Original article:

Correlation of clinical features and bacillary index in the accurate diagnosis of leprosy

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Abstract:

After sustained exposure to Mycobacterium leprae, only a subset of exposed individuals develops clinical leprosy. Moreover, leprosy patients show a wide spectrum of clinical manifestations that extend from the paucibacillary (PB) to the multibacillary (MB) form of the disease. This "polarization" of leprosy has long been a major focus of investigation for immunologists because of the different immune response in these two forms. But while leprosy per se has been shown to be under tight human genetic control, few epidemiological or genetic studies have focused on leprosy subtypes.(PLOS TROPICAL DISEASE).

Introduction

Leprosy is a chronic infectious disease caused by Mycobacterium leprae, with over 200,000 new cases detected each year, often leading to severe disabilities and social stigma. After sustained exposure to M. leprae, only a subset of individuals develops clinical leprosy. From the early observations of familial aggregation of leprosy cases to the most recent genome-wide association studies identifying genetic polymorphisms associated with leprosy, there is strong evidence that the development of the disease is under tight human genetic control .(PLOS TROPICAL DISEASE)

From a genetic perspective, polarization can thus be regarded as a neglected phenotype. To address this shortcoming, we have conducted a review of epidemiological studies addressing leprosy subtypes and polarization. We argue that the poor understanding of the genetic architecture of leprosy polarization is mainly caused by inappropriate study designs. We provide suggestions for future research on PB and MB leprosy and propose that the use of the armadillo model will be of pivotal importance for the study of these phenotypes.(PLOS TROPICAL DISEASE)

Material and methods:

The present study was undertaken in department of Pathology, JJM Medical College , Davangere, over a period of 2 years from August 2014 to July 2016.

Source of Data :

Materials for the study of consisted of skin biopsies obtained from patients clinically diagnosed as leprosy who attended either OPD or leprosy clinics of Chigateri District Hospital, and Bapuji Hospitals that are attached to JJM Medical College, Davangere.

Method of collection of data :

Skin biopsies for the study were obtained by incisional biopsy which was performed by the Dermatologist. These biopsies were sent to the Department of Pathology in 10 % formalin. After

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adequate fixation for about 8-12 hours, the biopsies were submitted in toto for routine processing , following which the paraffin embedded sections were stained with H and E for morphological analysis and Wade Fite staining for identifying the baciliI.

The procedure followed for Fite Faraco Stain was WADE –FITE METHOD FOR M.LEPRAE IN PARAFFIN SECTION (MODIFIED FROM KLADE , 1957)[74]

Steps for the above mentioned stain is as follows -

- Wax was removed over 2 changes of Xylene – Peanut oil (3:1) mixture, 7 minutes for each change.
- Excess oil was blotted from the sections with fine filter paper 3 times.

Sections were washed in running water for 5 minutes and then rinsed in distilled water

Sections were stained with strong carbol fuchsin for 30 minutes.

- Sections were washed in running tap water for 2 minutes .
- Sections were decolourised in 1 % acid alcohol to a pale pink colour.
- Sections were washed in running tap water for 2 minutes .
- Sections were counterstained in 0.15% methylene blue 5-6 dips .
- Sections were washed in running water until sections become pale blue.
- Sections were dehydrated quickly in absolute alcohol over three changes.
- Sections were cleared in xylene over 2 changes and mounted.

The above procedure was slightly modified in the present study.

Coconut oil instead of peanut oil and 5 % sulphuric acid instead of 1% acid alcohol were used.

The sections which were stained with the above modifications were observed under oil immersion using 100x objectives. The bacillary index was assessed in exactly the same way as the one followed for smear . The entire dermis was observed to assess the logarithmic index of bacilli .

Following was the scale used to calculate the bacillary index (BI) [75]

- 1+ = 1-10 bacilli in 100 OIF (Oil immersion field)- examine 100 OIF
- 2+ = 1-10 bacilli in 10 OIF – examine 100 OIF
- 3+ = 1-10 bacilli in 1 OIF – examine 25 OIF
- 4+ = 10- 100 bacilli in 1 OIF –examine 25 OIF
- 5+= 100 – 1000 bacilli in 1 OIF – examine 25 OIF
- 6+ = > 1000 bacilli in 1 OIF- examine 25 OIF
- H and E stained sections were studied to observe the various changes that occurred in the epidermis, papillary, reticular, deep dermis, neurovascular bundles and adnexa.
- After studying the histopathological features and noting the bacteriological status, the diagnosis of leprosy was confirmed and classified according to Ridley and Jopling classification.
- Clinicopathological correlation was done whenever possible.

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Results:

TABLE 1 : AGE AND SEX DISTRIBUTION

Age group (in years)	**Type of leprosy**														**Total M &F**	
	TT=2		BT=57		BB=7		BL=17		LL=16		IL=1		Total			
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	No	%
0-9	0	1	1	0	0	0	0	0	0	0	0	0	1	1	2	2%
10-19	0	0	3	2	0	1	0	1	0	0	0	0	3	4	7	7%
20-29	0	0	8	7	0	1	5	2	1	1	0	0	14	11	25	25%
30-39	0	0	7	4	0	1	3	0	4	0	0	0	14	5	19	19%
40-49	0	0	9	3	1	2	3	1	1	1	1	0	15	7	22	22%
50-59	1	0	2	4	0	0	1	0	5	1	0	0	9	5	14	14%
60	0	0	4	3	1	0	1	0	1	1	0	0	7	4	11	11%
Total	**1**	**1**	**34**	**23**	**2**	**5**	**13**	**4**	**12**	**4**	**1**	**0**	**63**	**37**	**100**	**100%**

GRAPH 1 : AGE AND SEX DISTRIBUTION

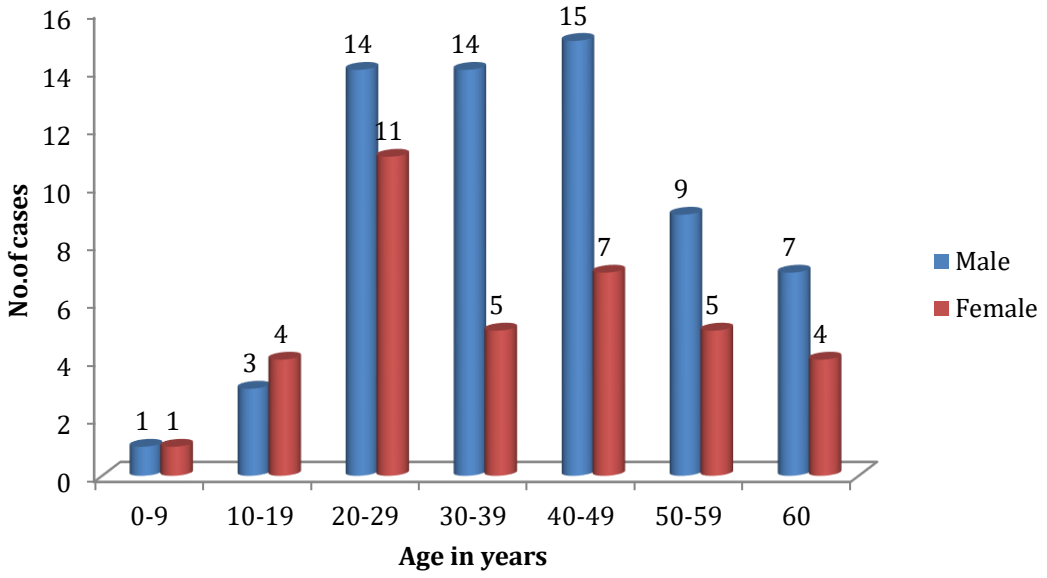

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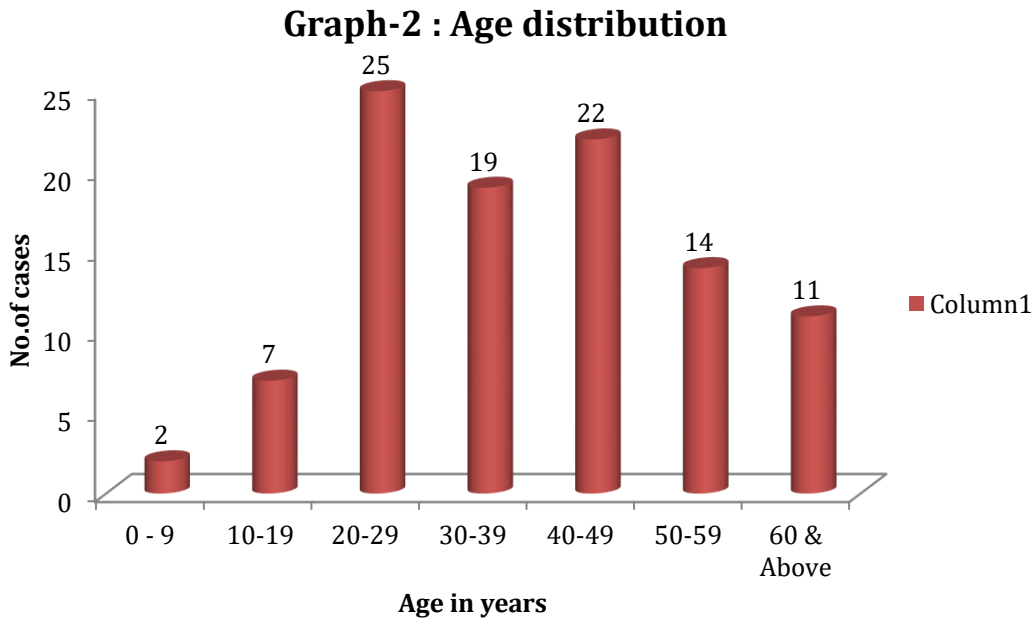
TABLE 2 : AGE DISTRIBUTION

Age	Frequency	Percent
0 - 9	2	2.0
10-19	7	7.0
20-29	25	25.0
30-39	19	19.0
40-49	22	22.0
50-59	14	14.0
60 & Above	11	11.0
Total	**100**	**100.0**



In the present study patients age ranged from 6 years to 70 years. Among them 2 (2%) were in first decade, 7 (7%) were in second decade, 25 (25%) were in third decade, 19 (19%) were in fourth decade, 22(22%) were in fifth decade, 14 (14%) were in sixth decade and 11(11%) were above 60 years of age .

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TABLE 3 : SEX DISTRIBUTION

Sex	**Frequency**	**Percent**
Male	63	63%
Female	37	37%
Total	**100**	**100%**

There were 63 male patients and 37 female patients . with a male :female ratio of 1.70:1

Graph-3 : Sex distribution

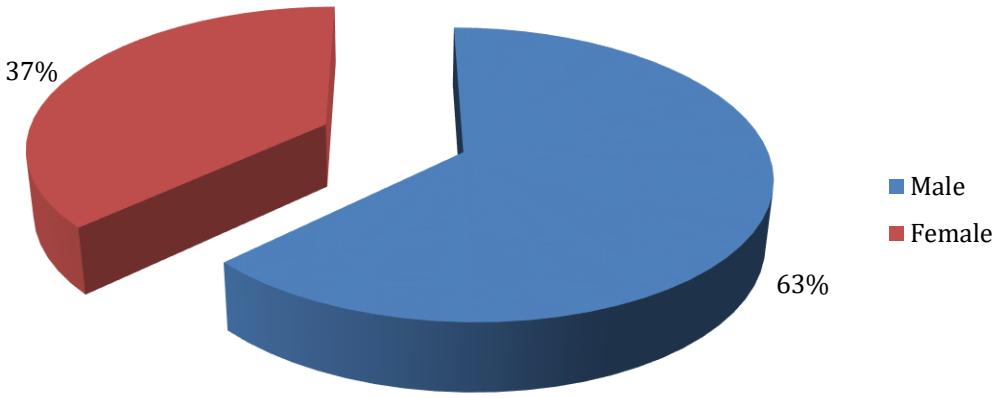

TABLE 4 : TYPES OF LEPROSY

Types of Leprosy	**No of cases**	**Percent**
TT	2	2.0
BT	57	57.0
BB	7	7.0
BL	17	17.0
LL	16	16.0
IL	1	1.0
Total	**100**	**100.0**

Among the 100 biopsies studied, 57 (57%) were of BT type, 17(17%) were BL type, 16 (16%) were LL type, 7(7%) were BB type, 2(2%) were TT type and 1(1%) was IL type.

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Graph-4 : Types of leprosy

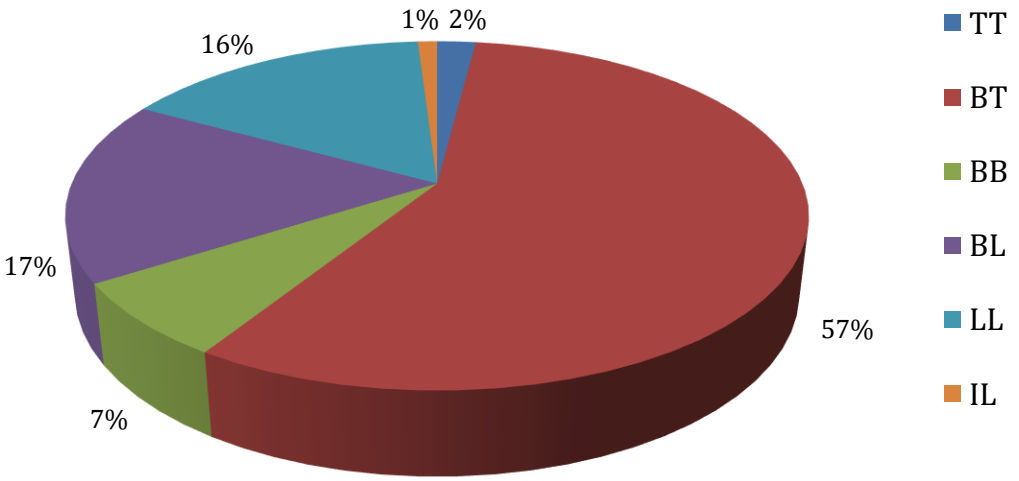

TABLE 5 : SIGNS AND SYMPTOMS

Signs & Symptoms	Types of Leprosy						Total
	TT (n=2)	BT (n=57)	BB (n=7)	BL (n=17)	LL (n=16)	IL (n=1)	
Hypopigmented Lesions	2 (100%)	37 (64.91%)	7 (100%)	14 (82.35%)	14 (87.5%)	1 (100%)	75 (75%)
Erythematous Lesions	0	28 (49.12%)	2 (28.57%)	7 (41.17%)	4 (25%)	0	41 (41%)
Combinations of lesions	0	15 (26.31%)	1 (14.28%)	6 (35.29%)	5 (31.25%)	0	27 (27%)
Anesthesia	2 (100%)	47 (82.45%)	6 (85.71%)	14 (82.35%)	16 (100%)	1 (100%)	86 (86%)
Nerve Thickening	2 (100%)	39 (68.42%)	6 (85.71%)	15 (88.23%)	12 (75%)	1 (100%)	75 (75%)
Nodules	0	0	0	7 (41.17%)	9 (56.25%)	0	16 (16%)
Trophic Ulcer	0	3(5.26%)	0	0	1(6.25%)	0	4(4%)

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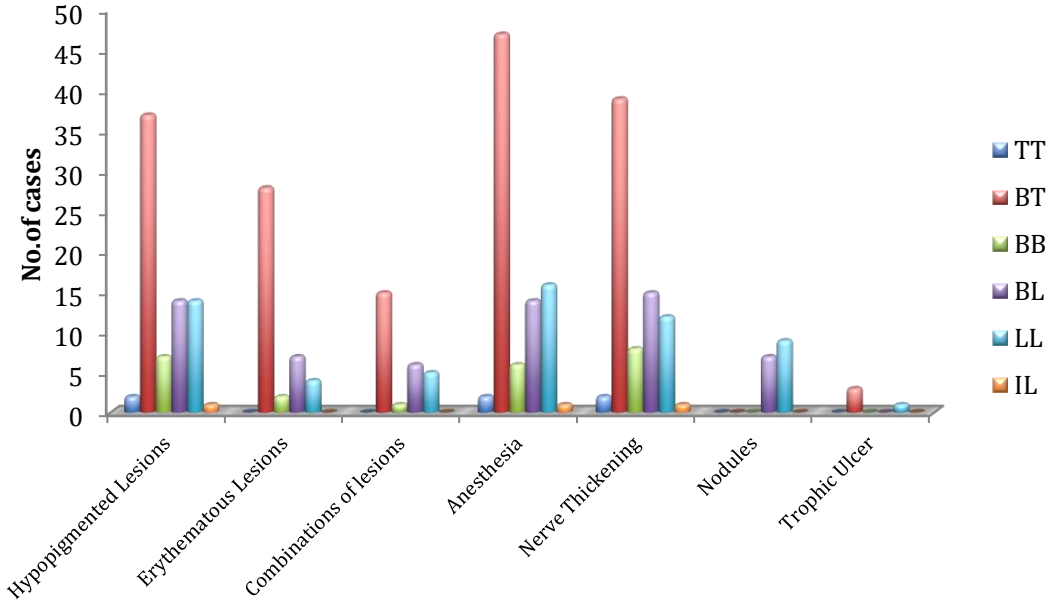
Graph-5 : Signs and symptoms



In the present study, 86 (86%) had clinical features suggestive of anesthesia (loss of sensation), 75(75%) were having nerve thickening, 75(75%) had hypopigmented lesions, 41(41 %) had erythematous skin lesions. 27(27%) had combination of lesions, 16(16%) had nodules and 4 (4%) of them had trophic ulcer.

TABLE 6 : BACTERIOLOGICAL INDEX

Types	**Total No**	**Bacillary Index**						
		Paucibacillary (PB)	**Multi bacillary (MB)**					
			1+	**2+**	**3+**	**4+**	**5+**	**6+**
TT	2	2	0	0	0	0	0	0
BT	57	56	1	0	0	0	0	0
BB	7	0	0	3	4	0	0	0
BL	17	0	0	1	4	7	5	0
LL	16	0	0	1	0	0	9	6
IL	1	1	0	0	0	0	0	0
Total	**100**	**59**	1	5	8	7	14	6
			41					

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Graph-7: Bacteriological index

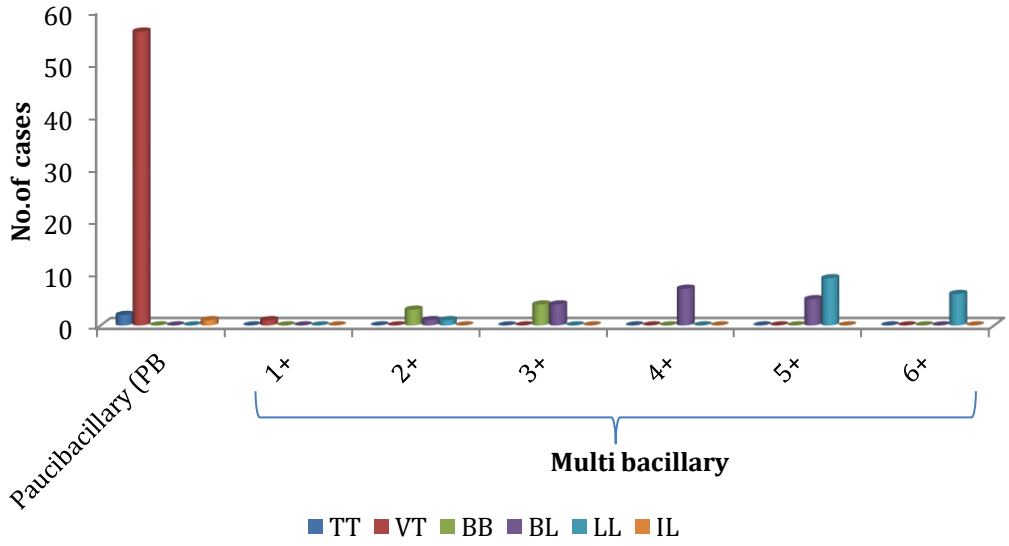

TABLE 7 : LOGARITHMIC INDEX OF BACILLI IN TT

Type	No	Bacillary Index						
		Paucibacillary	Multibacillary					
TT			1+	2+	3+	4+	5+	6+
	2	2(100%)	0	0	0	0	0	0

Of the 2 biopsies in this category, both (100%) were paucibacillary type.

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TABLE 8: LOGARITHMIC INDEX OF BACILLI IN BT

Type	No	Bacillary Index						
		Paucibacillary	Multibacillary					
BT			1+	2+	3+	4+	5+	6+
	57	56(98.24%)	1(1.75%)	0	0	0	0	0

Of the 57 biopsies in this category, 56(98.24%) were paucibacillary type, and 1(1.75%) was multibacillary which showed a BI of 1+.

TABLE 9: LOGARITHMIC INDEX OF BACILLI IN BB

Type	No	Bacillary Index						
		Paucibacillary	Multibacillary					
BB			1+	2+	3+	4+	5+	6+
	7	0	0	3(42.85%)	4(57.14%)	0	0	0

Of the 7 biopsies in this category , all were multibacillary type . 3(42.85%) showed BI of 2+ and 4 (57.14%) showed BI of 3+.

TABLE 10: LOGARITHMIC INDEX OF BACILLI IN BL

Type	No	Bacillary Index						
		Paucibacillary	Multibacillary					
BL			1+	2+	3+	4+	5+	6+
	17	0	0	1(5.88%)	4(23.52%)	7(41.17%)	5(29.41%)	0

Of the 17 biopsies in this category , all were multibacillary type . 1 (5.88%) showed BI of 2+ , 4(23.52%) showed BI of 3+ , 7(41.17%) showed BI of 4+ and 5(29.41%) showed BI of 5+ .

TABLE 11 : LOGARITHMIC INDEX OF BACILLI IN LL

Type	No	Bacillary Index						
		Paucibacillary	Multibacillary					
LL			1+	2+	3+	4+	5+	6+
	16	0	0	1(6.25%)	0	0	9(56.25%)	6(37.5%)

Of the 16 biopsies in this category , all were multibacillary type . 1(6.25%) showed BI of 2+ , 9(56.25%) showed BI of 5+ and 6(37.5%) showed BI of 6+

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TABLE 12 : LOGARITHMIC INDEX OF BACILLI IN IL

Type	No	Bacillary Index						
		Paucibacillary	Multibacillary					
IL			1+	2+	3+	4+	5+	6+
	1	1(100%)	0	0	0	0	0	0

Only 1(100%) biopsy was studied in this category and it was paucibacillary.

Discussion:

From a genetic perspective, polarization can thus be regarded as a neglected phenotype. To address this shortcoming, we have conducted a review of epidemiological studies addressing leprosy subtypes and polarization. We argue that the poor understanding of the genetic architecture of leprosy polarization is mainly caused by inappropriate study designs. We provide suggestions for future research on PB and MB leprosy and propose that the use of the armadillo model will be of pivotal importance for the study of these phenotypes.(PLOS TROPICAL DISEASE)

Here, we summarized the accumulated evidence for susceptibility genes acting at the polarization level that do not influence the onset of leprosy disease. We highlighted that precise phenotype characterization is crucial for the identification of genes driving polarization. Since there is no conventional animal model for leprosy while armadillo is not widely available, the " reverse genetics" approach through genetic epidemiology remains an attractive strategy to decipher the physiopathology of this neglected tropical disease, of which polarization is a major aspect. Importantly, such progress can only come from a close collaboration between physicians, biologists, epidemiologists, and geneticists. Leprosy, so long a cause of social isolation and stigmatization of those who suffer the disease, will only be eradicated by a joint effort of all components of the scientific community.

Conclusion:

Leprosy, so long a cause of social isolation and stigmatization of those who suffer the disease, will only be eradicated by a joint effort of all components of the scientific community.

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