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Prevalence of Non Tuberculosis Mycobacteria in Southeast of Iran

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The present study was decided to study the prevalence of isolation of NTM in this area which undoubtedly has special value on treatment decision and present diagnostic procedure. This study was done in two different years in 2000 and 2004 in patients referring to BOU-Ali Hospital with pulmonary symptoms which had a smear positive in ZNCF staining and positive culture results in cultivation on Lowenstein-Johnson and Middle brook 7H10 agar. The isolated mycobacteria were identified on the basis of their growth rate, pigmentation and biochemical testes. The results (2002) of cultural characteristics (i.e., growth rate and pigmentation) and biochemical testes in 91 specimens (60.7%) lead to isolation of *Mycobacterium tuberculosis* and in 59 specimens (39.3%) to isolation of NTM. In NTM isolates, 38 specimens (64.4%) belongs to females and 21 specimens (35.6%) belongs to males. The results (2004) of cultural characteristics (i.e., growth rate and pigmentation) and biochemical testes in 60 specimens (66.6%) lead to isolation of *Mycobacterium tuberculosis* and in 20 specimens (33.3%) to isolation of NTM and from these 20 NTM specimens, 13 specimens (65%) belongs to females and 7 specimens (35%) belongs to males. In both studies (2000 and 2004), the most amount of isolation of NTM was in upper 60 years old group. Due to reported anti TB drugs resistance of NTM and with respect to our findings, it is suggested that in the first visit of patients suspected to tuberculosis, simultaneous study of smear staining, cultivation, species identification and anti-biogram, specially in this region and in similar geographic conditions be done and treatment with the results of ZNCF staining alone may be not a suitable diagnostic test.

Key words: Non Tuberculosis Mycobacteria, pulmonary tuberculosis, acid fast bacilli, Zahedan

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INTRODUCTION

Tuberculosis is one of the most important and ancient human diseases and the World Health Organization has estimated that one third of the world's population is infected with *Mycobacterium tuberculosis*, with approximately 8 million new cases diagnosed annually (Dye *et al*., 1999; WHO, 2000).

With spread of drug resistance strains of *Mycobacterium tuberculosis* and emergence of HIV, Tuberculosis as a major health and treatment problem of human societies become again under serious considerations (Brown *et al*., 2000; Hart *et al*., 1996). Pulmonary NTM is a problem with differing rates in various parts of the world. North American rates of infection and disease have been reported to range from approximately 1-15 per 100,000 and 0.1-2 per 100,000, respectively (Marras and Daley, 2002).

Human encounters with Non Tuberculosis Mycobacteria (NTM) continuously in nature especially in the areas which non tuberculosis mycobacteria are more frequent in the soil and environment. Other studies have also shown marked geographic variability in prevalence of NTM (Marras and Daley, 2002). Also, inhibitory effects of environmental mycobacteria on the protective efficacy of BCG have been reported (Demangel *et al*., 2005).

Non Tuberculosis Mycobacteria may also produce diseases in healthy people, but are more frequent in people with underlying diseases such as Fibrosis, Bronchechtasis, Cavity damage of branches or Immunodeficiency (Brown *et al*., 2000) and so these bacilli have found special importance (Rateldge and Stanford, 1982; Jabbari *et al*., 2000; Yui, 2004).

Specific risk factors have been identified in several studies. CF and HIV, underlying chronic lung disease, work in the mining industry, warm climate, advancing age and male sex (Marras and Dakey, 2002).

Also, it has been reported that geographic distribution of NTM species is in relation with the diseases which these bacteria have been produced (Jabbari *et al*., 2000). Reviewed English-language reports and abstracts, probably leading to fewer data from non-English speaking regions, which may explain the paucity of studies from Africa, Eastern Europe and most Asian nations (Marras and Delay, 2002).

The heterogeneity of study methods in identifying cases and the lack of a uniformly applied definition of disease makes it difficult to compare rates between studies. Finally, the lack of systematic reporting of NTM infection in most nations limits the ability to derive accurate estimates of infection and disease. Regardless, there are more than adequate data to conclude that NTM disease rates vary widely depending on population and geographic location (Marras and Daley, 2002).

So, this study was done to declare the prevalence of NTM in this province which has a Tuberculosis rate multiple times over than Iranian National rate of Tuberculosis.

MATERIALS AND METHODS

This study was done in two different years with 4 years spacing (2000-2004) on the patients referring to Bou-Ali Hospital with smear positive ZNCF staining and culture positive results and without any consideration to sex, age and race of the patients.

After necessary incubation on Lowenstein-Johnson medium and growth rate, pigmentation and biochemical tests, NTM separated from Tuberculosis mycobacteria (Kent and Kubica, 1985; Sommers *et al*., 1985).We used Chi-Square and t-test for interpreting our data.

RESULTS

In the 2000 study, from 150 studied specimens, 91 specimens (60.7%) were *Mycobacterium tuberculosis* and 59 specimens (39.3%) were NTM. From patients with NTM, 21 had a history of underlying pulmonary disease including Chronic Bronchitis, Bronchechtasis or Asthma and in the 2004 study, from 60 smear positive and culture positive specimens, 40 specimens (66.6%) were *Mycobacterium tuberculosis* and 20 specimens (33.3%) were NTM. From these NTM, 13 specimens (65%) isolated from females and 7 specimens (35%) isolated from males. The most amount of NTM isolation was in upper 60 years old.

As it has been showed in Table 1. the most infected age group was over 60 years group and Mean of age of the patients in two groups was not significant with t-student test (p = 0.28).

Table 1: Frequency distribution of isolation of tuberculosis and non tuberculosis mycobacteria by age of the patients

	Group	
Age	Non Tuberculosis Mycobacteria	*Mycobacterium tuberculosis*
20-29	14(23.73%)	7(8.34%)
30-39	10(16.95%)	17(20.24%)
40-49	9(15.25%)	22(26.19%)
50-59	10(16.95%)	12(14.28%)
>60	16(27.12%)	26(30.95%)
Mean(SD)	45(17.2)	48(13.2%)
Total	59	84

Table 2: Frequency distribution of isolation of tuberculosis and non tuberculosis mycobacteria by gender

	Group	
Sex	Non tuberculosis mycobacteria	*Mycobacterium tuberculosis*
Males	21(35.6%)	34(37.36%)
Females	38(64.4%)	57(62.64%)
Total	59	91

As it has been showed this (Table 2) in our study, females are more susceptible to NTM or TB infections than males.

Study the history of the patients with underlying pulmonary disease showed that from 53 with available history, 21(39.6%) have not any underlying pulmonary disease. From 91 patients with *Mycobacterium tuberculosis*, 34(37.36%) were male and 57(62.44%) were females.

DISCUSSION

As it is mentioned in results, in the 2000 study from 150 smear positive, culture positive mycobacterial samples, 59 specimens (39.3%) were correspond to NTM and 91 specimens (60.7%) were correspond to *Mycobacterium tuberculosis* and in our 2004 study from 60 smear positive and culture positive specimens, 40 specimens (66.6%) were correspond to *Mycobacterium tuberculosis* and 20 specimens (33.3%) were correspond to NTM.

Study on different specimens collected from different areas of Khorasan province (second biggest province of Iran after) in 1984, showed that these mycobacteria are palmated in the area and made hidden infections (Mohammadi, 1990). Also different study in 1989 in laboratory of Isfahan center for pulmonary disease and treatment (the second most industrialized province of Iran) showed that from 82 culture positive mycobacterial specimens, 37.5% were NTM (Abtahi, 1990).

In 1992, in a study on different soil specimens collected from diverse part of Sistan and Baluchestan province (the biggest province of Iran and the only one in southeast of Iran), in several specimens NTM isolated and it has been showed that like Khorasan study (Mohammadi, 1990), NTM are exist in the soil of this region extensively (Karbassciyan, 1992).

On the other hand, as it is mentioned in the introduction, the heterogeneity of study methods in identifying cases and the lack of a uniformly applied definition of disease make it difficult to compare rates between studies and so many different rates reported for the prevalence of NTM in different studies.

In Marras report from USA (Marras and Daley, 2002), North American rates of infection and disease have been reported to range from approximately 1-15 per 100,000 and 0.1-2 per 100,000, respectively and in other study Martin *et al.* (2002) reported that one hundred twenty-two isolates of nine different types of mycobacteria and nocardia were cultured from 117 patients. The predominant isolates were *Mycobacterium avium-complex* (MAC) (60%) and *Mycobacterium tuberculosis* (Mtb) (21.3%).

Barnes *et al.* (2004) reported that among 716 culture positive cases, 684 (95.5%) were due to *Mycobacterium tuberculosis* complex and 32 to environmental mycobacteria. Suzuki *et al.* (2004) reported that the mean rate of atypical mycobacteria was 13.6 percent in the six years between 1996 and 2001 and in another study Imaizumi (1997) reported that during 1989-94 twenty-five out of 118 cases (21.8%) admitted to the tuberculosis ward of the Fuji City Central hospital were atypical mycobacteriosis.

Karak *et al.* (1996) reported that the prevalence rate of atypical mycobacteria was 17.4% which was substantially higher than that reported from other parts of India (0-8.4%) and Shankar *et al.* (1989) reported that 7.9% from his studied patients had atypical mycobacteria.

Choudhri *et al.* (1995) reported that of 80 patients who met the inclusion criteria of his study, 17 had definite NTM disease and 23 had probable NTM disease and Bollert *et al.* (1995) from Scotland reported that of all pulmonary isolates of mycobacteria in Lothian 53% were non-tuberculosis strains compared with 18% for Scotland outside Lothian. There are also other reports in them different rates of isolation of NTM from 1.3 to 16.7% have been reported by Pineda *et al.* (1997), Hosker *et al.* (1995) and Probst *et al.* (1994).

High incidence of Smear positive sputum samples (several times upper than Iranian National rate), existence of NTM in the soil of different parts of this province (Karbasciyan, 1992), resistance of NTM to the first line of anti drugs, emergence of diseases due to these species, seasonal sand storm and usage of narcotics, produce a complex situation in which determination of the prevalence of NTM found it's value.

Changes in clinician awareness leading to increased investigations, or laboratory methods leading to isolation and identification of previously unnoticed organisms, could play a role in this trend and studies have been published that support and refute The rates of NTM infection and disease are increasing, so the problem will likely continue to grow and become a far more important issue than current rates suggest (Marras and Daley, 2002).

Existence of Non Tuberculosis Mycobacteria in the environment and other underlying conditions including malnutrition, geographic situation, individual immunological status and many other known and unknown affecting factors make an individual prone to the infections with NTM and so there is a need to educate physicians about the diagnosis and management of NTM infections (Chudhari *et al.*, 1995).

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