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Original article

Polymerase Chain Reaction versus Slit Skin Smear in Diagnosis of Leprosy; A Cross Sectional Study

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ABSTRACT

Background: Leprosy is a chronic infectious disease where delay in diagnosis or treatment can lead to deformities and disabilities for the rest of the patient's life. Definite diagnosis of leprosy has long been based on clinical picture, histopathology and/or the detection of acid-fast bacilli (AFB) from tissue smears or tissue sections stained by Ziehl-Neelsen.

Aim of the work: We evaluated in this study, the usefulness of PCR for detection of *M.leprae* in the serum, as less invasive technique, in comparison to skin slit smear for the diagnosis of leprosy.

Patients and Methods: This study included 30 non-treated patients of leprosy (9 multibacillary, 21 paucibacillary) skin slit smears had been taken. In addition, serum samples had been collected for polymerase chain reaction [PCR] test.

Results: Negative Slit Skin Smear for AFB had been obtained in 20 cases with a rate of positivity of 33%. Serum PCR was positive in 20 patients giving an overall rate of positivity of 67% which confirm the diagnosis in 11 out of 20 cases with slit skin smear [SSS] negative.

Conclusion: PCR does show to be more sensitive than slit skin smear [SSS] indicating its future use for diagnostic purposes especially in early leprosy cases.

Keywords: Leprosy; Polymerase Chain Reaction; Slit Skin Smear; *Mycobacterium Leprae;* Acid-Fast Bacilli

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* Main subject and any subcategories have been classified according to the research topic.

INTRODUCTION

Leprosy is a chronic infectious disease which affects basically the skin, nasal mucosa, and peripheral nerves. Leprosy is caused by *Mycobacterium Leprae [M. leprae]*, an acid-fast bacillus transmitted through droplets from the mouth and nose during close contact with untreated cases[1].

Eventually if untreated, leprosy leads to disability and disfigurement. Thus, early detection of *M. leprae* is important to timely identification and treatment of patients [2].

Clinical manifestations and classification of leprosy are associated with the type of immune response generated by the host. Tuberculoid leprosy (TT) or paucibacillary on one pole of the spectrum; results from having an intense cell-mediated immune response and characterized by an abundance of Th1 cytokines, presented clinically by 1-5 lesions, no nerve affection or only one nerve trunk involvement. On the opposite pole of the spectrum, patients with lepromatous leprosy (LL) or multi-bacillary, is characterized by a predominant humoral immune response and lack of *M.Leprae* cell mediated immunity, presented clinically by ≥ 5 skin lesions and more than one nerve trunk involvement. Patients with the borderline forms of leprosy fall between the two poles (TT and LL), are immunologically unstable, and are more prone to complicating reactions[3]

The widespread use of multi-drug therapy (MDT) has reduced the disease burden globally. The number of new patients diagnosed with leprosy is still significant, at more than 200000 in 2016. New case detection (a proxy for incidence rate) is slowly declining; leprosy continues to be a significant health concern especially in areas like India, Brazil, and Africa[4].

Leprosy diagnosis is based on clinical criteria, slit skin smear either positive or negative (patients whose smears are positive are more infectious than those are not), bacterial index, histopathological findings and serological tests [5].

In early suspected cases, routine histopathological examination by Hematoxylin and Eosin and Fite-Faraco stains can confirm diagnosis in only about 35٪ of such early cases, because of paucity of acid fast bacilli and absence of infiltration inside the dermal nerves. Instead, the slides show nonspecific histopathology in the form of chronic inflammatory cell infiltrate at various locations, which is not specific for leprosy alone[6].

The diagnosis of early cases requires additional methods as demonstrating nucleic acid sequences specific to the pathogen by using in-situ hybridization and amplification by in situ polymerase chain reaction [PCR][7].

AIM OF THE WORK

The aim of this study is to assess the utility of the PCR on serum samples using RLEP gene (the *M. Leprae* specific repetitive element) and compare it to traditional slit skin smear [SSS] in diagnosis of leprosy.

PATIENTS AND METHODS

Ethical approval: written informed consents had been signed by all patients with approval of the local Research Ethics Committee. The study had been carried out in line with declaration of Helsenki.

Inclusion criteria: The study included thirty patients , all suspected of leprosy of any age and either gender having one or more of these features: typical hypothetic or anesthetic, erythematous, hypo-pigmented scaly skin lesions, nodules and plaques on any part of the body, thickened peripheral nerves, silent neuropathy, selected from Dermatology Outpatient Department (OPD) at Mansoura and Damietta Dermatology and Leprosy Hospitals. The study had been performed from December 2018 to December 2019.

Exclusion criteria: All patients who have already received treatment for leprosy and patients with lepra reactions were excluded from this study.

Skin slit smears were taken from the patients from the border of their ear lobules according to **Kamble *et al*.** [8]. Collected blood samples (five milliliters of venous blood) had been drawn into vacutainers tubes containing no anticoagulant. Tubes had been incubated in upright position, at room temperature for 30-45 minutes (no longer than 60 minutes) to allow clotting. Blood samples were centrifuged for 15 minutes according to manufacturer's recommended speed (1000-2000 RCF[relative centrifugal force]) for serum separation. DNA was extracted and purified using a Master Pure DNA extraction Kit ***Quick*-DNA™ Miniprep Kit, USA®** according to the manufacturer instructions, followed by amplification which was carried out using

RLEP primers sequences PS1 50-TGC ATG TCA TGG CCT TGA GG-30 and PS 2 50-CAC CGA TAC CAG CGG CAG AA-30[9]. Amplification was achieved by a combined procedure on Light Cycler Instrument using **(PTC-100** Programmable Thermal Controller, **MJ RESEARCH, INC, USA®**) consisted of first stage of single cycle of denaturation at 95°C for 5 minutes followed by second stage of 35–45 cycles of 30 seconds at 94°C, 30 seconds at 58°C and 1 minute at 72°C, and final stage of single cycle of 10 minutes at 72°C[10].

Statistics: Statistical presentation and analysis were calculated using mean and standard deviation for numerical data, frequency and percent distribution for categorical data. Groups compared by student [*t*] and chi-square tests. Also, to assess the strength of association between two quantitative variables, Pearson correlation coefficient [*r*] was calculated. All analyses were done by statistical package for social science [SPSS Inc., USA]. *P* is significant if <0.05 at confidence interval 95%.

RESULTS

Our study included 30 untreated leprosy patients, with average age 37.3, ranged from 15 to 70 years. Male patients represented 70%, while females represented 30%. All studied patients had been classified according to World Health Organization [WHO] classification; 70% had Paucibacillary [PB] and 30% had Multibacillary [MB]. In addition, they were also classified according to Riedley and Jopling classification; 50% had Tuberculoid leprosy [TT], 6.7% had Borderline tuberculoid [BT], 10% had Borderline lepromatous [BL], 16.7% had Lepromatous leprosy [LL], 13.3% had Pure neural [PN] and 3.3% had **Histoid** leprosy (a rare variant of LL) which may appear *de novo* or as a manifestation of drug resistance following irregular or inadequate treatment) [Table 1].

Seven patients had history of contact with leprosy patients. SSS was negative in 66.7% (20 cases) and positive in 33.3%. While PCR was negative in 33.3% (10 cases) and positive in 66.7% of all study cases. All MP cases were positive SSS (100%), while negative SSS cases were significantly associated with PB type [Table 2].

PCR could diagnose 3 out of 4 cases of pure neural leprosy (75%). None of BT type could be diagnoses by neither PCR or SSS [Table 3].

Only one patient out of 21 patients of PB was SSS positive. Patients with both negative SSS and PCR were 9; all were PB type; both positive SSS and PCR were 9; (8 MP+ 1PB), while 11 PB patients had positive PCR and negative SSS. Only one MP case showed positive SSS and negative PCR [Table 4].

Table [1]: Types of leprosy in all cases

		Leprosy	
		N	%
Type of leprosy according to WHO	PB	21	70%
	MB	9	30%
Type of leprosy according to R and J	TT	15	50.0%
	BT	2	6.7%
	BL	3	10.0%
	LL	5	16.7%
	PN	4	13.3%
	H	1	3.3%

PB: Paucibacillary; MB: Multibacillary; TT: Tuberculoid leprosy; BT: Borderline tuberculoid; BL: Borderline lepromatous; LL: Lepromatous leprosy; PN: Pure neural; H: histoid leprosy

Table [2]: Comparison between negative and positive slit skin smear [SSS] in all studied leprosy cases.

		Negative SSS [N=20]		Positive SSS[N=10]		*p*
Type of leprosy according to WHO class	**PB**	20	100%	1	10%	**<0.001**
	MB	0	0%	9	90%	
Type of leprosy according to R and J class	**TT**	14	70%	1	10%	**<0.001**
	BT	2	10%	0	0%	
	BL	0	0%	3	30%	
	LL	0	0%	5	50%	
	PN	4	20%	0	0%	
	H	0	0%	1	10%	

Table [3]: Comparison between negative and positive PCR in all studied leprosy cases

		Negative PCR N=10		Positive PCR N=20		*p*
Type of leprosy according to WHO class	**PB**	9	90%	12	65%	0.210
	MB	1	10%	8	35%	
Type of leprosy according to R and J class	**TT**	6	60%	9	45%	0.234
	BT	2	20%	0	0%	
	BL	0	0%	3	15%	
	LL	1	10%	4	20%	
	PN	1	10%	3	15%	
	H	0	0%	1	5%	

PB: Paucibacillary; MB: Multibacillary; TT: Tuberculoid leprosy; BT: Borderline tuberculoid; BL: Borderline lepromatous; LL: Lepromatous leprosy; PN: Pure neural; H: histoid leprosy

Table [4]: Agreement between SSS and PCR in all studied cases

		PCR		*k*	Agreement
		Negative	Positive		
SSS	**Negative**	9	11	0.280	Fair
	Positive	1	9		

DISUCSSION

Although leprosy is one of the ancient infectious diseases, many questions on modes of entry, incubation period and early manifestations are still unknown. Over the last few years many studies have been published on PCR-mediated detection and amplification of *M. Leprae* DNA, suggesting that PCR is a useful tool for diagnosis of this disease. Identification of *M. Leprae* is difficult from a biological sample because of inability of the bacillus to grow in vitro. Diagnosis of leprosy is based mainly on microscopic detection of AFB in tissue smears, histopathological study and clinical evaluation. Because acid-fast staining requires at least the presence of 10^4 organisms per gram of tissue for credible detection with low rate of sensitivity, especially for the majority of PB patients wherein AFB are rare or absent[11].

In the past 30 years, ultimate identification of *M. Leprae* has been possible through using PCR technique on different samples such as skin biopsy, skin smears, nerve sheath biopsy, urine, mouth washing, nasal swabs, blood, and ocular lesions [12].

Few studies focused on revealing *M. Leprae* from whole-blood samples. The aim of our study was to assess the sensitivity of serum PCR as an assay to detect *M leprae* in comparison to SSS. The study has adopted a simple PCR assay using **RLEP** primer for detection of *M. Leprae*, RLEP was found to be highly sensitive, specific and can detect 10 fg of purified *M. Leprae* DNA according to **Truman *et al.*[13].**

These results agree with study done by **Lavania *et al.*** [14] in which targeting RLEP gene was able to detect the highest number (53%) of BI (bacterio-logical index) negative leprosy patients and (83%) of blood samples in BI-positive leprosy patients. On the other hand, **Reis *et al.*[15]** presented a study applying quantitative real-time PCR (qPCR) for the detection of *M. leprae* DNA in peripheral blood samples of 200 untreated leprosy patients using ML0024 genomic region, which detected the presence of DNA bacillus in 22.0% (44/200) of the leprosy patients: 23.2% (16/69) in paucibacillary (PB), and 21.4% (28/131) in multibacillary (MB) patients; claiming that: although the specificity of the PCR in blood was very high (99.1%), the sensitivity was low (11.5%) and no test alone would be worthwhile with such low sensitivity, unless combined with other tests supporting the diagnosis.

It is important to confirm that blood is not the best sample for the diagnostic detection of leprae bacilli; meanwhile we depended on serum samples only as less invasive method. On the other hand **Tatipally *et al.*[16]** published a paper presents a systematic review analysis suggesting that, PCR on skin biopsy is the ideal diagnostic test . The highest percentage of PCR sensitivity was observed using multiplex PCR technique (82%) followed by RT-PCR (78%) and conventional PCR (63%).

The limitations of this study were: the lack of different samples from the same individual collected at the same time (SSS, skin biopsy, nerve sheath biopsy) for detection of *M. Leprae* DNA as we depended on serum samples only as less invasive method, our study was based on conventional PCR

method and also small number of patients.

From the present study, we can conclude that, PCR test in diagnosis of leprosy is more sensitive than SSS, and can detect many false negative cases, so when available, it is better to combine PCR with other tests in diagnosis of early cases of leprosy.

Financial and Non-Financial Relationships and Activities of Interest

None

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