Available online at www.iponlinejournal.com

Journal homepage: www.innovativepublication.com/journal/ijpo

Original Research Article

Pattern Analysis of granulomatous inflammatory lesions

Chandrashekhar T. N[1], Sateesh Chavan S[2*]

[1]Associate Professor, Dept. of Pathology, [1]Shivamogga Institute of Medical Sciences, Shivamogga, Karnataka, [2]Karnataka Institute of Medical Sciences, Hubballi, Karnataka, India

Article Info

Received: 15th November, 2018

Accepted: 1st February, 2019

Published Online: 9th August, 2019

Keywords: Patterns, Granulomatous, Inflammation, Epithelioid, Tuberculosis, Histiocytic.

Abstract

Aim: To study patterns of granulomatous inflammatory lesions and role of various diagnostic modalities in their diagnosis.

Introduction: Granulomatous inflammatory lesions encompass a wide spectrum of common and rare etiologies with diagnostic and management dilemma. Pattern analysis of granulamatous response aid in understanding their pathogenesis, immune responses to infection, regulation of such responses, and their effects on tissues. Newer diagnostic modalities like immunohistochemistry, special stains, polarizing microscopy, serum assays and ELISA aid in establishing the final diagnosis.

Materials and Methods: Histopathological study of 300 granulamatous lesions was done. Special stains like ZN, GMS, PAS, FiteFaraco, Auramine Rhodamine stain and polarizing microscopy, tools of molecular biology like IHC, PCR, and ELISA were used wherever required to establish the etiology.

Observations: Mean age at presentation was 31.85±14.64 years (range: 2-68yrs) with male preponderance (M: F=1.47:1). Majority of patients were in the 3rd decade between 21-30years (108 cases, 36%). Tuberculosis was the most common cause of granulomatous inflammation followed by tumors, fungal lesions, leprosy, inert substances, parasitic and least was granulomas with unknown etiology.

Conclusion: Present study is an attempt to describe the histopathology of various granulomatous inflammatory lesions and their accurate etiological diagnosis. Molecular diagnostic methodology is essential for accurate etiological diagnosis and understanding the pathogenesis of granulomatous inflammation.

Introduction

Granulomatous inflammatory lesions encompass a wide spectrum of common and rare etiologies leading to diagnostic and management dilemma. Common causes include infectious (such as *M.tuberculosis, fungal*), non infectious (foreign *body, drugs*), autoimmune (*Sarcoidosis, Rheumatoid arthritis*), neoplastic (Hodgkin's *disease*) and inherited disease (Chronic *granulomatous disease*). Histopathologically granulomatous inflammatory lesions presents with distinct patterns which include 'foreign body granulomas' (such as Talc, suture material), 'necrotizing granulomas' (such as *M.tuberculosis, Coccidioidomycosis, Histoplasmosis*), 'non necrotizing granulomas' (*M.tuberculosis, Candida Albicans, Cytomegalovirus, Sarcoidosis, Tuberculoid leprosy*), 'suppurative granulomas' (such as seen in *Actinomycosis, Phaeohyphomycosis, Sporotrichosis*) and 'Histiocytic collections' (such as due to *Tropheryma Whipplei, lepromatous leprosy, H.Capsulatum, Langerhan's cell histiocytosis, Mycosis fungoides*). Granulamatous inflammatory response offers a model which is uniquely suited for the study of many facets of pathogenesis, including immune responses to infection, the regulation of such responses, and their effects on host tissues. Good clinical history, close histological examination and clinico-pathological correlation along with tools of molecular biology are essential in making a final diagnosis.

In this study we have employed newer diagnostic modalities like immunohistochemistry, special stains, polarizing microscopy, serum assays and ELISA wherever needed to establish the final diagnosis, thus contributing to the better understanding of the granulomatous response and histopathological features in relation to etiology.[1-6]

Materials and Methods

All biopsies and surgical specimens which showed granulomas in heamatoxylin & eosin stained paraffin sections were included in the study and were evaluated histologically to know the possible etiology of granuloma. Granulomas are categorized into five types.

1. 'Epithelioid' type- well formed epithelioid cell aggregates with lymphoplasmacytic and fibroblastic cuffing, with or without necrosis
2. 'Histiocytic'- loose collections of 'histiocytes', but no epithelioid cells, variable number of lymphocytes.
3. 'Mixed inflammatory'- also called 'suppurative' or 'neutrophil' rich granuloma; collection of histiocytes or epithelioid cells, rich neutrophilic infiltration, variable lymphocytes and plasma cells.

*Corresponding Author: Sateesh Chavan S, Associate Professor, Dept. of Pathology, Karnataka Institute of Medical Sciences, Hubballi, Karnataka, India
Email: sat_sc@yahoo.com
http://doi.org/10.18231/j.ijpo.2019.069

4. 'Lymphocytic'- epithelioid cells/ or histiocytes aggregation, rich infiltration with lymphocytes and plasma cells.
5. 'Necrobiotic'- histiocytic/or epithelioid cells surrounding central zone of connective tissue destruction/ or deposition of degenerated proteinaceous material (fibrinoid necrosis), variable neutrophils and lymphocytes.

Wherever necessary special techniques including special stains such as GMS (Gomori's methenamine silver), PAS (Periodic acid Schiff), ZN stain (Ziehl neelsen), FF (Fite farracco), Mucicarmine, Auramine-Rhodamine stain, immune florescence with polorising microscopy were used to establish the diagnosis. In some cases molecular biology tools like immunohistochemistry. Nucleic acid amplification testing (NAT) and enzyme lnked immunosorbent assay (ELISA) were utilized to establish the diagnosis. Relavent clinical details were obtained from medical records and correlated with histopathology.

Observations

Three hundred cases of granulamatous lesions were diagnosed. Mean age at presentation was 31.85±14.64 years (range: 2-68yrs) with male preponderance (M: F=1.47:1). Majority of patients were in the 3rd decade between 21-30years (36%, 108 cases) followed by 5th decade (20%, 60cases), 4th decade (16%, 48cases), 6th decade (10.33%, 31cases), 2nd decade (8%, 24cases), first decade (5.66%, 17 cases) with only few cases beyond 7th decade (4%, 12cases)(Table 1).

Tuberculosis was the most common cause of granulomatous inflammation (52%, 156 cases) followed by tumors (13.33%, 40 cases), fungal lesions (12.66%, 38 cases), leprosy (7.66%, 23 cases), inert substances (6%, 18 cases), parasitic (2%, 6 cases) and least was granulomas with unknown etiology (6.3%, 19 cases)(Table 2).

Lymph nodes were the most common site of granulamatous lesion (40%, 120 cases), followed by skin and subcutaneous location (21.3%, 64 cases), respiratory system (9.6%, 29 cases), bones (6.3%, 19cases), male reproductive system (5.6%, 17 cases), central nervous system (3.3%,10 cases), liver (2.6%, 8 cases), breast (3%, 9 cases), female reproductive system (2%, 6 cases), intestine & urinary tract (1.3%, 4 cases each), vessel (0.6%, 2 cases), omentum (1%, 3 cases), peripheral nerve & abdominal wall (0.6%, 2 cases each),and least was parotid (0.3%, 1 case) (Table 3).

Histologically epithelioid cell granulomas were the most common type of granulomas (63.66%, 191 cases) cases followed by histiocytic granulomas (18.66%, 56 cases), lymphocytic granulomas (8.66%, 26 cases), mixed inflammatory granulomas (6.33%, 19 cases) and least was necrobiotic granulomas (2.66%, 8 cases)(Table 2).

Majority of granulomas (86.6%, 260 cases) showed the presence of giant cells. Langhan's type giant cells were the most common giant cells (58.84%, 153 cases) followed by both Langhan's and foreign body type (30.38%, 79 cases), foreign body (28.84%, 75 cases), tumour giant cell (10.38%, 27 cases) and touton type of giant cells (1.92%, 5 cases).

Majority of granulomas showed necrosis (62.66%, 188 cases). Caseous necrosis was the most common type of necrosis (60.1%, 113 cases) followed by coagulative necrosis (35.63%, 67 cases) and least was fibrinoid necrosis (4.26%, 8 cases).

Granulomas showed other accompanying inflammatory infiltrates like neutrophils, plasma cells, eosinophils (Table 3).

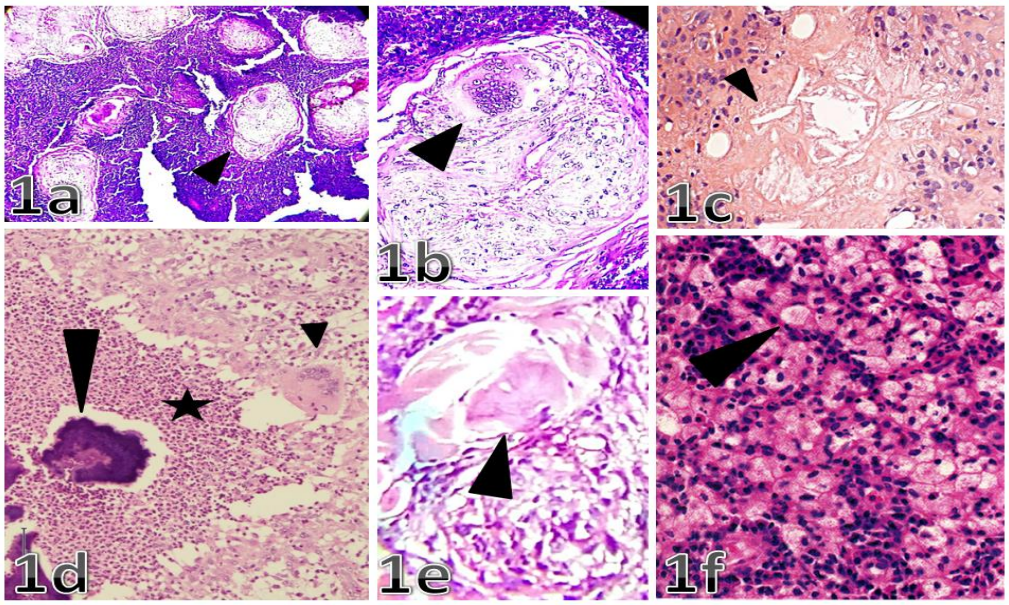

Fig. 1: Showing patterns of granulomatous inflammation. 1a-"*epithelioid cell granulomas*" (black arrow head) with multinucleated giant cell [black arrow head] [inset:1b][x40, H & E]. 1c- cholesterol crystals [black arrow head] an example of '*foreign body granuloma'*[x40, H & E]. 1d- '*mixed inflammatory granuloma'* showing 'microabscesses'(asterix) with *actinomycotic colonies*(large black arrow head) and a multinucleated giant cell (small black arrow head) [x40, H & E]. 1e- 'collagen destruction'(black arrow head) surrounded by histiocytic collections (*granuloma annulare*) an example of ' *necrobiotic granuloma'*[x40, H & E]. 1f- diffuse foamy histiocytic infiltration in '*histioid leprosy'*(black arrow head) an example of ' *histiocytic granulomas'*[x40, H & E].

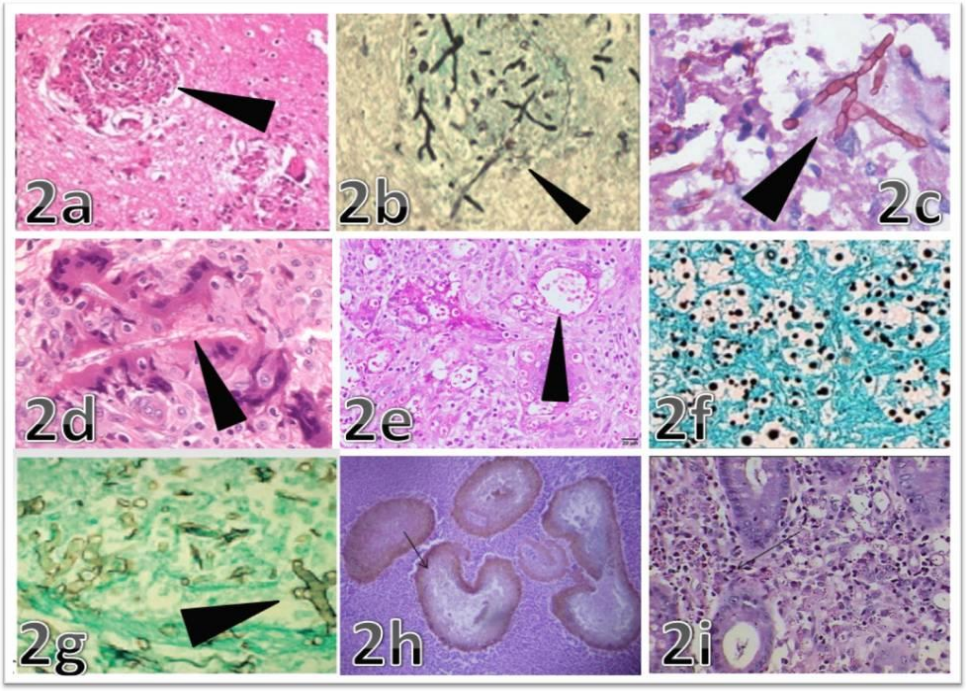

Fig. 2: Showing '*angiocentric granuloma'* with *aspergillus hyphae* invading the vessel wall [black arrow head][x10, H& E] (a), which are GMS positive showing narrow septate acute angled branching hyphae [black arrow head][x40 , GMS](b), PAS positive aspergillus hyphae[black arrow head] [x40. PAS] (c), Aspergillus hyphae within multinucleated giant cell [black arrow head][x 40, H& E](d), *cryptococcal colonies* [black arrow head][x40, H&E](e) which are GMS positive[black arrow head][x 40,GMS](f), *mucormycosis* with broad irregular obtuse angled branching hyphae[black arrow head][x 40, GMS](g), eumycetoma with 'pale grains' of *Pseuadallescheria Boydii* colonies within microabscesses[black arrow][x 10,H&E](h) and gastric biopsy showing numerous GMS positive small yeasts of ' *histoplasmosis*' within histiocytes in mucosa[black arrow][x 40, GMS-H&E](i).

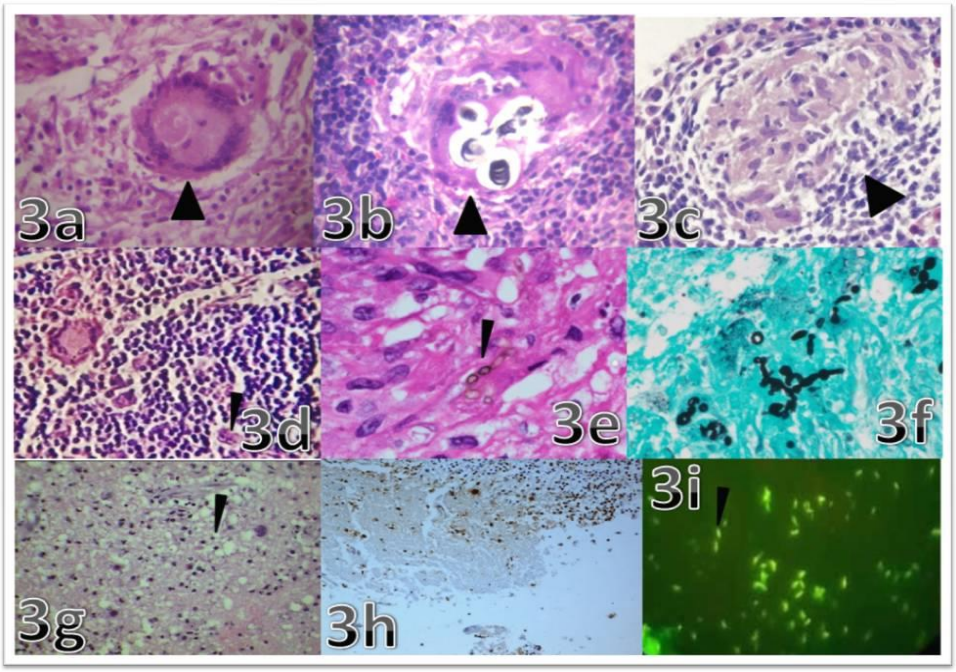

Fig. 3: Showing '*asteroid body'* within a multinucleated giant cell in non caseating granuloma of *sarcoidosis*[black arrow head][x40, H&E](a), cross section of '*filarial worm'* in multinucleated giant cell [black arrow head][x40, H&E](b), *Hodgkin's disease* with epithelioid cell granuloma with rich lymphocytic collection an example of *'lymphocytic & histiocytic granuloma'*[black arrow head][x40, H&E](c),'Reed Sternberg cell'[black arrow head]and multinucleated giant cell [x40,H&E](d), *chromoblastomycosis* with brown colored spores and some hyphal forms within multinucleated giant cell [black arrow head][x40, H&E](e) and are GMS positive[x40, GMS](f), section of brain tissue with extra- and intracellular '*tachyzoites'* of toxoplasmosis[x40,H&E](g) and are positive on immunohistochemistry[x40, immunohistochemistry](h) and flouroscent microscopy with *Auramine & Rhodamine* staining technique showing flourecsing M.tuberculosis bacilli [x40, Auramine & Rhodamine with fluorescent microscopy](i).

Discussion

Granulomatous inflammation occurs when (1) The provocative agents /organisms are partially digestible or completely non degradable (2) there is high local concentration of provocative agents /organisms (3) highly replicating organism within phagolysosome. In these circumstances neutrophils (first line of defence) non activated macrophages(or monocytes) and secreted cytokines/ chemokines (which influence tissue damage) are insufficient to completely digest or eradicate the offending agent. In such situations macrophages gets activated with augmented enzymatic capabilities and perhaps gets converted to epithelioid cells.[1,6,7] Histopathologically, granulomas are spectrum of discrete or confluent lesions, range from just loose collections of histiocytes with other inflammatory cells (the purpose of which is mainly healing in chronic inflammation with resultant fibrosis) to well formed granulomas with distinct collection of epithelioid cells, peripheral cuff of lymphoplasmacytes with or without necrosis, and with presence or absence of other features are distinctive and soft pointers towards etiology. Well formed granulomas can be non immune (foreign body granuloma) or immune granuloma with or without necrosis.[6] Over the past decade, use of the tools of molecular biology has led to major advances in our understanding of granulomatous disorders. Granulomatous inflammation is initiated and maintained by sensitized CD4 T cells that exhibit a T helper type 1 (TH-1) pattern of cytokine production. In infectious diseases, granulomas form a focus that isolates the pathogen and promotes the development of protective immunity by allowing cross-talk between T lymphocytes and macrophages.[7] A knowledge of the basic pathophysiology of this distinctive tissue reaction is therefore of fundamental importance in the understanding of many disease processes. However, the challenge remains for the clinician and scientist to understand the complex genetic, microbial, immunologic, and environmental factors that are responsible for the varied histological presentation, clinical manifestations and disparate outcomes of these disorders.

Patterns of Granulomas

'Epithelioid' pattern was the most common pattern observed in all granulomatous lesions. '*Histiocytic'*pattern were commonly found in fungal infectins, lepromatous leprosy and granuloma due to inert substances. '*Lymphocyte*' rich pattern were commonly seen in granulomatous reactions to tumours. The least common pattern was '*necrobiotic*' type observed in all cases of granuloma annulare, some cases of tuberculosis and fungal infections (Table 2).

Table 1: Demographic features of various granulamatous lesions

Age group	Male	Female	Etiology of granulomatous inflammation	Total
Birth-10yrs	10	7	T=12,Tu=2,Fu=2, P=1	17(5.66%)
11-20 yrs	16	8	T=16,Tu=3,Fu=1, Le=2, I=1, P=1,	24(8%)
21-30 yrs	64	44	T=63,Tu=3,Fu=20, Le=9, I=9, P=3, U=1	108(36%)
31-40 yrs	30	18	T=30,Tu=2,Fu=6, Le=4, I=4, P=1, U=1	48(16%)
41-50 yrs	37	23	T=22,Tu=10,Fu=7, Le=5, I=3, U=13	60(20%)
51-60 yrs	15	16	T=8,Tu=15,Fu=2, Le=3, I=1, U=2	31(10.33%)
>60yrs	7	5	T=5,Tu=5, U=2	12(4%)
Total	179(59.6%)	121(40.3%)	T=156,Tu=40,Fu=38, Le=23, I=18, P=6, U=19	300(100%)

T= Tuberculosis, Tu= Tumor, Fu= Fungal, Le=Leprosy, I= Inert substances, P=Parasitic, U=unknown

Table 2: Patterns of granulomatous inflammatory lesions and their etiology

	Etiology , No. of cases and percentage							
Pattern of granulomas	**Tuberculosis (156 cases)**	**Tumor (40 cases)**	**Fungal infections (38 cases)**	**Leprosy (23 cases)**	**Inert substances (18cases)**	**Parasite (6 cases)**	**Unknown etiology (19 cases)**	**Total**
'Epithelioid granuloma'	117 (75%)	18 (45%)	20(52.6%)	15(65.2%)	12(66.66%)	3(50%)	6(31.57%)	191 cases (63.66%)
'Histiocytic;	20 (12.8%)	4(10%)	13(34.2%)	7(30.43%)	6(33.33%)	1(16.66%)	5(26.31%)	56 cases (18.66%)
Mixed inflammatory ('neutrophilic')	8 (5.13%)	4(10%)	2(5.26%)	1(4.35%)	-	1(16.66%)	3(15.8%)	19 cases (6.33%)
'Lymphocytic'	6 (3.8%)	14(35%)	2(5.26%)	-	-	1(16.66%)	3(15.78%)	26 cases (8.66%)
'Necrobiotic'	5 (3.2%)	-	1(2.63%)	-	-	-	2 (10.52%)	8 cases (2.66%)
Total	**156 cases (52%)**	**40 cases (13.33%)**	**38 cases (12.66%)**	**23 cases (7.66%)**	**18 cases (6%)**	**6 cases (2%)**	**19 cases (6.33%)**	**300 cases (100%)**

Table 3: Histopathological features of various granulomatous lesions

Etiology (Number, %)	Site	Type of granulomas	Giant cells	Necrosis	Other accompanying cells	Other feature
Tuberculosis (156, 52%)	Ln-107, Rs-12, Bo-9, Mgs-9, CNS-5, S-4, In-3, Fgs-3, Ut-2, Li-1, Pa-1.	Ep-117, Hi-20, Mi-8, Ly -6, Ne-5	Lh-117 Fb-23	Cs-109 Cg-17	N-18 PL-2 Eo-4	Fs-35, A-4 AFB-45.
Tumor (40, 13.33%)	S-11, Rs-12, Ln=5, Mgs-4, Li-3, Fgs-3, Ut-2.	Ep-18, Ly-14, Hi-4, Mi-4	Tu-27, Fb-13	Cg-17	PL-17 , N-13, Eo-3	Fs-8
Fungal & fungal like lesions (38, 12.66%) [As -16 ,Mu-10, Hp-2, Cr-2, Chr-2, Ac-1, Ma-2, Rh-2, Pb-1]	S-13, Bo-10,Ln-5, CNS-5, Li-2, Rs-3.	Ep-20 ,Hi-13, Ly -2, Mi-2, Ne-1	Lh-17, Fb-12	Cg-22, Fn-7	PL-17, N-13, Eo-3	Fs-21, PAS-26,GMS-28,MC-2, A-10
Leprosy (23, 7.66%)	S-19, Ln-2, Nr-2.	Ep-15, Hi-7, Mi-1	Lh-12, Fb-7	Cs-4	N-8	FF-12, Fs-6
Inert substances (18, 6%) [Ke-5,Su-5,Ch-5, Li-2, Bi-1]	S-9, Om-3, Br-3, Ab-2, Li-1.	Ep-12,Hi-6	Fb-11, Tt-5	Cg-6	N-7, PL-7, Eo-3,	F-5
Parasitic (6, 2%) [Fi-3,Ro-2 To-1]	S-2, In-1, Li-1, Ln-1, CNS-1	Ep-3,Hi-1,Ly-1,Mi-1	Lh- 2, Fb-4	Cg-5	E0-6, PL-4, N-2,	Fs-1
Unknown etiology (19, 6.3%) GM-6, Sa-2, GAn-5, GO-4, GA-2	Br-6, V-2, Mgs-4, Rs-2, S-5.	Ep-6, Hi-5, Ly-3, Mi-3, Ne-2	Lh-5, Fb-5.	Fn-1	N-2, PL-2,Eo-1,	Fs-2
Total (300,100%)	Ln-120, Rs-29, Bo-19, Mgs-17, CNS-10, S-64, In-4, Fgs-6,Ut-4, Li-8,Pa-1, N-2, Br-9, Om-3, Ab-2, V-2	Ep-191,Hi-56,Ly-26,Mi-19, Ne-8	Lh-153, Tt-5, Fb-75, Tr-27,	Cs-113, Cg-67, Fn-8	N-58, PL-49, Eo-20	Fs-73, A-14, AFB-45, PAS-26, GMS-28, MC-2, FF-12.

Ac- Actinimycosis, As –Aspergilosis, AFB- acid fast bacilli, A- Angio-destruction, Bi-Bile, Bo- bone and soft tissue, Br-breast, Cg-coagulative, Ch-Cholesterol, Chr- chromomycosis & chromoblastomycosis, CNS- central nervous system, Cr-Cryptoccoci, Cs-caseous, Ep- Epithelioid, Eo-eosinophil, Fb- foreign body, FF- Fite Faroco, Fgs- female genital system, Fi-Filaria, Fn-fibrinoid, Fs-Fibrosis, GM-granulomatous mastitis, GO-Granulamatousorchitits, GAn-granuloma annularae, GA-Giant cell arteritis, GMS-Gomori'smethenamine silver, Hi-Histiocytic, Hp- Histoplasmosis, In- Intestine, Ke-Keratin, Lp-Lipid, Ly-lymphocytic, Lh-Langhan's, Li- liver, , Ln- lymphnode, Mu-Mucormycosis, Ma-Maduramucosis, Mi-Mixed inflammatory, MC- mucicarmine, Mgs- male genital system, N-Neutrophil, Ne- Necrobiotic, Nr- nerve, Om-omentum, Pa-parotid, Pb-P. Boydi, PL- plasma cell, PAS-Periodic acid Schiff, Rh- Rhinosporidiosis, Rs- respiratory system, Ro-round warm, S- skin and subcutaneous tissue, Sa-Sarcoidosis, Su-Suture material, Tt- touton, Tu- tumour, Ut- urinary tract.

Tubercuosis

Tuberculosis is one of the important cause of granuloma in clinical practice. Various studies observed tubercular granuloma as the most common granulomatous inflammatory lesion. In the present study over half of all granulomatous lesions were of tuberculous etiology (52%, 156 cases). Although the initial site of tuberculous infection is lung, various studies including the present study found lymph node as the most common site of tubercular granulamatous lesion.[3,8] Histologically various types of granulomas can be seen in tuberculosis most common being caseating epitheliod cell granulomas and other less common types are 'non caseating granulomas' rich in macrophages, 'neutrophilic 'granulomas, 'lymphocyte rich granulomas' and 'necrobiotic' and completely' fibrotic' granulomas [9](Table 2&3). Though granuloma is the hallmark of tuberculosis and expected to control the spread and limit the bacillary proliferation, it also provides an advantage for some bacilli a chance to survive amidst the granuloma for many years (dormant bacilli) and it could be the source of reactivation of infection(latent infection).[9]

Many macrophages of granulomas are uninfected and typically sorrounds the infected ones and their main role is to limit the infection. The infected macrophages are activated by both Th1 cells (CAMs, 'classically activated macrophages') and Th2 cells (AAMs;' alternatively activated macrophages). Th1induced CAMs are endowed with high microbicidal property owing to IFNγ driven increased iNOS (inducible nitric oxide synthase), whereas Th2 induced AAMs counteract iNOS owing to release of anti-inflammatory cytokines (IL-10 & TGF β) and arginase. Hence AAMs could actually facilitate survival of bacilli and are the source of 'dormant' bacilli.[9] As a result of TLR-2 signaling or Th1 signaling or both, activated macrophages fuse to form multinucleated giant cells, loose their phagocytic property but retain capacity to present antigens. In this way bacilli might escape phagocytosis by inducing fusion process.[9]

Caseous necrosis is characteristic of tuberculosis is actually coagulative type to begin with owing to hypoxia due to reduced vascularisation. The cheesy appearance is due to high lipid content (mycolic acid of the wall of bacilli) and the fact that presence of high lipid metabolism in necrotic centre points a possible role of foamy macrophages.[10,11] Apoptosis of infected cells but not the necrosis, kills the bacilli and antigen presentation to T cells is facilitated. Necrosis of infected cells (in susceptible individuals) releases bacilli, stimulates inflammation and promote tissue damage.[12,13]

Tubercular granulomas typically show lympho plasmacytic cuff rich in CD4+ T cells[14] and other less common features include infiltrates like neutrophils and eosinophils (though their significance is not clear), fibrosis and angiodestruction[15]and fibrosis as a part of tissue destruction and healing process in progressive cases.

Tumor

Tumors are known to be associated with granulomas either seen within the stroma of tumors or in the draining lymph nodes or other lymphoid organs spleen and liver. In the present study most common site of distribution of tumors associated with granulomas was respiratory system and least was urinary tract (Table 3). Majority of these were primary squamous cell carcinoma (SCC), metastatic SCC and adenocarcinoma to lymphnodes, primary Hodgkins disease of lymphnode, hepatocellular carcinomas, seminomas and ovarian dysgerminomas. Granulomatous response in tumor is a T cell immunologic response to tumor products. Although granulomatous inflammation has no prognostic implications, in some cases of SCC of lung it has influenced partial regression of tumor and those sites of regression histologically showed tumor debri with only few tumor cells surrounded by abundant granulomatous response.[16] Some tumors such as seminomas, Hodgkin's disease do show granulomatous response in draining lymphnodes or lymphoid organs such as liver and spleen without metastatic deposits and the response is related to soluble tumor products reaching those sites.[17] Problem arises when draining lymphnode shows caseating granulomas with AFB positivity as well as tumor deposits. Tumor progression is known to be associated with immunosupression and this could be the reason for reactivation of latent tuberculosis.[17]

Leprosy

Leprosy accounted for 7.66% (23 cases) of all granulomatous lesions commonly affecting skin and subcutaneous tissue (82.6%, 19 cases) followed by lymph node and nerve (8.6%, 2 cases each). Histologically epithelioid granulomas were the most common granuloma (65.2%, 15 cases) and are all seen in tuberculoid leprosy cases, followed by histiocytic granulomas (30.4%, 7 cases all are lepromatous leprosy) and least was mixed inflammatory granulomas (4.3%, a case of reactional leprosy)(Table 2&3). Definitive diagnosis of early lesions of leprosy is difficult. Two ends of spectrum, lepromatous and tuberculoid leprosy are easy to recognize histologically with large number of macrophages in the dermis with parasitized acid fast bacilli in the former and aggregates of epithelioid cells, multinucleate giant cells and lymphocyte with scanty or no bacilli in the latter. Epithelioid cell formation is inhibited if phagocytosed material cannot either be digested or extruded. Thus the lepra cells in lepromatous leprosy which are stuffed with lepra bacilli form diffuse sheets of macrophages. Likewise foam cells found in xanthoma do not become epithelioid cells. Conversely, in tuberculoid leprosy where immunity is high and bacilli are scanty, the reaction is epithelioid granulomas.[18]

Fungal & Fungal Like Lesions

Fungi is an important cause of granulomatous inflammation accounting to 12.66% (38 cases) of all granulomatous lesions and commonly affects skin and subcutaneous tissue. Histologically fungal elements (budding yeasts/ hyphae/ spores) were seen in the centre of granulomas or inside the giant cells. In the present study epithelioid granulomas were the most common type of granulomas (52.6%, 20 cases) followed by histiocytic granulomas (34.2%, 13 cases)(Table 2&3).

Inert Substances

In the present study granulomas due to inert substances (such as keratin, suture material, etc) accounted for 6% (18 cases) of all granulomatous lesions and commonly affected skin and subcutaneous tissue (50%, 9 cases)[table 2&3]. Histologically epithelioid granulomas were common type (66.6%, 12 cases) and rest were histiocytic granulomas (33.3%, 6 cases). Most common substances which evoked granuloma formation were keratin, suture material and cholesterol and less common substances are lipid and bile (Table 2&3).

Parasites

In the present study Parasitic granulomas accounted for 2% (6 cases) of all granulomatous lesions, majority of them were seen in skin and subcutaneous tissue (50%, 3 cases)(Table 2&3). Parasites commonly elicit epitheliod cell granulomas with foreign body type giant cells and necrosis, if seen are commonly coagulative type. Other frequent accompanying features are fibrosis and infiltration by eosinophils. In the present study commonly identified parasites were filarial warm, round worm and least was Schistosomiasis (Table 3).

Unknown Etiology

In a good number of cases of granulomatous inflammation it is very difficult to identify the etiologic agent. In the present study about 6.3% (19 cases) of granulamatous lesions the etiology was unknown. Most common site was breast followed by skin and subcutaneous location (Table 2&3). Morphologically in most of these cases granulomas are of both epithelioid and histiocytic type. In the present study histopathological diagnosis offered were granulomatous mastitis, granuloma annulare, granulomatous orchitis, Giant cell arteritis and sarcoidosis (Table 3).

Diagnosis of Granulomatous Lesions

In H&E stained sections the etiologic agents such as foreign bodies (suture material, keratin etc) or tumor deposits can be easily identified, while organisms such as fungal hyphae/ spores/ yeasts, tubercular organisms require careful examination, often supported by special stains (such as GMS, PAS, Mucicarmine, ZN stain, Fite Farracco stain, etc), serology (IgA and IgG levels), enzyme assays (such as adenosine de aminase levels), immunoflourescent and immunohistochemical techniques. Advantage of H&E stained sections is that distinctive morphology of fungi and associated tissue reactions can be recognized. Fungal hyphae can be seen freely or inside the giant cells. Notable exception to this general rule is *Histoplasma*, which is virtually impossible to detect *within necrotizing granulomas* on H&E-stained sections.[19] Tissue reactions may give clue to type of organisms but often are nonspecific. The presence of eosinophils gives clue about parasites,' neutrophil rich' granulomas gives clue about fungus such as *Blastomyces*, while the association of eosinophils with granulomas often indicates the presence of *Coccidioides*. "Infarct like" necrosis may be seen in granulomas caused by *Histoplasma* or *M tuberculosis*, while a bubbly appearance of the cytoplasm of histiocytes and multinucleated giant cells is a clue to the presence of *Cryptococcus*.

Tissue is an important material for *diagnosis of tuberculosis* and when required diagnosis is supported by

demonstration of acid fast bacilli by *Ziehl Neelsen stain*, IgA estimation by *enzyme linked immunosorbent assay* and culture methods. A presumptive diagnosis of tuberculosis can be made if a necrotizing, confluent epithelioid cell granuloma is demonstrated. Caseating granulomas are characteristically but not exclusively found in tuberculosis. Non caseating granulomas are also observed in other conditions such as sarcoidosis. In such situations although valuable other diagnostic methods such as culture and ZN staining are not always be useful. *ZN staining* though relatively simple AFB is not always demonstrable, even if AFB is positive it cannot identify the species of bacillus. Present study observed AFB positivity in only 28.8% (45 cases). In the present study the diagnosis of tuberculosis was supported by *Serum IgA and IgM levels* in two cases (with strong clinical suspicion presented with cervical lymphadenopathy, chronic fever and chronic cough but sputum negative for AFB) and both of them showed significantly elevated levels. Lymph node aspirations were cellular with lymphoid cells and collections of epithelioid cells but necrosis was absent. Estimation of serum IgA and IgM levels will only give supportive evidence in strong suspicion but are not specific. *Nucleic acid amplification testing (NAT)* was done in one case and was positive. *Culture* though 'gold standard 'fails to detect dead bacilli. Less frequently used methods include fluorescence with *Auramine - Rhodomine staining, immunohistochemistry and Nucleic acid amplification* testing for rapid diagnosis. Immunohistochemistry lacks the simplicity and is more expensive.[20] *Nucleic acid amplification testing* is expensive, has low sensitivity and cannot distinguish dead bacilli from viable.[21] *Mycobacteria Growth Indicator Tube* (MGIT) system using liquid media yields high sensitivity and rapidity. Even with progress in molecular biology and other ancillary techniques, smear microcopy and culture still remains 'gold standard'.

Though tedious, modified FiteFaraco stain is valuable special stain in the *diagnosis of leprosy* in tissue sections. In the present study all lepromatous leprosy cases showed histiocytic granulomas with strong Fite Farraco stain positivity and tuberculoid cases showed epithelioid granulomas and FF stain negativity. Fluorescent microscopy is a useful technique used for rapid screening[22] and helped us to establish diagnosis in one case in our study.

Gomori's methenamine silver (GMS) and Per-iodic acid Schiff (PAS) are two most commonly used special stains to establish *diagnosis of fungal granulomas* in difficult cases. In the present study majority of the fungal granulomas were positive with special stains such as GMS and PAS (96.55%, 28 cases; 89.65%, 26 cases respectively) and all cases of cryptococcosis also showed positivity with mucicarmine (6.89%, 2 cases) (Table 3). Culture was done in three cases and yielded nonspecific results. GMS stain is preferable, primarily because Periodic acid–Schiff provides less contrast between fungi and background debris. Also it is important to remember that GMS is not a specific stain for microorganisms, as it often stains mucin, elastic tissue, and dust particles in the background lung. However, these particles are smaller in size and lack the characteristic morphology associated with fungal organisms. Diagnosis may be missed due to many reasons including low numbers of organisms, artifactual distortion, necrosis, degenerative processes, or by other ongoing processes, such as dense cellular inflammatory infiltrates which may mask the typical morphologic appearance of some agents. Some organisms may lack typical staining characteristics or structural features because of factors such as intercurrent drug therapy, subspecies level variations in genomic makeup or phenotypic expression, or variations in staining reagents, tissue processing, or laboratory techniques used. Histologically organisms may be detected, but classification to the genus or species level may not always be impossible.

Finally, some agents are invisible in routine special cytochemical stains requiring time consuming and cumbersome culture methods.[19] *Parasitic* lesions generally show eosinophilic infiltration in addition to granulomas.[23] *Foreign body* granulomas usually show foreign material within macrophages and giant cells, a finding that of course is of great diagnostic value.[24] *Immunohistochemical techniques* may be required to identify the type of tumor in secondaries. Other methods employed for identification of the specific etiologic agent include *polaroscopy* of tissue section, *histochemistry*, *use of special optical filters*, *micro incineration of tissue*, *X-ray defraction*, and *tissue chromatography*. In the present study polarizing microscopy has helped establish the diagnosis in two cases.[24]

Conclusions

Granulomatous lesions have diverse etiology; serve to limit the etiologic agent from dissemination. Morphological Diagnosis may be hampered by low numbers of organisms, artifactual distortion, necrosis, degenerative processes, or by other ongoing processes, such as dense cellular inflammatory infiltrates which may mask the typical morphologic appearance of some organisms/agents. The detailed clinical history, histological examination often supported by special stains (GMS, PAS, ZN, FF, and Mucicarmine), special techniques (Auramine-Rhodamine), culture, polarizing microscopy, immunohistochemistry, molecular techniques and clinicopathological correlation are required for accurate etiological diagnosis.

Conflict of Interest: None.

Source of Funding: None.

References

1. Hirsh BC, Johnson W C. Concepts of granulomatous inflammation. *Int J Dermatol* 1984;24(2):90-100.
2. Warren KS. A functional classification of granulomatous inflammation. *Ann Newyork Acad Sci* 1976;278:7-18.
3. Baydur A. The spectrum of extra pulmonary tuberculosis. *West J Med* 1977;126:253-62.
4. Krishnaswamy H, Job CK. The role of ZiehlNeelsen and Flourescent stains in tissue sections in the diagnosis of tuberculosis. *Indian J Tuberc* 1974;21(10):18-21.
5. The granulomatous disorders. Edited by D. Geraint James and Alimuddin Zumla. 616 pp., illustrated. New York, Cambridge University Press, 1999.
6. KK Shah, Bobbi SP, Marium PA. Histopathologic review of granulomatous inflammation. *J Clin Tub Mycobac Dis* 2017:7:1-12
7. Romagnani S. T-cell subsets (Th1 versus Th2). *Ann Allergy Asthma Immunol* 2000;85:9–18.
8. Hooper AA. Tuberculous peripheral lymphadenitis. *Br J Surg* 1972;59(5):353-9.
9. MayraSilva M, Adrien B, Sophie A, Florece D and Frederic A. the Tuberculous granuloma: An unsuccessful host defence

mechanism providing safety shelter for the bacteria? *Clin Develop Immunol* 2012:2012:139127
10. D. G. Russell, P. J. Cardona, M. J. Kim, S. Allain, and F. Altare "Foamy macrophages and the progression of the human tuberculosis granuloma. *Nature Immunol* 2009:10(9):943–8.
11. M. J. Kim, H. C.Wainwright, M. Locketz. "Caseation of human tuberculosis granulomas correlates with elevated host lipid metabolism. *EMBO Mol Med*, 2010:2(7):258–74.
12. A. M. Dannenberg Jr., Delayed-type hypersensitivity and cell-mediated immunity in the pathogenesis of tuberculosis. *Immunol Today* 1991:12(7):228–33.
13. A. Fayyazi, B. Eichmeyer, A. Soruri. Apoptosis of macrophages and T cells in tuberculosis associated caseous necrosis. *J Pathol* 2000:191(4):417–25.
14. M. C. Tsai, S. Chakravarty, G. Zhu., Characterization of the tuberculous granuloma in murine and human lungs: cellular composition and relative tissue oxygen tension. *Cell Microbiol* 2006:8(2):218–32.
15. J. L. Taylor, O. C. Turner, R. J. Basaraba, J. T. Belisle, K. Huygen, and I. M. Ormel, Pulmonary necrosis resulting from DNA vaccination against tuberculosis. *Infect Immun* 2003:71(4):2192–8.
16. Shogo T, Kenji K. granulomatous inflammation of pulmonary squamous cell carcinoma: a rare phenomenon. *Int J Clin Exp Pathol* 2015;8(6):7547-52.
17. Chhabra S, Mohan H, Bal A. granulomas in association with neoplasm. Reaction or a different primary process? *J Postgrad Med* 2009;55:234-6.
18. Spector WG. Epithelioid cells, giant cells and sarcoidosis. Annals New York academy of sciences 1976;278: 3-6.
19. Sanjay Mukhopadhyay and Anthony A. Gal. Granulomatous Lung Disease: An Approach to the Differential Diagnosis. *Arch Pathol Laby Med* 2010;134;5:667-90.
20. Gupta SD, Ray R, Gill S S. Pathology. In: Sharma SK, Mohan A editors. Tuberculosis. New Delhi: Jaypee brothers; 2001.
21. Ichiyama S, Suzuki K. Clinical application of testing methods on acid-fast bacteria. *Kekkaku* 2005;80(2):95-111.
22. Nayak SV, Shivrudrappa AS, Mukamil AS. Role of fluorescent microscopy in detecting Mycobacterium leprae in tissue sections. *Ann Diagn Pathol* 2003;7(2):78-81.
23. Madri JA. Inflammation and healing. In: Kissane JM editor. Anderson's Pathology.9th ed. Vol1. London: Mosby; 1990.
24. Shapiro PE. Non infectious Granulomas. In: Elder D editor. Lever's histopathology of the skin.8th ed. Philadelphia: Lippincott-raven; 1997.

How to cite this article: Chandrashekhar TN, Chavan SS. Pattern Analysis of granulomatous inflammatory lesions. *Indian J Pathol Oncol* 2019;6(3):357-64.