

A Histopathological study of Granulomatous Inflammations with an attempt to find the Aetiology

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ABSTRACT

Granulomas are the commonest lesions that the pathologists come across in routine practice. In order to treat these lesions, definitive diagnosis by the demonstration of the aetiological agent is essential, which will bear an impact on the patient management and outcome.

Our aim was to find the aetiology in all the granulomatous lesions, on histopathologically evaluated biopsies.

A two year prospective study was done in KIMS; Hubli. The biopsies of the cases which were diagnosed as granulomas on H and E stained sections from all the sites were selected. Special stains like Ziehl-Neelsen stain, Gomori's Methenamine silver, Fite Faraco and Auramine Rhodamine stain were done wherever required.

A total of 170 granulomatous lesions were studied. Granulomas with different aetiologies were seen. The commonest were the granulomas due to tuberculosis with 84 (49.41%) cases, followed by those with leprosy, rhinoscleroma, actinomycosis and

fungal infections and foreign body granulomas and granulomas with unknown aetiology.

An attempt has to be made to put these granulomas into specific aetiological categories for specific treatment.

The morphology of the lesions and the use of special stains helped us to diagnose 159 out of the 170 cases.

KEY MESSAGE

1. Granulomas are the commonest lesions that the pathologists come across in routine practice.
2. Tuberculosis is the commonest cause of granuloma.
3. The morphology of the lesions and special stains helped us to identify the aetiology.

INTRODUCTION

The term, 'Granulomatous inflammation' defines a pattern of reaction to a wide range of aetiological agents, organic and inorganic, with certain morphological correlates [1].

Granuloma is "a focal chronic inflammatory response to a tissue injury, which is evoked by a poorly soluble substance which is characterized by the accumulation and proliferation of leukocytes, principally of the mononuclear type" [1].

The provocative agents of granulomatous inflammation appear to be non-degradable by both neutrophils and non-active macrophages. The actions of polymorphonuclear leucocytes, non-activated macrophages and chemical mediators which are associated with the tissue injury are insufficient to completely digest and eradicate the offending agents. For such a degradation, the action of transformed macrophages which are formed with the help of the CD4+T cells, is required. The CD4+T cells secrete various mediators such as IL2, IF γ , TNF and lymphotoxin for the transformation of the macrophages into epithelioid cells and giant cells, which are the components of granulomas [2].

Classification of granulomas based on the aetiology:

1. Bacterial
2. Metal induced
3. Fungal
4. Viral / Chlamydial
 - a. Cat scratch fever
 - b. Lymphogranuloma venerum
5. Helminthic
6. Foreign body type
7. Unknown cause [3].

Key Words: Granulomas, Tuberculosis, Fungal

Classification based on the morphological criteria:

1. Epithelioid
2. Histiocytic
3. Foreign body
4. Necrobiotic / Palisading
5. Mixed inflammatory [1].

Granulomatous inflammations are a common and intriguing problem. The arrival at a proper diagnosis is mandatory, so that the appropriate treatment can be meted out. Histopathology is a tool which can be used for establishing a correct diagnosis like in many other diseases, pertaining to the various organ systems of the body [4].

Good clinical history, a close histological examination and a clinicopathological correlation are essential in making a final diagnosis.

By combining all the available information, one should be able to arrive at a reasonable differential diagnosis on which to proceed. However in a minority of the cases, it will not be possible to make a definitive diagnosis, even with all the clinical information being available.

A rational histological diagnostic approach to granulomatous inflammation is also not present without its problems. Special stains may also be required to reach a diagnosis. In a small percentage of cases, no definitive diagnosis can be given, other than that of granulomatous inflammation [5].

However, no comparative study, to our knowledge, has been carried out to determine the frequency and the types of different gran-

ulomatous lesions in India and hence this study was carried out.

MATERIALS AND METHODS

The present study was undertaken from October 2004 to April 2006 in the Department of Pathology, Karnataka Institute of Medical Sciences, Hubli.

The biopsies were received from various departments of the KIMS hospital, Hubli and a few were received from other hospitals in and around Hubli.

A histopathological study of 170 granulomatous lesions was done. A majority of the granulomas were seen in skin and sub cutaneous tissues with 53 cases, followed by the involvement of lymph node in 31, the respiratory system in 29, bones and joints in 13, the gastrointestinal tract in 11, the breast in 10, the female reproductive system in 07, the male reproductive system in 05, the ear in 4, the gall bladder in 2 and 1 case each in the oral cavity, the liver, omentum, the brain and the urinary tract.

The biopsies of the cases which were diagnosed as granulomas from all the sites, on haemotoxylene and eosin stained sections were selected. Special stains like ZN, GMS, PAS, Fite Faraco and the Auramine Rhodamine stain were used whenever required. The relevant clinical findings and lab investigation details were collected by a personal interview and the examination of the patient, or from the hospital case sheets.

RESULTS

The ages of the patients with the 170 granulomatous lesions ranged from 2 to 70 yrs, with a mean age of 31.26±14.64 years. A majority of patients were in the age group of 20-29 years.

A majority of them were males. Males accounted for 92 (54.12%) cases of the total 170 cases with male to female ratio of 1.18:1 [Table/Fig 1]

Age group (years)	Male	Female	Total (percentage)
0-9	4	6	10(5.88%)
10-19	15	11	26(15.29%)
20-29	25	21	46(27.06%)
30-39	18	17	35(20.59%)
40-49	16	14	30(17.65%)
50-59	8	7	15(8.82%)
60-69	6	2	8(4.71%)
Total	92	78	170(100%)

[Table/Fig 1]: Age and sex distribution of the patients

Granulomas of different aetiologies were seen. Tuberculosis was the most common cause of granulomas with 84 (49.41%) cases, followed by leprosy with 30 cases (17.65%) and rhinoscleroma with 20 cases (11.76%), foreign body granulomas -13 (7.65%), granulomas with unknown aetiology- 11(6.47%) and those with fungal infections- 10 (5.88%) and actinomycosis -2 (1.18%). [Table/Fig 2]

Sl. No	Granulomas	No. of cases	Percentage
1	Tuberculosis	84	49.41
2	Leprosy	30	17.65
3	Rhinoscleroma	20	11.76
4	Actinomycosis	02	1.18
5	Fungal	10	5.88
6	Foreign body granulomas with known etiology	13	7.65

7	Granulomas with unknown etiology	11	6.47
Total		170	100

[Table/Fig 2]: Granulomas with different etiology

In tuberculosis, the ZN stain was done for all the 84 cases, out of which 19 (22.62%) cases were ZN positive and 65 (77.38%) were negative.

Twenty five cases were randomly selected for the Auramine Rhodamine staining. Out of these, 19 cases which were negative for the ZN stain, were positive for Auramine Rhodamine and 5 cases which were negative for the ZN stain, were negative for Auramine and Rhodamine also. One case, which was positive for the ZN stain, was also positive for Auramine Rhodamine. [Table/Fig 3]

Z-N stain	A R +Ve	A R - Ve	Total
Z-N +Ve	01	00	01
Z-N -Ve	19	05	24
Total	20	05	25

[Table/Fig 3]: Comparison of ZN and Auramine Rhodamine stains in diagnosis of tuberculosis

Though ZN stain and Auramine Rhodamine was negative in 46 cases, due to the presence of caseous necrosis, raised ESR and other relevant clinical findings, they were classified as tuberculous lesions.

In the present study, all the 30 cases of leprosy were stained with the Fite Faraco staining.

Seventeen cases (56.66%) were positive for the Fite faraco stain and 13 cases (43.33%) were negative.

All the 20 (100%) cases of rhinoscleroma showed histiocytic granulomas, with the predominance of histiocytes. Epithelioid cells and giant cells were absent in all the cases.

The Rhinoscleroma cases were confirmed by their typical histology.

In two cases, actinomycotic colonies were seen on H and E staining and these were confirmed by Grams staining.

Ten cases of fungal lesions were identified either by histology or with the help of special stains like GMS, with a majority being maduramycosis.

The interesting cases were P.Boydii and histoplasmosis involving the skin and subcutaneous tissues. One case of cerebral aspergilliosis infection was also seen. [Table/Fig 4]

Thirteen cases of foreign body granulomas were encountered, where the aetiology was identified. The most common among them were xanthomas with 6 (46.15%) cases, followed by bile induced granulomas of the gall bladder in 2 (15.38%) and epidermal cysts with granulomas in 2 (15.38%). In all these cases, foreign bodies could be identified in the giant cells. [Table/Fig 4]

Sl. No	Diagnosis	No. of cases	Percentage
1	Maduramycosis	03	30
2	P.boydii	02	20
3	Rhinosporidiosis	02	20
4	Histoplasmosis	01	10
5	Aspergilliosis	01	10
6	Mucormycosis	01	10

Total		10	100
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[Table/Fig 4]: Distribution of fungal granulomas

One (7.69%) case each of seminoma with granuloma, squamous cell carcinoma with granuloma and benign cystic teratoma of the ovary with granuloma were seen.

Eleven cases of granulomas of unknown aetiology were noted. Granulomatous mastitis was seen in a majority of the cases [with 5(45.45%) cases], followed by granuloma annulare in 2(18.18%). One case (9.09%) each of sarcoidosis, granulomatous orchitis, granuloma in the pleura and in the mastoid antrum were also noted. The aetiology was not identified in these cases, even on special staining like ZN and GMS. So, they were just grouped as granulomatous lesions.

Different morphological patterns of the granulomatous lesions were seen. The commonest was the tuberculoid type with 98(57.65) cases, followed by histiocytic granulomas in 29(17.06%), foreign body granulomas in 17(10.00%), ill defined granulomas in 13(7.65%), mixed inflammatory granulomas in 11(6.47%) and necrobiotic granulomas in 2(1.18%)

DISCUSSION

Out of the 170 cases,

- a) Special staining was not done in 33 cases
 1. 20 cases were Rhinoscleromas
 2. 11 cases were Foreign body granulomas
 - 06 were Xanthomas -
 - 02 were Bile induced -
 - 03 were Keratin induced-
 3. 2 cases were Granuloma annulare -
- b) Special stains were done in 137 cases

In the present study, the ZN stain was positive in 19 (22.62%) out of 84 cases, whereas it was positive in 91(71%) out of 128 cases in the study of Krishnaswamy H et al [6]. [Table/Fig 5]

ZN stain	Krishnaswamy H et al	Present study
ZN +ve	91(71.09%)	19(22.62%)
ZN-ve	37(28.90%)	65(77.38%)
Total	128	84

[Table/Fig 5]: Showing the comparison of results of ZN stain in Tuberculosis in the present with other study

Twenty five cases were randomly selected for the Auramine Rhodamine staining. Out of the 25 cases, the ZN stain was positive in 1(4%) case and it was negative in the rest of the 24 cases. The Auramine Rhodamine staining was positive in 20(80%) cases, whereas in Krishna Swamy H et al's study, out of 128 cases, the ZN stain was positive in 91 (71.1%) cases and the Auramine Rhodamine stain was positive in 102(79.69%) cases [6]. [Table/Fig 6]

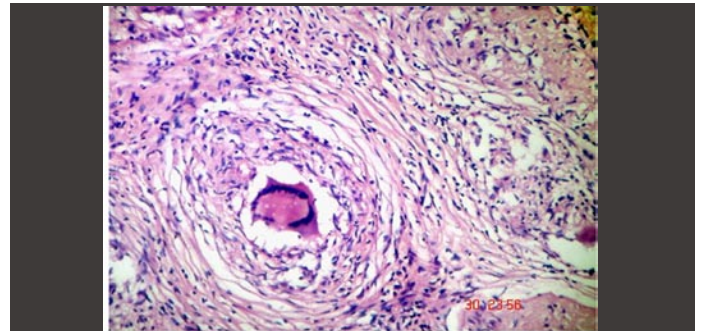
ZN stain	Krishnaswamy H et al			Present study		
	AR+ve	AR-ve	Total	AR+ve	AR-ve	Total
ZN +ve	84	07	91(71.1%)	01	00	01(4%)
ZN -ve	18	19	37(28.91%)	19	05	24(96%)
Total	102	26	128	20	5	25
%	79.69	20.31		80	20	

[Table/Fig 6]: Showing the comparison of Auramine Rhodamine and ZN stain in tissue sections in the present study with other study

Out of the 30 cases of leprosy, the Fite Faraco stain was positive in 17(56.66%) cases and negative in 13(43.33%) cases, whereas in the study which was done by Nayak SV et al, it was positive in

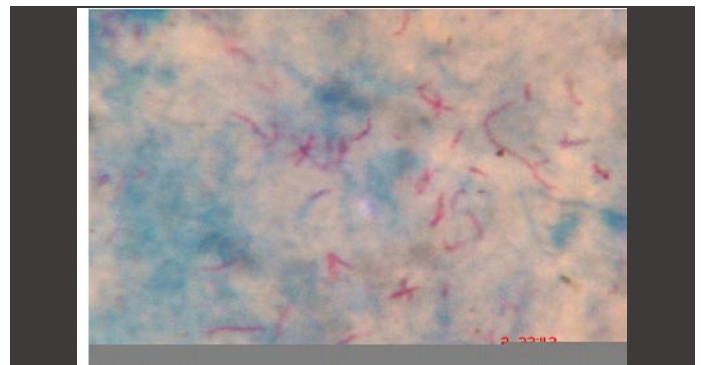
25(44.64%) cases and negative in 31(55.35%) cases [7].

There was a predominance of histiocytes and plasma cells in a majority of the rhinoscleroma cases. This was similar to the observation which was made by Meyer PR et al in their study on 9 rhinoscleroma cases [8]. [Table/Fig 7]



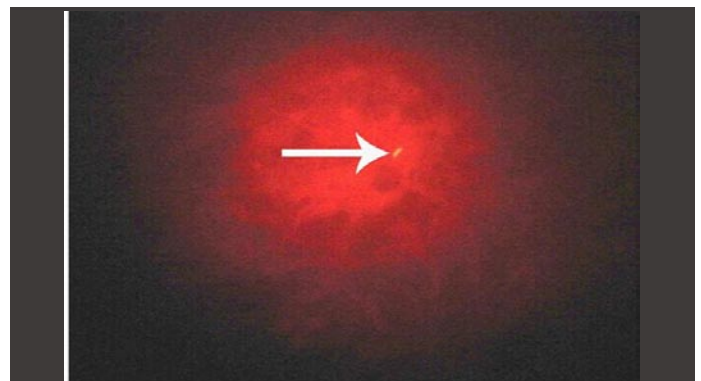
[Table/Fig 7]: Epithelioid cell granuloma with Langhan's giant cell in tuberclosis of lymphnode (H&E,x100)

[Table/Fig 8]



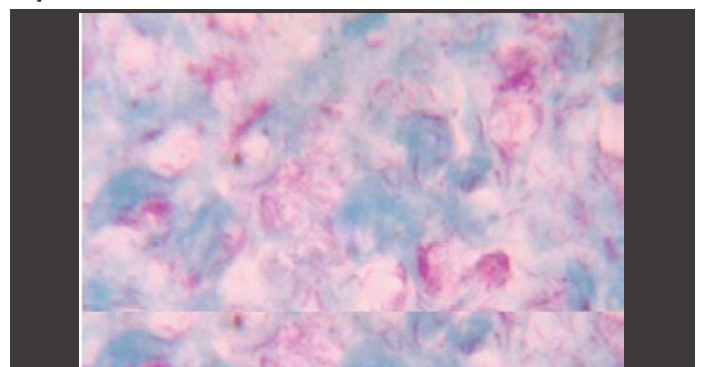
[Table/Fig 8]: Acid fast bacilli in caseating tuberclosis of limph node (ZN, x 1000)

[Table/Fig 9]



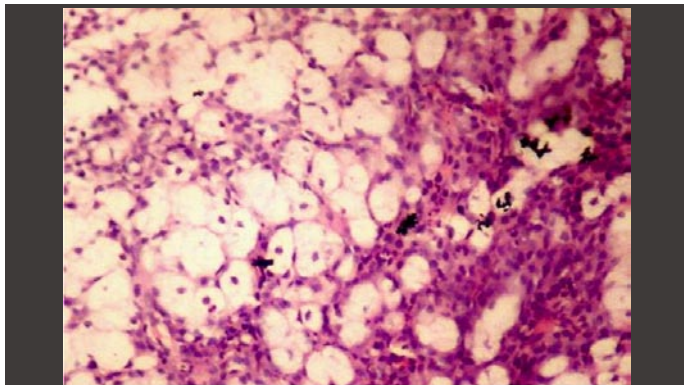
[Table/Fig 9]: Flourescent bacilli in tuberculosis of limphnode (AR, x 1000)

Fibrosis was seen in 5 (25%) cases and Russel bodies in 2(10%), which were comparable to Meyer PR et al's study showing fibrosis in 2(22.22%) cases and Russel bodies in 2 (22.22%) [8]. [Table/Fig 10]



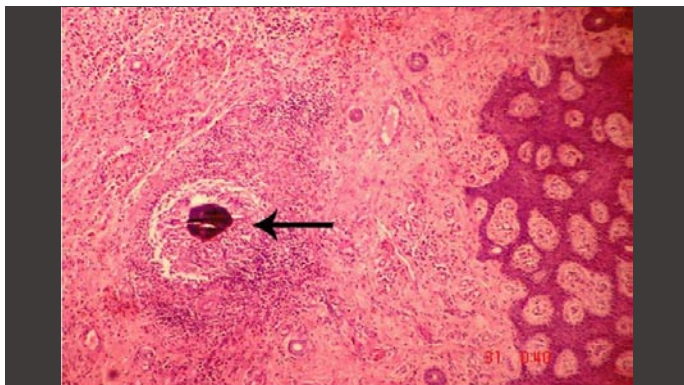
[Table/Fig 10]: Acid fast bacilli in lepomatous leprosy (FF, x 1000)

[Table/Fig 11]



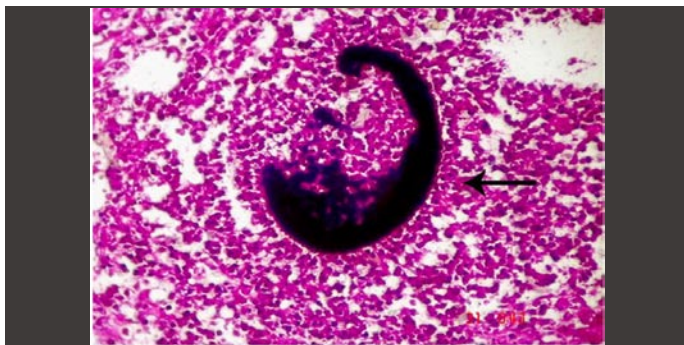
[Table/Fig 11]: Histiocytic granulomas in rhinoscleroma (H&E, x 40)

[Table/Fig 12]



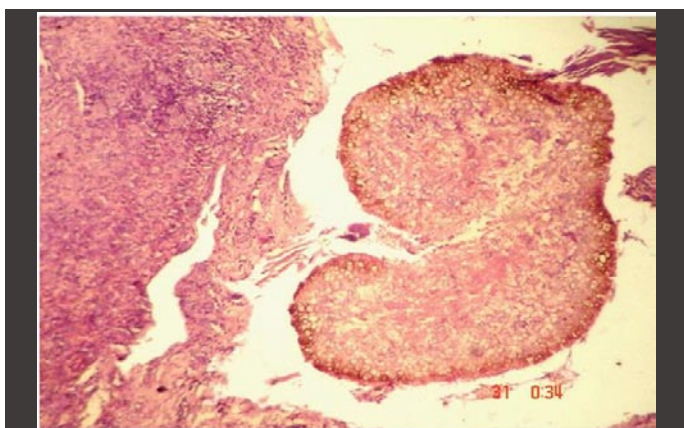
[Table/Fig 12]: Mixed Inflammatory granuloma with actinomycotic colonies (H&E, x40)

In a case of Actinomycosis, the histopathology revealed skin and subcutaneous tissues with a number of suppurative granulomas having central actinomycotic colonies, which was comparable to that seen in Mirza M et al's study [9]. [Table/Fig 13]



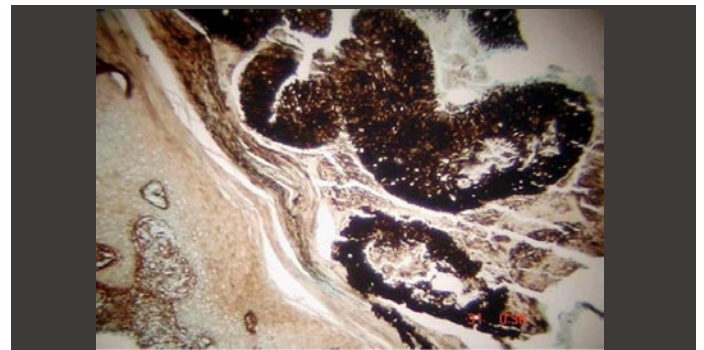
[Table/Fig 13]: Gram positive actinomycotic colonies (Gram Stain, x100)

[Table/Fig 14]



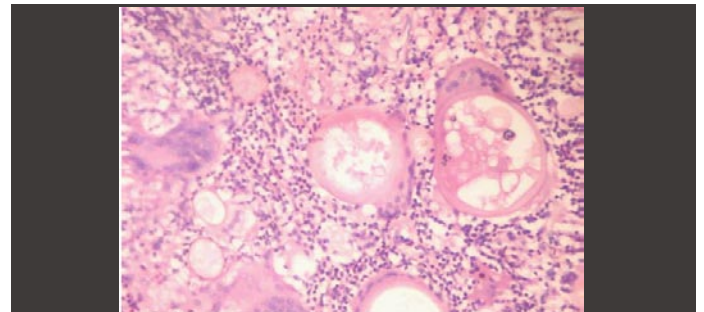
[Table/Fig 14]: Oval brownish granule in maduramycosis (H&E, x40)

[Table/Fig 15]



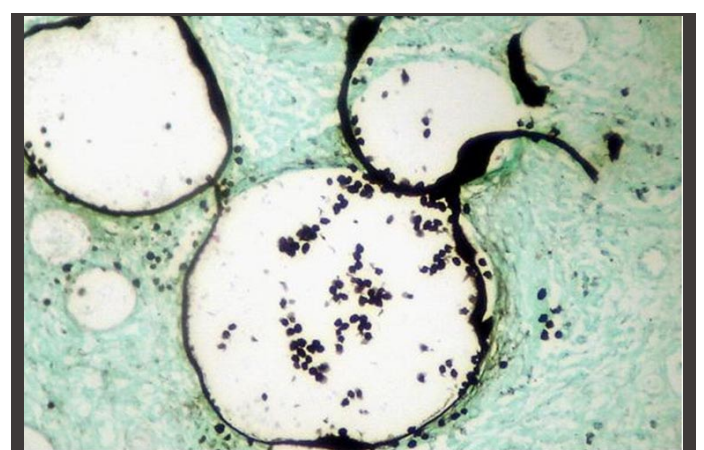
[Table/Fig 15]: Granules stained intense black in maduramycosis (GMS, x 40)

[Table/Fig 16]



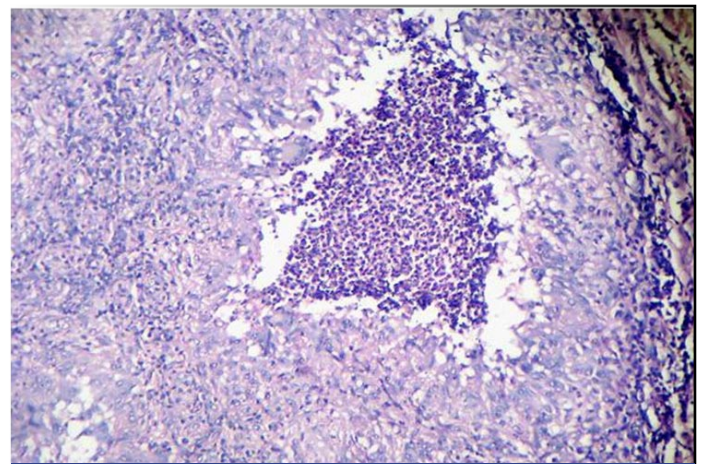
[Table/Fig 16]: Mixed inflammatory granuloma with FB giant cells in rhinosporidiosis (H&E, x40)

[Table/Fig 17]



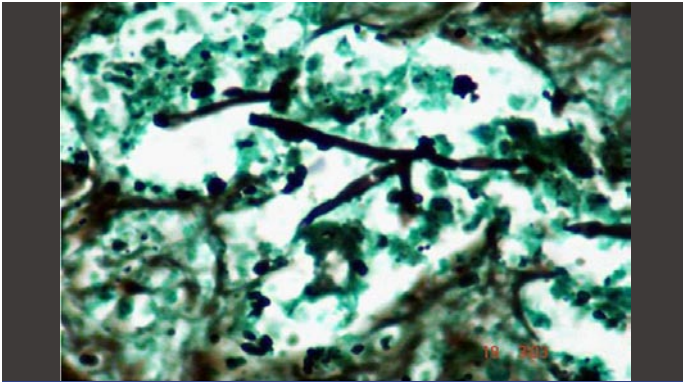
[Table/Fig 17]: Rhinosporidium seebri with its wall and spores (GMS, x100)

[Table/Fig 18]



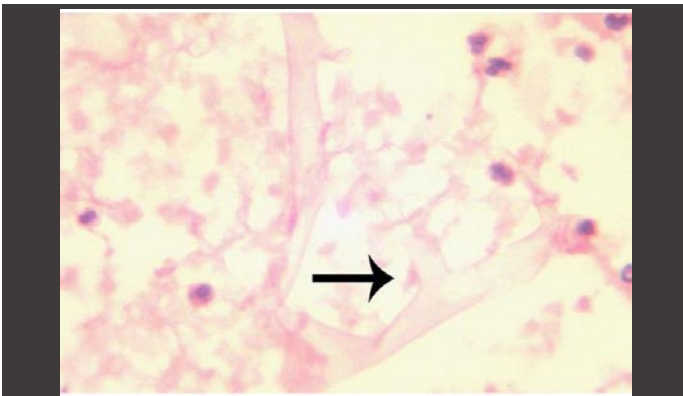
[Table/Fig 18]: Mixed inflammatory granuloma in P.boydi (H&E, x100)

[Table/Fig 19]



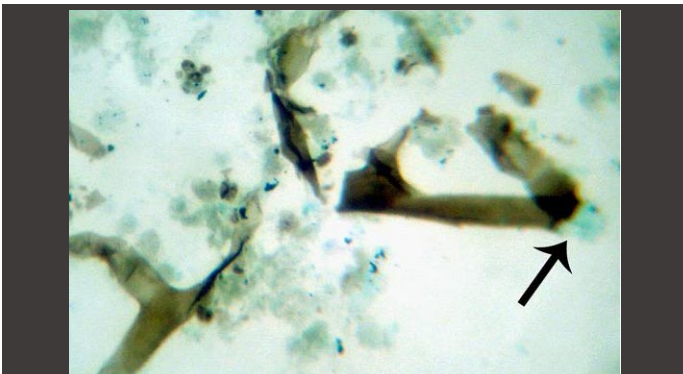
[Table/Fig 19]: P. boydii showing obtuse angle branching and beaded hyphae

The commonest fungal lesion which was observed in our study was maduramycosis, which constituted 3(30%) cases, followed by rhinosporidiosis in 2(20%) cases, whereas in the study done by Chavan SS et al, there was a predominance of rhinosporidiosis [34(68%)] cases, followed by maduramycosis [8(16%)] cases [10]. [Table/Fig 20]



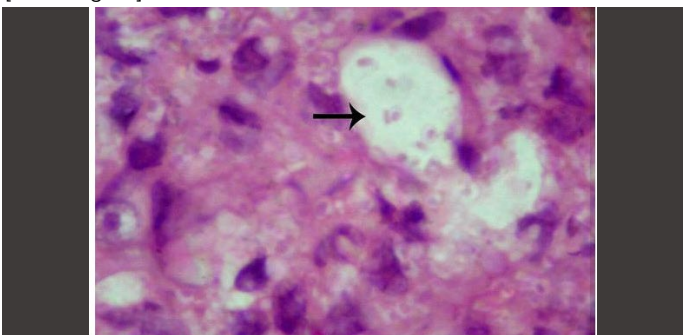
[Table/Fig 20]: Mucormycosis with broad hyphae and right angle branching (Mucorram osissimus) (H&E, x 400)

[Table/Fig 21]



[Table/Fig 21]: Folded and twisted hyphae in mucormycosis (GMS, x 400)

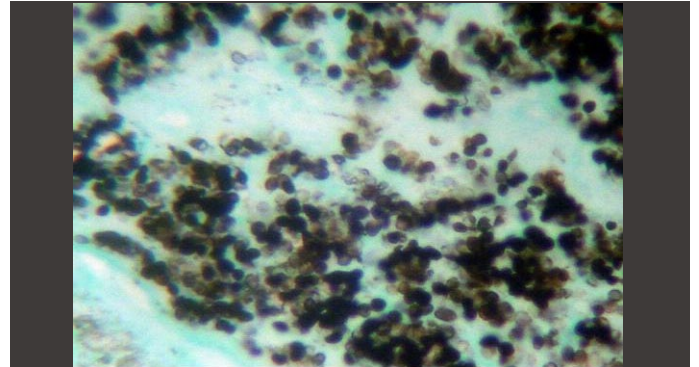
[Table/Fig 22]



[Table/Fig 22]: Histocyte packed with Histoplasma capsulati (H&E, x 400)

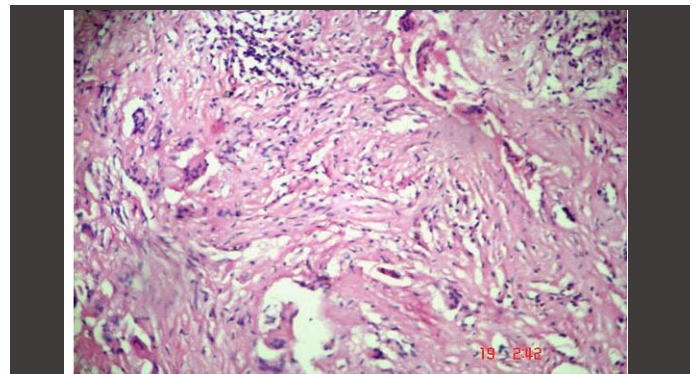
Two (20%) cases of P.boydii and one (10%) case each of histoplasmosis and mucormycosis were seen. However; granulomas caused by these three fungi were not reported in the study which was done by Chavan SS et al [10].

In our study, 5 cases of granulomas were seen in the breast, where the aetiology was not identified. Out of these, 3 were epithelioid cell granulomas and 2 were foreign body granulomas. In a study by Fletcher A et al, 7 cases of granulomas were seen and all were epithelioid granulomas [11]. [Table/Fig 23]



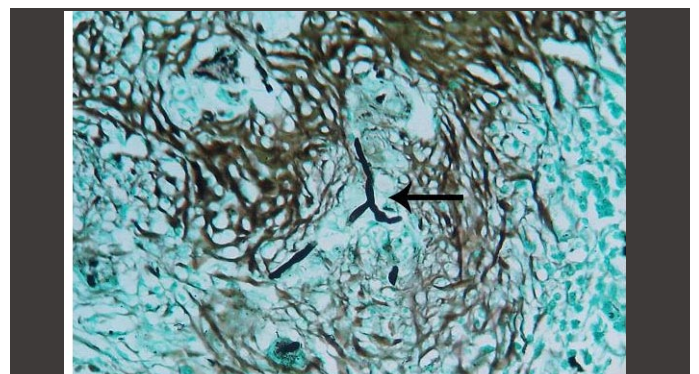
[Table/Fig 23]: Small round yeast like cells in Histoplasmosis (GMS, x400)

[Table/Fig 24]



[Table/Fig 24]: Mixed inflammatory granuloma with marked sclerosis in aspergillosis (H&E, x100)

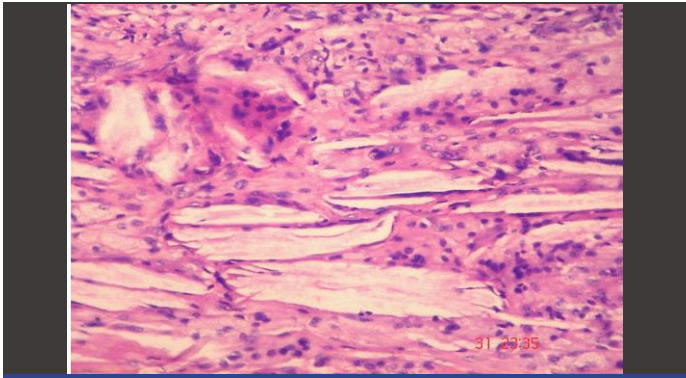
[Table/Fig 25]



[Table/Fig 25]: Showing acute angle branching in aspergillosis (GMS, x100)

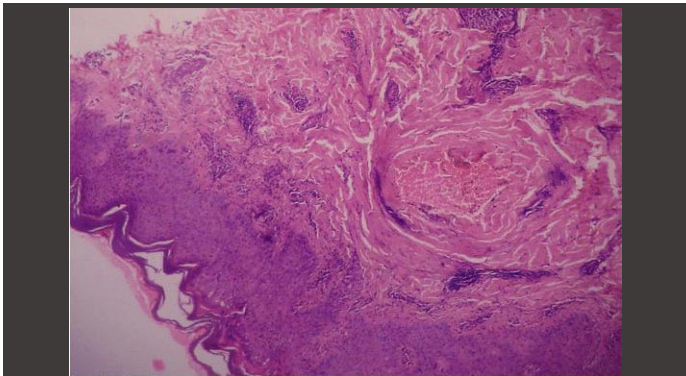
Two cases of granuloma annulare were identified. Both cases revealed several small granulomatous lesions in the upper and mid dermis which were composed of small foci of necrobiotic collagen, surrounded by histiocytes in a palisading arrangement and intermingled with the lymphoid cells.

All these findings were similar to the observation which was made by Khatri ML et al in his case study on generalized granuloma annulare [12]. [Table/Fig 26]



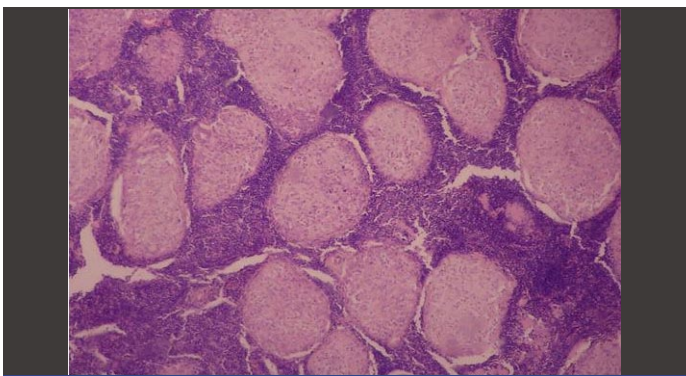
[Table/Fig 26]: Cholesterol clefts and touton giant cell in tuberculous xanthoma (H&E, x 100)

[Table/Fig 27]



[Table/Fig 27]: Necrobiotic granulomas in granuloma annulare (H&E, x 40)

[Table/Fig 28]



[Table/Fig 28]: Uniform, non-caseating, back to back granulomas in sarcoidosis (H&E, x 40)

In the present study, one case of sarcoidosis of the lymph node was identified. It showed dense, discrete, small, uniform, noncaseating, back to back granulomas. No giant cells or necrosis was seen. The ZN stain for AFB was negative. The diagnosis was made by a typical histopathological examination. However, serum calcium levels

and the angiotensin converting enzyme were not contributory. Subsequently, a careful search for other causes of granuloma by using clinical data and laboratory tests showed no positive results. All these above findings were comparable to the results of Manonukul J et al's study [13].

CONCLUSION

Our study shows that granulomatous lesions are more common in males. Tuberculosis is one of the most commonest cause of granulomas.

Granulomatous lesions accounted for 2.1% of the non-neoplastic biopsies which were received in our department. Out of the 170 cases, a definite aetiological diagnosis could be made only in 159 cases. Even after relevant special stains, the aetiological diagnosis could be confirmed in only 93.52% cases.

Cooperation between the clinician and the pathologist is more important in the field of dermatology than in any other field, if the patient is to derive the greatest benefit from the biopsy. This percentage can be further consolidated, if culturing, serological investigations and PCR are done.

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