided as fair, moderate and heavy.[16]

Results

		Cellularity		
Cytology	No of Cases	Fair	Moderate	Heavy
TL	17	12	05	-
BB	04	04	-	-
BL	04	-	04	-
LL	04	-	-	04
Inadequate	11	-	-	-
Total	40	16	09	04

4. Granulomas - Presence of epiheloid cells granulomas and their composition was noted. Granulomas were described as cohesive and poorly cohesive.[16] Epitheloid cells were recognised as elongated or slender cells but spherical shape epitheloid cells also seen.[8]

Results

Cytology	No of Cases	Cellularity		
		Cohesive	Poorly Cohesive	Epitheloid Cells
TL	17	17	-	17
BB	04	-	04	04
BL	04	-	-	-
LL	04	-	-	-
Inadequate	11	-	-	-
Total	40	-	-	-

5. Other cells : They comprise of lymphocytes, macrophages and foamy cells. Foam cells are macrophages, the cytoplasm of which show many vacuoles.[8]

Results

		Lymphocytes			
Cytology	No of Cases	Few	Numerous	Foamy Cells	Macro-phages
TL	17	-	17	01	-
BB	04	04	-	-	04
BL	04	-	04	-	04
LL	04	04	-	04	-
Total	29	08	21	05	08

6. Bacillary index:

Results

Cytology	No of Cases	AFB		BI
		+ve	-ve	
TL	17	02	15	1+
BB	04	02	02	2+
BL	04	04	-	3+ to 4+
LL	04	04	-	5+
Total	29	12	17	-

7. Correlation of FNAC to Histopathology:

Results

Cytology	HPR available	Total histopathology available (29)				
		TT	BT	BB	BL	LL
TL (17)	12	08	04	-	-	-
BB (04)	03	-	-	03	-	-
BL (04)	03	-	-	-	03	-
LL (04)	03	-	-	-	-	03
Inaq. (11)	08	03	03	-	01	01
Total (40)	29	11	07	03	04	04

8. Association between FNAC and histopathology

Results

		Histopathology		Total
		Available	Not Available	
FNAC	Adequate	21	08	29
	Inadequate	08	03	11
	Total	29	11	40

Discussion

1. *Age and sex distribution:* Majority of patients were between 21 and 30 years of age. Males 26(65%) and females 14(35%). Male to female ratio seen was 2:1(approx). Similar findings are reported by Noorden et al.[9]
2. *Adequacy:* Samples obtained by FNAC in 29 cases out of 40 in present study. In a similar study done by Singh N. et al[16] the number was 26, out of 30 cases and in work done by Dayal S. et al[5] was 18, out 25 cases.

   The percentage of adequacy in all three studies were Present study-72.5%, Singh N et al[16] 86.6%,Dayal S.et al.[5] - 72%.

   The percentage of adequate cellularity

in the present study is comparable with the results of the previous study performed by Dayal S.et al[5] but is less as compared with the study by Singh N.et al.[16]

3. *Cellularity*

   In the present study the cellularity was assessed, based on the criteria used by Singh N.et al.[16]

   Cellularity could be assessed in 29 cases.

   The findings are in agreement with the study carried out by Singh N.et al.[15,16]

4. *Cytologic Features*

   The different types of cells present in the inflammatory infiltrate and their arrangement form the basis of placement of the lesion on the Ridley-Jopling scale.

   In the study done by Singh N et al,[16] all the 5 cases of BL in which cytologic sub-classification was possible, intracellular 'negative images' were observed. This disparity could not be explained in the present study.

   The high cellularity, presence of many foamy macrophages; intracellular and extracellular 'negative images' and few lymphocytes were the features of all the four cases of LL. These findings; are in concurrence with the findings of Singh N et al[14,15,16,17] Jain S et al [7] and Dayal S et al.[5]

5. *Correlation with Histopathology:* All the 12 cases of TL showed correlation with histopathology.

   8 cases were of TT and 4 cases were of BT on histopathology.

   So, in all the cases where histopathology was available in this tuberculoid pole, there was 100% correlation.

   Similar findings have been mentioned by Singh N.et al [16] and Dayal S.et al.[5]

   All the 3 cases diagnosed on cytology as BB showed correlation with histological findings.

   Similar findings were observed by Singh N.et al.[16] Dayal S et al[5] have not commented on this point in their study.

   Out of 3 cases in borderline leprosy in whom histopathology was available; all the 3 cases showed correlation with histopathology.

   Similar findings by Singh N et al[16] and Dayal S.et al.[5]

   All the 3 cases of LL showed correlation with histopathology.

   Singh N.et al[16] had similar findings.

6. *In total*, the number of cases in which both cytology and histopathology were available was 21. The remaining 8 cases were diagnosed on histopathology as the FNAC was inadequate.

   The diagnosis given for these cases were as follows TT-03, BT-03, BL-01, LL-01. The remaining three cases in which FNAC was inadequate and histopathology was not available, could not be concluded.

Conclusion

- FNAC is a rapid, simple, cost and labour effective tool in diagnosing leprosy lesions.
- FNAC can be used to classify leprosy lesions as per the Ridley-Jopling scale.
- Expertise/exposure, in doing FNAC's of leprosy lesions is required as it is a

new technique.

- FNAC can be used in endemic areas, where facilities for histopathology are not available.

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