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Original Research Article

Immunoexpression of TGF-β: An utility marker in hansen's disease

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ARTICLE INFO

Article history:
Received 09-02-2023
Accepted 16-02-2023
Available online 11-03-2023

Keywords:
Hansen's disease
Immunoexpression
Prognosis
TGF
Biomarker β

ABSTRACT

Background: TGF-β is a pleiotropic cytokine that deploys several functions on different types of cells. In the lepromatous (LL) form of Leprosy, there is predominance of M2 macrophages, which induce the production of growth factors, such as TGF-β which is important for the mechanisms that cause apoptosis and healing.
Aims: To study the immunoexpression of TGF-β in Hansen's disease and to assess the severity of infection with potential response to treatment.
Materials and Methods: The present study was carried out in 45 patients of Hansen's disease after a detailed history and thorough physical examination performed in every subject and punch biopsies were performed. The specimens were fixed, paraffin embedded and sections of 3-5 microns thickness were cut and stained with hematoxylin and eosin stains. Additional sections of 3-4 microns thickness were cut and immunohistochemical staining by TGF-β antibody was performed.
Results: Of the 45 cases of Hansen's disease, most of the cases were of Borderline Tuberculoid and Borderline Lepromatous, constituting 14 (31.1%) cases each, followed by 9 (20.0%) cases of Lepromatous Leprosy. Maximum cases, 12 (26.7%) each with intense reaction were of Borderline Tuberculoid and Borderline Lepromatous, followed by 6 (13.3%) cases of Lepromatous Leprosy. Most of the cases, 9 (20.0%) were of Borderline Lepromatous category with highest proportion score, followed by 7 cases (15.6%) of Borderline Tuberculoid. Highest number of cases, 9 (20.0%) with high immunoreactivity score were in Borderline Lepromatous, whereas most of the cases, 7(15.6%) with low immunoreactivity score were in Borderline Tuberculoid.
Conclusion: The immunoexpression of TGF-β was more intense in lepromatous side of the spectrum as compared to the tuberculoid, which indicates the severity of the disease with less cure potential.

1. Introduction

TGF-β is a pleiotropic cytokine that deploys several functions on different types of cells.[1] Experimental studies have demonstrated that TGF-β also plays a role in the suppression of macrophages.[2] In the TT form of leprosy, there is activation of classical pathway by M1 macrophages which induces the production of tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and nitric oxide synthase, which causes the generation of free radicals that destroy the bacillus.[3] In the LL form, there is predominance of M2 macrophages, which induce the production of growth factors, such as TGF-β and FGF-β. These factors are important for the mechanisms that cause apoptosis and healing through the regulation of extracellular matrix by

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https://doi.org/10.18231/j.achr.2023.008

fibroblasts, proliferation of endothelial cells and induction of angiogenesis in tissue lesions.[4–8]

Many studies have shown a significant difference in TGF- β immunostaining between the TT and LL forms, with higher expression in lesions of patients with the lepromatous form.[3,9]This type of activity of the cytokine TGF-β can be explained by the fact that it plays an important role in the inhibition of the T cell response, suppressing INF- γ, TNF- α and IL-2. In addition, TGF-β causes an inhibitory effect on the lytic ability of macrophages, thus suppressing the production of reactive oxygen and nitrogen intermediates. TGF-β is considered as one of the most potent endogenous suppressors. Several studies have shown that macrophages found in skin lesions of patients with lepromatous leprosy produce larger amounts of TGF-β than macrophages present in skin lesions of patients with tuberculoid leprosy.[10–12]

2. Materials and Methods

The present study was carried out in 45 patients of Hansen's disease after ethical clearance from the Institutional ethics committee (No: IECJNMC/559). A detailed history and thorough physical examination was performed in every subject and punch biopsies were done in every subject.

2.1. Study design

For histopathological study, specimens were fixed, grossed, processed and paraffin embedded. Sections of 3-5 microns thickness were cut, stained with hematoxylin and eosin stains and were studied under light microscopy. Additional sections of 3-4 microns thickness were cut, placed on glass slide coated with poly L-lysine and immunohistochemical staining by TGF-β was performed. Positive control was normal human skin and negative control was tissue section stained without primary antibody.

2.2. Evaluation of immunohistochemistry:[13]

Five most representative fields of high magnification (400X) in each individual case were selected. The staining intensity was categorized as 0:Negative, 1+:Weak expression, 2+ :Moderate expression and 3+ :Intense expression. The proportion score was categorised as 0: None, 1+ : <25%, 2+ :25-50%, 3+ :51-75% and 4+:>75%.

Immunoreactivity score (IRS) (0-12) was calculated by multiplying the intensity and proportion score. A score of 0-6 was considered as low expression and 7-12 was considered as high expression. All data was tabulated and analyzed and appropriate statistical tests (Chi square tests) were applied wherever necessary. p value of <0.05 was considered significant.

3. Results

Our study comprised of 45 cases of Hansen's disease which were histopathologically classified according to Ridley Jopling's classification into five different types of the lesion. Majority of the cases, 23 (51.1%) were in the age group of 20-40 years, followed by 11 (24.4%) cases in 41-60 years of age. Males comprised 25 (55.6%) cases whereas, females comprised of 20 (44.4%) cases in our study.

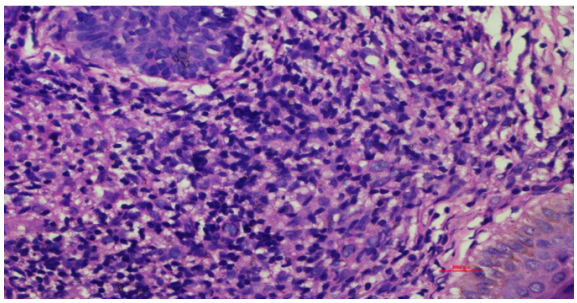

Fig. 1: BorderlineTuberculoid (BT): Photomicrograph shows mature lymphocytes admixed with few epithelioid cells abutting the basal epidermis. Hematoxylin and Eosinx 40X.

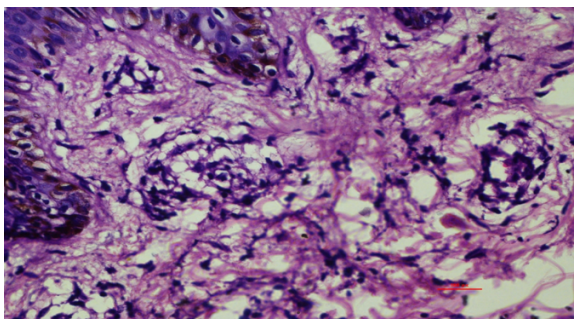

Fig. 2: Lepromatous Leprosy (LL): Photomicrograph shows foamy histiocytes in the dermis with clear zone of unna. Hematoxylin and Eosinx 40X.

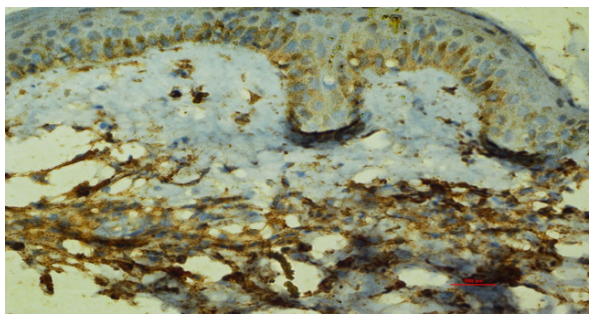

Fig. 3: Lepromatous Leprosy (LL): Sections shows intense immunoexpression of TGF-β in the virchows cells. IHC TGF-βx40X.

Table 1: Different subtypes of hansen's disease

Spectrum of Hansen's Disease	No. of patients	Percentage
Tuberculoid (TT)	7	15.6
Borderline Tuberculoid (BT)	14	31.1
Mid- Borderline (BB)	1	2.2
Borderline lepromatous (BL)	14	31.1
Lepromtaous (LL)	9	20.0
Total	45	100.0

Table 2: Immunohistochemical staining intensity of TGF-β in patients of Hansen's disease

Spectrum of Hansen's Disease	No. of cases	Staining intensity of TGF-β				p value
		0	1+ (Mild)	2+ (Moderate)	3+ (Intense)	
Tuberculoid (TT)	7	-	-	2 (4.4%)	5 (11.1%)	0.691
Borderline Tuberculoid (BT)	14	-	-	2 (4.4%)	12 (26.7%)	
Mid- Borderline (BB)	1	-	-	-	1 (2.2%)	
Borderline lepromatous (BL)	14	-	-	2 (4.4%)	12 (26.7%)	
Lepromtaous (LL)	9	-	-	3 (6.7%)	6 (13.3%)	
Total	45	-	-	9 (20%)	36 (80%)	

Table 3: Immunohistochemical proportion score of TGF-β in patients of Hansen's disease:

Spectrum of Hansen's Disease	No. of cases	Proportion score of TGF-β					p value
		0	1+ (<25%)	2+ (25-50%)	3+ (51-75%)	4+ (>75%)	
Tuberculoid (TT)	7	-	5 (11.1%)	-	-	2 (4.4%)	<0.001
Borderline Tuberculoid (BT)	14	-	7 (15.6%)	-	-	7 (15.6%)	
Mid Borderline (BB)	1	-	-	-	-	1 (2.2%)	
Borderline Lepromatous (BL)	14	-	-	5 (11.1%)	-	9 (20.0%)	
Lepromatous (LL)	9	-	3 (6.7%)	-	3 (6.7%)	3 (6.7%)	
Total	45	-	15(33.3%)	5(11.1%)	3 (6.7%)	22 48.9%)	

Table 4: Immunoreactivity score of TGF-β in different cases of hansen's disease

Spectrum of Hansen's Disease	No. of cases	Immunoreactivity Score of TGF-β				p value
		0-3	4-6	7-9	10-12	
Tuberculoid (TT)	7	5 (11.1%)	-	-	2 (4.4%)	<0.001
Borderline Tuberculoid (BT)	14	7 (15.6%)	-	-	7 (15.6%)	
Mid- Borderline (BB)	1	-	-	-	1 (2.2%)	
Borderline lepromatous (BL)	14	-	5 (11.1%)	-	9 (20.0%)	
Lepromtaous (LL)	9	3 (6.7%)	-	3 (6.7%)	3 (6.7%)	
Total	45	15(33.3%)	5 (11.1%)	3 (6.7%)	22 (48.9%)	

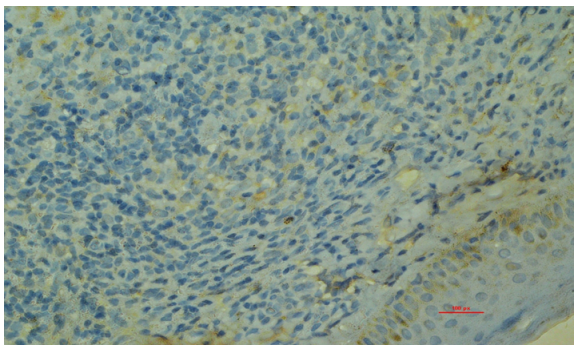

Fig. 4: Borderline Tuberculoid (BT): Sections shows faint immunoexpression of TGF-β in the histiocytes. IHC TGF-*B*x40X

Most common categories were Borderline Tuberculoid (BT) (Figure 1) and Borderline Lepromatous (BL) which comprised of 14 (31.1%) cases each, followed by 9 (20.0%) cases in Lepromatous Leprosy (LL) (Figure 2) Table 1. Majority of the cases, 30 (66.7%) were paucibacillary, followed by multibacillary cases, 15 (33.3%).

Highest number of cases, with intense TGF-β immunoreaction were seen in Borderline Tuberculoid (BT) and Borderline Lepromatous (BL) group, 12 (26.7%) each, followed by 6(13.3%) cases in Lepromatous Leprosy (LL) group (Figure 3), which was statistically insignificant (p = 0.691) Table 2.

Maximum number of cases,9 (20.0%) were in Borderline Lepromatous (BL) category with highest proportion score of TGF-β followed by 7 (15.6%) cases in Borderline Tuberculoid (BT), which was statistically significant (p = <0.001) Table 3.

A high number of cases, 9(20.0%) with intense immunoreactivity score was seen in Borderline Lepromatous (BL), whereas maximum number of cases, 7(15.6%) with low immunoreactivity score was seen in Borderline Tuberculoid (BT) group (Figure 4), which was statistically significant (p = <0.001) Table 4.

4. Discussion

In our study, majority of the cases, 23 (51.1%) were in the age group of 20-40 years, whereas in a study conducted by Quaresma et al in 2012, patients' age ranged from 25-57 years.[3] Roy et al in 2019 found maximum number of cases, 14 (28%) in the age group of 21-30 years followed by the 4^{th} and 5^{th} decades of life, 10 (20%) cases each.[14] Rather et al in 2022 reported mean age of the patients as 34.46 ± 15.64 with an age range of 8 years to 70 years.[15]

Our study showed a male preponderance with 25 (55.6%) cases and 20 (44.4%) females. Similarly, Aarão et al in 2014 reported 31 (62.0%) males and19 (38.0%) females.[16] Poudel et al in 2019 reported 44 (64.7%) males and 24 (35.3%) females in their study.[17] Atram et al in 2020 reported 113 (59.8%) males and 76 (42.2%) females.[18]

Borderline Tuberculoid (BT) and Borderline Lepromatous (BL) category comprised of most cases in our study, 14 (31.1%), followed by 9 (20.0%) cases in Lepromatous Leprosy (LL). Aarão et al in 2014 also reported 34.0% cases in the Mid Borderline category followed by 32.0% cases in Tuberculoid category.[16] Jindal et al in 2021 observed 49.5% cases in Borderline Tuberculoid (BT) followed by 21.3% in Lepromatous Leprosy (LL), 14.5% in Borderline Lepromatous (BL), 5.3% in Mid Borderline (BB) and 1.9% cases of Tuberculoid Leprosy (TT).[19]

In our study, paucibacillary cases comprised of 30 (66.7%), followed by multibacillary cases; 15 (33.3%). Quaresma et al in 2012 reported equal percentage of paucibacillary and multibacillary cases.[3] Rather et al in 2022 reported 88.9% multibacillary and 11.1% paucibacillary cases.[15]

We observed that highest number of cases, 12 (26.7%) each with intense TGF-β immunoexpression in Borderline Tuberculoid (BT) and Borderline Lepromatous (BL) group followed by 6 (13.3%) cases in Lepromatous Leprosy (LL) group with marked TGF- β expression. Our study demonstrated high number of cases, 9 (20.0%) in Borderline Lepromatous (BL) category followed by 7 cases (15.6%) in Borderline Tuberculoid (BT) with highest proportion score. Sousa et al in 2016 on quantitative analysis of immunostaining revealed significant differences between the different groups studied. An increase in the expression of TGF-β was observed in the Lepromatous form of the disease when compared with Tuberculoid form (p<0.0001).[20] Quaresma et al in 2012 showed a significant difference in mean TGF-β levels between the Tuberculoid and Lepromatous Leprosy (339 ± 99.4 versus 519.2 ± 68.2 cells/field), indicating a predominance of TGF-β in the Lepromatous Leprosy.[3] Aarão et al in 2014 also observed significantly higher expression of TGF-β in Lepromatous Leprosy (p=0.0285).[16] Mean number of positive events in their study was 2.00 ± 2.21 in the Indeterminate Leprosy, 5.53 ± 10.67 in Tuberculoid, 4.93 ± 6.30 in Mid-Borderline, and 9 ± 3.08 in the Lepromatous Leprosy.[16]

A high immunoreactivity score for TGF-β was seen in Borderline Lepromatous (BL) in 9 (20.0%) cases and Borderline Tuberculoid (BT) showed 7(15.6%) cases of low immunoreactivity score in our study.

5. Conclusions

TGF-β has an ability to induce apoptosis in immune cells which causes inhibition of the cellular response and is therefore a determinant marker of chronicity in leprosy. The immunoexpression of TGF-β waned from Borderline Lepromatous to Tuberculoid spectrum, indicating the severity of infection.

6. Conflict of Interest

None.

7. Source of Funding

None.

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Cite this article: Warsi S, Lubna F, Adil M, Akhtar K. Immunoexpression of TGF-β: An utility marker in hansen's disease. *IP Arch Cytol Histopathology Res* 2023;8(1):35-39.