Recent Advancements in the Treatment of Leprosy

Article *in* Current topics in medicinal chemistry · October 2018

DOI: 10.2174/1568026618666181025100434

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REVIEW ARTICLE

Recent Advancement in the Diagnosis and Treatment of Leprosy

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ARTICLE HISTORY

Received: May 20, 2018
Revised: October 11, 2018
Accepted: October 12, 2018

DOI:
10.2174/1568026618666181025100434

Abstract: ***Background*:** Many of the tropical diseases are neglected by the researchers and medicinal companies due to lack of profit and other interests. The Drugs for Neglected Diseases Initiative (DNDi) is established to overcome the problems associated with these neglected diseases. According to a report published by the WHO, leprosy (Hansen's disease) is also a neglected infectious disease.

***Methods*:** A negligible amount of advancements has been made in last few decades which includes the tools of diagnosis, causes, treatment, and genetic studies of the bacterium (*Mycobacterium leprae*) that causes leprosy. The diagnosis of leprosy at earlier stages is important for its effective treatment. Recent studies on Vitamin D and its receptors make leprosy diagnosis easier at earlier stages. Skin biopsies and qPCR are the other tools to identify the disease at its initial stages.

***Results*:** Until now a specific drug for the treatment of leprosy is not available, therefore, multi-drug therapy (MDT) is used, which is hazardous to health. Besides *Mycobacterium leprae*, recently a new bacterium *Mycobacterium lepromatosis* was also identified as a cause of leprosy. During the last few years the genetic studies of *Mycobacterium leprae*, the role of vitamin D and vitamin D receptors (VDR), and the skin biopsies made the treatment and diagnosis of leprosy easier at early stages. The studies of micro RNAs (miRNAs) made it easy to differentiate leprosy from other diseases especially from tuberculosis.

***Conclusion*:** Leprosy can be distinguished from sarcoidosis by quantitative study of reticulin fibers present in skin. The treatment used until now for leprosy is multi-drug treatment. The complete genome identification of *Mycobacterium leprae* makes the research easy to develop target specified drugs for leprosy. Rifampicin, identified as a potent drug, along with other drugs in uniform multi-drug treatment, has a significant effect when given to leprosy patients at initial stages. These are effective treatments but a specific drug for leprosy is still needed to be identified. The current review highlights the use of modern methods for the identification of leprosy at its earlier stages and the effective use of drugs alone as well as in combination.

Keywords: Leprosy, *Mycobacterium leprae*, *Mycobacterium lepromatosis*, neglected disease, Hansen's disease.

1. INTRODUCTION

Neglected diseases are the diseases that are ignored by the researchers or the medicine companies due to many reasons. The main reason of ignorance is the financial status of different regions. Therefore, most of the neglected diseases prevail in under developed countries [1]. Neglected tropical diseases are the diseases that are found within a tropical belt. From 1975-2004, only 21 drugs (1.3%) were synthesized for neglected tropical diseases [2]. However, a new strategy was required to solve the problem of such diseases that are limited to tropical countries, are of no tactical or military concern to rich countries, and are overlooked by markets or patients' organizations due to lack of interest and incapability of attracting the politicians' notice [3]. An all new non-profit organization - The Drugs for Neglected Diseases Initiative (DNDi) was established, in order to make correction in the ongoing imbalance for diseases, which relies on the development of new drugs for patients suffering from neglected diseases [4]. This formula was presented by DNDi to resolve the matter [5]. It is not that these diseases are totally forgotten and there is no hope left for the patients suffering from neglected diseases. During the last few years several attempts were made on research and development in order to resolve the problem of neglected diseases [6].

Another program for neglected diseases named as Special Program for Research and Training in Tropical Diseases (TDR) was initiated and financed by World Health Organization (WHO), the World Bank, and the United Nations De-

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1568-0266/18 $58.00+.00

velopment Program (UNDP) [7]. This program made a good progress with several important achievements in its fight against vector borne malarial and leishmanial diseases [8]. Considering the poorer or underdeveloped countries for their health issues, on average, over 350 million people were reported to be suffering from neglected diseases [9]. At present, the available treatments are insufficient and to some extent are fictional, therefore, new remedies and solutions are required immediately [10]. In this regard, DNDi is trying to do as much work as possible to facilitate the neglected populations by utilizing the advancements being carried out in the field of science that have benefited the wealthy nations in providing health and comfort [1]. The WHO made a list of tropical neglected diseases (Fig. **1**) which need an urgent attention for their prevention [11]. The neglected diseases are ranked as type I, II, and III by the WHO. The international organization *Medecins Sans Frontieres* (MSF) relates this classification as global, neglected, and most neglected diseases in its vocabulary [12]. The type I/global diseases are not confined to a particular region around the world, however, type II-III/ neglected-most neglected are absolutely frequent among developing countries [13]. Being widespread in underdeveloped or poor regions, the type II and III diseases (hereinafter "neglected diseases") are not of any interest for pharmaceutical or biotechnology industries, therefore, there is a huge lack of vaccines, drugs, and detection methods or kits for such diseases [14].

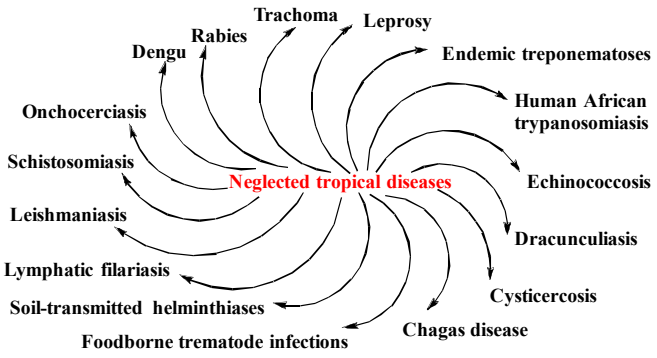

Fig. (1). Neglected tropical diseases in the world today.

1.1. Leprosy

In 2008, 213,000 cases were reported for leprosy, among which only 17 countries reported more than 1000 new cases, accounting for 94% of the new cases detected globally [11]. According to a 2010 report, out of 122 countries, considered as native to leprosy, 119 have been successful in eliminating the disease as a public-health problem (defined as a problem attaining a frequency of less than 1 case in a population of 10,000 people) [15]. Leprosy disease arises due to gradual growth of the bacterial infection caused by *Mycobacterium leprae* [16]. The main target areas of this disease are the skin, peripheral nerves, respiratory tract, eyes, and other vital organs. It develops through granulomas of the nerves. Leprosy is not restricted to a particular age or sex [17]. The treatment for leprosy was suggested and prescribed by a WHO study group in 1981 as a chemotherapy for the disease. The treatment is a multi-drug therapy which relies on combination of 3 drugs – rifampicin, dapsone, and clofazimine [18]. Multi-drug therapy was successful for the treatment of leprosy instead of mono-drug therapy that was based on dapsone only. The multi-drug therapy helps to inhibit the development of any disabilities and acts as an early remedy; it also suppresses the development of drug resistance [11]. When left unattended and untreated, leprosy leads to persistent damage to skin, nerves, eyes and complete deterioration of limbs in extreme cases.

For several centuries, the people affected by leprosy were considered as plagued and were disgraced, stigmatized, discriminated and banished from the locality [16]. Despite control programs, the disease still prevails in various parts of the world. The WHO report (Table **1**) was alarming for a possible outbreak of the disease in the developed world [17]. Kamath *et al.* investigated the cases of leprosy in the U.S. in 2014. They observed 3 types of leprosy reactions in their study. Type 1 (reversal reaction), type 2 (erythema nodosum leprosum) and type 3 (Lucio phenomenon) were found responsible for long life disability. At present, there are no treatments to reduce long lasting disability due to leprosy [19]. Various types of leprosy are classified according to bacteriological index and skin-smear positivity (Fig. **2**) [18].

2. RECENT ADVANCEMENTS

2.1. Advancements in the Diagnosis of Leprosy

Information, Education, and Communication (IEC) program, along with socio-economic rehabilitation designs, proposed different procedures for leprosy diagnosis in 2014. According to IEC, different methods for diagnosis were used in which specific area for activity was targeted and compared for results with other activities [20]. Duthie *et al.* carried out a research using recombinant proteins of *M. leprae* for T-cell responses and observed that several antigens were immunogenic, and leprosy specified. The overall result of the research concluded that several antigens were potent candidates that could be useful in future either for diagnosis or even vaccination against leprosy [21]. Bochud *et al.* aimed at studying the effect of Toll-like receptor 2 (TLR-2) responses against leprosy. Three types of polymorphism in TLR-2 were analyzed for leprosy patients and a group of people under the controlled disease. Results of the study revealed that both microsatellite and the 597C→T polymorphisms affected the susceptibility to reversal reaction and its occurrence, and had the ability to bring new information regarding immunogenetics related to leprosy [22]. Oca *et al.* determined the interconnection between leprosy and 3 single nucleotide polymorphisms (SNPs) in β-defensin 1 gene (DEFB1). The results concluded that DEFB1 can be used for earlier detection and as marker for lepromatous leprosy (L-lep), and also could be useful in designing new alternative cures against leprosy [23]. Elias *et al.* investigated the applicability of ulnar nerve sonography in leprosy using electrophysiological correlation. A total of 21 infected and 20 control patients were analyzed by sonography and a conclusion was drawn that both sonography and electrophysiology were compatible for the identification of leprosy [24]. Geluk *et al.* analyzed the *M. leprae* antigens for their potential in inducing cytokine secretions using peripheral blood mononuclear cells from leprosy infected patients. T-cell responses specified to leprosy and healthy close contacts were analyzed for ML2283- and ML0126-derived peptides, showing that *M.*

Table 1. The WHO report on annually detected leprosy cases (excluding the European regions) 2005-2009.

S. No.	WHO region	Annually detected cases of leprosy				
		2005	**2006**	**2007**	**2008**	**2009**
1	African	45179	34480	34468	29814	28935
2	America	41952	47612	42135	41891	40474
3	South-East Asia	201635	174118	171576	167505	166115
4	Eastern Mediterranean	3133	3261	4091	3938	4029
5	Western Pacific	7137	6190	5863	5859	5243
	Total	**299036**	**265661**	**258133**	**249007**	**244796**

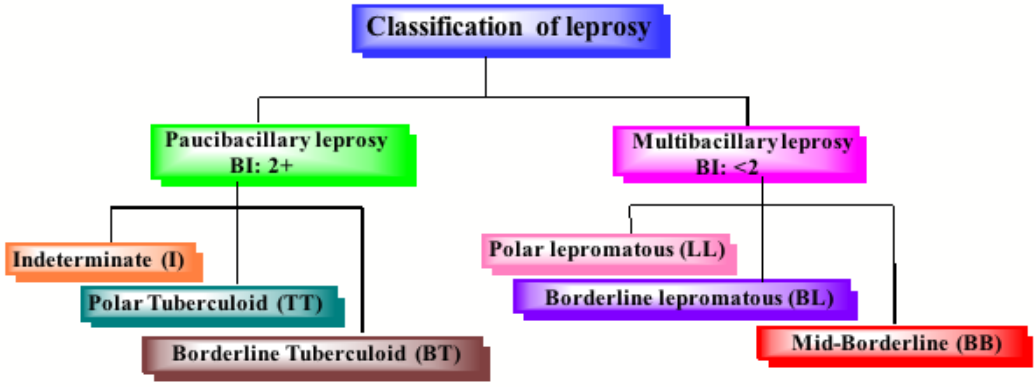

Fig. (2). Classification of leprosy according to bacteriological index and skin-smear positivity.

leprae peptides are potential candidates for diagnosis [25]. de Messias *et al.* evaluated the polymorphisms of gene encoding ficolin-2 (FCN2) - a soluble pattern recognition molecule. Results showed that the administration of functional FCN2 haplotypes was significantly different for infected and control leprosy subjects. It was concluded that FCN2 plays an immunogenetic role in the host against *M. leprae* [26]. Schuring *et al.* explored the connection between polymorphism of TLR1 N248S and its liability to leprosy. The study showed that TLR1 N248S lowers TLR1 signals and the following leprosy disease [27]. Duthie *et al.* inspected the development of antibodies within armadillos by infecting them with *M. leprae* to identify the antigen-specific immunoglobulin. The results discovered that several antigens are capable of early diagnosis of *M. leprae* and their combination can help in accurate diagnosis of leprosy [28]. Geluk *et al.* investigated new biomarkers for leprosy recognition. They determined cytokines generated by *M. leprae* proteins in blood samples of infected and endemic controls (EC) of leprosy from Bangladesh, Brazil, Ethopia, and South Korea. The study was successful in identification of *M. leprae*-unique Ags, specifically ML2478, as biomarker using IFN-g or IFN-induced protein-10 [29]. Kumar *et al.* also investigated the BL/LL cases in 2013. In their study, T-reg cells were found responsible for leprosy. A mechanism for observing the behavior of these cells was suggested. TGF was initiated by increasing phosphorylation-mediated-nuclear-import of SMAD3 and NFAT that help for FoxP3, responsible for the production of CD4+CD25+IL-10+sub class of T cells [30]. Lini *et al.* developed the assay based on real-time PCR for quantifying the number of bacterial DNA copied and hsp18 mRNA from patients employing paraffin-embedded biopsy samples. The approach was applied for monitoring the chemotherapy of leprosy. The results of the study revealed that real-time PCR could be a preferable technique for monitoring bacillary DNA and mRNA in lesions for a better diagnosis and treatment of the disease [31].

2.1.1. Diagnosis by Skin

Lima *et al.* presented a new leprosy diagnosis method through the detection of lipid markers from skin by using silica plates. The silica plates were gently pressed with the skin of affected and healthy people and the printed silica plates were analyzed by HR-ESI-MS. The results confirmed mycobacterial mycolic and apoptotic elements as markers for leprosy patients, and gangliosides and phospholipids as markers for healthy skin [32]. Iyer *et al.* examined the interconnection between leprosy and chitotriosidase in serum as well as *in situ* in biopsies of skin lesions of the patients. According to their findings, the serum chitotriosidase activity was related with MB leprosy and it could be potentially used for monitoring the therapy against erythema nodosum leprosum (ENL) reactions as well as for distinguishing between MB and PB leprosy [33]. Mathur *et al.* conducted a research for interdependence of histopathological and clinical diagnosis of leprosy via hospital-based studies. Skin biopsies

of infected patients stained with Hematoxylin and Eosin, and modified Fite-Ferraco were used for recognition of *Mycobacterium leprae* of 156 patients. The overall results indicated that clinical and histopathological diagnosis were in conjunction for 115 cases with maximum correlation of 95.2% in LL patients. Skin biopsy was confirmed as a most successful tool for the confirmation of leprosy [34]. Otsuka *et al.* examined other symptoms of leprosy by comparison of LL and BL cases. A specific inflammation caused by receptor CCR3 that produced Eotaxin 1 and 3 was observed. The inflammation appeared on the patient's skin containing anticoagulant heparin [35].

2.1.2. Role of Vitamin D

Mandel *et al.* reported the role of Vitamin D and Vitamin D receptor in leprosy patients and stated that most of the patients of leprosy have low level of Vitamin D and Vitamin D receptor. The bacilli index range of these patients was within +3 and +5, hence by using this information the severity of leprosy progression can be determined [36]. Neela *et al.* studied the relationship of leprosy with three Vitamin D receptors. They analyzed TaqI rs731236, FokI rs2228570, and ApaI rs7975232 of 222 leprosy patients and 182 healthy controls. All the three VDR genes showed positive relationship with leprosy [37].

2.1.3. Differentiation with Other Infectious Diseases

Vieira *et al.* reported that the regulatory T-cells play a vital role in the reactions of ML. They studied frequency of regulatory and *in situ* T-cells in type 1 and type 2 reactions and reported that T1R patients and controls have higher number of regulatory and in *situ* T-cells than T2R patients. Their study provides support to the hypothesis that decrease in T-cells increases the T-helper-17 cells response [38]. As micro RNAs (miRNAs) are biomarkers for different infectious diseases, Jorge *et al.* analyzed 377 miRNAs of TB and leprosy patients. Their analysis on TaqMan low-density array (TLDA) and transcription-quantitative PCR (qRT-PCR) along with the miRNAs of healthy controls revealed 4 miRNAs by which leprosy patients can be identified from normal and TB patients [39]. Utino *et al.* recently performed a study to differentiate two diseases (tuberculoid leprosy (TL) and sarcoidosis). They studied skin of 33 people having TL and 24 having sarcoidosis and reported that patients having sarcoidosis have high number of reticulin fibers than the patients having TL [40]. Bührer-Sékula *et al.* studied the merits of using ML flow test for classifying different types of leprosy. They concluded that ML flow test can be useful for field studies under unavailability of professional dermatologists. Compared to other classification methods having limitations, ML flow test was robust, efficient, and reliable [41]. It is very important, in controlling and eliminating leprosy, to diagnose the disease at initial stages using selective and sensitive tools. Although, some clinics perform serological tests, that proved helpful in detecting MB in patients, but the early stage diagnosis is not possible because only few laboratories have facilities of clinical tests for leprosy [42]. Lobato *et al.* compared 2 immunological (PGL-1, ND-O-HAS ELISA) and one lateral flow (ML flow) tests for leprosy diagnosis taking 152 infected patients, 191 close contacts, and 52 healthy volunteers. The results showed that ELISA tests were both sensitive and selective with 68.83% sensitive and 98% selective, however the ML flow assay did not show appreciable results in this perspective. The ML flow test was efficient for differentiation of pausibacillary (PB) and multibacillary (MB) forms of leprosy [43].

2.1.4. Mycobacterium lepromatosis

A new specie *Mycobacterium lepromatosis,* which could produce adult Schwann cells during lipid metabolism, was identified in 2008 [44]. It showed reactions with different organs such as receptor 4, NOD2, CD163, and interferons, responsible for leprosy [45].

2.2. Advancements in Treatment of Leprosy

Recently, various researchers developed many techniques to combat leprosy. Pardillo *et al.* studied the efficacy of moxifloxacin against multi-bacillary (MB) leprosy patients. The drug was able to kill the bacteria almost 82 to 99% and no workable bacilli were detected on further 3-week treatment. Using this therapy, skin lesions as well as resolution of leprosy patients improved rapidly with mild to almost no side effects [46]. Hagge *et al.* conducted a research for analyzing the effect of lymphotoxin-α (LTα) on leprosy control by infecting mice with low and high doses of *Mycobacterium leprae* foot pad (FP) infections. The study gave the verification that leprosy is dependent on genetic vulnerability of the host [47]. Balagon *et al.* in 2010 conducted a research based on comparison of potency of 4-week based treatment using ofloxacin with the WHO standard multi-drug therapy (WHO-MDT) against leprosy, involving 124 PB patients. Results showed that patients following ofloxacin treatment had a follow-up of 10.8 years whereas the WHO-MDT treatment had follow-up of 11.3 years with one relapse at 3^{rd} year and two late relapses at 8^{th} and 12^{th} year of treatment, respectively. Both treatments proved effective with very less number of relapses [48]. Hight resolution ultrasound (HRUS), a tool used for the diagnosis of leprosy, is used at primary level [49]. In a case study involving 2 patients with leprosy were examined. In the first clinical test, symptoms such as basophil, fever, and adenopathy were analyzed and active tuberculosis (TB) infection was diagnosed by AFB test. It was treated with multi-drug that showed response against the symptoms [50]. Kumar *et al.* investigated the role of FoxP3 in the inhibition of T cells that are responsible for leprosy. It showed strong binding interactions with deacetylase 7/9 and histone acetyl transferase that is helpful in inhibiting the T and $CD4^+$ $CD25^+$ cells as well as the CTLA-4 and CD25 genes that were isolated from BL/LL patients [51]. The T alleles are considered responsible for leprosy and tuberculosis. The major purpose of leprosy treatment is the reduction or inhibition of T cells production in the body. Carriers of $IFNG^+$874T allele are also used to inhibit leprosy [52]. Platelet-rich plasma (PRP) treatment has a wide use in treating chronic wounds. Conde *et al.* used this treatment on 2 patients having neuropathic leprosy ulcers. They reported that PRP had positive effect in the treatment of leprosy [53]. Kamal *et al.* performed a double blind case study on two groups in which they applied a new vaccine "Mycobacterium Indicus Pranii (MIT)" along with multi-drug therapy (MDT) on one group and MDT with placebo on the other group. The results showed that MDT was more potent along with MIT [54].

2.2.1. Treatment along with Rifampicin

Kroger *et al.* designed a uniform multi-drug therapy (U-MDT) for all types of leprosy patients using a combination of 3 drugs, clofazimine, dispone, and rifampicin. The aim of study was to observe the effect of U-MDT towards multi-bacillary (MB) and pauci-bacillary (PB) groups. The study concluded that PB patients responded much better than MB patients using U-MDT and it was also a promising therapy for skin lesion leprosy [55]. Moet *et al.* studied the efficacy of rifampicin for the inhibition of leprosy, in people who were in close contacts with patients of newly diagnosed leprosy, by using single and double blind, and placebo-controlled trials in Bangladesh. The results concluded that a single dose of rifampicin was potent against the development of leprosy at two-year stage, for the close contacts of patients [56]. Schuring *et al.* developed a new strategy of using Bacille Calmette-Guérin (BCG) vaccination in combination with rifampicin for the treatment of leprosy. The joint effect of BCG vaccination and rifampicin against leprosy was 80% which concluded that combination therapy could lower the prevalence of leprosy in future [57].

2.3. Genetic Studies

According to the family-based studies by de Sales *et al*., leprosy was considered a genetic disease, transferred from one generation to the next and was conformed from patients containing 248S amino acid in mononuclear blood cells, named as receptor 1. Infection in blood cells caused by Bacillus Calmette-Guerin strain is also a reason for leprosy [58]. Sapkota *et al*. in 2010 validated the association of leprosy with genetic variants in tumor necrosis factor (TNF), mannose binding lectin (MBL), and the vitamin D receptor (VDR) employing case-control study with 933 patients and compared their genotype frequencies. The outcome of study indicated that TNF-308 is linked to protection from leprosy (odd ratio 0.52), MBL polymorphism was connected to protection from lepromatous leprosy (odd ratio 0.33), however, negative results for VDR association with disease phenotypes were seen [59]. Ochoa *et al.* investigated the role of interleukin-5 (IL-5) in production of T-cells within lepromatous leprosy (L-lep) leisons for enhanced production of B-cell of immuoglobulin M (IgM). For this study, gene exprssions of lepromatous (L-lep) and tuberculoid leprosy (T-lep) leisons were compared *via* bioinformatics analysis. The results of the study showed that about 8% more IgM positive cells were present in L-lep leisons than T-lep, confirming the role of IL-5 in increased production of IgM [60]. Zhang *et al*. conducted a genome based study for the identification of new agents responsible for leprosy. The two new loci at IL23R an RAB32 were identified by taking 706 patients under examination. Also the association between NOD2 and RIPK2 was located and the vulnerability of IL23R for leprosy was revealed [61]. Liu *et al*. identified about 13 microRNAs (miRNAs) in lesions of L-lep and T-lep. With the help of bioinformatics tools, a prominent increase in L-lep specified miRNAs, responsible for reduction of immune gene towards leprosy, was observed. The new miRNA, has-mir-21, was found to upgrade the *M. leprae* infected monocytes. It was also responsible for the inhibition of gene encodings of 2 antimicrobial Vit-D dependent peptides (CAMP and DEFB4A) due to increased interleukin-10. Thus it was concluded that miRNA-21 is responsible for targeting Vit-D dependent antimicrobial routes in leprosy [62]. Liu *et al*. reported the vital role of IL12/IL18 as lepsory regulators. A study was conducted on 133 patients for the determination of multiple-gene interlinakge between inflamatory bowel disease (IBD) and leprosy. The results revealed 2 associations at rs2058660 and rs6871626 indicating IL18RAP/IL18R1 and IL12B as vulnerable genes for leprosy, thus confirming the association between IBD and leprosy [63]. Ali *et al.* examined 2345 people via MassArray platform for the functioning of 23 single nucleotide polymorphisms (SNPs) in IL12B and IL12RB2, and 257 people for IL23R, IL12RB2 and IL10 using PCR for copy number variations analysis, for the determination of their association to leprosy. The results indicated that SNP rs2853694 in IL12B gene was associated to leprosy whereas copy number variation anaysis indicated the increase of IL23R gene linked to PB leprosy [64]. Eichelmann *et al*. investigated leprosy in Brazil, using single nucleotide polymorphisms (SNPs) technique. It was observed that IL10 gene, responsible for haplotypes formation, was present [65, 66]. Garcia *et al.* investigated the same method and suggested that different genotypes such as A-1082G, C-819T, and C-592A were formed. At that time, it was the only method used for the protection of leprosy [67]. Liu *et al*. performed a three-stage genome-wide association study of leprosy in China and reported six new susceptibility loci. They further analyzed these loci under gene prioritization and reported that BATF3, CCDC88B and CIITA-SOSI have high affinity to get effected by these loci [68]. In a study conducted by Singh *et al*., the complete genome sequence of *M. lepromatosis* from the skin of a Mexican patient was obtained. This genome sequence was then compared with the genome sequence of *M. leprae* which showed size similarity (~3.27 Mb). The genome showed 93% nucleotide sequence similarity and 82% similar pseudogenes. Among 227 patients of leprosy, 221 were affected by *M. leprae* and only six had leprosy due to *M. lepromatosis* [69]. Naqvi *et al*. provided a brief report of hypothetical proteins present in the strain Br4923 of *M. Leprae* which helps to understand the pathogenic mechanism and in finding out possible therapies for leprosy. Among the reported 1604 proteins, the role of 632 hypothetical proteins is still not known. They proposed the possible roles of 312 hypothetical proteins by dividing them in to families (enzymes, binding proteins, and transporters) according to the sequence of similarities [70]. Pereira *et al*. carried out a combined disease and controlled case study with meta-analysis to analyze epidemiological and physiological relation of interleukin-10 (IL-10) genetic markers in leprosy. The research showed that low level of IL-10 through disease can lead the patients to a more chronic and susceptive response that intensifies with leprosy [71].

2.4. Leprosy Case Studies

Attia *et. al*., in 2010 used flow cytometry to study the effect and frequency of $CD4^+$ CD25 high $FoxP3^+$ T-cell regulators (T-reg) in 38 leprosy infected patients and 38 healthy controls classified in 4 different groups for lepsory type. The results showed that T-regs and FoxP3 expression increased in leprosy patients compared to healthy volunteers. However, T-reg frequency was lower in patients with

lepromatous (LL) and ENL, thus T-regs were concluded to be favourable instead of being deleterious [72]. A case study published in 2016 suggested that almost 43% of the leprosy patients in Ethiopia feel nociceptive pain and 11% feel pure neuropathic pain. Same case was reported in India where 21.8% patients felt neuropathic pain in the early stages of leprosy, hence, a tool can be developed so that leprosy can be diagnosed in the early stages [73]. In 2017, Gorge *et al*. analyzed histopathology of sural nerve and diagnosed pure neuritic leprosy (PNL) in 13 (52%) out of 25 patients. They also studied anaesthetic skin and identified 10 (40%) patients. The combined (sural nerve, anaesthetic skin) sensitivity of diagnosing PNL was 68%, therefore, they concluded that sural nerve and anesthetic skin biopsy can be used as a diagnostic tool for PNL [74]. Berrington *et al*. conducted a case-control study in Nepal with 933 patients, and found that polymorphism of nucleotide-binding oligomerization domain 2 (NOD2) gene is interconnected to vulnerability of leprosy. It was concluded that 4 polymorphisms were linked to leprosy liability, 8 with genotype frequencies, 5 were related to protection and reversal reaction, 7 were linked to reversal reactions, 4 with enhanced vulnerability to erythema nodosum leprosum, and other 7 out of 32 were in association with a dominant model and an overall relation of leprosy with NOD2 was confirmed [75].

2.5. Transmission of *Mycobacterium leprae*

Job *et al.* studied the transmission of leprosy via direct microscopic evaluation and PCR for the DNA of *Mycobacterium leprae*. The studies by PCR revealed that about 17% transmission of *M. leprae* was due to skin contact of healthy patients with the infected ones and about 4% transmission was due to nasal mucosa. They concluded that both skin as well as the nasal epithelia of untreated patients of MB leprosy are responsible for the distribution and shedding of bacteria to the environment [76]. Queiroz *et al.* used applied spatial statistics combined with geographic information systems (GIS) for surveying the distribution of leprosy in Brazil using 808 samples out of 1,293 cases. The study concluded that the conjunction of GIS and spatial analysis could determine the clustering of dieases, that are transmissible, indicating the areas to be targetted for disease control [77]. Mattos *et al*. explored the formation of foamy macrophages in leprosy. The research revealed that macrophages in dermal leison of lepromatous leprosy (LL) are positive for adipose differentiation related protein (ADRP). *In vivo* and *in vitro* studies showed that *Mycobacterium leprae* (ML) was capable of causing lipid droplet (LD) formation. The LD induction is transmitted due to infected cells. The research indicated that LDs produced by ML are responsible for eicosanoid synthesis due to which the immune repsonse in leprosy is disrupted [78]. Sergio *et al*. explored the route of *Mycobacterium leprae* through which it diffuses into the blood stream. The ML DNA was analyzed in nasal vestibule, nasal turbinate, and blood of 113 leprosy patients. Positive results were shown by all the samples (nasal swab 71.7%, nasal turbinate 19.5%, and blood 62.8%). These studies confirmed that aerosol route is predominant in the transfer of *Mycobacterium leprae* [79]. Sharma *et al.* investigated that *M. leprae* not only affects humans but also other living organism such as *Dasypus novemcinctus* (armadillo) by attacking nervous system thus causing nervous disorder for a long time, however, the mechanism of action of bacilli is still unknown. Researchers are trying to design a new drug that might be helpful against *M. leprae* in armadillos [80].

2.6. New Case Detections

Han *et al.* discovered a new species of *Mycobacterium* from patients died due diffuse lepromatous leprosy (DLL). The research resulted in unrevealing a new species *Mycobacterium lepromatosis* sp nov that could be useful in accounting the clinical as well as geographic variation of leprosy [44]. de Souza Sales *et al.* explained the functioning of indoleamine 2,3-dioxygenase (IDO) by studying the distribution and activity of IDO positive cells in skin and sera of leprosy patients. Test results via PCR and flow cytometry showed that IDO message and IDO expression of both healthy control and LL patients increased. These findings were also confirmed by *in vitro* studies. It was concluded that IDO molecule gets activated due to *M. leprae* and contributes to immunosuppression in LL leprosy [81]. Sausa *et al.* investigated the vulnerability of interleukin 6 (IL-6) for type 2 leprosy reactions. A group of 409 patients were examined for T1R and T2R with two controlled case studies. An interconnection between T2R and IL-6 polymorphisms was observed and also the IL-6 plasma levels of T2R leprosy patients were found in correlation with genotypes of IL-6. However, no linkage between IL-6 and T1R was observed [82]. Andrade *et al.* analyzed the serum of leprosy patients having (n¼9) and lack of (n¼8) acute neuritis, which showed that almost all patients with neuritis have demyelination. According to their findings *Mycobacterium leprae* can produce tumor necrosis factor mediated inflammation [83]. Meima *et al.* explored the relation between occurrence and future frequency of WHO grade-2 damage caused by leprosy. The study revealed that in future there will be significant number of people with impairment caused by leprosy and will need care, treatment, and training for self-care and prevention of leprosy [84]. Reis *et al.* observed the epidemiological studies of leprosy in Brazil and concluded that it can be detected through DNA of MP patients under quantitative PCR (qPCR). The qPCR targets the ML0024 genomic area giving positive results, thus helping in identification of bacillus DNA in leprosy patients [85].

CONCLUSION

The diagnosis of leprosy at earlier stages is important for its effective treatment. Recent studies on Vitamin D and its receptors make leprosy diagnosis at earlier stages easier. Skin biopsies, HRUS and qPCR are the other tools to identify the disease in its initial stages. There is a challenge to differentiate leprosy from other infectious diseases, especially from tuberculosis and sarcoidosis. Some of the miRNAs show variations in leprosy patients than those present in TB patients. Leprosy can be distinguished from sarcoidosis by quantitative study of reticulin fibers present in skin as sarcoidosis patients have high number of reticulin fibers than leprosy patients. The treatment used until now for leprosy is multi-drug treatment which has many side effects, therefore, it is required to develop a target-oriented drug for leprosy.

The complete genome identification of *Mycobacterium leprae* makes the research easy to develop target specified drugs for leprosy. FoxP3 shows strong binding interactions with deacetylase 7/9 and histone acetyl transferase, so it inhibits the growth of T cells that are responsible for leprosy and TB. Rifampicin, identified as a potent drug when given along with other drugs in uniform multi-drug treatment, has a significant effect when given to leprosy patients at initial stages. These are effective treatments but a specific drug for leprosy is still needed to be identified.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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