Original Article

Fine Needle Aspiration Cytology of Granulomatous Inflammation with Special Attention to Tuberculosis

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ABSTRACT

Background: Granulomatous inflammation is a special type of chronic inflammation due to a wide range of etiologic factors involving almost all organs of the body. Newer techniques (like Xpert gene test by polymerase chain reaction) are being developed for diagnosis of granulomatous inflammation and detecting the aetiologic factors with special attention to the commonest cause of tuberculosis.

Method: We reviewed slides of 75 cases of granulomatous inflammation diagnosed at cytopathology department of Sher-E-Bangla Medical College and Islami Bank Hospital, Barisal between March 2019 and June 2021. We examined sex, age, site of aspiration, presence of epithelioid histiocytes in scattered and clustered form, other supporting evidence of granulomatous inflammation such as giant cell formation, presence of caseation necrosis, polymorphs, plasma cells, eosinophils, result of Ziehl-Neelsen stain and final cytological diagnosis.

Results: Our sample compromised of 27 males and 48 females. Most of the cases (n=20, 26.7%) were between the age of 21 to 30 years. Lymph nodes (n=58,77.3%) were the commonest site of granulomatous inflammation ahead of breast (12), skin (4) and thyroid (1). Scattered epithelioid histiocytes were found in 41 cases (54.7%) while clustered epithelioid histiocytes were found in 32 cases (42.7%) and two cases had no visible epithelioid histiocytes. On the other hand, accessory evidence such as giant cell, caseation necrosis, polymorphs, plasma cells and eosinophils were present in 8 (10.66%), 8 (10.66%), 37 (49.33%), 8 (10.66%) and 5 (6.66%) cases respectively. Eight cases (10.66%) cases were cytologically consistent with tuberculosis while another 8 (10.66%) cases were suggestive of tuberculosis.

Conclusion: We should cautiously examine granulomatous inflammation with suspected tuberculosis in addition to other diagnostic tools for tuberculosis.

Keywords: FNAC, Granulomatous Inflammation, Tuberculosis

INTRODUCTION

Granulomatous inflammation is chronic inflammation characterized by formation of granuloma where aggregation of macrophages transformed into epithelioid histiocytes surrounded by mononuclear lymphocytes and other inflammatory cells are found, sometimes having multinucleated giant cells and central caseation necrosis.[1] The most important cause of granulomatous inflammation in a developing country like Bangladesh is tuberculosis.[2] As the burden of tuberculosis is increasing not only in Bangladesh but also in developed countries.[3] It is becoming more challenging for a cytopathologist to diagnose granulomatous inflammation.

Due to a wide range of aetiological factors including some malignancies like Hodgkin and Non Hodgkin lymphoma and mestatatic squamous and adenocarcinoma, sometimes it may become difficult for cytopathologists to diagnose granuloma. Although demonstration of Acid fast bacilli in the specimen by Ziehl-Neelsen stain is confirmatory for diagnosis of mycobacterium tuberculosis, it's detection rate is low. Even histopathology cannot confirm diagnosis of tuberculosis without demonstration of acid fast bacilli.

Epithelioid histiocytes is the hallmark of granulomatous inflammation.[4] Only scattered epithelioid histiocyte is sufficient

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to prove granulomatous inflammation. But cytopathologist always search more evidence because a sinus histiocytes, a fibroblast or an endothelial cell can mimic epithelioid histiocytes.[5] Along with scattered epithelioid histiocytes, presence of clustered epithelioid cells, formation of giant cells, caseation necrosis, presence of other cells of chronic inflammation such as plasma cells, eosinophils or abscess are supporting evidence of a granulomatous inflammation.

Because inflammation, repair and tissue destruction coexist at varying degrees in chronic inflammation, presence of endothelial cells as a part of angiogenesis (interleukin), fibroblast as part of fibrous tissue proliferation (interleukin) and sinus histiocytes and other resident histiocytes as component of lymph node and other tissues respectively are almost inevitably present in a granulomatous inflammation.[6]

The above scenario led us to review the granulomatous inflammation reports keeping in mind that tuberculosis might be an important association. So, we reviewed some cases of granulomatous inflammation to assess its association with tuberculosis.

METHODS

We conducted this retrospective study of 75 records of granulomatous inflammation in the department of cytopathology, Sher E Bangla Medical College (SBMC) and Islami Bank Hospital (IBH), Barishal. between March 2019 and June 2021. After approval by the ethical committee of the Institutional review board (IRB) of SBMC, we secured the confidentiality of the data with no disclosure of the information that might be harmful to the patient. We excluded cases other than granulomatous inflammation.

FNAC samples were collected from selected patients with all aseptic precaution at department of cytopathology. FNAC sampling was performed with a 21-gauge needle on a syringe with application of vacuum. FNAC aspirates were immediately smeared in glass slides and fixed in 95% alcohol and were sent to the laboratory for staining. In the laboratory, Hematoxyllin & Eosin staining and Ziehl-Neelsen staining of the slides were done according to the standard protocol followed at SBMC and IBH, Barishal and all the prepared slides were assessed for cytological diagnosis. We did the Hematoxyllin and Eosin staining by a) fixation of the prepared smear in 95% alcohol for >30 minutes, b) air drying of the slides, c) dip into Harris Hematoxyllin for 1-3 minutes, d) rinsing in tap water for 10 minutes, e) dip into 95% alcohol 10 times, f) dip into Eosin Azzure 65 ten times, g) dip into 95% ethanol 10 times, 2 changes, g) dip into 100% ethanol 1 minute h) clearing into 2 changes of xylene 2 minutes, i) permanent mounting with DPX. Steps of Ziehl-Neelsen staining were a) preparation of smear, b) air drying for 30 minutes, c) heat fixed dried smear, d) application of carbol fuschin, e) heat the smear until vapor begins to rise at 60 degree centigrade, f) wash off the stain with clean water, g) application of 20% sulfuric acid as decolorizer, g) wash with clean water. h) counter stain with malachite green, i) wash off with clean water, j) wipe the back of the slide, place it in a draining rack and air dry.

All the samples were collected from database of the department of pathology SBMC and IBH, Barishal in prescribed forma. Then we entered the data in Statistical Packages for Social Sciences (SPSS-17) where the analysis was done. We classified the data according to age, sex and the diagnosis, site of inflammation and other related variables.

The results were presented in tables, where absolute and relative frequency were given. In addition, we also presented figures and pictures of photomicrograph.

RESULTS

We recorded 27 males (36.0%) and 48 females (64.0%). When we looked at the age distribution shown in fig. 1.

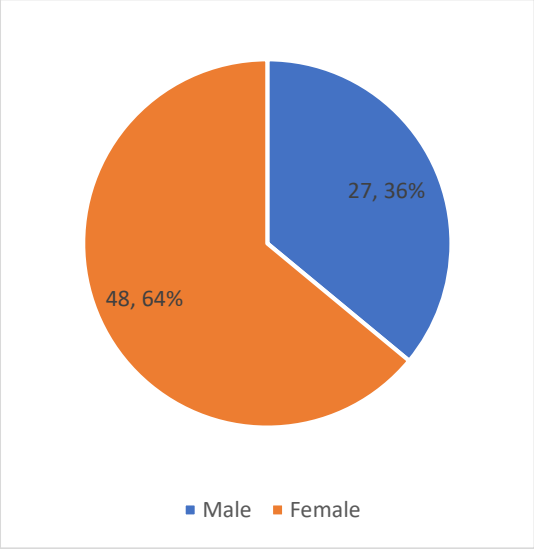
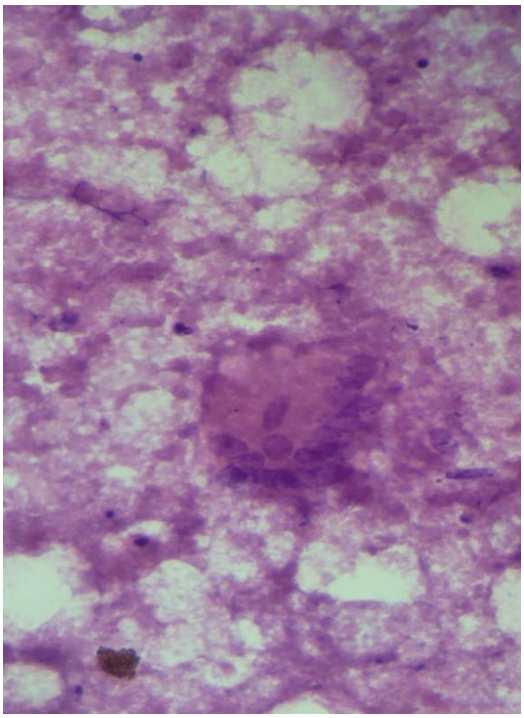
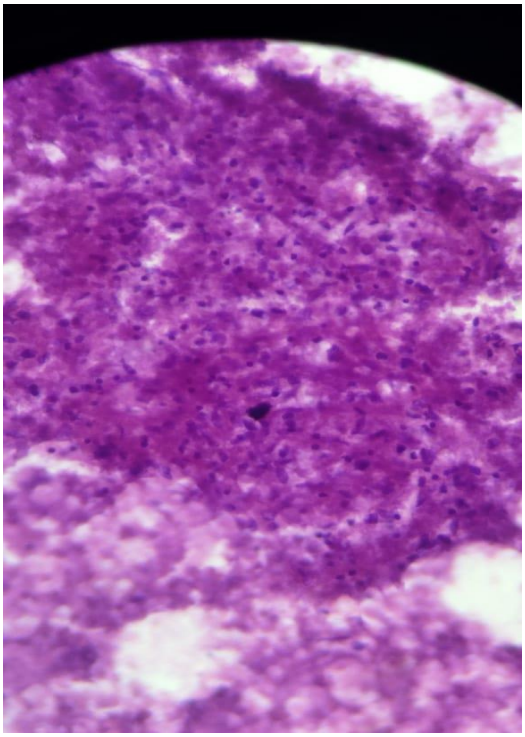


Fig. 1: Pie chart showing gender distribution.

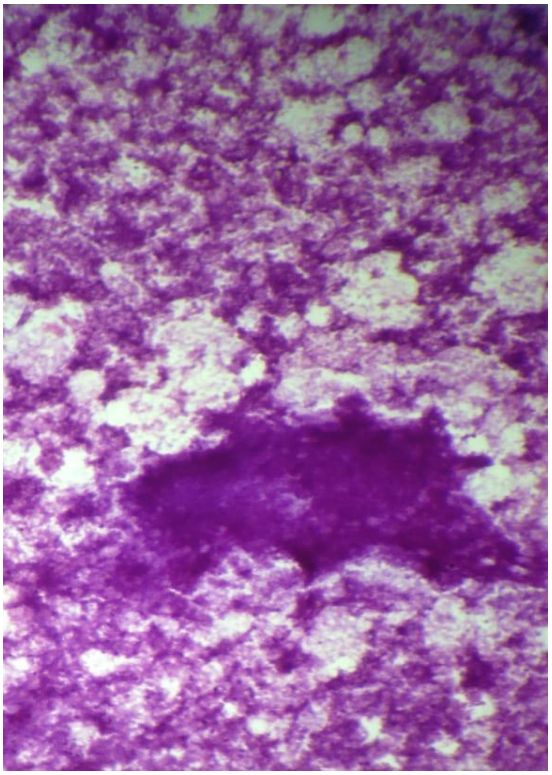
We found the ages of 21 to 30 years to be more prevalent (n=20) shown in fig. 2.



Pic.1 Photomicrograph showing giant cell



Pic.2 Phtomicrograph showing scattered and clutered epithelioid histiocytes



Pic.3 Photomicrograph showing caseation necrosis

Only scattered epithelioid histiocytes without any other evidence were found in only 2 cases while along with other findings

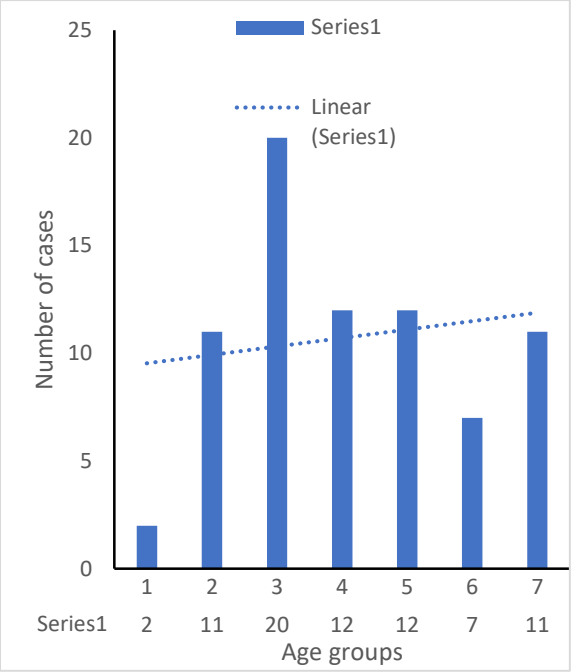


Fig. 2: Bar diagram of age distribution

epithelioid cells were found in 32 cases. Scattered as well as clusters of epithelioid histiocytes were observed in 41 Cases. Caseation necrosis was present in 8 cases. On the other hand, other evidence of granuloma such as giant cells, presence of polymorphs, plasma cells, eosinophils were present in 8, 37, 8, and 5 cases respectively (shown in Fig. 3).

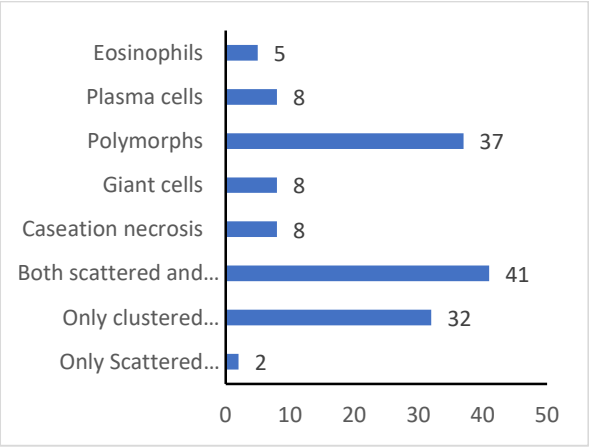


Fig.3 Microscopic Findings of Study Cases

Among the 75 cases, 19 cases were diagnosed as tuberculosis (Acid fast bacilli positive) (25.33%), 4 (5.33%) cases were diagnosed as cytologically consistent with tuberculosis while 8 (10.66%) cases were diagnosed as suggestive of tuberculosis. All the other cases (44) (58.67%) were recommended for further workup to rule out possibility of tuberculosis (shown in Fig.4).

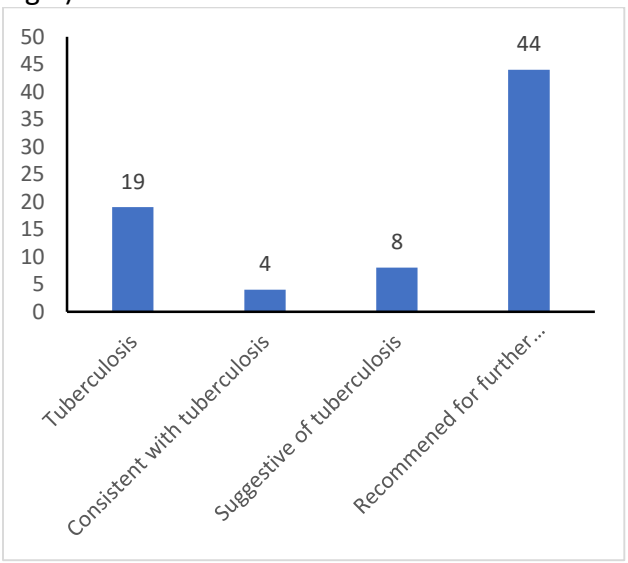


Fig. 4: Distribution of the Study Population According to Diagnosis

Among the 75 cases, 19 (25.33%) cases were found acid fast bacilli positive.

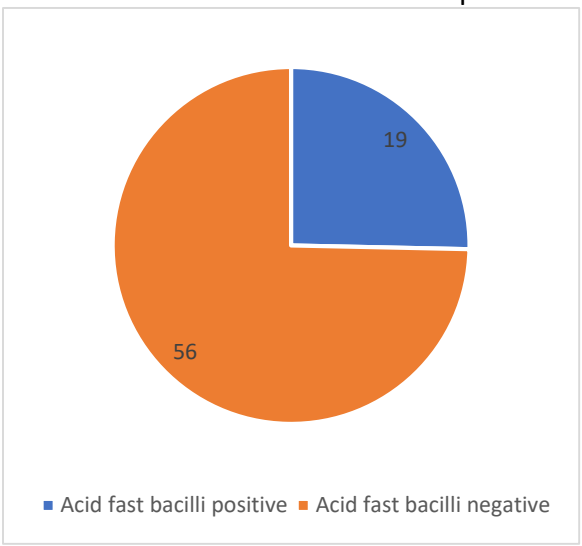


Fig. 5: Distribution of the Study population According to Acid Fast Bacilli Positivity

All the other cases 56 (74.67%) were acid fast bacilli negative shown in fig. 5.

DISCUSSION

This study was aimed to evaluate cytologic findings spectrum of granulomatous inflammation. From this study, epithelioid histiocytes both scattered along with

cluster were found in highest number of cases. On the other hand, only scattered epithelioid histiocytes were found in 2 cases only. Ziehl-Neelsen stain was positive in nineteen cases (25.33%). From this study, we understand that only scattered epithelioid histiocytes are not sufficient for diagnosis of granulomatous inflammation, rather we can expect scattered and clustered form of epithelioid histiocytes in majority of the cases. Again, although supporting evidence like multinucleated giant cells, polymorphs, plasma cells, eosinophils and caseation necrosis play an important role in diagnosis of granulomatous inflammation, we may not get these evidences in most of the instances.

In a study by V Koo et al. 14 out of 22 (64%) had their diagnosis confirmed through histological assessment,[7] though we could not go for histology. On the other hand, our study included only FNAC result, rather than histopathology and other sites rather than lymph nodes. Nineteen cases (25.33%) cases were Ziehl-Neelsen positive hence diagnosed as tuberculosis. However, other cases of granulomatous inflammation remained undiagnosed due to absence of histopathological examination of the excised lymph node. Here we observe that a large number of cases will be undiagnosed without the availability of lymph node biopsy.

Again, although histopathology is the gold standard of tissue diagnosis, it cannot confirm diagnosis of tuberculosis without demonstration of mycobacterium tuberculosis bacilli on Ziehl-Neelsen stain. In addition, lymph node biopsy is contraindicated in metastatic cases. Even a small lymph node biopsy may have adverse consequences in patient. Now days, Xpert gene test can confirm the presence of tuberculosis bacilli.

Majeed and Bukhari examined 100 cervical lymph node enlargement cases in Lahore, where 78% cases were below 30 years of age. Mean age was 25.14± 12.75 years.[8] We encountered age of 21 to 30 years to be highest (n=20, 26.7%) in our study. As females are more prone to develop granulomatous inflammation due to poor nutritional status, our study reported the fact by giving 68% females to be affected more than males. In our study among the 75 cases diagnosed as granulomatous inflammation, 27 were male and 48 (64%) were female. Majeed and Bukhari found 44 out of 100 patients of granulomatous inflammation were positive for AFB. In our study, among the 75 cases, 19 (25.33%) cases were found Ziehl-Neelsen stain positive. All the other cases 56 (74.67%) were Ziehl-Neelsen stain negative. Our study yielded similar results except positivity of acid fast bacilli. Only 19 cases (25.33%) were positive for Ziehl-Neelsen stain. Due to inclusion of other sites rather than cervical lymph nodes and possibility of wide range of etiologies other than tuberculosis, our study showed different result in acid fast bacilli positivity.

Limitations: FNAC cannot confirm the diagnosis of granuloma from wide range of etiologies. It can only be used as a screening tool for granulomatous inflammation as it is simple, cheap, safe and quick. We could not confirm the diagnosis by relating with our cytopathology finding hence leaving an important limitation of this study.

CONCLUSION

Our study revealed around 21% of granulomatous inflammation suspected for tuberculosis. Though granulomatous inflammation does not necessarily indicate the presence of tuberculosis as it is difficult for both clinicians and pathologists, we should correlate the finding with other ancillary tests like ESR, tuberculin test, sputum for AFB and Gene Xpert test. An online data registry system between clinical and pathology department can be used to

follow up the treatment outcome hence confirm the diagnosis of these cases.

REFERENCES

1. Muller-Quernheim J. Sarcoidosis: immunopathogenetic concepts and their clinical application. European Respiratory Journal. 1998 Sep 1;12(3):716-38.

2. Khan MK, Islam MN, Ferdous J, Alam MM. An Overview on Epidemiology of Tuberculosis. Mymensingh Medical Journal: MMJ. 2019 Jan; 28 (1): 259-266. PMID: 30755580.

3, Zaman K. Tuberculosis: a global health problem. J Health Popul Nutr. 2010 Apr;28(2):111-3. doi: 10.3329/jhpn.v28i2.4879. PMID: 20411672; PMCID: PMC2980871.)

4. Park SH, Chi JG. Chronic granulomatous disease: an ultrastructural study of the pigment laden histiocytes. Pediatric Pathology. 1992 Jan 1;12(6):839-47.

5. Silverman JS, Glusac EJ. Epithelioid cell histiocytoma–histogenetic and kinetics analysis of dermal microvascular unit dendritic cell subpopulations. Journal of cutaneous pathology. 2003 Aug;30(7):415-22.

6. Barron DA, Rowley DR. The reactive stroma microenvironment and prostate cancer progression. Endocrine-related cancer. 2012 Dec 1;19(6):R187-204.

7. Koo V, Lioe TF, Spence RA. Fine needle aspiration cytology (FNAC) in the diagnosis of granulomatous lymphadenitis. The Ulster medical journal. 2006 Jan;75(1):59.

8. Majeed MM, Bukhari MH. Evaluation for granulomatous inflammation on fine needle aspiration cytology using special stains. Pathology Research International.