



Evaluating the effect of 8-week behavioral counselling on smokers with low dependence and correlating with salivary cotinine levels in South Indian working men – A preliminary study

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Abstract

Context: The behavioural counselling is an effective method to bring awareness and prevent tobacco-associated cancers in subjects with low dependence. A pilot study was done to evaluate the effectiveness of an 8-week-based behavioural counselling, in which salivary cotinine among lower nicotine dependence smoking men (as per Fagerstrom scale) was assessed before and after counselling sessions.

Aims: This study aims to compare salivary cotinine levels in male smokers with lower dependence before and after 8-week behavioral counselling sessions.

Setting and Design: This was an observational pilot study.

Materials and Methods: The study involved 46 smokers, recruited after subjecting to inclusion and exclusion criteria. The individuals were scored based on Fagerstrom test of nicotine dependence (FTND) as “low nicotine dependent and very low dependence.” The unstimulated saliva was collected from the participants in the pre-counselling sessions and subsequently on the 1st, 3rd, 7th, and 8th week post-quit date. The samples were analyzed for cotinine levels using enzyme-linked immunosorbent assay before and after the counselling sessions.

Statistical Analysis: Percentages and proportions; Chi-square (χ^2) test, Student’s independent *t*-test, and one-way analysis of variance.

Results: Among sample of 46 smokers, 74% ($n = 43$) belonged to “very low dependence” and 26% ($n = 12$) with “low dependence.” The post-counselling salivary cotinine values were significantly low (19.54 ± 10.2) against the pre-counselling values of the (26.7 ± 10.1) samples ($p < 0.05$).

Conclusion: This study proved that a non-invasive, non-pharmacological technique such as an 8-week behavioural counselling helped smokers with lower dependence (FTND scores < 6) of tobacco. Future studies can be considered in adopting this method for smokers with high tobacco dependence.

Introduction

Tobacco smoking is a global epidemic. The World Health Organization has reported that over 3.5 million people throughout the world are killed by tobacco and its products every year. It is foreseen that by 2030, tobacco will kill 10 million people in a year.^[1] India is the second largest producer and third

largest consumer of tobacco in the world. The overall number of adult smokers has reached over 100 million thereby making it the second country after china to have the maximum number of smokers in the world.^[2] Tobacco is consumed in various forms, such as cigarettes, cigars, cigarillos, bidis, kreteks, pipe tobacco, snuff (oral and nasal), and chewing formulations.^[3] Nicotine

is an alkaloid found in *Nicotiana tabacum* (*Solanaceae*) which constitutes approximately 0.6–3.0% of dry weight of tobacco. It is the principal agent which is toxic causing cancer and responsible for addiction.^[4] Cotinine is the most important metabolite of nicotine and is obtained from the metabolism of nicotine by cytochrome 2A6 enzyme system in the liver. Cotinine levels are one of the most sensitive and non-invasive biomarkers of tobacco exposure that gives information about cotinine deposition in the body and nicotine intake.^[5] The serum cotinine is obtained by blood collection whilst the salivary cotinine is an effective and non-invasive alternative. Salivary cotinine is correlated with recent nicotine exposure (3–4 days) and smoking status (active smoker, passive smoker, and non-smoker).^[6] The cotinine-based enzyme-linked immunosorbent assay (ELISA) can be a practical biomarker of exposure to tobacco smoke.^[7]

Individual behavioral counselling from a smoking cessation specialist may help smokers to make a successful attempt to stop smoking.^[8] Individualized and more intensive counselling is more effective in promoting smoking cessation.^[8,9] These methods have considerable success in low-dependent smokers while a range of cessation techniques from simple counselling to pharmacological and combined therapies has been reported for high-dependent smokers but with varying and inconsistent results.^[10] The placebo-controlled trial on various pharmacological therapies had minimal benefits when reviewed^[10] on heavy smokers, thus this group was avoided and we attempted a pilot study on lower dependence smokers initially. The current study adopted an 8-week behavioral counselling and correlating with salivary cotinine levels for lower dependent smokers.

Materials and Methods

The study was conducted from 1st December 2016, to 30th October 2018, under the Department of Oral Medicine and Radiology, Indira Gandhi Institute of Dental Sciences, Sri Balaji Vidiyapeeth, Pondicherry. (counselling sessions, data collection, and storage) and department of Microbiology, Mahatma Gandhi Institute of Medical Sciences, Sri Balaji Vidiyapeeth, Pondicherry (ELISA). A total of 46 employees working at XXX University aged 15–75 years with the habit of smoking were recruited by after sample estimation. The sample size was calculated based on the previous salivary cotinine value^[11] by keeping power at 80% and attrition rate of 10% in standard formula. The study was conducted on employees of university housed within campus. A minimal attrition of three subjects was present (one passed away, two did not respond for all sessions). However, we considered an attrition rate of 10% as it was follow-up study. The final sample evaluated was 46, which was categorized based on Fagerstrom test of nicotine dependence (FTND) questionnaire, into group of very low dependence (FTND scores 1, 2) and low dependence (FTND score 3). Written informed consent was obtained from all the subjects who were enrolled for the study. FTND is a pre-validated, structured questionnaire containing closed-ended questions.

The inclusion criteria set were – (i) University employees, above the age of 18 years and below 75 years, (ii) gender: Male.

(iii) Habitual smoker for more than 6 months (any form of smoked tobacco) with the frequency of smoking <10 CPD. (iv) Very low and low dependence on the Fagerstrom scale of nicotine dependence (score <6). Exclusion criteria set were – (i) Employees with habit of using non-smoking or chewable form of tobacco, (ii) history of significant psychosis/cognitive impairment, (iii) patients with hyposalivation leading to xerostomia, (iv) FTND scores above 6 or smokers with higher dependence on tobacco, and (v) uncooperative and unwilling patients. The study was approved by Institutional Review Board and ethical clearance was obtained (Reference code: IGIDSIEC2016NDP19PGMDOMR). Study pro forma was designed as per guidelines given by the WHO in its manual for tobacco cessation.

The unstimulated saliva was collected as per standards, from each consented participants for assessing pre-salivary cotinine levels using ELISA. Similar estimates were done after giving behavioral counselling sessions on the 1st, 3rd, 7th, and 8th week post-quit date. At the 8th week, post-salivary samples were collected labeled and immediately frozen at –80°C until further evaluation cotinine by ELISA. All the collection methods were done by requesting the participants to rinse mouth thoroughly with water for 10 min before collection of sample. The unstimulated whole saliva (0.5 ml) was taken from each group in a separate sterile container as per standards. The ELISA was done using the respective kits (Cotinine ELISA Kit Elabscience). The ELISA kit had sensitivity: 0.38 ng/mL;^[5] a detection range between 0.63 ng/mL and 40 ng/mL.^[6] It could specifically recognize cotinine in salivary samples with no significant cross-reactivity or interference between cotinine and analogues. The repeatability: coefficient of variation is <10% for the kit. Saliva cotinine values >10 ng/mL were labeled as positive. This ELISA kit uses the competitive ELISA principle; wherein the microplate has been pre-coated with cotinine. During the reaction, cotinine in the sample competes with a fixed amount of cotinine on the solid phase supporter for sites on the biotinylated detection Ab specific to cotinine. Excess conjugate and unbound sample or standard are washed from the plate, and avidin conjugated to horseradish peroxidase is added to each microplate well and incubated. Then, a tetramethylbenzidine substrate solution is added to each well. The enzyme substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of cotinine in the samples is then determined by comparing the optical density of the samples to the standard curve [Figure 1].

The 5 A's was a brief intervention method or approach used as a guide for the clinician in tobacco cessation counselling. The counselling sessions were carried out as per standard guidelines^[12] and as per the WHO in its manual for tobacco cessation. The 8 weeks sessions were considered based on national and international current guidelines, targeting increasing the motivation to quit smoking, the identification of barriers, and prevention of relapse.^[13] The five major types in the intervention were as follows: (1) Ask – about tobacco use; (2) Advise – to quit; (3) Assess – commitment and barriers to change; (4) Assist – users committed to change; and (5) Arrange – follow-up

to monitor progress. All sessions were given for 30 min in local language by same experts (Associate Professor in psychiatry with 7 years' experience assisted by oral medicine specialist certified by Department of psycho-oncology and Resource Center for Tobacco Control, Adyar, Chennai). The study employed a triple blinding strategy where in the principal investigators, biochemical analyst and statistical expert were blinded from each other. The study protocol is summarized in Figure 2.

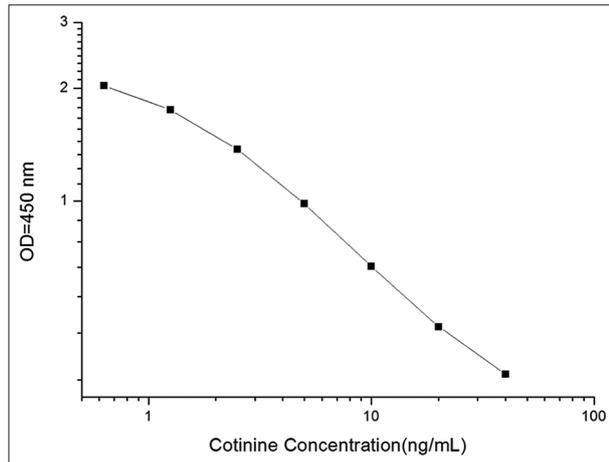


Figure 1: Enzyme-linked immunosorbent assay interpretation from the standard curve

Statistical analysis

Descriptive data for frequencies are presented as percentages and proportions. Chi-square test for trends was applied to see significant differences and associations of various categorical parameters. All the comparisons before and after counselling were evaluated by Student's *t*-test. The one-way analysis of variance (ANOVA) test was applied wherever comparison of two or more mean cotinine values was necessary. All the analyses were done in EPI Info (version 7, Centre for Disease Control and prevention, Geneva, Switzerland). For all tests, $p < 0.05$ was considered for statistical significance.

Results

The FTND-based questionnaire had noted on smoking intensity (pack years) and that was proportionally raised and highly correlated with increasing age ($p < 0.001$ ANOVA), Table 1. The mean age of the study participants was 49.48 ± 9.8 years. Majority of the study participants belonged to the 46–55 years age group (34.8%) [Figure 3]. There was less representation of younger age group men in the study sample (8.7%). Among them, majority were primary school level educated literates [Table 1]. The FTND scores had classified the sample into two groups – very low dependence $n = 43$ (74%) and low dependence $n = 12$ (26%). The overall pre-cotinine level and post-cotinine levels were 26.7

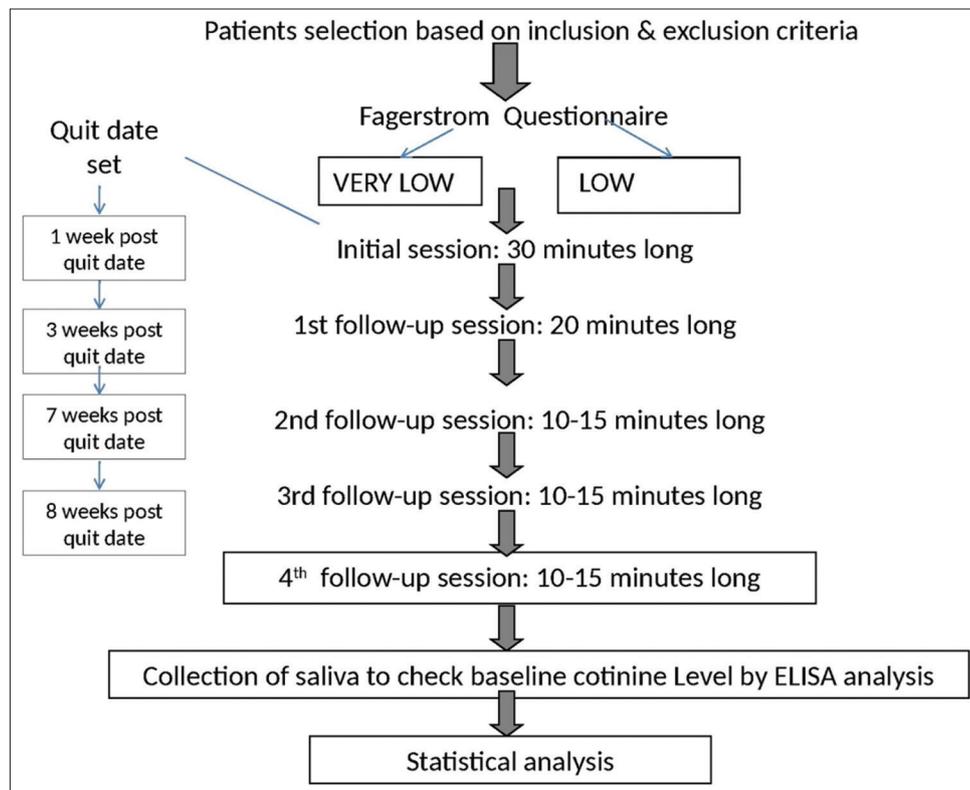


Figure 2: Methodology indicating the 8-week behavioural counselling and assessments

± 10.1 and 19.54 ± 10.2 for before and after counselling sessions, respectively (p < 0.001), Table 2.

Discussion

Age is an important factor when tobacco usage is considered. As per the current study, those aged between 25 and 44 and between 45 and 64 were approximately 20% less likely to make a major quit attempt than persons in either the younger or older categories. Those in the median age groups were not ready to make a quit attempt. The age groups considered and predilection for quit attempts are consistent with studies reported by Kumar et al. (2010)^[14] and Gichuki et al. (2014)^[15] in India and Kenya, respectively.

The socioeconomic and educational status of individual was the next important factor pertaining to tobacco additions and quitting habits. Lee et al. (2016)^[16] concluded that high level of

education was related to success in smoking cessation. Lower education group demonstrated higher intensity of smoking (p < 0.001). The current study had results consistent with that of observations made by Aniwada et al. (2018)^[17] wherein secondary education or above was less likely to smoke cigarettes than those with primary education. An inverse relation of education to smoking is reported in literature.

The FTND also known as revised description of “Fagerström Tolerance Questionnaire” was designed by Por Karl-Olav Fagerström in 1978. It is used for measuring the smokers degree of nicotine dependence through six questions, where the scores range from 0 to 10. The present study showed that 74% of the population had very low dependence (FTND score 0–2), 26.1% of population had low dependence (FTND score 3–4). The study clearly indicated that there was a significant difference across the two FTND scores in pre- and post-cotinine values across the two FTND scores (P < 0.001). The severity of dependence increased with that the serum cotinine values simultaneously. Furthermore, for the lower dependence subjects (low or very low as per FTND), the intervention used (counselling) made a significant impact shown by reduction in cotinine levels. The methodology and intervention used in our study, that is, classification of dependence based on FTND, period counselling was earlier used by Etter and Schneider (2000)^[18] who reported the scale to have reliability and validity. Pérez-Ríos et al. (2009)^[19] and Patel et al. (2017)^[20] also had reported similar observations in heavy dependent smokers using FTND scale. The correlation of FTND scores was done with change in cotinine, a major metabolite obtained from the nicotine absorbed by a smoker. It is excreted mainly by the kidneys, perspiration, maternal milk, and oral fluids. Most of the nicotine is transformed to cotinine, whose half-life of cotinine is found to be 15-20 hours whereas half-life of nicotine is 3 hours. Chadwick and Keevil (2007)^[21] reported that cotinine is proved to be a better biomarker compared to nicotine for measurement in blood serum, urine, semen, hair, and saliva due to its longer half-life.

The results of the present study showed that increase in dependence resulted in simultaneous increase in salivary cotinine values. Nosratchehi et al. (2015)^[22] reported similar relation between cotinine and dependence, however, their study involved hookah users unlike smokers in our study. Pai and Prasad (2012)^[23] made similar observation in their study. In the present study, a strong correlation between FTND and pre- and post-salivary cotinine levels were observed which was consistent with the observations made by Etter et al. (2000);^[24] while Asha

Table 1: Association of age, education status, and smoking intensity

Variable	frequency	No. of cigarettes per day (mean±SD)	Number of years of smoking (mean±SD)	Pack-years (mean±SD)
Age groups				
20–35 years	4	2.75±0.96	1.5±0.58	4.25±2.6
36–45 years	11	4.1±1.4	6.4±3.4	27.2±19.1
46–55 years	16	4.1±2.2	6.9±4.1	29.6±2.6
>55 years	15	4.1±2.1	7.8±4.9	32.7±2.4
Total	46	3.98±1.8	6.59±4.3	27.8±2.2
(ANOVA test) p-value		<0.001	<0.001	<0.001
Education level				
Primary school	26	4.04±1.6	6.96±3.4	28.6±6.9
Middle school	5	5.2±1.8	9.2±6.3	47.6±3.9
Higher secondary	12	3.6±2.4	6.3±2.6	32.9±9.6
Degree	3	2.6±0.5	9±0.9	26.3±2.4
Total	46	3.98±1.8	6.59±4.3	27.8±2.2
(ANOVA test) p-value		<0.001	<0.05	<0.001

ANOVA: Analysis of variance

Table 2: FTND and cotinine level scores before and after 8-week behavioural counselling method

FTND score	Pre-counselling cotinine value	Post-counselling cotinine value	p-value*
Very low dependence (1, 2)	23.9±9.5	17.0±9.3	0.001
Low dependence (3)	34.7±6.8	26.75±9.6	0.001
Overall	26.7±10.1	19.54±10.2	<0.001

*Paired t-test for comparison of two means horizontally (rows), **ANOVA test for comparison of three means vertically (columns), FTND: Fagerstrom test of nicotine dependence

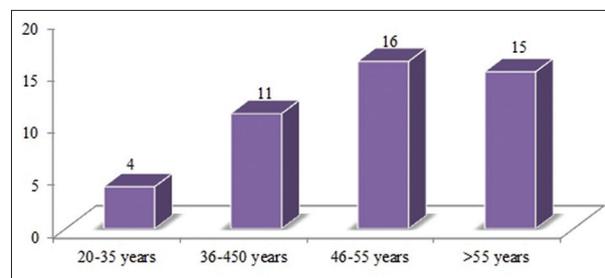


Figure 3: Age distribution of study population

and Dhanya (2015)^[5] have reported paradoxical observations in tobacco chewers. Mottillo *et al.* (2005)^[25] as part of Cochrane database of systematic reviews had shown that 'brief advice' from physicians as a part of behavioral counselling had shown to improve rates of smoking cessation alone or in combination with pharmacotherapy.

The FTND scores corresponding to high or very high dependence was set as exclusion owing to studies reporting need of pharmacological or combined therapies and variable success rates for this group of patients.^[10] Furthermore, identification of lower dependent smokers and targeting counselling sessions not only avoids adversities from pharmacological therapies but also may have a higher success rate. The advantages of the study are employing a single trained counselling expert, multiple non-invasive saliva collections after counselling sessions, and triple blinding methodology. Limitations of the study include a small sample size, women not being included, and inclusion of lower dependent smokers only. The future direction of this pilot study is to perform a study to evaluate the method in higher dependent smokers, in multiple centers with larger samples in men and women.

Conclusion

The study shows that 8-week behavioral counselling is a significant tool in reducing salivary cotinine levels in smokers with lower nicotine dependence. This method of behavioral counselling can be tested in smokers with high nicotine dependence to quit the habit.

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