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Lighting up of m.leprae

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Introduction

Leprosy or Hansen's disease is a slowly progressive infection caused by Mycobacterium Leprae that mainly affects the skin and peripheral nerves and results in disabling deformities. Despite its low communicability, leprosy remains endemic among an estimated 10 to15 million people living in poor tropical countries.

As far as tropical countries like India are concerned, it is still one of the major problems of public health importance.

The immune response of patient and the density of bacteria in the lesion (bacterial index) determine the clinical manifestation and the infectivity of the disease. Accordingly the disease manifests as a spectrum beginning from lesions having low immunity and infectivity to those having high immunity and low infectivity[1]. This clinicopathological spectrum determines the treatment regimen[2,3].

Diagnosis of leprosy is by demonstration of lepra bacilli in slit skin smears and skin biopsies[4,5]. Ziehl-neelsen (ZN) staining is the old and conventional method of detection of the organism in clinical specimens[6]. FF staining is more sensitive than ZN method in detection of Mycobacterium leprae in tissue section, it is not free from flaws[7,8]. The density of bacilli should be 1000 per cubic millimeter of the tissue to pick single bacilli in the section[1]. The laborious search for the bacilli is tiresome leading to increased chances of false negativity, under diagnosis and under grading of the disease. Many studies have been done on fluorescent techniques in this direction but its impact on bacteriological index and thus the clinical grade has been lacking in literature[7-9].

Aim

To compare the efficacy of auraminerhodamine stain with fitefaraco stain in diagnosing M.leprae in tissue sections.

Review of Literature

Deeepa sowurkaran and rama adiga et al published that fluorescent method is more sensitive than modified fitefaraco method in detecting lepra bacilli in tissue sections especially in cases with bacillary index less than three. With its higher sensitivity, paucibacilary cases could be upgraded to multi bacillary thus affecting treatment decisions.

Harshadrai J jariwala et al published fluorescent method compared with fitefaraco method for detecting acid fast micro organisms in paraffin sections of cases of leprosy. Biopsies were obtained from 50 cases of leprosy covering all varieties and at varying stages of treatment. The fluorescence method was better than the fitefaraco method; 22 biopsies showing acid fast

organisms in fluorescence microscopy and 20 in fitefaraco method. Its superiority was evidenced in 2 cases in which the organisms were very scanty. Fluorescence microscopy can also be used to determine the bacterial index and the morphological index of organisms. The morphological index, however, was one and a half times higher than that obtained by fitefaraco technique. The ease and speed of fluorescence microscopy appear to be a great advantage

Materials and Methods

The current study is a retrospective one spanning 18 months, including 65 skin biopsies from patients clinically diagnosed as leprosy. Ethical approval was obtained from institutional ethical clearance committee. The disease was classified according to clinical, histopathological and modified Fite Faraco staining results into indeterminate (IL), tuberculoid (TT), borderline tuberculoid (BT), mid borderline (BB), borderline lepromatous(BL), and lepromatous leprosy (LL). Skin biopsies received were routinely processed and after embedding in paraffin blocks, 5 micrometers thick sections were cut. One section each were stained with haematoxylin and eosin (H&E), FF and fluorescent stain using standard mthods.

For fluorescent staining ribbons containing two to three serial sections were taken on clean scratch free slide. Adhesive used was egg albumin. After deparaffinisation in xylene, the auraminerhodamine staining was done according to the procedure of Kuper and May. For each batch of sections that were stained, sections from a skin biopsy of a typical lepromatous leprosy patient and a skin biopsy from a normal individual were used as controls.

Study Design

Experimental, cross sectional and retrospective study to be carried out over a period of 18 months.

Duration of Study

Eighteen months

Site of Study

Department of histopathology of a tertiary care hospital and medical college

Sample Size: 65

Inclusion and Exclusion Criteria

All the skin punch biopsies suggestive of Hansen's disease on histopathological examination will be included in the study

Determination of Bacteriological Index

The stained tissue sections were observed immediately under the fluorescent microscope. All sections were screened under 10X and 40X objectives. Sections showing organisms with typical morphology of *Mycobacterium leprae* bacilli by the 40X objective were confirmed using 100X objective. Only strongly fluorescing organisms were considered for a definitive diagnosis. Bacillary fragments were not taken into consideration. *Mycobacterium leprae*that appeared as solid bright yellow green fluorescing rod shaped organisms and only when interspersed with the light staining artifacts was considered diagnostic for *Mycobacterium leprae*. Bacteriological index (BI) was calculated under oil immersion field. According to Ridleys logarithmic scale, it is graded from zero to six +, which is based on the number of bacilli seen on an average microscopic field under 100X objective.

Ridley's logarithmic scale (applies to both skin biopsies and slit skin smears)

BI=0: no bacilli observed

BI=1: 1-10 bacilli in 10-100 high power fields

BI=2: 1-10 bacilli in 1-10 high power fields

BI=3: 1-10 bacilli per high power field

BI=4: 10-100 bacilli per high power field

BI=5: 100-1000 bacilli per high power field

BI=6: >1000 bacilli per high power field

Statistical Analysis

SPSS v 17 (IBM, New York) will be used for data analysis. Chi square test will be used to calculate significance between differences.

Results

age group	Frequency	Percent
10 to 20 years	6	9.4
21 to 30 years	18	28.1
31 to 40 years	18	28.1
41 to 50 years	9	14.1
51 to 60 years	7	10.9
more than 60 years	6	9.4
Total	64	100.0

Sex	Frequency	Percent
Female	29	45.3
Male	35	54.7
Total	64	100.0

Histopath	Frequency	Percent
Borderline Borderline	4	6.3
Borderline Lepromatous	4	6.3
Borderline Tuberculoid Leprosy	15	23.4
Histiod Leprosy	3	4.7
Indeterminate Leprosy	5	7.8
LepraRection Type 2	1	1.6
Lepromatous Leprosy	12	18.8
Lupus Vulgaris	2	3.1
Tuberculoid Leprosy	18	28.1
Total	64	100.0

AR	Frequency	Percent
1+	18	28.1
2+	10	15.6
3+	10	15.6
4+	3	4.7
5+	2	3.1
6+	15	23.4
negative	6	9.4
Total	64	100.0

AR	Frequency	Percent
negative	6	9.4
Positive	58	90.6
Total	64	100.0

FF	Frequency	Percent
1+	19	29.7
2+	6	9.4
3+	1	1.6
4+	1	1.6
5+	9	14.1
6+	7	10.9
negative	21	32.8
Total	64	100.0

FF	Frequency	Percent
negative	21	32.8
Positive	43	67.2
Total	64	100.0

			FF		
			Positive	negative	Total
AR	Positive	Count	43	15	58
		% within FF	100.0%	71.4%	90.6%
	negative	Count	0	6	6
		% within FF	0.0%	28.6%	9.4%
Total		Count	43	21	64
		% within FF	100.0%	100.0%	100.0%

Kappa test

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.350	.112	3.682	.000

The sensitivity, specificity, PPV, NPV and accuracy of AR was 100%, 28.57%, 74.14%, 100% and 76.56%.

		FF							Total
		1+	2+	3+	4+	5+	6+	negative	
AR	1+	8	0	0	0	0	0	10	18
	2+	7	0	0	0	0	0	3	10
	3+	4	4	0	0	0	0	2	10
	4+	0	2	1	0	0	0	0	3
	5+	0	0	0	1	1	0	0	2
	6+	0	0	0	0	8	7	0	15
	negative	0	0	0	0	0	0	6	6
Total		19	6	1	1	9	7	21	64

Chi square test- 157, P value – 0.0001

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.217	.062	4.314	.000

	Sensitivity		Specificity		PPV		NPV		Accuracy	
	FF	AR	FF	AR	FF	AR	FF	AR	FF	AR
IL	50	83.33	31.03	8.62	6.98	8.62	85.71	83.33	32.81	15.62
TT	41.18	76.47	23.4	4.26	16.28	22.41	52.38	33.33	28.12	23.44
BT	61.11	94.44	30.43	10.87	25.58	29.31	66.67	83.33	39.06	34.38
BB	80	100	33.9	10.17	9.3	8.62	95.24	100	37.5	17.19
BL	100	100	34.43	9.84	6.98	5.17	100	100	37.5	14.06
LL	100	100	42.86	12.24	34.88	25.86	100	100	56.25	32.81

	Positive	Negative	Total
FF	43	21	64
AR	58	6	64
Total	101	27	128

Chi square test- 10.56, P value -0.001

Conclusion

In borderline lepromatous, borderline tuberculoid, and borderline borderline cases the determination of leprabacilli is increased by auramine rhodamine fluorescent staining as compared to fite faracco staining method.

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