

Effect of Inhaled Corticosteroids on Salival Composition: A Cross-Sectional Study in Patients with Bronchial Asthma

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Abstract

Background To date, no evidence exists in the literature as to the effects of inhaled corticosteroids (ICs) on salivary composition in patients with bronchial asthma.

Objective The aim of this study was to assess the effect of ICs on salivary composition.

Methods Adult patients attending an outpatient respiratory clinic who were classified into two groups (controls and patients with bronchial asthma receiving ICs), were recruited in this cross-sectional study. For each participant, data of clinical records, baseline history of asthma, and regular IC dose were recorded. A sample of stimulated saliva was collected and processed for investigation of mucin 5B (MUC5B), lipoxigenase (LPO), total antioxidant capacity, and 8-hydroxydeoxyguanosine (8-OHdG) levels.

Results Overall, 103 patients (49 controls and 54 patients receiving regular treatment with ICs) were recruited. No differences in comorbidities or smoking habits were observed. Patients treated with high-doses of ICs showed lower levels of salivary MUC5B compared with those treated with medium IC doses or those not treated with ICs (1.60 vs. 2.20 vs. 2.53 ng/mL; $p = 0.042$).

Conclusion In patients with asthma, treatment with high-doses of ICs is associated with reduced levels of salivary MUC5B. This effect can explain some of the effects of ICs on oral health.

Key Points

High-doses of inhaled corticosteroids (ICs) have a deleterious effect on the oral mucosa.

High-doses of ICs reduce mucin 5B in saliva.

Higher antioxidant capacity may be associated with higher IC dose.

1 Introduction

Bronchial asthma is defined as a chronic inflammatory disease of the respiratory system [1, 2]. Diverse cells and mediators are involved in its pathology, partly conditioned by genetic factors, which cause bronchial hyperresponsiveness and varying airflow obstruction. This is partially or completely reversible, either spontaneously or as a result of medication [3].

When it comes to evaluation and management of asthma patients, all clinical guides recommend the continuous use of inhaled corticosteroids (ICs), alone or accompanied by β_2 -adrenergic agonists, as a basic treatment for controlling the disease's symptoms and reducing aggravation [4, 5]. High-doses and prolonged use of ICs have been closely linked to adverse effects in the oral cavity [6], the explanation for which is based on the greater part of the inhaled

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drug remaining in the mouth and oropharynx, which can interfere in buccal physiology [7]. These disorders begin mainly with the appearance of xerostomia or subjective dry mouth sensation; however, the underlying physiopathological mechanism that triggers this sensation is not really understood. Saliva is a complex body fluid that contains a mixture of organic and inorganic molecules involved in functions that protect the mouth.

Mucins are high-molecular-weight glycoproteins secreted from sublingual, submandibular and minor salivary glands. Mucin 7 (MUC7) and mucin 5B (MUC5B) are the two major mucins in saliva. MUC5B is known as a high-molecular-weight, oligomeric mucin with a total molecular mass of 2–46,104 kDa. It is highly C-, N- and O-glycosylated, and 40–80 % of their sugar chains are O-linked oligosaccharides capped with sialic acids. These mucins in human saliva are potent lubricants and provide an effective barrier against desiccation. The larger salivary mucin MUC5B, present in (sero) mucous secretions, is thought to be related to the perception of dry mouth by retaining moisture in the mucosa. Among the salivary molecules, salivary mucins, particularly MUC5B, have a special relevance as a protective factor of oral mucosa integrity and subject comfort [8–10].

To date, no evidence exists in the literature as to the effects of ICs on salivary composition in patients with respiratory pathologies requiring IC use, particularly patients with bronchial asthma. The hypothesis of this study was that use of these drugs produces an alteration in the concentration of specific salivary proteins responsible for oral comfort due to their capacity to coat the oral mucosa, in particular MUC5B. Due to its molecular weight, this protein adheres to the oral tissues and protects their integrity. As well as evaluating changes to the cellular structure of salivary proteins, it was important to analyze the effect of the drug on internal cell structures, particularly nuclear damage. To do this, the study analyzed alterations that these drugs produce in terms of antioxidant capacity; this made it

necessary to determine the total antioxidant capacity and to monitor an exclusive marker of cell damage—8-hydroxydeoxyguanosine (8-OHdG), a marker of oxidative stress related to cellular DNA and mitochondrial damage [11]. The most important oxygen-free radical causing damage to basic biomolecules (proteins, membrane lipids, and DNA) is the hydroxyl radical ($\text{HO}\cdot$), which attacks DNA strands when it is produced adjacent to cellular and mitochondrial DNA, causing the addition of DNA bases new radicals, which lead to the generation of a variety of oxidation products. Interaction of the hydroxyl radical with the nucleobases of the DNA strand, such as guanine, leads to the formation of C8-hydroxyguanine (8-OHGua) or its nucleoside form deoxyguanosine (8-hydroxy-2'-deoxyguanosine). Initially, the reaction of the $\text{HO}\cdot$ addition leads to the generation of radical adducts, then by one electron abstraction, the 8-OHdG is formed [11].

Lipoxygenase (LPO) metabolites such as leukotrienes and lipoxins are involved in signalling or inducing structural or metabolic changes in the cell. Furthermore, LPO can attack low-density lipoproteins directly, with major implications for the onset of atherosclerosis.

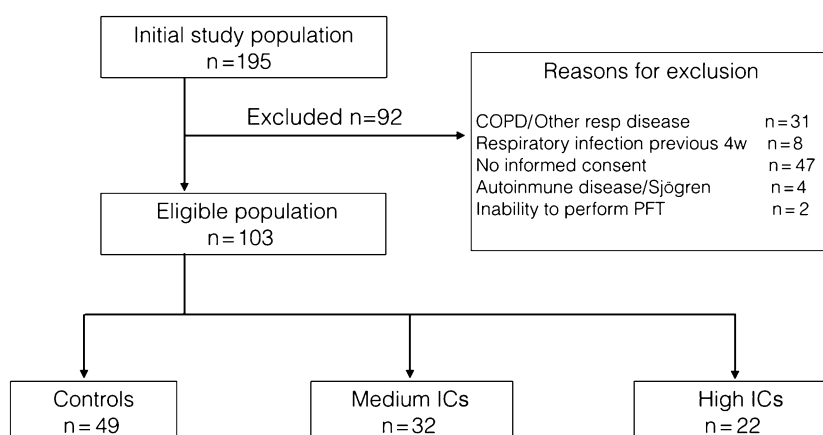
The aim of this study was to analyze the effect of ICs on salivary composition by determining MUC5B, cell damage, and total antioxidant capacity of saliva in a population of hospital outpatients with bronchial asthma.

2 Methods

2.1 Study Design

This research project took the form of an observational, transversal study, with a control group and patients with bronchial asthma receiving ICs over the age of 18 years who were consecutively attending a hospital pneumology clinic as outpatients (Hospital de Alta Resolución, Loja, Granada, Spain) (Fig. 1).

Fig. 1 Derivation of the study sample. *COPD* chronic obstructive pulmonary disease, *resp* respiratory, *PFT* pulmonary function test, *ICs* inhaled corticosteroids



2.2 Ethical Approval

The research protocol was approved by the Ethics and Research Committee of the Faculty of Medicine and Dentistry of the University of Granada (Spain), and followed guidelines established by the Declaration of Helsinki for scientific research involving human subjects. Research data collection fulfilled current data protection legislation (Data Protection Law 15, 13 December 1999) and patient rights (Patient Autonomy, Rights, and Obligations in Clinical Information and Documentation Management Law 41, 14 November 2002).

2.3 Study Population

Patients were categorized according to previous treatment with ICs: those who had not received ICs, patients receiving medium-doses of ICs, and patients receiving high-doses of ICs, measured as doses equivalent to beclomethasone (medium-doses 500–1000 µg/day of beclomethasone; high-doses >1000 µg/day) [1, 2].

All participants were given full information about the study and its purpose, and gave their consent to take part by signing an informed consent form. Biological samples and data tables for each patient were coded to guarantee confidentiality. All subjects fulfilled the following inclusion criteria: aged over 18 years, willingness to take part expressed by providing informed consent, and able to undergo functional respiratory exploration and to provide saliva samples. Exclusion criteria included patients with some respiratory disease other than bronchial asthma, patients having presented with respiratory infection within the previous 4 weeks, or patients presenting any other condition that could affect salivary function (such as autoimmune diseases or radiotherapy to the head or neck).

2.4 Study Variables

Data were collected from each patient detailing pulmonary function, diseases and concomitant treatments, toxic habits (smoking and alcohol intake), as well as each patient's history of bronchial asthma, habitual IC dose, and accumulated dose. For each participant, nitric oxide in exhaled air (FeNO) and pulmonary function were measured as recommended by national and international guidelines [12, 13]. Saliva samples were taken between 8 and 9 a.m., on an empty stomach, with the patient sedentary and at rest, after saliva stimulation (chewing a 1 g paraffin tablet for 5 min, discarding the saliva obtained in the first minute). Patients were requested not to take any medication on the morning of the study and not to take any liquids after 3 a.m. the night before, or to clean their teeth. When the samples had been collected, they were immediately frozen at -20°C

and stored for later processing. When the field work was completed, saliva samples were unfrozen and placed in sterile Eppendorf tubes; the samples were then centrifuged (Unicen21[®], Ortoalresa Ltd, Madrid, Spain) at 3500 rpm for 5 min, collecting the supernatant and discarding the cellular portion. For each saliva sample, MUC5B concentration, total antioxidant capacity, LPO, and 8-OHdG were evaluated. MUC5B samples were analyzed using an ELISA kit (ELISA Kit E01M0358, Blue Gene Biotech Co., Ltd, Shanghai, China). Antioxidant capacity was evaluated by a colorimetric method (measured in comparison with mmol/L of Trolox, a soluble analogue of vitamin E, using Kit CS0790 [SIGMA Aldrich, St. Louis, MO, USA]). LPO levels were evaluated by a fluorescence method (TBARS, thiobarbituric acid reactive substances assay) [14]. Lastly, 8-OHdG concentration was measured using an ELISA kit ('Highly Sensitive 8-OHdG Check' from the Japan Institute for the Control of Aging [JalCA], Nikken SEIL Co. Ltd, Fukuroi, Shizuoka, Japan).

2.5 Statistical Analysis

A descriptive analysis of the study variables was performed, using absolute and relative frequencies in the case of qualitative variables. According to whether or not they followed a normal distribution (applying the Kolmogorov–Smirnov test or the Shapiro–Wilk test [if $n < 50$]), quantitative variables were grouped by mean $\pm$ standard deviation (SD) and range (minimum and maximum) or P50 [P25–P75] (median, interquartile range), respectively. Comparison of quantitative variables by study group was performed applying analysis of variance (ANOVA) for independent samples (divided by IC dose groups) or the Kruskal–Wallis H test (depending on whether or not they followed a normal distribution). Dichotomous variables were analyzed using the Chi-square test. Depending on ANOVA and/or Mann–Whitney *U* test results, post hoc tests were applied using Bonferroni correction. The level of statistical significance was set at $p < 0.05$. Statistical analysis was performed using the Statistical Package for Social Sciences version 15.0 (SPSS Inc., Chicago, IL, USA).

3 Results

The total study sample consisted of 103 patients, of whom 49 were control subjects not receiving ICs, 32 were asthmatic patients treated with medium-doses of ICs, and 22 were asthmatic patients treated with high-doses of ICs. Table 1 shows the study population characteristics, as well as the quantitative variables analyzed. As expected, comorbidities were common, with depression accounting for one-quarter of the cases. Normal lung function values

Table 1 Characteristics of the study population

Characteristic	Controls (<i>n</i> = 49)	Medium-dose ICs (<i>n</i> = 32)	High-dose ICs (<i>n</i> = 22)
Age (years)	61.43 ± 14.30	59.88 ± 14.61	61.09 ± 14.82
Sex (male/female)	24/25	9/23	7/15
BMI (kg/m ²)	32.23 ± 5.65	30.23 ± 4.47	31.20 ± 6.50
Smoking (%)	3 (6.1)	3 (9.4)	2 (9.1)
Accumulated smoking (packets/year)	9.82 ± 13.19	5.09 ± 9.93	9.82 ± 15.65
Asthma (%)	15 (30.6)	32 (100)	22 (100)
Comorbidities [<i>n</i> (%)]			
DM	4 (8.2)	5 (15.6)	4 (18.2)
Cardiac insufficiency	10 (20.4)	4 (12.5)	3 (13.6)
Depression	9 (18.4)	6 (18.8)	5 (22.7)
GERD	7 (14.3)	3 (9.4)	6 (27.3)
Osteoporosis	7 (14.3)	2 (6.2)	4 (18.2)
Pulmonary function			
FEV ₁ postBD (%)	98.32 ± 16.25	98.88 ± 20.48	81.31 ± 20.58
FVC postBD (%)	99.25 ± 14.47	102.15 ± 13.66	92.96 ± 22.56
FEV ₁ /FVC postBD	79.99 ± 7.18	77.64 ± 7.17	71.99 ± 10.65
FeNO (ppb)	29.53 ± 31.11	24.82 ± 11.62	27.47 ± 19.97

Quantitative variables are expressed as mean ± SD

BMI body mass index, *DM* diabetes mellitus, *GERD* gastroesophageal reflux disease, *FEV₁ postBD* forced expiratory volume in 1 s post-bronchodilator, *FVC postBD* forced vital capacity post-bronchodilator, *FeNO* nitric oxide in exhaled air, *SD* standard deviation, *ppb* parts per billion

were observed except for the high IC dose group. With regard to the biochemical evaluations of saliva samples, it was found that those subjects treated with high-doses of ICs showed lower levels of salivary MUC5B compared with control subjects, with a statistically significant difference ($p = 0.042$) (Table 2). The other biochemical parameters evaluated did not present statistically significant differences between patients treated with medium- or high-doses of ICs compared with control subjects (1.31 vs. 1.75 vs. 1.74 ng/mL for 8-OHdG in controls vs. medium-dose ICs vs. high-dose ICs, respectively [$p = 0.468$]; 1.61 vs. 2.06 vs. 2.29 μ M malondialdehyde for LPO [$p = 0.601$]). However, there was a tendency towards statistical significance for total antioxidant capacity in the high-dose IC group compared with the control group ($p = 0.093$). The graphs in Fig. 2 represent the different salivary biochemical parameters analyzed in relation to IC dose.

4 Discussion

The results of this study show that the use of ICs at high-doses is related to a significant reduction in salivary MUC5B levels; other biochemical saliva characteristics were also slightly affected, although these effects did not reach statistical significance. In relation to salivary biochemical parameters, the study focused on four markers

related to the physiopathological mechanisms relevant to oral mucosa damage: cellular DNA damage (8-OHdG, LPO, and total antioxidant capacity) and MUC5B production by the salivary glands as a protective factor of the mouth. MUC5B plays an important role in the many functions of saliva [15]. Changes in MUC5B concentration have been associated with the appearance of xerostomia in patients subjected to radiotherapy of the head and neck [16] and in patients with Sjögren's syndrome [17]. The present results concur with research previously published by our research team that related the presence of xerostomia to poor clinical management of asthma and high IC use [18].

In this study population, patients receiving medium or high IC doses showed numerically higher values corresponding to 8-OHdG levels. These data suggest the possibility of greater cell damage to the oral mucosa as a result of IC use. At the same time, these findings concurred with the values obtained for LPO and total oxidative capacity, which, although they did not reach statistical significance, were higher in patients treated with medium- or high-doses of ICs compared with untreated control subjects. As far as the authors are aware, to date these markers have not been evaluated in patients with bronchial asthma.

The reduction observed in MUC5B concentration deriving from IC use can cause the incidence of xerostomia, causing patient discomfort, and therefore could be a

Table 2 Levels of biochemical parameters in saliva samples

Biochemical parameter	Controls (<i>n</i> = 49)	Medium-dose ICs (<i>n</i> = 32)	High-dose ICs (<i>n</i> = 22)
Proteins (mg/mL)	1.24 ± 0.71	1.42 ± 0.86	1.45 ± 0.97
Mucin 5B (ng/mL)	2.53 ± 2.02	2.20 ± 2.15	1.60 ± 0.88 ^a
Antioxidant capacity (mmol Trolox)	0.39 ± 0.20	0.46 ± 0.30	0.50 ± 0.23
8-OHdG (ng/mL)	1.31 ± 1.45	1.75 ± 1.95	1.74 ± 1.96
LPO (μM MDA)	1.61 ± 2.24	2.06 ± 2.80	2.29 ± 3.65

Data are expressed as mean ± SD

8-OHdG 8-hydroxydeoxyguanosine, LPO lipoxigenase, SD standard deviation, ANOVA analysis of variance, MDA malondialdehyde

^a *p* = 0.042 (high-dose ICs vs. controls, ANOVA)

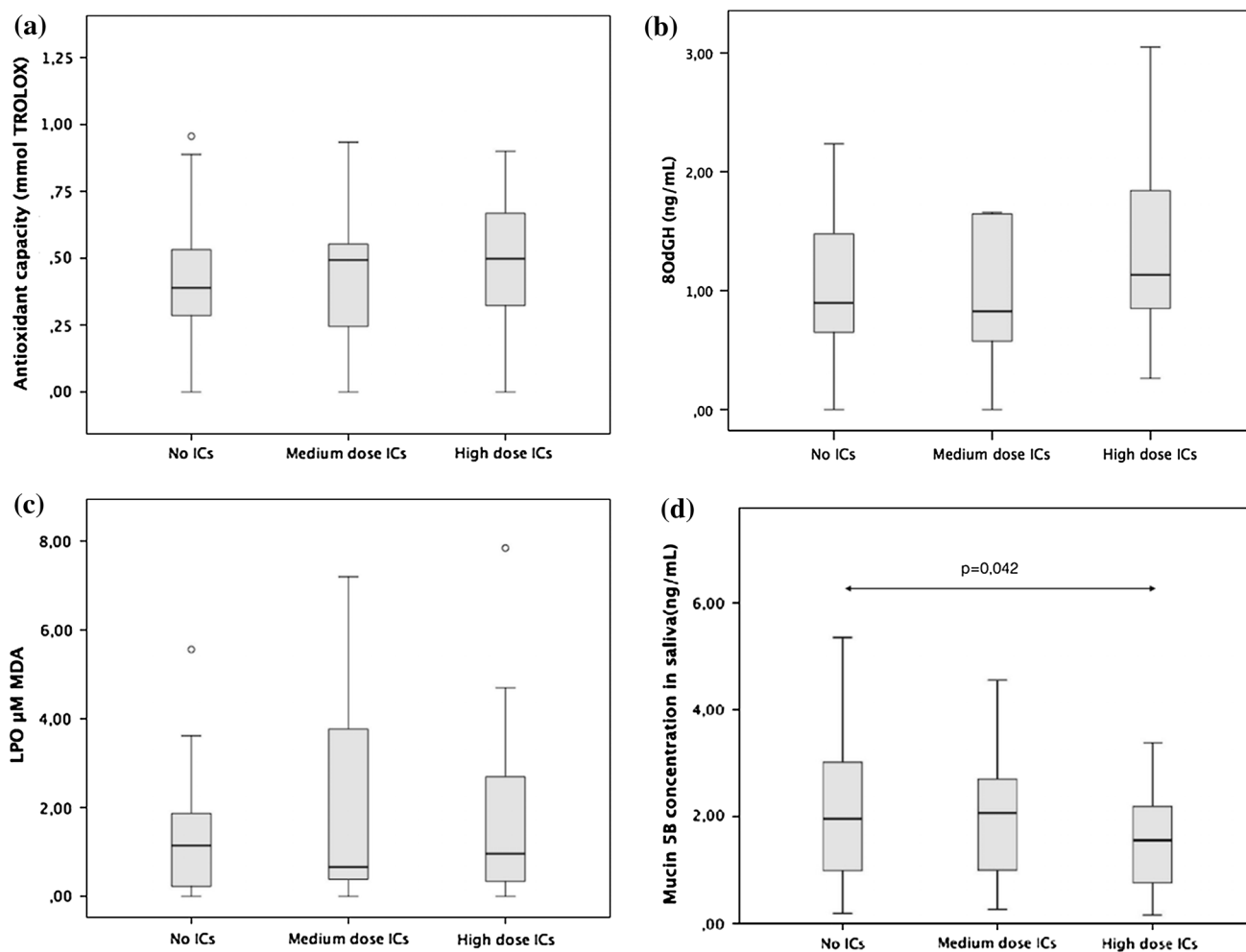


Fig. 2 Biochemical parameter levels in saliva in relation to IC dose: **a** antioxidant capacity of saliva (measured in units relative to mmol of Trolox); **b** 8-OHdG levels (ng/mL); **c** salivary LPO levels; **d** mucin 5B levels in saliva. Bars represent medians and interquartile

ranges, minimum and maximum values. ICs inhaled corticosteroids, 8-OHdG 8-hydroxydeoxyguanosine, LPO lipoxigenase, MDA malondialdehyde

factor that makes it difficult for patients to adhere to the IC treatment regimen. In addition, some local treatments can improve xerostomia, making it possible to lessen the effects of ICs on mouth health [19, 20]. On the basis of

these findings, it might be advisable to introduce questions relating to the state of the mouth into clinical examination and medical notes for patients with bronchial asthma. At the same time, long-term follow-up of patients using ICs at

high-doses, as well as periodic assessments of oral conditions, might be effective in preventing complications and ensuring correct adherence to the treatment regimen.

The strength of this study lies in the selection of an homogeneous and well-characterized study population, in which any patients with respiratory diseases other than bronchial asthma or systemic or local diseases were excluded, therefore avoiding bias. Also of note is the interdisciplinary character of the research team, which allowed a broad approach to this pathology. Although the study population was representative of the type of patient attending pneumology clinics, the presence of comorbidities could have affected the results. Another weakness of the study is that the cumulative dose was not able to ensure that the exact doses were measured correctly because of the nature of these data. Meanwhile, the proportion of patients receiving ICs in lower doses was not large, and therefore it was not possible to extract sufficient data to characterize this group adequately.

5 Conclusion

The data obtained in this study showed a deleterious effect of high-doses of ICs on the oral mucosa, expressed as a reduction of MUC5B in saliva. This fact could interfere in adherence to treatment by those patients with bronchial asthma, in addition to the discomfort and dry mouth sensation they suffer.

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Compliance with Ethical Standards

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Conflict of interest Bernardino Alcázar Navarrete reports receiving personal fees from Novartis AG, Boehringer-Ingelheim, GlaxoSmithKline, Almirall, and Astra-Zeneca, as well as grants and personal fees from Menarini, outside the submitted work. Pedro José Romero Palacios, Antonio Aguilar-Salvatierra, Javier Guardia, and Gerardo Gómez-Moreno have no conflicts of interest to disclose.

Ethical approval This study was performed in accordance with the Declaration of Helsinki 1964 and its amendments. The study protocol, patient information and informed consent form were reviewed and approved by the responsible Ethics Committee of the Faculty of Medicine and Dentistry, University of Granada, Granada, Spain.

Informed consent Informed consent was obtained from all individual participants in this study.

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