

LEPROSY: DISEASE PREVAILING FROM PAST TO PRESENT

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ABSTRACT

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, an acid-fast, rod-shaped bacilli. The newest research suggests that atleast as early as 4000 B.C. individuals had been infected with *M. leprae*, while the first known written reference to the disease was found on Egyptian papyrus in about 1550 B.C. The disease was well recognized in ancient China, Egypt, and India. At present many countries are in a way to achieve "world without leprosy" before 2105. India records the highest number of new leprosy cases in the world. The latest figures estimated by WHO states that out of around 35% of new leprosy cases in India, 48,000 are women and 13,610 children newly detected with leprosy. Skin, nose, blood are the potential reservoirs for growth of *M. leprae*, from the reservoirs *M. leprae* was thrashed forward to the extremity. Though leprosy can be treated by MDT, the formed deformities remains and prevention with vaccine can be beneficial. This review article on leprosy enlightens the existence of *M. leprae*, requirements for nourishment of *M. leprae*, core causes for deformities, effectiveness of treatment regimens, prevention of leprosy and antioxidant status in leprosy patients.

Keywords: Aerosols, Cardinal features, Drug resistance, Free radicals, Nasal stuffiness.

INTRODUCTION

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, an acid-fast, rod-shaped bacillus. *M. leprae* was discovered by Norwegian physician, Gerhard Henrik Armauer Hansen in 1873, it was the first bacterium to be identified as causing disease in man¹. Leprosy has long latent, induction period, usually three to six years in the tuberculoid form of disease and three to ten years in the multi bacillary form. Its transmission rate greatly exceeds the clinical attack rate.

1. Microbiology of *M. leprae*

In size and shape it closely resembles the tubercle bacillus. It occurs in large numbers in the lesions of lepromatous leprosy, chiefly in masses within the lepra cells, often grouped together like bundles of cigars or arranged in a palisade. Most striking are the intracellular and extra-cellular masses, known as globi, which consist of clumps of bacilli in capsular material. It is believed that only leprosy bacilli which stain evenly or uniformly with carbol-fuchsin are viable solid acid-fast rods and that bacilli which stain irregularly are probably dead and degenerating. The differences are

valuable pointers in biopsy specimens to the effects of treatment.



Fig. 1: Microscopical view of Mycobacterium leprae

2. Cardinal features of leprosy

a) Paucibacillary patches

- Hypopigmentation.
- Anaesthesia (Loss of sensation).
- Dry (Loss of sweating).
- Loss of hair growth (Not scalp hair).
- Macular/ Elevated/ Erythematous.

b) Multibacillary patches

- Nasal stuffiness: Difficulty in breathing, Musty repulsive odor, Epistaxis (nasal bleeding).
- Nasal bridge collapse (Advanced cases).
- Involvement of peripheral nerves.
- Upper palate perforation.
- Scanty beard/ moustache.
- Loss of eyebrows (Madarosis).
- Lagophthalmos (unilateral/bilateral).
- Gynecomastia.

Nose forms one of the potential reservoirs of *M. leprae* from where the *M. leprae* dissipate to the atmosphere and transmits infection to the close contacts while the MB patient is talking, coughing, sneezing and nose-blowing. The first symptom normally noticed in the advanced cases of leprosy is the nasal stuffiness as in them the nostrils may be packed with the plugs, clots of debris and *M. leprae*. Such patients find it difficult to breathe. *M. leprae* that lodge in the nose releases a variety of autacoids which are responsible for the emission of intolerable foul smell, upper palate perforation, and inflammation of the nose. During inflammation the blood vessels get dilated so the *M. leprae*, depending upon their size, can penetrate beneath the skin to reach different parts of the body from the nose to various peripheral sites. *M. leprae* are nonmotile organisms but are thrusted forward

from the site of higher density that is the nose to different parts of the body, will reach the peripheral sites like ear pinnae, fingers, toes and the scrotum in the male patients. Moreover *M. leprae* can enter into the eye through the naso-lacrima duct to cause irido cystitis, dryness of the eye, lagophthalmos (unilateral/bilateral) and ultimately blindness². The involvement of skin in lepromatous cases parallels the density of melanocytes (the cells that produce dopa) in the tissue. Melanocytes present not only in the skin but also in other areas such as the mucosa membrane of nose, the eyes and in the endothelium of the blood vessels. Unlike many other infectious diseases, in MB patients the *M. leprae* are found at many sites to which the blood circulation could be either poor (skin) or completely absent (nasal plugs). It is well established that the blood supply to the skin is only 7% of cardiac output³, which may be compromised further during winter or in cold countries.

Nerve damage in leprosy results from *M. leprae* invasion of Schwann cell of the peripheral nerves and is responsible for most of the deformity and disability of this disease. Surface proteins (ML-LBP21) of *M. leprae* cell wall that bind laminin-2 are crucial for bacterial targeting to the peripheral nerves^{4,5}. The degree of infection was greater in epineural tissues than in the intraneural compartment (i.e., Schwann cells) at all levels. The infection of nerves by *M. leprae* was associated with focal interstitial, mononuclear cell infiltration of involved nerves. Although patients can be cured of infection by multidrug therapy, the immunopathological sequelae responsible for the characteristic deformities of leprosy can continue during and even after antimicrobial therapy. Such therapeutic intervention has so far prevented only one third of infected individuals from suffering new disabilities. Blood forms yet another reservoir of *M. leprae* in MB patients. In leprosy, we have the most unusual situation where more than hundred thousand bacilli per millitre are found circulating in the blood. Many researchers demonstrated the presence of *M. leprae* in the blood, a condition called "Bacteraemia"⁶⁻¹⁰. Single or Multidrug do not seem to influence much the *M. leprae* in the blood^{11,12}.

c) Immune sites

The *M. leprae* can migrate from nose to toes beneath the skin, in the extracellular fluid but at certain sites their movement is either restricted or prevented by the hair roots, as in the case of scalp hair, axilla and groin, the so called immune sites or spares sites.

3. Classification of leprosy based on skin smears

Leprosy can be classified on the basis of clinical manifestations and skin smear results as:

- a) Patients showing negative smears at all sites are grouped as paucibacillary leprosy (PB).
- b) Patients showing positive smears at any site are grouped as multibacillary leprosy (MB).

WHO classification of Leprosy (in vogue)

- 1) Paucibacillary (PB)
 - Indeterminate.
 - Tuberculoid (1-5 patches).
- 2) Multibacillary (MB)
 - Tuberculoid (> 5 patches).
 - Lepromatus (LL) – Skin smears positive at all sites.

Borderline with skin smear positive to AAFB (BL).

4. Transmission of leprosy

Leprosy is known to occur at all ages ranging from early infancy to very old age.

a) Method of transmission of leprosy

The exact mechanism of transmission of leprosy is not known. The most widely held belief was that the disease was transmitted by contact between cases of leprosy and healthy persons. The possibility of transmission by the respiratory route is gaining ground. There are also other possibilities such as transmission through insects which cannot be completely ruled out. In addition, mother's milk may contain enormous number of *M. leprae* to be imbibed by the suckling infant, although the importance of this route is unknown.

b) Sex distribution

Although leprosy affects both sexes, in most parts of the world males are affected more frequently than females often in the ratio of 2:1. Women are more likely to delay the disease and to present with impairment¹³. Women are more likely to delay and to present with impairment. Women experience greater social impact than males.

c) The prevalence pool

The prevalence pool of leprosy in a population in general is in a constant flux resulting from inflow and outflow. The inflow is contributed by the occurrence of new cases, relapse of cured cases and immigration of cases. The outflow is mainly through cure or inactivation of cases, death of cases and emigration of cases. Of the various factors that influence the prevalence pool, the importance of inactivation of disease and mortality are less well recognized.

d) Reservoir of infection

The human being is the only known reservoir of infection in leprosy except for the fact that naturally occurring disease with organisms indistinguishable from *M. leprae* has also been detected among wild armadillos in parts of the southern United States. The epidemiological significance of the armadillo is generally considered to be negligible inspite of occasional cases reported among individuals giving history of handling armadillos. The occurrence of acid alcohol fast bacilli (AAFB) in the skin nasal mucosa of healthy subjects and blood have also been reported.

e) Portal of exit of *M. leprae*

The two portals of exit of *M. leprae* often described are the skin and the nasal mucosa. It is true that the lepromatous cases show large numbers of organisms in the dermis. Although there are reports of AAFB being found in the desquamating epithelium of the skin, AAFB cannot be seen in the epidermis even after examining a very large number of specimens from patients and their contacts.

f) Viability of *M. leprae* outside the human host

The possibility of discharge of *M. leprae* from the nasal mucosa raises the question of survival of the discharged organisms outside the human host. *M. leprae* from the nasal secretions can survive up to 36 hours or more and upto nine days under tropical conditions¹⁴. Such survival of the organisms suggests the possibility of contaminated clothing and other fomites acting as sources of infection.

g) Portal of entry of *M. leprae*

The portal of entry of *M. leprae* into the human body is not definitely known. The two portals of entry seriously considered are the skin and the upper respiratory tract. With regard to the respiratory route of entry of *M. leprae*, the evidence in its favour is on the increase inspite of the long-held belief that the skin was the exclusive portal of entry. Experimental transmission of leprosy through aerosols containing *M. leprae* in immune-suppressed mice, suggesting a similar possibility in humans. Successful results have also been reported on experiments with nude mice when *M. leprae* were introduced into the nasal cavity through topical application.

h) Sub-clinical infection in leprosy

Inspite of the fact that as yet there is no simple immunological test to identify sub-clinical infection with sufficient specificity and sensitivity. The *in-vitro* tests for cell-mediated

immunity (CMI) such as the lymphocyte transformation test (LTT) and serological tests for detecting humoral antibodies such as phenolic glycolipid I-based ELISA can be used. Lymphocyte transformation test values to *M. leprae* antigens are produced strongly in patients with lepromatous leprosy and production was low in patients with tuberculoid leprosy. More sophisticated immunological studies such as those on cytokines production and toll like receptors which again show that T-cell activation is high in tuberculoid end of spectrum and low at lepromatous end. *M. leprae* activates TLR2 and TLR1, which are found on the surface of Schwann cells, especially with tuberculoid leprosy. Although this cell-mediated immune defense is most active in mild forms of leprosy, it is also likely responsible for the activation of apoptosis genes and, consequently, the hastened onset of nerve damage found in persons with mild disease¹⁵.

In addition to the above, skin tests with various preparations of lepromin, and more recently with soluble antigens from *M. leprae*, have also provided useful information on the occurrence of sub-clinical infection, using a soluble skin test antigen prepared by the Convit method, have found that skin test positivity. In India no difference was seen in the distribution of skin test reactions to soluble antigens among cases, contacts and general population.

i) Transmission by contact

Individuals who are in close association or proximity with leprosy patients have a greater chance of acquiring the disease. The possibility of transmission of leprosy through the respiratory route is gaining increasing attention in recent years.

The possibility of transmission through the respiratory route is based on

- (a) The inability of the organisms to be found on the surface of the skin.
- (b) The demonstration of a large number of organisms in the nasal discharge.
- (c) The high proportion of morphologically intact bacilli in the nasal secretions.
- (d) The evidence that *M. leprae* could survive outside the human host for several hours or days.

The transmission involved some kind of 'inunction' or rubbing in of the organisms from the skin of affected persons into the skin of healthy subjects.

a) Diagnostic Tests

Positive skin smears

Lepromin test often is erroneously considered a diagnostic test for Hansen's disease, in light of WHO observation that the skin smears procedure is riddled with the problems; it is poor in quality, risky and expensive.

Based on the extent of distribution of *M. leprae* at different sites in the skin, the duration of disease perhaps can be assessed. Thus when a fresh MB patient has the bacilli only in the ear lobe and forehead or chin and nowhere else in the body, he can be considered as one whose disease was recently contracted, and the one who has the bacilli in the skin all over the body can be considered to have the disease for considerable span of the time. Skin smears should be taken atleast at six different sites in MB patients for proper evaluation¹⁶.

b) Bacteriological examination

Two indices which depend on observation of *M. leprae* in smears from skin or nasal smears are useful in assessing the amount of infection, and the viability of the organisms and also the progress of the patient under treatment. They are the morphological index and the bacteriological index.

The bacteriological index (BI)

This is an expression of the extent of bacterial loads. It is calculated by counting six to eight stained smears under the 100 x oil immersion lens in a smear made by nicking the skin with a sharp scalpel and scraping it; the fluid and tissue obtained are spread fairly thickly on a slide and stained by the Ziehl-Neelsen method and decolorized (but not completely) with 1% acid alcohol. The results are expressed on a logarithmic scale.

The bacteriological index is valuable because it is simple and is representative of many lesions but is affected by the depth of the skin incision, the thoroughness of the scrape and the thickness of the film. A more accurate and reliable index of the bacillary content of a lesion is given by the logarithmic index of biopsies (LIB). These indices help to assess the state of patients at the beginning of treatment and to assess progress.

5. Diagnosis of leprosy

The morphological index (MI)

This is calculated by counting the numbers of solid-staining acid-fast rods. Only the solid-staining bacilli are viable. It is not unusual for solid-staining *M. leprae* to reappear for short periods in patients being successfully treated with drugs. It is important to recognize that measurement of MI is liable for observer variations.

c) Skin slit smears method

- Slit skin smears; 5 mm long and 2-3 mm deep.
- Stained with Carbol Fuschin by method of Zeihl-Neelsen.
- Bacteriological Index (BI): Density of leprosy bacilli in smears; include both living and dead bacilli.
- Morphological Index (MI): Percentage of living bacilli in smears. Higher the MI, greater the infectivity.

6. Treatment of leprosy

For purposes of treatment, leprosy is divided into two types:

a) Paucibacillary (PB) leprosy : 1-5 skin lesions – Regimen of two drugs – Rifampicin and Dapsone for 6 months

b) Multibacillary (MB) leprosy: >5 skin lesions – Regimen of three drugs – Rifampicin, Clofazimine and Dapsone for 12 months.

WHO provides MDT drugs free of charge to all endemic countries since 1995. From 1995-2000, the free supply was sponsored by The Nippon Foundation of Japan and from 2000 to date by the Novartis Foundation for Sustainable Development. Novartis has assured the continuation of the free supply until 2010, through WHO.

For the economical treatment of PB patients when a tiny tuberculoid patch was observed on the skin it can be well treated with local Rifampicin and Clofazimine Ointment . The *M. leprae* that lodge in the nose can be treated with Rifampicin 1% nasal drops. Neuritis due to myelin sheath degradation of nerves can be treated with neem oil.

There are several effective chemotherapeutic agents against *M. leprae*. Dapsone (diaphenylsulfone, DDS), Rifampicin (RFP), Clofazimine (CLF), Ofloxacin (OFLX) and Minocycline (MINO) constitute the backbone of the multidrug therapy (MDT) regimen recommended by WHO. Other chemotherapeutic agents, like Levofloxacin (LVFX), Sparfloxacin (SPFX), and Clarithromycin (CAM) are also effective against *M. leprae*^{17,18}.

a) Rifampicin (RFP)

Rifampicin is highly bactericidal against *M. leprae* by inhibiting bacterial RNA synthesis and blocks RNA transcription by binding to the beta subunit of DNA dependent RNA polymerase, a single dose of 600 mg of RFP is capable of killing 99.9% or more of viable organisms and should not produce significant toxic effects. Currently rifampicin is undergoing trials with new regimen. Rifabutin is seldom used against *M. leprae* whilst trials with rifapentine in MDT regime are being carried out.

b) Clofazimine (CLF)

CLF binds to mycobacterial DNA inhibits both mycobacterial growth and exerts a slow bactericidal effect on *M. leprae*¹⁹. Because CLF is a repository drug, stored in the body after administration and slowly excreted, it is given as a loading dose of 300 mg once a month to ensure that the optimal amount of CLF is maintained in the body tissue. Patients starting the MDT regimen for MB leprosy should be informed of side effects including brownish black discoloration (coppery) and dryness of skin. These usually disappear within a few months of treatment suspension. It is used to treat dapsone resistant leprosy and erythema nodosum leprosum reactions. There is no well-documented resistance against *M. leprae* hence it is a safe drug for monotherapy in special circumstances.

c) Ofloxacin (OFLX)

OFLX, a synthetic fluoroquinolone, acts as a specific inhibitor of bacterial DNA gyrase and has shown efficiency in the treatment of *M. leprae*²⁰. Ofloxacin is a bactericidal drug for *M. leprae* and a good alternative to rifampicin. A combination regime for four months with ofloxacin, rifampicin, dapsone and clofazimine was tried out in leprosy cases in Sabah. WHO is conducting trials with regimes elsewhere with ofloxacin. Moxifloxacin is a more potent and safer drug than ofloxacin and are undergoing trials in combination regimes.

d) Minocycline (MINO)

Minocycline (MINO) is a semisynthetic tetracycline²¹. Minocycline inhibits bacterial protein synthesis by binding into 30s and 50s ribosomal subunits of susceptible bacteria. However, from the curative and cost-effectiveness points of view, the WHO-recommended, time-honored MDT remains to date the best combination regimen of the worldwide leprosy-control programs.

e) Clarithromycin

It is a macrolide antibiotic. It exerts antibacterial action by binding to 50s ribosomal subunits resulting in inhibition of protein synthesis. Clarithromycin has been found - to be effective against *M. leprae*.

f) Diaminodiphenylsulfone (DDS, dapsone)

Dapsone is bacteriostatic or weakly bactericidal against *M. leprae*²², its use in combination with other drugs has become essential to slow or prevent the development of resistance. Patients known to be allergic to any of the sulpha drugs should be spared dapsone. Anemia, hemolysis and methemoglobinemia may develop but are more significant in patients deficient for glucose-6-phosphodihydrogenase (G6PD). Dapsone was used as monotherapy and later as a standard drug in the MDT regimen. Both secondary and primary resistance has been reported from Sungei Buloh making them no longer safe to be administered as monotherapy. Acedapsone has been used in the past for chemoprophylaxis.

According to various researchers, a new regimen consisting of rifampicin 600mg + Sparfloxacin 200mg + Minocycline 100mg + Clarithromycin 500mg can be an effective, safe and acceptable with minimal side effects. This regimen also reduces the channels of development of drug resistance.

g) MDT and Drug-resistance

Resistance of *M. leprae* to existing major anti-leprosy drugs has been worldwide reported. The magnitude of which was selective. Actually resistance to dapsone was the most reported. Subsequently regimens of the MDT were designed on the principle that they would be effective against all the strains of *M. leprae* regardless of their susceptibility to dapsone. Recently, genetic profiles of drug-resistant strains have been elucidated.

h) Leprosy reaction and its treatment

In patients receiving standard multidrug therapy (MDT), a very high proportion of bacilli are killed within days, which suggests that many of the manifestations of leprosy, including reactions of the erythema nodosum type, which follow initial treatment, must be due in part to antigens from dead organisms rather than living bacilli.

Type 1 reaction

Under treatment, there is a rapid increase in cell mediated immunity (CMI), with a proliferation of "T-Type" lymphocytes, hence reaction can also be called as reaction as "Up-grading" or "Reversal" reaction. PB and MB

cases develop type 1 reaction (reverse reaction: RR), Reversal Reaction is most likely to happen early, during the first six months of treatment.

Type 2 reaction

Erythema nodosum leprosum ENL occurs in MB (LL type) patients first occurs later in the course of treatment²³. In relation to Lepra Reaction, it is important to remember that the two types of Immunity are involved - "Humoral Antibody Response" and "Cell Mediated Immunity".

In MB leprosy patients on MDT a reduction in the frequency and severity of ENL may occur. These may be attributable to the anti-inflammatory effect of CLF. On the other hand, a temporary increase in the reversal reactions (type 1) has been noted in MB leprosy patients in their first year of MDT.

Occasionally, they may appear even on the palms and soles of the feet. Some of these ENL lesions may rupture and become necrotic. "Type 2" reaction may also be accompanied by severe eye problems, especially Iritis which may be confused with conjunctivitis. The testes (cooler) may be swollen and extremely tender. There may be softening of the bones, particularly in hands, feet and tibiae. Muscle pain (Myositis) and swelling of the joints give the impression of severe rheumatism. These reactions respond satisfactorily to prednisolone along with thalidomide or CLF.

i) MDT and HIV, Pregnancy and TB

Existing data has shown that the response to MDT by leprosy patients infected with HIV has been similar to that of all other leprosy patients²⁴. Hence, HIV infection in leprosy patients is not a contraindication for MDT. Leprosy management remains the same as in non-HIV-infected leprosy patients. Experts say that AIDS drugs cause the immune system to recover. It then generates new white blood cells that carry the bacterium from old, silent leprosy infections to the skin of the face, hands and feet and leads to immune reconstructive inflammatory syndrome.

It is established that pregnancy exacerbates leprosy. Fortunately, MDT during pregnancy appears to be safe; no contraindications have been established currently²⁵. CLF is excreted through breast milk and can cause mild discoloration of the infant. MDT is not contraindicated in patients suffering from tuberculosis. However, because WHO's MDT for leprosy is not the ideal treatment for tuberculosis, an appropriate antitubercular regimen should be added to the antileprosy MDT in patients in whom the two diagnoses

are confirmed. If daily RFP is part of the antituberculosis treatment, there is no need to administer monthly RFP as part of the leprosy MDT.

7. Vaccination

The word 'vaccine' is derived from the French 'la vacche' meaning the cow, a reference to the cowpox extract used by Jenner in 1812 to prevent smallpox in humans. A mixed vaccine containing BCG and ICRC bacilli or their immunogenic 'sub-units' could be the future polyvalent mycobacterial vaccine that might offer protection against a wide spectrum of mycobacterial diseases. Such a polyvalent mycobacterial vaccine would reduce the number of vaccinations and thus would be of tremendous operational advantage to health authorities especially in the Third World. Vaccination against the leprosy bacillus may be considered. BCG vaccination is reported to be partially effective for protection against leprosy²⁶. Worldwide BCG vaccination program against *M. leprae* is not economically feasible; a cost-effective DNA vaccine could become a promising substitute²⁷.

8. Status of free radicals in leprosy patients

Oxidative stress is a condition associated with an increased rate of cellular damage induced by the oxygen derived oxidants commonly known as reactive oxygen species (ROS). Imbalance between production of oxygen free radicals and anti-oxidant defenses can result in oxidative stress leading to metabolic anti-oxidants such as glutathione, ascorbate or α -tocopherol or due to deficiency of anti-oxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase and or from increased levels of oxygen free radicals (OFRs) cause glutathione depletion, lipid peroxidation, membrane damage, DNA damage, activation of enzymes like proteases, nucleases etc. Any one or more of these biochemical changes could result in a clinical disorder. Reactive oxygen intermediates play significant role in leprosy. The reactive species like nitric oxide (NO^-) and peroxynitrite (ONOO^-) that are produced by macrophages in the skin lesions are shown to be involved in nerve damage in borderline leprosy. The microbicidal ability of phagocytes through reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-) and hydroxyl radical (OH^-) is a basic defence mechanism of the human host against microbial infection. Such ROS could also play a significant role in an infection with *M. leprae* in leprosy. The ROS can diffuse from the site of generation and damage the structural and

functional integrity of cells causing tissue damage. Thus, the oxidant force that kills pathogens is also cytotoxic to the host tissue. The possible role of ROS in the causation of renal damage in experimental leprosy has been investigated in mice infected with *M. leprae*.

The peroxidations of lipid component of the cells by ROS generate toxic species like lipid peroxides, lipid hydroperoxides and aldehyde breakdown products. Antioxidant systems exist in the body to protect against ROS toxicity. Super oxide dismutase (SOD), one of the important intracellular antioxidant enzymes present in aerobic cells has an antitoxic effect against superoxide anion. The presence of SOD in various fractions such as cytosol (Cupro zinc-SOD), mitochondria (Mangano SOD) and plasma (Cupro-SOD) enables SOD to eliminate super oxide radicals immediately and protect cells from accumulation of H_2O_2 by decomposing it to H_2O and O_2 .

9. Cultivation of *M. Leprae in-vitro*

Mycobacterium leprae the causative agent for leprosy belongs to the family of *mycobacteriaceae* and is an acid fast rod that cannot be cultured on an artificial media or tissue culture. However it can be consistently propagated in the footpads of mice. The bacillus divides exceedingly slow with an estimated optimal doubling time of 11 to 13 days during the logarithmic growth phase in mouse foot pads. The mouse model has been used extensively in the Sungai Buloh Leprosy Research Unit for the study of anti-leprosy drugs. The high bacterial yield from armadillos has been crucial for immunological studies in Carville, USA. More recently the nude mice have been used to obtain significant quantities of physiologically active *M. leprae* for genetic and physiological studies. Recent sequencing of *M. leprae* genome revealed it has lost one third of the genes possessed by *M. tuberculosis* and is well adapted for growth in humans. Nose contains some nutrients which favour the *M. leprae* to flourish and multiply at that site. So a natural medium containing nasal secretions can be used for growing *M. leprae*. It was found that *M. leprae* were multiplying in this medium²⁸.

10. Association between leprosy and genetic markers

The involvement of hereditary factor in susceptibility of leprosy patients is not new. It is supported by the well known facts that

- Disease fail to manifest in the majority of exposed subjects even when they

had been submitted to prolonged intimate contact.

- The risk of leprosy being contracted by the relatives of index cases increases according their degree of consanguinity.
- Environmental changes are not capable of changing one form of leprosy to another.

So new approaches are made by the genetists to approach the problem.

The frequency of RH negative factor is more in leprosy patients than general population. Based on the study on Negroids most of 'O' blood group population are prone to tuberculoid leprosy than lepromatous leprosy. It may reflect geographical variation in blood groups.

Leprosy and Glucose-6-phosphate dehydrogenase deficiency (G6PD)

The common genetic variations that occur in G6PD in human erythrocytes result in mutation of X-chromosome. Males with enzyme deficient gene will disclose the trait, but homozygous females will have low G6PD levels, but it is harmless. Lepromatous patients may be prone to exhibit haemolytic anaemia when treated with sulfones. Haptoglobulins, Transferrins and leprosy. There is no significant variation in haptoglobulins and transferrins in leprosy patients.

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