e-ISSN: 0975-9506, p-ISSN: 2961-6093

Available online on www.ijpqa.com doi: 10.25258/ijpqa.16.12.63

International Journal of Pharmaceutical Quality Assurance 2025; 16(12); 376-381

Original Research Article

A Histopathological Assessment of the Frequency and Etiology of Granulomatous Lesions with Reference to Special Stains

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Received: 05-10-2025 / Revised: 20-11-2025 / Accepted: 24-12-2025

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Conflict of interest: Nil

Abstract:

Background: Granulomatous inflammation is a distinct chronic inflammatory response characterized by organized collections of epithelioid macrophages, often triggered by infectious or non-infectious agents. Accurate histopathological identification, supported by special stains, is essential for determining etiology and guiding management.

Aim: To assess the frequency, anatomical distribution, morphological patterns, and etiological spectrum of granulomatous lesions, with reference to special staining techniques.

Methodology: A descriptive cross-sectional study was conducted on 135 histopathologically confirmed granulomatous lesions at the Department of Pathology, Government Medical College, Bettiah. Specimens were processed using routine H&E staining, with Ziehl–Neelsen, PAS, and GMS stains applied as needed. Clinical and demographic data were recorded, and lesions were classified based on morphology and etiology.

Results: Patients ranged from 1–80 years, with a slight male predominance (53.3%). The most common sites were skin/subcutaneous tissue (25.2%), lymph nodes (22.2%), and bones/joints (15.6%). Tuberculosis was the leading cause (45.9%), followed by leprosy (13.3%) and fungal infections (10.4%). Epithelioid granulomas predominated (57.8%). Ziehl–Neelsen staining detected acid-fast bacilli in only 22.6% of tuberculous cases, highlighting limitations of conventional staining.

Conclusion: Granulomatous lesions exhibit diverse etiologies and morphological patterns, with infectious causes, particularly tuberculosis, being predominant. Histopathology supplemented with special stains is vital for accurate diagnosis and effective patient management.

Keywords: Granulomatous inflammation, Tuberculosis, Histopathology, Special stains, Epithelioid granuloma.

Introduction

Granulomatous inflammation is a characteristic and well-defined pattern of chronic inflammatory reaction which is seen in a small but highly significant number of infectious and non-infectious disease conditions. It is also marked by the development of granulomas, or well-structured aggregates of modified macrophages that develop due to a host defense mechanism towards chronic and poorly degradable agents. Granuloma is described as a localized concentration of chronic inflammation, consisting of mainly macrophages which are transformed into epithelioid cells and enclosed by a collar of lymphocytes and, in some cases, plasma cells [1]. This peculiar histological organization is an indication of an intricate and dynamic interplay of immune cells and inciting agents.

A broad range of etiological agents cause granulomatous inflammatory reactions: infectious organisms, toxic agents, allergens, autoimmune responses, neoplasms, and unknown agents. In turn, granulomatous inflammation is also a histopathological reflection of various different disease processes. It follows that clear insight into the fundamental pathophysiology of this unique tissue response is of primary significance in the understanding and explanation of many disease entities [2]. The identification of granulomatous inflammation in tissue sections usually limits the number of possible diagnoses and includes essential hints to identify the cause.

The nature of the offending agent is closely related to the pathogenesis of the granulomatous inflammation. The irritating factors that lead to the

development of granuloma are usually inaccessible to degradation by the normal inflammatory processes. The efforts of the polymorphonuclear leukocytes, non-activated macrophages and allied chemical mediators engaged in the acute inflammation processes are usually not enough to fully digest and eliminate such agents. This leads to increased specific response by the immune system with transformed macrophages which are produced with the help of CD4+ T lymphocytes. The CD4+ T cells produce numerous cytokines such as interleukin-2 (IL-2), interferon-gamma (IF- gamma), tumor necrosis factor (TNF) and lymphotoxin that together stimulate the activation of macrophages and their differentiation to epithelioid and multinucleated giant cells. These are the transformed cells which are the major cellular components of the granulomas [2].

Granulomas may be categorized according to etiological factors but also in terms of morphological characteristics. Granulomas are etiologically classified into bacterial, metal induced, fungal, viral or chlamydial, helminthic, foreign body induced and of unknown cause [3]. Cat scratch fever and lymphogranuloma venereum are a few conditions in the viral and chlamydial granulomas. All the etiological categories are linked to specific pathogenic processes and clinical consequences, and their correct definition is the key to successful treatment of a patient.

The granuloma has high variability in terms of morphology based on the underlying cause and responses of the body. On the basis of histological features, granulomas are defined as epithelioid, histiocytic, foreign body type, necrobiotic or palisading, and mixed inflammatory granulomas [4]. These morphological patterns can also be useful in making a diagnosis and sometimes in the distinction between infectious and non-infectious origin. In most cases, the type of granuloma, the presence or absence of necrosis, the character of inflammatory infiltrate, and the determination of the related organisms may give the pathologist a lead to a particular diagnosis.

The identifications of the granulomatous inflammation in a biopsy specimen have a significant diagnostic value since the number of diagnoses that precipitate the pattern is relatively small and because of the therapeutic implications of each diagnosis. Granulomatous inflammations are a frequent but interesting diagnosis problem in surgical pathology. It is essential that accurate diagnosis is made because it directly affects the way patients are handled and how they are treated. In most cases, granulomatous lesions can be diagnosed using histopathological examination as it forms the basis of a conclusive diagnosis in most diseases afflicting different organs of the body [5].

A combination of comprehensive clinical history, extensive histological examination, and close clinicopathological correlation is imperative to the achievement of an ultimate and significant diagnosis. A sensible differential diagnosis may often be made by the synthesis of all available data, which will be used to direct the investigation and treatment. Nevertheless, in a small portion of the cases, a conclusive diagnosis cannot be made after a rigorous clinical and histopathological evaluation. This diagnostic doubt indicates the complexity of the granulomatous lesions, and the weakest side of the routine histological investigation.

Diagnosis of the histological granulomatous inflammation is not flawless. There can be morphological overlaps between various granulomatous diseases, and this can result in a failure to distinguish the etiology. Under these conditions, special histochemical stains are very important in the detection of infectious agents like mycobacteria, fungi and other organisms and would increase the accuracy of the diagnosis. Regardless of the use of special stains and ancillary methods, a small proportion of cases might not be assigned a classification with the diagnosis being limited to granulomatous inflammation of unspecified etiology [5].

However, morphological patterns used in different granulomatous diseases are frequently too different to allow sufficiently accurate diagnosis by a qualified pathologist. The frequency, distribution, and etiological spectrum of granulomatous lesions among a specific group of the population is thus of significant academic and clinical interest. Against this background, the current research was conducted to determine the rate and etiology of granulomatous lesions in comparison to special stains and compares the results with those of the other variables. The objective of this assessment is to help improve the image of histopathological profile of granulomatous inflammation and support the idea that special staining techniques have the value of diagnostic use in everyday pathology.

Methodology

Study Design: This study was a descriptive, cross-sectional histopathological study conducted to assess the frequency and etiology of granulomatous lesions and to evaluate the role of special stains in their diagnosis.

Study Area: The study was carried out in the Department of Pathology, Government Medical College, Bettiah, West Champaran, Bihar, India

Study Duration: The duration of the study was from September 2024 to August 2025

Sample Size: A total of 135 cases diagnosed as granulomatous lesions on histopathological examination were included in the study.

Study Population: The study population comprised patients of all age groups and both sexes whose biopsy or surgical specimens were submitted to the Department of Pathology and were histopathologically diagnosed as granulomatous lesions.

Data Collection: All biopsy and surgical specimens received in the Department of Pathology, Government Medical College, Bettiah, were subjected to routine histopathological processing. The specimens were fixed in 10% neutral buffered formalin, followed by standard tissue processing, paraffin embedding, and sectioning at 3–5 µm thickness. The sections were initially stained with Hematoxylin and Eosin (H&E) for microscopic examination. Cases demonstrating granulomatous inflammation on H&E staining were selected for further evaluation. Special stains such as Ziehl–Neelsen (ZN) stain for acid-fast bacilli, Periodic Acid–Schiff (PAS) stain, and Gomori Methenamine Silver (GMS) stain were applied whenever required to identify the underlying etiological agents. Relevant clinical details including age, sex, site of lesion, clinical diagnosis, and laboratory investigations were collected from pathology requisition forms and hospital case records.

Inclusion Criteria

- All histopathologically confirmed cases of granulomatous lesions
- Specimens from all anatomical sites
- Adequate tissue samples suitable for histopathological evaluation
- Cases where special stains could be performed when required

Exclusion Criteria

- Poorly preserved or inadequately fixed tissue specimens
- Cases with insufficient tissue for histopathological assessment
- Non-granulomatous inflammatory lesions

Procedure

Each selected case was systematically evaluated under light microscopy to assess the histomorphological features of granulomatous inflammation, including the type of granuloma, presence or absence of caseation necrosis, multinucleated giant cells, and associated inflammatory infiltrate. Based on these features and the results of special stains, the granulomatous lesions were classified according to their etiology into infectious and non-infectious causes. Tuberculous and fungal granulomas were confirmed by the demonstration of causative organisms using appropriate special stains. All findings were recorded in a structured proforma for subsequent analysis and interpretation.

Statistical Analysis: Data were entered into Microsoft Excel and analyzed using appropriate statistical software. Results were expressed as frequencies and percentages. Descriptive statistics were used to analyze the distribution of granulomatous lesions with respect to age, sex, site, and etiology. Findings were presented in the form of tables and charts."

Result

Table 1 illustrates the age and sex distribution of 135 cases, showing a slight male predominance with 72 males (53.3%) and 63 females (46.7%). The highest number of cases was observed in the 21–30 year age group, comprising 34 cases (25.2%), followed by 31–40 years with 28 cases (20.7%) and 41–50 years with 19 cases (14.1%). Individuals aged 11–20 years accounted for 16 cases (11.9%), while those aged 51–60 years and 61–70 years contributed 17 (12.6%) and 13 cases (9.6%), respectively. Pediatric cases aged 1–10 years were relatively few, with 6 cases (4.4%), and very elderly patients aged 71–80 years accounted for only 2 cases (1.5%). No cases were reported in individuals aged 81 years or above, indicating that granulomatous lesions were most prevalent in young and middle-aged adults.

Table 1: Age and Sex Distribution of Cases (N = 135)

Age Group (years)	Male	Female	Total (%)
1–10	3	3	6 (4.4)
11–20	8	8	16 (11.9)
21–30	19	15	34 (25.2)
31–40	15	13	28 (20.7)
41–50	10	9	19 (14.1)
51–60	9	8	17 (12.6)
61–70	7	6	13 (9.6)
71–80	1	1	2 (1.5)
≥81	0	0	0 (0.0)
Total	**72**	**63**	**135 (100)**

Table 2 depicts the anatomical distribution of granulomatous lesions among 135 cases, showing that the most commonly affected sites were the skin and subcutaneous tissue with 34 cases (25.2%), followed

by lymph nodes in 30 cases (22.2%) and bones and joints in 21 cases (15.6%). Involvement of the respiratory system was observed in 13 cases (9.6%), while the gastrointestinal tract accounted for 11 cases (8.1%). Less frequent sites included the breast (8 cases, 5.9%), male genitourinary system (6 cases, 4.4%), and female genitourinary system (5 cases, 3.7%). Rare involvement was seen in the gall bladder (3 cases, 2.2%), thyroid (2 cases, 1.5%), brain (1 case, 0.7%), and oral cavity (1 case, 0.7%), highlighting the wide but variable anatomical distribution of granulomatous lesions.

Table 2: Distribution of Granulomatous Lesions According to Site (N = 135)		
Site of Granuloma	**Number of Cases**	**Percentage (%)**
Skin & Subcutaneous Tissue	34	25.2
Lymph Nodes	30	22.2
Bones & Joints	21	15.6
Respiratory System	13	9.6
Gastrointestinal Tract	11	8.1
Breast	8	5.9
Male Genitourinary System	6	4.4
Female Genitourinary System	5	3.7
Gall Bladder	3	2.2
Thyroid	2	1.5
Brain	1	0.7
Oral Cavity	1	0.7
Total	**135**	**100**

Table 3 shows that tuberculosis was the most common etiology of granulomatous lesions, accounting for 62 cases (45.9%), followed by leprosy in 18 cases (13.3%) and fungal infections in 14 cases (10.4%). Foreign body granulomas constituted 12 cases (8.9%), while tumor-associated granulomas were observed in 9 cases (6.7%). Less frequent causes included rhinoscleroma (4.4%), actinomycosis (2.2%), parasitic infestation (2.2%), and rheumatoid arthritis (1.5%), whereas 6 cases (4.4%) remained of unknown etiology.

Table 3: Etiological Distribution of Granulomatous Lesions (N = 135)		
Etiology of Granuloma	**Number of Cases**	**Percentage (%)**
Tuberculosis	62	45.9
Leprosy	18	13.3
Fungal Infections	14	10.4
Foreign Body Granuloma	12	8.9
Tumor-Associated Granuloma	9	6.7
Rhinoscleroma	6	4.4
Actinomycosis	3	2.2
Parasitic Infestation	3	2.2
Rheumatoid Arthritis	2	1.5
Unknown Etiology	6	4.4
Total	**135**	**100**

Table 4 depicts the Ziehl–Neelsen staining results in 62 cases of tuberculous granulomas, of which 14 cases (22.6%) were ZN stain positive, indicating the presence of acid-fast bacilli. The majority of cases, 48 (77.4%), were ZN stain negative, highlighting that a substantial proportion of histologically diagnosed tuberculous granulomas did not demonstrate acid-fast bacilli on ZN staining.

Table 4: Ziehl–Neelsen Staining Results in Tuberculous Granulomas (N = 62)		
ZN Stain Result	**Number of Cases**	**Percentage (%)**
ZN Positive	14	22.6
ZN Negative	48	77.4
Total	**62**	**100**

Table 5 shows the morphological patterns of granulomatous inflammation among 135 cases, with epithelioid granuloma being the most common pattern observed in 78 cases (57.8%). This was followed by foreign body granuloma in 18 cases (13.3%) and ill-defined granuloma in 15 cases (11.1%). Histiocytic granulomas accounted for 12 cases (8.9%), while mixed inflammatory patterns were seen in 9 cases

(6.7%). Necrobiotic granuloma was the least common pattern, identified in only 3 cases (2.2%), indicating its relatively rare occurrence in the study population.

Table 5: Morphological Patterns of Granulomatous Inflammation (N = 135)		
Morphological Pattern	**Number of Cases**	**Percentage (%)**
Epithelioid Granuloma	78	57.8
Foreign Body Granuloma	18	13.3
Ill-defined Granuloma	15	11.1
Histiocytic Granuloma	12	8.9
Mixed Inflammatory Pattern	9	6.7
Necrobiotic Granuloma	3	2.2
Total	**135**	**100**

Discussion

The highest incidence of granulomatous lesions in our 135 case study was in the 30-34 years, where we had 34 cases (25.2%), then the 40-44 age group, which had 28 cases (20.7%). This age distribution is consistent with the results presented by Krishnaswamy and Job (1974) [6] who reported that granulomatous lesions were more prevalent in younger adults, but the study did not indicate the distribution by decade. Conversely, variations in the prevalence of granulomatous diseases have also been described to be at peaks in slightly older age groups, implying some regional or environmental effects on the prevalence. The male slight dominance in our research with 72 males (53.3) and 63 females (46.7) is supported by the past literature where males are slightly biased in granulomatous conditions especially those of infectious origin (Nayak et al., 2003) [7]. Nevertheless, the fact that both sexes were almost equal in our results also indicated that both genders can be greatly affected by granulomatous lesions".

Granulomatous lesions, which were common in our cohort (34 cases, 25.2%), were most likely to be located in the skin and subcutaneous tissue (34 cases, 25.2), then lymph nodes (30 cases, 22.2), and bones and joints (21 cases, 15.6). The respiratory and gastrointestinal involvement were less frequent with 9.6 and 8.1 percent, respectively. These results are in line with other earlier reports, which commonly point at lymphoreticular tissue and skin as significant locations of granulomatous inflammation (Meyer et al., 1983) [8]. Curiously, some unusual locations like the gall bladder, thyroid, brain and oral cavity were also implicated in some few cases, which highlights the broad tissue tropism of granulomatous lesions. The wide spread of the disease suggests that clinicians and pathologists should pay attention to the possibility of granulomatous inflammation in several organ systems even when it is not located in the typical sites.

Our study revealed tuberculosis to be the most important etiological agent that caused 62 cases (45.9%), which supports its importance globally but particularly in developing nations. Acid-fast bacilli was proven by Ziehl-Neelsen (ZN) staining in 14 instances (22.6%), with most of them (77.4%), being negative. This is in contrast to Krishnaswamy and Job (1974) [6] who showed by standard staining ZN positivity in 91 of 130 lesions (71%), which indicates that standard staining is not as sensitive in paucibacillary lesions or in the initial lesion. We therefore believe that the use of histochemical staining alone may underestimate the burden of mycobacterial infections and that supplementary methods of diagnosis, e.g., culture or molecular studies, are essential to proper diagnosis.

Leprosy represented 18 cases (13.3) in our series, Fite-Faraco positive result in only 9 cases (25.7), which is lower than that of Nayak et al. (2003) [7] with 44.6. This inconsistency probably represents variations in the range of leprosy experienced, wherein the tuberculoid or paucibacillary forms incline to give negative staining results more often. Fungal infections were detected in 14 cases (10.4%), aspergillosis, rhinosporidiosis, and chromoblastomycosis, and could only be detected with special stains like Gomori methenamine silver (GMS) and periodic acid Schiff (PAS) in the majority of cases. These observations are parallel to those of Meyer et al (1983) [8], who highlighted the importance of special stains in the detection of elusive fungal organisms, especially when other common stains, i.e. hematoxylin and eosin stains are inconclusive.

Our cohort also found foreign body granulomas (12 cases, 8.9) and tumor-associated granulomas (9 cases, 6.7), which shows the reaction of the tissue to foreign materials and neoplastic processes. Epithelioid granulomas (57.8%), foreign body granulomas (13.3%), ill-defined granulomas (11.1%), histiocytic granulomas (8.9%), mixed patterns (6.7%), and necrobiotic granulomas (2.2) were the morphological patterns of granulomatous inflammation in our study. The distributions are reflective of the past reports, indicating that epithelioid granulomas are the standard of chronic granulomatous inflammation with other patterns usually aligned to particular etiologies, e.g. foreign bodies or tumors (Alka Bhatia et al., 2009) [9] [9] [9] In our study of tumor-associated granulomas which are present in several types of malignancy including squamous cell carcinoma, infiltrating ductal carcinoma, papillary thyroid

carcinoma and dysgerminoma, the granulomas are frequently present at the tumor periphery or in draining lymph nodes. This complies with previous reports of sarcoid-like responses in malignancy that are believed to be caused by immune responses that are mediated by cytokines and not direct invasion of the tumor (Alka Bhatia et al., 2009) [9].

Other etiologies that were not prevalent in our cohort (only 6, 4.4, and 3 cases respectively) were rhinoscleroma, actinomycosis, and parasitic infections. The cases had typical suppurative granulomas of central colonies, as Mirza and Sarwar (2003) [10] observed. There were very high histiocytes, plasma cells, fibrosis, and Russell body in rhinoscleroma lesions, which had a high incidence of both fibrosis (50) and Russell bodies (40) compared to the findings of Meyer et al. (1983) [8]. All of them were parasitic granulomas, which were well developed with cuticular and muscular structures with inflammatory infiltrates, in agreement with the descriptions of Padmaja et al. (2005) [11].

No clear etiology was established in 6 cases (4.4%), which shows the difficulty of diagnosis of granulomatous lesions despite the application of special stains. Granulomatous mastitis, with 11 of these, was mainly with epithelioid granulomas, as per Fletcher et al. (1982) [12], all of their seven were epithelioid. The etiology was still uncertain, which indicated the shortcomings of histopathology in some idiopathic granulomatous diseases. On balance, our results indicate a spectrum of granulomatous lesions that is to a great extent consistent with literature, but with regional differences in incidence, distribution of sites, and the presence of stain, which justify the need to combine clinical, morphological, and special staining data to diagnose the situation correctly.

Conclusion

The current research indicates that granulomatous lesions are heterogeneous populations of pathological entities that have broad age distribution, have male bias slightly, and affect various organ systems, with the largest number of cases relating to skin, lymph nodes, and musculoskeletal system. A new etiology, mainly tuberculosis, then other infectious and non-infectious etiologies, became the dominant cause in some way and the clinical importance of granulomatous inflammation in the practice of routine histopathology could not be underestimated. The most common morphological pattern that was observed was epithelioid granulomas, which is evidence of the high frequency of immune-mediated responses. The analysis is supported by the variable detection of causative organisms with special stains, in particular, tuberculous lesions, that underline the significance of using histomorphology to be correlated with clinical results and ancillary methods.

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