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## Study of Bacterial Infections Among the Patients with Suspected Cutaneous Leishmaniasis

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**Abstract:** The main objective of this study was to evaluate the prevalence of secondary bacterial infections in the patients with cutaneous lesions. The patients admitted to leishmaniasis laboratory of faculty of health, Tehran university of medical sciences from October 2004 to June 2005 were subjected in this study. Clinical samples were analyzed using standard bacteriological and parasitological methods. One hundred seventy three patients were subjected to this study and leishmania was found in 84 (48.5%) cases. According to bacteriological experiments, 47 cases (55.9%) had been also infected by bacterial infections. The most prevalent bacterial isolates included group D *Streptococcus* (19.1%), *Enterococcus* spp. (19.1%) and *Staphylococcus aureus* (12.7%). The findings of current study indicated that the bacterial infections are still an important problem in the patients with cutaneous leishmaniasis and should be considered in treating these patients.

**Key words:** Cutaneous leishmaniasis, secondary bacterial infection, Iran

### INTRODUCTION

The leishmaniasis is considered as an endemic disease found in the most tropical and semitropical parts of the world. It has been estimated that 1 to 1.5 million people are infected with different species of this organism annually. Cutaneous leishmaniasis is one of the important endemic diseases in Iran (El-Safi *et al.*, 2004; Alrajhi and Fidsa, 2003; Shirzadi and Pedram, 2004). In a recent survey, 88 countries have been declared as leishmaniasis-endemic (Herwaldt, 1999).

As a rule, the cutaneous lesions in cutaneous leishmaniasis caused by the wet species are accompanied by many serositic secretions, which make them highly susceptible to infections caused by a variety of bacterial agents (El-On *et al.*, 1992). Many researchers in Iran, Ecuador and Mexico have reported the secondary bacterial infections among the patients with cutaneous leishmaniasis (Edrissian *et al.*, 1990; El-Safi *et al.*, 2004; Couppie *et al.*, 1997).

These secondary infections can lead to decrease the number of amastigotes in microscopic specimens provided from cutaneous lesions (Thomas *et al.*, 2004;

Edrissian *et al.*, 1990). Bacterial infections not only lead to the graveness and paroxysm of the disease, but also they have a bad influence on disease diagnosis (Andrade *et al.*, 1990; Van Der Vliet *et al.*, 2006).

The main aim of current study was to investigate the prevalence of secondary bacterial infections in the patients with cutaneous lesions admitted to Leishmaniasis Laboratory of Faculty of Health.

### MATERIALS AND METHODS

**Patients:** The patients with cutaneous leishmaniasis admitted to the Leishmaniasis Laboratory of Faculty of Health of Tehran Medical Sciences University during October 2004-June 2005 were subjected in this study.

**Sample collection and preparation:** The lesions were cleaned using sterile gauze and physiologic saline and disinfected five times in an outward circular motion with 70% alcohol-immersed cotton. Using a sterile lancet or sterile surgical blade, 2-3 mm long superficial incisions were made on the margins of the lesion and pressure was maintained with a finger to achieve hemostasis. Then

smears were studied by gram stain and direct microscopy techniques. Bacteriological culture then accomplished using streak method according to standard methods (Al-Jawabreh *et al.*, 2006; Antinori *et al.*, 2007).

The first smears of cutaneous leishmaniasis lesions were studied by the 100X lens of optical microscope and classified +1 to +5 on the basis of the number of amastigote parasites in microscopic field. 10-100 and 1-10 parasites in each microscopic field were considered as +5 and +4. 1-10 parasites in each 10 microscopic fields, 1-10 parasites in 100 microscopic fields and 1-10 parasites in 1000 microscopic fields was considered as +3, +2 and +1, respectively.

### RESULTS

According to the microscopic tests as well as the Giemsa staining of the smears, among 173 patients suspected of cutaneous leishmania referring to the Leishmaniasis Laboratory of the Faculty of Health and Institute of Public Health Research, it was found leishmania amastigote forms among 84 cases (48.55%). Among 84 positive leishmania cases, 47 cases (55.9%) having positive microbial culture on blood agar medium, had also bacterial infections including pathogen and opportunist bacteria as follows:

Nine cases of *Entrococcus* (19.1%), 9 cases of *Streptococcus* group D lansfield (19.1%), 6 cases of coagulase positive *staphylococcus* (*Staphylococcus aureus*) (12.7%), 6 cases of non hemolytic *Staphylococcus* (12.7%), 5 cases of some species of coryneform bacterium (10.6%), 5 cases of non hemolytic *Streptococcus* (10.6%), 4 cases of beta hemolytic

*Streptococcus* (8.5%), 1 case of *Pseudomonas aeruginosa* (2.1%), 1 case of *Proteus vulgaris* (2.1%), 1 case of *Nocardia* (2.1%) were found (Fig. 1). There were four patients infected with two bacteria species, one patient with three bacteria species and the others with one bacterium species. Among all people infected with positive leishmania and positive bacteria who referred to the leishmania laboratory of the faculty of health and institute of public health research from October 2004 to June 2005, there were 19 women and 28 men. The affluence of leishmaniasis and bacterial infection in the age range from 20 to 24 years old was more than other ages and there was no significant difference between the affluence of these infections in the age range below and above 24 years old.

In 47 cases, besides having lishman materials, they were also positive in bacterium. Among these cases, for the number of amastigote, 73.3% were +1, 6.6, 6.6, 10 and 3.3% were +2, +3, +4 and +5, respectively.

### DISCUSSION

The results of this study showed the difference in the affluence of a variety of aerobic bacteria in the lesions from which it was separated amastigote (24.2%) as well as in the cases from which it wasn't separated the parasite (5.2%).

The onset of organisms which make the infection happen in cutaneous leishmaniasis lesions is different in various endemic parts throughout the world, such that Zaini and Davies (1984) found out fungus infections such as Sporotrichosis in leishmaniasis lesions in Iran (Marquez and Lezama-Davila, 2003; Edrissian *et al.*, 1990; Zaini and Davies, 1984).

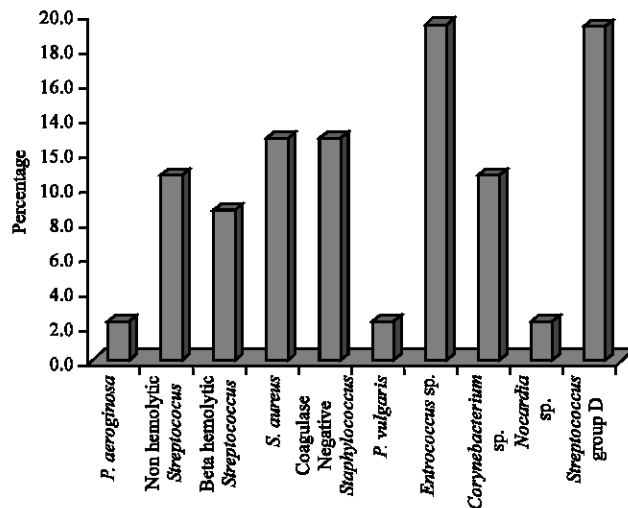


Fig. 1: Bacterial strains isolated from lesions of cutaneous leishmaniasis in the patients

Edrissian *et al.* (1990) showed the affluence of a variety of pathogenic bacteria such as beta hemolytic *Streptococcus* and coagulase positive *Staphylococcus* in cutaneous leishmaniasis lesions in Iran (Edrissian *et al.*, 1990). These bacteria are demonstrated to be present in our research too.

Patricia Angelica and Claudio Manuel (2003) detected in Mexico, the bacteria like *Staphylococcus aureus* and *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Enterococcus* and *Enterobacter* species in cutaneous leishmaniasis lesions.

Tabbara *et al.* (2004) discovered the alternative infection of *Listeria monocytogenes* in the leishmaniasis lesions.

In our study, it was observed the rate of bacterial *Staphylococcus* infections in the cases of positive leishmania more than other bacteria. After that, *Enterococcus* and *Streptococcus* group D lansfield occupied the second place. Beta hemolytic *Streptococcus* was also seen among the found pathogen bacteria in these lesions.

In this study, the infection rate of *staphylococcus aureus* was 12.7% and the one of hemolytic streptococcus was 8.5%. In one research the same infection rates (27.8 and 10.6%, respectively) reported by Edrissian *et al.* (1990) in Iran. The comparison of these findings to each other illustrates that the alternative infection of cutaneous leishmaniasis lesions with these pathogen bacteria has been reduced in the country. Since the beta hemolytic *Streptococcus* infections in children are important to the medicine, it is especially important to find and treat them in the CL lesions of endemic regions.

In further microscopic studies of the slides having both amastigote parasite and bacterium, in 80% of the cases, it was decreased the number of parasites and they were +1 and +2 and this often represents the negative effect of bacterium on the parasite. However, we only studied by using aerobic measurement methods and microscopic tests and gram staining of the smears provided from the lesions didn't specify anaerobic bacteria. Mainly, the results showed that the aerobic bacteria, particularly coagulase positive *Staphylococcus* and beta hemolytic *Streptococcus* were found among important pathogenic bacteria in the lesions suspected of leishmania in Iran and this must be taken into account when curing the disease.

The cutaneous leishmania is still considered as an important problem in Iran and furthermore, alternative infections of pathogenic or opportunist bacteria make this problem more complex and the bacteria make a negative effect on leishmania diagnosis and treatment. Thus, in cutaneous leishmania lesions having alternative infections, it is necessary that the diagnosis and the

treatment of the parasite are simultaneously accomplished by using suitable antibiotics. On the whole, health care should help this problem to be prevented and reduced and avoids alternative infections happening in the lesions.

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