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## Research Article

# IMMUNOCHROMATOGRAPHIC ASSESSMENT OF SALIVARY NICOTINE BY USING ONE (+) STEP STRIP TO VALIDATE SMOKING STATUS AMONG SMOKERS

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### ABSTRACT

**Aims and Objectives:** The aim of this study is to assess the presence of nicotine in saliva among smokers and non-smokers.

**Materials and Methods:** Unstimulated whole saliva was collected by asking the patients to spit in a cup that is able to measure 1ml of saliva. Testing was done with One (+) step strip as per the manufacturer's instructions, the presence of nicotine was identified after 10 minutes.

**Results:** The saliva One (+) step strip seems to be valid, highly sensitive and specific method for validating smoking status and may have clinical applications in selected medical settings. In this study the participants were divided into 3 groups and a review was conducted once in 5 days. The results obtained were statistically significant.

**Conclusion:** Smokers had significantly higher values of nicotine compared to that of non-smokers. Nicotine presence proved to be a useful biomarker of recent smoking and can be used in epidemiological studies and smoking cessation programs.

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## INTRODUCTION

Nicotine is the primary addictive component in tobacco products that acts on the brain. Cotinine is the major metabolite of nicotine and results from the metabolism of nicotine by the cytochrome 2A6 enzyme system in the liver.<sup>(1,9, 10)</sup> Nicotine is a particularly useful measure because it has a half-life of 15-40 hr and can detect tobacco use or second-hand smoke (SHS) exposure over the previous 2-3 days.<sup>(1, 11)</sup> Nicotine measurements from human body fluids can provide an assessment of recent exposure to tobacco, but they do not indicate the duration of exposure. Nicotine has been isolated in plasma, urine, saliva and gingival crevicular fluid. Nicotine, when smoked in cigarettes is absorbed across buccal and nasal membranes<sup>(1, 12)</sup>. Saliva was used in our study as it is non-invasive and it is easy to collect without causing much discomfort to the patients. Tobacco exposes the oral cavity to toxic carcinogens that may have a role in initiation and promotion of cancer. It is the major etiologic agent causing oral squamous cell carcinoma and is considered to be responsible for 50% to 90% of oral cancer cases worldwide.<sup>(2)</sup> A precise estimate of tobacco consumption and nicotine dependence in people is an important concern in cessation programmes. In most investigations, tobacco exposure is examined exclusively

via previous and present history of deleterious habit<sup>(6)</sup>. We have compared the intake of nicotine among two groups of smokers and non-smokers<sup>(2)</sup>.

## MATERIALS AND METHODS

The present study evaluated the diagnostic accuracy of nicotine in saliva. Unstimulated whole saliva was collected by asking the patient to spit in a cup of about 1ml of saliva. After 10 minutes, nicotine presence was identified by immune chromatographic assay using one (+) step strip test as per the manufacturer's instructions. The patient was advised to quit smoking for a period of 15 days, the nicotine presence was tested using dipstick strip at an interval of every 5 days. They are first day, fifth day, tenth day and fifteenth day. Sample size is 90 which was divided into 3 groups. Group I has 30 smokers (Smoking cessation advised patients), Group II has 30 smokers (Smoking cessation non-advised patient), Group III has 30 non-smokers. And the presence of nicotine was checked at 4 intervals in each group. Inclusion criteria is patients aged 20 years and above, all are out patients and males. Exclusion criteria patients with any systemic disease, other addictive habits like alcohol, chewing tobacco and patients who are

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taking medicines such as cocaine, benzodiazepines, ketamine, morphine, opiates.

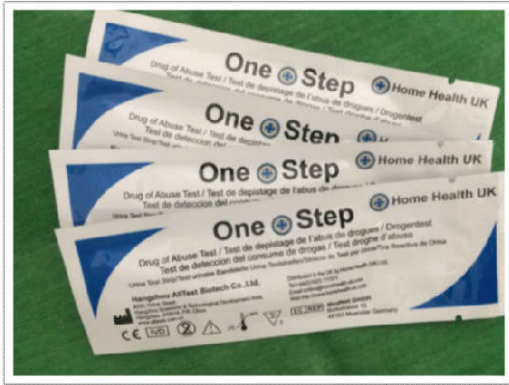
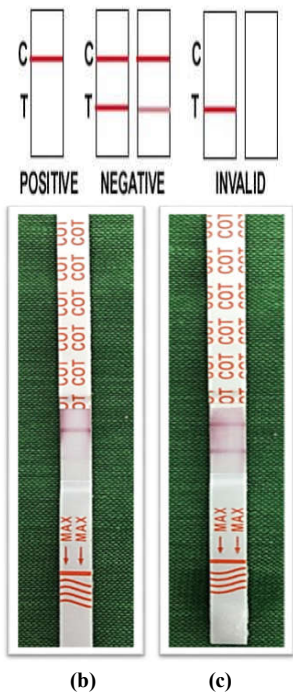


Figure 1 One plus test strips



b) Positive (presence of nicotine) c) Negative (absence of nicotine)

Figure 2 Test strip results {a,b,c}

**RESULTS**

Table 1 shows the total participants of the study were 90 which were divided into 3 groups, they are Group-1, Group -II, Group -III. By comparing these groups for first day. Nicotine presence in smoker group with and without counselling is 100%. Nicotine was absent in non-smokers group is 100%.

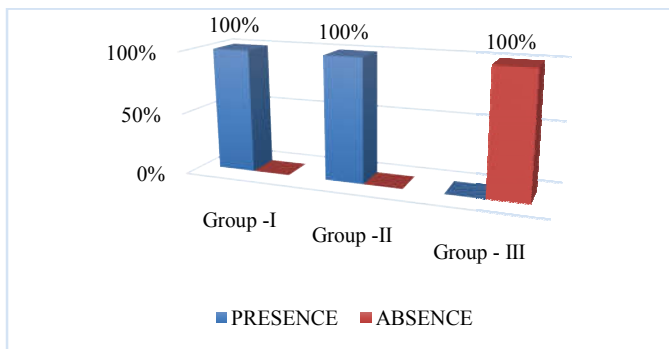


Table 1 First day

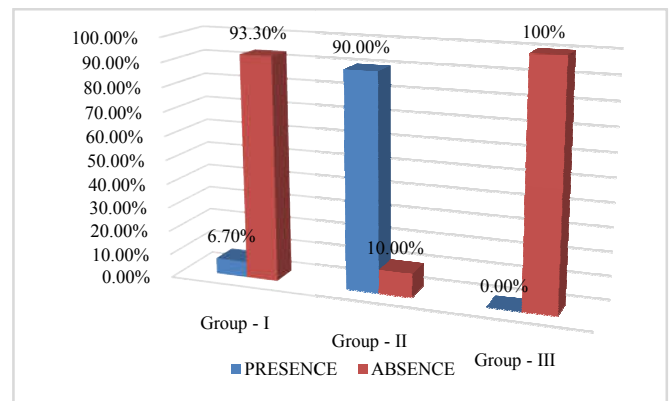


Table 2 Fifth day

While analysing on fifth day Group I shows nicotine presence is 6.70% and absence of nicotine in 93.30% out of 30 people. At the same time Group II shows 90% are continuing smoking and 10% are temporarily stopped smoking. Group III - 100% of people have absence of nicotine.

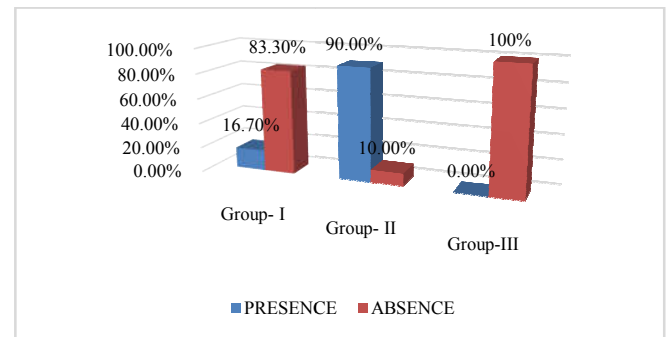


Table 3 Tenth day

Third review on tenth day, 16.70% are continued smoking and 83.30% are stopped smoking in Group I. In Group II continued smoking was increased by 10% and in third group there is no change in non-smokers group. Here the p-value is less than 0.001.

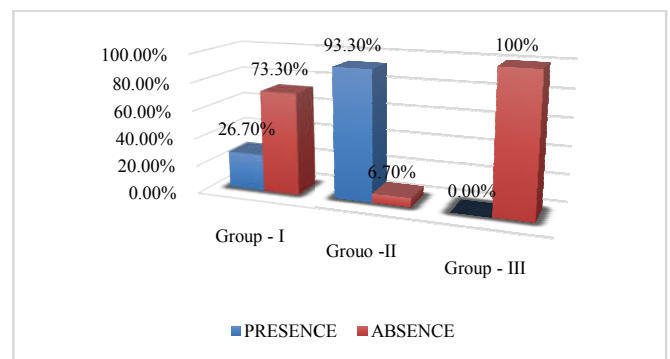


Table 4 Fifteenth day

Last review that is on fifteenth day. In smokers group with counselling, 73.30 % quitted smoking which is appreciable. At the same time we could see 26.70 % were not able to quit and in smokers group with counselling, it is clear that very few i.e. 6.70 % only stopped smoking by various reasons

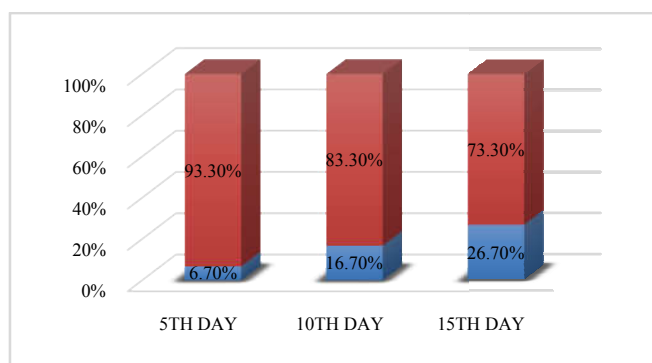


Table 5 Smoker group with counselling

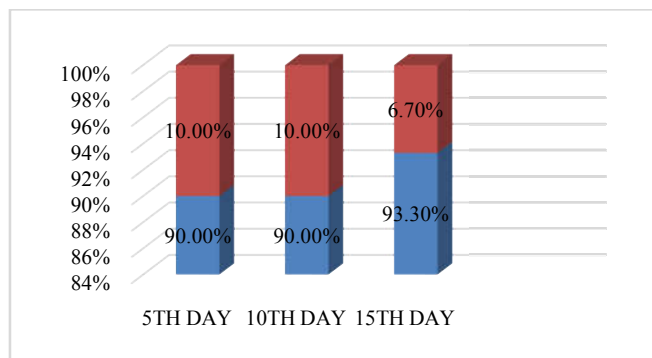


Table 6 Smoker group without counselling

Comparing these two tables (5 and 6) in a duration of 5<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup> day. In smokers group with counselling there is a remarkable change among smokers that is 73.3 % of people quit smoking within 15 days. Which is a better result for the efforts taken through counselling, at the same time it is clear that more than 93% of people continued smoking in the 2<sup>nd</sup> group. The p-value < 0.001 which is statistically significant.

## DISCUSSION

This study highlights the importance of standardising the salivary presence and absence of nicotine among smokers and non-smokers respectively and also showed a significant reduction of smoking and absence of nicotine in smoker group with counselling. The findings in this study could be explained by how drugs such as nicotine and cotinine are absorbed across the lipophilic oral mucosa (Kidwell *et al.*, 1998)<sup>(6)</sup>. A precise estimate of tobacco consumption and nicotine dependence in people is an important concern in cessation programmes. Though measures of nicotine presence have been developed and validated for cigarette smokers, few nicotine presence measures have been evaluated for clinical use among smokers.

Nicotine levels have earlier been used to validate the smoking status of an individual<sup>(13,17,18)</sup>. These biomarkers have also been used in epidemiological studies to assess the effects of tobacco use on human health<sup>(19)</sup> as measures to estimate the exposure to environmental tobacco smoking and for the assessment of efficacy in interventional methods on cessation of smoking<sup>(7)</sup>.

The saliva nicotine concentration was high in smokers, whereas the concentration was remarkably low in case of those who attempted to quit smoking, which is similar to JF Etter *et al.*, study<sup>(8)</sup>. Nicotine and cotinine concentrations are used to estimate tobacco consumption, to determine exposure to environmental smoke and to validate abstinence in smoking cessation programmes (Hatsukami *et al.*, 2003).

Nicotine has a fast onset of action with a half-life of 2 hours and can be detected in blood, saliva and urine<sup>[3]</sup>. Blood provides quantitative results that can be more accurately related to dosing trials, saliva collection is favoured over blood and urinary measures as it is easy to obtain and non-invasive methods (Hatsukami *et al.*, 2003)<sup>(6)</sup>. Saliva samples are useful for determining compliance with medication for analysing the concentration of free drugs and in situations where ever it is necessary<sup>(2)</sup>. Furthermore, Lea *et al.* demonstrated that nicotine ratio is stable in active smokers throughout the day<sup>(4)</sup>.

Unstimulated technique is recommended for salivary nicotine estimation a stimulated technique is recommended for salivary cotinine estimation (Robson *et al.* 2010)<sup>(7)</sup>. The measurement of salivary cotinine by immunochromatographic assay using one (+) step strip saliva test is a useful and convenient method for studying the nicotine dependence in smokers<sup>(1)</sup>.

Immunochromatography nicotine test strips are easy method of detecting salivary nicotine in a dental setup. Self-reported tobacco use among people can underestimate the actual prevalence of tobacco use especially in dental patients. Biochemical validation of self-reports is particularly recommended for intervention studies where cessation outcomes are to be measured. As the habit of quitting tobacco is gradual by using test strips can serve as simple, regular and chairside reminders in a dental office. Biochemical validation of self-reported tobacco use should be considered during prevention and cessation studies among dental patients in developing countries like India<sup>(1)</sup>.

Immediate feedback from a point-of-care test within 10 mins, possibly because the immediate and personalized feedback from the test helps reinforce the smoking cessation advice that smokers receive (Barnfather, Cope, &Chapple, 2005)<sup>(3)</sup>. In the present study salivary cotinine were measured in smokers with and without counselling and it was correlated with nicotine dependence. A significant association was found between the presence and absence of nicotine in 5<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup> day<sup>(F)</sup>.

## CONCLUSION

The present study assessed the determinants of nicotine concentration, smokers had significantly higher values of nicotine compared to non-smokers. Salivary nicotine is proved to be a useful biomarker of recent smoking and can be used in epidemiological studies and smoking cessation programs.

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