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ORIGINAL RESEARCH

Disease profile with its updated diagnostic assay in Leprosy patients attending IGIMS, Patna: A tertiary care hospital of Bihar

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ABSTRACT

Introduction: Leprosy is considered a public health issue in countries where the annual prevalence rate is greater than 1 case per 10,000 inhabitants. It is endemic in several countries with low levels of social and economic development, especially India, with the highest absolute number of cases in the former. In 2018, 208,619 new leprosy cases were registered worldwide by the World Health Organization (WHO).

Aims & Objectives: to assess bacteriological index in these sample, to correlate these findings with the newer techniques(ELIZA)m

Data and methods: A total of 106patients selected who were attending General Medicine Department & V.D. Department at IGIMS, Patna and investigated at Microbiology Department for slit skin smear examination. Blunt, narrow scalpel was introduced into the nose and a piece of mucous membrane was taken for nasal smear examination. The smears were examined under oil immersion lens to see red AFB arranged singly or in groups / bundles (Globi).

Results: The most common form of leprosy observed in the present study was borderline leprosy (BB) and Lepromatous leprosy (LL). Both the forms constituted equal proportion of patients (24.5%). The sensitivity and specificity of ELISA was 48.42% and 72.73% respectively.

Conclusions: It was found that sensitivity; specificity, positive predictive value, and negative predictive value of enzyme-linked immunosorbent assay (ELISA)

INTRODUCTION

Leprosy is one of six diseases the World Health Organization (WHO) considers a major threat in developing countries and often results in severe, life-long disabilities and deformities due to delayed diagnosis [1]. Notable epidemiological features of global leprosy today include a continued new case detection rate of approximately 225,000 per year and indirect evidence suggesting that millions of unreported cases linger undetected [2,3].

Leprosy is a chronic granulomatous infection that mainly affects the skin and peripheral nerves, presenting several clinical manifestations due to the pattern of the immune response established as a result of infection with *Mycobacterium leprae*.[4]

The disease is considered a public health issue in countries where the annual prevalence rate is greater than 1 case per 10,000 inhabitants. It is endemic in several countries with low levels

of social and economic development, especially India, with the highest absolute number of cases in the former. In 2018, 208,619 new leprosy cases were registered worldwide by the World Health Organization (WHO). Preliminary data for 2019 show 120,334 and 26,612 new leprosy cases for India and Brazil, respectively, both classified as high-load countries.[5]

GLOBAL LEPROSY SITUATION

The WHO launched a 5-year "Global leprosy strategy 2016– 2020' in April 2016 titled 'accelerating towards a leprosy-free world'."[6] This was built on the earlier 5-year strategy 2011–2015 that focused on early leprosy detection to reduce disabilities. The document states that the agenda of eliminating leprosy at the subnational level is still unfinished in many countries and will therefore continue to be pursued in the coming years. Other challenges remain – continued delay in detecting new patients, persisting discrimination against people affected by leprosy, and limited impact on transmission of leprosy. Perhaps, for above-mentioned reasons, the strategy for years 2016–2020 is built around three pillars: (i) to strengthen government ownership, coordination, and partnership; (ii) to stop leprosy and its complications; and (iii) to stop discrimination and promote inclusion. There is a special focus on women and children, strengthening referral systems, more effective contact tracing, assessing the value of chemoprophylaxis, and monitoring drug resistance.

In India, the National Leprosy Eradication Programme (NLEP) is the centrally sponsored health scheme of the Ministry of Health and Family Welfare, Government of India. While the NLEP strategies and plans are formulated centrally, the programme is implemented by states and union territories (UTs). The programme is also supported by WHO, ILEP, and few other nongovernmental organizations (NGOs). Due to their efforts, from a prevalence rate of 57.8/10,000 in 1983, India has succeeded with the implementation of MDT in bringing the national prevalence down to "elimination as a public health problem" of less than 1/10,000 in December 2005 and even further down to 0.66/10,000 in 2016. In addition to achieving the national elimination target by the end of 2005, India by the end of March 2011–2012 succeeded in achieving elimination at the state level in 34 states/UTs out of the total of 36 states/UTs. Only the state of Chhattisgarh and the UT of Dadra & Nagar Haveli were yet to achieve elimination. By the end of March 2016, 551 districts (82.36%), out of the total 669 in districts, in India had a prevalence of <1/10,000 population which is the target of elimination as a public health problem. The number of districts with prevalence between 1 and 2/10,000 were 76, number of districts with prevalence between >2 and 5/10,000 were 39, and those between 5 and 10 were 2.[7]

NLEP annual reports of the last 4 years have consistently observed that the four states/UTs (Orissa, Chandigarh, Delhi, and Lakshadweep), which achieved elimination earlier in 2011–2012, have shown a prevalence of >1 per 10,000 population, which is a matter of concern for the programme.[8] In addition, although the average national child leprosy rate is approximately 9%, the proportion of child cases was more than 10% of new cases detected in eleven states/UTs of India, with 6 of them (Tamil Nadu, Punjab, Dadra & Nagar haveli, Bihar, Mizoram, and Arunachal Pradesh) showing very high rates ranging from 14% to 23%. In a few of these states, the high multibacillary proportion, and in others a difficult to reach terrain could contribute to continued transmission.

ETIOPATHOGENESIS

The etiologic agent, *M. leprae*, was identified by Norwegian physician Gerhard Armauer Hansen in 1873. Therefore, it is also called Hansen's bacillus.Taxonomy, morphology, staining and biological characteristics of M. leprae *M. leprae's* scientific classification is as follows: class *Schizomycetes,* order *Actinomycetales,* family *Mycobacteriaceae,* and genus *Mycobacterium. M. leprae* is a straight or slightly curved rod, with rounded ends,

measuring 1.5-8 microns in length by 0.2-0.5 micron in diameter. In smears, it is red stained with fuchsin using the Ziehl-Neelsen (ZN) stain, and because of its high lipid content, it does not get discolored when washed with alcohol and acid, thus showing the characteristics of acid-alcohol-resistant bacil-li (AARB). *M. leprae* is different from other mycobacteria in terms of arrangement, since it is arranged in parallel chains, just like cigarettes in a pack, bound together forming the *globi*. When the Gram staining method is used, *M. leprae* is gram-invisible, appearing as negatively stained images, called ghosts, or as bead-like gram-positive bacilli.[9]

ULTRASTRUCTURAL CHARACTERISTICS OF M. LEPRAE

The ultrastructure of *M. leprae* is common in the genus *Mycobacterium.* Electron microscopy has shown that this bacillus has cytoplasm, plasma membrane, cell wall, and capsule. The cytoplasm contains common structures in gram-positive microorganisms. The plasma membrane has a permeable lipid bilayer containing interaction proteins, which are the protein surface antigens. The cell wall attached to the plasma membrane is composed of peptidoglycans bound to branched chain polysaccharides, consisting of arabinogalactans, which support mycolic acids, and lipoarabinomannan (LAM), similarly to other *mycobacteria*

THE GENOME OF M. LEPRAE

The genome of *M. leprae* was sequenced by Cole et al. in 2001.[10] It is circular. Its estimated molecular weight is 2.2 x 109 daltons, with 3,268,203 base pairs (bp) and guanine + cytosine content of 57.8%. When compared to the genome of *Mycobacterium tuberculosis,* which has 4,411,529 bp and guanine + cytosine content of 65.6%, it seems that *M. leprae* underwent reductive evolution, resulting in a smaller genome rich in inactive or entirely deleted genes. It has 2,770 genes, with coding percentage of 49.5%, that is, 1,604 genes encoding proteins (1,439 genes common to *M. leprae* and *M. tuberculosis*) and 1,116 (27%) pseudogenes.

MECHANISMS OF LEPROSY TRANSMISSION

It is believed that leprosy transmission occurs by close and prolonged contact between a susceptible individual and a bacillus-infected patient through inhalation of the bacilli contained in nasal secretion or *Flügge* droplets. The main route of transmission is the nasal mucosa.[11] Less commonly, transmission can occur by skin erosions.[12] Other transmission routes, such as blood, vertical transmission, breast milk, and insect bites, are also possible.[13,14]

It is assumed that infected individuals, even those who did not develop the disease, may have a transitional period of nasal release of bacilli.[15]

GENETIC FACTORS

Although the exact genes involved in leprosy are not known, it is accepted that different sets of genes of the human leukocyte antigen system (HLA) and non-HLA have an impact on the susceptibility to leprosy, both in infection per se control and in the definition of the clinical presentation. Changes in candidate genes, that is, genes whose product participates in the host response to the infectious agent, have been currently investigated. Genomic scan studies identified binding peaks for leprosy in chromosome regions 6p21, 17q22, 20p13, and 10p13.[16]

MATERIAL AND METHODS

TYPE OF STUDY & STUDY DESIGN

It was a prospective hospital based observational study.

SOURCE OF STUDY
Patients who were attending General Medicine Department & V.D. Department at IGIMS, Patna and investigated at Microbiology Department for slit skin smear examination.

DURATION OF STUDY
TOTAL DURATION
18 months

SAMPLE COLLECTION AND INVESTIGATION
12 months

DATA ANALYSIS AND THESIS WRITING
6 months

SAMPLE SIZE
Samples taken was 106

INCLUSION CRITERIA
Patient more than 14 years of age.
Either sex
All clinical types

EXCLUSION CRITERIA
Paediatric age group (age <14 years) were excluded from the study.
Patients not given consent

METHODOLOGY
The study was conducted after approval from Ethics Committee of IGIMS, Patna. Patients who were attending General Medicine Department & V.D. Department at IGIMS, Patna and investigated at Microbiology Department for slit skin smear examination were enrolled for the present study.

SAMPLE COLLECTION
Sample was collected from six sites out of which one is from nasal scrapping and the rest five from both ear lobules, both forehead, chin, shin of tibia and from the lesion. Skin was pinched and cut in length of about 5mm and a deep infiltrated layer was taken with a scalpel. Scalpel was rotated transversely.
Blunt, narrow scalpel was introduced into the nose and a piece of mucous membrane was taken for nasal smear examination.

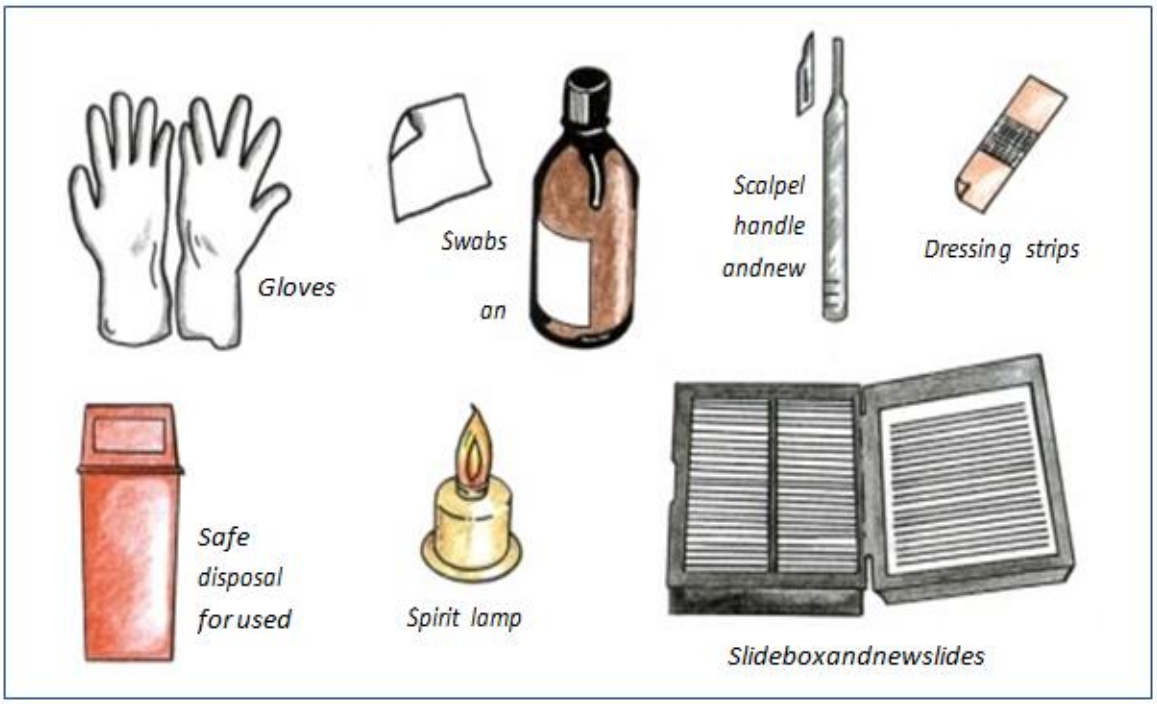
STAINING
Smears were prepared from slit skin and nasal mucosal samples. Th acid fast bacilli was demonstrated by performing an acid fast staining of skin lesions or nasal scrapings using 5% H2SO4 for decolourization. In Ziehl-Neelsen (ZN) stained smears the viable *M. Leprae.*was seen against blue background as uniformly and intensely red stained bacilli having length 4 times greater than breadth: they are described as solid stained (S) bacilli. Dead leprae bacill stain irregularly and are described as fragmented (F) or granular (G). The total number of the bacilli was counted using Ridley's logatithimic scale and bacteriological index was calculated.

RESULT CALCULATION

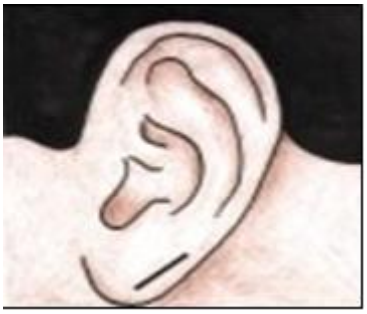
The smears were examined under oil immersion lens to see red AFB arranged singly or in groups / bundles (Globi)

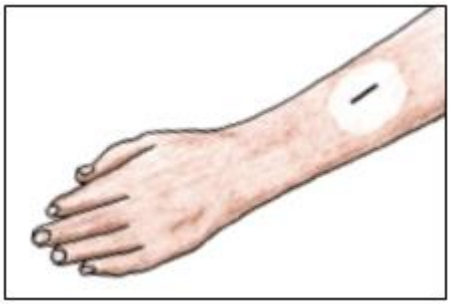
SMEARS WERE GRADED AS

1-10 acilli in 100 OIF = 1+
1-10 acilli in 10 OIF = 2+
1-10 bacilli per OIF = 3+
10-100 bacilli per OIF = 4+
100-1000 bacilli per OIF = 5+
>1000 bacilli or bacilli in clumps and Globi in each OIF =6+



PREPARING TO TAKE A SKIN SMEAR



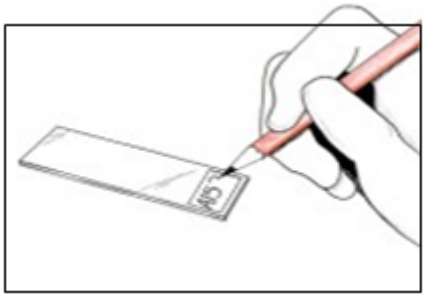


Place all the material on a clean table.
We have need a slide marker.

SELECT THE SITES

Take a smear from two sites only:

1. One earlobe.
2. Onelesion.Selectthemostactive-lookinglesion,butnot on the face. 'Active' means raised and reddish in colour. Take the smear in the most active area of the lesion (usually theedge).



If there is no suitable skin lesion, take the second smear from the other ear lobe, or from a site where active lesions were originally recorded or where a previous smear was positive. Many programmes traditionally took smears from four or even six sites, but two sites are now considered adequate in most cases

- Take a new, clean, unscratched microscope slide. Using a slide marker, write the patient identification (ID) number at the bottom of the slide. This number must be on the request form.
- Clean the skin at the smear sites with a cotton waddrenched in alcohol. Let it dry.
- Light the spirit burner.
- Put a new blade on the scalpel handle. If you put the scalpel down, make sure the blade does not touch anything.
- Pinch the skin firmly between your thumb and fore finger; maintain pressure to press out the blood.
- Make an incision in the skin about 5 mm long and 2 mm deep (**3**). Keep on pinching to make sure the cut remains bloodless. If bleeding, wipe the blood away with cotton wad.
- Turn the scalpel 90° and hold it at a right angle to the cut. Scrape inside the cut once or twice with the side of the scalpel, to collect tissue fluid and pulp. There should be no blood in the specimen, as this may interfere with staining and reading.

RESULTS AND DISCUSSION

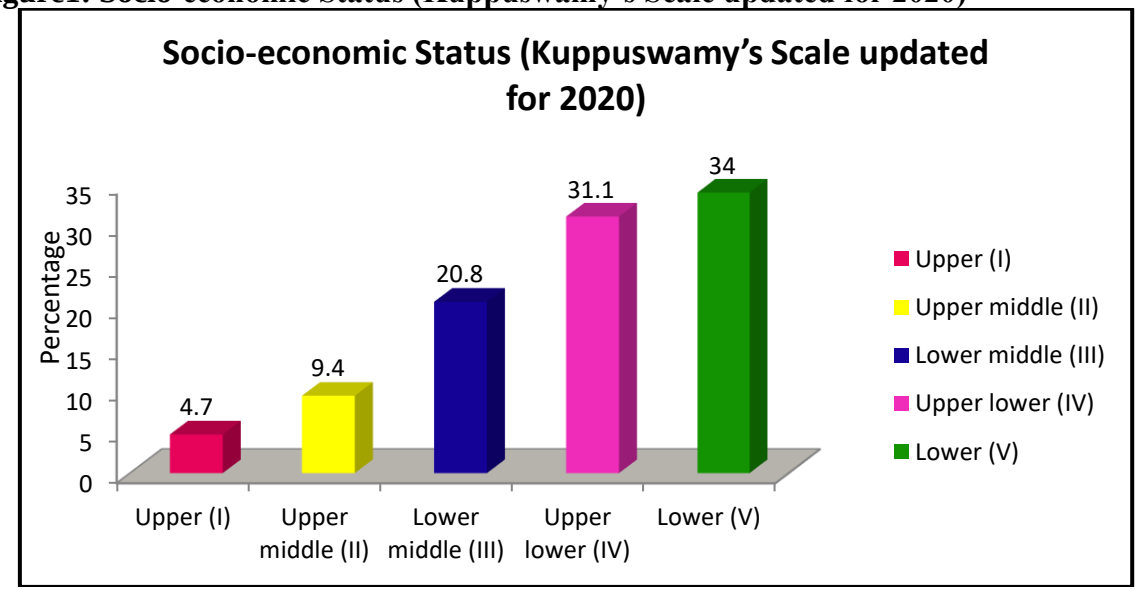
Table 1: Demographic profile of respondents

Demographic profile	Age Group	Frequency	Percentage
Age group	**14-30 years**	44	41.5
	31-40 years	20	18.9
	41-50 years	19	17.9
	51-60 years	14	13.2
	61-70 years	7	6.6
	>70 years	2	1.9
Gender	**Male**	68	64.2

	Female	38	35.8
Resident	**Urban**	15	14.2
	Rural	91	85.8
	Total	106	100.0

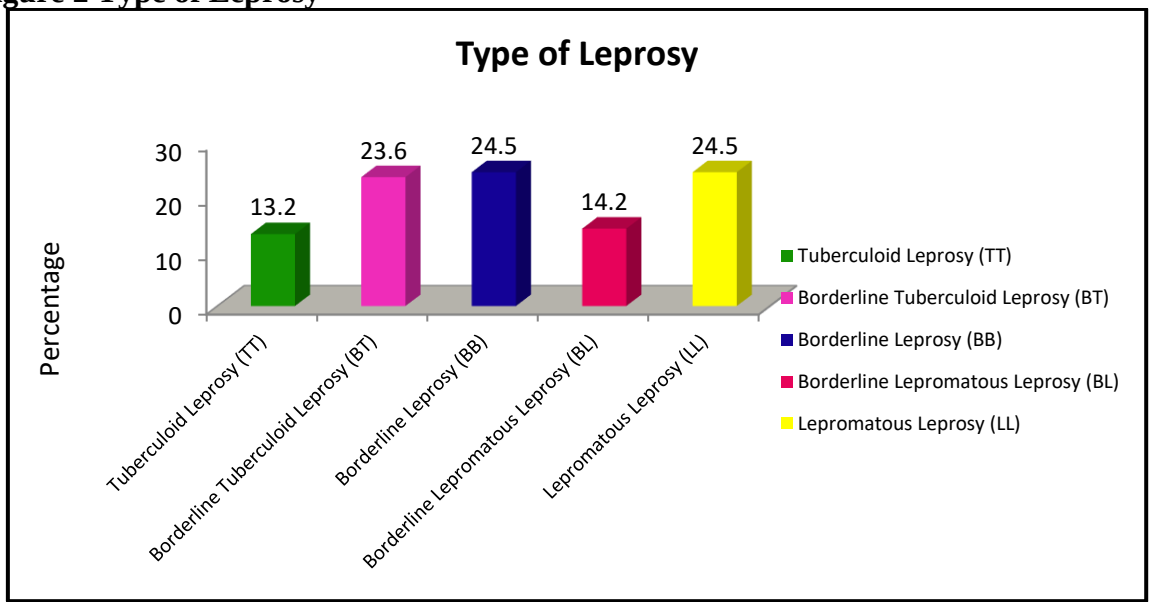
Table1 shows that demographic profile of the selected respondents. Majority of the respondents (41.5) were belonged to age group (14-30years) and followed by 18.9 % belong to age group (31-40 years). Majority of respondents (64.2%) were male whereas 35.8% were found female respondents, whereas mean age of respondents were observed 37.70 ±14.82. Majority of respondents (85.8%) were belonged to rural area whereas only 14.2% were belonged to urban area.

Figure1: Socio-economic Status (Kuppuswamy's Scale updated for 2020)



Socio-economic status of the study population according to Kuppuswamy's scale updated for 2020 of is presented in figure1. More than half of the patients belonged to lower socioeconomic status.

Figure 2 Type of Leprosy



The most common form of leprosy observed in the present study was borderline leprosy (BB) and Lepromatous leprosy (LL). Both the forms constituted equal proportion of patients

(24.5%). Borderline tuberculoid leprosy was also prevalent with 23.6% incidence. Data is provided in figure2.

Table 2 WHO clinical type of Leprosy

WHO clinical type of Leprosy	Frequency	Percentage
PB	11	10.4
MB	95	89.6
Total	106	100.0

According to the WHO operational classification, 89.6% of the study participants in the present study were classified as MB and 10.4% were classified as PB. Data is shown in Table 2.

Table 3 Slit Skin Smear Findings

Slit Skin Smear Findings	Frequency	Percentage
Positive	95	89.6
Negative	11	10.4
Total	106	100.0

Slit skin smear findings of the present study is shown in table 3. Out of 106 patients, microscopy was positive in 95 (89.6%) cases.

Table 4 Nasal Smear Findings

Nasal Smear Findings	Frequency	Percentage
Positive	42	39.6
Negative	64	60.4
Total	106	100.0

Nasal smear finding of the present study is shown in**t**able 4. Out of 106 patients, nasal smear was positive in 42 (39.6%) cases.

Table 5 ELISA Findings

ELISA	Frequency	Percentage
Positive	49	46.2
Negative	57	53.8
Total	106	100.0

ELISA finding of the present study showed 46.2% (49) positive and 53.8% (57) negative results. Data is depicted in table 5.

Table 6 Diagnostic Accuracy of Nasal Smear

Statistic	Value
Sensitivity	38.95%
Specificity	63.64%
Positive Predictive Value	90.24%
Negative Predictive Value	10.77%
Accuracy	41.51%

Considering slit skin smear as the gold standard we analysed the diagnostic accuracy of nasal smear finding for leprosy. The sensitivity and specificity of the nasal smear was 38.95% and 63.64% respectively. The positive predictive value and negative predictive value of nasal smear was 90.24% and 10.77% respectively while diagnostic accuracy of nasal smear was 41.51%. Data is shown in table 6.

Table 7 Diagnostic Accuracy of ELISA

Statistic	Value
Sensitivity	48.42%
Specificity	72.73%
Positive Predictive Value	93.88%
Negative Predictive Value	14.04%
Accuracy	50.94%

Considering slit skin smear as the gold standard we analysed the diagnostic accuracy of ELISA finding for leprosy. The sensitivity and specificity of ELISA was 48.42% and 72.73% respectively. The positive predictive value and negative predictive value of nasal smear was 93.88% and 14.04% respectively while diagnostic accuracy of ELISA was 50.94%. Data is shown in table 7.

DISCUSSION

In the present study, it was undertaken to detect Acid-fast bacillus (AFB) in nasal smear and slit skin smear of leprosy patients and also assess the bacteriological index in these samples. We also correlated these findings with the newer technique that is enzyme-linked immunosorbent assay (ELISA) to analyze the disease profile.

The observation of the present study regarding the clinical features, disease profile and the diagnostic accuracy of updated diagnostic assay (ELISA) are as follows:

The most common form of leprosy observed in the present study was borderline leprosy (BB) and Lepromatous leprosy (LL). Both the forms constituted equal proportion of patients (24.5%). Borderline tuberculoid leprosy was also prevalent with 23.6% incidence. According to the WHO operational classification, 89.6% of the study participants in the present study were classified as MB and 10.4% were classified as PB.

Out of 106 patients, microscopy was positive in 95 (89.6%) cases and nasal smear was positive in 42 (39.6%) cases.ELISA finding of the present study showed 46.2% (49) positive and 53.8% (57) negative results. Considering slit skin smear as the gold standard we analysed the diagnostic accuracy of nasal smear finding for leprosy. The sensitivity and specificity of the nasal smear was 38.95% and 63.64% respectively. The positive predictive value and negative predictive value of nasal smear was 90.24% and 10.77% respectively while diagnostic accuracy of nasal smear was 41.51%.

Considering slit skin smear as the gold standard we analysed the diagnostic accuracy of ELISA finding for leprosy. The sensitivity and specificity of ELISA was 48.42% and 72.73% respectively. The positive predictive value and negative predictive value of nasal smear was 93.88% and 14.04% respectively while diagnostic accuracy of ELISA was 50.94%.

Sensitivity values among studies from different regions and among studies from the same regions showed great differences, for both the MB and PB groups, as reported previously.[17] Even studies that were designed by the same authors and conducted in the same regions produced different sensitivity values.[18,19] Specificity values were more similar among the studies analyzed in both groups, MB and PB. Sensitivity and specificity found for each ELISA matched the accuracy reported by other authors.[20-23]

Serological tests are aimed at detecting specific antibodies against *M. leprae* that indicate infection. These tests can be useful in monitoring the effectiveness of therapy, determining the prevalence of the disease, and assessing the distribution of infection in a particular community.[24] The elucidation of the chemical structure of Phenolic glycolipid 1 (PGL-I), a specific antigen of *M. leprae*, in 1981 made it possible to create serological tests for diagnosis.[25]

According to **Frade et al**. (2017), the commercial rapid test NDO-LID (Orange Life, Rio de Janeiro, Brazil) was positive in 62.8% of patients clinically diagnosed with leprosy. However,

it showed less specificity than the anti-PGL-I and anti-LID-1 ELISAs.[26] Although this test can identify dominant responses to both the glycolipid (IgM anti-PGL-I) and protein (IgG anti-LID-1), the NDO-LID has the same limitation as other rapid diagnostic tests, highlighting the difficulty of using this test to monitor individuals in the early stages of the disease and/or PB. Regardless, the use of serological tests associated with clinical examination, can contribute to the early detection and treatment of leprosy. Serological tests perform better in the identification of MB patients, especially the BL and LL forms. Additionally, BL and LL patients produce high IgM titers against PGL-I, while TT patients have low levels of specific antibodies.[27]

A study carried out in a hyperendemic area of Brazil indicated that the anti-LID-1 assay has a sensitivity of 89% and a specificity of 42% for the diagnosis of leprosy. The low specificity is probably related to the presence of a large number of asymptomatic individuals infected with Mycobacterium.[28] Conversely, NDO-LID has a specificity of 85.89% and a sensitivity of 90.6% for MB and 27% for PB.[29] In that study, the authors correlated the ELISA results with the bacteriological index and the Ridley-Jopling classification since the lepromatous pole patients had higher responses. In contrast, in those of the tuberculoid pole, the antibody levels were lower. Other authors confirmed these results.[30] Additionally, the cases with high bacilloscopy index (BI) have high titers of anti-PGL-1 IgM anti-LID-1 IgG60, and anti-NDO-LID IgM and IgG.[31,32]

Hence at the end of our study we can suggest that early detection of leprosy is a strategy to interrupt the transmission of *M. leprae* and to prevent the occurrence of physical disability, a serious consequence. However, the diagnosis is still essentially defined by clinical examination. Slit skin smear and histopathology examinations are used to aid the clinical diagnosis and are useful in spectral and treatment categorization.[33]

CONCLUSIONS

In this study, we correlated the slit skin smear findings and nasal smear findings with ELISa and also determined the sensitivity; specificity, positive predictive value, and negative predictive value of enzyme-linked immunosorbent assay (ELISA).Considering slit skin smear as the gold standard we analysed the diagnostic accuracy of nasal smear finding for leprosy. The sensitivity and specificity of the nasal smear was 38.95% and 63.64% respectively. Considering slit skin smear as the gold standard we analysed the diagnostic accuracy of ELISA finding for leprosy. The sensitivity and specificity of ELISA was 48.42% and 72.73% respectively.

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