The effect of inhaled corticosteroid on oral conditions among asthmatic children

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ABSTRACT

Background: Inhalation therapy has been employed as the mainstay of the treatment in chronic respiratory diseases such as asthma, Patients who taking asthma medication may be at risk of many health problems including oral health .The purpose of this study was to assess the local effect of ICS on oral tissue by measuring *Candida albicans* count colonies in saliva among12 years old asthmatic children who were collected from AL- Zahra Center Advisory for Allergy and Asthma, and compares them with non asthmatic children of the same age and gender.

Material and Methods: The total sample involved sixty children of 12 years old, thirty asthmatic children who received medium dose of ICS/day (200-400 microgram/day) for 2 years and 30 non-asthmatic children. The unstimulated saliva was collected under standard condition and then analyzed for *Candida albicans* colony counts assessment.

Results: The mean rank of the colony counts were found to be higher among asthmatic than non- asthmatic children with statistically significant difference (P<0.05). Concerning each gender, the results illustrated that the difference for boys was statistically non significant (P>0.05), while for girls the difference was statistically significant (P<0.05). Concerning gender differences, data analysis showed that the mean rank of Colony counts were found to be higher among girls than boys in both groups with statistically non-significant difference (P>0.05)

Conclusions: The findings of the present study showed that the asthmatic disease and ICS treatment play an important role in elevation of the *candida* prevalence in oral cavity.

Keywords: asthma, *candida*, inhaled corticosteroid. (J Bagh Coll Dentistry 2015; 27(1):169-174).

INTRODUCTION

Inhaled corticosteroid (ICS) therapy is commonly used for treatment of allergic phenomenon such as asthma [(1)]. Patients taking asthma medication may be at risk of oral candidiasis [(2)]. The local mode of action of ICS lead to local adverse effects of ICS on oral tissue [(3, 4)], the one most common local adverse effect of ICS is oral candidiasis [(5)]. Previous studies which regarding the incidence of *Candida* in inhaled corticosteroid treated patients reported divergent results. Dubus et al. [(6)], Ellepola and Samaranayake [(7)], Fukushima et al. [(8)], and Fukushima et al. [(9)], suggested that the inhaled corticosteroids in addition to other host factors could potentially increase the risk of oral candidiasis.

On the other hand, Komiyama et al. [(10)], reported that the percentage of *Candida* were 43.33% in thirty asthmatic children of 4 -12 years old who treated with corticosteroids for period range between 2-48 months and were 30% in thirty control children with no significant difference between them and no correlation was observed between the number of colony-forming units of *Candida* per ml of saliva (CFU/ml), dose of medication and time of treatment. While Adams et al. [(11)], Rachelefsky et al. [(12)], and van Boven et al. [(13)], had been conducted the association between ICS and the occurrence of oral candidiasis regardless of the dose.

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Söderling et al. [(14)], and Cortelli et al. [(15)], reported that females tended to have higher *Candida* prevalence than males. While Lotti et al. [(16)], and Reynaud et al. [(17)], found no relationship between the *Candida* counts and age or gender.

Saliva is a diagnostic and monitoring method for many infectious diseases[(18)], saliva contain a large numbers of proteins that participate in protection of oral tissue in addition to several peptides with fungal killing activity that had been identified [(19)], in this way saliva determined the composition of the oral micro flora and controlled oral health[(20)], by maintaining the integrity of the oral hard tissues and soft tissues through the salivary immune and non-immune defense proteins [(21-23)].

This study was performed to provide greater visibility to the harmful effects of beclamethasone inhaler on oral pictures among asthmatic children aged 12 years in comparison to control group to evaluate the association between ICS, asthma and oral health which include salivary *Candida* prevalence.

MATERIALS AND METHODS

In the present investigation, the study group included 30 asthmatic children aged 12 years old who received medium dose of ICS/day (200-400 microgram/day) for 2 years, they were examined in AL- Zahra Center Advisory for Allergy and Asthma during the period from 20 December 2013 till the end of March 2013. The control group included 30 non asthmatic children who possess as much as similarity as possible to the

study group with regard to age, gender, social structure and geographic position except in asthmatic condition. Both groups should not possess any systemic disease that could effect on salivary analysis.

The collection of unstimulated saliva was performed under standard condition according to the instructions cited by Navazesh and Kumer [24] and immediately placed it in ice box until reach the microbiological laboratory. At the Ministry of Science and Technology fungal laboratory, each salivary sample of control and study group was dispersed using vortex mixer for 1 minute and then tenfold dilutions were performed by transferring 0.1 ml of each suspension from each tube of the control and study to 0.9 ml of sterile phosphate buffer saline (pH 7.0), then from dilution 10^{-1} ,10^{-2} ,10^{-3} salivary samples, 0.1 ml was taken and spread on the Sabourauds dextrose agar (SDA), the plates were incubated aerobically for 48 hr at 37°C, then the colony–forming unit per milliliter was counted (CFU/ ml) for all the plates.

The Identification of *Candida albicans* (*C.albicans*) can be done through: (A) Colony morphology: Colonies of *C. albicans* appeared smooth creamy in color with a yeast odor and typically medium size (1.5-2) mm diameter which later developed into high convex, off-white larger colonies after 2 days [25,26]. (B) Gram stain: A small inoculum from a discrete, singly isolated colony was picked up from SDA plates separately under sterilized conditions and subjected to gram's stain according to Koneman et al. [27].(C) Germ tubes formation: Very small inoculums from isolated colonies was suspended in 0.5 ml of normal human serum. The inoculated tubes were incubated at 37°C for 3hr. after incubation, a drop of yeast suspension was placed on clean microscopic slide covered with cover slip and examined under low power magnification [28].

Intra and inter calibration were performed to overcome any problems that could faced during the research. Statistical analysis and processing of the data were performed using the SPSS version 19. After exploring the data, it had been found that the data were not normally distributed. The non-parametric test Mann-Whitney test was utilized for the parameters of data which were not normally distributed, in this test the median and mean rank were used to determine and analyze the differences between the study and control groups.

The confidence level was accepted at the level of less than or equal to 5%. The highly confidence level was accepted at the level of less than or equal to 1%.

RESULTS

The description of the samples is illustrated in Table (1). The *C. albicans* carrier group of asthmatic children was represented by 60%, while the *C. albicans* carrier group of non-asthmatic children was represented by 33.33%. The asthmatic children without *C. albicans* were represented by 40%, while the non-asthmatic children without *C. albicans* were represented by 66.67%.

Identification of *C. albicans*:

(A)Colony morphology: Colony of *C.albicans* appeared smooth, creamy in color with yeast odor and typically medium sized (1.5-2 mm) diameter which later develop into high convex, off- white large colonies after 2 days. Figure (1)

(B)Microscopic examination: The slide was examined under light microscope, the rounded or oval yeast cells were stained gram positive (gram staining test). Figure (2)

(C)Germ tube formation: Under light microscope (100 x magnification), the presence of germ tubes were the characteristic of *C.albicans*. Figure (3)

The differences in *C.albicans* $x10^2$ quantities (CFU /ml) between asthmatic and non- asthmatic children are demonstrated in Table (2). Results reported that the mean rank of the colony counts were found to be higher among asthmatic than non- asthmatic children with statistically significant difference (Mann Whitney=311.5, Z= -2.180, P=0.029). Concerning each gender, the results revealed that the difference for boys was statistically non significant (Mann Whitney=99, Z= -1.130, P=0.259), while for girls the difference was statistically significant (Mann Whitney=58, Z= -2.087, P=0.037).

Table (3) shows comparison between genders in asthmatic and non-asthmatic children, the mean rank of colony counts were found to be higher among girls than boys with statistically non-significant difference (P>0.05).

Table 1: Description of the experimental samples

Groups	With *Candida* (carrier)		Without *Candida*	
	No.	%	No.	%
Asthmatic	18	60	12	40
Non-asthmatic	10	33.33	20	66.67

Table 2: Difference in salivary *C.albicans* x 10^2 quantities (CFU /ml) between the asthmatic and non- asthmatic children

Variables	Genders	Asthmatic				Non- asthmatic				Difference		
		No.	Median	Mean± S.D.	Mean rank	No.	Median	Mean±S.D.	Mean rank	U test	Z-value	p-value
C. albicans x10^2 (CFU /ml)	Boys	16	2	24.25±69.07	18.31	16	1	1.81±2.83	14.68	99	-1.130	0.259
	Girls	14	1	15.21±29.97	17.36	14	0	1±2.94	11.64	58	-2.087	0.037*
	Total	30	1	20.03±53.77	35.12	30	0	1.43±2.86	25.88	311.5	-2.180	0.029*

(Non Sig. at P>0.05;*S: Sig. at P<0.05 between asthmatic and non- asthmatic children)

Table 3: Genders difference for asthmatic and non-asthmatic children

		Asthmatic						Non- asthmatic					
Variables	Genders	No.	Median	Mean rank	U-test	z-value	p-value	No.	Median	Mean rank	U-test	z-value	p-value
C. albicans x10^2 (CFU)	Boys	10	10.5	9.4	39	-0.090	0.929	7	1	5.14	8	-0.610	0.542
	Girls	8	11.5	9.62				3	2	6.33			

(Non Sig. at P>0.05 between asthmatic and non- asthmatic children)

Figure 1: *C. albicans* colonies on SDA (15x magnification)

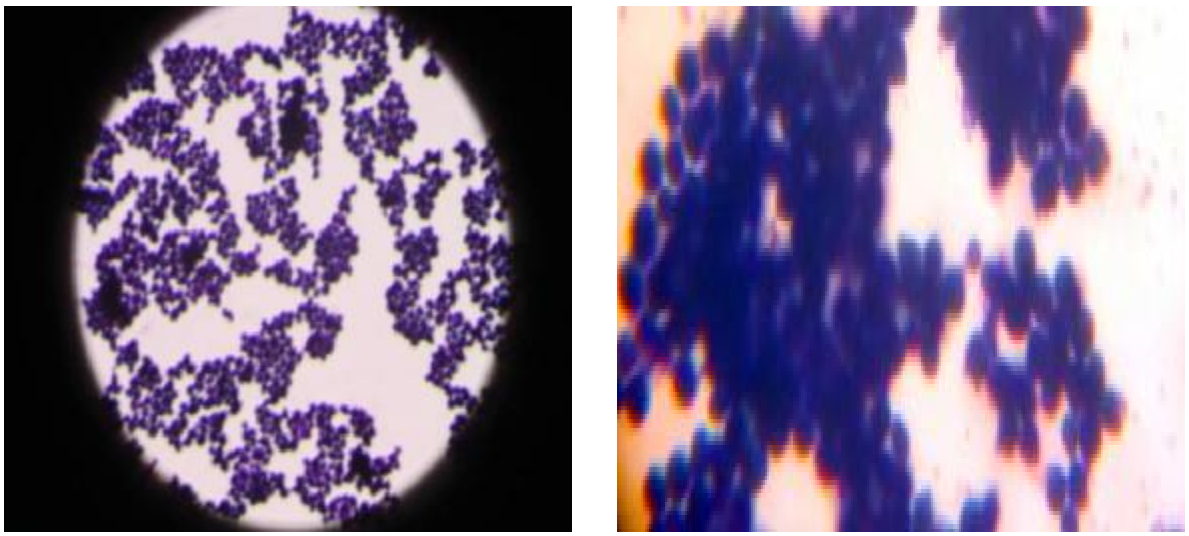

Figure 2: Gram's stain of *C. albicans* colonies showing gram positive stains (100x magnification).

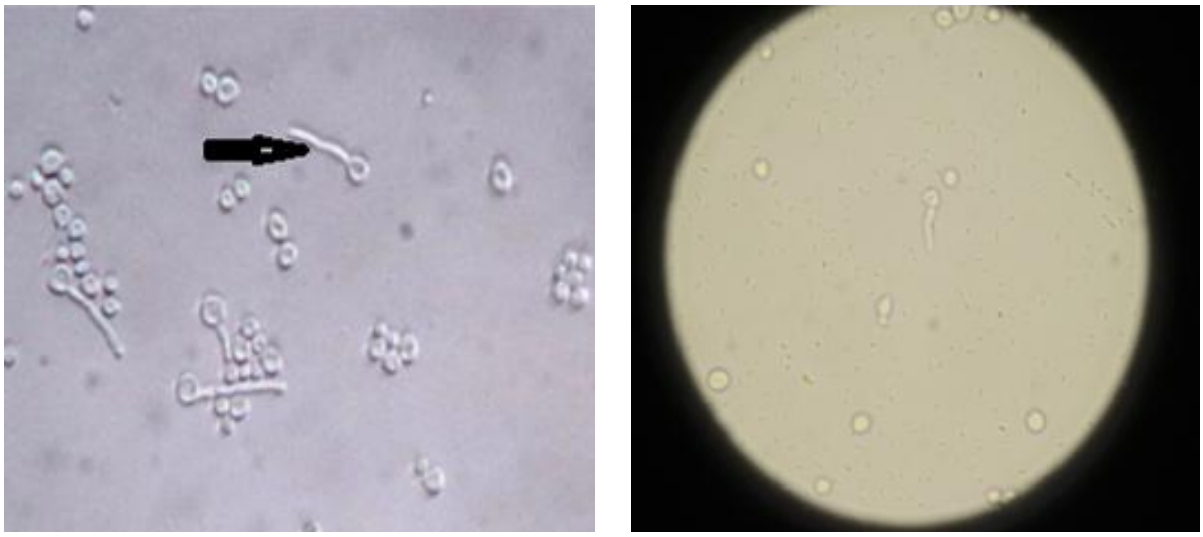

Figure 3: Germ tube formation

DISCUSSION

Data analysis of the current study concluded that the percentage of *C. albicans* in carrier group of asthmatic children were higher than non-asthmatic children and the mean rank of the colonies counts were found to be higher among asthmatic than non- asthmatic children with statistically significant difference. These finding were in Angreement with Dubus et al. [6], Ellepola and Samaranayake [7], Fukushima et al. [8], Adams et al. [11], Fukushima et al. [9], Rachelefsky et al. [12], and van Boven et al. [13],and in disagreement with Komiyama et al. [10], generally it is difficult to compare the prevalence of *C. albicans* (CFU/ml) reported by different studies in literature with that of the present study, this might be due to variability in the type and dose of ICS used, frequency of the use of medication, patient compliance with instructions for administration, duration of drug therapy and the mode of delivery (direct or with spacer) or due to the hospital-based population of children with moderate asthma or due to methodologic issues such as study design, sample size, age, gender and length of observations .The findings of the current study can be explained by the fact that the mechanisms by which ICS cause local adverse effects have appear to be related to the deposition of the active ICS into the oral cavity, since the major proportion of the inhaled drug is retained in the oral cavity and oropharynx and only 10-20% reach to the lung during drug administration [29], so it might interfere with normal physiology of oral tissues[3], and it might interferes with the cell-mediated immunity and involve the inhibition of normal host defense functions of neutrophils, macrophages and T lymphocytes at the oral mucosal surface and the esophagus that cause local immunosuppressant in oral cavity[7,30], the decreased efficiency of the immune system may in turn allow an opportunistic infection of *Candida* [31], or ICS might cause an increase in salivary glucose levels, which stimulate growth, proliferation and adhesion of *Candida* to oral mucosa [32] and these events accompanied by acid production and a significant concomitant reduction in pH to very low levels [33,34].

However, the reduced pH levels may potentiate *Candida* virulence by enhancing acidic proteases and phospholipases enzymes of the yeast [35]. In addition the results of the this study might be due to the presence of some predisposing factors in asthmatic children who treated with ICS that influence *Candida* carriage more than the non- asthmatics which include the lack of salivary flushing action and absence of antifungal salivary constituents such as lactoferrin and lysozyme which was attributed to underlying disease and medication intake, the deficiency of salivary IgA which caused by ICS[36,9], the significant alterations in the microbial flora which occur with ICS, the intake of medication at night before going to bed due to poor patient awareness, no oral hygiene measures after medication, the diminution of salivation and lack of masticatory movements during the night might increased *C. albicans* prevalence which predisposed to candidosis [37] .

Furthermore this study showed that the prevalence of colonies counts was higher among girls as compared with boys among asthmatic and non asthmatic children with non-significant difference, these results were in agreement with Söderling et al. [14], and Cortelli et al. [15] and in disagreement with Lotti et al. [16], and Reynaud et al. [17], the disagreement with these studies could be due to ethnic differences, sample size and

selection differences. This result might be attributed to the lower salivary secretion in females than males even after controlling other variables such as underlying disease and medications [38], which might be attributed to different volumes of salivary glands as described by Inoue et al. [39].

In conclusion this study found clinically relevant increased *Candida* prevalence in asthmatic patient who received ICS treatment. This study stresses the need for patient education and inhalation instruction, in order to avoid this local adverse effect, thereby increasing therapy effectiveness and patients' quality of life.

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الخلاصه

الخلفية :ان علاج الاستنشاق يستخدم كدعامة اساسية لعلاج الأمراض التنفسية المزمنة مثل الربو. ان المرضى الذين يتناولون أدوية الربو قد يكونون عرضة للكثير من المشاكل الصحية بما في ذلك صحة الفم. ان الهدف من هذه الدراسه هوتقييم تاثير استنشاق كورتيكوستيرويد (ICS) على انسجه الفم وذلك بواسطه عد مستعمرات المبيضات في لعاب الأطفال المصابين بالربو بعمر12 سنة الذين يراجعون مركز الزهراء الاستشاري للحساسيه والربو ومقارنتهم مع الأطفال غير المصابين بالربو من نفس العمر والجنس.

المواد والطرق: تتكون العينة الكلية من 60 طفلا بعمر12 سنة (30 مصابين بالربو والذين حصلوعلى جرعة متوسطة من ICS (200-400µg / اليوم) لسنتين و 30 طفلا غير مصابين بالربو). وقد تم جمع اللعاب الغير المحفز من العينة الكلية تحت ظروف موحدة و بعد ذلك تم تحليله لعد وحدة مستعمرات المبيضات .

النتائج: إن رتبة متوسط المستعمرات أعلى بكثير بين الاطفال المصابين بالربو عنه لغيرالمصابين مع وجود فروق ذات قيمه معنويه وتم الحصول على نفس النتائج لكلا الجنسين مع عدم وجود فروق ذات قيمه معنويه بالنسبه للذكور بينما كانت ذات قيمه معنويه للاناث .

الاستنتاج: أظهرت نتائج هذه الدراسة أن مرض الربو واستنشاق كورتيكوستيرويد يلعب دورا هاما في ارتفاع انتشار المبيضات في تجويف الفم.

الكلمات الرئيسيه: الربو، المبيضات، استنشاق كورتيكوستيرويد.