



Research Article

Granulomatous Inflammation: A Comparative Study Using Special Stains on FNAC Smears

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Abstract

Background: Tuberculosis is the most common infectious disease in the developing countries. In spite of newer modalities for diagnosis and treatment million of people are still suffering and dying from this disease. Many diagnostic tests are devised for its detection including FNAC. This study was designed to discuss the role of Fine Needle Aspiration Cytology using special stains in classifying and diagnosing the cause of granulomatous inflammation in patients diagnosed to have granulomatous inflammation on FNAC.

Materials and Methods: A descriptive cross-sectional survey was done on 200 cases of granulomatous inflammation consistent with tuberculosis diagnosed on fine needle aspiration cytology at the Department of Pathology, of Cure Surgical Hospital & Research Center, Solan, H.P, India.

All FNAC aspirates showing granulomatous inflammation on light microscopy with Hematoxylin & Eosin staining were subjected to special stains, like ZN, GMS, and PAS. Cases positive for AFB on ZN stain and fungus on GMS/PAS were noted down along with their frequency and percentages.

Results: One hundred and four cases (52%) of AFB positive smears were reported in granulomatous inflammation while only 4% cases of fungus were reported down. Cervical lymph nodes were the most commonly involved site (81%), and females were affected more (55%) than males. Most cases of AFB-positive smears were associated with caseation necrosis (96%).

Conclusion: Special stains should be done on all granulomatous inflammation cases seen on FNAC for confirmation of TB and ruling out other infectious causes.

Introduction

Tuberculosis is one of the most ancient disease of mankind and has co-evolved with human for many thousand of years or perhaps for several million years ^[1].

The global burden of TB remains enormous, mainly because of poor control in S-E Asia, sub-

Saharan Africa and Eastern Europe and because of high rates of Mycobacterium TB & HIV coinfection in some African countries ^[2].

In spite of newer modalities for diagnosis and treatment of TB, Unfortunately, millions of people are still suffering and dying from this disease. TB is one of the top 3 infectious killing disease in the

world: HIV/AIDS kills 3 million people each year, TB kills 2 million people each year and malaria kills 1 million ^[3].

According to WHO, TB is a world wide pandemic. Among the 15 countries with the highest estimated TB incidence rates, 13 are in Africa, while half of all new cases are in 6 Asian countries viz. Bangladesh, China, India, Indonesia, Pakistan and Philippines ^[3].

A WHO fact sheet dated March 2010 on TB stated that overall one third of the world's population (over 2 billion) is currently infected with the TB bacillus ^[3].

The average prevalence of all forms of TB in India is 5.05/1000 and prevalence of smear positive cases is 2.27/1000 ^[4].

Tuberculosis (TB) carries a high risk of morbidity and mortality. TB has widespread involvement and rarely any tissue or organ is not involved by it. Most common is the pulmonary involvement ^[5] which has caused numerous deaths in the past. It can also involve the appendix ^[6], small and large intestine ^[7], skin ^[8], soft tissues, lymph nodes ^[9], genitourinary tract ^[10], and brain ^[11]. The dilemma does not end here and many other unusual organs are also involved ^[12].

The histology of TB is characteristic showing granuloma formation by epithelioid histiocytes and Langhan's type of Giant cells with or without caseation necrosis. This pattern is also preserved in cytology specimens. ^[13] Infectious causes most notably presenting with granulomatous inflammation is Mycobacterium Tuberculosis with a reported frequency of 59.4% ^[13] and fungal causes ^[14, 15] with a reported frequency of 20.4% ^[16].

Other common causes include Sarcoidosis ^[17], Wegener's granulomatosis ^[18], Actinomycosis ^[19], Crohn's diseases ^[20], Histoplasmosis ^[21], foreign body, and Langerhans cell histiocytosis ^[22]

Pertaining to a broad differential diagnosis, the diagnosis of tuberculosis remains a challenge. History and clinical examination are always very important and helpful. Many diagnostic tests are in practice. Every test has its own sensitivity and specificity and limitations. The commonly performed tests include examination of sputum for

Acid Fast Bacilli ^[23], Cultures for Mycobacterium tuberculosis ^[24], Fine Needle Aspiration Cytology (FNAC) ^[25], Biopsy and PCR ^[26]. Fine Needle Aspiration Cytology is a minimally invasive and time-saving procedure, which helps in the diagnosis of number of diseases especially in palpable nodules of breast, lymph node disorders ^[27], thyroid ^[28], and palpable skin and subcutaneous nodules. It has become very popular now-a-days among physicians and surgeons because of its many benefits.

In clinical practice, it helps them to reach a diagnosis or at least plan beforehand the proper management of the patient. As we have already discussed that granulomatous inflammation is not diagnostic of TB, many others causes must be ruled out before starting ATT. However, in the clinical scenario if a patient is diagnosed as granulomatous inflammation, then antituberculous treatment (ATT) is started at the first point in our setup. Statistically this behavior may be right but this is not in accordance with the reality. We come to encounter cases which have taken ATT for at least 9 months but still these symptoms persist. Reassessment is done, and later the patient is diagnosed as suffering from fungus, sarcoidosis, or some other granulomatous disease.

Some special stains are very helpful in this regard, like Gomori Methenamine silver stains (GMS), Giemsa stain, Periodic acid Schiff (PAS), and Ziehl Neelson's stain (ZN stain) ^[29].

In the present study, granulomatous inflammation consistent with Tuberculosis diagnosed on FNAC will be analyzed using special stains like ZN (Ziehl Neelson's) and GMS (Gomori Methenamine Silver) stains. This will help to confirm tuberculosis in cases which will be positive for Acid Fast Bacilli on ZN staining. Positive GMS/PAS staining will confirm the fungal causes of granulomatous inflammation including Mucormycosis, Blastomycosis, Cryptococcosis, and Candidiasis. There is a limitation of this study that not all causes of granulomatous inflammation can be ruled out since the ancillary investigations needed to diagnose them are not available in our setup.

The rationale of this study is that cases diagnosed wrongly as TB can turn out to be fungus and can be picked by GMS/PAS. These patients can thus be saved from long painful and harmful side effects of expensive ATT (Antituberculous therapy).

Another important point of concern is that :In India, it is felt that the phenomenon of MDR-TB is on the rise and is bound to reach much more menacing proportions^[30]. Hence it is important to curb the overusage of ATT which could be one of the causes of MDR-TB.

These benefits prove the usefulness of this study, and this would be further reaffirmed by those who have experienced taking ATT for 9 months in their life without having TB. On ZN staining, the positive AFB cases would help the physicians to start treatment of TB, very confidently. Moreover, the work done to assess the frequency of different infectious agents in granulomatous inflammation especially fungal causes is very old, and this study would bridge a gap between newer studies done on this topic.

Material and Methods

2.1 Setting: The study was conducted at Pathology Department of Cure Surgical Hospital & Research Center, Solan, H.P., India.

2.2. Duration: One Year.

2.3. Sample Size: Sample size of 200 cases was calculated with 95% confidence level, 8% margin of error, and taking expected percentage of positive cases of fungus on GMS/PAS that is, 20.4% in diagnosed cases of granulomatous inflammation.

2.4. Sampling Technique: Nonprobability purposive sampling.

2.5. Inclusion Criteria

- (1) Cases diagnosed as granulomatous inflammation on FNAC consistent with tuberculosis as per operational definitions.

- (2) Cases in which FNAC was done on Lymph nodes, skin swellings, subcutaneous swellings, and Breast lumps.

2.6. Exclusion Criteria

- 1) Pyogenic inflammation observed on microscopy as extensive neutrophilic infiltration.
- 2) Acellular smears/smears with crushed morphology or poorly stained slides will be excluded.
- 3) Previously diagnosed cases and cases already getting ATT.

2.7. Study Design: Descriptive cross-sectional survey.

2.8. Operational Definitions

2.8.1. Granulomatous Inflammation: It is defined on cytology as aggregates of epitheloid cells forming a granuloma with or without necrosis. Sometimes multinucleated giant cells are also seen.

2.8.2. Positive for AFB: On ZN staining the acid fast bacilli would be labeled when pink, beaded, and rod-shaped organisms are found in smears against a blue background after comparing with control samples.

2.8.3. Positive for Fungus: On GMS staining, presence of black colored septated or nonseptated hyphae (depending upon the species of Fungus) or spores against a greenish background would be labeled as positive for fungus. On PAS stain, presence of red- or purple-colored septated or nonseptated hyphae or spores would be labeled as positive for fungus.

2.9. Data Collection Procedure: Patients fulfilling inclusion and exclusion criterion were selected from Fine needle aspiration cytology specimens received during the study period. After informed consent of patients and noting down the demographic data, the *Hematoxylin* and Eosin

staining was done. Two extra unstained slides were smeared from aspiration material. One slide was stained by GMS stain. Some cases were stained with Periodic Acid Schiff stain (PAS), similarly second unstained slide was stained with Ziehl Neelson's stain.

Table -1 Distribution of age of Patients

Age	Group	Granulomatous	% of
1-10		6	3
11-20		30	15
21-30		80	40
31-40		40	20
41-50		30	15
51-60		10	5
61-70		2	1
71-80		2	1
Total Mean =31.8		200	100

Table -2 Distribution of gender of patients

Gender	Frequency	Percentage (%)
Male	110	55
Female	90	45
Total	200	100

Table- 3 Frequency of Positive smears of Acid fast Bacilli

AFB	Frequency	Percentage (%)
Positive for AFB	104	52
Negative for AFB	96	48
Total	200	100

Table-4 Frequency of Fungus and Granulomatous Inflammation

Fungus	Frequency	Percentage (%)
Negative for Fungus	192	96
Positive for Fungus	08	4
Total	200	100

Table-5 Distribution of Lesions according to site of FNAC

Site of FNAC	Frequen cy	Perce ntage	Valid Percentage	Cummulative Percentage(%)
Cervical L.N.	146	73	73	73
Pre-auricular L.N.	10	5	5	78
Super clavicular L.N.	16	8	8	86
Axillary L.N.	12	6	6	92
Inguinal L.N.	10	5	5	97
Skin/subcutane ous lesion	2	1	1	98
Other sites	4	2	2	100
Total	200	100	100	

Table-6 Relationship of AFB with Caseation Necrosis

Caseation necrosis	Acid Fast Bacilli		Total
	Negative for AFB	Positive for AFB	
Not Present	60	2	62
Present	42	96	138
Total	102	98	200

Table-7 Frequency of Caseation Necrosis with Fungus

Caseation Necrosis	Fungus		Total
	Negative	Positive	
Not Present	58	4	62
Present	134	4	138
Total	192	8	200

Table-8 Frequency of Acid Fast bacilli positivity with Giant cells

Giant cells	Acid Fast Bacill		Total
	Negative For AFB	Positive For AFB	
Not Present	104	46	150
Present	16	34	50
Total	120	80	200

These smears were examined under the light microscope by a histopathologist. The findings of Hematoxylin and eosin staining were categorized as epitheloid granuloma with necrosis and epitheloid granuloma without necrosis. The finding of ZN staining was labeled as positive for AFB or negative for AFB. The finding of GMS was recorded as positive for fungus or negative for fungus.

2.10. Data Analysis: Data was analyzed by IBM SPSS Statistics.

Age of patient was presented as mean and standard deviation.

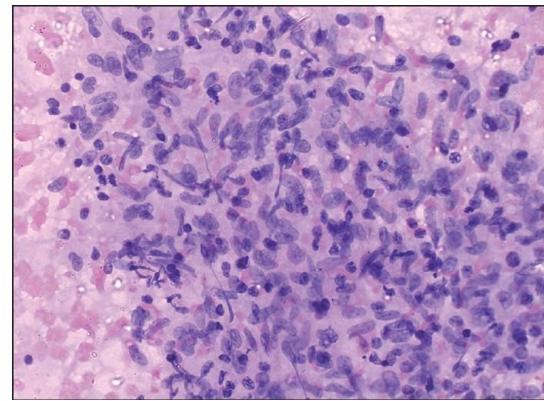
Gender, positive cases of AFB, and positive cases of fungus were presented as frequency and percentages.

Results

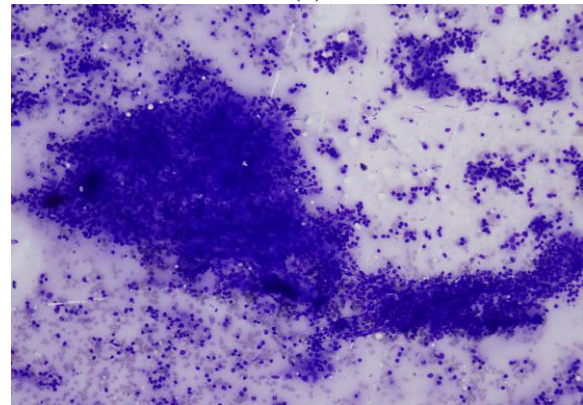
Two hundred patients of granulomatous inflammation diagnosed on FNAC were taken. Granulomas were described as comprising of pale staining epithelioid cells which were round to oval to spindle against an eosinophilic background (Figure 1a). Few degenerated epithelioid histiocytes were also seen in long-standing mycobacterial infection with caseation necrosis in the background (Figure 1 a,b,c). Some cases showed Langhans type of Giant cells (Figure 1 d). On Ziehl Neelson's staining, mycobacterium tuberculosis appeared as red/pink beaded rod-shaped bacteria against a blue background (Figures 2(a) and 2(b)). On PAS staining, fungus appears as purple hyphae which were segmented or nonsegmented depending on the species. Few spore forms with budding were also seen (Figure 3b). On GMS stain, fungal hyphae appeared as black-colored forms which showed segmentation and some were nonsegmented (Figure 3a).

In this study, 58% patients were below 30 years of age (Table 1). Mean age was 31.8 with Females were affected more (55%) than males (table 2)

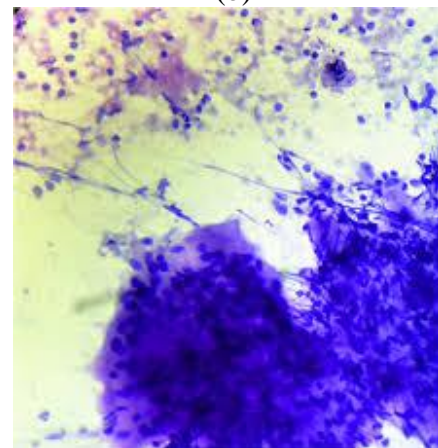
104 out of 200 patients of granulomatous inflammation are positive for AFB (table 3). There was an association between AFB positivity and caseation necrosis. we have found 96 out of total 98 AFB positive cases (97.9%) with caseation necrosis (Table 6), while 50% cases of fungus were related to caseation (Table 7). No definite relationship was seen between AFB and giant cells since 34 out of total 80 AFB positive cases were seen with caseation while rest 57.5% were without giant cells. Another finding was the involvement of specific lymph nodes regions. In 81% of cases, the most commonly involved group of lymph nodes was cervical lymph node (combining cervical and supraclavicular lymph nodes). If pre auricular lymph nodes were included, then in 86% of cases the head and neck was the primary site of TB involvement (Table 5).



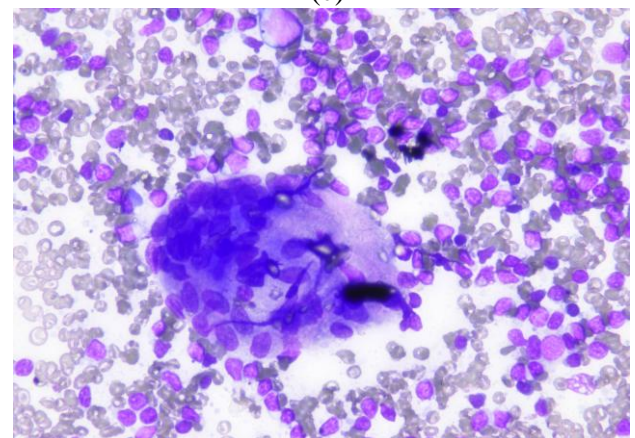
1 (a)



1(b)



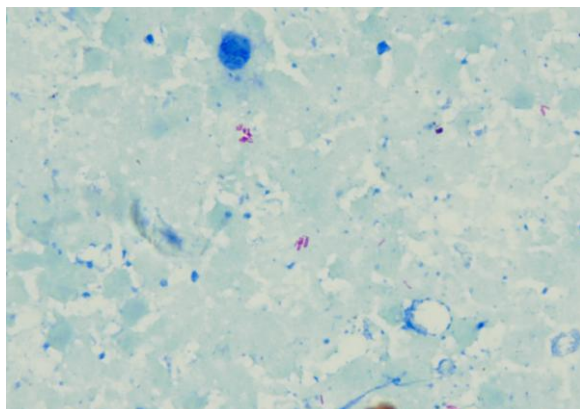
1(c)



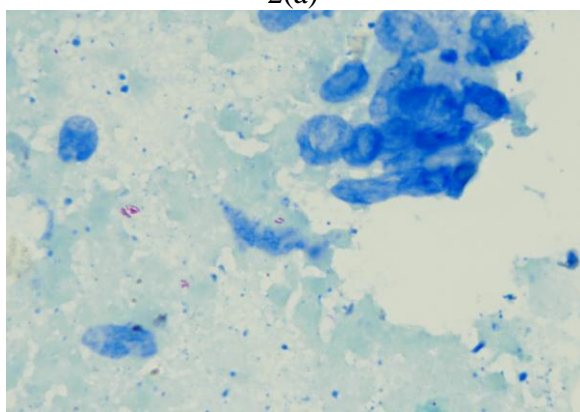
1(d)

Figure 1: (a) Photomicrographs showing granuloma with caseation necrosis (H & E stain, 400x), (b) and (c) Granulomas comprising of

epithelioid histiocytes with caseation necrosis (H & E stain, 200x), (d) Giant Cell in Granulomatous inflammation on FNAC (H & E stain, 200x)

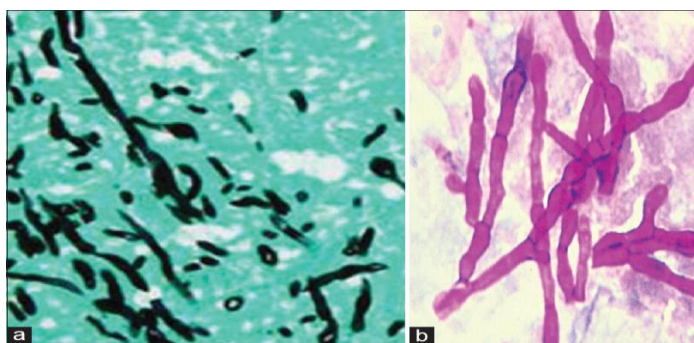


2(a)



2(b)

Figure 2: Photomicrographs in (a) Acid Fast Bacilli (Ziehl Neelson's stain, 400x), (b) Pink beaded rod against a blue background (ZN stain, 400x).



3(a)

3(b)

Figure 3 (a) Black fungal hyphae against greenish background (GMS, 400x). (b) Septated fungal hyphae and budding spore (PAS, 400x)

Discussion

Early and precise diagnosis together with effective TB treatment is the mainstay to prevent morbidity and mortality due to tuberculosis. A confirmed diagnosis of TB can only be given on isolating the

M. tuberculosis or finding specific DNA sequence of the bacteria in aspirates. In developing countries, these tests are not within the reach of common man.

In these countries, cost-effective techniques for example, sputum smear microscopy and morphological features on FNAC smears are the most reliable and cost effective for the diagnosis of TB. In cases of extra pulmonary tuberculosis, fine needle aspiration cytology (FNAC) is a very useful and reliable test. Granulomatous inflammation is the common histological presentation of tuberculosis. However, there are many other infectious and noninfectious causes which can lead to granulomatous inflammation. Another common and important infectious cause of granulomatous inflammation is Fungal infection. In the present study, we tried to differentiate between granulomatous inflammation caused by TB and fungus, by using special stains. Now a days, FNAC is most important and reliable tool in diagnosing safely the superficial lesions, viz. lymph nodes, skin, soft tissue nodules and breast lumps. In our study, 194 (97%) cases were from lymph nodes. Many studies have diagnosed TB by FNAC of lymph nodes^[9,13,28,31,32]. Cervical group of lymph nodes was the most common site of involvement in studies followed by axillary lymph nodes^[13,23]. Our study was also consistent with above studies in terms of cervical lymph node involvement (81%) as the most common anatomic site of granulomatous inflammation. Axillary group of lymph nodes were involved in 6% cases in our study and was the second most commonly

Involved which is in accordance with study of Bezabih et al^[13] where axillary group of lymph nodes were involved in 20% cases. Female gender was a slightly more affected (55%) in current study and was in concordance with other studies^[28]. However, there was slight male predominance in a study of Bezabih et al.^[13]. Out of 200, 58% patients in this study were of 30 years of age or below. This finding was in accordance with Bezabih et al, in which 69% were below 30. Based on the facts, it can be inferred that

tuberculosis was more commonly seen in young population^[13].

Two cases of granulomatous inflammation was from skin (Table 5). Older studies from our country have also discussed this aspect^[33]. Numerous morphological variations in the granulomatous inflammation are seen. There were 69% cases with necrosis. The rest (31%) of cases were granulomatous inflammation without necrosis. International data also supports this findings and studies tried to correlate morphological findings with the AFB staining^[13,34].

The Acid Fast Bacilli positivity was labeled after finding red or pink rod-shaped bacteria with beaded appearance (Figures 2(a) and 2(b)). Regarding AFB positivity variable, results were seen and frequency ranges from 10% to 70%^[28,29,33,34].

In current study, out of 200 cases, 96 cases were positive for AFB (48%). This was in concordance with the international data of a large-scale study of 328 cases, out of which 152 cases (46.4%) were positive for AFB^[23].

Similarly, our findings agree with Lau et al. who report 47% sensitivity for tuberculous abscess cases^[28] and with Das et al. showing overall 45.8% rate of AFB positivity^[35]. A recent study conducted in our country shows an overall 54% AFB positivity^[36]. Some studies report higher frequency of AFB positivity than ours like Bezabih et al. reported 59.4% of overall AFB positivity^[13], and Vignesh et al. reported 53.3% sensitivity for single AFB smear^[29].

In regions where TB is very common, the morphological findings of granulomatous inflammation is consistent with tuberculosis^[33,34]. India comes under this category along with other countries like Ethiopia, Pakistan, Bangladesh and other African countries. Since epithelioid granulomas, caseation necrosis, giant cells, and AFB positivity are specific for TB, so in these countries excision biopsy can be avoided and antituberculous treatment can be given straightaway^[28]. Excision is not free of complication and is expensive and time consuming, thus it can delay

the treatment. Above findings conclude that FNAC with special stains can solely help the physician to start the treatment.

Another important feature we found that caseation necrosis was more commonly associated with AFB. In our study 96 out of 98 AFB positive cases were associated with caseation necrosis. This finding is consistently seen in previous studies^[13,23,34].

One interesting finding derived from our study was that the acid fast bacillus was usually found extracellularly in areas of microscopic degeneration, within or at the periphery of the granulomas. The morphology of these bacilli was short and stumpy rods with red beaded appearance. These findings correlated with those given by Rajasekaran et al.^[37] and Ahmad et al.^[23]. For early lesions of tuberculous lymphadenopathy, there is no evidence that chemotherapy (ATT) plus excision is superior than chemotherapy alone^[28]. Moreover, the excision biopsy in tuberculous lymph nodes is hazardous since it may cause sinus formation. Therefore, FNAC finding of granulomatous inflammation and detection of AFB would be very specific and help the physicians to start ATT confidently, immediately as it is cost effective and economical. The special stains GMS and PAS were used to detect the fungus, since it may present with same morphology as TB^[16,19,38]. In this study, we found 4% cases of fungus presenting with granulomatous inflammation. After extensive search of the literature, only one study was found in which 20.4% cases of fungus occurred among 245 subjects^[16]. Yet many other studies discussed fungus as a cause of granulomatous inflammation and published them as case reports^[16,38-41]. But these studies did not mention frequency or percentage of positive case of fungus. In this regard, the present study would bridge a gap and may become a source of future reference for further studies in this aspect. The main benefit we gained from this study was that these patients were diagnosed morphologically as "consistent with tuberculosis". However, the results via special stains established that it can be caused by

fungus and not only by mycobacterium tuberculosis. Many benefits of differentiating between granulomatous inflammation due to TB and due to other causes would be :

- a) Timely and precise treatment of the main cause.
- b) The patients with fungal infection would be saved from long ATT treatment and its harmful side effects. They can get antifungal treatment, and the disease can be cured.
- c) Another useful benefit of this study is that over diagnosis of TB and overuse of ATT in cases of granulomatous inflammation can be prevented as MDR-TB is on the rise. In India, it is felt that the phenomenon of MDR-TB is on the rise and is bound to reach much more menacing proportions^[30].

Limitation of Study

- (1) This study does not include comparison with histology and other microbiological detection methods like culture and PCR, because of cost and unavailability issues.
- (2) Our study did not comment on all the possible differential diagnosis of granulomatous inflammation, which requires sophisticated techniques and tertiary care laboratory services which are currently not available in our setup.

Conclusion

In present scenario the FNAC plays an important role in diagnosing the superficial swellings of the body and if granulomatous inflammation is seen on FNAC we must subject it to special stains like ZN, GMS, and PAS, as it will help to differentiate between many infectious causes of granulomatous inflammation and its accurate diagnosis and timely treatment.

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