CLINICAL STUDY

OROPHARYNGEAL BACTERİAL FLORA İN ASTHMATİC PATİENTS USİNG STEROİD THERAPY

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SUMMARY

Background: The aim of this study was to evaluate the differences in oropharyngeal microbiota in patients with asthma who were using inhaled corticosteroids (ICS) compared to those in healthy individuals.

Materials and Methods: A sample consisting of 100 oropharyngeal swabs was analyzed between April 2016 and May 2016. Due to recurrence, 1 asthmatic patient was excluded from the study. Common bacteriological culture techniques and the Vitek version 2.0 (Biomerieux, France) automatization systems were used to obtain the bacteriological diagnosis.

Results: The isolation of transitory flora bacteria in the control group was 88%, and 79.6 % in asthmatic group. There were no significant differences between the groups in respect of the cultured bacteria. Bacteria were recovered in 10/49 samples from the asthmatic patients and in 6/50 samples from the non-asthmatic subjects. In the asthmatic group all the growths were those of female patients and in the control group all were male. A statistically significant association was determined in respect of gender and the presence of growth bacteria in the asthmatic patients (p:0.042). Cultured bacteria were observed to increase with age in the control group (p:0.021). There was no significant association in respect of the use of ICS and the presence of growth bacteria in the asthmatic patients (p = 0.838).

Conclusions: E. coli, Klebsiella spp., Pseudomonas spp., Acinetobacter spp., Methicillin-resistant Staphylococcus aureus (MRSA) and Methicillin-susceptible Staphylococcus aureus (MSSA), Streptococcus pyogenes were found in the oropharyngeal swab specimens of the asthmatic subjects, as well as in some of the non-asthmatic group. Inhaled corticosteroids were not seen to have any obvious effect on oropharyngeal flora in the patients with asthma, which suggested that inhaled corticosteroids are safe for the treatment of bronchial asthma.

Keywords: Asthma, oropharynx, infection, corticosteroid

STEROİD TEDAVİSİ ALAN ASTIMLI HASTALARDA OROFARİNGEAL BAKTERİYEL FLORA

ÖZET

Giriş: Bu çalışmada, inhaler steroid alan astımlı hastaların sağlıklı bireylere göre orofaringeal mikrobial farklılıklarının değerlendirilmesi amaçlanmıştır.

Materyal ve Metod:100 orofaringeal svab örneği Nisan 2016 - Mayis 2016 tarihleri arasında analiz edildi. Bir astımlı hasta mükerrer olması nedeni ile çalışma dışı bırakıldı. Konvansiyonel bakteriyolojik kültür yöntemleri ve Vitek 2.0 (Biomerieux, Fransa) otomatize sistem bakteriyolojik tanıyı elde etmek için kullanıldı.

Sonuçlar: Kontrol grubunda geçici bakteriyel flora izolasyonu %88, astımlı grupta %79,6 idi. Üreyen bakteri açısından gruplar arasında istatistiksel önemli bir farklılık yoktu. Astımlı hastalarda 49 örnekten 10'unda, astımlı olmayan bireylerde 50 örnekten 6'sında bakteri üredi. Astımlı grupta tüm üremeler kadın iken kontrol grubunda hepsi erkek idi. Astımlı hastalardaki cinsiyet ile bakteri üremesi durumu arasında istatistiksel anlamlı bir ilişki saptandı (p:0.042). Kontrol grubunda üreme durumunun yaş ile yükseldiği gözlendi(p:0.021). Astımlı hastalarda bakteri üremesi durumu ile inhaler steroid kullanımı arasında anlamlı bir ilişki gözlenmedi (p=0.838).

Sonuç: Astımlı hastalar ile kontrol grubu bireylerin bazılarının orofaringeal svab örneklerinde E.coli, Klebsiella Spp., Pseudomonas Spp., Acinetobacter Spp., Metisilin-Dirençli Stafilococcus Aureus (MRSA), Methicillin-Duyarlı Stafilococcus Aureus (MSSA) ve Streptococcus Pyogenes ürediği gözlendi. İnhaler steroid kullanımının astımlı hastaların orofaringeal florası üzerine belirgin etkisi gözlenmemiştir. Böylece inhaler kortikosteroid kullanımının bronşial astım tedavisi için güvenli olduğunu düşünülmüştür.

Anahtar Sözcükler: Astım, orofarenks, enfeksiyon, kortikosteroid

INTRODUCTION

Asthma is a widely seen chronic inflammatory disease of the airways, with an ever-increasing prevalence and mortality rate [1].

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The World Health Organization (WHO) has stated that 235 million people per year are affected by asthma and there are approximately 250,000 asthma-related deaths per year worldwide [2]. Clinical studies of chronic respiratory diseases have mostly focused on the relevance of common bacterial infections, which are factors that play a part in the development of asthma. Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis have been

clinically proven to contribute to asthma exacerbations [1-2]. Chlamydophila pneumoniae and Mycoplasma pneumoniae have also been associated with asthma [3,4,5]. Human microbial flora varies between individuals and in different parts of the body. Oral microbiotas have been reported to be involved in upper respiratory tract infections which may also lead to atopic airway disease such as bronchial asthma [6]. Oral microbiota can trigger critical stages in bronchial asthma-like conditions [7], especially with the consideration that the earlier assumption of the lung as a sterile organ in normal health conditions is not true [8]. Previous studies have examined the relationship of airway microbial flora and asthmatic diseases, using samples from the lower respiratory tract such as sputum and bronchoalveolar lavage (BAL) [7,9,10]. However, there has been insufficient definition of the microbial inhabitants of the upper respiratory tract in asthma patients. A widely-used treatment for respiratory diseases such as bronchial asthma, which manifest with airway obstruction, is inhalation therapy [11]. Although these treatments are generally safe and well-tolerated in both adult and pediatric patients, recent studies have highlighted the hypothesis that ICS may alter oropharyngeal microflora in asthma patients [12].

The aim of this study was to determine any differences in the oropharyngeal microbial flora of asthma patients using inhaled corticosteroids (ICS) compared to a healthy control group.

MATERIAL and METHODS

1. Patient selection

Approval for the study was granted by the Ethics Committee of Sutcu Imam Medical Faculty, Kahramanmaraş, Turkey. The study included 49 asthma patients using inhaled corticosteroids who were diagnosed in the out-patient clinics of the Departments of Chest Diseases and Otorhinolaryngology. A comparison was made of these 49 asthma patients with 50 healthy volunteers. The absence of known diseases and normal pulmonary function was accepted as a status of normal health. Written informed consent for participation in the study was obtained from all participants.

The asthmatic group was corticosteroid-dependent and used inhaled corticosteroids for at least 3 months. Both the control and asthma group were chosen among patients whose oral hygiene are clean and have had no upper respitory infection minimum of 4 weeks.

2. Culture techniques

The sample of 100 oropharyngeal swabs was analyzed between April 2016 and May 2016. Due to recurrence, 1 asthmatic patient was excluded. Thus, evaluation was made of 49 asthmatic patients and a control group of 50 healthy individuals. The mean age of patients was 54.5± 14.5 years (range 27-81 years) in the asthma group and 46.8± 14.3 years (range 20-82 years) in the control group.

The oropharyngeal swabs specimens were obtained from the posterior wall of the oropharynx with a sterile cotton tipped swab. The swabs were smeared onto 5% sheep blood agar (RTA,Turkey), eosin-methylene blue agar (RTA,Turkey) and chocolate agar (RTA,Turkey) and were incubated aerobically at 37◦C for 24 hours. All samples were stained gram. For the identification of microorganisms, the Vitek version 2.0 (Biomerieux, France) automatization system was used in addition to conventional methods when necessary. Antibiotic sensitivity tests were applied in compliance with Clinical and Laboratory Standards Institute (CLSI) standards with the Kirby-Bauer disc diffusion susceptibility test.

3. Statistical analysis

Statistical analyses of the study data were performed using the Statistical Package for Social Sciences Statistical Software, version 21 (SPSS Windows Version 21, SPSS, Inc., Chicago, IL, USA). Descriptive data were stated as mean+-standard deviation (SD) and median (min-max) for continuous variables, and as number (n) and percentage (%) for categorical variables. The Mann Whitney U test was used for independent samples and the differences between categorical variables were analyzed with the Chi-square test. A value of $p<0.05$ was considered statistically significant.

RESULTS

The average age of patients in the asthmatic group was 54.5±14.5 years, (median 58 years, range 27-81 years) and in the non-asthmatic group, 46.8±14.3 years, (median 45.5 years, range 20-82 years). The mean age of the asthmatic patients was determined to be significantly higher than than that of the control group (p:0.009). Bacterial culture was positive in 10/50 (20.4 %) oropharyngeal specimens from the asthmatic patients and in 6/49 (12%) from the control group. The pathogens isolated in both groups are presented in Table 1.

The most frequent isolated bacteria in the asthmatic patient group were Klebsiella spp.(18.2%), and Escherichia coli (E.coli) (%57.1) was the most

frequent bacteria in the control group. In the asthmatic group all the growths were from female patients and in the control group all were male. A statistically significant association was determined in respect of gender and the presence of growth bacteria in the asthmatic patients (p:0.042). Cultured bacteria were determined to increase with age in the control group (p:0.021). The average age of patients with growth in the asthmatic patients was 58.3±16.3 years, (median 59 years, range 30-78 years) and the mean age of those with non-growth was 53.6±14.03 years, (median 56 years, range 27-81 years). The average age of the healthy individuals with growth in the control group was 59.17±17.27 years, (median 62 years, range 34-82 years) and the mean age of those with non-growth was 45.07±13.17 years, (median 43.5 years, range 20-65 years). The average age of those with growth was statistically significantly higher than those with non-growth in the control group (p:0.042).

Transient flora is composed of the following bacteria; Alpha-hemolytic Streptococci, Neisseria spp. and Corynobacterium spp. The rate of isolation of transitory flora bacteria in asthmatic patients was 79.6%, which was not significantly different from the rate of 88% in the control group (p:0.256). The results of bacteria growth according to groups and gender are presented in Tables 2 and 3.

The prevalence of oropharyngeal growth of different types of bacteria was no higher in the patients using ICS than in those not using ICS. No statistically significant relationship was determined in respect of ICS and the presence of growth bacteria in the asthmatic patients (p = 0.838). Inhaled corticosteroids were determined not to have any obvious effect on oropharyngeal flora in patients with asthma. The growth results according to the use of ICS are presented in Table 4.

Table 1: Microbiological findings of the oropharyngeal swab specimens

	Non asthmatic (N:50)	Asthmatic Patients (N:49)	Total
Klebsiella Oxytoca	_	2 (18.2%)	2 (10.5%)
Enterobacter cloaqa complex	_	2(18.2%)	2 (10.5%)
Pseudomonas spp.	_	1 (9.1%)	2 (10.5%)
Klebsiella pneumonia	_	1(9.1%)	1(5.3%)
Acinetobacter spp.	_	1(9.1%)	1(5.3%)
E. coli	4 (57.1%)	1(9.1%)	5 (26.3%)
MSSA	3 (42.9%)	1(9.1%)	4 (21%)
MRSA	_	1(9.1%)	1(5.3%)
Streptecoccus pyogenes	_	1(9.1%)	1(5.3%)
Total	7(100%)	11(100%)	19 (100%)

MSSA: Methicillin-susceptible Staphylococcus aureus; MRSA: Methicillin-resistant Staphylococcus aureus;

Table 2: Growth results according to groups.

	Asthmatic	Non-Asthmatic	Total
No growth (n %)	39 (79.6%)	44 (88%)	83 (83.8%)
Growth (n %)	10 (20.4%)	6 (12%)	16 (16.2%)
Total	49(100%)	50(100%)	99 (100%)

Table 3: Growth results according to gender

		Female (n%)	**Male (n%)**	**Total (n%)**
Asthmatic	No growth	12 (100%)	27 (73%)	39 (79.6%)
	Growth	0 (0%)	10 (27%)	10 (20.4%)
	Total	12 (100%)	37 (100%)	49 (100%)
Non-asthmatic	No growth	22 (78.6%)	22 (100%)	44 (88%)
	Growth	6 (21.4%)	0(0%)	6 (12%)
	Total	28 (100%)	22 (100%)	50 (100%)

Table 4: Growth results according to ICS use

		No growth	**Growth**	**Total**
Inhaled Corticosteroid	Patients not using ICS	17 (43,6%)	4 (40%)	21 (42,9%)
	Patients using ICS	22(56,4%)	6 (60%)	28 (57,1%)
Total		39 (100%)	10 (100%)	49 (100%)

ICS: Inhaled corticosteroids.

DISCUSSION

The aim of this study was to determine the status of oropharyngeal flora in patients with asthma, as this is an area which has not yet been clarified in understanding the role of the microbial population in upper respiratory tract conditions [13-14].

The results of this prospective study demonstrated data about the microbial diversity of the upper respiratory tract. Analysis of the microorganisms isolated from the oropharyngeal swab samples of diseased and control subjects indicated differing amounts of E.coli, Klebsiella spp, Enterobacter spp, Acinetobacter spp., Pseudomonas spp, MSSA and MRSA. Compared to the non-asthmatic group, the oropharyngeal airway microbiota of the asthma patients were more diverse but there were only a few differences between the asthmatic and non-asthmatic individuals. In the asthma patient group, although Klebsiella spp, Pseudomonas spp., E.coli, and MSSA were the most predominant populations, these were also detected in the healthy oropharynx of the control group subjects. In addition, Streptococcus pyogenes as member of the viridans streptococci. Haemophilus spp. was found in the non-asthmatic group but not in the asthma patient group.

In the non-asthmatic group, organisms from Streptococcus spp., Corynobacterium spp. and Neisseria spp. were determined at a higher rate than in the asthma patient group. Under normal circumstances, Streptococcus species are considered

to be oral cavity commensal bacteria. The results of the current study showing a lower rate of Neisseria spp. in asthmatic patients, suggests that Neisseria spp. is a significant indication of upper respiratory tract health.

Garzoni et al.[10] examined the oropharyngeal airway of patients with asthma and determined a predominance of Pseudomonas spp, Stenotrophomonas, and Lactobacillus.

Dang et al.[13] analysed the microbial populations of asthma patients and a healthy control group. In the asthma patient group, Firmicutes comprised 45.6% of the total microbial diversity detected in the oropharynx, whereas in the control group 44.0% of the oropharyngeal microbiota consisted of Proteobacteria. In both groups, the presence of Bacteroidetes, Fusobacteria, Actinobacteria, Cyanobacteria and unclassified bacteria was determined [13].

In a study by Park et al [15], a high abundance of Pseudomonas spp. and Lactobacillus spp. was determined in asthma patients, whereas in the healthy oropharynx of the control group subjects, there was a predominance of Streptococcus, Veillonella, Prevotella, and Neisseria of Bacteroidetes.

In this prospective study inhaled corticosteroids were not determined to have had any obvious effect on the oropharyngeal flora of asthma patients, which suggested that inhaled corticosteroids are safe in the treatment of bronchial asthma. Further studies are required to confirm these findings that ICS did not alter the oropharyngeal microbial population of the respiratory tract in asthmatic patients. To determine the effects of different types and doses of ICS on the microbial flora of the respiratory tract as a whole, there is need for longitudinal studies.

A limitation of the current study was that the standard culture methods used are recognized to have much lower sensitivity than molecular-based methods.

In conclusion, the results of this study showed a consistent distribution of microbiota between the oropharynx and the bronchi in asthma patients. As chronic respiratory diseases progress, it is known that the pathogenic role of commensal bacteria in the oropharynx may change. From these findings, it can be concluded that oropharyngeal airway microbiota are important to be able to make a full evaluation of asthma through an examination of the associations between different sections of the respiratory tract. Further studies are required for a better understanding of the pathogenesis of diseases at different anatomic sites of the respiratory tract.

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