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ROLE OF FLUROCHROME STAIN IN THE DIAGNOSIS OF LEPROSY

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Abstract

Leprosy is a chronic infective disease, caused by Mycobacterium leprae, an acid fast bacillus, discovered by Dr. Armouer Hansen in 1873[1]. Early detection of leprosy is important to prevent severe handicaps, spread of infection and economic loss. For diagnosis of leprosy, slit skin smear method is the widely used conventional procedure. Though it is simple but traumatic procedure. Fine needle aspiration (FNA) smear is simple and easy procedure and almost no trauma to the patient. For identification of M. leprae, modified Ziehl-Neelsen (Z-N) stain (Fite stain) has become the solely dependant method so far. In this study flurochrome staining (auramine- phenol stain) technique is applied to stain the FNA smears from lymphnode in leprosy patients to see ability to detect the M.leprae in modified Z-N stain negative cases. The study was carried out at the Department of Pathology, Bangabandhu Sheik Mujib Medical University, Dhaka, during May 2002 to January 2003. In this study, 15 modified Z-N stain negative FNA smears of lymph node and 04 positive smear as control, were included. Smears of all the 04 modified Z-N stained positive cases (control group) showed positive fluorescence of bacilli. Out of 15 smears under study, 04(26.66%) smears showed positive fluorescence. Flurochrome (auramine- phenol stain) stained FNA smears of lymph node appears to have higher detection rate of M.leprae than the widely and routinely practiced modified Z-N stain.

Introduction

Leprosy is a chronic infective disorder caused by Mycobacterium leprae. Early detection of leprosy is important to prevent severe handicaps, spread of infection and economic loss. There are several tools/ tests for diagnosis of leprosy. Clinical findings, accompanied by demonstration of Mycobacterium leprae in skin lesions through slit skin smears in conjunction with histopatholgy, remain the only available standard for the diagnosis of leprosy[2]. Skin test through slit skin smear though simple, lack the required sensitivity and specificity to serve as diagnostic tool for Mycobacterium leprae infection[3].

Fine needle aspiration (FNA) technique is very simple, easy procedure and almost no trauma to the patient in comparison to the traumatic slit skin smear procedure[4]. Modified Z-N stained (Fite stain) slit skin smear is still the conventional method for detection of M.leprae. In this study flurochrome staining (auramine- phenol stain) technique is applied to stain the FNA smears from lymphnode in leprosy patients to see ability to detect the M.leprae in modified Z-N stain negative cases. Flurochrome stain (auramine- phenol stain) technique is simple and speedy in observation[5].

Materials and methods

This study was carried out at the Department of Pathology, Bangabandhu Sheik Mujib Medical University (BSMMU), Dhaka, during the period from May 2002 to January 2003. A total 42 clinically and/or bacteriologically diagnosed or suspected leprosy patients with palpable lymph node, were under study. Out of 42 patients, 27 patients showed M.leprae in modified Z-N stained smear of lymph node and remaining 15 patients showed negative results. From 27 positive smears, 4 smears randomly selected as control and all negative i.e 15 smears of lymph node were included in the study.

FNA smears were taken from the best palpable lymph node. A 26 gauge size needle fitted with 5ml syringe was introduced into lymph node. Aspiration and smear preparation were performed according to the usual methods of FNA as described by Bruitt –Marie Ljung[6]. Smears were fixed by a brief exposure to the flame. All fixed smears were stained by flurochrome (auramine- phenol) stain. Stained smears were evaluated under fluorescence microscopy immediately and or the same day of staining. Smears were examined at 10x objectives to confirm M.leprae bacilli. Yellow golden or lemon yellow rods having morphology of M.leprae were

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taken as positive and fluroscent round masses as globi. The smears were recorded as positive or negative if smear showed or not showed M.leprae.

Result

A total 19 clinically diagnosed leprosy patients with palpable lymph node were included in the study. Out of 19 patients, 15 patients showed no M.leprae in the modified Z-N stained FNA smears of lymph node (study group) and 4 patients showed M.leprae in modified Z-N stained smears (positive control group). Smears of all the 4 modified Z-N stained positive patients (control group) showed positive fluorescence of bacilli. Out of 15 smears under this study, 4 (26.66%) smears showed positive fluorescence. (Table –I)

Table I : Results of Auramine – Phenol (A-P) stained smears.

A-P stain	Number	Percentage
Positive	4	26.66
Negative	11	73.34
Total	15	100

Clinically 4 positive smear patients (control group) classified as lepromatous leprosy (LL). Out of 15 patients under study, clinically 1 patient classified as borderline tuberculoid (BT), 7 patients as borderline lepromatous (BL) and remaining 7 as lepromatous leprosy (LL). In 4 fluorescence positive patients, two were BL type and another two were LL type. (Table –II).

Table II : Comparative results of Auramine – Phenol (A-P) stained and modified Z-N stained smears.

Type of Leprosy	Z-N negative smears (No.)	A-P stain Positive		A-P stain negative		Total	
		No.	%	No.	%	No.	%
TT	00	00	00	00	00	00	00
BT	01	00	00	01	6.67	01	6.67
BB	00	00	00	00	00	00	00
BL	07	02	13.33	05	33.33	07	46.66
LL	07	02	13.33	05	33.33	07	46.66
Total	15	04	26.66	11	73.34	15	100

Discussion

The conventional slit skin smears for the detection of M.leprae is the recommended method at present. Slit skin smears need multiple skin cuts which is traumatic to the patient. Regarding the staining technique, modified Z-N stain (Fite stain) is the solely dependant method so far as it is simple and cheaper[3]. In this study, fine needle aspiration (FNA) technique is applied to obtain material from the lymph node in leprosy and flurochrome (auramine - phenol) staining was used instead of modified Z-N stain (Fite stain). This findings are consistent with the findings of Chhetri and Rahman[7], and Ahmed, Ahamad and Rahman[8].

Conclusion

Leprosy is a fairly common disease which needs early diagnosis to treat the patient as well as to prevent deformities and relapse. For this purpose, modified Z-N stained (Fite stain) slit skin smears is the recommended and widely practiced method. In this study flurochrome (auramine- phenol stain) stained FNA smears of lymph node appears to have higher detection rate of M.leprae bacilli than the widely and routinely practiced modified Z-N (Fite stain) stain. So, it is recommended that flurochrome (auramine -phenol stain) stain may be routine technique in those laboratories where fluorescence microscope is available for the detection of M.leprae.

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