ORIGINAL ARTICLE

Non-Caseating Granulomas in Skin Biopsies of Leishmania Cases

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ABSTRACT

Objective: To determine the frequencies of different types of granulomas in patients suffering from cutaneous leishmaniasis.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: Armed Forces Institute of Pathology, Combined Military Hospital, Rawalpindi, Dec 2021 to Oct 2022.

Materials and Methods: This study was conducted on 290 patients suffering from cutaneous leishmaniasis. Patients aged between 18 and 70 years of both genders were included, while those who had already received treatment for leishmaniasis, or suffered from mucocutaneous or visceral leishmaniasis were excluded. All patients underwent a punch biopsy, tissue which was stained with hematoxylin and eosin, and then studied for the presence of granulomas and Parasitic Index. PCR was done to confirm species of Leishmania causing disease. Data was analyzed using SPSS 26.0.

Results: Our study population had a median age of 38.00 (16 - 63) years, with a male majority: 185 (63.8%). Granuloma formation was seen in 185 (63.8%) patients: 121 (41.7%) had a tuberculoid granuloma and 13 (4.5%) had suppurative granulomas, while 51 (17.6%) had caseating ones. L. Tropica was the most seen organism accounting for 255 (87.9%) cases, L. Major was the infective organism in 26 (9.0%) patients, while L. Infantum was found in 9 (3.1%) cases. Factors such as gender (p=0.273), age of the patient (p=0.901), disease duration (p=0.366), site of lesion (p=0.669), type of skin lesion (p=0.490), parasite index (p=0.297) and species of Leishmania (p=0.870) did not have any significant association with the development of non-caseating granulomas.

Conclusion: Chronic granulomatous inflammation is a common finding on histopathology in cutaneous leishmaniasis and vigilance is required to avoid confusion with other endemic granulomatous skin conditions.

Key Words: *Cutaneous Leishmaniasis, Mycobacterium Tuberculosis, Non Caseating Granulomas, Polymerase Chain Reaction, Ridley Modified Parasitic Index.*

Introduction

Leishmaniasis is a common tropical disorder attributed to an obligate intracellular protozoan which is transmitted to humans by the bite of the Phlebotomus or Lutzomyia sand-fly and causes manifestations by which it is classified into the cutaneous, mucocutaneous, and visceral disease, with just the cutaneous form having an estimated incidence of one million new cases per annum.[1,2] Pakistan accounts for approximately 10% of the global disease burden for all forms of leishmaniasis which amounts to an estimated half a million cases.[3] Leishmania is diagnosed using a wide variety of tests such as direct visualization of the parasite in a tissue specimen, skin hypersensitivity testing, antibody detection and molecular methods such as polymerase chain reaction, however, such definitive but expensive methods are not readily available in resource-poor countries, and diagnosis is based on clinical and tissue examination.[4,5]

However, this diagnostic approach is not without risk: Leishmania is known for its ability to imitate different skin conditions not only macroscopically, but microscopically as well.[6] Histologically, the disease may present in wide variety of ways including

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parasitized macrophages with lymphocytic infiltration or non-specific chronic inflammation.[7] It is also associated with the formation of granulomas: these can vary from non-specific ill-defined granulomas to epithelioid granulomas.[7,8] Cases of cutaneous leishmaniasis have been demonstrated to show caseous necrosis as well, and have the potential to be misdiagnosed as tuberculosis,[9] or may be mischaracterized as a non-infectious disease such as pyoderma gangrenosum.[10] We conducted this study with the aim of determining frequencies of different types of granulomatous inflammation in patients suffering from cutaneous leishmaniasis. Understanding the nature of the myriad types of histological pictures that leishmaniasis can present with is an important concept to understand, the lack of which can result in the misdiagnosis of the disease, and the institution of inappropriate management, with potentially dire consequences for the patient and the treating team. Recognition that leishmaniasis can mimic the histological picture of other diseases and remaining sentient to the possibility of the presence of this endemic parasite will go a long way towards reducing the morbidity associated with it.

Materials and Methods

We conducted this descriptive cross-sectional study between 1st Dec 2021 and 31st Oct 2022 in Armed Forces Institute of Pathology, Combined Military Hospital, Rawalpindi, on 290 patients suffering from cutaneous leishmaniasis, after obtaining informed consent. Approval from the institutional review board of the hospital was obtained. Sample selection was carried out via non-probability, consecutive sampling. The WHO sample size calculator was used to calculate the sample size keeping a confidence level of (1-α) of 95%, an absolute precision (d) of 0.05 and an anticipated population proportion (P) of 0.748, which was the percentage of patients with cutaneous leishmaniasis suffering from non-caseating granulomas, from Aoun et al.[11]. For convenient sampling patients aged between 15 and 70 years of both genders were included in the study. Patients who had received previous treatment for leishmaniasis, or suffered from mucocutaneous or visceral leishmaniasis, or those who were suffering from malignancies, immunosuppressed states, drugs that caused immunosuppression, or patients who had a positive PCR for Mycobacterium Tuberculosis were excluded.

All patients' details were documented for demographic data such as age and gender on enrollment, as well as for duration of disease, site of lesion and type of skin eruption. Each patient underwent a punch biopsy of about 5 mm, which was processed and preserved as tissue blocks. Subsequent slides were prepared using hematoxylin and eosin stain. At this point, patients were documented for type of granuloma, if any. Diagnosis of leishmaniasis as well as confirmation of species was done by Polymerase Chain Reaction (PCR) targeting kinetoplast DNA.

In a brief, the formalin-fixed, paraffin-embedded tissue blocks were used to extract DNA. Leishmania ribosomal internal transcribed spacer 1 (ITS1) was amplified using primers. This was done in a 50 mL amplification reaction. The following stages and conditions were employed for amplification on the Px2 thermal cycler: 95 C for 2 min, 35 cycles of (95 C for 20 sec, 53 C for 30 sec, 72 C for 1 min), and 72 C for 6 min.[11]

Furthermore, Ridley modified parasitic index quantifies the parasitic load of amastigotes in cutaneous lesions and has a numerical score from 1 to 6 as displayed in table I.[11]

Table I : Ridley Modified Parasitic Index

Parasitic Index	Number of Amastigotes Per Standard Section
6+	≥100, 000
5+	≥100, 00
4+	≥100, 0
3+	≥100
2+	≥10
1+	≥1

Data was analyzed using the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows version 26, IBM Corp; Armonk, USA). Mean and standard deviation was calculated for quantitative variables specifically patient age and duration of disease. Qualitative variables like gender, site of skin lesion, type of eruption, Parasitic Index, species of Leishmania, presence and type of granuloma were recorded in terms of frequency and percentage. Patients were divided into three groups: one with caseating granulomas, one without and one with no

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granuloma formation. Quantitative variables were compared across groups using the independent samples *t*-test / One-Way ANOVA test while the Chi square test / Fischer exact test was used for qualitative variables and a *p*-value of ≤ 0.05 was considered significant.

Results

This study was conducted on a total of 290 patients with a median age of 38.00 (16 - 63) years. Most of the patients were male i.e., 185 (63.8%), while the median duration from onset of skin lesion to presentation was 27.00 (6 - 49) weeks. A total of 112 (38.6%) had a lesion present on the upper limbs, the lower limbs were affected in 89 (30.7%), while the head and neck region and the trunk were involved in 80 (27.6%) and 9 (3.1%) cases, respectively. Nodules were seen in 159 (54.8%) i.e., most of our cases, followed by ulcers: 97 (33.4%) and 34 (11.7%) had papular lesions at presentation.

Table-II shows the patient characteristics distributed according to gender at the time of presentation.

Table II: Patients Characteristics According to Gender

Variable	Male (n=185)	Female (n=105)
Gender	185 (63.8%)	105 (36.2%)
Age (years)	37.75 ± 13.87	39.21 ± 13.51
Duration of Disease (weeks)	27.74 ± 11.84	26.51 ± 10.92
Site of Lesion		
Upper Limbs	67 (36.2%)	45 (42.9%)
Lower Limbs	62 (33.5%)	27 (25.7%)
Head and Neck	51 (27.6%)	29 (27.6%)
Trunk	5 (2.7%)	4 (3.8%)
Type of Skin Lesion		
Nodule	103 (55.7%)	56 (53.3%)
Ulcer	62 (33.5%)	35 (33.3%)
Papule	20 (10.8%)	14 (13.4%)

Table-III displays the histological characteristics of the leishmaniasis for the sample according to gender. A Parasite Index of One was seen in 27 (9.3%) patients, cases with an index of Two were 45 (15.5%), those with a Parasite Index of Three accounted for 59 (20.3%) cases, while an index of Four, Five and Six were seen in 67 (23.1%), 63 (21.7%), and 29 (10.0%) cases, respectively. Leishmania Tropica (L. Tropica) was the most seen organism accounting for 255 (87.9%) cases, while Leishmania Major (L. Major) was the infective organism in 26 (9.0%) patients, while Leishmania Infantum (L. Infantum) were found in the least number of patients i.e., 9 (3.1%). Granuloma formation was seen in 185 (63.8%) patients: 121 (41.7%) had a tuberculoid granuloma while 51 (17.6%) and 13 (4.5%) had caseating and suppurative granulomas, respectively. Thus, a total of 134 (46.2%) patients had non-caseating granulomas while and 105 (36.2%) had no evidence of any granuloma on histology.

Table III: Histological Characteristics According to Gender

Variable	Male (n=185)	Female (n=105)
Parasite Index		
One	15 (8.1%)	12 (11.4%)
Two	24 (12.9%)	21 (20.0%)
Three	35 (18.9%)	24 (22.9%)
Four	48 (25.9%)	19 (18.1%)
Five	47 (25.4%)	16 (15.2%)
Six	16 (8.6%)	13 (12.4%)
Species of Leishmania		
L. Tropica	161 (87.0%)	94 (90.5%)
L. Major	19 (10.3%)	7 (6.7%)
L. Infantum	5 (2.7%)	4 (3.8%)
Granuloma Formation	123 (66.5%)	62 (59.0%)
Granuloma Type		
Tuberculoid	85 (45.9%)	36 (34.4%)
Caseating	31 (16.8%)	20 (19.0%)
Suppurative	7 (3.8%)	6 (5.7%)
None	62 (33.5%)	43 (40.9%)

Table-IV shows the comparison between patients with non-caseating granulomas, those without and those patients who did not have granulomas. Factors such as gender, age of the patient, disease duration, site of lesion, type of skin lesion, parasite index and species of Leishmania were not associated with the development of non-caseating granulomas.

Discussion

Granuloma formation occurs in response to persistent inflammatory stimulation which results in the aggregation of inflammatory cells (primarily macrophages): an immune protective mechanism which neutralizes foreign/pathogenic matter by encapsulation and neutralization.[12] Cutaneous leishmaniasis, in its limited form, is usually cleared spontaneously by the immune system without

Table IV: Comparison of Different Variables with and Without Chronic Granulomatous Inflammation

Variable	Non-Caseating (n=134)	Caseating (n=51)	None (n=105)	*p* value
Gender				
Male	92 (68.7%)	31 (60.8%)	62 (59.0%)	0.273
Female	42 (31.3%)	20 (39.2%)	43 (41.0%)	
Age (years)	38.62 ± 14.19	37.61 ± 12.42	38.14 ± 13.86	0.901
Duration of Illness (weeks)	28.33 ± 11.51	26.49 ± 11.87	26.36 ± 11.33	0.366
Site of Lesion				
Upper Limbs	48 (35.8%)	24 (47.1%)	40 (38.1%)	0.669
Lower Limbs	47 (35.1%)	13 (25.5%)	29 (27.6%)	
Head and Neck	34 (25.4%)	13 (25.5%)	33 (31.4%)	
Trunk	5 (3.7%)	1 (1.9%)	3 (2.9%)	
Type of Skin Lesion				
Nodule	76 (56.8%)	31 (60.8%)	52 (49.5%)	0.490
Ulcer	42 (31.3%)	17 (33.3%)	38 (36.2%)	
Papule	16 (11.9%)	3 (5.9%)	15 (14.3%)	
Parasite Index				
One	11 (8.2%)	7 (13.7%)	9 (8.6%)	0.297
Two	21 (15.7%)	6 (11.8%)	18 (17.1%)	
Three	22 (16.4%)	16 (31.4%)	21 (20.0%)	
Four	30 (22.4%)	8 (15.7%)	29 (27.6%)	
Five	37 (27.6%)	8 (15.7%)	18 (17.1%)	
Six	13 (9.7%)	6 (11.7%)	10 (9.6%)	
Species of Leishmania				
L. Tropica	119 (88.8%)	44 (86.3%)	92 (87.6%)	0.870
L. Major	10 (7.5%)	6 (11.8%)	10 (9.5%)	
L. Infantum	5 (3.7%)	1 (1.9%)	3 (2.9%)	

intervention in about a month, however, that is not always necessarily the case and the disease may take as long as six years to clear, necessitating confirmation of diagnosis and institution of appropriate treatment.[13] However, the process of diagnosis may be difficult in areas without molecular methods of disease confirmation i.e., reliant on histology for establishing presence of the disease: cutaneous leishmaniasis is known as the great imitator and it has the ability to mimic almost any dermatosis, hence the requirement for this study.[14]

Granulomas were seen in 63.8% (46.2% non-caseating, 17.6% caseating), while 36.2% did not appear to have any granulomas on histological examination in our study. Most of the non-caseating granulomas were tuberculoid, while a minority were suppurative. Cardozo et al reported that 84.0% of their cases had granuloma formation, with the majority of these having non-caseating granulomas, while less than 1.0% had caseating ones.[15] Most of their patients had ill-defined histiocytic aggregates rather than the well-formed granulomas seen in our study.[15] Aoun et al reported granuloma formation in 61.5% of their study, of which 46.1% were non-caseating, while 15.4% demonstrated caseation,[11] while Andrade-Narvaez et reported that 43.8% had granulomas in their study, none of whom demonstrated caseation.[16] The variability in results may be attributable to a number of reasons: granuloma formation may be dependent on a number of host and disease factors which were not homogenous across the studies mentioned above, which may account for the differences in histological patterns seen.

Our study had a median age of 38.00 (16 - 63) years, age did not appear to have any association with the visualization of non-caseating granulomas, (*p*=0.901). This figure is in keeping with existing studies on the subject such as Debash et al who reported that the maximum number of cases of cutaneous leishmaniasis were seen in the range of 15 to 49 years in their study, while Bisetegn et al reported a mean age of 31.9 ± 14.29 years for their sample.[17,18] Moreover, Aoun et al also reported that there was no association between the development of different types of granulomas and age.[11]

The most of our study sample was male i.e., 185 (63.8%). Gender did not appear to have an association with the formation of non-caseating granulomas, (*p*=0.273). Wijesinghe et al and Solomon et al both noted that cutaneous leishmaniasis was more likely to be present in males, which is likely due to increase outdoor activities and differences in clothing when compared to females.[19,20] In addition, Aoun et al also concluded that gender did not appear to be associated with the development and type of granuloma.[11]

Most patients i.e., 38.6% had a lesion on the upper limbs, while the lower limbs had lesions in 30.7% cases. The head and neck region were affected in

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27.6% patients while the trunk was least affected: 3.1% cases, in our study. Anatomical location of the lesion did not appear to have an association with the development of non-caseating granulomas, (p=0.669). Rather et al noted in their study that lesions tended to occur on exposed areas, while Aoun et al also confirmed that the bites were most commonly found on the extremities and the head and neck region as in our study, and that location did not have any effect on the development of non-caseating granulomas, a fact that was also concurred with by Aguado et al.[11,22]

The principle macroscopic skin manifestation of disease were nodules in our study which accounted for 54.8% cases. Ulcers were seen in 33.4% patients while papular lesions were the least common and occurred in 11.7%. The type of macroscopic skin lesion did not appear to have a significant association with the development of non-caseating granulomas, (p=0.490). Our results were in contrast to Wijesinghe et al who noted that the occurrence of papules, nodules and ulcers occurred at the roughly same frequencies of 34.7%, 32.7% and 30.6%, respectively,[19] while Aoun et al reported that the majority of patients had nodular lesions (49.2%), while ulcerative lesions were also common (49.2%), which was in agreement with our results.[11]Variability in results between our study and Wijesinghe may be due to the delayed presentation seen in the latter study which may lead to worsening of the skin lesion. Aoun et al, like our study, also noted that the type of macroscopic lesion was not associated with the development of non-caseating granulomas.[11]

The Parasitic Index did not appear to have a relationship with the development of non-caseating granulomas, (p=0.297), in our study, which was concurred with by Aoun et al (p=0.09),[11] however, Wijesinghe et al reported that having a higher Parasitic Index was associated with a higher risk of developing granulomas, (p=0.027), which was at odds with our study.[19] This aspect of research requires further study before concrete conclusions can be drawn, but studies have shown that the number of organisms reduce as the cutaneous leishmaniasis becomes chronic but the granulomas form regardless.[23]

Lastly, L. Tropica was the most seen species in our study, accounting for 87.9% of our sample, followed by L. Major, which affected 9.0% patients, while L. Infantum was isolated in 3.1% cases. None of the species had any propensity towards the development of non-caseating granulomas, (p=0.870). Previous studies have shown the most common organism in Pakistan is L.Tropica in patients with cutaneous leishmaniasis which forms the bulk of cases, while L. Major accounts for a minor percentage, and L. Infantum is rarely seen.[24] Studies have also previously demonstrated that no species is associated with the development of non-caseating granulomas.[11]

Study Limitations

This research protocol was conducted as a single-center study and was limited by its small sample size. Secondly, this study was performed on military personnel and their wards which has robust screening programs which may have resulted in early detection of disease which may or may not have affected the frequency of granuloma formation. Lastly, a case-control study would give a better idea of the association of different disease and patient factors with the formation of different types of granulomas and should be the subject of future research.

Conclusion

Cutaneous Leishmaniasis is a commonly encountered disease that may present as a diagnostic dilemma in our part of the world due to its ability to mimic other common skin conditions such as tuberculosis and leprosy in terms of histological features. Recognition that the disease can commonly cause manifestations such as non-caseating and caseating granulomas, the pathologist must remain vigilant in making the correct diagnosis and institute appropriate and timely management. Future research should focus on the effect of granuloma formation on response to treatment and prognosis in terms of scarring.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.