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Original Article

# A HISTOPATHOLOGICAL STUDY OF GRANULOMATOUS INFLAMMATION

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Abstract :
Granulomatous inflammation is a distinctive pattern of chronic inflammation that is encountered in a limited number of infectious and non-infectious conditions. Recognisation of granulomatous pattern and finding the etiology in a biopsy specimen is very important for specific treatment and outcome of the disease. We aimed at finding the etiology of all granulomatous lesions on tissue biopsy sent for histopathogical examination. A study was done at K S Hegde Medical Academy of Nitte University, Mangalore from January 2009 to December 2010. All the cases which were diagnosed as granulomas on Hematoxylin and Eosin stained sections were selected. Special stains like Ziehl-Neelsen stain, Gomori's Methenamine silver, PAS, Fite Faraco were done whenever required. We encountered 275 granulomatous lesions in our study. The most common sites were skin and subcutaneous tissue, lymph nodes, bones and joints. The commonest cause of granulomas was tuberculosis, followed by leprosy, foreign body granulomas, fungal infections, rhinoscleroma, parasites, tumor granulomas and actinomycosis. The morphological features and special staining helped us to find the specific etiology of granulomas in 253 cases whereas it could not be determined in 22 cases even after special stains. Thus we conclude that histopathological examination of granulomatous lesions helped us to find the exact etiology of granulomas in 92 % of cases. The correlation of histopathology with polymerase chain reaction (PCR) serological tests and culture correlation would have helped to find the specific etiology in the remainder of cases.

Keywords : Granuloma, histopathology, special stains, etiology.

## Introduction :

Granulomatous inflammation is a distinctive pattern of chronic inflammation that is encountered in a limited number of infectious and non-infectious conditions. Granuloma is a focus of chronic inflammation consisting of microscopic aggregation of macrophages that are transformed into epithelial like cells, surrounded by a collar of lymphocytes and occasionally plasma cells. [1] The granulomatous inflammatory response is a manifestation of many infective, toxic, allergic, autoimmune, neoplasm and conditions of unknown aetiology. A knowledge of the basic pathophysiology of this distinctive tissue reaction is therefore of fundamental importance in the understanding many disease processes. [2] 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 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, IFγ, TNF and lymphotoxin for the transformation of the macrophages into epithelioid cells and giant cells, which are the components of granulomas. [2] Etiological 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] Classification based on the morphologic criteria: 1. Epithelioid 2. Histiocytic 3.Foreign body 4. Necrobiotic / Palisading 5. Mixed inflammatory. [4] Recognisation of the granulomatous pattern in a biopsy specimen is important because of the limited number of possible conditions that cause it and the significance of the

diagnosis associated with it. 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. [5] Good clinical history, a close histological examination and a clinicopathological correlation is 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] The morphologic pattern in the various granulomatous diseases may be sufficiently different to allow reasonable accurate diagnosis by the pathologist. Hence the present study was undertaken to find the frequency and etiology of granulomatous lesions and to compare with other studies.

## Materials and Methods :

A study was done from January 2009 to December 2010 in the Department of Pathology, K S Hegde Medical Academy Mangalore. The biopsy samples were received from various departments of the K S Hegde Charitable hospital and Dental college hospital. A histopathological study of 275 granulomatous lesions was done. The cases diagnosed as granulomas from all the sites, on haematoxylin and eosin stained sections were selected. Special stains like ZN, GMS, PAS, were used whenever required. The relevant clinical details and laboratory investigations were collected from the hospital case sheets.

## Results :

The ages of the patients ranged from 1 to 87 yrs, with a mean age 33.26 +/-12.64 years. Majority of patients were seen in the age group of 21-30 years in 65 (23.64%) cases. Males were affected predominantly in 144 (52.36%) cases and females in 131 (47.64%) cases. [Table 1] Male to female ratio was 1.09:1. Majority of the granulomas were seen in skin and subcutaneous tissues with 68 (24.72%) cases, followed by the involvement of lymph node in 59 (21.46%), bones and joints in 50 (18.18%), respiratory system in 26 (9.46%), gastrointestinal tract in 22 (8%), breast in 16 (5.8%), female genitourinary system in 07 (2.54%), male genitourinary system in 10 (3.62%), gall bladder in 5 (1.8%), thyroid in 4 (1.46%), brain in 2 (0.72%), alveolus in 2 (0.72%), floor of mouth in 1 (0.36%), buccal mucosa in 1 (0.36%), gingival in 1(0.36%) and tongue in 1(0.36%). [Table 2] The most common cause of granulomas was tuberculosis seen in 130 (47.26%) cases, followed by leprosy in 35 (12.72%) cases, foreign body granulomas in 23 (8.36%), rhinoscleroma in 14 (5.1%), fungal infections in 24 (8.72%), actinomycosis in 4 (1.44%), parasitic infestation in 4 (1.44%), rheumatoid arthritis in 3 (1.1%), tumor associated granulomas in 16 (5.82%) cases, granulomas with unknown etiology in 22 (8%). [Table-3] Out of 130 cases of tuberculosis, lymph nodes were involved in 53 (40.76%), followed by bones and synovial tissue in 35 (26.92%), cutaneous 4 (3.08%), intestinal 14 (10.76%), larynx in 11 (8.46%), sinus tract in 4 (3.08%), fallopian tube in 2 (1.54%), bladder in 3 (2.30%) and kidney, breast, lung, epididymis in 1 (0.76%) case each. Males were affected predominantly in 76 cases and females in 54 cases. ZN stain demonstrated tubercle bacilli in 27 (20.74%) out of 130 cases. In 35 cases of leprosy, tuberculoid leprosy was seen in 22 (62.86%), followed by lepromatous leprosy in 7 (20%), borderline tuberculoid in 5 (14.28%) and indeterminate leprosy in 1 (2.86%). Foreign body granulomas were encountered in 23 cases where the etiologic agent was identified. The most common among them were epidermal cyst with keratin and cholesterol granulomas in 13 (56.52%) cases, followed by xanthomas in 4 (17.4%) cases, sinus tract in 2(8.7%), bile induced granulomas of the gall bladder in 2(8.7%) and gouty arthritis in 2 (8.7%). The fungal infections were identified on histopathology sections with H&E, GMS and PAS in 24 cases. Aspergillus was seen in 6 (25%), followed by Rhinosporidiosis in 4 (16.6%), Chromoblastomycosis in 3 ( 12.5%), Pseudolleshcheria boydii in 2 (8.33%), subcutaneous

entomphothormycosis in 1 (4.16%), phaeohyphomycosis in 2 (8.33%), mucormycosis in 2 (8.33%), cryptococci in 2 (8.33%), Madura mycosis in 1 (4.16%) and Candida in 1 (4.16%). Parasitic infestation was seen in 4 (1.44%) cases of subcutaneous Dirofilariasis. In 4 (1.44%) cases of actinomycosis, the colonies were surrounded by suppurative granulomas on H and E staining and confirmed by Grams staining. Histiocytic granuloma was seen in all the 14 (5.1%) cases of rhinoscleroma with absence of epithelioid and giant cells. Granulomatous response to tumors was seen in 16 cases. The tumors were squamous cell carcinoma in 3 (18.75%), infiltrating ductal carcinoma breast in 3 (18.75%), papillary carcinoma thyroid in 3 (18.75%) , dysgerminoma in 2 (12.5%) , Hodgkin's lymphoma in 2 (12.5%), seminoma in 1 (6.25%), ameloblastoma in 1 (6.25%) and benign cystic teratoma in 1 (6.25%). In 22 (8%) cases, granulomas were of unknown etiology. They were Granulomatous mastitis in 12 (54.54%), granulomatous orchitis in 2 (9.09%), cat scratch disease in 2 (9.09%), vasculitis in 2 (9.09%), granulomatous osteomyelitis in 2 (9.09%), granulomatous hepatitis in 1(4.54%) and granuloma annulare in 1(4.54%). The commonest morphological pattern of the granulomatous inflammation was of epitheloid type in 165 (60%) cases, followed by foreign body in 35 (12.72%), ill defined in 29 (10.54%), histiocytic in 23 (8.36%), mixed inflammatory in 19 (6.9%) and necrobiotic granulomas in 4 (1.45%) cases.

Discussion :

Granulomatous inflammation is a distinctive pattern of chronic inflammation that is encountered in a limited number of infectious and non-infectious conditions. In our study, granulomatous lesions were seen predominantly in the 3rd decade and in males. The commonest site was skin and subcutaneous tissues followed by lymph nodes, bones and joints, respiratory system, gastrointestinal tract, breast female reproductive system, urinary tract, male reproductive system, gall bladder and brain. In our study most common cause of granuloma was tuberculosis followed by leprosy, foreign body granulomas, rhinoscleroma, fungal infections, actinomycosis, parasitic infestation, tumor associated granulomas, and granulomas of unknown etiology. In our study, ZN stain demonstrated tubercle bacilli in 27 (20.74%) out of 130 cases, whereas it was 91(71%), out of 84 cases in a study by Krishnaswamy H et al [6]. In our study on 35 cases of leprosy, Fite Faraco stain demonstrated lepra bacilli in only 9 (25.72%) cases and was negative in 26 (74.28%) cases, possibly due to increase in number of tuberculoid leprosy cases where it is paucibacillary type, in contrast to study by Nayak SV et al, [7] on 56 cases of leprosy, Fite Faraco stain demonstrated lepra bacilli in 25 (44.64%) cases and was negative in 31(55.36%) cases. All the cases of Actinomycosis showed suppurative granulomas having central actinomycotic colonies, which was comparable to the findings reported in a study by Mirza M et al [7]. The commonest fungal infection was aspergillosis followed by rhinosporidiosis and chromoblastomycosis. In few cases fungus was demonstrated in H&E stained sections. But majority of cases needed special stains, GMS and PAS to identify the fungus. In 14 cases of rhinoscleroma, histiocytes and plasma cells were seen predominantly similar to the observation made by Meyer PR et al [9] in their study on 9 rhinoscleroma cases. Fibrosis was seen in 7 (50 %) cases and Russel bodies in 6 (40%), which was higher than seen in a study by Meyer PR et al [9] study showing fibrosis in 2 (22.22%) cases and Russel bodies in 2 (22.22%) cases. Parasitic granulomas were due to subcutaneous Dirofilariasis seen in four cases. Dirofilaria show smooth thick multilayered cuticle. The outer most layers had prominent wavy longitudinal ridges, each separated from the next by a distance greater than the width of the ridge and also transverse striations. [10] The muscle layer below the cuticle was well developed and the body cavity showed uteri and intestinal tube. The surrounding soft tissue showed infiltration by eosinophils, lymphocytes, and occasional foreign body granulomas. In 26 cases, granulomas were of unknown etiology. They were granulomatous mastitis in 11, granulomatous orchitis, cat scratch disease, vasculitis, granulomatous osteomyelitis in 2 cases each, and granulomatous hepatitis, granuloma annulare was seen in one case each. In 11 cases of granulomatous mastitis 8 cases showed epitheloid granulomas and 3 cases showed foreign body granulomas.

In a report by Fletcher et al, [11] all the seven cases of granulomatous mastitis showed epitheloid granulomas. The etiology could not be determined in all these cases even on special staining like ZN, PAS and GMS. So they were reported as granulomatous inflammatory lesions. In our study, Squamous cell carcinoma of skin, infiltrating ductal carcinoma breast, papillary carcinoma thyroid, dysgerminoma, Hodgkin's lymphoma and seminoma, ameloblastoma and benign cystic teratoma were the 16 tumors which showed granulomatous inflammation. Neoplasms are known to be associated with a granulomatous response in the parenchyma mainly seen in Hodgkin's disease, on-Hodgkin T cell lymphomas, seminoma of the testis, renal cell carcinoma, nasopharyngeal carcinoma and ovarian dysgerminoma. The presence of granulomas in tumour parenchyma has largely been attributed to the cytokine milieu of either the main tumour or the other cells composing the tumour background. [12] In other cases the granulomatous inflammation may be found in the lymph nodes draining the primary tumour either with or without metastatic cancer. It has been labelled as sarcoid reaction or sarcoid like lymphadenopathy. It has been observed in many malignancies arising from breast, stomach, colon and larynx can be associated with a granulomatous response in the draining lymph nodes and should be included in the list of differential diagnosis of causes of granulomatous inflammation. Whenever a granuloma is seen in the draining lymph node in a known case of cancer it should be carefully scrutinized for the presence of malignant cells. [12] In our cases granulomatous response was seen at the periphery of the tumor area and also in the draining metastatic lymph nodes.

Conclusion :

We conclude that granulomatous inflammation is more common in males with tuberculosis being most common cause of granulomas. Granulomatous inflammation accounted for 2.1% of the non-neoplastic biopsies which were received in our department. Out of the 275 cases, a definite aetiological diagnosis of granulomas was made in 253 cases (92%).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 microbial culture, serological investigations and PCR are done.

Table 1) Showing age and sex distribution of cases

| Age group | 1-10 | | 11-20 | | 21-30 | | 31-40 | | 41-50 | | 51-60 | | 61-70 | | 71-80 | | 81-90 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M F | 4 | 7 | 13 | 18 | 30 | 35 | 23 | 27 | 26 | 15 | 33 | 18 | 14 | 10 | 00 | 01 | 01 | 00 |
| Total (275) | 11(4%) | | 31(11.4%) | | 65(23.60%) | | 50(18.16% ) | | 41(14.9% ) | | 51(18.52%) | | 24(8.70%) | | 01(0.36%) | | 01(0.36%) | |

Table 2) Showing the sites of granuloma

| Sites of granuloma | No of cases (%) |
|---|---|
| Skin &Subcutaneous | 68 (24.72%) |
| Lymph nodes | 59 (21.46%) |
| Bones & Joints | 50 (18.18%) |
| Intestine | 22 (8.00%) |
| Breast | 16 (5.82%) |
| Respiratory system | 26 (9.46%) |
| Female genitourinary | 07 (2.55%) |
| Gall bladder | 05 (1.83%) |
| Thyroid | 04 (1.46%) |
| Male genitourinary | 10 (3.64%) |
| Brain | 02 (0.72%) |
| Alveolus | 02 (0.72%) |
| Floor of mouth | 01 (0.36%) |
| Gingiva | 01 (0.36%) |
| Buccal mucosa | 01 (0.36%) |
| Tongue | 01 (0.36%) |
| Total | 275 (100%) |

Table 3) Showing causes of granuloma

| No. | Cause of granuloma | No of cases (%) 275 (100%) |
|---|---|---|
| 1) | Tuberculosis | 130 (47.26%) |
| 2) | Leprosy | 35 (12.72 %) |
| 3) | Foreign body | 23 (8.36%) |
| 4) | Tumors | 16 (5.83%) |
| 5) | Fungal | 24 (8.73%) |
| 6) | Rhinoscleroma | 14 (5.10%) |
| 7) | Actinomycosis | 04 (1.45%) |
| 8) | Parasites | 04 (1.45%) |
| 9) | Rheumatoid arthritis | 03 (1.10%) |
| 10) | Unknown etiology | 22 (8.00%) |

Table 4) Showing comparison of ZN staining to demonstrate AFB in various studies

| ZN stain | Krishnaswamy H et al | Present study |
|---|---|---|
| ZN positive | 91 (71.09%) | 27 (20.74%) |
| ZN negative | 37 (28.91%) | 103 (79.26%) |
| Total | 128 | 130 |

Table 5) Showing comparison of Fite Faraco staining to demonstrate lepra bacilli in various studies

| Fite Faraco stain | Nayak SV et al | Present study |
|---|---|---|
| Positive | 25 (44.64%) | 09 (25.74%) |
| Negative | 31 (55.36%) | 26 (74.28%) |
| Total | 56 | 35 |

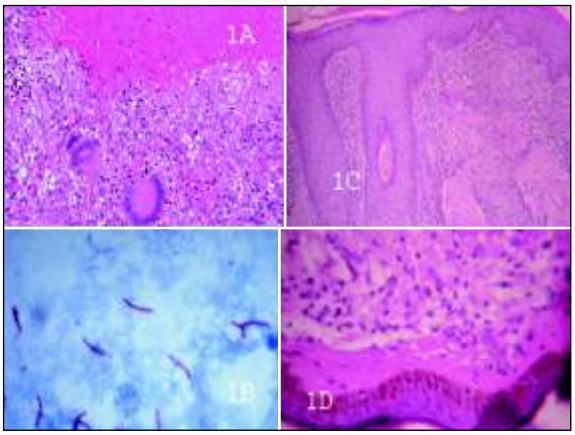


Figure 1 - A) Microscopy showing epitheloid granuloma, Langhan's giant cell & caseous necrosis seen in tuberculosis. (H&E X400). B) Presence of pink coloured tubercle bacilli. (ZN X400). C) Section from skin showing granuloma with giant cell at the epidermo-dermal junction seen in tuberculoid leprosy. (H&E X400). D) Section from skin showing subepidermal clear zone and aggregation of foamy macrophages seen in lepromatous leprosy. (H&E X400)

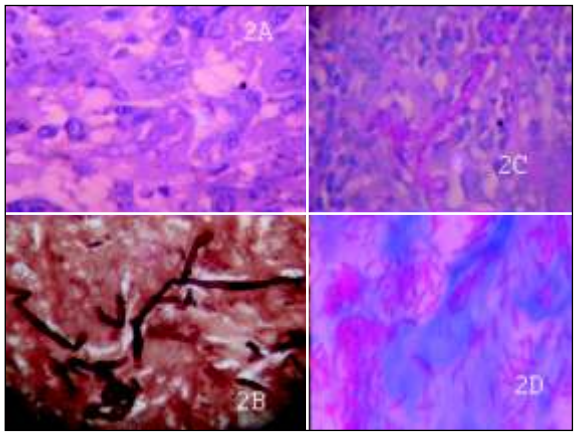


Figure 2 –A) Microscopy showing regular septate fungus with granuloma. (H&E X400). B) Presence of PAS positive regular septate branching fungus with granuloma. (PAS X400). C) Presence of irregular septate branching fungus. (GMS X400). D) Presence of pink coloured bacilli in bundles seen in lepromatous leprosy. (FF X400)

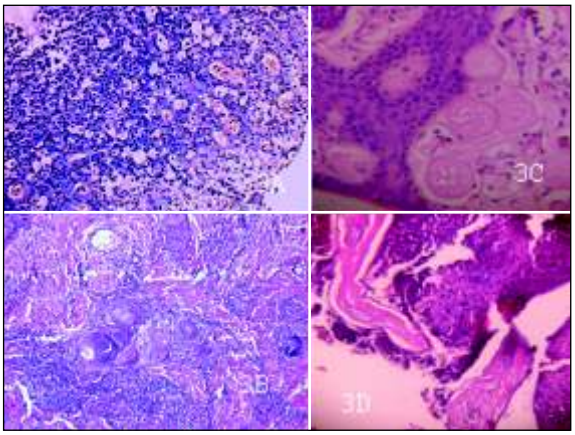


Figure 3 – A) Microscopy showing aggregation of foamy macrophages, lymphocytes and plasma cells seen in rhinoscleroma. (H&E X400). B) Showing foreign body surrounded by granulomas, giant cells and lymphocytes. (H&E X400). C) Showing stratified squamous epithelium and mature sporangium with spores seen in rhinosporidiosis. (H&E X400). D) Showing Dirofilaria parasite surrounded by granulomas, giant cells and lymphocytes. (H&E X400)

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