# Value of Polymerase Chain Reaction (PCR) In Diagnosis and Follow-up of Paucibacillary Leprosy Patients

## Thesis

Submitted for partial fulfillment of
Master Degree in Dermatology, Venereology and Andrology


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# List of Abbreviations

| | | |
|---|---|---|
| **AFB** | **:** | **Acid Fast Bacilli** |
| **APC** | **:** | **Antigen Presenting Cells** |
| **BB** | **:** | **Borderline Leprosy** |
| **BI** | **:** | **Bacterial Index** |
| **BL** | **:** | **Borderline Lepromatous Leprosy** |
| **BT** | **:** | **Borderline Tuberculoid Leprosy** |
| **CMI** | **:** | **Cell mediated Immunity** |
| **DC** | **:** | **Dendritic Cells** |
| **DNA** | **:** | **Deoxyribo Nucleic Acid** |
| **DTH** | **:** | **Delayed Type Hypersensitivity** |
| **ENL** | **:** | **Erythema Nodosum Leprosum** |
| **H&E** | **:** | **Haematoxyline – Eiosine** |
| **HD** | **:** | **Hansen's Disease** |
| **IFN-y** | **:** | **Interferon Gamma** |
| **IL – ١٠** | **:** | **Interleukin-١٠** |
| **IL – ١٢** | **:** | **Interleukin – ١٢** |
| **LL** | **:** | **Lepromatous Leprosy** |
| **LC-PCR** | **:** | **Light Cycler Polymerase Chain Reaction** |
| **LIB** | **:** | **Logarthmic Index of Biopsies** |
| **M. leprae** | **:** | **Mycobacterium Leprae** |
| **MB** | **:** | **Multibacillary** |
| **MDT** | **:** | **Multi Drug Therapy** |

| | | |
|---|---|---|
| **MI** | **:** | **Morphological Index** |
| **NAPCS** | **:** | **New – Antigen Presenting Cells** |
| **PB** | **:** | **Paucibacillary** |
| **PCR** | **:** | **Polymerase Chain Reaction** |
| **PGL-l** | **:** | **Phenolic Glycolipid-one** |
| **QPCR** | **:** | **Quantitative Polymerase Chain Reaction** |
| **RT. PCR** | **:** | **Real- Time Polymerase Chain Reaction** |
| **SGPSI** | **:** | **Stocking- Glove Pattern Sensory Impairment** |
| **TLRs** | **:** | **Toll Like Receptors** |
| **Th – ١** | **:** | **T .lymphocytes Helper Cells** |
| **TNF- &** | **:** | **Tumour Necrosis Factor Alpha** |
| **TT** | **:** | **Tuberculoid Leprosy** |
| **VC** | **:** | **Virchow Cells** |
| **WHO** | **:** | **World Health Organization** |

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# Acknowledgment

*Praise and thanks to Allah the all mighty who provided me with patience and determination to complete this work.*

*I would tike to express my deepest gratitude and appreciation to Dr.* ***Ahmad IBrahim Rasheed****, Professor of Dermatology, Andrology and Venereotogy, Ain Shams University, for his close valuable guidance all through the work and for his advice and sincere support during supervision of this work. He provided me with valuable comments, knowledge, and experience for achieving this work.*

*Also, I would like to express my great thanks and gratitude to Dr.* ***Heba Mahmoud Diab****, Assistant Professor of Dermatology, Andrology and Venereology, Ain Shams University, for her general helps and her indispensable advice, precious observations, and support all through supervision of this work.*

Omar Mohammad Abd El-Moneim

# Introduction

Leprosy, a chronic granulomatous disease which primarily affects the peripheral nervous system and skin, has a worldwide distribution; the total number of cases is estimated to be around ٥٣٤٠٠. India accounts for ٦٤٪ of leprosy prevalence and ٧٦٪ of the new cases detected worldwide ***(Dayal et al,*** ***)***.

Leprosy is characterized by two polar forms, a paucibacillary tuberculoid and a multibacillary lepromatous (LL) form. Between these, three intermediate forms are described: the borderline tuberculoid (BT), borderline borderline (BB), and borderline lepromatous (BL) forms. Another rare form is the pure neural leprosy (PNL), whish is characterized by the uniqueness of nerve lesions and is difficult to diagnose because skin lesions and acid-fast bacilli (AFB) in slit smears are absent ***(Riedly and Jopling*** ***).***

The diagnosis of paucibacillary leprosy is primarily clinical. Anesthetic skin lesions with or without thickened peripheral nerves are virtually pathognomonic of paucibacillary leprosy, a full thickness skin biopsy from an anesthetic lesion showing granuloma and lymphocytic infiltration of nerves essentially confirms the diagnosis. Acid fast bacilli (AFBs) are rarely found in patients with indeterminate or tuberculoid disease. In the established form of the disease, the diagnosis is relatively simple. However, the established

forms are decreasing in number due to widespread use of multi drug therapy (MDT) and early disease is being reported more frequently ***(Buhrer-Sekula et al, ).***

In early suspected cases, routine histopathological examination by Hematoxylin and Eosin and Fite-Faraco staining of sections can confirm the diagnosis in only about ٣٥٪ of such early cases, because of paucity of acid fast bacilli and absence of infiltration inside the dermal nerves . Instead, they show non-specific histopathology in the form of chronic inflammatory cell infiltrate at various locations, which is not specific for leprosy alone ***(Ramu et al, ).***

The diagnosis of these early cases requires additional methods as demonstrating nucleic acid sequences specific to the pathogen by e.g. using in-situ hybridization and amplification by in situ polymerase chain reaction ***(Jardim et al, ).*** This technique allows cellular localization of low-level of nucleic acid within the tissue and cells and can aid in a significant way to augment the sensitivity of histopathological diagnosis, and provide a solid base for follow up of these patients ***(Komminoth et al, ).***

# Aim of the work

The aim of this work is to evaluate the technique of Real-time polymerase chain reaction (RT-PCR) in the diagnosis of paucibacillary leprosy i.e tuberculoid leprosy, borderline tuberculoid and the pure neural leprosy. Another aim is to assess the value of (PCR) technique in the follow up of the patients for the efficacy of the multidrug therapy (MDT).

# Introduction

Leprosy is a chronic granulomatous disease principally affecting the skin and peripheral nerves caused by the infection with the obligate intracellular Mycobacterium leprae. ***(scollard et al., ٢٠٠٦)***

Leprosy is best understood as two conjoined diseases. The first is a chronic mycobacterial infection that elicits an extraordinary range of cellular immune response in humans. The second is a peripheral neuropathy that is initiated by the infection and the accompanying immunological events. ***(Sunaina et al.,  )***

## Epidemiology

The prevalence of leprosy fell by ٩٠ percent in the last two decades because patients completing a course of multiple-drug therapy(MDT) have been considered to be cured, but the incidence of the disease, which varies in direct proportion to case-finding efforts, has yet to be reduced convincingly. ***(WHO,  . )***

The highest new case detection rates are in India, Brazil, Democratic Republic of Congo, Tanzania, Nepal, Mozambique, Madagascar, Angola and the Central African Republic. The disease burden in India alone represents ٦٤٪ of all new cases worldwide. ***(WHO,  . )***

At the beginning of ٢٠١٠, the registered prevalence of leprosy globally was ٢١١،٩٠٣ cases and the number of new cases detected during ٢٠٠٩ was ٢٤٤،٧٩٦ cases. ***(WHO,*** ***)***(Table ١)

Table (١): Prevalence of leprosy and number of new cases detected, by WHO region, beginning of ٢٠١٠. ***(WHO,*** ***)***

| **WHO region** | **No of cases registered (prevalence rate*) Beginning of ٢٠١٠** | **No of new cases detected (case-detection rate**) ٢٠٠٩** |
|---|---|---|
| African | ٣٠،٩٤٧ (٠,٤٠) | ٢٨،٩٣٥ (٣,٧٥) |
| Americas | ٤٣،٣٧٠ (٠,٤٩) | ٤٠،٤٧٤ (٤,٥٨) |
| South-East Asia | ١٢٠،٤٥٦ (٠,٦٨) | ١٦٦،١١٥ (٩,٣٩) |
| Eastern Mediterranean | ٨،٤٩٥ (٠,١٥) | ٤،٠٢٩ (٠,٧٠) |
| Western Pacific | ٨،٦٣٥ (٠,٠٥) | ٥،٢٤٣ (٠,٢٩) |
| Total | ٢١١،٩٠٣ | ٢٤٤،٧٩٦ |

* *Prevalence rate = number of cases / ¤ population*
** *Case-detection rate = number of cases / ¤ population*

In all populations studied, lepromatous disease is more common in men than in women by a ٢:١ ratio. If leprosy is common, the tuberculoid forms dominate; if leprosy is uncommon, the lepromatous forms predominate. Leprosy is said to be a disease of rural incidence, but of urban prevalence. The median age of onset is less in tuberculoid than in lepromatous patients, but in both groups,

the median age of onset being less than ٣٥ years old. However, old age does not protect. ***(WHO,*** ***)***

In Egypt, the highest new case detection rates are present in the Upper Egypt Governorates (Qena, Sohag, Aswan and Assiut, respectively). ***(WHO,*** ***.)***

**Table (٢):** *Leprosy situation in Egypt, beginning of* ***(WHO,*** ***).***

| Registered prevalence | No. of new cases detected ٢٠٠٩ | No. of new cases of multibacillary leprosy | No. of new female cases | No. of new cases among children | No. of new cases with grade ٢ disabilities | No. of relapses ٢٠٠٩ |
|---|---|---|---|---|---|---|
| ٩١٢ | ٧٠٠ | ٦١٦ | ٢٦٥ | ٤٨ | ٤٢ | ٥ |

## Mode of transmission:

The route of infection of leprosy is unproven, but current evidence favors respiratory transmission: evidence for congenital and percutaneous transmission has been presented, but examples are rare. ***(Truman,*** ***)***

The incubation period for tuberculoid leprosy is up to ٥ years and for lepromatous disease may be ٢٠ years or longer. ***(Irgens,*** ***)*** Untreated multibacillary (MB) patients are probably the most important source of Mycobacterium leprae transmission. It is estimated that household contacts of MB patients have a relative

risk of developing leprosy that is ٥- to ١٠-fold greater than that of the general population. However, in many areas, the number of MB patients is very small, and they may not represent the most important source of infection. ***(Cole et al., ٢٠٠٦)*** There is increasing evidence that subclinical transmission may occur, since even in countries where leprosy is highly endemic, for many patients, no history of close contact with a leprosy patient can be established. ***(International Leprosy Association Technical Forum, )***

As Mycobacterium leprae can persist and possibly proliferate in the environment in association with certain plants and animals, it is conceivable that infection may result through prolonged or repeated exposure to an environmental source containing viable bacilli. This is difficult to investigate experimentally because M. leprae cannot be cultivated in vitro and evidence can only be obtained indirectly through epidemiological studies. ***(Desikan and Sreevatsa, )***

***<u>Genetics of susceptibility:</u>***

A twin study has provided compelling evidence that both genetic and environmental factors are important in determining disease susceptibility and disease expression. The study demonstrated higher concordance rates for leprosy among monozygotic compared with dizygotic twins. ***(Chakravartti and***

***Vogel, ١٩٧٣)*** Various genes and regions in the human genome have been linked to or associated with susceptibility to leprosy per se or with a particular type of leprosy. These are not all reproducible in different populations, which may be unsurprising ***(Ciechanover, ٢٠٠٦).***

Mira et al.(٢٠٠٤) have identified a certain locus within the gene PARK٢\ PACRG which is located on chromosome ٦ and was identified to be associated with overall susceptibility of human populations to M. leprae ***(Mira et al., ).*** This is the first example of the use of positional cloning to identify a human gene associated with susceptibility to an infectious disease ***(Skamene., ).*** In their initial association scan of a linkage peak identified through a genome scan of a Vietnamese patient population, the investigators identified a locus within this gene that was highly associated with leprosy (leprosy per se), regardless of the subtype of this disease. These results were confirmed by a second analysis of Brazilian families with one or more persons affected by leprosy. The specific locus is a promoter region of PARK٢ and a coregulated gene, PACRG, located on chromosome ٦q٢٥-q٢٧. PARKIN was named for its association with an early-onset form of Parkinson's disease, and the finding that a locus within this gene is also associated with susceptibility to leprosy is very unexpected ***(Liu et al., ٢٠٠٦)***.