Nasal and systemic inflammatory profile after short term smoking cessation

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KEYWORDS
Inflammation; Mucociliary clearance; Smoking cessation; Smoking

Summary
Introduction: Smoking cessation promotes health benefits and, despite cigarette smoking be an important pro inflammatory stimulus, there are few studies concerning the nasal and systemic inflammation; as well as the mucociliary clearance behavior in smokers after short period of smoking cessation.

Aim: To evaluate the nasal and systemic inflammatory markers and mucociliary clearance behavior after 30 days of cigarette smoking abstinence.

Methods: Twenty-five smokers were included and divided into two groups: abstinent smokers (n = 14) and current smokers (n = 11). Tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, IL-8 and IL-10 were measured on nasal lavage and blood serum samples by ELISA at baseline and after 30 days. The mucociliary clearance, exhaled carbon monoxide (exCO) and carboxy-hemoglobin (HbCO) were also measured at the same moments.

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**Introduction**

Smoking cessation services are crucial tools in public health, once tobacco use is the major cause of preventable morbidity and mortality. It is responsible of nearly 6 million of deaths and causes hundreds of billions of dollars of economic damage worldwide each year. Over the course of the 21st century, the number of deaths can reach a billion, unless urgent action is taken [1].

There are known substantial benefits of smoking cessation, such as decreased risk of death, coronary heart disease and stroke, decreased lung function decline, improved quality of life, as well as changes in immune response with increased anti-inflammatory mediators [2–4].

Quitting smoking interrupts the continuous exposure to several chemicals present in the cigarette smoke that cause carcinogenic, mutagenic, toxic and irritant effects and a sustained inflammatory response, which can lead in respiratory epithelium’s damage [5–7].

The respiratory epithelium, besides acting as a mechanical barrier, also participates in the immune response of the individual, playing an essential role in the defense of the respiratory system [8–10]. Many cells are involved in the immune response: epithelial cells, macrophages, neutrophils, B and T lymphocytes, which secrete substances responsible for host protection [11–13].

Cytokines are extracellular signaling proteins formed by various cells types in the body [14] that are released at the site of inflammation, resulting in the local inflammatory response, which is accompanied by a systemic response known as the acute-phase response [13].

Some cytokines [13] are pro-inflammatory and other, anti-inflammatory mediators [15] and the balanced action of these two patterns is crucial for the proper functioning of the immune system [16–20].

Cigarette smoking is a potent pro-inflammatory stimulus and has been implicated in the activation of a complex inflammatory cascade resulting in the production of a variety of potent chemokines and cytokines [21,22] interfering in the balance of pro-and anti-inflammatory cytokines [23].

Several studies have been shown increased levels of cytokines such as interleukin 6 (IL-6), interleukin 8 (IL-8) and tumor necrosis factor alpha (TNF-α) in induced sputum, mononuclear cells and serum of healthy smokers compared to non-smokers [14,23,24]. However, there are few studies on the differences between local and systemic inflammation behavior in healthy smokers after smoking cessation.

Besides interfering with immune function, chronic exposure to cigarette smoke causes respiratory tract epithelial remodeling, with an increase of goblet cells and hypertrophy of mucous cells, which leads to a considerable increase in the amount of mucus to be transported and, in addition, ciliated cells also undergo changes, with reduced number and beat frequency of their cilia. These changes imply in mucociliary clearance impairment [25–27].

Mucociliary clearance is an important defense mechanism of the respiratory system, since entraps and expels the inhaled aggressor agents. Some studies have reported that chronic smokers have decreased mucociliary clearance [28–31]. However, a previous study noted that this mechanism can recover in a period of 15 days without exposure to cigarette smoke [32].

Thus, in summary, smoking has an alarming comprehensiveness worldwide and promotes important health hazards, as the impairment of lung defense mechanisms. Nevertheless, it is known that smoking cessation can bring health benefits, but there are few studies concerning the nasal and systemic inflammation; as well as the mucociliary clearance behavior in smokers after short period of smoking cessation.

The purpose of this study was to evaluate the systemic and nasal inflammatory markers and mucociliary clearance behavior in 30 days of cigarette smoking abstinence.

**Methods**

**Study sample**

Twenty-five smokers of both genders, with more than 20 years of smoking history, who were enrolled in the Smoking Cessation Programme [32] of São Paulo State University (UNESP) from November, 2010 to January, 2011 were included in this study. The sample size required for the study was based on a preliminary study [21]. Our analysis indicated a sample size of 25 participants to detect changes in TNF-α after smoke cessation with the significance level of 5% and the absolute error of 2.5. During the treatment of smoking cessation, those individuals who quit smoking composed the abstinent group and those who could not quit composed the current smoker group. All subjects received the same intervention for smoking cessation with the significance level of 5% and the absolute error of 2.5.

All participants were notified in advance about the objectives and procedures of the study and after their approval, signed an informed consent in accordance with the Declaration of Helsinki of the World Medical Association to become part of effective research. This study was approved by the Ethics Committee in Research (13/2010) of the UNESP, Presidente Prudente, Brazil and by Brazilian Clinical Trials Registry (RBR-6rzhnh). Individuals with cystic fibrosis, bronchiectasis, asthma, COPD, immotile cilia syndrome, history of nasal surgery or trauma, upper airway

**Results:** There was a decrease of TNF-α level only in blood serum at 30 days of abstinence compared to current smokers. The mucociliary clearance improved and there was a reduction in exCO and HbCO (p < 0.05 for all) after 30 days of smoking cessation.

**Conclusion:** The short term smoking abstinence decreased systemic inflammation and improved nasal mucociliary clearance, despite not having changed the nasal inflammation.

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inflammatory and tobacco-related diseases, clinically or certified by spirometry were excluded.

Study design and protocol

Subjects included in this study were evaluated at the Physical Therapy and Rehabilitation Clinic at UNESP by a first interview conducted to obtain personal data, smoking history (years of smoking, cigarettes/day and pack years index) and level of dependence according to Fagerstrom questionnaire, [33] besides to assess the lung function by spirometry [34].

The levels of exhaled carbon monoxide (exCO), carboxyhemoglobin (HbCO) and mucociliary clearance were assessed and the amount of cytokines: tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), interleukin 8 (IL-8) and interleukin 10 (IL-10) were determined through nasal lavage and blood plasma samples. These evaluations were carried out in two moments: at baseline (when all subjects still were maintaining their smoking habits, but under 12 h of abstinence) and after 30 days. The evaluations of current smokers and abstinent smokers groups were performed at the same moments, but the current smokers subjects did not change their smoking habits (they just were under 12 h of abstinence), and the abstinent were under 30 days of abstinence.

All subjects were instructed not to use pharmacological agents such as anesthetics, analgesics, barbiturates, tranquilizers, antidepressants, as well as alcohol and caffeine-based substances during the last 12 h preceding the test. Current smokers were also asked not smoke during the last 12 h preceding the evaluations in order to exclude the acute effect of smoking.

Evaluations were performed in the morning (from 8 to 9 am); the temperature was set at 24 °C and the relative humidity from 50 to 60% to avoid variation in the analyzed parameters.

Lung function

Spirometry was performed according to the guidelines of the American Thoracic Society, using a portable spirometer (Spirobank-MIR®, Italy, version 3.6) [34]. We used specific reference values for the Brazilian population [35].

Exhaled carbon monoxide and carboxyhemoglobin

The exCO and HbCO were measured using a CO analyser (Micro CO Meter®, Cardinal Health, UK) [36]. Subjects were instructed to take a deep breath, remain in apnea for 20 s, engage the mouthpiece of the device in his mouth and then perform a slow exhalation [37].

Mucociliary clearance

For the measurement of nasal mucociliary clearance, saccharin transit time test (STT) was used, as previously described by Salah et al., 1988 [38]. The subjects were seated with their head extended at 10° and granulated sodium saccharin (250 µg) was deposited under visual control at about 2 cm inside of the right nostril. Nasal mucociliary clearance was measured as the time it took for subjects to perceive a sweet taste. If no response was reported after 30 min, the test was concluded after confirming the subject had normal sweet taste perception by placing saccharin powder directly on the tongue. Individuals were instructed not to breathe deeply, talk, cough, sneeze or sniff during the test.

Determination of the amount of cytokines

Nasal lavage

For nasal lavage collection it was asked patients to incline their head back and then make a pause in breathing for ten seconds. Three milliliters and half of saline at room temperature were instilled into each nostril using a 10 ml disposable syringe. After 10 s the patients flexed their neck and the liquid were expelled into a sterile plastic container [39]. The material was transferred to graduated conical tubes immersed in ice, and then homogenized in a Vortex for 15 s. After this the samples were placed in a refrigerated centrifuge at 4 °C under rotation 1000 × g for 10 min and the supernatant aspirated and stored in 1.5 ml eppendorf tubes at −70 °C.

Blood plasma

Two milliliters of blood were collected from the antecubital vein in dry tube by a skilled professional, left undisturbed for 15 min at room temperature and centrifuge at 600 g for total separation of serum which was collected, coded and frozen at −70 °C.

Levels of TNF-α, IL-6, IL-8 and IL-10 in blood plasma and nasal lavage were measured by using an enzyme linked immunosorbent assay (ELISA) kits (Duo Set, R & D System®, Minneapolis, MN) according to the manufacturer’s instruction.

Statistical analysis

The descriptive statistics consisted of mean values, standard deviation, median and interquartile range. Comparisons between groups were made by Student’s t test for independent data and Levene’s test was used to correct distortions caused by different sample sizes. In the statistical analyzes concerning the behavior of variables after 30 days, we compared the differences between the two periods of study, using the formula: Final Value - Initial Value, which were named numeric deltas (Δ). Data analysis was performed using SPSS® (Version 13.0) and all procedures considered significance values (p) of less than 5%.

Results

Twenty-five individuals were divided into two groups: abstinent smokers (n = 14, 49 ± 11 years) and current smokers (n = 11, 41 ± 10 years). At baseline abstinent smokers and smokers were similar in age, BMI (27 ± 4 x 27 ± 4 kg/m²), lung function (FEV1/FVC (%) = 79 ± 9 x 80 ± 13; FEV1 (% prev) = 92 ± 13 x 96 ± 17), smoking history (Pack/year = 30 [20–44] x 21 [14–29]) and nicotine dependence data (Fagerstrom score 5 [4–7] x 5 [3–6]), inflammatory markers concentration on nasal lavage (TNF-α = 11 ± 4 x 10 ± 5; IL-6 = 31 ± 11 x 30 ± 10;
IL8 = 107 ± 37 × 127 ± 74; IL-10 = 11 ± 5 × 12 ± 7 pg/ml) and blood serum samples (TNF-α = 11 ± 3 × 7 ± 3; IL-6 = 0.18 ± 0.15 × 0.36 ± 0.17; IL-8 = 9 ± 5 × 7 ± 7; IL-10 = 13 ± 4 × 12 ± 7 pg/ml), as well as STT (16 ± 7 × 10 ± 4 min), exCO (17 ± 7 × 20 ± 8 ppm) and HbCO values (3 ± 2 × 3 ± 1 (%)). Baseline characteristics are summarized in Table 1. After 30 days, seven individuals remained abstinent, five individuals relapsed before reaching 15 days of abstinence and two individuals relapsed after seven days of abstinence. Among the current smokers, seven individuals completed the 30 days of follow up (Fig. 1). After 30 days, the variables behavior (△) were compared and abstinent smokers presented lower values of

<table>
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<td>exCO (ppm)</td>
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<td>HbCO (%)</td>
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SD: standard deviation; BMI: Body Mass Index; FEV₁: forced expiratory volume in first second; FVC: forced vital capacity; exCO: exhaled carbon monoxide; HbCO: carboxyhemoglobin; STT: saccharin transit time test; TNF-α: tumor necrosis factor alpha; IL-6: interleukin 6; IL-8: interleukin 8; IL-10: interleukin 10.

TNF-α in blood serum compared to current smokers (–6 ± 5 × 3 ± 4); as well as nasal mucociliary clearance (–8 ± 8 × 1 ± 6), exCO (–15 ± 8 × 0 ± 2) and HbCO (–2 ± 1 × 0 ± 0.32) (p < 0.05 for all) (Fig. 2). There were no differences between groups in the behavior of others inflammatory markers in blood serum and in none of them in nasal lavage (Table 2).

Discussion

This study showed that 30 days of abstinence was sufficient for decrease delta concentration of TNF-α in blood serum of abstinent smokers and improve their numeric delta of mucociliary clearance when compared to smokers who maintained the habit, despite no changes on the nasal lavage TNF-α levels’.

Smoking exerts its primary effects in the respiratory tract and leads to the activation of an inflammatory cascade in the upper and lower airway epithelium that is associated with the development of various diseases such as cancer and COPD [40–42]. Even the asymptomatic smokers exhibit signs of inflammation as manifested by increased numbers of macrophages, neutrophils, and CD8+ T cells in the airway epithelium when compared with non-smokers [43–45].

Although there are some differences among the studies [21,23,46,47], there is a tendency of the results to show a greater inflammatory picture in healthy smokers, with higher levels of TNF-α, IL-8 and IL-6 in the bronchoalveolar lavage fluid (BALF), incubated blood cells and blood serum than in non-smokers.

At baseline, the concentrations of inflammatory markers (TNF-α, IL-6, IL-8 and IL-10) in blood serum and nasal lavage were similar between the groups, what was expected, since no subjects had quit smoking at this moment. However, in the 30 days of abstinence there was a significant decrease in the TNF-α level in the blood serum of abstinent smokers when compared to active smokers.
The TNF-α is a cytokine mainly released by macrophages, monocytes and Th1 cells and plays an essential role in the inflammatory response, being considered an important factor in the development of lung injury secondary to smoking [17,48,49].

Despite divergences in the literature regarding the concentration of inflammatory markers in healthy smokers, smoking is an important pro-inflammatory stimulus, which leads smokers to have higher levels of systemic inflammation than those who do not smoke, with higher concentrations of circulating TNF-α. As this cytokine appears to play a key role in the pathogenesis of COPD, a disease potentially caused by smoking, downregulation of this cytokine may bring benefits to the COPD management or even prevent its development [24,49].

The fact that the decrease of the TNF-α have occurred only in blood serum without concomitance with the nasal lavage can be related to the findings of Pelegrino et al., [24] where regression analyses indicated that serum levels of TNF-α are mainly affected by active smoking, whereas sputum concentrations are affected by COPD. Thus, as our sample was composed by smokers without lung function impairment, the smoking cessation only interfered with the serum levels of these cytokine.

The study conducted by Ohkawara et al. [50] showed that after an antigen challenge, there was a rise in TNF-α, with peak levels detected at 3 h in serum and 6 h in BALF of a mice model. It is of interest to note that TNF-α levels in BALF were 6-fold greater than those in serum suggesting considerable compartmentalization. Despite the differences between systemic and local levels of TNF-α at baseline had not been found in our study the TNF-α changes after 30 days of quitting smoking needed a lower time to be detected.

Regarding the anti-inflammatory response, IL-10 has been described the major cytokine released from Th2 cells and is decreased in the BALF of rats exposed to cigarette smoke. Further, IL-10 levels in sputum of healthy smokers, asthmatic and COPD patients are lower compared with control subjects, suggesting that inhibition of IL-10 induces inflammatory patterns in lung diseases [15,41,52,53].

However, in this study, there was no change in nasal and systemic release of IL-10 after a month of tobacco smoking abstinence. Other studies also found no change in this pattern after interventions as performing strenuous exercise in patients with COPD and using systemic corticosteroids combined with inhaled β2 agonists and anticholinergics in the management of COPD exacerbation [54].

It is feasible that the concentration of IL-10, as of others pro-inflammatory cytokines, IL-6 and IL-8 did not change possibly due to the short interval of smoking abstinence so there would be change in the pattern of release of such
mediators. Also, the lack of a nonsmoker group prevented us from knowing if these markers values in healthy smokers were at normal levels.

The impairment in smokers’ mucociliary clearance has been observed in previous studies. Cigarette smoking alters the ciliary beat frequency and thus its efficiency. Deficiency in this clearance system leads to increased frequency and severity of respiratory tract infections [28,59–59].

The mucociliary clearance improvement in 30 days of abstinence may have occurred because of reversibility in cilia acting or mucus physical chemical composition, since their perfect interaction is determinant in mucociliary functioning [60]. Cigarette smoke is highly toxic and generates the release of reactive oxygen species that modify the physiological condition of the respiratory epithelium and therefore mucociliary clearance [61,62]. When this chronic exposure to aggressive agents present in cigarette smoke is ceased, this defense mechanism can be recovered. This finding corroborates the results of Ramos et al., 2011 [29] that also presented improvement in mucociliary clearance after smoking cessation.

It was also observed a decrease in exCO and HbCO levels in abstinent smokers compared to those who continued smoking. They reached the literature acceptable cut levels [63], proving they really maintained smoking abstinence during the evaluation period.

The measurement of exCO is an immediate, noninvasive and well established method to distinguish smokers from non-smokers. However, the cutoff for determination of smoking vary in the literature, often established between 8 and 9 parts per million (ppm) in accordance with personal characteristics [37,64–66]. It has been reported that patients with asthma and chronic obstructive pulmonary disease (COPD) have higher limits of exCO (10–11 ppm) for classification as non-smokers compared with individuals without lung disease (6 ppm) [63,67].

Although we evaluated the smokers who kept smoking habits as a form of control for the analysis of inflammatory markers in the smoking abstinence, we can point out as a limitation of the study the lack of a non-smoker group. Such group could add information about the condition of inflammatory markers in the absence of tobacco smoke exposure and strengthen the inferences related to the no change in the concentrations of cytokines IL-6, IL-8 and IL-10.

Conclusion

The short term smoking abstinence decreased systemic inflammation and improved nasal mucociliary clearance, despite not having changed the nasal inflammation.

Conflicts of interest statement

The authors have declared no conflicts of interest.

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All authors participated in the results discussion and the manuscript writing. Ms. Rodrigues, Ms Xavier and Ms Ito also participated in the data collection. Dr Souza, Dr Cecchin and Dr Guarnier performed data analyses. Statistical analyses were made by Dr Fernandes. Dr. D. Ramos, Dr Silva, Dr Macchione and Dr. Toledo, participated in the discussion and contributed intellectually in the manuscript drafting. Dr. E.M. Ramos participated substantially in all stages of the study development.

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