ORIGINAL RESEARCH PAPER

Pathology

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EVALUATION OF DIAGNOSTIC ROLE OF SLIT SKIN SMEAR IN LEPROSY

Dr. Versha Prasad Assistant Professor, School Of Health Sciences, C.S.J.M. University, Kanpur.

Rudra Pratap Singh Research Student, M.Sc. MLT, Medical Laboratory Technology, School Of Health Sciences, C.S.J.M. University Kanpur

ABSTRACT

Objective: To study the role of Slit-Skin Smear in the diagnosis of suspected cases of leprosy. **Background:** India is the country with the most leprosy cases in the world. According to data from 2020, India has 65,147 leprosy cases, or around 51.09% of all leprosy cases worldwide. Leprosy's eradication depends significantly on early diagnosis. Slit skin smear (SSS) diagnosis of leprosy is a quick and easy procedure. This is crucial for the early detection of leprosy in countries like India where there are more cases. Slit skin smear (SSS) testing is a crucial component of India's National Leprosy Eradication Programme (NLEP). In this study, the usefulness and sensitivity of slit skin smear (SSS) as a diagnostic tool for leprosy were investigated. **Methods:** This study includes 50 suspected cases of leprosy who visited the Department of Microbiology GSVM Medical College for diagnosis. Slit skin smears (SSS) were prepared from the active skin lesions such as earlobes, eyebrows, forehead, elbows, knees and at the junction of the healthy skin and affected skin. These smears were stained with modified Ziehl-Neelsen staining (ZN staining) or modified AFB (acid fast bacilli) staining and examine microscopically for Mycobacterium leprae. Mycobacterium leprae stained pink coloured in a cigar-bundle appearance or arranged in a regular clump. **Results:** Microscopic examination of 50 patients was done with the help of Slit Skin Smear (SSS), age group between 10-65 years. Out of 50 patients, there were 35 male patients and 15 female patients. Out of 35 male patients, there were 4 Slit Skin Smear (SSS) positive patients while 31 were Slit Skin Smear (SSS) negative patients. Out of 15 female patients, there was 1 Slit Skin Smear (SSS) positive patient while 14 were Slit Skin Smear (SSS) negative patients. Male positivity rate for Slit Skin Smear (SSS) is 11.42% out of 35 patients, while female positivity rate for Slit Skin Smear (SSS) is 6.66% out of 15 patients. Slit Skin Smear (SSS) confirmed the clinical diagnosis in 10% cases only, while 90% cases were negative (out of 50 cases). **Conclusion:** Microscopic examination of Slit Skin Smear (SSS) is a rapid, accurate, easy to carry out and important diagnostic tool especially in early and doubtful cases of leprosy. However, sometimes some cases required some other diagnostic method to detect mycobacterium leprae bacilli.

INTRODUCTION

G. H. Armauer Hansen discovered the Mycobacterium leprae in 1873. Leprosy originated in East Africa or the near East.[3] Leprosy is a chronic infectious disease that affects the humans. This is caused by the bacteria Mycobacterium leprae and Mycobacterium lepromatosis.[4] Leprosy is caused by cold loving bacteria or slow growing bacteria. This is also known as Hansen's disease. It is a granulomatous infection which affects the nose, eyes, lymph nodes, ears, bone marrow, skin, nerves, internal organs.[1] The incubation period of the disease is variable can range from 1 to 20 years.[1]

Morphologically lepra bacilli is a slightly curved rod or straight, 1-8X0.2-0.5 micrometer in size. It is gram positive and decolourized by 5% sulphuric acid. Lepra bacilli have been found to remain viable in a warm humid environment for 9-16 days and in moist soil for 46 days. They survive exposure to direct sunlight for two hours and ultraviolet light for 30 minutes.[2]

The lepra bacilli are transmitted through close contact with patients affected with leprosy, droplets from the nose and mouth during coughing and sneezing. If leprosy left untreated it can cause serious damage to limbs, eyes, skin and nerve.[1-2]

Common symptoms of the leprosy may include: Skin nodules, Rough skin, Loss of eyebrows, Discolored skin lesions, Numbness on affected part, Paralysis, Blurred vision, Multiple skin lesions on hands, feet, back and neck, nerve damage.[2-4]

The disease affects the nerves, skin, eyes, and lining of the nose (nasal lining). The bacteria attack the nerve, which can swell under the skin. This can cause the affected area to lose its ability to feel touch and pain, which can lead to injuries such as cuts and burns. Typically, the affected skin changes color and becomes-lighter or darker, usually dry or scaly, with loss of sensation or Redness due to skin irritation.[2]

In very severe cases, a person may suffer multiple injuries due to lack of sensation, and eventually the body may reaccept the affected digit over time, seemingly resulting in loss of toes and fingers.

The majority of leprosy problems are brought on by nerve injury. The M. leprae bacteria directly invade the nerves, causing inflammation as a result of the immune system's reaction. Although the exact chemical process by which M. leprae causes leprosy symptoms is unknown, it has been demonstrated that M. leprae binds to Schwann cells, which may cause nerve damage, including demyelination and a loss of nerve function (more specifically, a loss of axonal conductance). Numerous laminin-binding proteins including the glycoconjugate (PGL-1) on the surface of M. leprae, which can bind to laminin on peripheral nerves, have been linked to this nerve injury among other molecular pathways. White blood cell-derived macrophages may phagocytose M. leprae as part of the human immune response.[3]

Long-term untreated conditions may result in- Vision loss, Hand malformation and facial disfigurement, Erectile dysfunction and infertility in males, Kidney disease, Permanent damage to the nerves outside the brain and spinal cord, Permanent injury to the inside of the nose, which can cause nosebleeds.

People with Hansen's disease can continue to work and enjoy active lives if they receive early diagnosis and treatment, which typically prevents disability that can be caused by the condition. After receiving therapy, the patient is no longer contagious. However, it is crucial to follow the doctor's instructions and complete the entire course of treatment.[2]

A cytodiagnostic method called a slit-skin smear (SSS) is employed as a supplementary laboratory procedure in the diagnosis of certain cutaneous dermatoses. It is a simple and secure method in which a sample is taken from a very small

wound in the lesional skin, dyed, and viewed under a microscope. SSS may be applied as a diagnostic tool in the following situations: Leprosy

1. As a screening method for all individuals in whom leprosy is suspected to be the underlying disease. One of the primary indications of leprosy,
2. To identify leprosy relapse
3. To categorise leprosy and determine the prognosis. [6]

Leprosy was diagnosed in 1884 by Patrick Manson using the squeeze and pierce method. Later, Alvarez surgically removed the nodules and ground them to create smears. Muir prepared smears using the skin clip process. Wade finally introduced the SSS method for leprosy diagnosis in 1963. [7] Slit skin smear (SSS) Mycobacterium leprae detection is the gold standard method for leprosy diagnosis. Clinical examinations and skin samples have traditionally been used to diagnose leprosy. Microscopy has the benefit of being widely accessible in outlying and referral centres, but its detection limit is just 104 bacteria per milliliter.[7]

MATERIAL AND METHODS

It will be a cross sectional study to be done on the patient of Leprosy enrolled at G.S.V.M Medical College, Kanpur. Study will conducted at School of Health Sciences, C.S.J.M. University, Kanpur and collaboration with G.S.V.M Medical College Kanpur. Total number of patient will be 50. These will be selected as per inclusion & exclusion criteria. This study will be done in duration 6 Months.

Inclusion Criteria: Patient of either sex, suspected patients of leprosy of all ages, patients with multiple chronic skin lesions, patients who have given informed consent.

Exclusion Criteria: Patients diagnosed with leprosy were excluded from the study, patients who are taking multidrug therapy, patients who have not given consent to participate in this study.

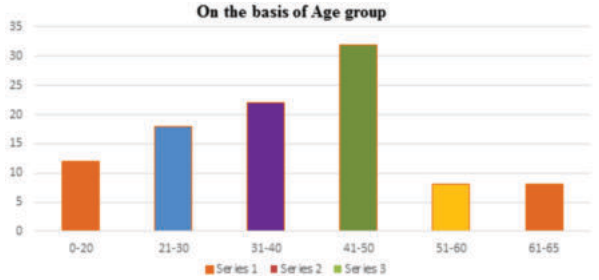
Investigational Strategy: Initial skin samples are often obtained from 6 "routine sites" (both earlobes, elbows, and knees) as well as a few common lesions from the patient in order to diagnose Mycobacterium leprae. To gauge improvement, repeat smears are taken from three to four of the most active locations previously evaluated.

OBSERVATION AND RESULTS:

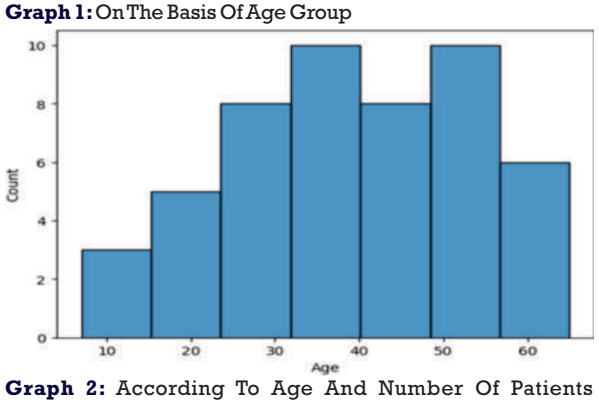
In the present study, microscopic examinations of 50 patients were done with the help of Slit Skin Smear, age group between 10-65 years. Out of 50 patients, there were 35 male patients and 15 female patients. Maximum number of the cases presented with hypo-pigmented, ill-defined and macular skin lesions presents both over covered and uncovered parts of the body. Out of 35 male patients, there were 4 Slit Skin Smear positive patients while 31 were Slit Skin Smear negative patients. Out of 15 female patients, there was 1 Slit Skin Smear positive patient while 14 were Slit Skin Smear negative patients. Male positivity rate for Slit Skin Smear is 11.42% out of 35 patients, while female positivity rate for Slit Skin Smear is 6.66% out of 15 patients. Slit Skin Smear confirmed the clinical diagnosis in 10% cases only, while 90% cases were negative (out of 50 cases). Slit Skin Smear confirmed the clinical diagnosis in 10% cases only, while 90% cases were negative.

Table No 3 – On The Basis Of Age Group

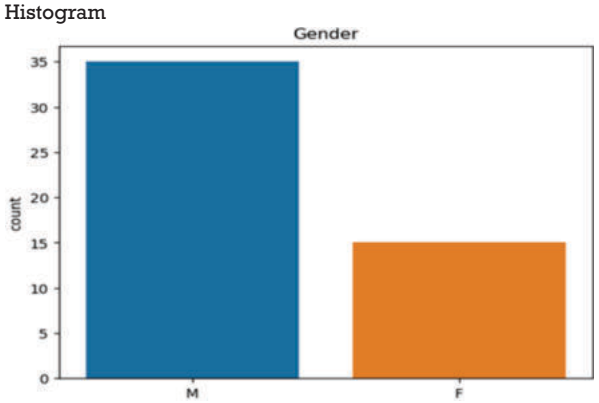
AGE groups	No. of cases = 50	Percentage
10-20	06	12%
21-30	09	18%
31-40	11	22%
41-50	16	32%
51-60	04	08%
61-65	04	08%



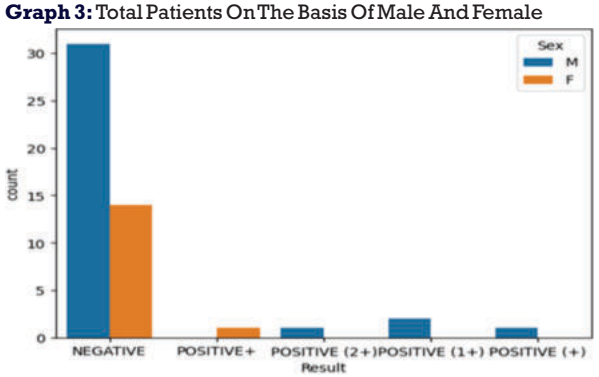
Graph 1: On The Basis Of Age Group



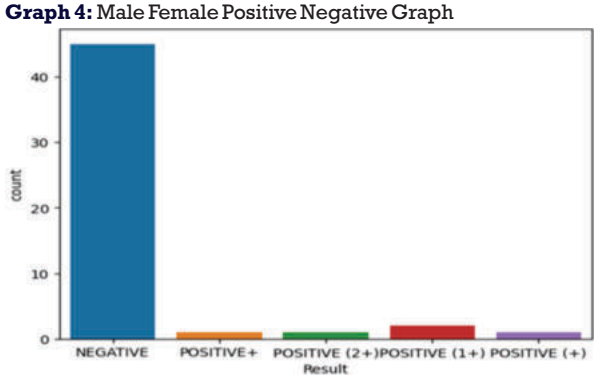
Graph 2: According To Age And Number Of Patients Histogram



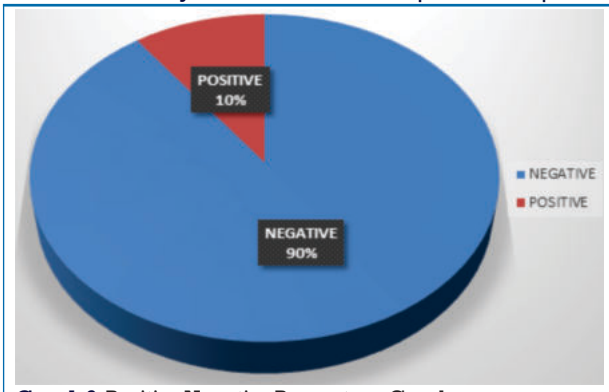
Graph 3: Total Patients On The Basis Of Male And Female



Graph 4: Male Female Positive Negative Graph



Graph 5: Result Positive Negative Graph



Graph 6: Positive Negative Percentage Graph

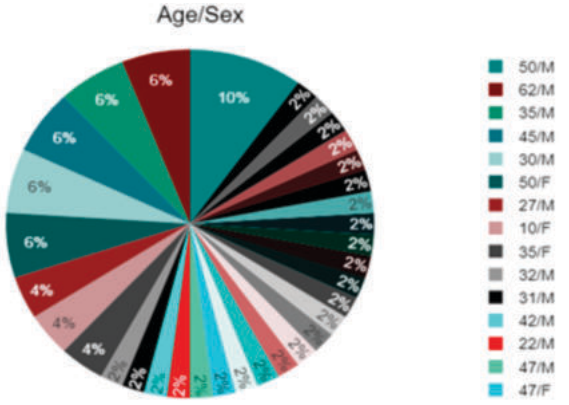


Figure 7: Percentage Of No. Of Cases According To Age And Sex

Table 03: Show The Frequency Of Result And Also Show The Mean, Sd Minimum And Maximum Valuse Of Age According To Result.

		Result		
		NEGATIVE	POSITIVE	POSITIVE+
Age	Frequency	45	4	1
	Mean	39.49	36.75	42
	Std. Deviation	14.48	14.57	NaN
	Minimum	10	17	42
	Maximum	65	50	42

Point Biserial Correlation

Label	Value
M	0
F	1

Summary

A point-biserial correlation was run to determine the relationship between Age and Sex. There was a negative correlation between Age and Sex, which was statistically not significant (rpb = -0.15, n = 50, p = .294).

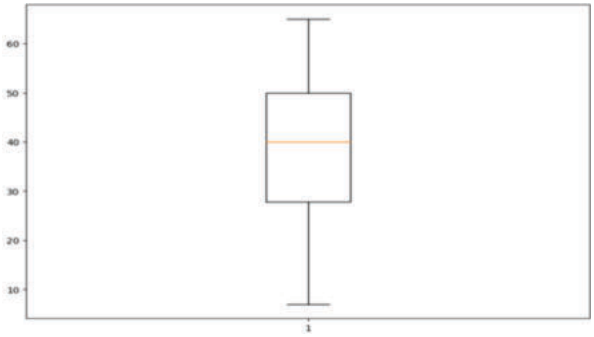


Figure 5: Outlair

DISCUSSION

The present study includes 50 suspected cases of leprosy (age group between 10-65 year) who visited the Department of Microbiology GSVM Medical College for diagnosis. Slit skin smears were prepared according to WHO protocol from the active skin lesions such as earlobes, eyebrows, forehead, elbows, knees and at the junction of the healthy skin and affected skin. This reflects a good trend of reporting early for diagnosis and treatment. Maximum number had skin problems such as skin lesions or numbness present over both covered and uncovered parts of the body. This study includes 35 male patients and 15 female patients. Out of 50 patients only 5 patients had leprosy, diagnosed by slit skin smear including 4 male patients and 1 female patient. Skin smears were positive in 10% cases. Diagnosis of leprosy is easy when cardinal signs are present. Leprosy is not highly communicable. The disease develops in only about 5 percent of spouses living with leprosy patients. The incubation period is very long and averages 2-5 years. It has been estimated to vary from a few months to as long as 30 years. It is generally held that intimate and prolonged contact is necessary for infection to take place. The disease is more likely if contact occurs during childhood.

CONCLUSION

Microscopic examination of Slit Skin Smear (SSS) is a rapid, accurate, easy to carry out and important diagnostic tool especially in early and doubtful cases of leprosy. However, sometimes some cases required some other diagnostic method to detect mycobacterium lepra bacilli. Slit Skin Smear can play an important role in early and accurate diagnosis of leprosy in low or medium income and densely populated country such as India because it required minimum instrumentation and it is cost effective.

REFERENCES

1. www.who.int/health-topics/leprosy#tab=tab_2
2. www.cdc.gov/leprosy/transmission/index.html
3. en.wikipedia.org/wiki/Leprosy
4. www.nhp.gov.in/disease/skin/leprosy
5. cltri.gov.in/AboutLeprosy/default.html
6. Banerjee S, Biswas N, Kanti Das N, Sil A, Ghosh P, Hasanoor Raja AH, Dasgupta S, Kanti Datta P, Bhattacharya B. Diagnosing leprosy: revisiting the role of the slit-skin smear with critical analysis of the applicability of polymerase chain reaction in diagnosis. Int J Dermatol. 2011 Dec;50(12):1522-7. doi: 10.1111/j.1365-4632.2011.04994.x. PMID: 22097999.
7. Siwakoti S, Rai K, Bhattarai NR, Agarwal S, Khanal B (2016) Evaluation of Polymerase Chain Reaction (PCR) with Slit Skin Smear Examination (SSS) to Confirm Clinical Diagnosis of Leprosy in Eastern Nepal. PLOS Neglected Tropical Diseases 10(12): e0005220. https://doi.org/10.1371/journal.pntd.0005220.