Original Research Article

The Role of Slit Skin Smear in the Diagnosis of Doubtful Cases of Hansen's Disease

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A B S T R A C T

Leprosy remains one of the major public health hazards in the world even today. It is caused by *Mycobacterium leprae*, which is an acid-fast and rod-shaped bacillus. It is mainly transmitted by close and intimate contact with untreated cases via droplets from nasal and oral secretions and rarely through the skin. It is endemic in tropical countries, especially in developing countries in South-East Asia, America, Africa, Eastern Pacific and Western Mediterranean. Early case detection and prompt treatment have been identified as key strategies for effective control and elimination of leprosy disease. This article aims to study the role of Slit Skin Smear in the diagnosis of doubtful cases of Hansen's disease. After enrolment of doubtful cases of the leprosy patients, a detailed history and demographic information were collected with their consent in addition to obtaining slit skin smear (SSS) as per the standard protocol. The SSS were stained with modified Ziehl- Neelson stain (5% sulphuric acid) and viewed under an oil immersion microscope. From a total of 120 clinically doubtful cases of leprosy, 23 (19.1%) were SSS positive. These cases were neither previously diagnosed as leprosy nor had undergone any treatment for leprosy. Among the 23 SSS positives, there was a male predominance (78.2%); 78.3% were from rural area and 65.2% were from economically low background. The most predominant age group among SSS positives were 30-40 years (43.4%) followed by 40-50 years (34.7%). In the 23 positive cases, BI 1+ was present in 70% and borderline tuberculoid in 66.7%. Among 120 doubtful cases, paucibacillary (PB) accounted for 80.9% being the highest number against 19.1% of cases with multibacillary (MB) disease. Further treatment and follow up action on the cases of SSS positive as per the result of the study were intimated to Dermatology department. Hansen's disease is still a threat and challenge to human life. Early detection, treatment, awareness building and community education will reduce the prevalence consequently leading to eradicate the disease altogether.

Introduction

Leprosy is an age old chronic infectious disease caused by *Mycobacterium leprae*, an acid-fast, rod-shaped bacillus. It predominantly affects skin and peripheral nerves. (Ashwini S Patil *et al.,* 2021) Generally, leprosy infection occurs through the droplets from the nose and mouth of the persons

affected, who is medically untreated. Persons who establish close and frequent contact with untreated diseased persons are likely to get affected. (WHO, Leprosy Key facts, 2021) Normally the incubation period is considered to be five years. After exposure, the first lesion may appear in the duration of twenty years. (Sapna Sonija *et al.,* 2016) Leprae affects the cooler parts of the body like skin, peripheral nerves, mucosa of upper respiratory tract and sometimes eyes. (Sapna Sonija *et al.,* 2016) It is contagious in some cases leading to disease and in most cases, it may not cause the disease due to their effective immune system. (Pooja Chaoubey *et al.,* 2019)

In 1955, National Leprosy Control Program (NECP) was launched in India based on Dapsone Mono-therapy. In 1983, National Leprosy Eradication Program (NCEP) was launched with introduction of multi drug therapy (MDT) in phases as recommended by WHO. (Parik Shit Sharma *et al.,* 2017) All types of leprosy can be cured by Multi-drug therapy (MDT) as recommended by the WHO and these drugs are available for free of cost since 1995, worldwide. (Shahi Duzzaman *et al.,* 2009) However it is important to note that Slit Skin Smear (SSS), the method available in Developing countries for the diagnosis of leprosy is strengthened by the direction of Indian Government. Directorate General of Health services, central leprosy division has issued guidelines to this effect with an intention of strengthening leprosy control activities and program. However, the directorate has cautioned all the Stakeholders for continuous supervision and monitoring the collection and processing of SSS at reference centers to ensure uniformity, reliability and high levels of quality and performance standards. Despite the prevalence rate of the disease in India although appear to be reducing in the late 1990s, have been shooting up from 2000s due to untreated leprosy patients. It is reported in 2020, that it continues to home more than 50% of the global leprosy cases. As of the reason SSS remains as gold standard for all diagnostic techniques in all developing countries as per the report of International Leprosy association technical forum 2002. (Mahajan, 2013)

India is the home to the highest number of new cases in the world followed by Brazil and Indonesia. As per the World Health Organization's (WHO) 2020 report, out of 202,189 new cases globally, 114,451 cases (57%) were contributed by India.

Also, there are more than three million people with leprosy deformities needing attention and care. (Narasimha Rao, 2021) Although India had started leprosy control program in 1955, known as National Leprosy Eradication Program (NLEP) since 1983, it could not have breakthrough to attain the vision of NLEP: "Leprosy-free India." (NLEP, dghs 2016)

Materials and Methods

This is a prospective study conducted in the Department of Microbiology and Dermatology, Guntur Medical College (GMC) and Government General Hospital (GGH), Guntur, Andhra Pradesh for a period of 18 months from May 2020 to October 2021 with an approval from the Institutional Ethical Committee. A detailed history, clinical examination and demographic information were obtained from the doubtful cases of Hansen's disease patients. Those who were previously diagnosed and undergoing treatment for leprosy were excluded. After taking consent from the enrolled patients, slit skin smear (SSS) was obtained as per the standard protocol. Smears were stained with modified Ziehl-Neelson stain where 5% Sulphuric acid was used as decolorizer. Slides were examined under oil immersion microscope and Ridley's logarithmic scale was used for Bacteriological Index (BI).

Statistical Analysis

The statistical analyses were performed using Microsoft Excel Data Analysis Software. In this connection, various factors of the study relating to doubtful cases of Hansen's disease were analyzed by regression model using univariate analysis. In addition, all the P values reported were made on the basis of two tailed tests with the significance level of 0.05.

Results and Discussion

As per the table.1, out of the 120 doubtful cases, there was male predominance of about 68.3% and the rest 31.6% females. 37.5% of cases belonged to the age group between 30-40 years followed by 26.6% between 40-50 years, 20% between 20-30 years and 15.8% above 50 years of age. 79% of cases were from rural and the rest 21% from urban areas. The majority of cases belonged to the lower socio-economic status of about 55% followed by 26.7% middle and 18.3% upper socio-economic status. 96.7% of cases had no contact history whereas 3.3% had contact history of leprosy patients.

Most of the cases in this study were Paucibacillary (PB) 80.9% and the rest 19.1% Multibacillary (MB). All were newly identified cases rather than retreatment cases. For further treatment and follow up of all cases, intimation was given to the Department of Dermatology, Government General Hospital, Guntur.

The present study reported SSS positivity for AFB of about 19.1% which is nearly correlating with the study conducted by Dharmedra Kumar *et al.,* (2020) (20.83%); Suma Nalamada *et al.,* (2018) (24.7%). In consensus with other studies conducted by Parikshit Sharma *et al.,* (2017); Kilikdar *et al.,* (2018)*;* Marfatia *et al.,* (2020)*;* Vinay *et al.,* (2019)*;* Jitu Mani Kalita *et al.,* (2020) SSS positive males outnumbered females (M: F= 3.6:1). According to Parikshit Sharma *et al.,* (2017) the male predominance was due to self-report and female health issues often neglected particularly in rural populations. As per the present study, the majority of SSS positive patients were from rural areas (78.3%) which is nearly correlating with Borah *et al.,* (81.9%). According to Borah *et al.,* (2019) rural predominance is due to following reasons: lack of awareness, low accessibility to health care facilities, lack of adherence to therapy, lack of knowledge regarding the consequences of the disease and inhibition of reporting for treatment due to social taboos and customs. While comparing socio-

economic status, the majority of SSS positive patients belonged to lower socio-economic group (65.2%) in the present study with a statistically significant (p value of 0.039684) nearly correlating with the study conducted by Ashwini S. Patil *et al.,* (2020) (58.75%). 13% of SSS positive patients had contact/ family history of leprosy which is nearly correlating with Borah *et al.,* (2019) (12.5%).

In the present study, the majority of SSS positive patients were between 30-50 years of age (78.1%) with a statistically significant (p value of 0.041305) which is nearly correlating with Dharmedra Kumar *et al.,* (2020) (60%) and none of the patients were less than 20 years of age which is correlating with Mousumi Kilikdar *et al.,* (2018). Also, the majority of patients had Bacteriological index 1+ (70%) nearly correlating with the study by Ashwini S. Patil *et al.,* (2020) (41.25%). Whereas Ghosh *et al.,* (2014-2017) reported that Bacteriological index 6+ was most common. In the present study, borderline leprosy cases were found to be dominating the clinical types (BT- 66.7%, BB- 19.1%) followed by tuberculoid 14.1%.

Among the borderline spectrum, the most frequently observed clinical type was BT nearly correlating with the study conducted by Vinay *et al.,* (2019) (45.3%) and Suma Nalamada *et al.,* (2018) (33%). In addition, 97 (80.9%) cases were, as per the present study, smear-negative (Paucibacillary) and 23 (19.1%) smear- positive (Multibacillary) correlating with the observations of Rao *et al.,* (2016)*;* Mahajan *et al.,* (2003); Borah *et al.,* (2019)*;* Ashwini S. Patil *et al.,* (2020). Whereas Suma Nalamada *et al.,* (2018) and Mousumi Kilikdar *et al.,* (2018) reported more of multibacillary cases when compared to paucibacillary cases. Hansen's disease is still a threat and challenge to human life. As per the study, male predominance was more common in lower socio-economic group particularly in rural areas. Early detection, treatment, awareness building and community education will reduce the prevalence consequently leading to eradicate the disease altogether.

Table.1 Demographic Profile of Study Population

Variable	Total patients 120	Percentage % 100%
Gender		
Male	82	68.3%
Female	38	31.6%
Age group in years		
20-30	24	20%
30-40	45	37.5%
40-50	32	26.6%
>50	19	15.8%
Residence		
Rural	95	79%
Urban	25	21%
Socio-economic status		
Lower	66	55%
Middle	32	26.7%
Upper	22	18.3%
Family history/ contact history		
Yes	4	3.3%
No	116	96.7%

Table.2 Analysis of Slit Skin Smears and WHO Classification of Leprosy for the Rationale of Treatment

Number of Slit skin smear	Positive (Multibacillary)	Negative (Paucibacillary)
120	23 (19.1%)	97 (80.9%)

Table.3 Sex Distribution Among Doubtful Cases of Leprosy

Sex distribution	Total (N=120)	Positive SSS (n=23)
Male	82 (68.3%)	18 (78%)
Female	38 (31.6%)	5 (22%)

Table.4 Age wise Distribution Among the Doubtful Cases of Leprosy

Range	Total (N=120)	SSS positive (n=23)	p value
20-30 years	24 (20%)	3 (13%)	
30-40 years	45 (37.5%)	10 (43.4%)	
40-50 years	32 (26.6%)	8 (34.7%)	0.041305
>50 years	19 (15.8%)	2 (8.7%)	

Table.5 Distribution of Residence Among Doubtful Cases of Leprosy

Residence	Total (N=120)	Positive SSS (n=23)
Rural	95 (79%)	18 (78.3%)
Urban	25 (21%)	5 (21.7%)

Table.6 Socio-Economic Status Among Doubtful Cases of Leprosy

Socio-economic status	Total (%) (N=120)	SSS Positive (%) (n=23)	P value
Lower	66 (55%)	15 (65.2%)	
Middle	32 (26.7%)	5 (21.7%)	
Upper	22 (18.3%)	3 (13%)	0.039684

Fig.1 Distribution of Leprosy Cases   **Fig.2** Microbiological Study of Lesions
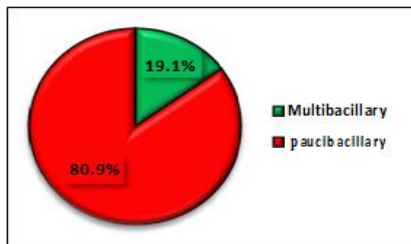
Out of 120 SSS, 23 (19.1%) were positive for AFB and the rest 80.9% negative.

Fig.3 Distribution of Sex among SSS Positivity
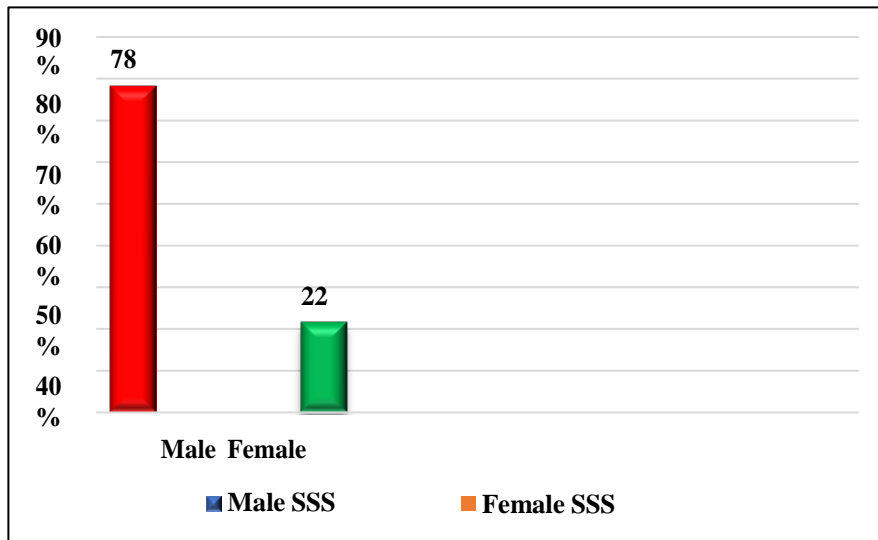
Among 23 positive cases, 78% were males and 22% females.

Fig.4 Relation Between AFB Positivity and Age
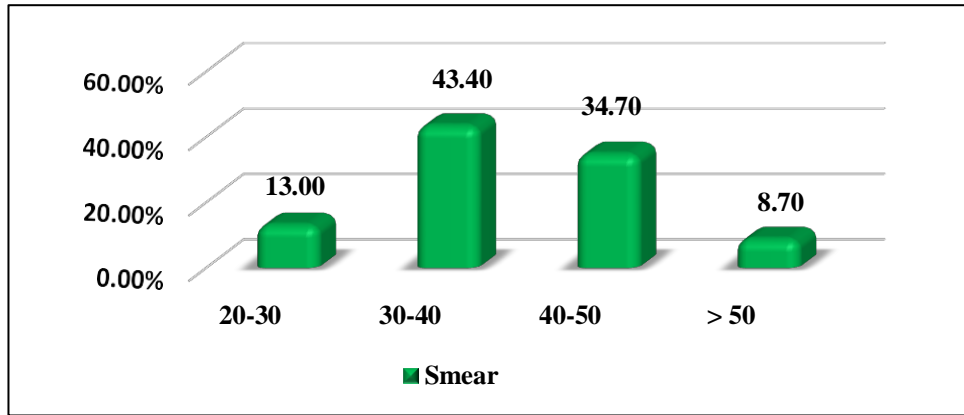
Among 23 positive cases, 43.4% were from 30-40 years, 34.7% from 40-50 years, 13% were from 20-30 years and 8.7% were above 50 years with statistically significant p- value of 0.041305.

Fig.5 Distribution of Residence Among SSS Positivity
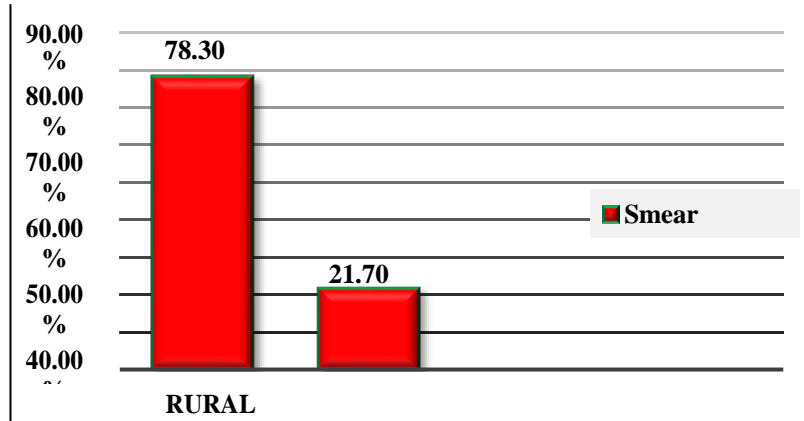
Among 23 SSS positive cases, 78.3% were from rural and the rest 21.7% from urban area.

Fig.6 Socio-Economic Status Among SSS Positivity
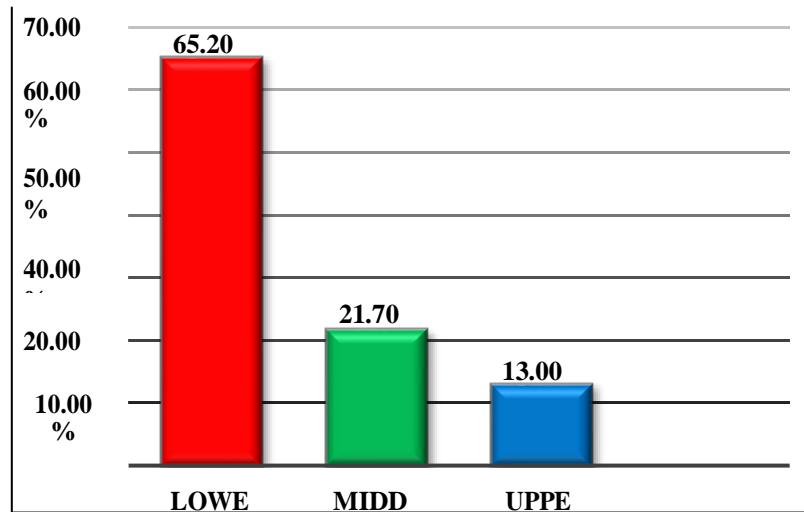
Among 23 SSS positive cases, 65.2% were from economically lower class; 21.7% from middle class; 13% from upper class with a statistically significant p- value of 0.039684.
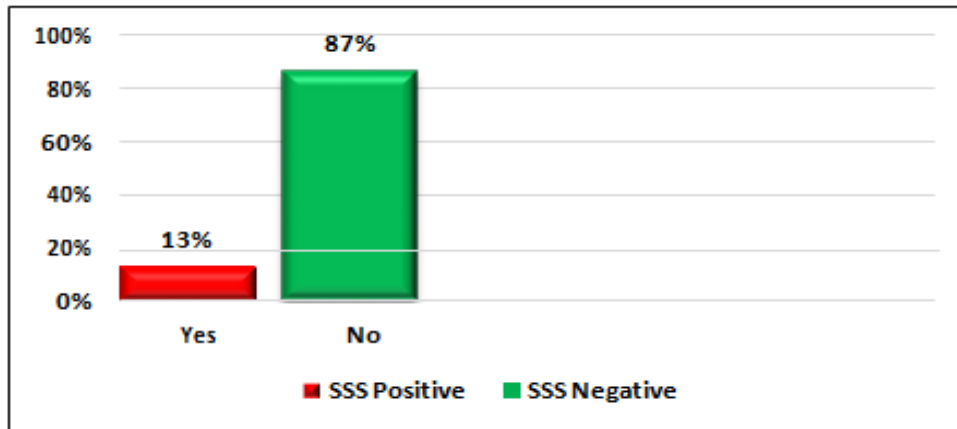
Table.7 Family history/Contact history Among Doubtful Cases of Leprosy

Family history/ Contact history	Total (%) (N=120)	SSS Positive (%) (n=23)
Yes	4 (3.3%)	3 (13%)
No	116 (96.7%)	20 (87%)

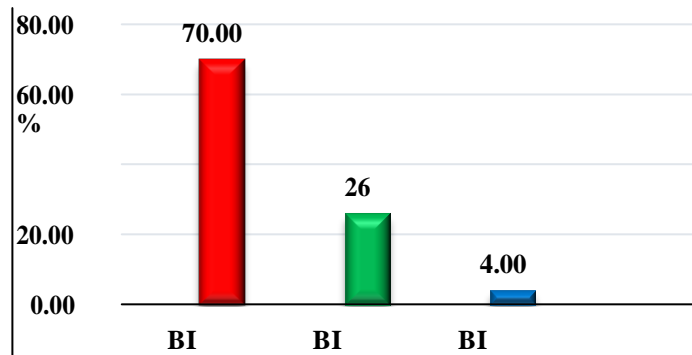**Table.8** Distribution of Bacteriological Index of Smear Among AFB Positive Patients

Bacteriological Index (BI)	No: of cases (%) (n=23)
BI 1+	16 (70%)
BI 2+	6 (26%)
BI 3+	1 (4%)

Fig.7 Family history/ Contact history Among SSS Positivity

Among 23 SSS positive cases, 13% had contact/ family history and the rest 87% had no history.

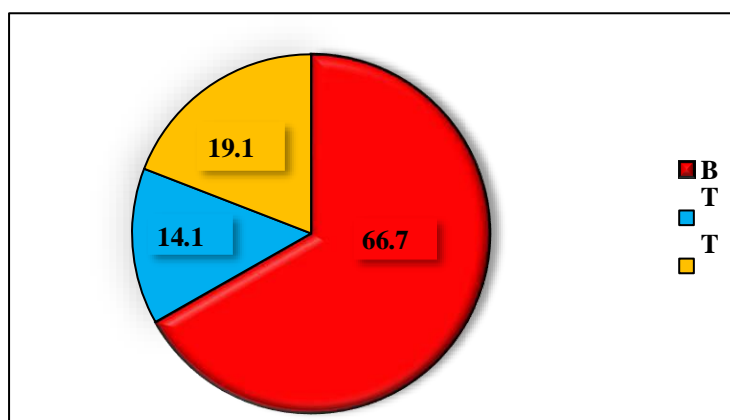**Fig.8** Distribution of Bacteriological index among SSS positive

Among 23 positive cases, 70% had BI 1+ and 26% BI 2+, 4% BI 3+.

Table.9 Clinical Classification of Leprosy in the Study

Total cases	Tuberculoid leprosy (TT)	Borderline tuberculoid leprosy (BT)	Borderline Border line (BB)
120	17 (14.1%)	80 (66.7%)	23 (19.1%)

Fig.9 Illustrates the Clinical Classification of Leprosy Cases

The most commonly encountered type of leprosy in the study was borderline tuberculoid (BT) 66.7% followed by borderline borderline leprosy (BB) 19.1% and tuberculoid leprosy (TT)14.1%.

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