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Original Article:

Histomorphological Spectrum of Hansen's Disease at Urban Teaching Hospital – A Prospective Study.

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Abstract:**Introduction**: Leprosy is an ancient, chronic, granulomatous, curable disease affecting the skin and peripheral nerves extensively and is caused by Mycobacterium (M) leprae. It remains a considerable health issue in many developing countries including India. Timely and accurate diagnosis is essential for leprosy management as it helps to spread and morbidity associated with it. **Materials and Methods**: The Cross-sectional Prospective study was conducted between period August 2020 to September 2022 in a teaching hospital from urban industrial area. A total of 100 Skin biopsies from clinically suspected cases of leprosy were included in this study. Detailed histomorphological features were studied. Bacteriological Index (BI) of acid fast bacilli was calculated on Fite ferraco stain. **Results**: The most common type of leprosy was BT (n=24). Males were commonly affected than females and commonest age group was 21-40 years (n=39). Majority of the cases in lepramatous leprosy (50%) had Grenz zone. Perineural lymphocytes (37.50%) and periadnexal lymphocytes (45.45%) were appreciated. **Conclusion: C**lassifying leprosy is vital to decide the treatment course. It should only not be on the basis of counting skin lesions, but also through clinical, bacteriological, histopathological examination of the skin biopsies.

Running Title: Spectrum of Hansen's Disease.

Keywords: Leprosy, mycobacteria, bacilli, AFB.

Introduction:

Leprosy, also known as Hansen's disease was first described by Sir Gerhard Armauer in 1873. It is an ancient, chronic, granulomatous, curable disease affecting the skin and peripheral nerves extensively and is caused by Mycobacterium (M) leprae. These are Acid-fast, non-motile bacilli arranged singly or in parallel bundles. It remains a considerable health issue in many developing

countries including India. [1] It presents as different clinicopathologic forms based on the basis of the hosts immune status. Timely and accurate diagnosis is essential for leprosy management. Histopathological examination is the gold standard in the classification and diagnosis of leprosy. Ridley and Jopling proposed the histologic classification of leprosy that is widely used today [2]In 1982, WHO classified leprosy as-Paucibacillary (PB) and Multibacillary (MB)

Detailed examination of peripheral nerves, skin lesions, and skin slit swabs are the basis for leprosy diagnosis. In addition to detecting this mycobacterium, proper histopathological examination of skin biopsies is important [3]

Over 80% of leprosy cases are non-infectious. There is no vaccine available for leprosy, but research is on under the National Leprosy Eradication Program (NLEP), which is dedicated to eradicating leprosy. [4]The aim of our prospective study is to study histomorphological spectrum of leprosy lesions in skin biopsies and to correlate with clinical features and bacterial index in various forms of leprosy.

Materials and Methods:

This Cross-sectional Prospective study was conducted between period August 2020 to September 2022 in a teaching hospital from urban industrial area.An institutional ethics committee clearance was obtained(Ref.No.:I.E.S.C./284/2021) before starting the study .(Research Protocol No .IESC/PGS/2020/196)

A total of 100 Skin biopsies from clinically suspected cases of leprosy were included in this study. Inclusion Criteria**:** Skin biopsies from clinically suspected cases of leprosy.

Exclusion Criteria**:** Skin biopsy for other infectious skin diseases.

Detailed history and clinical features of clinically suspected cases of leprosy were taken from the case sheets. Skin biopsies were sent to the Department of Pathology for detailed histopathological examination.The biopsies were 10% formalin fixed and paraffin processed three to five thick microns' sections were cut and stained with hematoxylin and eosine (H and E). Skin biopsies were looked for epidermal changes and dermal changes for epithelioid granulomas, foamy macrophages, giant cells other relevant inflammatory changes. Additional section was stained with Fite faraco stain for detecting AFB. Calculation of the Bacteriological Index (BI) and classification of lesions done by the Ridley Jopling Classification. Results were analyzed and presented in percentile and tabular form.

Results:

Our study included 100 skin biopsies of the patients diagnosed as leprosy clinically. The most common type of leprosy seen in the present study was BT (n=24). Table 1 show types of leprosy in 100 patients.

Males were commonly affected than females and commonest age group was 21-40 years (n=39). Table 2 show age and sex wise distribution in 100 cases.

In our study, most common clinical feature was loss of sensation(n=66), and least common were trophic lesions (n=4). Table 3 show detailed clinical features in 100 cases.

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In epidermis the most significant finding was atrophy in lepromatous leprosy (77.78%) found in 25 cases followed by ulceration in 11 cases. 13 cases showed erosion of basement membrane in cases of TT (52.38%). Table 4 shows detailed histomorphological changes in epidermis in 100 cases.

Majority of the cases in lepramatous leprosy (50%) had Grenz zone. Perineural lymphocytes (37.50%) and periadnexal lymphocytes (45.45%) were appreciated.

Epitheloid granuloma (66.67%) were mostly seen in borderline tuberculoid leprosy. Table 5 show detailed dermal changes in 100 leprosy cases.

It was observed that 1 case (4.76%) of TT and 1 case (4.17%) of BT showed leprae reaction 1 whereas 5 cases (25%) of LL and 2 cases (100%) of Histoid leprosy showed leprae reaction 2(Table 6).

Bacillary Index of TT and BT was maximally found to be 0 (TT =33.87%,(BT =37.10 %) and BI of 1+ was seen in BT (50%) and BB(50%). A BI of 3+ was mostly seen in BL. A BI of 4+ was seen in BL (40%) and LL (60%). A BI of 5+ was seen mostly in LL. Histoid leprosy showed a BI of 6+.

The co-relation between clinical diagnosis and histopathological diagnosis was done.

The highest clinico-pathological co relation was seen in histoid (100.00%) and borderline leprosy (100.00%), followed by lepramatous leprosy (95.00%), Borderline lepramatous (85.71%), Tuberculoid (85.71%), The least correlation was seen in borderline (79.17%) and indeterminate leprosy. (76.47%)

Discussion:

Leprosy is a deep-rooted, slow-growing infection caused by Mycobacterium leprae. It is a major public health problem in the world. Timely and accurate diagnosis is essential for leprosy management as it helps to spread and morbidity associated with it. Histopathological examination is the gold standard in the classification and diagnosis of leprosy.

Out of 100 cases, the most common form of leprosy on histopathology examination was BT (n=24), followed by TT (n=21), LL (n=20), IL (n=17), BL (n=14) and BB and Histioid leprosy (n=2) each. A similar observation was made in other studies[5]

In our study, patients in the 21-30 year old group were most affected (33%), followed by the 31-40 group (27%), followed by the 41-50 group. followed by a group of (13%). In the 51-to 60-year age group (10%). Males (63%) were commonly affected than females (37%). The prevalence of leprosy in men has increased due to industrialization, urbanization and increased outreach opportunities for men.Social customs and taboos have also contributed to the decline in the number of women going to hospital for treatment [6,7]

In our study, the most common clinical feature was anesthesia (66%), followed by hypo pigmented skin lesions (62%), followed by neural hypertrophy(n=4). A similar finding was reported by his Suri SK et al. [3]. A study by Murthy NB et al. A study conducted showed that hypopigmentation was the most common finding, followed by anesthesia. [8]

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A basic tool for the classification and diagnosis of leprosy remains an adequate skin biopsy. [9]The epidermis was atrophic and was observed in 25 cases, of which maximum (77.78%0 were lepromatous leprosy. Basement membrane ulceration and erosion were the least common features and were mostly restricted to TT and BT leprosy. Basement membrane erosion was the most frequently observed feature in TT. A study conducted by Suri SK found epidermal atrophy in the majority of biopsies (66.7%). In the rest (31.1%) the epidermis was unremarkable.

Granuloma location is also important. When associated with the deep and middle dermal nerves along with the neurovascular complex, they can cause the somatosensory and autonomic neuropathy symptoms of this disease. [81]The border zone is widely accepted as a hallmark of nontuberculous leprosy. Though it is not a diagnostic of leprosy, it is useful for reviewing diagnoses of leprosy and its variants.Perineural and peri-adrenal lymphocytes were more presumed in undetermined leprosy. Epithelioid granulomas were most common in borderline tubercular leprosy.

In our study, TT showedepithelioid granuloma that extends into the epidermis. Clusters of lymphocytes surrounding epithelioid cells, granulomas. Langhan giant cells may or may not be present. Granulomas eroded the epidermis in some cases.Granuloma of epithelioid cells in BT were more diffuse than TT. Giant cells were few and lymphocytes were often found in moderate numbers within the granuloma. BB showed **f**eatures of both TT and LL. No prominent granulomas were seen. Skin edema was seen in few cases.

IL showednon-specific chronic inflammation. Perivascular and peri-adnexal lymphocytes was seen. Lymphocytes are present around nerve fibers.Poorly defined hypopigmented skin lesions were considered for correct diagnosis. With IL, there are very few histopathological changes that can be overlooked. Therefore, the biopsy should include the entire length of the epidermis and sub cutis

BL: Showed diffuse infiltration of macrophages, foamy histiocytes, and few lymphocytes that affect the appendages and nerves.Multiple asymmetrically arranged vague, glossy skin lesions were seen. There may or may not be anesthesia.

LL showed **c**lear infiltration of foam cells, macrophagesand histiocytic around nerves. Lymphocytes may or may not be present.Multiple symmetrical, blurred, shiny skin lesions under anaesthesia. Nerve thickening may or may not be present. Histoid leprosy showed **n**odules seen as spindle cells.

Leprosy Reactions were observed in1 case with TT and 1 case with BT showed Leprosy Reaction 1 and 5 cases with LL and 2 cases with Histoid Leprosy. Of the 21 cases of TT, all 21 (100%) were paucibacillaryOf the 24 cases of borderline tuberculoid leprosy, 23 were paucibacillary (95.83) and 1 (4.17) was Multibacillary.Two borderline cases showed paucibacillary (50%) and Multibacillary. (50%), respectively.Fourteen borderline lepromatous leprosy cases (100%), 20 lepromatous leprosy cases (100%), and two histoid cases (100%) were all multibacillary.

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Bacteriological Indexwas0 in 62 cases.Bacteriological index was 1+ in 2 cases, 2+ in 0 cases.10 cases had 3+. 10 cases showed 4+ 9 cases showed 5+ .7 cases had a bacteriological index of 6.There is discrepancy between bacterial load and cell-mediated immunity as the spectrum of leprosy begins to shift from the pole of tuberculoid to that of lepramatous .. The present study supports this. Some of the LL and BL biopsies also showed bacilli in the appendages, sub epidermal regions around hair follicles, and vascular endothelial cells.

The highest clinicopathologic correlations were with histoid leprosy 100.00%) and BL (100.00%), followed by LL (95.00%), BL (85.71%), TT (85.71%). The lowest correlation was found in the borderline type (79.17%), where indeterminateleprosy (76.47%).Out of 24 cases that were given histpathologically as BT, the clinical diagnosis was given as TT for 2 cases, BL for two cases and BT for 19 cases.

Amongst the 20 cases given as lepramatous leprosy, 19 werediagnosed clinically as LL.Also separation from BL and LL is very difficult, hence strict criteria considering paucity in lymphocytes and clinical features was taken into account. [11,12]

Indeterminate leprosy cases can come across as problematic because the histology of their lesions is nonspecific. The diagnosis of IL depends on many factors, namely the nature and the depth of biopsy, quality of the section, the amount of acid fast stain observed. [82] The interobserver variation both clinically and his pathologically also should be considered.

The absolute diagnosis of IL is dependent on demonstration of acid fast bacilli and nerve lesions. But in endemic areas it can be diagnosed if the clinical and histopathological features are evident even without detecting a single bacillus. [11,13]

Conclusion:

It is essential to correlate both clinically and histopathologic ally to ascertain the different types of leprosy, as some degree of overlap can occur. Furthermore, classifying leprosy is vital to decide the treatment course. It should only not be on the basis of counting skin lesions, but also through clinical, bacteriological, histopathological examination of the skin biopsies. Studies should be done in depth to reevaluate the criteria, be weighing various signs and symptoms and histopathology whilediagnosis leprosy and subtypes

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Tables:

Table 1-Types of Leprosy in 100 patients

Types	**TT**	**BT**	**BB**	**BL**	**LL**	**IL**	**Histoid**	**Total**
No. of Cases	21	24	2	14	20	17	2	**100**
Percentage (%)	21.00%	24.00%	2.00%	14.00%	20.00%	17.00%	2.00%	

Table 2: Age and Sex distribution in 100 cases of leprosy

Age	**No of Cases (N)**		**%**		**Total**
	M	**F**	**M**	**F**	
0-10	**0**	**0**	**0%**	**0%**	**0**

11-20	**5**	**3**	**62.50%**	**37.50%**	**8**
21-30	**21**	**12**	**63.64%**	**36.36%**	**33**
31-40	**18**	**9**	**66.67%**	**33.33%**	**27**
41-50	**8**	**5**	**61.54%**	**38.46%**	**13**
51-60	**7**	**3**	**70.00%**	**30.00%**	**10**
61-70	**3**	**3**	**50.00%**	**50.00%**	**6**
>70	**1**	**2**	**33.33%**	**66.67%**	**3**
Total					**100**

Table 3: Clinical presentation in 100 cases of Hansens disease.

Symptoms	**No of Cases (N)**														
	TT		**BT**		**BB**		**BL**		**LL**		**IL**		**Histoid**		**Total**
	No of Cases (N)	**%**	**No of Cases (N)**	**%**	**No of Cases (N)**	**%**	**No of Cases (N)**	**%**	**No of Cases (N)**	**%**	**No of Cases (N)**	**%**	**No of Cases (N)**	**%**	
Total no of cases	**21**	**21.00 %**	**24**	**24.00 %**	**2**	**2.00 %**	**14**	**14.00 %**	**20**	**20.00 %**	**17**	**17.00 %**	**2**	**2.00 %**	**100**
Hypopigmented plaques	**8**	**22.86 %**	**20**	**26.32 %**	**4**	**30.77 %**	**7**	**25.00 %**	**10**	**18.52 %**	**13**	**37.14 %**	**0**	**0.00 %**	**62**
Erythromatous lesions	**6**	**17.14 %**	**8**	**10.53 %**	**1**	**7.69 %**	**3**	**10.71 %**	**6**	**11.11 %**	**4**	**11.43 %**	**0**	**0.00 %**	**28**
Combination of lesions	**2**	**5.71 %**	**3**	**3.95 %**	**1**	**7.69 %**	**2**	**7.14 %**	**1**	**1.85 %**	**1**	**2.86 %**	**0**	**0.00 %**	**10**
Anaestheisa	**10**	**28.57 %**	**23**	**30.26 %**	**4**	**30.77 %**	**7**	**25.00 %**	**13**	**24.07 %**	**9**	**25.71 %**	**0**	**0.00 %**	**66**
Nerve thickening	**9**	**25.71 %**	**19**	**25.00 %**	**3**	**23.08 %**	**6**	**21.43 %**	**12**	**22.22 %**	**8**	**22.86 %**	**1**	**50.00 %**	**58**
Nodules	**0**	**0.00 %**	**1**	**1.32 %**	**0**	**0.00 %**	**3**	**10.71 %**	**10**	**18.52 %**	**0**	**0.00 %**	**1**	**50.00 %**	**15**
Trophic ulcers	**0**	**0.00 %**	**2**	**2.63 %**	**0**	**0.00 %**	**0**	**0.00 %**	**2**	**3.70 %**	**0**	**0.00 %**	**0**	**0.00 %**	**4**
Total	**35**		**76**		**13**		**28**		**54**		**35**		**2**		**243**

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Table 4- Histomorphological features in Hansen's disease in epidermis

Symptoms	No of Cases (N)													Total
	TT		BT		BB		BL		LL		IL		Histoid		
	No of Cases (N)	%	No of Cases (N)	%	No of Cases (N)	%	No of Cases (N)	%	No of Cases (N)	%	No of Cases (N)	%	No of Cases (N)	%	
Unremarakable	4	19.05%	26	83.87%	4	80.00%	2	20.00%	0	0.00 %	15	100.00%	0	0.00 %	51
Atrophy	2	9.52 %	2	6.45 %	1	20.00%	6	60.00%	14	77.78%	0	0.00 %	2	100.00%	25
Basement membrane erosion	11	52.38%	2	6.45 %	0	0.00 %	0	0.00 %	0	0.00 %	0	0.00 %	0	0.00 %	13
Ulceration	4	19.05%	1	3.23 %	0	0.00 %	2	20.00%	4	22.22%	0	0.00 %	0	0.00 %	11
Total	21		31		5		10		18		15		2		100

Table 5: Dermal changes in 100 cases of Hansen's disease.

Symptoms	No of Cases (N)													
	TT		BT		BB		BL		LL		IL		Histoid	
	No of Cases (N)	%	No of cases	%	No of cases	%	No of cases	%	No of cases	%	No of cases	%	No of cases	%
Epitheloid granuloma	1	4.76 %	14	66.67 %	1	4.76 %	0	0.00 %	0	0.00 %	5	23.81 %	0	0.00 %
Foamy histiocytes	0	0.00 %	4	26.67 %	0	0.00 %	4	26.67 %	7	46.67 %	0	0.00 %	0	0.00 %
Giant cells	1	6.67 %	12	80.00 %	1	6.67 %	0	0.00 %	1	6.67 %	0	0.00 %	0	0.00 %

Grenz zone	**0**	**0.00 %**	**3**	**15.00 %**	**2**	**10.00 %**	**5**	**25.00 %**	**10**	**50.00 %**	**0**	**0.00 %**	**0**	**0.00 %**
Perineural lymphocytes	**1**	**6.25 %**	**4**	**25.00 %**	**1**	**6.25 %**	**2**	**12.50 %**	**0**	**0.00 %**	**6**	**37.50 %**	**2**	**12.50 %**
Periadnexal lymphocytes	**0**	**0.00 %**	**4**	**36.36 %**	**0**	**0.00 %**	**1**	**9.09 %**	**0**	**0.00 %**	**5**	**45.45 %**	**1**	**9.09 %**
Periappendageal lymphocytes	**1**	**9.09 %**	**6**	**54.55 %**	**1**	**9.09 %**	**1**	**9.09 %**	**0**	**0.00 %**	**1**	**9.09 %**	**1**	**9.09 %**

Table 6 : Leprae reaction in Hansen's disease –

Types	**No**	**Leprae reaction 1**		**Leprae reaction 2**	
		No of cases	%	No of cases	%
TT	21	1	4.76%	0	0.00%
BT	24	1	4.17%	0	0.00%
BB	2	0	0.00%	0	0.00%
BL	14	0	0.00%	0	0.00%
LL	20	0	0.00%	5	25.00%
Histoid	2	0	0.00%	2	100.00%
IL	17	0	0.00%	0	0.00%
Total	100				

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Legends to figures:

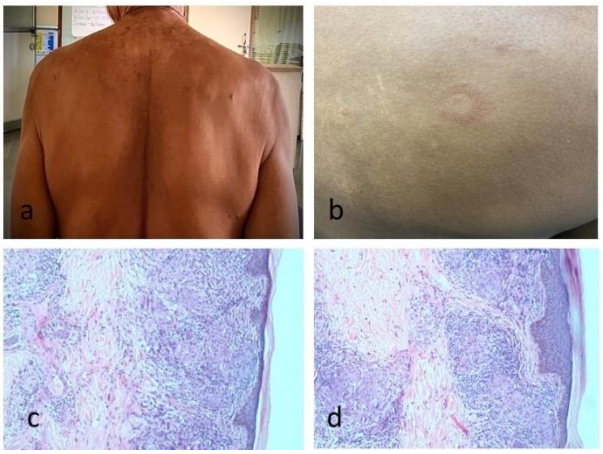

Figure 1: Tuberculoid leprosy- (a) Well defined ,flat hypopigmented lesion(b) Well defined lesion with central clearing and slightly raised margin (c) Histopathology of Tuberculoid leprosy showing Epithelial granuloma reaching up to epidermis.(H&E x100) (d) Histopathology of Tuberculoid leprosy showing Epithelioid granuloma surrounded by lymphocytes(H&E x400).

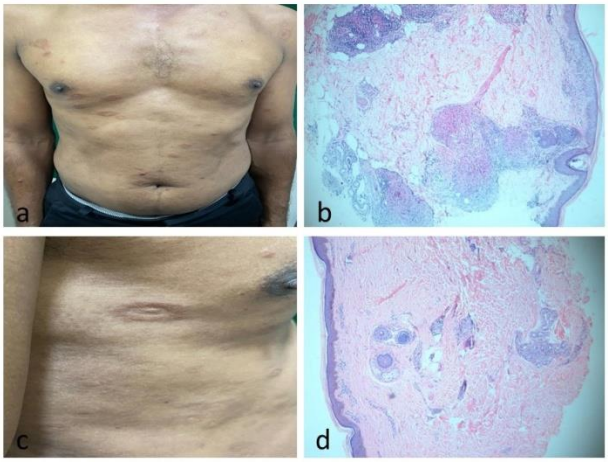

Figure 2: Borderline tuberculoid-(a) Multiple lesions of varying size with raised margin with hair loss over the lesion. Satellite lesions are seen. (b) Histopathology showing Epitheliod granuloma with Langhans giant cells. (H&E x100) (c)Histopathology of Midborderline leprosy-showing irregularly dispersed and shaped erythematous plaques with punched out centres. (d) Histopathology showing Perineural/Periadnexal lymphocytes. (H&E x100)

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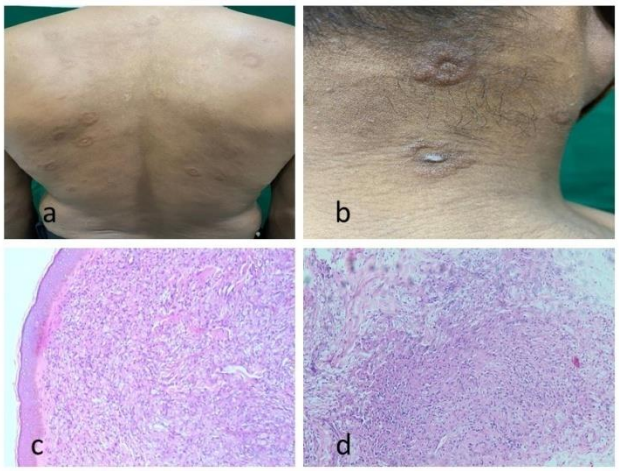

Figure 3: Lepromatous Leprosy –(a) Multiple, bilateral, flesh coloured nodules over back. (b) Infiltrative nodular type- Papules, nodules and diffuse dull red infiltrates seen.(c) Histopathology of Lepramatous leprosy showing Higher magnification-foamy histiocytes.(H&E x400)(d) Histopathology of Lepramatous leprosy showing Foamy Histiocytes in nerve(H&E x400).

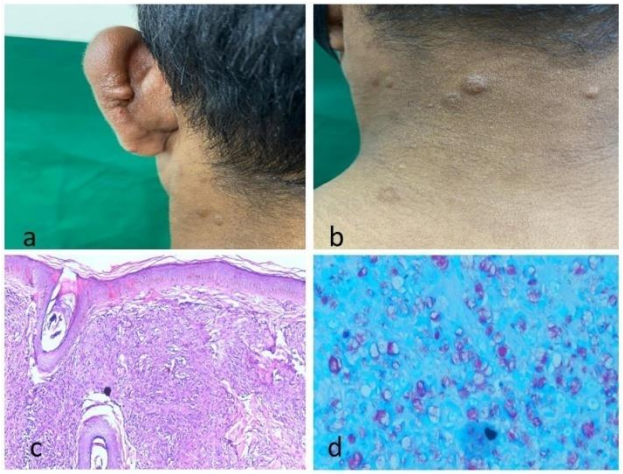

Figure 4: Histoid leprosy-(a) Skin thickening and nodules on the auricle. (b) Well demarcated cutaneous and subcutaneous nodules. (c) Histopathology of histoid leprosy showing Higher magnification- Nodules of spindle cells. (H&E x400) (d) Numerous Acid fast bacilli in Histioid leprosy (ZN x100).

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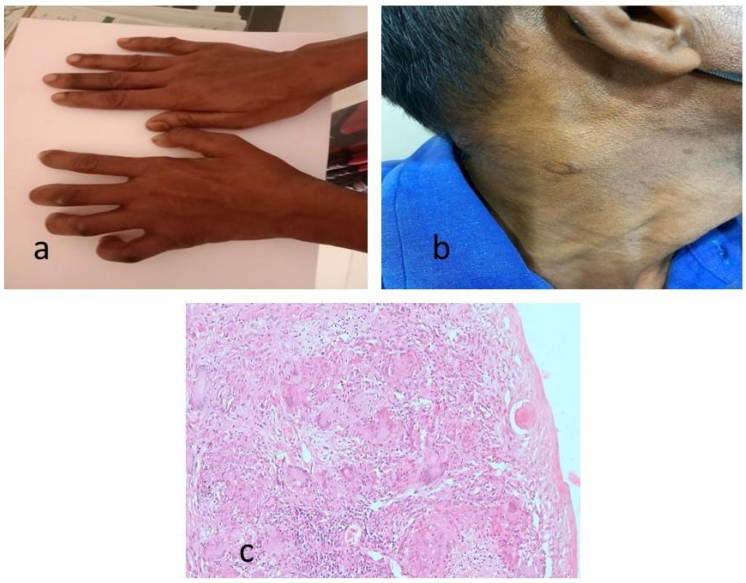

Figure 5: (a)Claw hand in leprosy (b) Thickened peripheral nerve in Leprosy (c)Higher magnification-type 1 leprae reaction. (H&E x400)