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Ecology and Epidemiology Aspects of *Mycobacterium leprae*

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Abstract: Leprosy is a chronic infectious disease also known as Hansen's disease, caused by *Mycobacterium leprae* that tends to be chronic and to compromise human societies by producing peripheral nerve damage, limb loss, blindness, and disfiguring skin lesions. Leprosy occupies a prominent position among infectious diseases due to its high frequency of disability and associated stigma. Detection of grade 2 disability (G2D) reflects a delay in the diagnosis of leprosy, which results in persistent neuritis leading to disability. There is also a possibility that there might be hidden cases in the population. *Mycobacterium leprae* has been reported for than 2000 years and today they are ubiquitous, occurring in every habitat and ecosystem of the world, perhaps except for the polar- regions. The first known representative of this group was discovered, under the name of Bacillus leprae, by Hansen in 1875. *M. leprae* is a non-cultivable obligate intracellular pathogen with a slow division time that targets peripheral nerves by predominantly infecting Schwann cells and histiocytes and keratinocytes in the skin. Leprosy is classified according to the WHO guidelines in 2012 to Paucibacillary leprosy (PB) and Multibacillary (MB).Up to 95% of patients exposed to *M. leprae* will not develop the disease, suggesting that host immunity plays an important role in disease progression and control. The incubation time is variable, ranging from 2 to 20 years, or longer cases have been reported supporting the possibility of transmission by different ways, and discussions on different ways of transmission are continuing. Today the prevalence of this ancient disease is declining in most around the world this decline is a direct effect of widespread administration by public health workers of multidrug therapy. However, emerging despite the use of multidrug therapy, identifying and monitoring resistance are still necessary.

Key wards: *Mycobacterium*, *M. leprae*, Ziehl-Neelsen (ZN) stain, Epidemiology, Ecology.

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Introduction

The genus *Mycobacterium* is part of the order *Actinomycetales* and the phylum *Actinobacteria* and belongs to a variety of environmental habitats, including natural waters, soils, and drinking water distribution systems (1), Mycobacterial species reside in a wide-variety of environments due to multiple adaptations. Some of the features include the presence of lipid-rich hydrophobic outer membrane, which is a major determinant of surface adherence, biofilm formation, aerosolization, and antibiotic/ disinfectant resistance (2,3). Additionally, *mycobacteria* have the ability to replicate at a low rate, providing them with a decreased susceptibility to most antimicrobial agents effective competitors in low-nutrient environments (oligotrophs) (3,4).From a large mycobacterial pool, some species have evolved into potential major human pathogens (5,6) (Figure 1). *Mycobacteria* may seed the skin and soft tissues during systemic dissemination in immunosuppressed individuals. There is some evidence of

potential human-to-human transmission of *Mycobacterium abscessus* subsp. *massiliense* among patients with cystic fibrosis (7,8).Cutaneous *mycobacterial* infections may be grouped into four major categories: (i) infection due to *Mycobacterium tuberculosis* complex, (ii) infection caused by *Mycobacterium leprae* and *M. lepromatosis*, (iii) infection caused by *Mycobacterium ulcerans* and other slowly growing *mycobacteria* and (iv) infection due to rapidly growing *mycobacteria* (9).

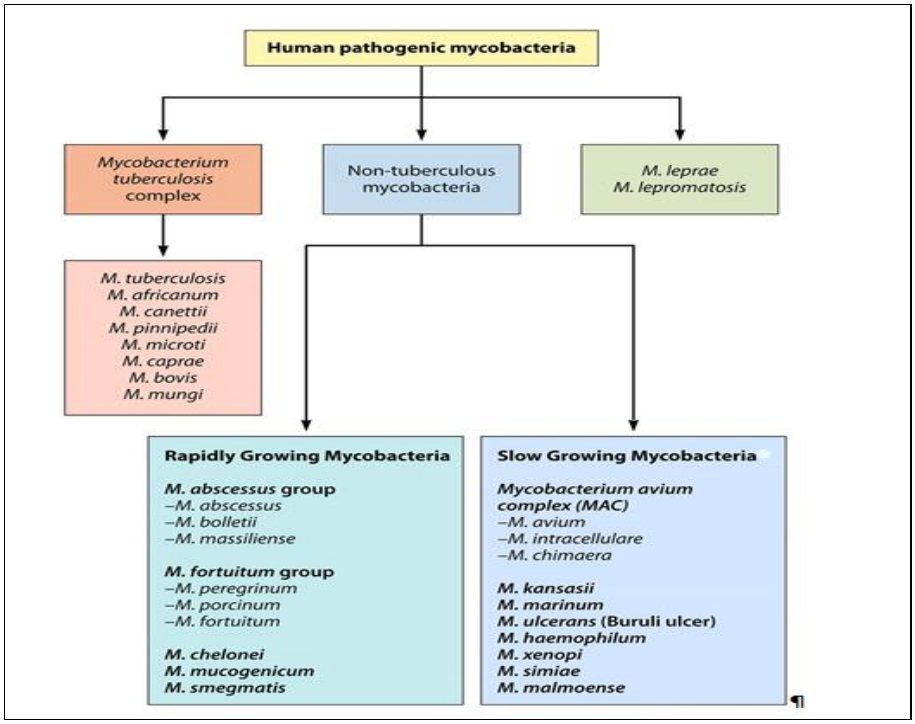

Figure (1): Classification of major pathogenic *mycobacteria* (9).

Leprosy is a chronic infectious disease also known as Hansen's disease, caused by *Mycobacterium leprae* that tends to be chronic and to compromise human societies by producing peripheral nerve damage, limb loss, blindness, and disfiguring skin lesions (10). Leprosy occupies a prominent position among infectious diseases due to its high frequency of disability and associated stigma (11,12). *M. leprae* is a non-cultivable obligate intracellular pathogen with a slow division time that targets peripheral nerves by predominantly infecting Schwann cells and histiocytes and keratinocytes in the skin (13). *M. leprae's* scientific classification is as follows: class *Schizomycetes*, order *Actinomycetales*, family *Mycobacteriaceae*, and genus *Mycobacterium. M leprae* is a straight or slightly curved rod, with rounded ends, measuring 1.5-8 microns in length by 0.2-0.5 micron in diameter. In smears, it is red stained with fuchsine using the Ziehl-Neelsen (ZN) stain and because of its high lipid content, it does not get discolored when washed with alcohol and acid, thus showing the characteristics of acid-alcohol-resistant bacilli (AARB) (14). *M. leprae* infects mainly macrophages and Schwann cells. It has never been grown in artificial media. Reproduction occurs by

binary fission and it grows slowly (about 12-14 days) in the footpads of mice. The temperature required for survival and proliferation is between 27 ºC and 30 ºC. This explains its higher incidence in surface areas, such as skin, peripheral nerves, testicles, and upper airways, and lower visceral involvement. *M. leprae* remains viable for 9 days in the environment (14, 15). The genome of *M. leprae* was sequenced by (16). Its estimated molecular weight is 2.2 x 109 Daltons, with 3,268,203 base pairs (bp) and guanine + cytosine content of 57.8%. When compared to the genome of *Mycobacterium tuberculosis*, which has 4,411,529 bp and guanine + cytosine content of 65.6%. It seems that *M. leprae* underwent reductive evolution, resulting in a smaller genome rich in inactive or entirely deleted genes. It has 2,770 genes, with a coding percentage of 49.5%, that is, 1,604 genes encoding proteins (1,439 genes common to *M. leprae* and *M. tuberculosis*) and 1,116 (27%) pseudogenes (16,17).

Mycobacterial Leprae Ecology

The precise mode of transmission of leprosy human-to-human via respiratory droplets of *M.leprae* infection has been traditionally considered the driving engine of transmission of leprosy while leprosy bacilli are present in the nasopharynx of individuals with multibacillary leprosy and from cutaneous lesions, and that these bacilli are able to infect other susceptible human hosts (18,19). Despite the fact leprosy is a very old disease, we still have a limited knowledge of contamination routes and reservoirs. Contamination usually occurs after prolonged contact with the nasal and oral secretions of lepromatous leprosy (LL) patients infected and untreated with *M. leprae* (20). However, many cases have been reported supporting the possibility of transmission in different ways, and discussions on different ways of transmission are continuing. There are reports that leprosy cases reported to develop by tattooing and accidental needle penetration support that they can be transmitted through damaged skin, there are also reports supporting undamaged skin contamination (21,22). Leprosy cases also are seen in the infant period also suggest a possible infection from the mother via blood or with breast milk (23,24). In addition, a leprosy case developed after blood transfusion has been reported (25).

The precise mechanism and route of transmission remain to be completely elucidated. Indeed, the current epidemiology of the persistent transmission of leprosy along with collected evidence made since the 19thcentury suggests that environmental factors such as soil and water, vegetation, arthropods, free-living amoebas, and animal reservoir host such as the nine-banded armadillo (*Dasypus novemcinctus*) play an influential role in the ongoing transmission of *M. leprae* (26,27). Environmental factors such as climate, type of soil and water, environmental degree of acidity, etc.; along with spillover of *M. leprae* from human cases (e.g., nasal discharges contaminating soil or water) may facilitate the amplification of the transmission cycle(28). Zoonotic transmission of *M. leprae* from armadillos in the Gulf Coast of the United States contributes to endemic human infections detected in this geographic area every year, supporting

the fact that leprosy is not exclusively transmitted person-to-person (21). Armadillos may also play a role in the transmission of leprosy in some areas in Colombia (29) and in Brazil (27). In some of the British Isles, red squirrels may develop leprosy-like lesions due to either *M. leprae* or *Mycobacterium lepromatosis.*

Spread of *Mycobacterium Leprae*

Humans are natural reservoirs in the transmission of *M. leprae* and therefore the global spread of leprosy is tied to historical milestones of human migration. Leprosy is nonrandom in its distribution. For example, a study among highly endemic island populations in Indonesia found that leprosy patients are extensively clustered and not equally distributed among islands; furthermore, within highly affected islands there was an unequal distribution among the houses (30). It is often stated that, in endemic countries, not more than 5% of those exposed to *M leprae* will develop clinical leprosy during their lifetime. Recent comparative genomic evidence points to the origin of leprosy in Eastern Africa (9,26). Overall, genomic comparisons of ancient and modern strains of *M. leprae* remain remarkably similar, indicating it was probably improvements in social conditions that led to a substantial reduction of leprosy in Europe in the 16th century (31,32). In modern times, it is likely that the clustering of cases of leprosy occurs among individuals living in resource poor areas with favorable ecological niches for *M. leprae* to thrive (33). Therefore, it is important to consider the larger social drivers that underlie the unequal distribution of life choices of individuals living in the highest endemic areas that place them at risk of suffering from leprosy and other neglected diseases (11).

The major sources of leprosy data are the WHO. At the global level, data are available from 1985 onward. In that year the registered prevalence of leprosy was about 4 million; in 2014 it had declined to 175,554 (Figure 2). The "elimination of leprosy as a public health problem" policy by the WHO, declared in 1991(34).

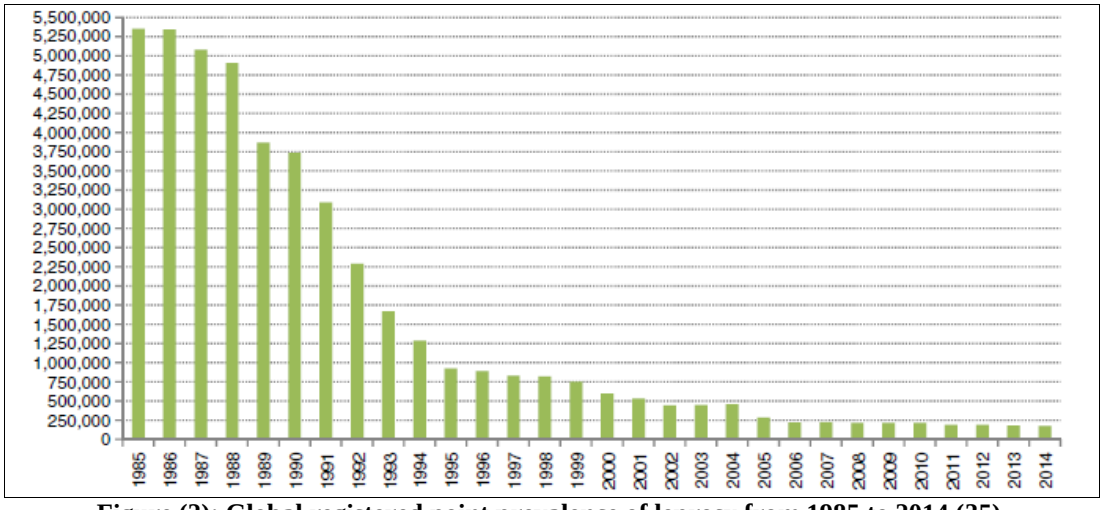

Figure (2): Global registered point prevalence of leprosy from 1985 to 2014 (35).

The disease is still an important public health problem in three regions, namely Southeast Asia, the Americas, and Africa. The global trend without India shows a more gradual decline since the year 2000 (9) (Figure 3). India contributes the highest number of leprosy patients to the burden of leprosy all over the world. A total number of new cases diagnosed in 2017 was 1, 26,164 (approximately 60% of the world's new leprosy cases). While the detection of new cases was almost stationary for the period 2008 – 2017 (35,36) (Table 1) (Table 2) (Table 3).

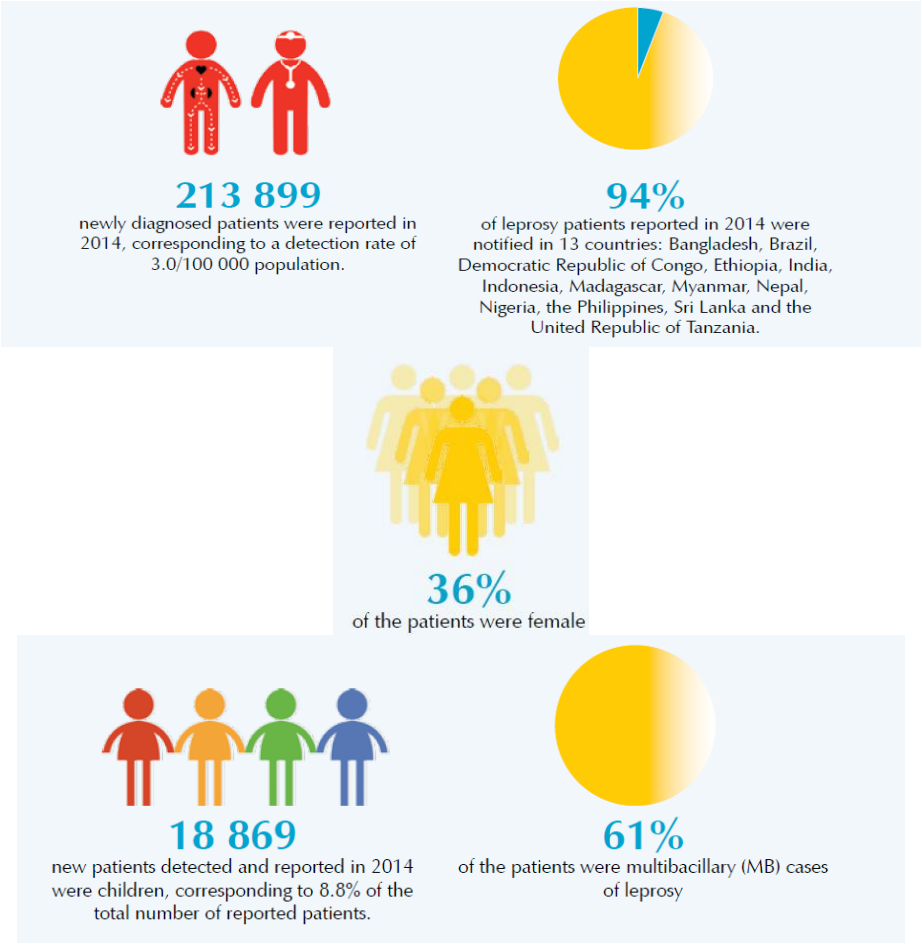

Figure (3): The "Global leprosy update, 2014. (35).

Table (1): Registered Prevalence at the end of 2017 and new case detection during 2017, by WHO (35,36).

Region	Number of cases registered (prevalence/10 000 population), end of 2017	Number of new cases detected (new case detection rate/100 000 population)during 2017
African	30 654 (0.28)	20 416 (1.90)
Americas	31 527 (0.31)	29 101 (2.86)
Eastern Mediterranean	4 405 (0.06)	3 550 (0.51)
South-East Asia	119 055 (0.6)	153 487 (7.72)
Western Pacific	7 040 (0.04)	4 084 (0.21)
Europe	32 (0)	33 (0)
Global total	192 713 (0.25)	210 671 (2.77)

Table (2): Presents the numbers of new cases reported annually between 2008 and 2017 by WHO (35,36).

Region	Number of new cases detected									
	2008	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**
African	29 814	28 935	25 345	20 213	20 599	20 911	18 597	20004	19 384	20 416
Americas	41 891	40 474	37 740	36 832	36 178	33 084	33 789	28806	27 356	29 101
Eastern Mediterranean	3 938	4 029	4 080	4 357	4 235	1 680	2 342	2167	2 834	3 550
South-East Asia	167505	166115	156254	160132	166445	155385	154834	156118	163095	153 487
Western Pacific	5 859	5 243	5 055	5 092	5 400	4 596	4 337	3645	3 914	4 084
Europe								18	32	33
Global total	**249007**	**244796**	**228474**	**226626**	**232857**	**215656**	**213899**	**210740**	**217968**	**210671**

Table (3): Trends in number (and rate per million population) of new cases with G2D, by WHO, 2008–2017(35,36).

Region	Number of new cases									
	2008	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**
African	3 458 (5.1)	3 146 (4.1)	2 685 (4.0)	2 300 (2.6)	2 709 (4.0)	2552 (4.3)	2726 (3.6)	2887 (4.1)	2 899 (2.8)	2 911 (2.7)
Americas	2 512 (2.9	2 645 (3.0)	2 423 (2.7)	2 382 (2.7)	2 420 (2.8)	2168 (2.5)	2222 (2.5)	1973 (3.5)	1940 (1.9)	2 149 (2.1)
Eastern Mediterranean	687 (1.4	608 (1.1)	729 (1.2)	753 (1.2)	700 (1.2)	191 (0.5)	300 (0.5)	315 (0.5)	299 (0.4)	316 (0.5)
South-East Asia	6 891 (3.9)	7 286 (4.1)	6 912 (3.9)	7 095 (3.9)	8 012 (4.3)	7964 (4.3)	8525 (4.5)	8572 (4.4)	7 538 (3.8)	6 513 (3.3)
Western Pacific	592 (0.3)	635 (0.4)	526 (0.3)	549 (0.3)	568 (0.3)	386 (0.2)	337 (0.2)	312 (0.2)	362 (0.2)	299 (0.2)
Europe									4 (0)	1 (0)
Global total	**14 140 (2.5)**	**14 320 (2.5)**	**13 275 (2.3)**	**13 079 (2.2)**	**14 409 (2.5**	**13 289 (2.3)**	**14 110 (2.5)**	**14 059 (2.5)**	**13 042 (1.8)**	**12 189 (1.6)**

Epidemiology of Disease in Iraq and neighboring countries

The prevalence of leprosy was registered in Iraq at 1975 was 610 case (37), the cases were declined gradually for the sequenced years to be zero cases at the period between 2005-2008, Leprosy, almost non-existent in Iraq recently, no new cases have been found in the last several years, even there is maybe a cases that not reported because leprosy does not exist in Iraq in the last several years, and this delay occurs outside leprosy endemic areas, when doctors fail to diagnose leprosy delay in the patient(38). According to WHO, one new cases has been reported in 2009, 2011 and 2012 to three cases reported in 2013 and 2014. The disease was starting to disappear from 2015-2018 and there was no reported case (Figure 4).

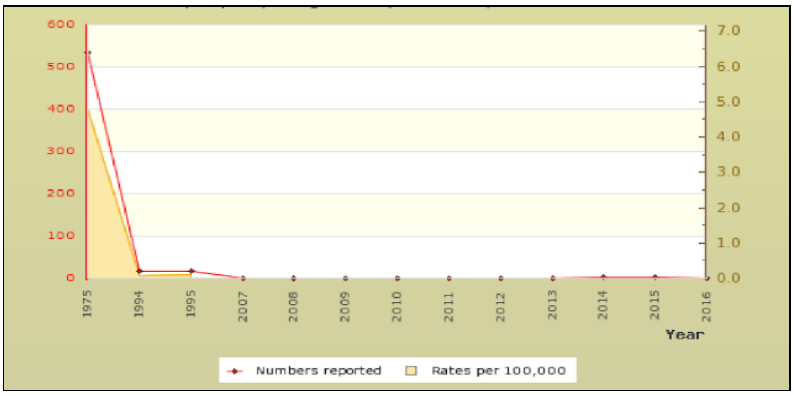

Figure (4): Iraq Leprosy cases 1975 – 2016 (37).

According to WHO, In Jordan that no leprosy case has been indicated at the period among 2005 - 2018, there was a decline in the cases that been reported from 1968-2002(Figure 5) (39) to zero case in 2005.

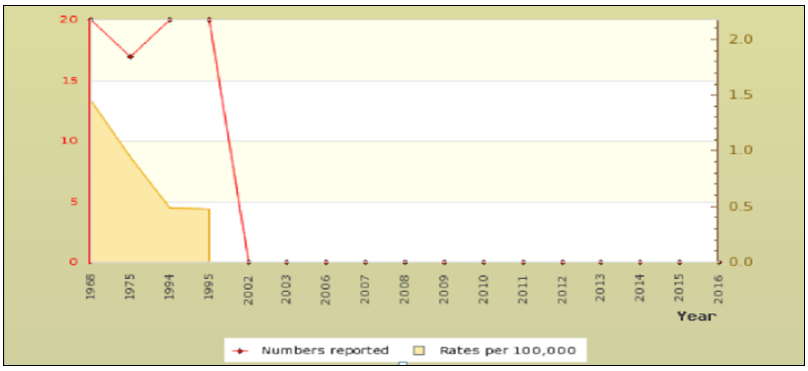

Figure (5): Jordan leprosy case 1986 – 2016 (37).

The few leprosy patients among the Kuwaiti and non-immigrant population suggest the rarity of the disease in the Gulf area since similar findings were reported from the Eastern Province of Saudi Arabia (40). Leprosy in Kuwait is almost exclusively an imported disease, and most patients came from areas of high endemicity. There were 121 new cases of leprosy (1983-23; 1984-20; 1985-25; 1986-21; 1987-19, 1988-13). Over 95% of the patients were foreign born (41)) (Figure 6). According to WHO, there was stability in the number of registered cases among 2005-2018 that has been recorded.

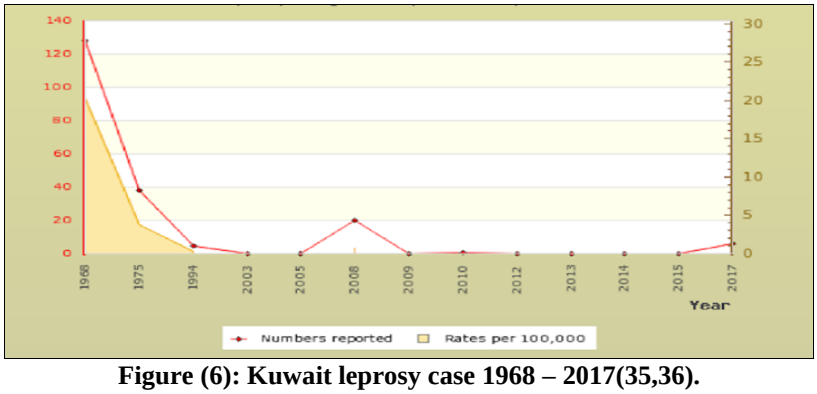

Figure (6): Kuwait leprosy case 1968 – 2017(35,36).

In Syria, the number of registered cases was a decline from the period 1968-2003(Figure 7) (40). According to WHO reported in 2019 about 35 cases were reported between 2005-2018 with no data at the years 2012, 2013and 2014. On 29 January 2019, WHO disease surveillance focal point in north-east Syria informed a suspected case of leprosy in Al-Hol camp, Al-Hasakeh governorate (36).

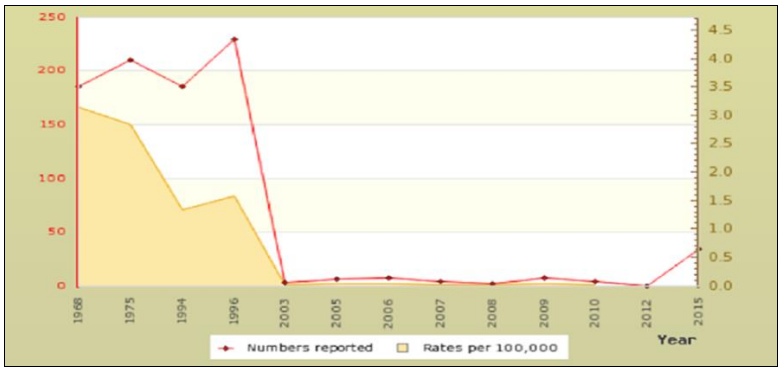

Figure (7): Syrian Arab Republic leprosy case 1968 – 2015(35,36).

In Saudi Arabia, during 1986 to 1992, 792 patients were registered in the country's single leprosarium 432 (54'55%) were non-Saudi and 360 (45 '45%) were Saudi (42) (Figure 8), In 1997 about 51.5% was multibacillary. and during 2003 to 2012, 242 cases were identified through active surveillance. According to WHO, there was a decline in the reported cases among 2005-2018.

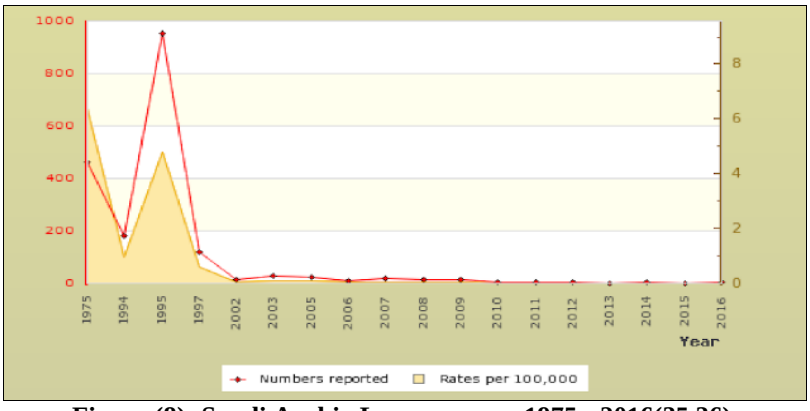

Figure (8): Saudi Arabia Leprosy cases 1975 - 2016(35,36).

Although Iran is currently an area in which leprosy is not a serious problem, new cases of leprosy are still diagnosed in Iran. Considering that Iran is attempting to eradicate the disease, careful attention to all aspects of the disease is essential of the 433 cases of leprosy diagnosed from 2005 to 2014, 87.1% were Iranian, and 56.2% of the Iranian cases were male (43). Furthermore, 82.5% of cases were multibacillary. The annual prevalence and case detection rates of leprosy (per 100,000 populations) significantly decreased in Iran between 2005 and 2015: from 0.2 to 0.02 and from 0.11 in

2005 to 0.02, respectively. The geographical distribution of leprosy cases in 2014 showed that leprosy is more common in the west, north, northwest, and south of Iran is essential (44) (Figure 9).

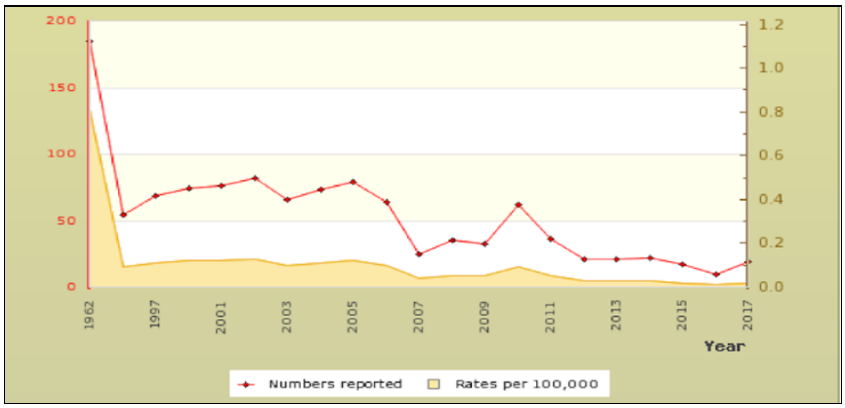

Figure (9): Iran leprosy case 1962 – 2017(35,36).

In Turkey, there are 2,414 patients with leprosy in Turkey, registered to Istanbul Leprosy Hospital and 829 of them are females. The mean age and duration of disease of our female leprosy patients are high. Most of them (97.2%) have an inactive disease. The disability degrees of patients are high. Patients with disability degrees over one constitute 54% of the total for eyes, 55% for hands and 51% for feet. A high percentage of multibacillary form and long duration of disease (45). According to WHO (Table 4), the reported cases of leprosy in turkey was five cases for the years 2008,2009 and 2010 while there was no reported data for the next years.

Table (4): Number of Leprosy cases patients according to WHO, in Turkey (35,36).

Indicator	Registered prevalence(end of 2017 or end of first quarter of 2018)	2017	2016	2015	2014	2013	2012	2011	2010	2009	2008	2007	2006	2005
Number of new Leprosy cases	NO data	NO data	NO data	NO data	NO data	NO data	NO data	NO data	5	5	5	NO data	NO data	NO data

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