Original Article

Estimation of salivary *Candida albicans* counts in asthmatic adult patients taking anti-asthmatic medication for 3–5 years

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Abstract

Background: Bronchial asthma is a chronic inflammatory disease of airways. The disease itself along with the principal medication used makes the oral cavity susceptible to most common opportunistic infection, i.e., oral candidiasis. There are many species of Candida causing oral candidiasis, but the most prevalent among them is *Candida albicans*. Hence, assessing *C. albicans* count in response to disease and its treatment is necessary. This enables us to educate asthma patients about side effects of medication and highlight the necessity for oral health care, thereby improving their quality of life.

Aims: The present study aims to evaluate the effects of asthma and its medication on *C. albicans* count in saliva samples of asthmatic adult patients taking medication for 3–5 years and compare *C. albicans* count in saliva samples among cases and controls.

Materials and Methods: Thirty asthmatic adults taking medication for asthma since 3–5 years' age ranging from 20 to 50 years and equal number of age- and sex-matched healthy participants were included in the study. In both groups, saliva was collected and inoculated on Sabouraud Dextrose Agar culture plates for estimation of *C. albicans* counts. *C. albicans* counts were assessed in colony-forming unit/milliliter.

Statistical Analysis: Mann–Whitney U-test and Fisher's exact *t*-test were used.

Results: The *C. albicans* count is significantly higher among asthmatics than healthy individuals.

Conclusions: The present study concludes that there is increased candidal growth among asthmatics as compared to their normal healthy counterpart.

Keywords: Asthma, *Candida albicans*, colony-forming units, corticosteroids, saliva, β2-agonist

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INTRODUCTION

Bronchial asthma is a chronic disorder characterized by airway inflammation, reversible airway obstruction and airway hyperresponsiveness.[1] Asthma is one of the most common diseases in industrialized countries, and there is convincing evidence, suggesting that its prevalence and morbidity are increasing despite better recognition.[2] This disease is characterized by increased responsiveness of airway to nonspecific stimuli, leading to symptoms such as wheezing, coughing, chest tightness and dyspnea.[3] Asthma is a growing public health problem affecting over 300 million people worldwide including almost all age groups.[4]

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Bronchial asthma is a common disease in India with a prevalence rate of about 1696 women and 1627 men per 100000 people in 2010 and is increasing.[5-7]

Apart from the morbidity caused by the disease *per se*, medication to prevent or treat asthma has more adverse effects on various body systems along with oral cavity.[8] The principle medication used in asthma is bronchodilators and β_2-agonists and inhalational therapy is the major way of drug delivery system in asthmatics.[4] Only 10%–20% of the dose from an inhaler reaches the lung and rest remains in the oral pharyngeal region. These remnant corticosteroids are exposed to oral tissues for the prolonged period of time, due to which oral defense system is suppressed.[10] Prolonged use of β_2-agonists is associated with diminished salivary production and secretion; in severe cases, it can lead to xerostomia, which alters the oral cavity environment.[11-13] Therefore, combined effect of these principal used medications in asthmatics reduces the local immunity and makes the oral cavity susceptible to opportunistic infections such as oral candidiasis.[14]

Oral candidiasis is by far the most common oral opportunistic infection in man.[15] The Candida genus is comprised over 150 species of asporogenous "yeast-like" fungi.[16] The majority of Candida species are unable to grow at 37°C and are, therefore, not normally associated with human colonization.[17-19] However, several species do persist as commensal microorganisms within humans in about 2%–70% of the general population and figures do vary depending on the population examined.[20,21] There are many species of Candida, but the most prevalent one which is recovered from the oral cavity, in both commensal state and in cases of oral candidiasis, is *Candida albicans* and it accounts for over 80% of all oral yeast isolates.[16,22] Common oral Candida species isolated from oral cavity are *C. albicans*, *Candida glabrata*, *Candida guilliermondii*, *Candida krusei*, *Candida parapsilosis*, *Candida pseudotropicalis* and *Candida stellatoidea*; these can act as opportunistic pathogens in immunocompromised host. A change from the harmless commensal existence of Candida to a pathogenic state can also occur following alteration of the oral cavity environment to one that favors the growth of Candida. The causes of such changes are the so-called predisposing factors for candidal infection (candidiasis) and most often these relate to a weakening of host immune defense.[23] The ability of Candida to convert from commensals to pathogenic from is attributed to its multiple virulence factors.[24]

Oral candidal colonization and candidiasis have recently received increased attention by the health-care providers and researchers alike, particularly following the widespread use of broad-spectrum antibiotics and immunosuppressant therapy in various diseases.[25] One of the common of such diseases is asthma, where inhalation therapy leads to local immunosuppression, leading to candidiasis, which can also precipitate asthmatic attack.[26]

Thus, evaluating the candidal superinfection in response to asthma and its treatment is necessary, to improve its management and improve the quality of life of asthmatics.

MATERIALS AND METHODS

Institutional ethical clearance was obtained and the present study was conducted in the Department of Oral Pathology and Microbiology, College of Dental Sciences, Davangere, along with the Department of Pulmonary Medicine, JJM Medical College and Hospital, Davangere.

A total of 60 participants comprising 30 asthmatics and 30 age- and sex-matched healthy controls were included in the study [Table 1] after obtaining an informed consent and a detailed case history. The mean age of the study participants was 34.23 years, and there were 22 females and 8 males in each group.

Sample collection

All the appointments for sample collection were given between 9.00 am to 11.00 am. Participants were informed not to eat prior giving sample. Before sample collection, participants were asked to rinse the mouth and sit upright and to swallow existing saliva. After a minimum of 5-min rest, unstimulated whole saliva was collected by asking the participants to spit the whole saliva into sterile container for 5–10 min, and samples were then carried immediately in a vaccine carrier with ice pack to the laboratory for further processing. The sample was immediately inoculated.

Table 1: Inclusion and exclusion criteria for cases and controls

Exclusion criteria (for both cases and controls)	Inclusion criteria	
	Cases	Controls
Individuals wearing oral removable prosthesis Poor oral hygiene Patients on long-term antibiotics Immunocompromised patients Chronic alcoholics and chronic smokers Febrile patients Pregnancy Anemia Chronic illness and systematic diseases such as diabetes and hypertension	Confirmed cases of asthmatic adults taking medication for 3-5 years. Age ranging from 20 to 50 years	Age- and gender-matched healthy individuals

Microbial procedure

After serial dilution of saliva sample, a standard dilution was taken and 50 μl of that standard dilution inoculated onto already prepared Sabouraud Dextrose Agar (SDA) culture medium using lawn culture method. One area of the culture plate was marked and known candidal organisms were inoculated [Figure 1]. The plates were incubated at 37°C for 24–48 h aerobically. After incubations, Candida colonies were examined morphologically, and germ tube test was done for confirmation of *C. albicans*. Colony counting was done using digital colony counter.

Statistical analysis

Obtained data were entered in Microsoft Excel and analyzed using SPSS version 21 (IBM Corp. Armork, NY: IBM Corp) using Mann–Whitney U-test, Chi-square test and Fisher's exact test.

RESULTS

In the present study, the cases with colony-forming unit (CFU)/ml <400 were considered as the carriers of Candida and >400 were considered as pathogenic.[27,28]

Assessment of salivary *Candida albicans* counts in colony-forming unit/milliliter in study groups

Among cases, 22 individuals showed candidal growth and 8 individuals did not show any candidal growth. Among controls, none of the individuals showed candidal growth [Table 2].

Assessment of salivary *Candida albicans* counts in colony-forming unit/milliliter among cases

There were 12 individuals in ≤400 CFU/ml group, and there were 18 individuals in ≥401 CFU/ml group. The median of ≤400 CFU/ml group was 0.00 and ≥401 CFU/ml group was 2600. There was a statistically significant difference between both the groups with regard to CFU/ml ($P = 0.00$) [Table 3].

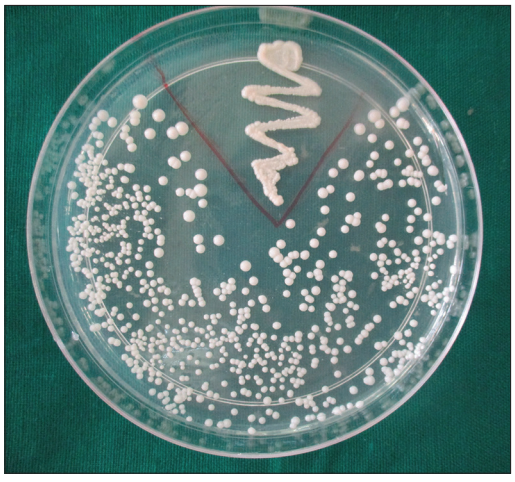

Figure 1: Colonies of Candida on Sabouraud Dextrose Agar with positive control

Intergroup comparison of salivary *Candida albicans* counts in median colony-forming unit/milliliter between asthmatics and controls

There was a statistically significant difference between cases and controls with regard to CFU/ml ($P = 0.00$) [Table 4].

Association of duration of disease with salivary *Candida albicans* counts in colony-forming unit/milliliter

For analysis, purpose duration of asthma was divided into 3, 4 and 5 years. Eleven (36.4%) were taking medication for 3 years, 11 (36.6%) were taking medication for 4 years and 8 (26.6%) were taking medication for 5 years. There was no significant association between duration of disease and CFU/ml of *C. albicans* counts ($P = 0.06$) [Table 5].

Association of doses of anti-asthmatic medication with salivary *Candida albicans* counts in colony-forming unit/milliliter

In the present study, patients were taking anti-asthmatic medication in the dose of 100 or 250 or 500 mg. There was no statistically significant association between doses of anti-asthmatic medication and CFU/ml of *C. albicans* counts ($P = 0.13$) [Table 6].

Association of severity of the disease with salivary *Candida albicans* counts in colony-forming unit/milliliter

In the present study, patients were having mild or moderate or severe disease. There was no statistically significant

Table 2: Assessment of salivary *Candida albicans* counts in colony-forming unit/ml in study groups

	No *C. albicans* growth	*C. albicans* growth	Total
Controls	30	0	30
Cases	8	22	30

C. albicans: *Candida albicans*

Table 3: Assessment of salivary *Candida albicans* counts in colony-forming unit/ml among cases

	n	Median	Mean rank	*U*	*P*
≤400 CFU/ml	12	0.00	6.50	0.00	0.00
≥400 CFU/ml	18	2600	21.50		

CFU: Colony-forming unit

Table 4: Intergroup comparison of salivary *Candida albicans* counts in median colony-forming unit/ml between asthmatics and controls

	n	Median	Mean rank	*U*	*P*
≤400 CFU/ml	12	0.00	6.50	0.00	0.000
≥401 CFU/ml	18	2600	21.50		
Cases	30	128.00	41.50	120.00	0.000
Control	30	0.00	19.50		

CFU: Colony-forming unit

Table 5: Association of duration of disease with salivary *Candida albicans* counts in colony-forming unit/ml

Duration of disease	CFU/ml		Total (%)
	≤400/ml (%)	≥401/ml (%)	
3 years	4 (36.4)	7 (63.6)	11 (100)
4 years	9 (81.8)	2 (18.2)	11 (100)
5 years	6 (75)	2 (25.0)	8 (100)

Chi-square test, $P=0.06$. CFU: Colony-forming unit

Table 6: Association of doses of anti-asthmatic medication with salivary *Candida albicans* counts in colony-forming unit/ml

Doses	CFU/ml		Total (%)
	≤400/ml (%)	≥401/ml (%)	
100 mg	11 (78.6)	3 (21.4)	14 (100)
250 mg	5 (41.7)	7 (58.3)	12 (100)
500 mg	3 (75)	1 (25)	4 (100)

Chi-square test, $P=0.13$. CFU: Colony-forming unit

Table 7: Association of severity of the disease with salivary *Candida albicans* count in colony-forming unit/ml

Severity of disease	≤400/ml (%)	≥401/ml (%)	Total (%)
Mild	11 (78.6)	3 (21.4)	14 (100)
Moderate	5 (45.5)	6 (54.5)	11 (100)
Severe	3 (60)	2 (40)	5 (100)

Chi-square test, $P=0.23$

association of severity of the disease with *C. albicans* counts in CFU/ml ($P = 0.23$) [Table 7].

DISCUSSION

Bronchial asthma is chronic debilitating disease, the effects of which are not always restricted to the pathogenesis and disease *per* se but also to the standard treatment protocol followed.[7] Pathogenesis involves genetically susceptible individuals on exposure to stimulus, or allergens show increased responsiveness leading to a hyperinflammatory reaction in the airway mucous membrane leading to a plethora of symptoms.[29] The treatment targets hyperresponsive inflammatory system using anti-inflammatory medication to avoid generalized immune suppressed state for prolong period of duration and local delivery is preferred, directly through nebulization. Any drug which is nebulized or inhaled will have their residual presence in the oronasal region for prolong duration.[30] Thus; the oral environment is susceptible to alteration due to nebulized drug usage. As in asthma, the various drugs used alter or suppress the immune system and are delivered by nebulization bringing them in contact with the oral cavity for a prolonged period of time which can lead to alteration in oral bioflora and facilitate the growth of opportunistic infections.[26]

With this background, our study was designed to estimate the prevalence of *C. albicans*, most common opportunistic organism in adult asthmatics, wherein nebulization is the choice of drug delivery and compares the CFU of *C. albicans* with age- and sex-matched controls.

To estimate the set objectives, 30 individuals diagnosed with asthma under medication who fulfilled the criteria previously mentioned and their age- and sex-matched healthy individuals were included in this study as cases and controls, respectively. CFU/ml of *C. albicans* of saliva was estimated among the cases and controls using standard methods on SDA. Among 30 cases, 22 (73.3%) of the individuals showed candidal growth and 8 (26%) showed no candidal growth, indicating that compared to healthy individual asthmatics under medication showed microfloral alteration facilitating candidal growth.

Among the asthmatics, the salivary *C. albicans* CFU/ml was analyzed and the median value obtained was 1000, and a statistically significant difference was demonstrated between two groups, i.e., cases and controls. Further among asthmatics with candidal growth, individuals having salivary Candida count in CFU/ml ≥401 were 18 and ≤401 were 12 (CFU/ml ≥401 was considered as pathogenic).[27,28]

All the above findings indicate that there is increased an incidence of *C. albicans* among asthmatics using anti-asthmatic medications such as corticosteroids and β_2-agonists. It was also noted that asthmatics showed CFU count similar to that of infective candidiasis, i.e., ≥401 CFU/ml of saliva.

Among above-mentioned results, it was noteworthy that although CFU counts were high and indicate candidal infection, none of the cases were symptomatic for oral candidiasis. This possibly suggests that the infection was subclinical or a microbiotic change, facilitating increased candidal growth without producing symptoms.

Numerous similar studies indicate and support the above-mentioned facts that there is increased candidal infectability among asthmatics using inhaled anti-asthmatic medication.[14,31-34] Various concepts for this finding have been explained which include an interplay between disease, medication and host.[16]

- Bronchial asthma facilitating candidal growth – Restricted salivary flow in asthmatics makes them susceptible to mouth breathing. Mouth breathing on a long duration induces xerostomia and an altered microbial environment of oral cavity both of which facilitate candidal adhesion and colonization among asthmatics
- Host factor affected by anti-asthmatic medication leading to increased candidal growth – Asthmatic

condition control is gained by medication which falls mainly under two categories, β_2-agonists which promote bronchial relaxation and corticosteroids which suppress the immune system.

Apart from the effects which ease the asthmatic condition, these drugs affect various systems and induce a favorable environment for candidal growth

1. β_2-agonists decreases the salivary flow which, in turns, leads to decreased salivary pH and also decreased biological active components such as amylase, salivary IgA, lysozyme and lactoferrin which favor the candidal growth
2. Corticosteroids suppress the immune system and induced alterations facilitate growth by the following mechanisms:
    a. Neutrophils are the first line of defense and it kills 20%–30% of Candida regardless of the number of Candida in the oral cavity. This activity is diminished in asthmatics due to suppressed immunity
    b. Intracellular killing of Candida by myeloperoxidase-hydrogen peroxide-halide system is greatly reduced due to immune suppression caused by corticosteroids used by asthmatics
    c. Activation of T-cells produces a wide range of lymphokines that can, in turn, modulate the functions of macrophages and other leukocytes. Interferon-gamma is the only lymphokine known to increase the microbicidal activities of macrophages, which is reduced in asthmatics due to suppressed immunity.

The present study revealed no candidal carriage in healthy individuals on the contrary to the other reports. Cohen *et al*.[35] reported the prevalence of yeast as 35% in the oropharynx in healthy volunteers. Hanan *et al*.[36] in their study reported an incidence of 30%–45% of oral candidiasis in healthy adults. Zaremba *et al*.[37] conducted a study on the oral carriage of Candida in healthy individuals and reported a prevalence rate of 63.1%. Samaranayake found *C. albicans* in the oral cavity in about 3%–48% of healthy adults.[38]

This stark variation could possibly be attributed to other variables which are to be considered, such as geographical variations, nature and size of the sample selected and also the method of sample collection. Estimates of the prevalence of Candida species as human commensal vary considerably according to the size of the sample, type of person sampled and method of sampling adopted.[39,40]

In the present study to eliminate the possible procedural error, a positive control was included in every culture plate. A colony of *C. albicans* stock culture confirmed by colony morphology on SDA and germ tube test was streaked in a corner along with the sample in the culture plate. In all the culture plates, the control streak produced candidal growth irrespective of sample yielding growth or not.

Among the asthmatics, the effect of duration of asthma, severity of disease and dosage of medication on salivary *C. albicans* count in CFU/ml was evaluated. There was no statistical evident association between the above parameters and *C. albicans* count. This suggests that the asthmatic individuals are prone to candidal carriage and/or infection irrespective of severity of disease, duration of disease or dosage of medication, and it is possible that they make the individual susceptible to candidal growth.

The exact effect of the above-mentioned parameters could not be evaluated from our study due to smaller sample and cross-sectional study design. A long-term follow-up study and larger sample can yield a clearer picture of the effect of the parameter on candidal susceptibility of asthmatics.

Hence, with our study, we could prove that there is an increased susceptibility of individuals with asthma to oral candidal growth. Although none of the asthmatic participants demonstrated clinically evident candidal lesion, candidal culture demonstrated levels of Candida equivalent to active candidal infection. These indicate oral environment in asthmatic facilitate candidal growth and colonization without any infection. Exact nature of change in the bacterial ecosystem, effects of the dose of medication, duration and severity of asthma needs further thorough evaluation.

CONCLUSIONS

The present study concludes that there is increased candidal growth among asthmatics as compared to their normal healthy counterpart.

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Conflicts of interest
There are no conflicts of interest.

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