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Original Research Article

Histopathological spectrum of granulomatous disorders: A hospital based study at B.P. Koirala Institute of Health Sciences

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Abstract

Background: Granulomatous disorders comprise a large family sharing histological denominator of granuloma formation. A granuloma is a focal compact collection of inflammatory cells, mononuclear cells predominating usually as a result of the persistence of non-degradable product and of active cell mediated hypersensitivity. Incidence and prevalence of granulomatous disorder differs with geographic location. Histopathology along with special stains serves as gold standard tool for the diagnosis and classification of granulomatous disorders. The present study was done to investigate the histopathological spectrum on the patients of eastern part of Nepal.
Objectives: To study on histopathological spectrum of the granulomatous disorders at B.P. Koirala Institute of Health Sciences, Dharan.
Methods: Descriptive type of observational study was done on total 80 cases during the period of July 2017 to July 2018 in department of pathology of BPKIHS, Dharan.
Results: In present study, granulomatous disorders are common in third decade of life with male predominance (55%). Most commonly skin and subcutaneous tissue (40%) was the site affected by granulomatous reaction followed by lymph node (17.5%). Tuberculosis was found the most common etiology (38.75%) while Tuberculoid being the most common type of granuloma (60%). Ziehl Neelsen stain is helpful in diagnosis of tuberculosis (positive: 61.30%). Similarly Fite Faraco stain for leprosy is 37.5% (6/16). Non-caseating granulomas are more common (43/80, 53.67%).
Conclusion: Granulomatous lesion seems to be common in third decade of life with male predominance. Tuberculosis is the most common cause. The information is useful in management of granulomatous disorders and also for preventive measures.
Keywords: AFB (TB), Caseous necrosis, Granuloma, Tuberculosis.

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1. Introduction

Granulomatous disorders comprise a large family sharing the histological denominator of granuloma formation. [1]

According to Robbins Pathologic basis of disease, “Granuloma is a focal area of Granulomatous inflammation. It consists of a microscopic aggregation of macrophages that are transformed into epithelium like cells surrounded by a collar of mononuclear leukocytes, principally lymphocytes & occasionally plasma cells.” [2]

Turk (1971) defined a Granuloma as a collection of cells of the macrophages-histiocyte series with or without the admixture of other inflammatory cells. [3]

Rodriguez S. defined granuloma as "A Granuloma is a compact (organized) collection of mature mononuclear phagocytes (macrophages and/or epithelioid cells) which may or may not be accompanied by accessory features such as necrosis or the infiltration of other inflammatory leukocytes”.[4]

Granulomatous inflammation is a form of chronic inflammation characterized by collections of activated macrophages, often with T lymphocytes, and sometimes associated with central necrosis. Granuloma formation is a cellular attempt to contain an offending agent that is difficult to eradicate. In this attempt there is often strong activation of T lymphocytes leading to macrophage

activation, which can cause injury to normal tissues. The activated macrophages may develop abundant cytoplasm and begin to resemble epithelial cells, and are called epithelioid cells. Some activated macrophages may fuse, forming multinucleate giant cells. [2]

Fully developed granulomas with sheets of epithelioid histiocytes and giant cells are easily recognized, but more subtle lesions containing a few epithelioid histiocytes still qualify as granulomatous. [5]

It is difficult to present a completely satisfactory classification of the granulomatous reaction. [6]

Six types of granulomatous disorders are identified according to cellular constitute and associated changes: 1. Tuberculoid; 2. Sarcoidal; 3. Necrobiotic; 4. Suppurative; 5. Foreign body; 6. Miscellaneous. [4, 7, 8]

Classification of granulomas based on the etiology can be done as: 1. Bacterial; 2. Metal induced; 3. Fungal; 4. Viral / Chlamydial (a. Cat scratch fever, b. Lymphogranuloma venerum); 5. Helminthic; 6. Foreign body type and 7. Unknown cause. [9]

The granulomatous inflammatory response is ubiquitous in pathology, being a manifestation of many infective, toxic, allergic, autoimmune and neoplastic diseases and also conditions of unknown etiology. [10] A knowledge of the basic pathophysiology of this distinctive tissue reaction is therefore of fundamental importance in the understanding of many disease processes. [11]

Incidence and prevalence of granulomatous disorder differs in geographic location. Site of occurrences, age and sex distribution, may differ in different geographical areas. Good clinical history, a close histological examination and a clinicopathological correlation is essential in making a final diagnosis. For skin, histopathology is established as gold standard investigation for diagnosis, categorization and clinico-pathological correlation of granulomatous skin lesions. [12]

There is often no single histological feature that distinguishes infectious necrotizing granulomas from other granulomas. Ancillary studies like special stains e.g. Ziehl-Neelsen can be performed to find the cause of granuloma. However, some granulomas remain unexplained even with ancillary studies and in these instances, good clinical history and clinicopathological correlation are essential in making a final diagnosis. [13]

Hence, the study was with following objectives:

1.1 Primary objective

To study the histopathological spectrum of the granulomatous disorders at B.P. Koirala Institute of Health Sciences, Dharan.

1.2 Secondary objectives

- To determine the various etiology of granulomatous disorders.
- To study the frequency of granulomatous disorders with respect to age, sex and site.

2. Methodology

This is a hospital based descriptive study. The histopathological study of various granulomatous disorders was carried out in histopathology laboratory of pathology department, BPKIHS, Dharan, Nepal during the period of August 2017 to July 2018. For histopathological study most of the specimens were received from O.P.D. and wards of clinical departments. However, some of the cases were received from nearby hospital with block preparation as well.

The consent for the study was taken from the Ethical clearance committee BPKIHS before the commencement of the study. Microscopic examination was done. The cases diagnosed as granulomatous reactions were selected. Special stains like Ziehl Neelsen, Fite Faraco, and Periodic Acid Schiff stain were used whenever required. The relevant clinical details and laboratory investigation was collected from the hospital case sheet and analysis was done considering the inclusion criteria.

Final diagnosis was made considering the histopathological findings and special stains. Etiology of various granulomas was determined wherever possible. Morphological classification was done at the same time.

2.1 Inclusion: All the biopsy cases revealing granuloma in histopathological examination.

2.2 Exclusion: Patients who refuse for valid consent.

2.3 Sample size: All biopsy samples reported as granuloma during the study period of 12 months. Estimated 55 cases.

2.4 Calculation of the sample size:

This study considers 95% confidence interval and 80% power to estimate the sample size. For this purpose, proportion of tuberculosis among Granulomatous disease as reported by a study by Bararia *et al*, 2015 (Granulomatous reaction: a histopathological study (a retrospective and prospective study of 5 years) was 56.33%.

P= 56.33%

Q=43.67% where, Q= (100-P)

Therefore this study uses corrected sample size formula for finite population,

For finite population, N=60

Revised sample size becomes,

$n = n / (1+n/N)$

$= 298 / (1+298/60)$

$= 50$

Adding 10% for non-response, sample size need to be 55.

So, **n=55.**

Study period: 1 year

Total 80 cases were diagnosed as granulomatous lesions. All the cases were included as none of the patient denied for the consent.

2.5 Data management and statistical analysis

a) **Data handling:** The collected data was entered in Microsoft Office Excel 2013 software and data

analysis was done by using SPSS 11.5 (Statistical Package for Social Sciences).

b) Coding: Data was appropriately coded.

c) Monitoring: After entering every 10 data, corrections were made.

d) Statistical method:

Percentage (%), proportion, ratio, and mean were calculated along with graphical and tabular presentations were made.

3. Results

This is a descriptive type of observational study, conducted from August 2017 to July 2018, over a period of 1 year. This study included a total of 80 cases and the study was carried out in histopathology section of Pathology department, BPKIHS, Dharan. Results are described below in details.

3.1 Age:

Age of the patient ranges from 12 years to 73 years with the mean age of 38.18 yrs. Majority of the patients were in the age group 20-29 years (25%). (Table 1) Among them, paediatric populations were 12.5%, adults were 68.5%, and elderly were 18.75%.

Table 1: Distribution of granulomatous disorders (GD) based on age groups

Age group(years)	No. of Cases	Percentage
0-9	0	0%
10-19	12	15%
20-29	20	25%
30-39	13	16.25%
40-49	12	15%
50-59	8	10%
60-69	12	15%
70-79	3	3.75%
Total	80	100%

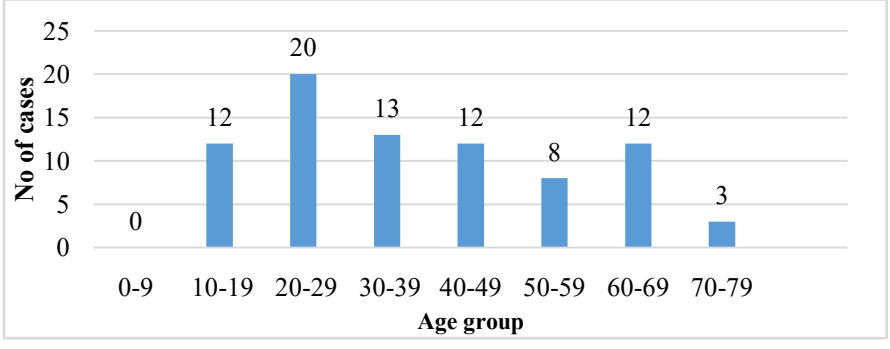

Figure 1: Distribution of GD based on age group.

Similarly, Skin and subcutaneous tissue was the most common (m/c) site in 2nd, 3rd, 4th, 6th decades of life. In 5th, 7th and 8th decades skin and subcutaneous tissue and lymph node were found in equal number.

Tuberculoid type of granuloma was predominant in all age groups.

Tuberculosis is the m/c etiology in 2nd, 3rd, 4th, 6th and 7th decade. In 5th decades of life, unknown cause was more in number than that of tuberculosis.

3.2 Sex:

Of the total 80 cases studied, 44 were males and 36 were females with male to female ratio of 1.22:1.

Table 2: Distribution of GD based on sex

Sex	Number	Percentage
Male	**44**	**55%**
Female	**36**	**45%**
Total	80	100%

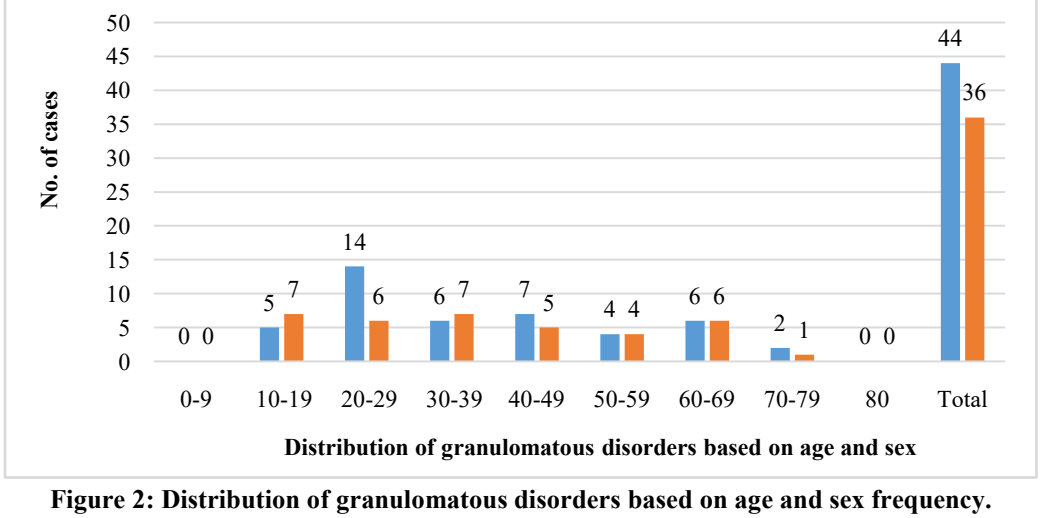

Figure 2: Distribution of granulomatous disorders based on age and sex frequency.

3.3 Site:

Most common site for GD was skin and subcutaneous tissue (40%), followed by lymph node (17.5%). Other uncommon sites were bone and bone marrow, breast, female reproductive system, respiratory system, etc (Table 3)

Table 3: Distribution of GD based on site

Site	No. of Cases	Percentage (%)
Skin and subcutaneous tissue	32	40
Lymph Node	14	17.50
Bone And Bone Marrow	6	7.50
Breast	4	5
Female Reproductive System	4	5
Male Reproductive System	3	3.75
Muscle	1	1.25
Respiratory System	3	3.75
Gastrointestinal Tract	3	3.75
Urinary Bladder	2	2.50
Pericardium	2	2.50
Nose, Mucosa	4	5
Omentum And Peritoneal Deposit	2	2.50
Total	80	100

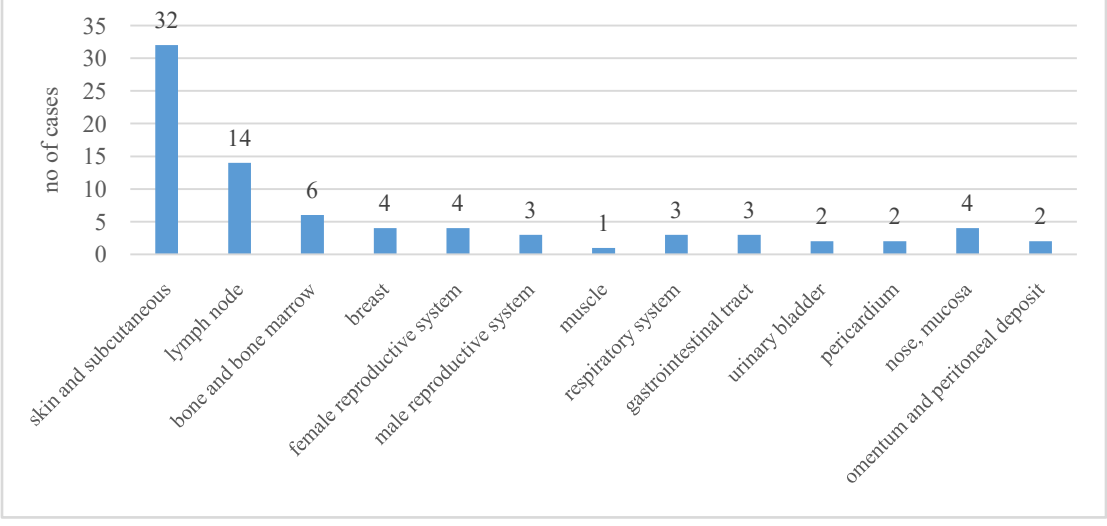

Figure 3: Distribution of GDs based on sites.

When most common site (skin and subcutaneous tissue) was taken into account, the most common age group affected by GDs was 20-29 years (27.58%) followed by 30-39 years (20.68%). Most common sex was male (51.72%). Most common cause was Leprosy (55.17%). Tuberculosis was lesser (10.34%) but granulomatous disorders with unknown etiology were 24.13%. Most common type of granuloma was Tuberculoid (65.51%).

3.4 Etiology

In this study tuberculosis was found to be the most common etiology (38.75%), followed by Granulomatous lesions of unknown aetiology (26.25%). Leprosy was found to be the third most common etiology of all GDs (20%). (Table 4)

Rare cases to cause GD are BCG effect, Crohn's disease, tumor and actinomycosis. Four cases of rhinosporidiosis in nasal mucosa were diagnosed. Three cases of GD coexisting with tumor were found. Two cases were diagnosed as Lymphoma and one case as Squamous cell carcinoma. Among two cases of Lymphoma, one was diagnosed as "Hodgkin's lymphoma, nodular sclerosis-syncytial variant" and the other case as Non-Hodgkin's Lymphoma, in which immunohistochemistry (IHC) was advised for confirmation. Due to unavailability of IHC in our department, these patients were referred to BPKMCH, Bharatpur for IHC.

Table 4: Distribution of GDs based on etiology

Etiological class	No. of Cases	Percentage
Actinomycosis	1	1.25%
BCG Effect	1	1.25%
Crohn's Disease	1	1.25%
Foreign Body	1	1.25%
Fungal	1	1.25%
Leprosy	16	20%
Parasite	4	5%
Tuberculosis	31	38.75%
Tumor	3	3.75%
GL of unknown etiology	21	26.25%
Total	80	100%

Among Tuberculosis cases (N=31), most common age group was 4th decade (25.8%) followed by 2nd and 3rd decade (19.35% each). Males were affected predominantly (64.5%) than females (35.58%). Most common site for tuberculosis was found to be lymph node (22.7%) followed by Bone and bone marrow (16.12%). Skin and subcutaneous tissue was the site affected by tuberculosis in 9.67%.

Special stain for Acid Fast Bacilli (AFB TB) was positive in 61.30% (19 cases out of 31 cases of tuberculosis). (Table 5)

Table 4: Status of special stain for Acid Fast Bacilli (AFB TB) in Tuberculosis

AFB(TB)	Cases	Percentage
Negative	**12**	**38.70%**
Positive	**19**	**61.30%**
Total	**31**	**100%**

3.5 Leprosy:

Out of 16 leprosy cases, BTHD was found to be the most common type (62.5%) followed by Mid-borderline HD (BB) and Erythema Nodosum Leprosum (ENL) (12.5% each). Tuberculoid Hansen's disease (TT) and Lepromatous leprosy (LL) were comprised of 6.25% each. Histioid leprosy was not seen in this study.

Table 6: Distribution of Leprosy based on subtypes of Leprosy

Types of Leprosy	BTHD	BB	ENL	TT	LL	Total
Number of patient	10	2	2	1	1	16
Percentage	62.5%	12.5%	12.5%	6.25%	6.25%	100%

AFB (Lepra) was positive in total six cases (37.5%). Out of six, three cases were of BTHD, rests three are of BB, ENL and LL each. Bacteriological index (BI) was ranged from 1 to 5. BI 5 was found in Lepromatous (LLHD) and one of the BTHD case. (Figure 4)

Table 7: Status of AFB (L) in leprosy

Fite Faraco Stain	No. of cases	Percentage
Positive	6	37.5%
Negative	10	62.5%
Total	16	100%

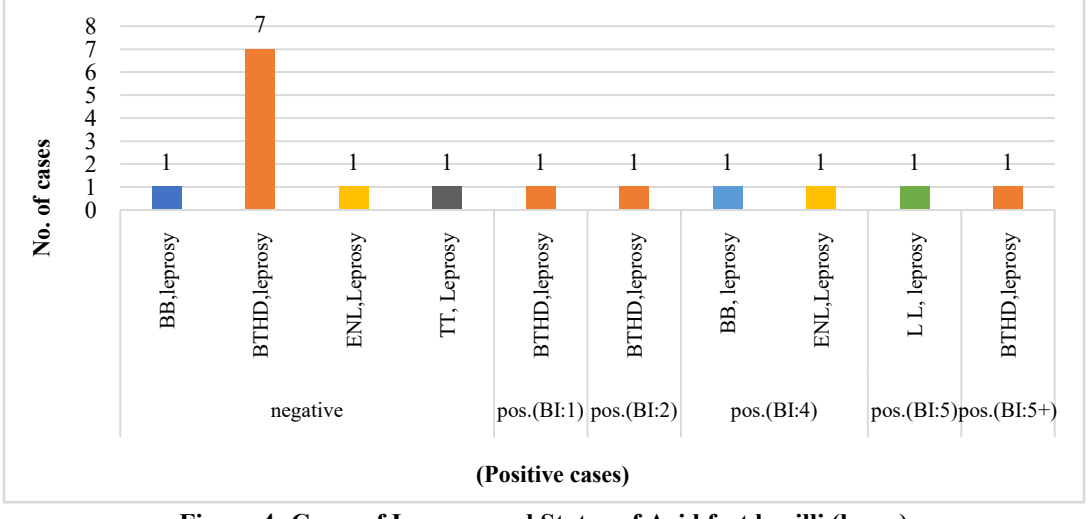

Figure 4: Cases of Leprosy and Status of Acid fast bacilli (lepra)

Two cases (2.5%) of granulomatous disorders were positive for both the special stains [AFB (TB) and AFB (Lepra)].

Table 8: Status of AFB stain in total cases:

Positive for AFB(TB)	Positive for AFB(L)	Positive for Both AFB(TB) and AFB(L)	Negative for Both AFB(TB) and AFB(L)
21	6	2	22

3.6 Granulomatous lesion of unknown aetiology:

21 cases (26.25%) of granulomatous lesion of unknown aetiology were noted. Among them, fair number of cases had been diagnosed as Granulomatous lesion (10%) and Granulomatous lymphadenitis (8.7%). Similarly, Granulomatous mastitis (2.5%) followed by one case (1.25%) each of granuloma annulare, Interstitial Granulomatous drug reaction, erythema nodosum, xanthogranulomatous lesion, was also noted. Even on special stains (ZN stain for AFB, GSM and PAS), these granulomatous lesions were found to be negative for the detection of any organisms.

Hence these cases were kept under Granulomatous lesions of unknown etiology and advised PCR. (Table 9)

Table 9: Granulomatous lesion of unknown aetiology

Granulomatous lesion of unknown aetiology	Cases	Percentage
Granulomatous lesion	8	10%
Granulomatous lymphadenitis	7	8.7%
Granulomatous mastitis	2	2.5%
Granuloma annulare	1	1.25%
Interstitial Granulomatous drug reaction	1	1.25%
Erythema nodosum	1	1.25%
Xanthogranulomatous lesion	1	1.25%
Total	21	100%

3.7 Morphological classification

This study showed that tuberculoid granuloma was the most common type of granulomas (63.75%). Tuberculoid types of granulomas were seen most commonly in tuberculosis (60.78%), leprosy (31.37%) and Crohn's disease (1.96%).

In this study, the neoplastic tumors like Hodgkin's lymphoma, Non- Hodgkin's lymphoma and squamous cell carcinoma also showed focus of granulomatous inflammation (1.96% each).

Among Tuberculoid cases (N=51), most common age group was 3rd decade (23.5%). Males (60.79%) were affected mostly than female and the most common site for tuberculoid type of GD was found to be skin and subcutaneous tissue (37.25%).

Other types of Granulomas other than tuberculoid type were Necrobiotic (12.5%), Suppurative (5%), Foreign Body (1.25%) and miscellaneous (Msc) (17.5%). (Table 10)

Table 10: Distribution based on morphological classification of GDs

Morphological subclass	No. of Cases	Percentage (%)
Foreign Body	1	1.25
Necrobiotic	10	12.5
Suppurative	4	5
Tuberculoid	51	63.75
Msc	14	17.5
Total	80	100

Single case of foreign-body giant cell granulomas revealed histiocytic reactions to refractile material. The diagnosis was given as "FB granuloma of unknown etiology". A single case of fungal infection (1.25%) was found to be revealing suppurative granuloma. Special stains like PAS and SM were negative, so the patients were advised for culture, which is a definitive tool for confirmation of causative fungus.

3.8 Types of granulomas based on caseation necrosis:

In this study caseating type of necrosis was found in 43.75% cases and non caseating type of granulomas was in 56.25% cases. Caseating type of granulomas were found in cases of tuberculosis. However non-caseating type of granulomas were seen in granuloma caused by tuberculosis, leprosy, foreign body, parasite, BCG intillation, fungus, Crohn's disease, and with GL of unknown etiology as well. However, special stain for AFB (TB) was positive with the cases having caseating type of necrosis than in cases with non caseating type of necrosis (Table 11).

Table 11: Distribution of granulomatous disorders based on necrosis

Granuloma	No. of cases	Percentage
Caseating granuloma	35	43.75%
Non-caseating granuloma	45	56.25%
Total	80	100%

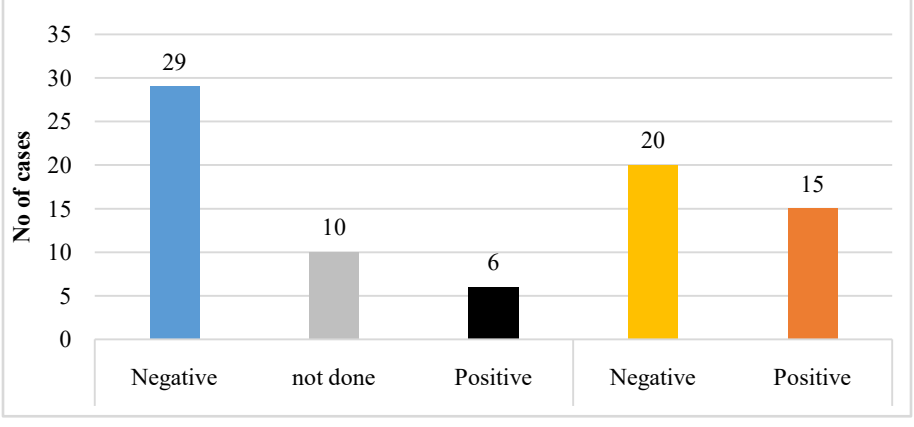

Figure 5: Status of AFB (TB) in cases with caseating granuloma

Four cases of rhinosporidiosis in nasal mucosa showed GMS and PAS positivity. (Figure 6)

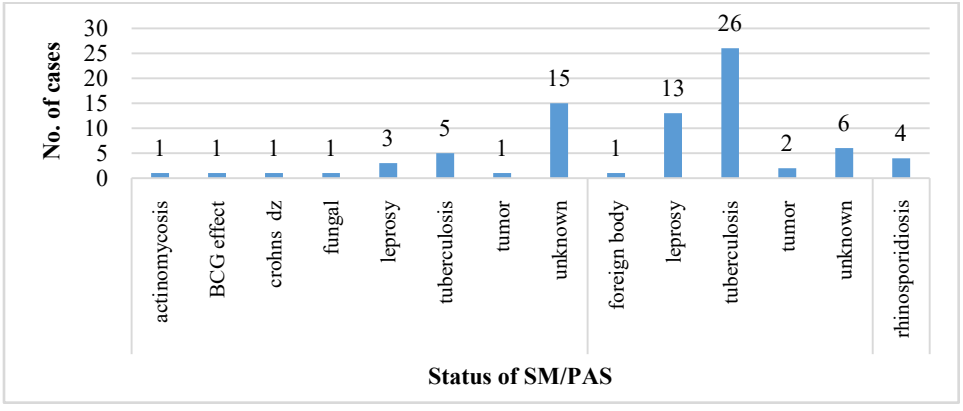

Figure 6: Status of special stain SM/PAS in granulomatous disorders.

Summarizing the result, GDs were found in age ranging from 12 years to 73 years with male to female ratio of 1.22:1. GD was common in third decade of life (25%), closely followed by fourth decade (20%). Most common site affected by granulomatous reaction was skin and subcutaneous tissue (40%) followed by lymph node (17.5%). Tuberculosis was found to be the most common etiology (38.75%) and Tuberculoid being the most common type of granuloma (63.5%).

Non-caseating type of granuloma (56.25%) was more common than caseating type. Ziehl-Neelsen stain is helpful in diagnosis of tuberculosis (positive: 61.30%). Similarly, Fite Faraco stain for leprosy was positive in 37.5%. Among leprosy cases, BTHD was found to be most common type (62.5%).

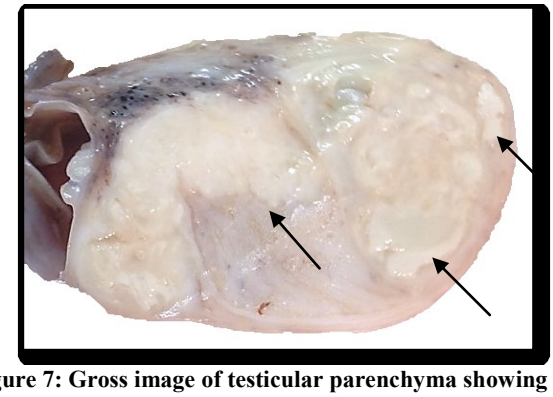

Figure 7: Gross image of testicular parenchyma showing multiple foci of grey white granular lesion of necrosis (arrows). Microscopically the lesions were of tuberculoid granuloma

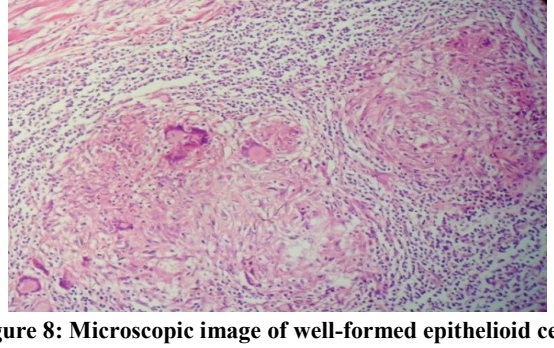

Figure 8: Microscopic image of well-formed epithelioid cells granulomas and Langhans giant cells in a case of tuberculoid granuloma. (40x, H&E stain)

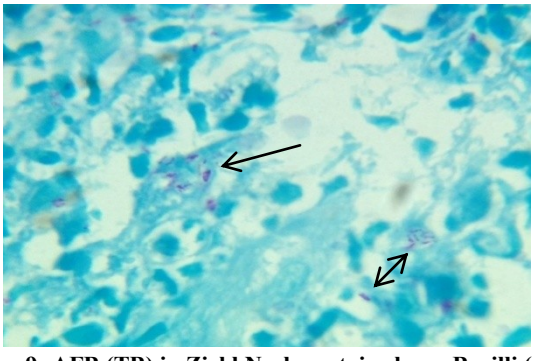

Figure 9: AFB (TB) in Ziehl Neelsen stain shows Bacilli (pink slender beaded rods, single and in clusters, shown by arrows). (1000x oil immersion)

4. Discussion

In the present study 80 cases of GDs were studied over a period of 1 year, from August 2017 to July 2018. Histopathology remains a time-tested tool for establishing a correct diagnosis like in many other diseases pertaining to various organ systems of the body. [19] So, with this study we aimed to have clear picture regarding the histopathological spectrum of granulomatous disorders in eastern part of Nepal.

4.1 Age

Various studies conducted show the distribution of granulomatous disorders commonly in 3rd decade of life. (Table 12)

In this study, majority of the patients were in the age group of 20-29 years (25%) followed by 30-39 years (20.00%) with the age range of 12 years to 73 years. This finding is similar to the various studies recorded by Babaria *et al* (29.3%), Adhikari *et al* (27.8%), Permi *et al* (23.64%), and Pawale *et al* (27.06%) (Table 11). Age group was ranged from 4 month to 85 years, 1 to 83 years, 1 to 87 years, 2 to 70 years in studies done by Barbara *et al* [17], Adhikari *et al* [12], Permi *et al* [16] and Pawale *et al* [9] respectively. So granulomatous disorders may present at any age from infancy to older age group. Mean age was 38.18 years in this study which is similar to the study done by), Permi *et al* (33.26 years) [17] and Pawale *et al* (31.26 years). [9]

Table 12: Comparison of most common age group of granulomatous reaction

Studies	Present study	Babaria *et al* [19]	Permi *et al* [17]	Pawale *et al* [9]	Adhikari *et al* [13]
Most common age group	20-29yrs (25%)	21-30yrs (29.3%)	21-30yrs (23.64%)	20-29 yrs (27.06%)	20-29yrs (27.8%)
Age range	12 to 73 years	4 month to 85 years	1 to 87 years	2 to 70 years	1 to 83 years

4.2 Sex

Granulomatous disorders can affect both males and females of all age group. In the present study, males (55%) were affected more commonly than the females (45%) with male to female ratio of 1.22:1. Similar findings were reported by Barbara *et al*, Permi *et al*, Pawale *et al* and Ahikari *et al* (Table 13).

All of above mentioned studies showed that males are more susceptible to develop granulomatous lesions of skin. However this finding was not concordant to the study by Zafar *et al* [15], a study done in Pakistan, where he found females (57.3%) to be involved more frequently than males.

Table 13: Comparison of sex distribution of granulomatous reaction

Studies	Present study	Babaria *et al* [19]	Permi *et al* [17]	Pawale *et al* [9]	Adhikari *et al* [13]	Zafar *et al* [15]
Male %	55	55.67	52.36	54.12	54.54	42.7
Female %	45	44.33	47.64	45.88	45.45	57.3
Male: Female ratio	1.22:1	1.25:1	1.09:1	1.18:1	1.2:1	1:1.36

4.3 Site

In this study most common site to be affected by GDs was skin and subcutaneous tissue (40%), followed by lymph node (17.5%). This finding is similar to the studies done by Permi *et al* (24.72%), Pawale *et al* (53%) and Babaria *et al* (35%). (Table 14)

However, in a study done by Adhikari *et al*, the most common site of granulomatous lesion was Lymph node (41.1%). (Table 14)

This may be due to fact that BPKIHS being the popular treatment centre for all types of the skin diseases among all the centres located in the eastern part of Nepal in comparison to various centres for all other disciplines. Other sites were bone and bone marrow, breast, female reproductive system and others. (Table 3)

Granuloma can mimic tumour of various organs in radiology. [38] Boo-Kyung *et al* [38] found granulomatous lesion in mastectomy specimen with the radiological impression of malignancy. So, he states that "although the imaging findings strongly suggested malignancy, preoperative histological confirmation by means of large core-needle biopsy helped prevent unnecessary radical mastectomy and led to appropriate treatment." In this study two cases of granulomas were found in testis, two in breast and one in ovary mimicking tumors radiologically and for those cases surgery was performed and later in histopathology they were diagnosed as granulomatous lesions. This also states the importance of this study to put granulomatous disorders as differential diagnosis of tumor like growth.

Table 14: Comparison of various sites for granulomatous disorders

Sites	Present study (%)	Babaria *et al* [17] (%)	Permi *et al* [16] (%)	Pawale *et al* [9] (%)	Adhikari *et al* [12] (%)
Skin and subcutaneous tissue	40	35	24.72	53	22
Lymph node	17.5	11	21.46	28.97	41.1
Bone and marrow	7.5	8.67	18.18	12.14	11.5
Gastrointestinal tract	3.75	21.33	8.00	10.28	5.5
Respiratory system	3.75	10.67	9.46	27.10	7.7

(Color code: Red: m/c, yellow: 2nd m/c, blue: 3rd m/c)

4.4 Etiological classification

Tuberculosis

The worldwide incidence of tuberculosis varies from 0.1 to 1% of all cutaneous conditions.[7] In this study, among all the cases of GDs, Tuberculosis was found to be the most common etiology (38.75%) which is similar to the studies done by Permi *et al* (47.26%), Pawale *et al* (49.41%), Adhikari *et al* (61.7%) and Babaria *et al* (56.75%). (Table 14) Tuberculosis is much common in our population in spite of the active immunization programme through BCG vaccination (93% coverage). [39] So, our study support a study which states the use of BCG vaccine has been limited because a) its effectiveness in preventing infectious forms of TB is uncertain and b) the reactivity to tuberculin that occurs after vaccination interferes with the management. With emergence of anti-tuberculosis drug-resistant strains and AIDS epidemic, there has been a worldwide rise of tuberculosis in the recent years more so ever in poverty-struck areas of the world due to poor nutrition, poverty, non-availability of diagnostic aids and treatment, overcrowding and ignorance about the disease. [40].

Table 15: Comparison in relation to etiological classification

Cause	Present study	Babaria *et al* [19]	Permi *et al* [17]	Pawale *et al* [9]	Adhikari *et al* [13]
Tuberculosis	38.75%	56.75%	47.26%	49.41%	61.7%
Leprosy	20%	17.67%	12.72%	17.65%	0.5%
Parasite	5%	0.33%	1.45%	-	0.7%
Tumor	3.75%	-	5.83%	-	0.2%
Unknown etiology	26.25%	11.34%	8.0%	-	28.9%
Foreign body granuloma	1.25%	12.67%	8.36%	14.12%	1.7%
Fungal	1.25%	1.33%	8.73%	5.88%	3.1%
Crohn's disease	1.25%	-	-	-	0.5%
Actinomycosis	1.25%	0.33%	1.45%	1.18%	-
BCG instillation	1.25%	-	-	-	0.2%

In a study done by Adhikari *et al* [13], ZN stain demonstrated AFB (TB) only in 11.11% cases. However, AFB (TB) demonstrated in a study done by Permi *et al* [17] was 20.74%, 22.62% in a study of Pawale *et al* [9] and 71% in a study of Krishnaswamy *et al* [41]. In this study the overall Acid Fast Bacilli [AFB (TB)] positive cases were seen in 61.30%. Looking for AFB in ZN staining of paraffin block slide is a time consuming and laborious procedure. But if AFB is positive, diagnosing the case would be easier. So, this study highlights the importance of AFB staining in the diagnosis of granulomatous disorders.

Table 16: Comparison of results of Ziehl-Neelsen stain [AFB (TB)] in Tuberculosis cases

Studies	AFB (TB) Positive (%)	AFB (TB) Negative (%)	Total (%)
Present study	19(61.30)	12(38.70)	31 (100)
Babaria *et al* [19]	35(20.71)	134(79.29)	169(100)
Permi *et al* [17]	27(20.76)	103(79.23)	130(100)
Krishnaswamy *et al* [41]	91(71.09)	37(28.90)	128(100)
Jayalaxmi *et al* [42]	29(49.15)	30(50.84)	59(100)
Adhikari *et al* [13]	10(11.11)	80(88.88)	90(100)
Pawale *et al* [9]	19(22.62)	65(77.38)	84(100)

Granulomatous Lesion of Unknown etiology: The granulomatous inflammatory response is ubiquitous in pathology, being a manifestation of many infective, toxic, allergic, autoimmune and neoplastic diseases and also conditions of unknown etiology. [19]

Some granulomas remain unexplained even with ancillary studies and in these instances, good clinical history and clinic-pathological correlation are essential in making a final diagnosis.[13] 21 cases (26.25%) of granulomas of unknown aetiology were noted which is similar to the study done by Adhikari *et al* (28.9%). [13]

Fair number of cases in this study has been diagnosed as Granulomatous lesion [N=8, (10%)] and Granulomatous lymphadenitis [N=7, (8.7%)]. Similarly, Granulomatous mastitis [N=2(2.5%)] followed by one case (1.25%) each of granuloma annulare. Interstitial Granulomatous drug reaction, erythema nodosum and xanthogranulomatous lesion, were also noted. The exact etiology could not be identified in these cases, even on special stains like ZN, SM and PAS. These cases were finally diagnosed as granulomatous lesions and kept under category of granulomatous lesions with unknown etiology. It may be due to limited special stains and unavailability of PCR or ISH like techniques. The use of auramine / auramine-rhodamine using fluorescence technique, in-situ hybridization and real- time PCR may provide specific diagnosis. [13]

Leprosy: In this study, out of 16 total leprosy cases, BTHD was found to be the m/c sub type of leprosy (62.5%). This finding is similar to the study done by Gautam *et al* (47.6%) [7] and Manandar *et al* (40%) [43] and Jha *et al* (38.88%) [21]. In a study conducted by Gupta *et al* [26], majority of the cases (32.38%) of Leprosy were of Borderline tuberculoid type followed by Tuberculoid Type (TT). However in this study Tuberculoid Type (TT) and Lepromatous (LLHD) comprise of 6.25% each and cases mid-borderline (BB) and ENL comprise of 12.5% each. This might be due to small number of leprosy cases in this study as Nepal is in elimination phase of Leprosy since 2010. Histioid leprosy were found to have no contribution, which is the least common type in other similar studies. In a study by Gautam *et al* [7], least common were histioid leprosy and Hansen's disease without clinical sub-classification (1 case each, 1.6%).

AFB (Leprae) was found to be positive in six cases (37.5%) of leprosy in this study. This finding was concordant to the study done by Babaria *et al* (20.75%), Permi *et al* (25.72%) [17], Nayak *et al* (44.64%) [44] and U. Manandhar *et al* (25%) [43]. (Table 17). Out of six AFB (Lepra) positive cases, three cases were of BTHD; rest three is of BB, ENL and LL each. Bacteriological index (BI) was found to be ranged from 1 to 5 in this study. BI of 5 was found in one case of LLHD and in one BTHD case. So, bacteriological index was highest, upto 5+ towards lepromatous pole and lowest towards Tuberculoid pole having 0, which is similar to the study done by Jha *et al*. [21] Any case of leprosy being detected is good news for that individual. This will help in early treatment as well as prevent the handicapped population as a complication of leprosy.

Table 17: Comparison of results of Fite Faraco stain [AFB (Lepra)] in Leprosy

Studies	Fite Faraco stain Positive (%)	Fite Faraco stain Negative (%)	Total
Present study	37.5	62.5	100%
Babaria et al [19]	20.75	79.25	100%
Permi *et al* [17]	25.72	74.28	100%
Nayak *et al* [44]	44.64	55.36	100%
Manandhar *et al* [43]	25	75	100%

Other cause for granulomatous disorders in this study are Parasitic infection (5%) in which Rhinosporidiosis was identified as parasite, Foreign body granuloma (1.25%), Fungal (1.25%) and some rare cases, which were associated with BCG effect, Crohn's disease, tumor and actinomycosis, and were found to have little contribution in this study.

In this study, Hodgkin's lymphoma, Non-Hodgkin's lymphoma and squamous cell carcinoma were seen co-existing with granulomatous inflammation as well. According to a study done by Bhatia *et al* [34] certain neoplasms are known to be associated with a granulomatous response in the parenchyma e.g. Hodgkin's disease and non-Hodgkin T cell lymphomas, seminoma of the testis, renal cell carcinoma, nasopharyngeal carcinoma and ovarian dysgerminoma. In other cases the granulomatous inflammation may be found in the lymph nodes draining the primary tumour (sarcoid reaction or sarcoid like lymphadenopathy). It has been observed in many other malignancies e.g. breast carcinoma, gastric, colonic and laryngeal cancer etc. Sometimes a co-incidental association of a systemic granulomatous disease and a malignant neoplasm may be the cause. [34]

In a study done by Permi *et al* [17] granulomas were seen in squamous cell carcinoma of skin, infiltrating ductal carcinoma of breast, papillary carcinoma of thyroid gland, dysgerminoma, Hodgkin lymphoma, seminoma, ameloblastoma and benign cystic teratoma. In this study, two biopsy specimens were of cervical lymphnodes. One of them was diagnosed as "Necrotizing granulomatous lymphadenitis, possibly tubercular in a case Well-differentiated Squamous cell carcinoma of floor of mouth". Another was diagnosed as Classical Hodgkin's Lymphoma, Nodular Sclerosis- Syncytial variant. Next case was of Bone marrow biopsy along with cervical lymph node diagnosed as "Non-Hodgkin's lymphoma", in which advice for immunohistochemistry was given for confirmation.

Morphological classification: In this study, Tuberculoid granuloma was found to be the most common type of granulomas (63.75%) which is similar to the studies done by Pawale *et al* (57.65%)(9), Aoun *et al* (43.2%) [14] and by Gupta *et al* (80%) [26].

In this study, Tuberculoid type of granulomas was seen most commonly in tuberculosis (60.78%), leprosy (31.37%), tumors (5.88%) and Crohn's disease (1.96%). If skin cases were taken into account, most common type of granuloma was Tuberculoid (65.51%), similar to the study done by Zafar *et al* [15] in GL of skin, where 92.7% showed tuberculoid granulomas. Similarly, in a study done by Gautam *et al*[7], out of total 106 cases of GL of skin, 73 (68.9%) showed tuberculoid type of granulomas.

In our study, tuberculosis was the most common aetiology of GDs in which tuberculoid type of granulomas was resent. Possibly this could be the reason for Tuberculoid being the most common type of GDs. Other types of Granulomas were Necrobiotic (12.5%), Suppurative (5%), Foreign Body (1.25%) and miscellaneous (17.5%).

Necrobiotic granulomas contributed less in this study and were found in Granuloma annulare and xanthogranulomatous lesion (N=1, 10% each) and Necrotizing granulomatous lesions (N=8). This finding is similar to the study done by Zafar *et al* [15], where one case (0.8%) of Necrobiotic granuloma was a case of granuloma annulare. Suppurative type of granulomas were the cases of rhinosporidiosis, fungal granuloma, Granulomatous lymphadenitis with panniculitis and suppurative GL (25% each). In this, fungal etiology had contribution similar to the study done by Zafar *et al* [15], in which 2 cases of suppurative granulomas were suggestive of aspergillosis and chromoblastomycosis.

Single case of foreign body granuloma (1.25%) was present in this study which is similar to the study done by Adhikari *et al* [13], in which there was 1.7% of foreign body granulomas.

Other types of granulomas were present which did not fit in the classification of granulomas were taken as miscellaneous type (17.5%), e.g. Interstitial Granulomatous drug reaction, Granulomatous cystitis, Actinomycosis, BCG instillation, erythema nodosum, rhinosporidiosis, and granulomatous lesions. In a study by Gupta *et al* [26], 3.8 % of granulomatous lesions were kept under miscellaneous category, 1 case (0.95%) each of Cryptococcus and Histoplasmosis along with 2 cases (1.90%) showing granulomatous reaction pattern not fitting into any of the above mentioned categories and no identifiable etiology despite diligent search. He stated that, it might have resolved the miscellaneous category if large sample size was studied. [(26)]. Similar reason can be applied to this study as well.

Types of granulomas based on necrosis: Necrosis refers to dead cells that, under the microscope, appear as a mass of formless debris with no nuclei present. A related term, "caseation" (literally: turning to cheese) refers to a form of necrosis that, to the unaided eye (i.e., without a microscope), appears cheese-like ("caseous"), and is

typically (but not uniquely) a feature of the granulomas of tuberculosis. The identification of necrosis in granulomas is important because granulomas with necrosis tend to have infectious causes. [29]

In this study caseating type of necrosis were found in 43.75% of GDs. Caseating type of granuloma was found to be more in the study done by Babaria *et al* (54.67%) but non-caseating type of granulomas were found to be the most common type of granulomas (56.25%) in this study. Similarly, in a study done by Zafar [15], 79 cases (69.3%) of tuberculoid granulomas showed caseating and 35 (30.7%) showed non-caseating type of granulomas. (Table 18).

In this study, special stain for AFB (TB) was more commonly found positive in granulomatous inflammation with caseation necrosis. 15 out of 21 AFB(TB) positive cases were associated with caseation necrosis (71.42%) which is similar to a study done by Majeed *et al* (93%)[46] and Ahmed *et al* (68.7%).[47]

Table 18: Comparison of Granulomatous reaction based on caseation necrosis

Study	Caseating granuloma	Non-Caseating granuloma	Total
Present study	35(43.75%)	45(56.25%)	80(100%)
Barbaria *et al*[19]	164(54.67%)	136(45.33%)	300(100%)
Zafar *et al*[15]	79(69.7%)	35(30.7%)	114(100%)

Other Special stains (GMS and PAS): The histochemical stains commonly used for the pathological evaluation of infective organisms are the GMS and PAS stain for fungi. The PAS stain is also a useful histochemical stain for fungi. The PAS stain can detect the cell walls of living fungi, whereas the GMS stain detects the cell walls of both living and dead fungal organisms. [48]

In this study, all the four cases of rhinosporidiosis in nasal mucosa showed GMS and PAS positivity. Thick-walled sporangia and endospores stain positively with various special stains like PAS, GMS (as used in our study), mucicarmine, Grocott's stain, etc.

A study done by Hussein *et al* [49] also says that the organism can also be identified with fungal stains such as GMS, PAS as well as with standard hematoxylin and eosin (H&E) staining.

Limitation and future scope of this study

In this study, 21 cases (26.25%) of granulomas of unknown aetiology were noted. This may be due to limited special stains and unavailability of PCR or ISH like techniques. BPKIHS is a tertiary level health centre. Services like immunohistochemistry, PCR and conventional karyotyping are still lacking. Time has come for introduction of new techniques. It is an utmost need of pathologist, clinicians and institute. Cooperation between clinician and pathologist is more important in diagnosing granulomatous disorders if the patient is to derive the greatest benefit from the biopsy.

This study reflects the high contribution of tuberculosis in granulomatous lesions in eastern part of Nepal. With further study, various relative and absolute histopathologic criteria with clinical, bacteriologic parameters can be established to diagnose tuberculous granuloma.

Further study on granulomatous lesion to identify the tuberculosis load and treatment effect is very important. This is helpful to allocate preventive programs in this region of Nepal.

5. Conclusion

This study was able to determine the aetiology and spectrum of granulomatous disorders in eastern part of Nepal.

Present study confirms that the most common cause of granulomatous disorder is tuberculosis and most common affected site is skin and subcutaneous tissue. Our study being a descriptive type of study about granulomatous disorders in relation to age, sex, site and aetiology, the information is useful in management of granulomatous disorders and also for preventive measures.

With increasing level of awareness of disease and in health seeking behaviour the number of biopsies related to granulomatous disorders are on rise day by day.

Biopsy is easy to obtain and an economic procedure and gives us an idea of the underlying disease process. So, any patient with granulomatous disorders of longer duration should be subjected to biopsy for a definite diagnosis and treatment.

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