Oral carriage of cariogenic bacteria and Candida albicans in asthmatic adults before and after anti-asthma medication: A longitudinal study

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Abstract

Background: Antiasthmatic medications such as β2 agonists and corticosteroids have shown potential side effects such as increased caries risk and oral candidiasis. Studies evaluating microbial changes in adult asthmatics are very scanty in the literature. The present study aimed to evaluate the effects of asthma and its medication on cariogenic bacteria and Candida albicans in adult asthmatics.

Aim and Objectives: Our aim was to evaluate and compare counts of Streptococcus mutans (SM) and lactobacilli in plaque and C. albicans in saliva samples of adult asthmatics with controls and during the course of medication longitudinally.

Methodology: Samples were collected from twenty recently diagnosed asthmatic adults and twenty controls for estimation of microbial counts at baseline and at 3rd and 6th month after initiation of medication among cases.

Results: Asthmatics at baseline had higher microbial counts than controls, but the difference was not statistically significant. Comparison between asthmatics at baseline and 3rd month after initiation of medication showed an increase in counts of SM, lactobacilli and decreased C. albicans counts though the difference was not significant. Comparison between asthmatics at baseline and 6th month and also between 3rd and 6th month showed significantly increased counts of SM. Although there was an increase in counts of lactobacilli and decreased C. albicans counts, significant results were not noted. Asthmatics showed increased microbial counts than controls overall.

Conclusion: Asthmatics were found to have higher microbial counts than controls at baseline. Increase in SM and lactobacilli counts in asthmatics after medication emphasizes the need to monitor these patients regularly.

Keywords: Asthma, Candida albicans, colony forming units, corticosteroids, lactobacilli, microbial counts, plaque, saliva, Streptococcus mutans, β2 agonists

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INTRODUCTION

Bronchial asthma is a chronic airway disorder. Very few studies evaluating the microbial changes in the oral cavity of asthmatic subjects have been reported.β2 agonists have shown potential side effects such as hyposalivation and altered salivary composition, leading to increased caries risk. Use of corticosteroids increases the risk of oral candidiasis. Drug treatment in asthmatics has a stronger effect on oral health than the disease per se. Evaluating microbial changes in response to disease and its treatment is necessary to emphasize oral hygiene.

The present study aimed at evaluating the effects of asthma and its medication on cariogenic bacteria and Candida albicans. Our aim was to estimate and compare counts of Streptococcus mutans (SM), lactobacilli in plaque and C. albicans in saliva samples of healthy controls and asthmatic adults before and after initiation of standard prescribed medication.

METHODOLOGY

A total number of forty subjects with twenty each in case and control groups were included in the study. Recently diagnosed twenty cases of asthmatic adults confirmed by a pulmonologist in the age range of 20–45 years, reporting to Department of Pulmonary Medicine, J.J.M. Medical College, were included in the study. An equal number of age- and sex-matched healthy volunteers were included as controls. Informed patient consent was obtained from every subject for inclusion in the study and for performing tests. Institutional ethical clearance was also obtained. Individuals on long-term antibiotic therapy or any medication that would alter the oral microbial environment, diabetes mellitus, pregnancy, malnourishment, obesity, denture wearers, smokers, alcoholics and individuals having decayed missing filled teeth >15 were excluded from the study.

Supragingival plaque samples were collected using a sterile toothpick from cervical third of lingual surface of mandibular first or second molar of either side, and toothpick was immediately immersed in 1 ml of phosphate-buffered saline and carried in a vaccine carrier with ice pack to the laboratory (Figure 1a and b).

For a collection of saliva samples, subjects were advised not to eat 1 h before saliva sample collection. Subjects 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 subjects to spit the whole saliva into a sterile container for 5–10 min (Figure 2), and samples were then carried immediately in a vaccine carrier with ice pack to the laboratory for further processing. As far as possible sample was immediately inoculated. Subsequently, samples were collected in asthmatics at an interval of 3 months, and 6 months after, initiation of medication and microbial counts were assessed for SM, lactobacilli and C. albicans. Baseline samples of plaque and saliva were collected from age- and sex-matched controls for microbial counts. All samples were collected at 1–2 pm before lunch. After serial dilution, a dilution of 10⁻¹ was selected for inoculation. Fifty microliters of diluted sample was inoculated using sterile L-shaped glass rods by spread method for lawn culture (Figure 3).

Samples were inoculated on different media as follows: samples were inoculated on mitis salivarius bacitracin agar medium (MSB) for SM (Figure 4a) and in Rogosa selective lactobacilli agar (RSL) media for lactobacilli (Figure 4b). The MSB agar plates and RSL plates were incubated in candle jars at 37°C for 2 days anaerobically. Similarly, 50 μl of diluted sample was inoculated on Sabouraud's Dextrose Agar for C. albicans and were incubated at 37°C for 3–5 days aerobically (Figure 4c).

![Figure 1: Collection of plaque from the patient using wooden toothpick (a) and toothpick dipped in the phosphate buffer saline (b)](image-url)
For SM, confirmation was done by characteristic colony morphology, catalase test and mannitol fermentation test. All colonies on RSL medium were considered to be lactobacilli [Figure 4b], and colony morphology was also studied. Gram’s staining was done for both SM [Figure 5a] and lactobacilli [Figure 5b]. For confirmation of C. albicans, Gram’s staining [Figure 6], Periodic acid–Schiff stain [Figure 7] and germ tube test were done. All colonies formed were counted using digital colony counter. The counts were tabulated as colony-forming units per milliliter (CFU/ml). Microbial quantification was performed using the following formula:

\[
\text{Number of colonies} \times \frac{\text{Dilution of plate}}{\text{Volume of sample}} = \frac{X}{\text{ml}}
\]

Where X = Quantification of microbes in CFU
Wilcoxon signed-rank test was used for intragroup comparison within asthmatics and Mann–Whitney test was used for intergroup comparison between asthmatics and controls.

RESULTS

A total number of forty subjects comprising twenty asthmatics and an equal number of age- and sex-matched healthy controls with the age range of 20–45 years were included in the study. Cases were followed longitudinally for a period of 6 months [Table 1].

The mean CFU/ml counts of SM, lactobacilli and C. albicans among controls and asthmatics at different time intervals were as shown in Table 2.

Comparison of asthmatics without medication (at baseline) and at 3rd month after initiation of medication revealed the mean difference of SM, lactobacilli and C. albicans counts as 18.1 ± 203.7, 43.7 ± 230.4 and 18.1 ± 55.4, respectively. The difference for SM (P = 0.41), lactobacilli (P = 0.27) and C. albicans (P = 0.10) counts in CFU/ml was not statistically significant [Table 3].

Comparison of asthmatics at 3rd month after initiation of medication and asthmatics at 6th month showed the mean difference for SM, lactobacilli and C. albicans counts to be 27.9 ± 41.6, 4.3 ± 168.1 and 13.6 ± 48.6, respectively. The mean difference for SM was statistically significant (P = 0.009), whereas the difference for lactobacilli (P = 0.07) and C. albicans (P = 0.11) counts was not significant [Table 3].

Comparison of mean microbial counts between asthmatics and controls revealed statistically insignificant result. However, SM, lactobacilli and C. albicans counts were found to be higher in asthmatics when compared to controls overall [Table 4].

DISCUSSION

The present study was conducted to evaluate microbial counts in adult asthmatics when compared to healthy controls and also during the course of medication among cases. The prevalence of SM, lactobacilli (L) and C. albicans (C) in controls was 50%, 80% and 5% respectively in this study. Hirasawa and Takada[9] and Wu et al[10] detected SM in 58.3% and 75.45% in caries-free children and adults, respectively. Sigurjóns et al. found SM in 96.7% and lactobacilli in 62% of the subjects in their study.[9] Samaranayake found the oral carriage of C. albicans in about 3%–48% of healthy adults.[10]

The prevalence of SM, lactobacilli and C. albicans was 55%, 55% and 10%, respectively, in asthmatics in the present study. Dubus et al. found 10% prevalence of C. albicans in asthmatics.[11]
When microbial counts were compared between asthmatics at baseline (without medication) and controls, though the counts were higher in cases than controls, the difference was not statistically significant. Our result could not be compared with any other published data as we could not find any other data pertaining to asthmatics before medication. It remains uncertain whether higher counts in asthmatics are due to disease *per se* or any other unknown factors.

Comparison of SM counts in asthmatics at baseline and 3rd month showed no difference. This could be attributed to the oral prophylaxis performed just before initiation of medication and time taken by microbes to recolonize themselves. However, comparison of SM counts at 3rd month and 6th month and also between baseline and 6th month showed statistically significant results (*P* = 0.009 and *P* = 0.023, respectively). This could be due to an increase in microbes after recolonization or in response to medication use.

Use of medications such as β₂ agonists has shown to cause a reduction in salivary flow rate, altered salivary composition, reduced pH, reduced buffering capacity and increase in cariogenic bacteria which may be due to the medications containing fermentable carbohydrates and sugar.[2,4]

The decreased salivary flow and altered salivary composition may be due to other reasons other than β₂ agonist use. In a study, subjects were mainly treated with inhaled corticosteroids and showed reduced salivary flow rates.[12]

In the present study, lactobacilli counts did not show a statistical difference between cases and controls though counts were higher in asthmatics. Comparison of lactobacilli counts among asthmatics at different intervals also showed no statistical difference. However, counts were increased at 3rd month and 6th month after initiation of medication compared to baseline. Possible reasons for this increase would be the use of medications containing lactose or fermentable carbohydrates and sugar causing acidic pH environment[13–15] and onset of root caries and deep dentinal caries which favor colonization of lactobacilli.[16,17]

Brigie *et al.* observed no significant difference in concentration of SM between asthmatic children (7–14 years with 2-year medication) and controls. They concluded that antiasthma drugs do not cause hyposalivation and thus do not favor increased concentration of SM.[18] On the contrary, Venkatesh observed SM to be higher in asthmatic children (5–12 years), whereas lactobacillus was similar in both the study groups[19] which is in accordance with the present study. However, our study subjects were adults.

Increased dental biofilm and salivary SM levels and no difference in lactobacilli levels were also noticed by Botelho *et al.* in asthmatic children (3–15 years). They observed a correlation between SM levels and treatment duration. Authors concluded that asthma should be considered as risk factor caries experience, especially in older children (11–15 years) since it increases levels of SM.[38] Children using medications three times a day and in combination with corticosteroids had increased levels of SM and lactobacilli compared to other asthmatics.[21]

A negative correlation between duration of medication and a positive correlation between duration of disease and salivary SM levels was noted in young asthmatics (6–19 years). Authors concluded that asthma through its disease status and its medication carries some risk factors for caries development in these patients.[22]

Effects of inhaled corticosteroids and long-acting sympathicomimetics on dental health in adult asthmatics (20–55 years) were studied by Karova and Christoff, and they observed a significantly higher decayed, missing and filled teeth index among asthmatics compared to the controls.[23]

Stensson *et al.* in their study in asthmatics (18–24 years) on long duration of medication (13.5 years) concluded that initial caries was more common in asthmatics compared to the controls.[11]

Candidal counts did not show significant difference among cases and controls at baseline in the present study. Among three cases positive for candida, one presented with
Our data were limited to microbial quantification as a possible indication for higher caries risk. However, analysis of salivary flow rate, composition, buffering capacity and pH should also be considered in future studies. Multifactorial etiology of dental caries is a well-established fact. Just the mere increase in microbial counts does not necessarily put asthmatics at increased caries risk. Nevertheless, the oral physiological changes and changing oral environment may also result in fluctuation of bacterial counts making it difficult to study the effects of drugs and the disease itself.\[13,20\]

Our study has shown that definite microbial changes do occur in asthmatics which further get modified by antiasthma medications, thus emphasizing the need for patient education on proper oral health care, technique of drug use, noncariogenic dietary regimen, fluoride supplementation and regular checkups. This would reduce oral health problems in asthmatics, thereby improving the quality of life.

CONCLUSION

Based on the present study, it can be concluded that subjects with asthma have slightly higher microbial counts than controls at baseline. Whether this points to a tendency of the disease to modify or increase the bacterial counts or is a chance occurrence needs to be studied in a larger population study. A significant increase in microbial counts of cariogenic bacteria, especially SM was found in asthma patients after initiation of medication. Although lactobacilli counts were higher after initiation of medication, results were not significant. Simultaneously, no significant change was found in the number of opportunistic candidal counts. Whether a direct cause to effect relation exists with the medication itself causing an increased bacterial count or an indirect effect of the medications on salivary function could not be ascertained in this study.

Asthmatic patients commonly face oral problems due to lack of awareness regarding oral hygiene measures. Special preventive and educational measures will be required to prevent dental caries and other oral diseases in asthmatic patients.

Limitations and future scope

Longitudinal studies at further time intervals are needed. Determining the microbial counts among controls in a corresponding time pattern as the case group could also be considered to take into account the possible physiological variations. Conducting the study with larger sample size in future can examine the effect of the disease itself rather than the treatment.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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