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*Original Research*

# A Comparative Study to Estimate levels of salivary catalase, alpha- amylase and cotinine levels in levels in chronic smokers and non smokers

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***ABSTRACT:***

**Background:** Smoking is considered a health problem at present all over the world. Smoking is known to have potential effect on body's immune system, antioxidants level and salivary cotinine levels. Hence; the present study was planned to estimate levels of salivary catalase, alpha- amylase and cotinine levels in levels in chronic smokers and non smokers. **Materials & Methods:** The present cross-sectional study included assessment of salivary parameters of smokers and non- smokers. A total of 200 subjects were analysed out of which 100 were active smokers and 100 were non- smokers. Unstimulated salivary samples were taken and assessment of alpha- amylase levels was done using biochemical kit and spectrophotometer. For assessment of salivary catalase activity was done using Luck method. For the determination of cotinine levels, Bioassay Technology Laboratory kit was used using ELISA technique. Data obtained was statistically analyzed. **Results:** Levels of α- Amylase in smokers and non- smokers group was 204.45 and 165.05 U/ ml respectively. While comparing the salivary α- Amylase levels among the two study groups, we observed non- significant results (**P- value > 0.05**). Salivary catalase levels in the smokers group and in the non- smokers group was found to be 6.25 and 10.12 U/ ml respectively. Non- significant results were obtained while comparing salivary catalase levels among the smokers and non- smokers group (**P- value > 0.05**). Values of salivary cotinine levels among smokers and non- smokers group was found to be 16.20 and 0.76 pg/ ml respectively. We observed statistically significant results while comparing mean cotinine levels among smokers group and non- smokers group. **Conclusion:** Based on the results of the present study, salivary cotinine levels were higher in smokers than non- smokers.

**Key words:** α- Amylase, Catalase, Cotinine, Smoking

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## INTRODUCTION:

Smoking is considered a health problem at present all over the world. Based on estimates, smoking will be the cause of 1 death in every 3 deaths by 2020.1 Research has shown that cigarette, and recently hookah, is the most important etiologic factors for oral squamous cell carcinamas.[1] Smoking is classified byWorld Health Organization (WHO) as a chronic and progressive pathology which is 'contagious', also every body part is prone to damage by smoking.[2] Cigarettes are the most common form of smoking employed by a majority of smokers. Other commonly used forms of smoking include pipe smoking, cigar smoking, bidis, bongs etc.[3] Tobacco is the most widespread substance present in smoking.

Tobacco is the plant belonging to genus Nicotiana of the Solanaceae family.[4] Literature supports the fact that relaxation and calmness feelings are provided by smoking. It is also hypothesized to reduce the appetite and increase the metabolism of the body. As a result, there is also sometimes weight loss seen in smokers. α-amylase is heterogeneous enzyme which requires calcium and chloride ions for its action. It plays an important role in the physiologic digestion of starches.[5,6] Protection against free radicals is provided by antioxidants in the body. Antioxidants in the saliva are chiefly composed of uric acid, catalase (CAT), peroxidase (POX) and some other important enzymes. Antioxidant system of the body is attached by the cigarette smoking.[7-9]

The most commonly used biomarker of tobacco exposure is cotinine. Measurement of cotinine, a primary metabolite of nicotine having a half-life of 16–18 h, provides a reliable means of determining smoking status and other tobacco product uses or exposures. Cotinine concentration in various biological fluids such as urine, saliva, or serum is directly proportional to the degree of nicotine exposure. The advantage of using cotinine as a biomarker of tobacco smoke and environmental tobacco smoke (ETS) is the fact that 72% of nicotine is converted to cotinine, and it has a longer half-life (17 h) in comparison to nicotine.[8,9]

Literature quotes paucity in data highlighting the changes occurring in anti-oxidative levels in smokers. Hence; present cross sectional comparative study was designed to estimate levels of salivary catalase, alpha- amylase and cotinine levels in levels in chronic smokers and non smokers.

## MATERIALS & METHODS:

The present study was conducted in 200 subjects to estimate salivary parameters among smokers and non-smokers. Out of total subjects 100 were active smokers and 100 were non- smokers. All the patients belonged to the age group of 25 to 50 years with approximately similar mean age. For avoiding discrepancy in the results, only male smokers were included in the present study. Smokers were categorized as subjects having 5 or more cigarettes per day from past a minimum of five years.[10] Subjects that had never smoked even a single cigarette were categorized as non- smokers. Patients with history of any systemic illness, with any known drug allergy, alcohol drinking habit, new smokers were excluded from the present study.

### Collection of salivary sample

Drool (resting) technique was used for the collection of unstimulated whole saliva.Any kind of oral stimulation was prohibited in all the subjects two hours before the collection of saliva. In patients belonging to the smoker group, they were given institution of smoking one hour before the starting of the experiment. In the floor of the oral cavity, salivary pooling was allowed, out of which, collection of five ml of saliva (unstimulated) was done in a sterile tube. For the elimination of effect of dietary supplements, collection of salivary sample was done after one hour of fasting.Centrifugation of the salivary sample was done immediately at 4 degree centigrade for removing of squamous cells and other remaining cellular debris. Isolation of the resultant supernatant solution was done. Until collection of all the samples was done, all the samples were stored at minus eighty degree centigrade.

### Assessment of Alpha- amylase, Catalase and Cotinine levels

Centrifugation of salivary samples was done for three to five minutes for the purpose of acquiring pure saliva.Assessment of alpha- amylase levels was done using biochemical kit (Salimetrics Salivary Alpha-Amylase Assay Ki) and spectrophotometer. For assessment of salivary catalase activity was done using Luck method as described previously in the literature by Karincaogluet al.[11]

For the determination of cotinine levels, Bioassay Technology Laboratory kit was used using ELISA technique. After assessment of levels of all the salivary parameters, all the data were recorded and compiled. All the results were analysed by SPSS software. Chi- square test and one way ANOVA was used for the assessment of level of significance. P- value of less than 0.05 was taken as significant.

## RESULTS

In the present study, we assessed and compared the salivary α- Amylase, catalase and cotinine levels in smokers and non- smoker subjects. We observed that levels of α- Amylase in smokers and non- smokers group was 204.45 and 165.05 U/ ml respectively (**Table 1**). While comparing the salivary α- Amylase levels among the two study groups, we observed non- significant results (**P- value > 0.05**). Salivary catalase levels in the smokers group and in the non- smokers group was found to be 6.25 and 10.12 U/ ml respectively. Non- significant results were obtained while comparing salivary catalase levels among the smokers and non- smokers group (**P-value > 0.05**). Values of salivary cotinine levels among smokers and non- smokers group was found to be 16.20 and 0.76 pg/ ml respectively. We observed statistically significant results while comparing mean cotinine levels among smokers group and non- smokers group (**P- value < 0.05**).

**Table 1:** Correlation of salivary parameters in between smokers and non- smokers

| Parameter | Smokers group | Non- smokers | p- value |
|---|---|---|---|
| **α- Amylase (U/ ml)** | 204.45 | 165.05 | 0.28 |
| **Catalase (U/ ml)** | 6.25 | 10.12 | 0.22 |
| **Cotinine (pg/ ml)** | 16.20 | 0.76 | 0.01* |

*: Significant

## DISCUSSION

Saliva is a proper alternative diagnostic tool for other body fluids because salivary tests are cost-effective, simple and non-invasive. A correlation has been demonstrated between salivary and plasma cotinine levels.[11-14] Present cross sectional comparative study was designed to estimate levels of salivary catalase, alpha-amylase and cotinine levels in levels in chronic smokers and non smokers. In the present study, we didn't observed any significant difference in the levels of salivary α-Amylase and catalase in between smokers and non-smokers group (**P-value < 0.05**) (**Table 1**). In relation to salivary cotinine levels in between smokers group and non- smokers group, we observed statistically significant difference (**P-value > 0.05**) (**Table 1**). These results highlighted the effect of smoking on the salivary antioxidants and salivary cotinine levels. Our results were in correlation with the results obtained by Nosratzehi T et al and Ahmadi-Motamayel F et al who also reported similar findings in their respective studies.[15,16] Ahmadi-Motamayel F et al evaluated the impact of cigarette smoking on salivary levels of catalase, vitamin C, and α-amylase. This research was done in Hamadan on 510 patients; 259 were smokers and 251 were non-smokers. Spitting method was used for collection of 5µl of unstimulated salivation. Spectrophotometric assays were used for the measurement of salivary Catalase, vitamin C, and α-amylase levels. In comparison to non- smokers, Vitamin C level in smokers was fundamentally lower. In smokers, the salivary catalase levels were decreased and α-amylase levels were elevated, yet the distinctions were not factually critical. Smokers were more youthful than non-smokers. Smoking brought about a change in salivary cell reinforcement levels. Changes in cancer prevention agent levels can impact the malicious impacts of smoking on oral mucosa; it may likewise show systemic changes and changes in the serum levels of oxidative operators.[16] Etter JF et al gathered via mail self-detailed information on smoking propensities and saliva tests that were investigated for cotinine focus in smokers and non-smokers. Members were individuals from the University of Geneva. The 207 cigarette-just smokers smoked by and more than 10.7 cigarettes/day and had a middle grouping of cotinine of 113 ng/ml. The cotinine focus was tolerably connected with the quantity of cigarettes smoked every day and was 54 ng/ml higher in men than in women after alteration for cigarettes every day and for the Fagerström Test for Nicotine Dependence. The cotinine level was not related with the nicotine yield of cigarettes. In nonsmokers, the middle grouping of cotinine was 2.4 ng/ml. The cotinine fixation was 1.5 times higher in non smokers whose dear companions/life partners were smokers than in nonsmokers whose dear companions/life partners were non smokers. This investigation gave proof to the build legitimacy of both surveys and salivation cotinine for the appraisal of dynamic and latent presentation to tobacco smoke.[17]

Cotinine levels have earlier been used to validate the smoking status of an individual. The current work on cotinine can distinguish between nonsmokers, passive smokers, and smokers based on tobacco smoke exposure. Not much research has been focused on defining a cut off value for passive smokers and nonsmokers in India and Southeast Asian region.

Sharma et al observed that the mean cotinine levels of urine for smokers, passive smokers, and non smokers were 1043.7, 36.63, and 13.6 ng/ml, respectively, while in saliva, it was 327.39, 18.31, and 9.53 ng/ml, respectively. Analysis of variance showed that cotinine levels (urine and saliva) of smokers were significantly higher levels than passive smokers and nonsmokers ($P < 0.01$). Similarly, passive smokers also had significantly higher cotinine levels (urine and saliva) than nonsmokers ($P < 0.001$)[18]

## CONCLUSION

The current work establishes the potency of cotinine in the context of smoking and exposure. Harmful effects of smoking on the oral mucous membrane are influenced by alteration in the antioxidant levels. Further research relating to smoking and second hand exposure with other health issues can be planned.

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