Histopathological Spectrum of Granulomatous Inflammation : A Prospective Study

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Abstract

Aims: To study histomorphological patterns in granulomas detected in various granulomatous lesions on tissue specimens. To assess the role of ancillary techniques like fluorescent microscopy and polarized microscopy in finding the etiology

Materials and Methods: Prospective study of 135 cases which had granulomatous inflammation were included and duration of study was 2years. All granulomatous lesions confirmed on H&E stained sections were subjected to special stains like Ziehl-Neelsen, FiteFaraco and Periodic acid Schiff, Fluorescent microscopy (Auramine rhodamine) and Polarised microscopy to identify the specific etiological factors.

Results: In present study tuberculosis was the most common cause with 64 (47.4%) cases, followed by leprosy 38 (28.1%). Eight (5.9%) were of fungal infection and 4 (3%) cases of actinomycosis. Foreign body granuloma was seen in 17 (12.6%) cases, suture material was the commonest seen in 8 cases (47.1%). Majority of cases were in age group of 21-30 years with slight male 52.6% preponderance. ZN stain was positive in 17 (26.56%) cases and FF stain in 12 (31.57%). AR stain increased the detection of AFB from 26.56% to 40.62% in cases of tuberculosis and in cases of leprosy from 31.57% to 47.36%.

Conclusion: The most common etiology for granulomatous lesion was tuberculosis followed by leprosy in the present study. AR stain increases the detection of AFB. Utilizing these simple, ancillary techniques optimizes and improves the accuracy of histopathology reporting in cases having granulomatous disorders.

Key words: Granuloma, special stain, polarized microscopy

Introduction

Granulomatous disorders are a group of disorders with granulomatous inflammatory response leading to common histological denominator of granuloma formation. Granulomatous inflammation has been of research interest since 19th century, when it was recognized as a distinct inflammatory pattern. Numerous infectious diseases and non-infectious conditions are associated with it[1].

Pathologically the granulomatous inflammatory response is a manifestation of many toxic substances, infections, autoimmune diseases, neoplasms, allergic diseases and also conditions of unknown etiology. Granulomas are usually formed as a result of the persistence of a non-degradable product or as a result of hypersensitivity responses and characterized by collection of activated macrophages (epithelioid cells) often with T lymphocytes and sometimes associated with central necrosis. At times activated macrophages may fuse to form giant cells. Identification of granuloma formation and its pattern in a biopsy specimen is necessay for diagnosis and treatment. This in turn influences the disease outcome[2].

In establishing a correct diagnosis histopathology is a very important tool. Advances in molecular diagnostic techniques in the recent past have helped in identification of organisms involved in granulomatous diseases which were not known previously[3].

A detailed clinical history, a detailed histological examination and a clinicopathological correlation are necessary in arriving at a final diagnosis. With all the available information, probable differential diagnoses should be enumerated. In few scenarios, even with all the available information a definitive diagnosis cannot be arrived at.

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Ancillary techniques like Ziehl-Neelsen, Fite Faraco and Periodic acid Schiff, Fluorescent microscopy (Auramine rhodamine) and Polarised microscopy stain, Polymerase chain reaction and Immunohistochemistryhelps in identifying the etiology of granulomatous lesion . The histopathological examination of various granulomatous reactions is mandatory to reach a definitive diagnosis[3,4]. In the present study, we identified the various etiological causes of granulomatous inflammation and attempted to determine the underlying causative agents responsible for the lesions. Additionally polarized microscopy was employed to detect foreign body material causing granulomatous inflammation which maybe overlooked on routine hematoxylin and eosin (H&E) stained sections. The use of polarized microscopy enhanced the detection of birefringent foreign body materials, thereby improving the diagnostic accuracy in selected cases.

Materials and Methods

It is a prospective study. One hundred thirty five samples with granulomatous lesion identified in the histopathological section two year study, referred to the Department of Pathology, BLDE (Deemed to be University) Shri B M Patil Medical College, Hospital and Research centre, Vijayapura were included in the study

The cases showing granulomatous inflammation on H&E section from all sites were included. Special stains like ZN stain, FF stain, PAS, AR stain under fluorescent microscope using the blue excitation filter and Polarized microscopy were used to find the etiology of granulomatous lesions on a case to case basis.

ZN and FF stained smears were examined for AFB using a bright field microscope in oil immersion (Labomed® 300i). Appropriate control slides were stained simultaneously along with the test slides in all the cases. Negative sections were subjected for additional six sections and stained with FF stain before declaring them as negative.AR stain was taken as a reference standard and AR stained smears were scanned under fluorescence microscope (ZEISS microscope of wavelength 450nm).

A polarizing microscope had two accessories polarizer and analyzer. Polarizer was placed below the condenser and analyzer was placed in the eye piece part of the microscope (Labomed Lx300i). Foreign body material was observed in 10X and 40X objectives

Inclusion Criteria:

All the cases with granulomas identified in histopathological examination due to any etiology.

Statistical Analysis

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean, standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries. Chi-square (χ^2)/ Freeman-HaltonFisher exact test was employed to determine the significance of differences between groups for categorical data. Sensitivity- specificity was done to check relative efficiency. If the p-value was < 0.05, then the results were considered to be statistically significant otherwise it was considered as not statistically significant. Data were analyzed using SPSS software v.23.0and Microsoft office.

Results

In this prospective study of 2 years duration, 135 cases with granulomatous lesion identified in the histopathological section from all the sites were included. Majority of cases were in the age group of 21-30 years followed by 31-40 years. Male to female ratio was 1.11:1 with a slight male 71 (52.6%) preponderance

The cases were stratified based on the etiology into broad categories like neoplastic, non-neoplastic and inflammatory lesions (shown in table 1). Predominant category was inflammatory. Among them infections etiology was most common with 114 (84.4%) cases.

Table 1: Distribution of cases according to etiology

ETIOLOGY	N	%
Tuberculosis	64	47.4
Leprosy	38	28.1
Foreign body	17	12.6
Fungal infection	8	5.9
Actinomycosis	4	3.0
Autoimmune	2	1.5
Cholesterol granuloma	1	0.7
Crohn's disease	1	0.7
Total	135	100

In the present study we had specimens from a wide number of organs and organ systems affected with tuberculosis. Among them lymphnodes were the most commonly affected in 22 (34.4%) cases (shown in table 2)

Table 2: Distribution of cases according to site and type of Fungal Infection.

UPPER LIMB	Fore arm	Phaeohypomycosis (1 case)
		Hyalohypomycosis (1 case)
	Right finger	Madura mycetoma (1 case)
LOWER LIMB	Foot	Madura mycetoma (1 case)
HEAD AND NECK	Nose and paranasal sinus	Mucor mycosis (2 cases)
	Nasal polyp	Aspergilosis (1 case)
	Ear	Aspergilosis (1 case)

Among 102 cases which included tubercular and leprosy as etiological agent, 17 (26.56%) cases were positive on ZN staining and 12 (31.57%) were positive on FF stain. Majority of the cases, that is 73 (71.56%) were negative on either ZN or FF stains (shown in table 2). All the ZN positive cases showed positive result with the AR stain with 100% correlation (Fig. 2). Among 47 (73.43%) ZN negative cases, additional 9 (14.06%) cases showed positive result with the AR stain. On the other hand, for all the leprosy cases FF stain and AR stain were done. FF positive was seen in 12 (31.57%) cases and negative in 26 (68.42%) cases. All the FF positive cases showed positive result with the AR stain with 100% correlation. Among 26 (68.42%) FF negative cases, additional 6 (15.78%) cases showed positive result with the AR stain

Taking AR stain as reference standard, the ZN stain showed a Sensitivity of 65.4%, Specificity of 100%, Positive predictive value (PPV) of 100% and Negative predictive value (NPV) of 80.9% and Accuracy of 85.9%. The FF showed a Sensitivity of 66.7%, Specificity of 100%, Positive predictive value (PPV) of 100% and Negative predictive value (NPV) of 76.9% and Accuracy of 84.2% (shown in table 3).

Table 3: Association of ZN stain and FF stain with AR stain

Special stain	Cases	AR-Negative		AR-Positive		p value
		N	%	N	%	
ZN-Positive	17	0	0.0	17	26.56	<0.001*
ZN-Negative	47	38	59.3	9	14.06	
FF-Positive	12	0	0.0	12	31.57	
FF-Negative	26	20	52.63	6	15.78	

Note: * means significant association between AR staining and ZN, FF staining with p value <0.05.

Seventeen cases (12.6%) of foreign body granuloma were seen in the present study. For the detection of foreign body, polarizing microscope was used. The most common foreign body material encountered in this study was suture material that is, 8 (47.1%)cases (Fig.5),vegetative material in 6 (35.3%)cases and ruptured cyst was seen in 3 cases (17.6%).

Total (5.9%) cases of fungal infection were seen in present study diagnosed based on histomorphology and special stains like PAS (Fig.3&4).

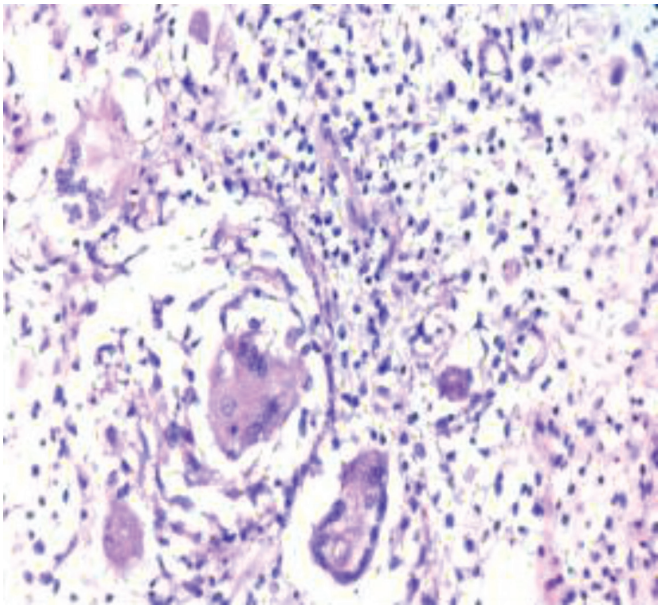

Figure 1: Microphotograph showing well formed granuloma with aggregates of epithelioid cell, lymphocytes and giant cells on H & E stain (400X).

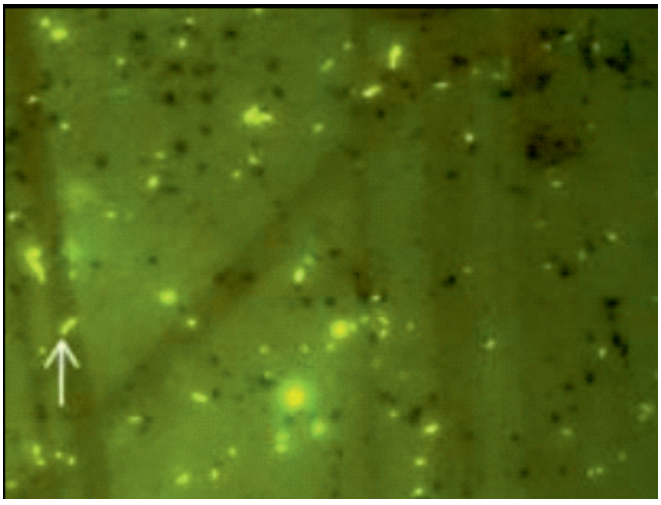

Figure 2: Microphotograph showing Plenty of slender yellow to orange rod shaped bacilli on AR (Auramine-Rhodamine, 400X)

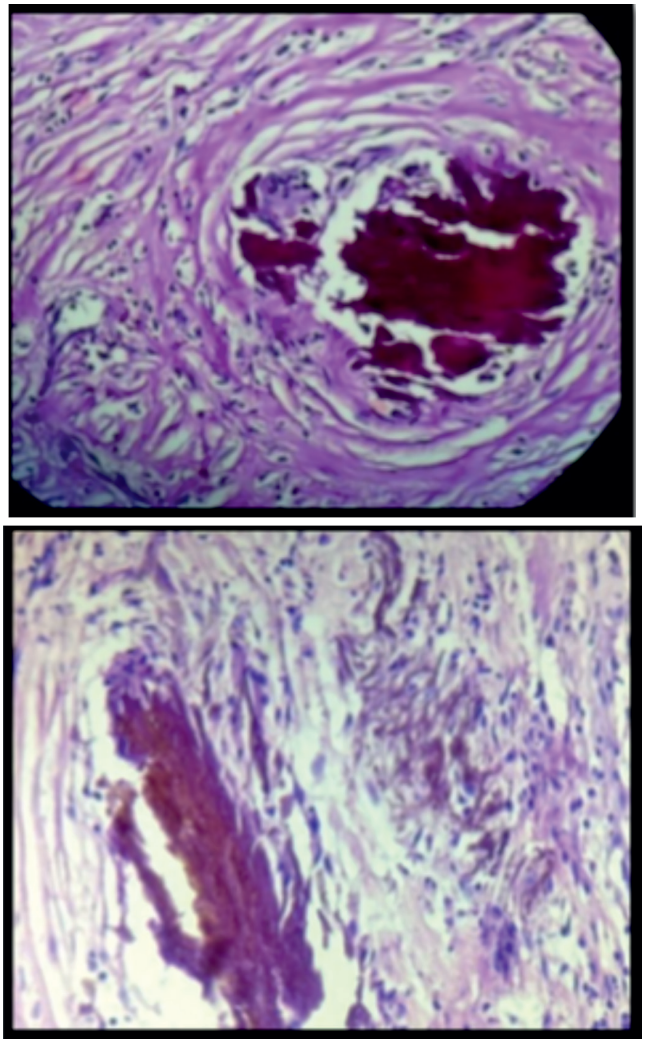

Figure 3: Microphotograph of Phaeohypomycosis on PAS stain (400X)

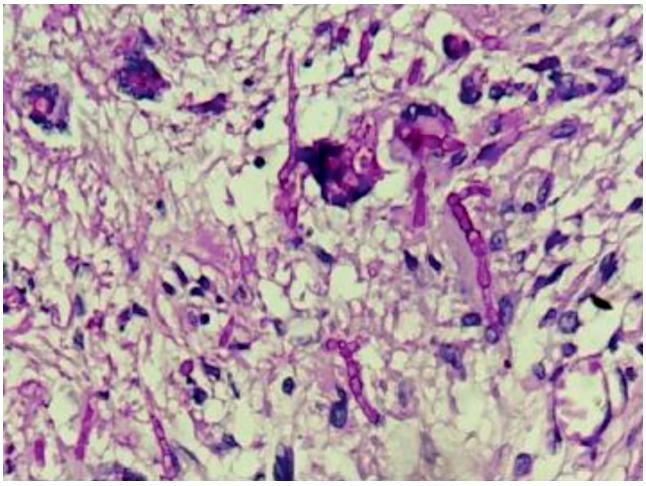

Figure 4: Microphotograph of Hyalohypomycosis on PAS stain (400X)

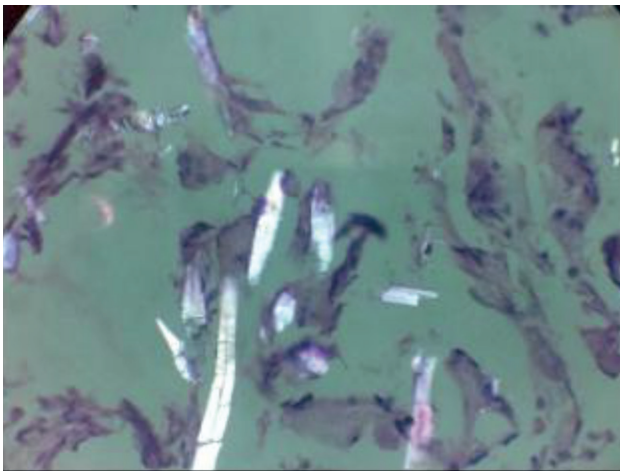

Figure 5: Microphotograph of foreign body on polarized microscope (400X)

Discussion

In the present study, the most common etiology for granulomatous inflammation was Tuberculosis in nearly half of the cases 47.4% (64 cases). Amongst these, 26.56% (17 cases) were positive for AFB on ZN staining. The ZN positivity is varied as the detection of AFB is having a wide range for sensitivity and specificity among the study groups. Fluorescent microscopy has been made available in various centers by the Revised National Tuberculosis Control Program (RNTCP) initiative wherein the equipment and the stains like Auramine Rhodamine (AR) are utilized. This technique is simple, faster, cost-effective and has improved the detection rates thereby making a difference in the management of Tuberculosis in developing countries. In the present study, with the help of this AR stain, an additional 14.06% (9cases) demonstrated AFB in case of Tuberculosis, thus detection of AFB had increased from 26.56% to 40.62%. Similarly in leprosy, an additional 15.78% (6 cases) has been detected, and the detection of lepra bacilli has increased from 31.57% to 47.36%.

The demonstration of the organisms was not possible in case of 59.3% (38 cases) even after performing ZN and AR, this is probably due to low bacillary load and large areas of caseous necrosis. Based on the clinical presentation and classical morphological appearance on the bright field, H& E staining these cases were offered a diagnosis of Tuberculosis. In leprosy organism could not be demonstrated in 52.63% (20 cases). Depending on past history, histomorphology patterns and leprosy spectrum these cases were diagnosed as Leprosy.

The studies done by Harish *et al*[5] and Jayashree*et al*[6] had nearly half of the cases caused by Tuberculosis in concordance with the present study. Babaria*et al*[1] had 56.33% of tuberculosis cases in their study with

a sample size of 300 cases. In developing countries, the diagnosis of TB is made on symptoms based algorithms. Initially the diagnosis was made based on certain patterns like Granulomatous inflammation, Caseous necrosis, Langhan's type of giant cells. But many viral infections, bacterial infections can also simulate the same histomorphological patterns. So laboratory test plays an important role to establish the cause of such granulomatous inflammation because the prognosis and treatment will differ.

Staining of ZN stain was done on thin sections (3µ) of the block, complete deparaffinization and positive control slides were taken for every batch. Extensive search for the bacilli was a prerequisite before reporting it as negative,which may give a false negative result.

The positivity of FF stain was comparatively high in the present study, this could be due to, as a part of protocol, minimum of six sections were examined before declaring them negative. According to Elder DE *et al*[7], there should be 1,000 bacilli per cubic centimeter of tissue in order to detect 1 bacillus in a section.

In the present study, Sixty four cases of tuberculosis were diagnosed on the H&E stain. All the cases were stained with ZN along with a positive control, wherein ZN stain was positive in 26.56% (17 cases) and rest 73.43% (47 cases) was negative. The AR stain was positive in all the cases of ZN positive cases and in ZN negative cases AR was positive in 14.06% (9 cases), thus the detection of AFB with the help of AR stain has increased to 40.62 % (26 cases). In the study done by JayashreePawal*et al*[6], 25 cases were randomly selected for AuramineRhodamine staining, out of these, 24 cases were negative for the ZN stain and 1 case was positive for ZN stain, 19 cases showed positive with AR stain and 5 cases were negative out of 24 negative ZN stained cases, One case which is positive for ZN stain showed positivity in AR stain too. The disparity in the detection of ZN positive and AR positive cases is probably due to the random selection of cases for AR staining in the study done by JayashreePawal*et al*[6].

Routinely performed ZN staining has low sensitivity as it rarely detects AFB when there is a low bacillary load. However, in the present study, we used AR stain for detection of AFB. It is observed that whenever there is scant AFB positivity, searching for them is a tedious process on conventional ZN and FF stain as compared to AR stain. On AR, AFB are easily detectable under low power view, the large area of the section can be screened within a short period of time, which reduces the turnaround time. Although the PCR technique was not done in the present study it gives positive result even with a low bacillary load. In a study conducted by Yoo Jin Lee *et al*[8], higher sensitivity of PCR was seen in specimens containing necrosis. They also stated that long duration of formalin exposure causes DNA fragmentation and decreases DNA's quality. Two to four hours of formalin exposure is enough for successful amplification. They also correlated PCR with histologic findings and noted that its sensitivity was higher in necrotizing granulomatous inflammation and lower in non specific inflammation.

In the present study, two cases each of Mucormycosis, Madura Mycetoma and Aspergillosis were noted in the infectious causes along with very rare cases like Phaeohypomycosis and Hyalohypomycosis. In all of these cases special stain PAS was done and showed positivity.

In the present study, a total of 25 cases were suspected to have foreign body material on H and E stain, out of which 17 cases had shown to have a foreign body on polarized microscopy. Most commonly encountered foreign material was suture materialfollowed byvegetative matter. Suture material mostly was observed in the fallopian tube. The vegetative matter was demonstrated in cases of fistula in ano. In a study done by Manjunatha BS *et al*[9], they stated that the vegetable matter examined under polarized light appeared as a birefringent refractile matter. The appearance of such material was characteristic on polarized microscopy as described in figure in the present study.

The foreign body material is sometimes very difficult to appreciate on the routine H and E stain, the tissue sections will show granulomas with many giant cells, which are nonspecific findings where a definite etiology cannot be ascribed without utilizing the polarized microscopy and special stains.

In the present study, on histomorphological examination, three cases showed thin fragments of foreign material on tissue sections stained with H and E stain. However, due to the lack of contrast and dense and diffuse inflammation on H & E examination their morphology was not appreciated. With the aid of polarized microscopy, foreign body material was appreciated with certainty by observing the birefringence and the characteristic appearance which was specific to few of the materials such as suture material, appeared as linear or curvilinear refractile material and vegetative matter as birefringent refractile matter.

In majority of the cases,the most common location of the foreign body materialwas within the cytoplasm of the giant cells or in the center of the granulomas.So

by using these simple polarized filters to the routine microscope, we can establish a confirmatory diagnosis in cases of suspected foreign body granuloma.

Based on the observation in the present study following recommendations have been proposed:

1. In suspected cases of Tuberculosis/ Leprosy on HPR, we recommend to do AR stain under IF microscope.
2. AR negative with histopathology showing suspected morphology of tuberculosis then subject the tissue for PCR.
3. Simple use of polarized lenses on routine microscopy will aid in the identifying the foreign body.
4. PAS stain and other fungal stain should be routinely practiced in suspected cause of fungal infections.

Conclusion

Histopathological examination of granulomatous lesions and to find out the cause of the granulomatous inflammation in the biopsy specimen is very important for diagnosis and treatment. An aid of ancillary techniques is very helpful in finding out the etiology of granulomatous inflammation which is previously of unknown etiology. The present study was undertaken to determine the utility of ancillary techniques like special stain, fluorescence microscopy and polarized microscopy in identifying etiology of the granulomatous lesion on histopathology.

AR stain can be used as a supplementary tool in detecting acid fast bacilli where the bacillary load is scant. AR stain helps in rapid detection of bacilli and helps to improve turnaround time. In this study, AR stain increased the detection of tubercle bacilli from 26.56% to 40.62%. On the other hand, AR stain increased the detection of lepra bacilli from 31.57% to 47.36%.

Polarizing filters are simple attachment used for the routine microscope, which helps in detection and identification of foreign body material. PAS stain helps in identifying the morphology of fungus.

Utilizing these simple ancillary techniques, this will optimize and improves the accuracy of histopathology reporting in granulomatous lesions.

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