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MICROBIOLOGICAL AND CLINICAL PROFILE OF PATIENTS SUSPECTED OF LEPROSY AT A TERTIARY CARE TEACHING HOSPITAL.

Clinical Microbiology

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ABSTRACT

Leprosy, also known as Hansen's disease, is a chronic infectious disease caused by Mycobacterium leprae. The disease affects the skin, the peripheral nerves, mucosal surfaces of the upper respiratory tract and the eyes. From the suspected patients, 2 or more Slit skin Smears were sent to the lab from different sites, the smears were stained with a modified Ziehl-Neelsen stain and the smears were graded by using the bacteriological index and morphological index. At our tertiary care teaching hospital which is a 750-bed hospital with a Leprosy care unit, catering diagnostic services to Leprosy patients under NLEP (National Leprosy Elimination Programme), we found an increase in cases of leprosy. Demonstration of acid-fast bacilli in slit skin smears by Ziehl-Nelsen's staining also aids in the diagnosis of leprosy. A reliable diagnosis hinges on a good histopathological diagnosis and demonstration of bacilli in the histopathological section So by keeping this in mind, the present study was undertaken to study the bacteriological and clinical profile of cases of leprosy at a tertiary care hospital in Pune, Maharashtra.

KEYWORDS

Leprosy, Bacteriological index, Lepromatous, Morphological index

INTRODUCTION

Leprosy, also known as Hansen's disease, is a chronic infectious disease caused by Mycobacterium leprae. The disease affects the skin, the peripheral nerves, mucosal surfaces of the upper respiratory tract and the eyes.[1]Leprosy transmission occurs by close and prolonged contact between a susceptible individual and a bacillus-infected patient through nasal secretion.[2]

The spectrum of leprosy includes multibacillary cases that present with more than five skin lesions or with nerve involvement or with the demonstrated presence of acid-fast bacilli in slit skin smear and paucibacillary cases presenting with 1-5 skin lesions without the presence of acid-fast bacilli in smear.[2]

Clinically leprosy cases can be divided into five types about detailed examination of skin lesions and peripheral nerves:[3] The **indeterminate group** is characterized by a small number of hypochromic spots, with a slight decrease in sensitivity, without increased nerve thickness. TT form predominantly affects the nerves and is characterized by erythematous plaques, often with elevated external borders and a hypochromic centre, presenting significant changes in sensitivity. **LL (Lepromatous Leprosy) form**, is characterized by multiple and symmetrical skin lesions which are hypochromic, erythematous, or bright brownish spots with indefinite borders. In the advanced stages of the disease, the patient's face has a peculiar appearance (leonine facies), characterized by diffuse infiltration and eyelash loss (madarosis). In the **Borderline Tuberculous subgroup,** the skin lesions resemble the Tuberculoid form in terms of appearance and loss of sensitivity, but they occur in a larger number and are smaller. Nerve thickening tends to be irregular, less intense, and appears in a larger number. **Borderline lepromatous** subgroup characterized by skin lesions that resemble the LL form, and in a large number, but not so symmetrical and with loss of sensation in some areas.[3]

Leprosy remains an important cause of disability despite of WHO's adoption of the resolution to "eliminate leprosy as a public health problem by the year 2000" in 1991. In 2011, of the total 219,075 new leprosy cases reported globally, 58.1% were detected only in India.[5,6]. Till 2018, the PR of leposy was calculated per 10,000, however, as per WHO Global leprosy (Hansen disease) update 2019.[5] India contributed 79,898 cases to this point prevalence (45.1%). Though, statistically, India has eliminated leprosy (PR 1/10,000) Leprosy cases still tend to be reported from various areas of India alone.

At our tertiary care teaching hospital which is a 750-bed hospital with a Leprosy care unit, catering diagnostic services to Leprosy patients under NLEP (National Leprosy Elimination Programme), we found an increase in cases of leprosy. Demonstration of acid-fast bacilli in slit skin smears by Ziehl-Nelsen's staining also aids in the diagnosis of leprosy. A reliable diagnosis hinges on a good histopathological diagnosis and demonstration of bacilli in the histopathological section So by keeping this in mind, the present study was undertaken to study the bacteriological and clinical profile of cases of leprosy at a tertiary care hospital in Pune, Maharashtra.

MATERIAL AND METHODS

A Prospective observational study was undertaken at a tertiary care hospital in the Department of Microbiology from September 2022 to August 2023 (12 months). All the suspected cases of leprosy who attended OPD irrespective of their age and sex were included in the study. From the patients, 2 or more Slit skin Smears were sent to the lab from different sites i.e., the left or right eyebrow, ear lobe, or from the site of lesion/active disease.

The smears were stained with modified Ziehl-Neelsen stain using 5% H2SO4 as a decolourizer and were examined under oil immersion to look for pink-coloured acid-fast bacilli. The smears were graded by using the bacteriological index and morphological index as stated under.

Bacteriological index:

The bacteriological index denotes the density of lepra bacilli both living (solid staining) and dead (fragmented or granular) in the smear. According to Ridley's logarithmic scale, it ranged from 0 to 6+ and was based on the number of bacilli seen in an average microscopic field of the smear using an oil-immersion objective.[6,8]

No AFB: No bacilli in any of the 100 oil-immersion fields.
1+: 1–10 bacilli, on an average, in 100 oil-immersion fields.
2+: 1–10 bacilli, on an average, in 10 oil-immersion fields.
3+: 1–10 bacilli, on an average, in each oil-immersion field.

4+: 10–100 bacilli, on average, in each oil-immersion field.
5+: 100–1000 bacilli, on average, in each oil-immersion field.
6+: more than 1000 bacilli, on average, in each oil-immersion field.

Morphological Index:
Morphological index is the percentage of solid staining bacilli in a stained smear. Average MI was calculated for the body as the total of the MIs for all sites divided by the number of sites.[10]

The following criteria were used for calling the bacilli solid rods are-
a. Uniform staining of the entire organism
b. Parallel sides
c. Rounded ends, and
d. Length 5 times that of the width.

Simultaneously clinical profile of patients was noted such as demographic characteristics, clinical presentation such as anatomical/morphological deformities, only skin lesions, nerve involvement and both skin & nerve lesions.

Data was entered in Microsoft Excel and analysed.

RESULTS

Demographic Characters-
A total of 250 smears were received in the Microbiology laboratory from a total of 120 clinically suspected cases of leprosy patients and were examined. Out of these 120 suspected leprosy patients, 75(62.5%) were males and the remaining 45 (37.5%) were females.

Age distribution of clinically suspected cases (n=120) revealed that a maximum number of patients i.e., 57(48%) belonged to the age group 21-40 years followed by 42(35%) and 14(11.6%) belonging to the age group 40-60 years and > 60 years respectively. Age group 0-20 years included the least number of patients i.e., 6(5%).

Out of 120 suspected cases of leprosy, 28(23.3%) were smear-positive and microbiologically confirmed cases of leprosy. Out of smear-positive cases,15 (53.5%) cases belonged to the age group 21–40 years. (Figure 1) Among 28 smear-positive cases, there were 18(64.2%) males and 10 (35.7%) females and most of them belonged to rural areas of lower and middle socioeconomic class.

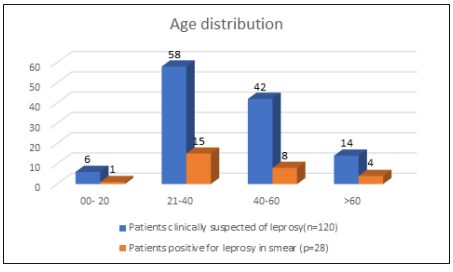


Fig 1: Age distribution of Patients suspected of leprosy and positive for leprosy

Clinical Profile of Patients-
Patients of leprosy presented with various clinical features like morphological lesions, nerve involvement or both lesions and nerve involvement. Some patients presented with anatomical/morphological deformities.

In clinically suspected 120 patients of leprosy maximum (45%) presented with morphological lesions followed by 30% presenting with both lesions and nerve involvement. Morphological or anatomical deformities were seen in 16.6% of patients while Pure nerve involvement was observed in only 8.3% of patients. (Table 1).

In the Microbiologically confirmed group (n=28) a similar trend was observed with 42.8% of patients presenting with morphological lesions followed by 28.5% presenting with both lesions and nerve involvement, 21.4% with deformities and only 7% with pure neuritis type. (Table 1)

Table 1: Clinical presentation in suspected and microbiologically confirmed patients

Type of leprosy	Patients clinically suspected of leprosy (n=120)	Microbiologically confirmed cases of leprosy (n=28)
Patients with anatomical/morphological deformities	20 (16.6%)	6(21.4%)
Patients with only morphological lesions	54(45%)	12 (42.8%)
Patients with only nerve involvement	10(8.3%)	2(7.1%)
Patients with both morphological and nerve involvement	36(30%)	8(28.5%)

Bacteriological index (B.I.)- (Table 2)
B.I. of 1+ to 6+ was calculated for all smear-positive cases. A total of 32.1% of smear-positive cases depicted B. I of 3+ followed by 21.4% cases showing B.I. of .4+ and 17.8% showed B.I. of 1+(Table 2). B. I of 2+ and 5+ was observed in 14.2% of cases. B.I. of 6+ could not be found in any case.

Table 2: Bacteriological index of smear-positive patients

Bacteriological index in smear-positive cases	No of patients positive (n=28)	Percentage (%)
1+	5	17.85
2+	4	14.28
3+	9	32.14
4+	6	21.42
5+	4	14.28
6+	0	0

Morphological Index (M.I.) –(Table 3)
Average M.I. was calculated for the body as the total of the M.I.s for all sites divided by the number of sites. A total of 15 patients (53.5%) had M.I. of 1-20 followed by 32.1%with M.I. of 21-40 and 14.2 % showed M.I. of 41-60. (Table 3). No patient showed M.I. of 61-80.

Table 3: Patient Distribution According To Morphological Index

Morphological index in smear-positive patients	**No of patients (n=28)**	**Percentage (%)**
1-20	**15**	**53.5**
21-40	**9**	**32.1**
41-60	**4**	**14.2**
61-80	**0**	**0**

DISCUSSION

Elimination of leprosy as a public health problem globally was achieved in 2000 and in most countries by 2010.[1,3] However, a few districts and blocks continue to have a prevalence of>1/10,000. Leprosy is a neglected tropical disease (NTD) that still occurs in more than 120 countries, with more than 200,000 new cases reported every year.

In the current study, the males were affected by the disease more than double the females, a male preponderance was found due to increased mobility and frequent interaction with the community leading to an increased opportunity for contact in males the results were supported by a few other studies.[1,3,5]

A total of 104 smears from clinically suspected cases of leprosy were examined. Out of all, 48% of suspected cases belonged to the age group 21-40 years, most of them from rural areas of lower and middle socioeconomic class Similar observations were also made by other researchers like R Gupta in 2019.[9]

In the present study, the clinical presentation of suspected patients ranged from Lepromatous Leprosy type to purely neuritis type. Also, clinical presentation varied from multibacillary to paucibacillary cases. In the present study most common type of clinical presentation i.e. 45.5 in the suspected group and 42,8% in the confirmed group was that of the lepromatous type showing only morphological lesions while morphological or anatomical deformities were seen in 16.6% of patients and 21.4% patients in suspected and confirmed group respectively. The percentage of lepromatous cases was also more common in a study conducted at Udaipur in 2015 and studies conducted by R Gupta,[9] Chhabra et al (2015),[10] Bajjaragi et al (2012)[11] also showed somewhat similar findings. The reason behind the low number of patients in TT and IL can be seen as misdiagnosis or spontaneous regression with good Cell-Mediated Immunity.

In the present study, pure nerve involvement was observed in only

8.3% of suspected and 7% of microbiologically confirmed patients.

There is an inverse proportion between the number of skin lesions and/or several body areas affected and the protective immunity. According to the degree of skin-smear positivity, the WHO Study Group on Chemotherapy of Leprosy Control Programme (1981) classified the disease into multibacillary and paucibacillary but later in 1987 the committee recommended that all the patients with smear-positive should be classified as multibacillary leprosy. In the present study, 28 patients out of 120 were smear-positive with multibacillary form. The findings of our study match with the observations of Rao et al[1] and Mahajan et al.[2] But other Authors like R Gupta,[9] Mathan and Devan 2016,[12] Relhan et al 2016,[13] have reported more multibacillary cases compared to paucibacillary cases.

BI is an expression of the extent of bacterial load in smears (includes both living and dead bacilli). B.I. is an important index of leprosy as it is simple to calculate and is representative of many lesions. The disadvantage of B.I. is that it is the result is affected by the depth of the skin incision, the thoroughness of the scrape, and the thickness of the film.

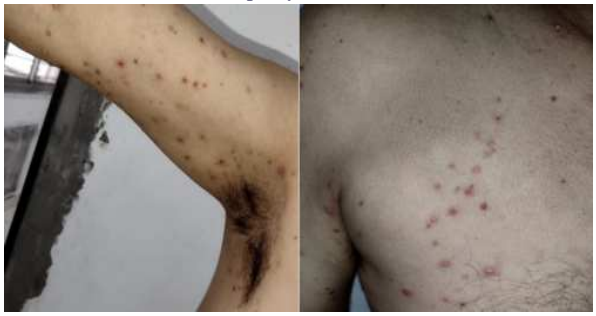
The bacteriological indices of smear-positive cases in our study ranged from 1+ to 6+ with the majority of cases (32.1%) having BI 3+ followed by 21.4% cases showing B.I. of .4+ and 17.8% showed B.I. of 1+(Table 2). B. I of 2+ and 5+ was observed in 14.2% of cases The findings of the present study are concomitant with that of Kilikdar et al. [7] The lower B. Is of 1+ or 2+ denotes low bacillary load and better prognosis, while high (BI 5+ and 6+) having high bacillary load, highly infectious, and are more likely to transmit the disease in the community.[1,11]

The percentage of solid staining bacilli in a stained smear is referred to as MI and it is one of the more sensitive parameters of therapeutic failure, non-compliance, drug resistance, or relapse and it has a high prognostic value for leprosy. However, the disadvantage is measurement of M.I. is liable for observer variation and it is not always reliable. As M.I. takes into consideration the morphological parameters of bacteria, it needs the expert opinion of a microbiologist. In the present study MI was found to be the maximum in the range of 1-20 in 53.5% of patients followed by 32.1%with M.I. of 21-40 and 14.2 % showed M.I. of 41-60. This finding was supported by another study by Patil et al. [15]

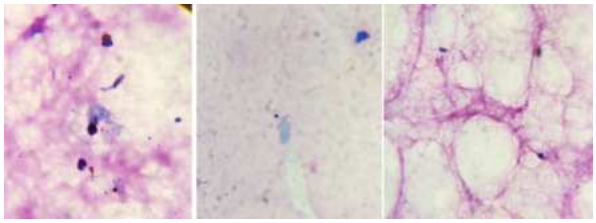
CONCLUSION

Efforts need to be made to reduce deformity through early detection clinically and microscopically. Timely detection proper bacteriological and morphological grading of leprosy cases along with good histopathological diagnosis is crucial which will lead to better treatment and prevention of the disease.[3,8,11]

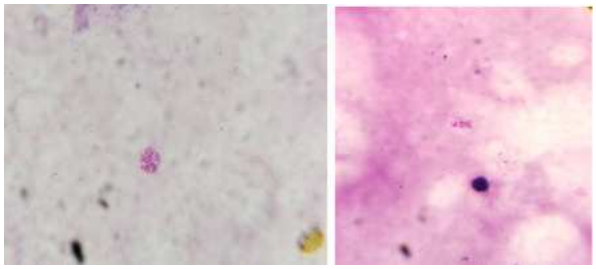
Pictures of Patients with Leprosy



Pictures showing a few Acid-fast bacilli scattered (Picture depicting BI of 2+)



Pictures showing acid-fast bacilli in a bunch (depicting BI of 5+)



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