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Original Research Article

Cutaneous mycobacterial lesions- A retrospective study in a tertiary care hospital

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ABSTRACT

Introduction: Mycobacterial dermal lesions pose diagnostic challenge and require histopathological examination for confirmation. Granuloma is an important histopathological feature present in most of the mycobacterial dermal lesions. Multiple etiologies can produce an identical histopathological picture. The present study was undertaken with the objective to study the spectrum of mycobacterial dermal lesions, their etiologies and their incidence in this geographical area.

Materials and Methods: This present study was carried out in Department of Pathology G.K General Hospital, Bhuj for a period of one year. A total of 50 cases mycobacterial dermal lesions of all etiologies are included in the present study. Hematoxylin and eosin stain was routinely done on all cases. Special stains like Ziehl-Neelsen, Fite Faraco, Periodic acid–Schiff stain were done whenever required.

Conclusion: Histopathology is gold standard to diagnose mycobacterial dermal lesions. Along with it, the clinical presentation of the patient is also very important in elucidating the exact etiology.

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1. Introduction

Mycobacterial infections occur most commonly in immunocompromised patients and also resurge in them. Necrotizing granulomatous inflammation is the commonest histopathological manifestation in mycobacterial skin infections.¹

Granulomatous inflammation of skin is a known histological pattern encountered commonly in skin biopsies. It possesses a diagnostic challenge to the pathologist because its etiopathogenesis is variable as well as the dermatologists. These granulomatous lesions share the histopathological denominators of the granuloma formation.² Infectious and non-infectious causes can lead to granulomatous lesions Mycobacterial infection, non mycobacterial of skin. infection, mycobacterial leprae infection, leishmaniasis and fungal infections are common etiologies.³ Cutaneous mycobacterial infections may causes a wide range of clinical manifestation viz, 1) Cutaneous manifestation of Mycobacterium Tuberculosis (MTB), 2) Buruli ulcer and M. ulcerans, 3) Leprosy caused by M.leprae, 4) Cutaneous

The term "granuloma" was coined by Virchow, to describe a tumor like mass or nodule of granulation tissue.⁴ The granulomatous reaction pattern is defined as a distinctive inflammatory pattern characterized by the presence of granulomas. Granulomas are relatively discrete collections of histiocytes or epithelioid histiocytes with variable numbers of admixed multinucleate giant cells of varying types and other inflammatory cells.³

Several etiologies can produce an identical histopathological pattern and conversely a single etiology can produce multiple histopathological pattern.

The present study was conducted with the aim to study the histopathological spectrum of pattern of mycobacterial dermal lesions and identification of their etiology.

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infection caused by rapidly growing mycobacteria. Though special stains are helpful in making a definite diagnosis, at times only the histopathological pattern and its correlation with the clinical picture that aids in making a definitive diagnosis.

2. Materials and Methods

This retrospective study was carried out in department of Pathology, G.K General Hospital, Bhuj for a period of one year. Skin biopsies were received from the department of Dermatology. A 4 mm punch biopsy is adequate for histopathological examination. It must show all the anatomical compartments viz skin epidermis, dermis, deep vascular plexus, sebaceous glands, eccrine glands and subcutaneous tissue. Slides were stained with routine Haematoxylin and eosinstain(H&E)(J.K. Diagnostics) and special stains like Ziehl-Neelsen stain(ZN stain)(HIMEDIA), Fite-Faraco stain, Periodic acid-Schiff stain and Congo red stain were done where ever required.

2.1. Exclusion criteria

Inadequate samples and cases of mycobacterial dermal lesions on treatment. The Ridley and Jopling classification system was used to asses the histopathological spectrum of dermal lesions of Hansen's disease.⁵ The classification system of Ridley and Jopling.⁵ divides leprosy cases into five groups according to their position on an immuno-histological scale: tuberculoid(TT), borderline tuberculoid(BL), borderline(BB), borderline lepromatous(BL) and lepromatous(LL). The neuritic type of leprosy does not find a place in the Ridley and Jopling classification system. Condition in which there is a diffuse infiltration of histiocytes within the dermis such as lepromatous leprae are not included in the granulomatous reaction pattern.

3. Observation

- 1. Distribution of cases according to gender and age group is shown in Table 1
- 2. The most common age group affected was 21-30 years. There was male preponderance in the present study.(Table 1)
- 3. The most common histopathological diagnosis was leprosy present in 56% of cases followed by tuberculosis in 44% of cases.Table 2
- 4. Classification of dermal lesions of leprosy (Hansen's disease) was done according to the Ridley-Jopling classification system that divided leprosy in five groups.(Table 2)
- 5. Lepromatous leprosy(20%) was most common lesion followed by tuberculoid leprosy(16%).(Table 2)
- In cutaneous tuberculosis, most common dermal lesion was lupus vulgaris (36%) followed by tuberculosis verrucosa cutis(8%).Table 2
- 7. The total granulomatous lesions were 80%.(40/50 cases). Distribution of the different types of granuloma along with special stains shown in Table 3.
- Well defined granulomas were mostly present in tuberculoid leprosy and lupus vulgaris. Strong positivity for Fite Faraco stain seen in lepromatous

leprosy and histoid lepromatous leprosy.(Table 3)

4. Discussion

In humans, pathogenic mycobacterial species may breach the innate immune system and cause respiratory tract, skin and soft tissue infection.

Mycobacteria are group of gram positive, acid fast rod, that do not take up gram staining due to their cell wall structure. There are three main groups according to taxonomy viz.

- 1. Mycobacterium tuberculosis complex-(Pathogen of tuberculosis) which includes Mycobacterium tuberculosis(M TB) and Mycobacterium bovis(M. Bovis).
- 2. Mycobacterium leprae(Pathogen of leprosy).
- 3. Non tuberculosis mycobacteria.⁶

Cutaneous tuberculosis is primarily caused by M TB and less frequently by M. Bovis.^{7,8} M. leprae is pathogen that causes leprosy(Hansen' s disease).⁹

The various type of cutaneous tuberculosis are distinguished according to immune status of the affected individual and depends on its first time infection or recurrence. The various type of cutaneous tuberculosis are Tuberculosis verrucosa cutis(TVC), lupus vulgaris(LV) and tuberculids.⁹ Tuberculids include lichen schrofulosorum, erythema induratum of Brazin, scrofuloderma(SD), tuberculosis cutis orificialis, tuberculoid chancre, miliary tuberculosis and tuberculoid gummata. The most relevant dermatological manifestations are seen TVC, LV, and SD.⁹

Leprosy is a disease bedevilled by many classifications, e.g., Ridley-Jopling classification,⁵ Madrid classification,¹⁰ the Indian classification¹¹ etc. These classifications are based on clinical, bacteriological, immunological and histological status of patients.

The Indian and Madrid classification is widely used in field leprosy programme. The Indian classification is different from Madrid classification as it has an additional type the pure neuritic type in which no skin lesion exists. The Ridley-Jopling classification is widely used in the histopathological classification. The neuritic type of leprosy does not find a place in the Ridley-Jopling classification system. M. leprae cannot be cultivated in vivo. Perineural infiltration is characteristic of all forms of leprosy. Intraneural pathogen detection is pathognomonic for detection of leprosy. Lepra bacilli along with being present in the macrophages can also be present in the endothelial cells. Cutaneous TB(TVC, LV, SD) and Leprosy(except lepromatous leprosy) are characterized by a distinguished granulomatous reaction characterized by presence of granuloma. Granulomas are relatively discrete collections of histiocytes or epithelioid histiocytes with variable numbers of admixed multinucleate giant cells of varying types and other inflammatory cells.² Lepromatous leprosy falls out of spectrum of granulomatous pattern as it contains a diffuse pattern of histiocytic infiltration in dermis. $^{\rm 2}$

Granulomatous lesions can be divided according to the etiology or on the basis of their reaction pattern. These include Sarcoidal granulomas, Tuberculoid granulomas, Necrobiotic granulomas, Suppurative granulomas, Foreign body granulomas, Xanthogranuloma and Combined granulomatous and lichenoid pattern.

The dermal manifestations of tuberculosis (TVC, LV) and Leprosy (TT, TL, BT, BL) are characterised by presence of tuberculoid granuloma.

Tuberculoid granulomas composed of epithelioid histiocytes, Langhans giant cells and foreign body type with a more substantial rim of lymphocytes and plasma cells and sometimes showing central caseation necrosis. These granulomas have a tendency to confluence.

Other condition in which tuberculoid granuloma can be present are late syphilis, fatal bacterial granuloma, leishmaniasis and crohn's disease.²

The necrotizing granuloma is the most common histopathological manifestation in patients with mycobacterial infections.¹ At times other less common types of tissue reaction like ill formed granulomas, non granulomatous acute inflammation and non necrotizing granulomas can also be present.¹ This present study focuses only on mycobacterial skin lesions and its infectious granulomatous causes. Various studies on different types of granulomatous lesions of skin have been done.^{12–14} But very few studies like ours have been done on histopathological spectrum of cutaneous TB and cutaneous Hansen's disease.^{15,16}

Leprosy was most common mycobacterial infection in the present study(56%) followed by cutaneous tuberculosis(44%). The findings were similar to various other studies where leprosy cases were more common than cases of cutaneous tuberculosis. ^{12,17,18} Bat et al ¹² found 74% cases of leprosy followed by 23.19% of cutaneous tuberculosis. Gautam et al found 79.7% cases of leprosy followed by 7.6% cases of cutaneous tuberculosis.

Chakrabarti¹⁹ et al found 57.52 % cases of leprosy followed by 24.73 % cases of cutaneous tuberculosis.

Tuberculoid granuloma was the most common granuloma in the present study comprising 80% of cases. Lepromatous leprosy is does not fall in this spectrum of granulomatous reaction pattern, as it is characterized by the present of diffuse histiocytic infiltration in the dermis. This finding was similar to other studies^{12,14,17,18} that found tuberculoid granuloma to be most common.

Bat et al,¹² Zafar et al,¹⁴ Dhar et al.¹⁷ and Gautam et al.¹⁸ found 87.7%, 92.7%, 77.3% and 68.9% of tuberculoid granuloma in their studies respectively.

Cases of leprosy in the present study were typed according to the Ridley and Jopling⁵ classification system and histopathological examination was done. Leprosy predominantly affects the skin and peripheral nerves. In

the present study, LL(20%) cases was the most common skin lesion of Hansen's disease in this geographic location. Table 2

Microscopic examination of lepromatous leprosy shows an extensive cellular infiltrate consisting predominately of macrophages in the dermis and separated from the flattened epidermis by a narrow Grenz zone of normal collagen. [Figure 1A(arrow)]

These macrophages have eccentrically located nucleus with eosinophilic cytoplasm containing bacilli.(solid or fragmented) There is no granuloma formation. Lepromatous leprosy indicates an absence of cellular response to M. Leprae antigens with the absence of macrophage activation and no epithelioid granuloma formation.¹⁹ [Figure 1B] In the later stages, macrophages are distended with large group of degenerated lepra bacilli in cytoplasm(globi). These are also called as lepra cells or Virchow cells.²⁰

Other studies had Borderline Tuberculoid leprosy ^{12,17,18} was the most common lesion followed by tuberculoid leprosy as the most common finding in few other studies. ^{21,22}

The second most common lesion of leprosy in our study was TL (16%) followed by BT (12%) and Borderline Lepromatous leprosy (6%). Other studies have reported 7.2%, 13.08%, 20% and 33.5% incidence of TL. 12,19,21,22

Microscopic examination of TL is characterized by the presence of compact granulomas present around skin appendages and eroding into the neural structures. (Granuloma neuritis) [Figure 2 A, B] Dermal nerves may either be absent or surrounded and eroded by dense lymphocytic cuff and granuloma. AFB are rarely found. These compact granulomas are comprised of large epithelioid cells with dense peripheral lymphocytic accumulation. There is absence of Langhans giant cell. At times these granulomas encroach upon the basal layer of epidermis.

The third most common lesion in the presence study was BTL present in 12% of cases. (Table 3)

However this finding deferred from other studies where BTL was the most common lesion in the leprosy spectrum. ^{12,18,19}

On microscopic examination BTL lesions are characterised by presence of granulomas with fewer lymphocytes and variable Langhans giant cells. The granulomas are present in the deeper dermis and around neurovascular bundles, sweat glands and along superficial vascular plexus. Acid fast bacilli is scant. (Figure 3 A, B)

Borderline Lepromatous leprosy was present in 6% cases (Table 3). This was nearly similar to 6.54% cases and 5.79% cases in other studies ^{19,21} This was lower than the study by Bal et ¹³ who found 15% of cases of Borderline Lepromatous leprosy. On microscopic examination, BLL is characterised by poorly defined granulomas with more lymphocytes as compared to LL where there is

Age Range	Male	Female	Total	Percentage
11-20	2	2	4	8%
21-30	12	8	20	40%
31-40	8	5	13	26%
41-50	2	4	6	12%
51-60	3	1	4	8%
61-70	3	0	3	6%
Total	30	20	50	100%

Table 1: Age distribution of patients

Table 2: Histological spectrum of cutaneous mycobacterial lesions

Leprosy			Tuberculosis				
S. no	Histological Type	No. of Cases	Percentage(%)	S. no	Histological Type	No. of Cases	Percentage(%)
1	Tuberculoid Leprosy	8	16%	1	Lupus vulgaris	18	36%
2	Borderline Tuberculoid Leprosy	6	12%	2	Tuberculosis verrucosa cutis	4	08%
3	Borderline Lepromatous Leprosy	3	06%				
4	Lepromatous Leprosy	10	20%				
5	Histoid lepromatous leprosy	1	2%				
	Total	28	56%		Total	22	44%

Table 3: Types of granulomas and special stains

		Granuloma				Special stain			
No.	Histological Type	Well		Doorly	No No	Ziehl-Neelsen		Fite Faraco	
			Moderate	FOOTY	Granuloma	Positive	Negative	Positive	Negative
1	Tuberculoid Leprosy	5	3	0	0				8
2	Borderline Tuberculoid	0	0	6	0			2	4
	Leprosy								
3	Borderline Lepromatous	0	0	3	0			2	1
	Leprosy								
4	Lepromatous Leprosy	0	0	0	10			10	
5	Histoid lepromatous leprosy	0	0	0	1			1	
6	Tuberculosis verrucosa cutis	0	0	4	0	2	2		
7	Lupus Vulgaris	12	6	0	0		12		

no granuloma formation. The typical histopathological features is perineural fibroblastic proliferation forming onion skin appearance on cross section.²⁰

The least common lesion was histoid leprosy found in 2% cases. (Table 2) Other studies found $0.93\%^{19}$ and $5.72\%.^{21}$ This type of leprosy shows highest load of bacilli followed by LL. On microscopic examination an unusual type of macrophage reaction take place in which the macrophages become spindle shaped and are oriented in a storiform pattern.

Granulomas of TT and BT are well formed granulomas with presence of epithelioid cells, lymphocytes and Langhans giant cell. (Table 3)

These need to be differentiated from the lesions with non caseating granuloma like sarcoidosis and foreign body giant cell reaction. In the present study the FF stain was positive in only 2 cases of BTL Moreover, as there are sparse bacilli, lepra stain is not very useful in such cases.^{23,24} Perineural infiltration is present in all forms of leprosy. Intraneural pathogen detection pathognomic of leprosy. As such a definitive subclassification of leprosy on histopathological examination is quite impossible, cliniopathological correlation is of particular importance. It is the combination of clinical picture along with the location of granuloma around neurovascular bundle and adnexal structures that aids in the diagnosis. Post kalaazar dermal leishmaniasis (PKDL) mimics LL. It is the demonstration of Leishmania donovani bodies on Giemsa stain along with plasma cells and histiocytic infiltrate that aids in diagnosis of PKDL.

The next frequently encountered mycobacterium skin lesion was cutaneous Tuberculosis (C.TB) present in 44% cases. This incidence was higher than two other studies ^{12,13} where they found an incidence of 23% and 24.73%



Fig. 1: (A): (H&E 100 X) Section shows extensive cellular infiltrate consisting predominately of macrophages in the dermis and separated from the flattened epidermis by a narrow Grenz zone of normal collagen.(arrow); (B): (H&E 400 X) Section shows extensive cellular infiltrate consisting predominately of macrophages in the dermis.



Fig. 2: (**A**): (H&E 100 X) Section shows the presence of compact granulomas in the dermis layer. Granulomas are composed of epithelioid cells with dense peripheral lymphocytic accumulation; (**B**): (H&E 400 X) Section shows the presence of granulomas surrounding neural vascular bundle (Granulomatous neuritis).

respectively. This could be due to geographical variation. The two types of C. TB encountered in present study is lupus vulgaris (36%) and TVC (8%) (Table 2). This finding similar to other studies^{12,17–19,21,22} were lupus vulgaris was the most frequent finding in the cutaneous TB spectrum. On microscopic examination lupus vulgaris is characterised by presence of well-defined granuloma as were seen in the presence study (Table 3). These tuberculoid granuloma were near confluent, non-necrotizing composed of epithelioid cells and giant cell with a rim of surrounding lymphocytes. Caseating necrosis is usually slight to absent within these tubercles.

However few other studies^{25,26} have found Scrofulosorum as the commonest form of cutaneous TB especially in childhood. The second most common lesion in cutaneous TB spectrum was TVC (8%).Table 2 Other studies also found TVC as common clinical pattern of cutaneous TB.^{13,18,19,21} TVC represent an inoculated exogenous infection of the skin. On histopathological examination an acute inflammatory infiltrate, with or without abscess formation may be observed in the upper dermis with downward extension. (Figure 4 A) Epidermal hyperplasia is also usually seen. Tuberculoid granulomas with necrosis are usually presence and tubercle bacilli more common than lupus vulgaris (Figure 4B). Sporotrichosis is an important differential diagnosis in this entity. Hence PCR and culture play animportant role in it diagnosis.²⁷LV, Scrofulosorum, TVC are represent reinfection and reactivation of the



Fig. 3: (A): (H&E 50 X) Section shows the presence of granuloma in deep dermis with fewer lymphocytes.(arrow); (B): (H&E 400 X) Section shows the presence of granulomas are comprised of large epithelioid cells with peripheral lymphocytic accumulation.



Fig. 4: (A): (H&E 100 X) Section shows the presence of an acute inflammatory infiltrate in the upper dermis with downward extension to the dermis; (B): (H&E 100 X) Section shows caseation necrosis with few epithelioid cells and Langhans giant cell (arrow).

tuberculous bacilli.²⁵ Acid fast bacilli examination is indicated in all suspected cases of cutaneous TB and FF stain in all cases of Hansen' s disease. (Table 3) In the present study ZN stain was positive in only 2 cases (11%) of TVC and none of LV. These findings are similar to Dhar at al.¹⁷ The fluorochrome stain-Auramine rhodamine stain is useful to determine AFB, but culture is most important diagnostic modality.¹ Though the presence of caseous necrosis within the epithelioid

granuloma is diagnostic, TB can not be rule out in its absence.¹² Literature^{12,27,28} has reported incidence of 5%, 13-15% AFB positivity in lupus vulgaris. However in the present study there was no AFB positivity in lupus vulgaris. Few studies.^{27,28} have also reported rates as high as 50% positivity of AFB in scrofulosorum. Non tuberculous mycobacteria (NTM) are human opportunistic pathogens. They usually have an environmental source on infection. On histopathology they are characterised by diffuse infiltration

of mixed inflammatory cells, small vessels proliferation in the dermis, dermal granulomas and epidermal proliferation. Suppurative granuloma may be present or absent.²⁹

5. Conclusion

Granuloma is an important histopathological feature present in most of the mycobacterial dermal lesions. Multiple etiologies can produce an identical histopathological picture. Mycobacterial and non mycobacterial dermal lesion required histopathological examination for confirmation along with clinical presentation. Mycobacteria is an important cause of granulomatous dermal lesion and needs to be differentiated from other infectious and fungal etiologies. Very few studies have been done on dermal manifestation of mycobacterial dermal lesions. The present study was undertaken with the objective to study the spectrum of mycobacterial dermal lesions, their etiologies and their incidence in this geographical area.

6. Conflicts of interest

The authors declare no conflicts of interest.

7. Funding

None.

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