e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(10); 792-798

Original Research Article

Immunohistochemical Characterization of M4 Macrophages in Tuberculoid and Lepromatous Leprosy: An Observational Study

Nausheen Sanaullah Khan[1], Shalini Suman[2], Mayank Anand[3], Javed Iqbal[4]

[1,4]Associate Professor, Department of Pathology, Integral Institute of Medical Sciences and Research, Lucknow

[2]Junior Resident, Department of Pathology, Integral Institute of Medical Sciences and Research Lucknow

[3]Assistant Professor, Department of Pathology, Integral Institute of Medical Sciences and Research Lucknow

Received: 21-07-2023 / Revised: 20-08-2023 / Accepted: 27-09-2023

Corresponding author: Dr Javed Iqbal

Conflict of interest: Nil

Abstract:

Background/Aim: To study the immunohistochemical expression of M4 macrophages in Tuberculoid and Lepromatous leprosy to substantiate the existing pool of knowledge and to assess its clinical significance.

Material and Methods: It was a single center, observational study conducted under a period of March 2021 to September 2022 which included skin biopsies of 62 cases of leprosy seen in the Department of Dermatology and reported in the histopathology section of the Department of Pathology. The histopathology slides were reviewed under light microscopy and classified according to the Ridley Jopling classification on the basis of BI index. Representative sections of each case were stained simultaneously by immunohistochemistry (IHC) for CD68, MMP7 and MRP8 in the Department of Pathology.

Results: CD68 expression was seen in 43.2% of Tuberculoid and 100% of Lepromatous cases. Moderate to strong expression (Score 2 and 3) was seen in 10.8% of Tuberculoid and 62.1% of Lepromatous cases.MMP7 expression was significantly higher in Lepromatous (93.1%) as compared to that in Tuberculoid leprosy (37.8%).MRP8 expression was significantly higher in Lepromatous (93.1%) as compared to that in Tuberculoid leprosy (43.2%).It was seen that TT type was associated with weak expression of these markers whereas LL type was associated with moderate to strong expression of these markers.

Conclusion: The findings of the study showed that expression of all the three M4 macrophage markers was significantly higher in lepromatous as compared to tuberculoid leprosy, thus showing different pathogenetic and progression pathways of the disease.

Conclusion: The findings of the study showed that expression of all the three M4 macrophage markers was significantly higher in lepromatous as compared to tuberculoid leprosy, thus showing different pathogenetic and progression pathways of the disease.

Keywords: Macrophages, M4, Tuberculoid, Lepromatous, Leprosy.

Introduction

Leprosy or Hansen's disease is perhaps one of the world's oldest and most dreaded diseases that has tormented humans throughout history, leaving lasting impressions on religion, literature and art. In addition to the physical effects of the disease, patients have also suffered severe social stigma and ostracism from their families, communities and even health professionals to such an extent that leprosy has been known since ancient times as "the death before death".[1]

Globally nearly 200,000 new cases of leprosy are detected each year with India contributing to almost 60% of total global burden of leprosy as per WHO report 2020. However, it is much below the two decade back scenario when each year nearly 400,000 new cases of leprosy were diagnosed, of which 80% used to be from India.[2] As per a WHO release, in the year 2020, a total of 127558 new leprosy cases were detected throughout the world.[3] In India too, during the period ranging from Jan-2020 to Sept.-2020, a total of 50505 cases and during the period ranging from January to March, 2021 a total of 22613 new leprosy cases were diagnosed.[4]

Throughout the world, leprosy has been highlighted as the most important cause of peripheral neuropathy. If left untreated, leprosy can cause nerve damage, leading to muscle weakness, atrophyand permanent disabilities.[5]Jopling has classified leprosy into five types, namely tuberculoid (TT), borderline tuberculoid (BT), borderline borderline (BB), borderline lepromatous (BL) and lepromatous

leprosy (LL), depending upon the nature of infection.[6]

M4 macrophages differentiate in the presence of colony-stimulating factor (CSF) and (CXCL4). M4 macrophage produce CD68, metalloproteinase 7 (MMP7), MMP12, and calcium-binding protein A8 (MRP 8 or S100A8) IL-6, TNF-α and thus they act as its markers. M4 macrophages highly express the low- density lipoprotein (LDL) receptor, and in chronic lesions, these cells form a large contingent of foam cells which are found responsible for inflammation[7-9]. M4 macrophages have been found to be associated with coronary atherosclerotic plaque instability and metabolic disorders too and its relationship with other conditions is also being explored. Most of these studies are in evolutionary stage and require further exploration and substantiation. As such, with respect to M4 expression in leprosy, there is extreme paucity of scientific studies. [10,11]Hence, the present study was planned to study the immunohistochemical expression of M4 macrophages in Tuberculoid and Lepromatous leprosy to substantiate the existing pool of knowledge and to assess its clinical significance.

Material and Methods

It was a single center, observational study conducted under a period of March 2021 to September 2022 which included skin biopsies of all cases of leprosy seen in the Department of Dermatology and reported in the histopathology section of the Department of Pathology.

Sample Collection: All the relevant materials (blocks and slides) were retrieved from the Department of Pathology of clinically diagnosed and histopathologically confirmed cases of Tuberculoid (TT, BT) and Lepromatous leprosy (LL, BL) attending Dermatology OPD from August 2018 to September 2022.

Sample Processing: The histopathology slides were reviewed under light microscopy and classified according to the Ridley Jopling classification on the basis of BI index. Representative sections of each case were stained simultaneously by immunohistochemistry (IHC) for CD68, MMP7 and MRP8 in the Department of Pathology.

Inclusion Criteria: Skin biopsy of all patients of Tuberculoid (TT, BT) and Lepromatous leprosy (LL, BL) from August 2018 to September 2022 were included.

Exclusion Criteria:

1. Patients with pure neuritic leprosy,
2. Histologically proven indeterminate leprosy,
3. Histologically proven mid borderline leprosy

Sample Size: 66

Sample size (n) = $\boldsymbol{z2*pq/d2}$

Calculation:

Where,

n - desirable sample size

p- expected prevalence or based on previous research = 54.5% q is 1-p = 100 – 54.5 = 45.5

d is margin of error or precision = 12%

(Z1-α/2)2 is a critical value and a standard value for corresponding level of confidence at 95%CI it is 1.96 and 99%CI it is 2.58.

Method

Relevant clinical details were noted from archivable material available. Tissue-specific Immunohistochemical study was performed using Rabbit monoclonal antibody to CD68 [PA5-78996], Rabbit polyclonal antibody to MMP7 [PA5-87486] and Rabbit monoclonal antibody to MRP8/S100A8

[MA5-31227]. Representative sections of 3-5 micron thickness were cut from the paraffin embedded blocks and subjected to immunohistochemical staining with CD68, MMP7 and MRP8.

First, tissue samples were deparaffinized and hydrated in alcohol. For antigen retrieval, the sections were incubated in citrate buffer. Blocking of endogenous peroxidase activity was done by 3% hydrogen peroxide diluted with buffer. Sections were then incubated with primary antibody followed by immersion in PBS buffer and further incubated with secondary antibody. Chromogen was applied and all sections were counter stained with hematoxylin. Positive control for CD68 used was human tonsil tissue and for MMP7 and MRP8 were colon carcinoma tissue and infected spleen tissue respectively. Negative control was done by omitting primary antibody. Each immunohistochemical stained slide was scanned by light microscope for positive staining and was graded.

Quantitative Analysis: Each immunohistochemical stained slide was scanned by light microscope for positive stain. The lesions were classified on the basis of number of positively stained cells/ field. Staining was evaluated according to the protocol developed by de Sousa et al [12], Boström MM et al [13], Jakubowska K et al[14], Sickert D et al[15] and Varsha et al.[16]

Positive staining of IHC markers expression were graded as:

0: no stained cell/hpf- No expression
1:< 25% stained cell/hpf- Weak/Mild expression
2: 25-50 %stained cell/hpf-Moderate expression
3:> 50% stained cell/hpf- Strong expression

[Grade 1, 2, 3 were counted in positive cases and Grade 0 in negative cases]

Grading of markers were done independently on the slides of each case and were analyzed for their correlation of expression and then association between expression of M4 macrophage markers and the morphological changes were studied in both Tuberculoid and Lepromatous leprosy.

Statistical Analysis: Data were stored in electronic spreadsheet of the excel 2010 programme. Diagnostic test was used to evaluate the efficacy of immunohistochemical expression of M4 macrophages in Tuberculoid and Lepromatous leprosy.

Results

The present study was carried out to immunohistochemically characterize the presence of M4 macrophages in tuberculoid and lepromatous leprosy. For this purpose, a total of 66 leprosy cases were enrolled. Majority of cases (n=37; 56.1%) were histopathologically diagnosed as tuberculoid whereas remaining 29 (43.9%) were diagnosed as lepromatous. Majority of cases (n=42; 63.6%) were aged between 15 and 40 years. Overall, majority (n=47/66; 71.2%) of cases were males. The sex-ratio (M:F) of the study population was 2.47. Out of 37 histopathologically confirmed tuberculoid cases, a total of 11 (29.7%) corresponded to TT type and 26 (70.3%) corresponded to BT type. Out of 29 histopathologically confirmed lepromatous cases, a total of 8 (27.6%) corresponded to BL type and 21 (72.4%) corresponded to LL type.

Out of 37 tuberculoid cases, there were 16 positive cases and 21 negative cases for CD68 expression. In 29 Lepromatous cases, there were 29 positive cases of CD68 expression and no negative case noted. Thus, it was seen that CD68 expression was high towards lepromatous leprosy cases, where, granulomatous infiltration was more by macrophages and plasma cells ($p<0.001$) (Table 1. Fig 1a, Fig 1b).

Amongst the cases of Tuberculoid leprosy, positive cases were 14, and negative cases were 23 of MMP7. In 29 cases of lepromatous leprosy, grade 0, 1, 2 showed 7.0%, 73% and 17% respectively. 3% cases were in grade 3. There were 27 positive and 2 negative cases. Thus, we were able to conclude that MMP7 expression was more in lepromatous group as compared to tuberculoid group ($p <0.001$) (Table 2. Fig 2a, Fig 2b).

It was noted that out of 37 cases of Tuberculoid leprosy, there were 16 positive and 21 negative cases for MRP8. In 29 LL cases, there were 27 positive and 2 negative cases. Therefore, expression of MRP8 was noted to be more in Lepromatous type of leprosy as compared to Tuberculoid type ($p<0.001$) (Table 3. Fig 3a, Fig 3b).

Accuracy of CD68 positive expression for differentiating between Lepromatous and Tuberculoid types was 75.8%.MMP7 was found to be 93.1% sensitive and 62.2% specific. Its positive and negative predictive values were found to be 65.9% and 92% respectively.MRP8 was found to be 93.1% sensitive and 56.8% specific. Accuracy of MRP8 positive expression for differentiating between Lepromatous and Tuberculoid types was 72.7%. CD68 expression scores did not show a significant correlation with MMP7 as well as MRP8 in both Tuberculoid and Lepromatous types.A mild positive and significant correlation was observed between MMP7 and MRP8 for both Tuberculoid and Lepromatous types.

Table 1: CD68 Expression in leprosy cases

CD68	Tuberculoid(n=37)			Lepromatous(n=29)		
	TT Cases (n=11)	BT Cases (n=26)	Expression in % cases of Tuberculoid	BL Cases (n=8)	LL Cases (n=21)	Expression in % cases of Lepromatous
0 (no stained cell)	05	16	56.8%	00	00	00%
1 (<25% Stained cell)	05	09	37.8%	07	05	41.4%
2(25-50% Stained cell)	01	00	2.7%	00	12	41.4%
3 (> 50% Stained cell)	00	01	2.7%	01	04	17.2%
Total(N)	11	26	37	8	21	29
Intra group comparison	χ^2=3.379; p=0.337			χ^2=10.39; p=0.006		
Inter group comparison	χ^2=32.64; p<0.001					

Table 2: MMP7 Expression in leprosy cases

MMP7	Tuberculoid(n=37)			Lepromatous(n=29)		
	TT Cases (n=11)	BT Cases (n=26)	Expression in % cases of Tuberculoid	BL Cases (n=8)	LL Cases (n=21)	Expression in % cases of Lepromatous
0(no stained cell)	1	22	62%	2	0	7.0%
1(<25% Stained cell)	8	4	32%	6	15	73%
2(25-50% Stained cell)	1	0	3.0%	0	5	17%
3 (>50% Stained cell)	1	0	3.0%	0	1	3.0%
Total(N)	11	26	37	8	21	29
Intra group comparison	χ^2=19.657; p<0.001			χ^2=7.546; p=0.056		
Inter group comparison	χ^2=22.116; p<0.001					

Table 3: MRP8 Expression in leprosy cases

MRP8	Tuberculoid(n=37)			Lepromatous(n=29)		
	TT Cases (n=11)	BT Cases (n=26)	Expression in % cases of Tuberculoid	BL Cases (n=8)	LL Cases (n=21)	Expression in % cases of Lepromatous
0 (no stained cell)	5	16	56.8	1	1	6.9
1(<25% Stained cell)	5	8	35.1	7	6	44.8
2(25-50% Stained cell)	1	1	5.4	0	11	37.9
3 (>50% Stained cell)	0	1	2.7	0	3	10.3
Total(N)	11	26	37	8	21	29
Intra group comparison	χ^2=1.643; p=0.650			χ^2=10.324; p=0.016		
Inter group comparison	χ^2=22.284; p<0.001					

Fig.1: (a) CD68 expression in LL (b) CD68 expression in TT

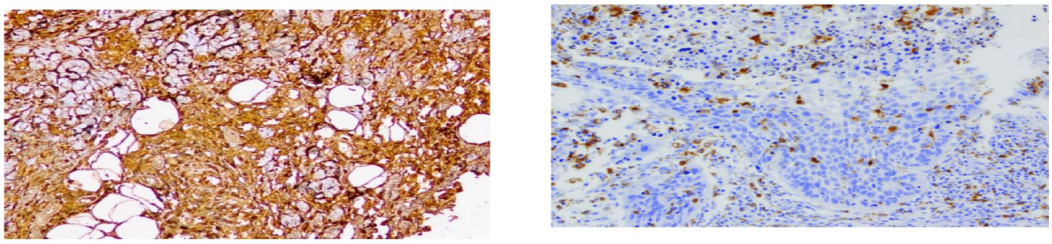
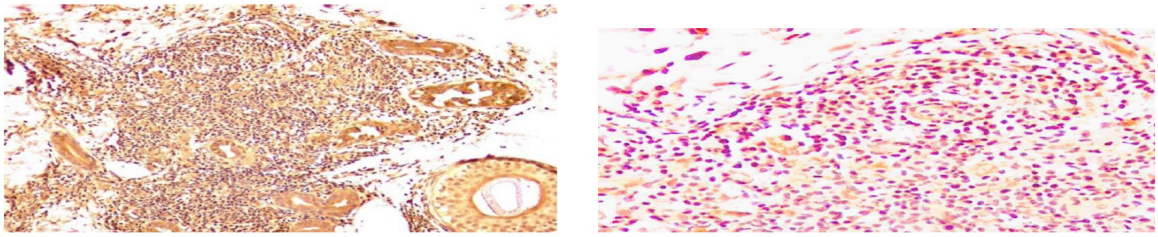


Fig.2: (a) MMP7 expression in LL (b) MMP7 expression in TT



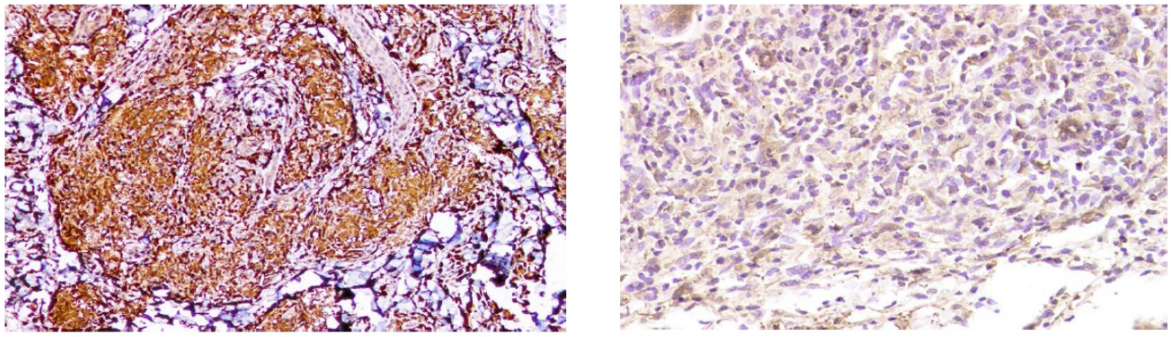


Fig.3: (a) MRP8 expression in LL (b) MRP8 expression in TT

Discussion

Macrophage plays the central role due to its characteristic plasticity and heterogenous behaviour. It can differentiate into distinct subtypes like M1, M2, Mox, M4 etc. based on host tissue microenvironment and produces receptors, enzyme, costimulatory molecule that induces development of suppressive or inflammatory response and thus determine clinical outcome. Based on tissue microenvironment and cytokines released, host immune response varies.[12, 17]

M4 macrophages are also seen to induce the establishment of a regenerative environment and remodelling of extracellular matrix, which are important for pathogen-host interaction. M4 macrophages produce CD68, MMP7, MRP8 or S100A8, IL6, TNF-α, MMP7 and MMP12, so they can be detected by immunolabelling with combination of markers.[18,19] With this background the present study was planned to immunohistochemically characterize the presence of M4 macrophages in Tuberculoid and Lepromatous leprosy.

In the present study, out of a total of 66 skin biopsy tissue specimen, 37 cases (56.1%) were identified as Tuberculoid while remaining 29 (43.9%) were identified as Lepromatous. Subsequently, among tuberculoid type, majority were identified as borderline tuberculoid cases (i.e. BT, 70.3%) and tuberculoid cases (i.e. TT, 29.7%) respectively. Among lepromatous cases, majority were identified as lepromatous LL type (i.e. 72.4%) and remaining 8 (27.6%) were identified as borderline lepromatous, BL type. Thus, a total of 34/66 (51.5%) of our patients were in borderline spectrum followed by LL type (n=21/66; 31.8%) and TT type (n=11/66; 16.7%).

Compared to the present study, Thakkar and Patel[20] in their study of 250 leprosy patients, found 40% patients in the borderline spectrum followed by tuberculoid leprosy (TT) (29.2%) and lepromatous leprosy (LL) (26.8%). In their study, there were 3.9% cases of indeterminate leprosy (IL) too, however, in the present study, there was no casewith indeterminate type. In another study, Jha et al[21] in their series of 72 leprosy patients, found the maximum cases (n=28; 38.9%) were BT followed by TT (n=15; 20.8%), BL (n=14; 19.4%), LL (n=12; 16.7%) and BB (n=1; 1.4%)respectively, thus showing a dominance of borderline types (n=43; 59.7%). In the present study, we also had BT (n=26/66; 39.4%) as the most common type and also had a dominance of borderline types. In the study by Singh et al[22] that included a total of 58 cases, borderline tuberculoid (BT) was the most common type seen in 37.9% cases. In a recent study by Joshi et al[23], out of 98 cases with confirmed histopathological diagnosis, maximum were diagnosed as LL (32.7%), followed by BT (29.6%), TT (17.3%) and BL (12.2%)respectively. In their study, a total of 4 (4.1%) cases were diagnosed as mid borderline and 4 cases (4.1%) as histioid respectively. Like the present study, they also found BT and LL to be the dominant types.

In the present study, bacillary index was found to be higher in LL as compared to TT cases. Similar to the present study, Poudel et al. also found higher bacillary indices to be associated with LL/BL type and lower with TT/BT types.[24] A similar observation was also made by Bhagya Lakshmi et al. who also observed higher bacillary indices for LL as compared to TT types.[25]

The present study observed higher expression of IHC markers of M4 macrophage activity in Lepromatous as compared to Tuberculoid leprosy. Expression of CD68, MMP7 and MRP8 was seen in 100%, 93.1% and 93.1% oflepromatous as compared to 43.2%, 37.8% and 43.2% of tuberculoid histopathological types. No significant difference in expression of CD68 and MRP8 was seen between BT and TT subtypes, however, there was a significant difference between BL and LL subtypes. For MMP7, a significant difference in expression was also seen between TT and BT cases whereas in lepromatous type, though expression was higher in LL as compared to BL yet it was not significant statistically. The trends showed minimal expression of different IHC markers in TT and BT types followed by BL and maximum expression in LL type. Thus, the markers were not only able to

differentiate between the lepromatous and tuberculoid types but were also able to differentiate the borderline cases within each of these two types.

The findings in the study are in agreement with the observations of de Sousa et al[12], however, Govindan et al[26] who evaluated the expression of CD68 did not find a significant difference in its expression between lepromatous and tuberculoid types and found the expression to be strong in all the cases in both the leprosy types. Compared to their study, in the present study, we found strong expression of CD68 (score 3) in only 1/37 (2.7%) of tuberculoid and 5/29 (17.24%) of lepromatous types. In the present study, even moderate to strong expression (scores 2 and 3) of CD68 were observed in only 2/37 (5.4%)cases of tuberculoid as compared to 17/29 (58.6%)cases of lepromatous type. CD68 activity in lepromatous leprosy has also been reported by Mi et al[27] in a recent study using RNA-sequencing, however, no such report regarding its association with tuberculoid leprosy has been reported in any other study excepting one by Govindan et al.[26]

Low expression of MRP8 in tuberculoid leprosy was seen in the present study. This has also been documented by Sunderkötter et al[28] in their study who reported that increased expression of MRP8 is characteristic for a macrophage subtype M4 associated with high inflammatory but low antimycobacterial activity. de Sousa et al[12] in their study, similar to the present study found higher expression of CD68, MMP7 and MRP8 in Lepromatous as compared to Tuberculoid types. A much higher differentiating ability in their study could be owing to absence of borderline subtypes in their study. In the present study, owing to a high proportion of borderline (BT and BL) cases, there was certain loss in the specificity, however, the sensitivity for detection of lepromatous type was higher for all the three markers. The present study adds on the previous studies with respect to the ability of these M4 macrophage markers in understanding the differences in pathogenesis and progression of the two histopathological types by incorporating the borderline cases that depict the transitionary line and thereby differentiate the role of M4 macrophages in tuberculoid and lepromatous types.

The present study is relevant in evaluating the role of macrophages, particularly M4 macrophage in leprosy. Despite being the only second study evaluating the specific role of M4 in differentiation of Tuberculoid and Lepromatous leprosy types, it was successful in highlighting its role. Apart fromthis it also highlighted the transitionary pattern of M4 expression in borderline types. These findings are helpful not only from the diagnostic purposes but to evaluate further immunopathogenesis which might help in the development of new targeted therapies.

Conclusion

The findings of the study showed that expression of all the three M4 macrophage markers (CD68, MMP7, MRP8) was significantly higher in lepromatous as compared to tuberculoid leprosy, thus showing different pathogenetic and progression pathways of the disease. It was seen that TT type was associated with weak expression of these markers whereas LL type was associated with moderate to strong expression of these markers. The findings of the study have therapeutic implications in view of difference in immune pathways of two entities and this may elucidate further researches to develop targeted therapies.

References

1. Pandya SS. Historical background. In: Kar HK, Kumar B, editors. IAL textbook of leprosy. New Delhi: Jaypee; 2008. p. 1-18.
2. Murthy PK. Current epidemiology of leprosy. J Indian Med Assoc. 2004 Dec;102(12):672-3, 683.
3. World Health Organization. Leprosy: Key Facts. Updated January 11, 2022. Available online at: https://www.who.int/news-room/fact-sheets/detail/leprosy, last accessed 26th February 2022.
4. Department of Health & Family Welfare, Ministry of Health & Family Welfare, Government of India. 2020-2021 Annual Report. Available online at: https://main.mohfw.gov.in/sites/default/files/Annual%20 Report%202020-21%20English.pdf, last accessed 26th February 2022.
5. Kar HK, Kumar B. IAL Textbook of Leprosy. Jaypee Brothers, Medical Publishers Pvt. Limited; 2010.
6. Jopling WH, McDougall AC. Definition, epidemiology and world distribution. In, W. H. Jopling, A.C. McDougall (edi). Handbook of leprosy, 1st Indian edition. Delhi, CBS publishers, 1992.1-7.
7. Hoeksema MA, Stöger JL, de Winther MP. Molecular pathways regulating macrophage polarization: implications for atherosclerosis. CurrAtheroscler Rep. 2012; 14:254–263.
8. Gleissner CA. Macrophage phenotype modulation by CXCL4 in atherosclerosis. Front Physiol. 2012; 3:1.
9. Van Overmeire E, Laoui D, Keirsse J, Van Ginderachter JA, Sarukhan A. Mechanisms driving macrophage diversity and specialization in distinct tumor microenvironments and parallelisms with other tissues. Front Immunol. 2014; 5:1–16.
10. Erbel C, Wolf A, Lasitschka F, Linden F, Domschke G, Akhavanpoor M, et al. Prevalence of

M4 macrophages within human coronary atherosclerotic plaques is associated with features of plaque instability. International journal of cardiology. 2015 May 1; 186:219-25.
11. Chinetti-Gbaguidi G, Staels B. Macrophage polarization in metabolic disorders: functions and regulation. CurrOpinLipidol. 2011;22(5):365-372.
12. de Sousa JR, Neto FD, Sotto MN, Quaresma JA. Immunohistochemical characterization of the M4 macrophage population in leprosy skin lesions. BMC Infectious Diseases 2018; 18:576.
13. Boström MM, Irjala H, Mirtti T, Taimen P, Kauko T, Ålgars A, et al. Tumor-Associated Macrophages Provide Significant Prognostic Information in Urothelial Bladder Cancer. PLoS One. 2015 Jul 21;10(7):e0133552.
14. Jakubowska K, Pryczynicz A, Iwanowicz P, Niewiński A, Maciorkowska E, Hapanowicz J, et al. Expressions of Matrix Metalloproteinases (MMP- 2, MMP-7, and MMP-9) and Their Inhibitors (TIMP-1, TIMP-2) in Inflammatory Bowel Diseases. Gastroenterol Res Pract. 2016; 2016:2456179.
15. Sickert D, Aust DE, Langer S, Baretton GB, Dieter P. Characterization of macrophage subpopulations and microvessel density in carcinomas of the gastrointestinal tract. Anticancer Res. 2007;27(3B):1693-700.
16. Vk V, Hallikeri K, Girish HC, Murgod S. Expression of CD34 and CD68 in peripheral giant cell granuloma and central giant cell granuloma: An immunohistochemical analysis. J Oral MaxillofacPathol. 2014 Sep- Dec;18(3):341-8.
17. Montoya D, Cruz D, Teles RM, Lee DJ, Ochoa MT, Krutzik SR, et al. Divergence of macrophage phagocytic and antimicrobial programs in leprosy. Cell Host Microbe. 2009 Oct 22;6(4):343-53.
18. Butcher MJ, Galkina EV. Phenotypic and functional heterogeneity of macrophages and dendritic cell subsets in the healthy and atherosclerosis- prone aorta. Front Physiol. 2012; 3:44.
19. de Paoli F, Staels B, Chinetti-Gbaguidi G. Macrophage phenotypes and their modulation in atherosclerosis. Circ J. 2014; 78:1775–81.
20. Thakkar S, Patel SV. Clinical profile of leprosy patients: a prospective study. Indian J Dermatol. 2014;59(2):158–162.
21. Jha SM, Dangol AKS, Shkaya S, Jha B. Clinicobacterial correlation of bacterial index in Hansen's disease. Journal of Pathology of Nepal 2016; 6: 998-1000.
22. Singh AK, Singh R, Singh S, Grover S. How accurately do clinical diagnosis correlate with biopsy findings in leprosy? Journal of Pakistan Association of Dermatologists. 2017;27 (1):23-29.
23. Joshi K, Rizvi G, Kaushik A, Joshi U. A Five-Year Retrospective Study of Profile of Leprosy Patients in a Teaching Tertiary Care Centre in Uttarakhand. Indian J Lepr 2022; 94: 141-152.
24. Poudel S, Ranabhat S, Pun G. Evaluation of agreement between clinical and histopathological diagnosis of subtype of leprosy by skin punch biopsy. Journal of Gandaki Medical College Nepal 2020; 13(1): 1-8.
25. Bhagya Lakshmi A, Rao N, Uma P, Himaja S, Kartheek BVS, Chowdari B. Incidence and clinic histopathological correlation of leprosy in coastal districts of Andhra Pradesh. National Journal of Basic Medical Sciences 2012; 3(1): 52-56.
26. Govindan A, Sasidharanpillai S, Ajithkumar K, Parambath SP, Abdul Latheef EN, Rahima S, et al. Immunohistochemistry of skin lesions in leprosy and leprosy reactions. Lepr Rev 2018; 89: 256–271.
27. Mi Z, Wang Z, Xue X, Liu T, Wang C, Sun L, et al. The immune-suppressive landscape in lepromatous leprosy revealed by single-cell RNA sequencing. Cell Discov. 2022;8(1):2.
28. Sunderkötter CH, Tomimori-Yamashita J, Nix V, Maeda SM, Sindrilaru A, Mariano M, et al. High expression of myeloid-related proteins 8 and 14 characterizes an inflammatorily active but ineffective response of macrophages during leprosy. Immunology. 2004 Apr;111(4):472-80.