Original Article

CONTRIBUTION OF SPECIAL STAINS IN DIFFERENTIAL DIAGNOSIS OF CHRONIC GRANULOMATOUS INFLAMMATION

Hamza Tahir[1], Tariq Sarfraz[1], Aaminah Hanif[1], Aiza Saadia[1], Nosheen Tariq[1], Muhammad Tahir Khadim[2]

[1]Army Medical College (National University of Medical Sciences), Rawalpindi Pakistan
[2]Armed Forces Institute of Pathology (National University of Medical Sciences), Rawalpindi Pakistan

ABSTRACT

Objective: To observe the results of special stains in determining the etiology of different granulomatous lesions in skin biopsies.

Material and Methods: This descriptive cross-sectional study was carried out at Army Medical College, Rawalpindi in the Department of Histopathology for one year from Jan to Dec 2018.

A total of one hundred skin biopsies displaying granulomatous inflammation were included in this study. The biopsies were subjected to special stains after staining with basic hematoxylin and eosin stain. The special stains included Giemsa, Periodic acid Schiff (PAS), and Ziehl Neelsen (ZN stain). After staining, slides were seen under the microscope to observe if they were positive or negative for these special stains and if they gave a clearer picture to aid in the final diagnosis. The data was entered and analyzed in SPSS version 20.

Results: Most of the patients presented with an erythematous plaque and others were with either chronic non-healing ulcer or erythematous rash or with a papule and only 6 presented with a nodular lesion. The age distribution was also observed and most of the patients were in the age group of 21-30 years.

Out of 100 cases, 69 % were diagnosed as cutaneous leishmaniasis. The most useful stain in this study was observed to be Giemsa, which was found to be positive in 54 cases for the diagnosis of LT bodies. Moreover, in 6 cases PAS was positive and for ZN staining only 1 case was positive. 5 cases showed negative results in all stains collectively.

Conclusion: In cases where conventional hematoxylin and eosin did not suffice in finding out the diagnosis, special stains were extremely useful and contributed towards a definitive diagnosis.

Key Words: Chronic Granulomatous Inflammation, Skin Biopsy, Special stains.

This article can be cited as: Tahir H, Sarfraz T, Hanif A, Saadia A, Tariq N, Khadim MT. Contribution of special stains in the differential diagnosis of chronic granulomatous inflammation. Pak J Pathol. 2019; 30(4): 108-112.

INTRODUCTION

Granulomatous inflammation is a very unique type of inflammatory response. This peculiar defensive mechanism of the human immune system involves the accumulation of a myriad of immune cells at the site where the inflammation is occurring. The cells include macrophages, epithelioid cells and multinucleated giant cells [1, 2]. It can also be defined as a unique type of inflammations that involves the formation of a necrotic core which is surrounded by a collar of inflammatory cells which include epithelioid cells forming a granuloma. This may also be without the presence of a necrotic core. Multinucleated giant cells are also seen in this inflammatory collar.

Granulomatous inflammation can be manifested - due to an infection, chemical toxins, allergens and even as an effect of some drugs [3]. Amongst the infection, the tuberculous infection caused by *Mycobacterium Tuberculosis* is the leading cause of granulomatous inflammation [4]. Moreover, it may also be due to chronic granulomatous disease, which is a group of hereditary diseases in which the human immune cells are unable to produce reactive oxygen species (ROS), hence are unable to eliminate the pathogens [5].

Granulomatous inflammation may involve many sites of the body, ranging from skin to something as deep as the lung parenchymal tissues. However, skin being the very first barrier of the body is one of the most commonly involved areas for this type of inflammation [6,7].

Cutaneous infections caused by leishmaniasis are amongst the most common diseases in traveling. Depending on the pathogens subtype and the patient's immunity, cutaneous forms (approximately 90 % of cases) can be distinguished from mucocutaneous and visceral leishmaniasis [8]. The protozoan parasites are transmitted by sandflies (Phlebotominae). Sandflies live in tropical and subtropical regions, as they can only survive in places where outside temperatures do not drop below 10°C. The pathogen subspecies vary depending on the region. More than 90 % of infections occur in countries of the "old world" (e. g. Afghanistan, Algeria, Saudi Arabia, Iran, Sudan, Syria). A corresponding travel history is therefore

Correspondence: Dr Hamza Tahir, Department of Pathology, Army Medical College, Rawalpindi Pakistan

Email: **hamza_tahir09@hotmail.com**

Received: ***22 Sep 2019****; Revised:* ***30 Oct 2019****; Accepted:* ***08 Dec 2019***

important for an accurate diagnosis. Cutaneous leishmaniasis is also common in endemic areas of Pakistan. It is clinically characterized by a nodule (Aleppo boil) with raised borders. There are also clinically less typical manifestations, presenting as indurated, sometimes eczematous erythema [8]. The acute phase is histologically typically characterized by a diffuse infiltrate of lymphocytes, macrophages, plasma cells, and granulomas [9,10]. Neutrophils are found in variable numbers, especially in the upper dermis. Following transmission of the pathogens by the sand fly, the promastigotes are phagocytosed by macrophages where they mature into amastigotes. In the hematoxylin-eosin (H&E) stain, the practiced eye is able to detect the amastigotes as small, “gray-blue pellets” within the macrophages [9,10]. The infiltrate near the epidermis should be examined particularly carefully, as pathogens can frequently be found there. Giemsa staining facilitates amastigote detection. The epidermis is frequently acanthotic, sometimes showing pseudoepitheliomatous hyperplasia.

There are many diagnostic methods used to identify the source of granulomatous inflammation. Usually, the process of diagnosis starts with clinical presentation and patient history that leads to an expert's decision of taking a biopsy of the skin area that is involved. Skin biopsy is considered a diagnostic sampling technique that is done at the site of the lesion. It may involve taking some part of the lesion or the entire lesion itself or sometimes it may also involve taking some surrounding healthy skin tissue. Skin biopsies are subjected to several staining techniques to get a clear histopathological review for a better diagnosis of the root cause of inflammation [11].

Basic staining methods like hematoxylin and eosin staining are first applied to these biopsies. Although this basic method of staining may suffice for most cases, however, this may present a challenge in certain other cases where basic staining is providing a vague picture. In such cases, special staining methods are used. These special stains include Giemsa stain, periodic acid Schiff (PAS), and Ziehl Neelsen's stain (ZN stain) [12].

In the present study, the practicality of these special staining techniques was observed. How vital they are in finding out the root cause of granulomatous inflammation in the skin biopsies?

MATERIAL AND METHODS

This was a descriptive cross-sectional study, which was conducted at Army Medical College, Rawalpindi in the Department of Pathology, National University of Medical Sciences from January 2018 till December 2018 after approval by the Institutional Review Board (IRB). The sample size was calculated via an online sample size calculator from the Australian Bureau of Statistics. The sample calculator was designed for simple random samples only. The confidence level of 95% was taken with a 5% margin of error. A total of 100 samples were collected according to the calculated sample size. The sampling technique was non-probability purposive sampling.

All cases of skin biopsies that showed the classical granulomatous type of inflammation on histopathological evaluation were included in this study. Cases that did not show clear features of granulomatous inflammation or showed any other pathologies such as malignancies on skin biopsies were excluded from the study. Poorly fixed tissues and specimen with scanty tumors were also excluded from this study.

The tissue of 5µm thickness was prepared by microtome from the selected blocks, then it was deparaffinized in xylene and rehydrated with a decreasing concentration of ethanol. The epitopes were retrieved by using the heat method in Tris/EDTA buffer at pH 9.0. The data were analyzed using the computer software program SPSS version 20. Mean +/- SD were calculated for continuous variables. Frequency and percentages were obtained for qualitative variables. The data was collected by first locating all the skin biopsy sample reports in the record of the department of pathology from the period of January to December 2018. The skin biopsies were carefully screened to pick out samples that included a granulomatous type of inflammation. After careful collection of samples, it was observed in any sample needed to go through further staining processes. All samples included in the study were already stained with hematoxylin and eosin stain after which, they were also subjected to special stains which included Giemsa stain, ZN and PAS stain. The prepared slides were carefully observed under the microscope and a definitive diagnosis was made

DATA ANALYSIS

Data was added to the software SPSS for analysis. The data were coded in order to deduce the percentages of desired results. The main aim was to find out the percentages of different diseases that involved the granulomatous type of inflammation. With each disease, the usefulness of the special stain that leads to the diagnosis was also observed. The usefulness of each special stain was also recorded as a percentage. This data was then tabulated and

also simplified in the form of figures such as a pie chart and a bar graph using the same software tools.

RESULTS

A total of 100 samples were collected and out of which 77 samples were taken from male patients and 23 from female patients. The age distribution of all the patients is given in Figure-1. It can be seen from the figure that most of the patients were in the age group of 21-30 years followed by 31-40 years, age group.

Table-1: Distribution of sample sites.

Area of biopsy	Number of Cases
Facial region	20
Arm	18
Lumbar region	12
Hand	12
Foot	12
Neck region	11
Leg	9
Scalp	6

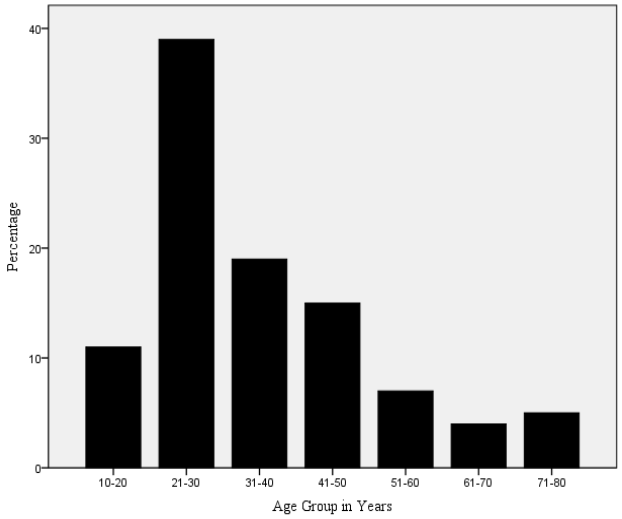

Figure-1: Percentage distribution of samples according to age groups.

The distribution of samples according to their site of infection is displayed in Table-1. It can be seen that most of the samples were collected from the face and arm. Figure-2 represents the percentages of different diseases in our study. It can be observed that the most common disease in the present study was cutaneous leishmaniasis that was accounted for 69 %, followed by lupus vulgaris that accounted for 13 %. Other less frequent diseases were sarcoidosis, tuberculosis, and fungal infections.

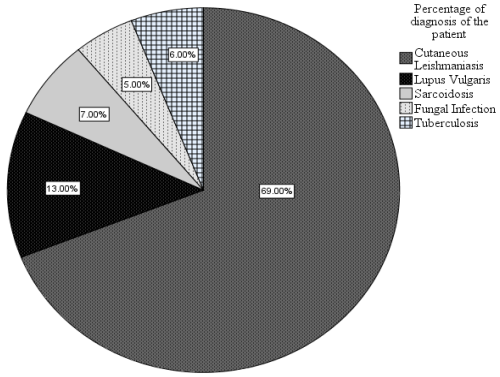

Figure-2: Percentages of different diseases in skin biopsies

Figure-3 shows the percentages of special stain used. It is evident that the Giemsa stain for LT bodies was the most frequently used stain. This reflects the fact that the frequency of cutaneous leishmaniasis was more and basic stain did not suffice in giving a clear diagnosis in such cases.

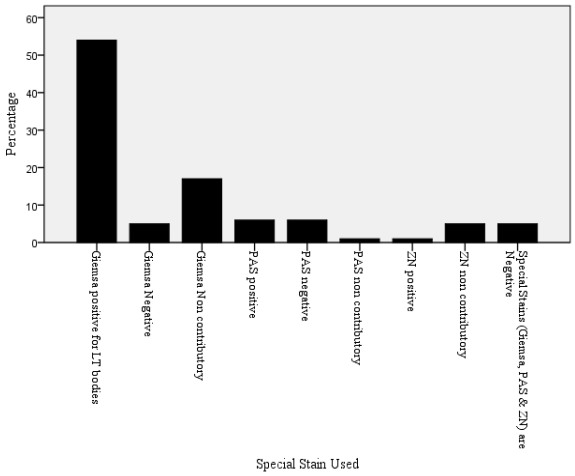

Figure 3: Percentages of special stain used.

Figure-4 represents the percentages of clinical lesions that the patients presented with. It is evident that the most common presentation was an erythematous plaque that accounted for 61 %. It was followed by erythematous rash and chronic non-healing ulcers

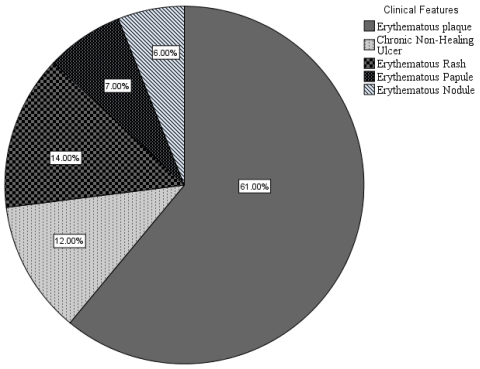

Figure-4: Percentage of clinical lesions of the cases.

DISCUSSION

The granulomatous inflammation is a distinctive type of inflammations that comprises a necrotic core with a surrounding collar of inflammatory cells and epithelioid cells forming a granuloma. This may also be presented without a necrotic core. Multinucleated giant cells are also seen in this inflammatory collar. Granuloma formation is typically seen in the process where the immune system fails to eliminate the pathogen and it may lead to fibrosis [22]. The present study focusses on exploring different etiologies of granulomatous inflammation using special stains.

In this study, male predominance was seen i.e. 77 out of 100 cases were males, which is in agreement with Dhar's study which showed comparable gender distribution of granulomatous disease [16]. A large number of cases in the current study were from the age group 21-30 years of age (39 cases39%) which is similar to the findings of another study conducted by Chakrabarti *et al*, (2016) [12].

The majority of the cases in the present study presented with cutaneous leishmaniasis i.e. 69 out of 100 (69 %), which is a unique finding in itself. In contrast, previous studies conducted by Bal *et al*. (2006) and Dhar *et al*. (2012) revealed tuberculoid granulomas as the largest etiology of lesions [13, 16]. Out of a total of 515 cases of infectious granuloma in Bal's study, 373 (72.4%) were leprosy, followed by 119 (23.1%) cases of cutaneous tuberculosis. Nonetheless, in the present study, 69 (69%) cases were of cutaneous leishmaniasis followed by 13 (13%) cases of tuberculosis and only 7 (7%) cases of sarcoidosis (13).

This unique finding may be due to the reason that the majority of the patients (64%) included in this study were serving in endemic areas of cutaneous leishmaniasis. Seven cases of cutaneous cases of sarcoidosis were diagnosed in our study that represents 7 % of the total study population. All the cases exhibited non-caseating granuloma with or without the presence of multinucleated giant cells. Findings in this study are in contrast to the results of the study conducted by Gautum *et al*. (2011) which showed a 1.88% prevalence of sarcoidosis in their study. [17]

In the present study, we found 5 cases of granulomatous inflammation due to fungal etiology. In the study conducted by Zafar *et al*, (2016) the most common site involved in granulomatous skin disease was head and neck region which is in concordance with the present study showing facial region (20%) as the most commonly involved region affected by the disease followed by arm (18%) [15].

In present study, Giemsa was found to be the most useful contributory stain in diagnosing granulomatous diseases of the skin as 54 cases showed positive LT bodies on Giemsa stain which is a disparity to the study conducted by Bal *et al*. (2006) which showed modified ZN as most valuable stain (36.4%) in their cases [13].. This difference is again due to the distribution of the majority of cases in both the studies. The second helpful stain in the present study was PAS.

In the present study, 7 cases of sarcoidosis were seen. The initial diagnosis was made on biopsy and was later confirmed on radiological evidence of pulmonary and mediastinal lymph nodes involvement and serum ACE (angiotensin-converting enzyme) and serum calcium levels. In a study conducted by Zafar (2016), there were few cases of sarcoidosis [15]. In another study conducted by Suri *et al*, (2017) there were 4 cases of sarcoidosis. [23]

Cutaneous leishmaniasis cases comprised the major bulk in our study for which clinical and histopathological concordance were analyzed. Skin lesions of cutaneous leishmaniasis were found more common in males (39%) than females (12%) in our study. These findings were also reported in another study by Jayalakshmi, revealing males to be affected more (73.68%) as compared to females, with a male to female ratio of 2.8: 1.7. In studies by Zafar *et al* and Bal *et al*, cutaneous leishmaniasis comprised (7.3%) and (1.16%) cases but, in contrast, our study comprised 68% of the cases studied. [13,14] All of 34(68%) cases involved upper extremity with itching and pain as common symptoms and were seen predominantly in males. Histopathology examination revealed LT bodies on routine H & E sections were further confirmed by Giemsa stain, thus confirming the diagnosis of cutaneous leishmaniasis. Although Leishmania skin test (positive in 80% cases) and Giemsa / Wrights staining of exudate can be performed as an ancillary test the definitive diagnosis rests on its isolation by culture or identification in smear/tissue sections [17]. Bal *et al* (2006) in his study were able to identify LT bodies in only 50% of the cases as it is usually difficult to detect LT bodies in paraffin sections, but plasma cell histiocytic infiltrate can suggest the diagnosis [13].

In our study, tuberculosis was diagnosed in 6% of skin biopsies. Although the worldwide incidence of tuberculosis varies from 0.1 to 1 % of all cutaneous conditions, in Pakistan, higher frequency (3.7 %) was also reported [18]. Although Tuberculosis is major disease-causing morbidity and

mortality in Pakistan, reliable data is deficient on the subject. Annually with an estimated incidence rate of 85-100/100,000 persons, around 120,000 new cases are being added to already existing infectious cases. However, the higher prevalence rate of 554/100,000 cases was also reported from Northern Pakistan [19,20]. Among different age groups, as in other developing countries, youngers are commonly affected and male gender outnumbers females except in adolescence. As per Burden of Disease estimates, tuberculosis accounts for 5 % of the total disability-adjusted life years (DALYs); indicating a higher burden of tuberculosis in Pakistan than the world average of 3 %. [12]

CONCLUSION

To conclude, in this study it is safe to deduce that the contribution of special stains in diagnosing granulomatous inflammation is of utmost importance. Basic staining techniques may also be useful as most of the cases may get revealed by simple techniques. However, when a challenge is faced in making a diagnosis, it is crucial to consider the option of a special stain.

AUTHORS CONTRIBUTION

Hamza Tahir: Data gathering & Data analysis, building questionnaire, writing introduction, results, discussion writing, Literature review & final editing of the entire article.

Tariq Sarfraz: Supervising and facilitating the research.

Aaminah Hanif: Contribution in discussion and literature review.

Aiza Saadia: Contribution in discussion, results and literature review.

Nosheen Tariq: Contribution in introduction and literature review.

Muhammad Tahir Khadim: Overall supervision.

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