

**MULTI COUNTRY STUDY ON DETERMINANTS OF SALIVARY COTININE  
LEVELS IN SMOKERS – MUMBAI STUDY**

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## **Background**

Cotinine is a major metabolite of nicotine and can be measured in different body fluids as a biomarker of exposure to inhaled or ingested nicotine. The absorbed dose of nicotine is best indicated by the concentration of cotinine in blood, but the blood levels can be estimated reasonably well by measuring cotinine levels in saliva or urine (Benowitz 1996). Levels of cotinine in the saliva, urine and blood are highly correlated, with the typical concentration ratio for saliva to blood of 1.1-1.4 and for urine to blood of 5 (Jarvis et al. 1984). Earlier studies have shown substantially different mean salivary and serum concentrations of cotinine among smokers of different ethnic background (Wagenknecht et al. 1990, Coultas et al. 1993). For example, among a sample of 298 smokers from New Mexico, the mean saliva cotinine level was 350.3 ng/ml (SD 208.6) among Hispanic men and 553.8 (228.6) among non-Hispanic men (Coultas et al. 1993). For women the corresponding means were 267.6 ng/ml (191.9) among Hispanics and 328.1 (224.0) among non-Hispanics. Among participants in the Coronary Artery Risk Development in (Young) Adults (CARDIA) Study, the mean serum cotinine concentration was among men: 210.2 ng/ml (SD 145.1) in whites and 244.8 (156.2) in blacks, among women: 176.4 (137.6) in whites and 251.2 (175.6) in blacks (Wagenknecht et al. 1990). These differences in levels may reflect differences in smoking habits, nicotine uptake and metabolism between smokers of different ethnic groups. Such differences may explain ethnic differences in smoking cessation rates. Identification of determinants of cotinine levels in body fluids may provide useful information for planning of smoking cessation programs.

Factors that have been suggested to influence nicotine uptake and metabolism in smokers include: 1) smoking rate; 2) characteristics of the cigarette, such as length, nicotine content, freshness, filter versus nonfilter, and menthol content; 3) smoking topography, including puff volume and duration, inhalation frequency, retention time in lung, depth of inhalation, and percent of available tobacco smoked; and 4) factors that determine metabolic state, such as age, gender, genetic sensitivity, physical fitness, and body composition (McCarthy et al 1992). Thus all these factors may influence the cotinine levels in body fluids, and it is likely that these factors also modify the effects of

each other. For example, the relation between the amount of cigarettes smoked and the cotinine concentration may differ between different ethnic groups, perhaps reflecting different metabolism of nicotine on a genetic basis or culturally determined differences in smoking patterns, or across countries because of differences in nicotine delivery by the cigarettes used nationally.

The possibility of differing nicotine delivery among different groups, whether defined racially and ethnically, culturally, or nationally, has relevance to nicotine replacement therapy. A nicotine-delivery system constructed and tested in one market may not be optimal in another; dosage might be too high or low or, perhaps, one temporal profile may be more appropriate than another. Potential markets where this problem of non-optimum dosing may emerge include China, Mexico and India. In China, information about tar and nicotine yields of local products indicates greater exposures to nicotine than from cigarettes currently smoked in the United States. In Mumbai, as in other parts of India, smokers tend to smoke fewer cigarettes daily than in the United States. Thus, there would appear to be a potential for either providing too little or too much nicotine in a replacement device based on U.S. smoking patterns.

### **Aims**

- 1) To characterize the distribution of salivary cotinine levels among smokers in different ethnic or national groups.

This objective will be met by taking salivary samples for determination of cotinine from smokers from population samples in China, Mexico, Brazil, India and ultimately other countries.

- 2) To investigate the determinants of salivary cotinine levels among smokers, with emphasis on assessing the relationship between number of cigarettes smoked and salivary cotinine level.

The following potential determinants will be studied: number of cigarettes smoked during the day of the sample collection and during the previous day, the number of cigarettes smoked daily on average, the duration of smoking, the brand of

the cigarette (giving information on tar/nicotine content, filter vs nonfilter, and menthol content), the depth and frequency of inhalation, age, gender, BMI, and ethnic background. Information on these determinants will be collected in an interview. Analysis of nicotine in the most commonly consumed brands will provide data on nicotine intake for use in these analyses. The role of ethnic/national group as a modifier of the relations will be also addressed, we will compare the relationship between number of cigarettes smoked and salivary cotinine concentration across national groups.

### **Study Design**

Population-based cross-sectional studies will be carried out initially in China, Mexico, Brazil, India and ultimately in other countries. The samples will be drawn from ongoing studies or from existing sampling frames. Emphasis will be placed on obtaining samples from defined populations.

### **Study Population (for the Mumbai Study)**

Participants would be selected mainly from the ongoing cohort study on tobacco attributable mortality in the city of Mumbai (1, 2). In this study, trained investigators approach participants on a house-to-house basis. Currently the follow-up phase is going on so only the individuals interviewed during the baseline survey are approached. After ascertaining the correctness of the address, an individual is identified as belonging to the cohort. If available, an interviewer-administered questionnaire is filled. These individuals generally belong to the middle and low socio-economic group; the buildings housing high socio-economic group were not included due to denial of access to investigators. During the baseline survey, in addition to obtaining information on tobacco use, measurements were made for height, weight, blood pressure and peak expiratory flow. The results on tobacco use showed that about a quarter of men were smokers. Nearly half of them were cigarette smokers and half were bidi smokers. Smoking was nearly absent among women.

## **Proposed study**

For the proposed study after obtaining the interview and taking measurements for the height and weight, saliva samples would be collected as per the standard protocol. Those smokers who use smokeless tobacco as well in addition to smoking would be excluded. The samples would be restricted to men only as very few women smoke. If an identified participant is not available, a revisit would be made.

## **Sample size**

The following table shows sample sizes needed to detect differences of 20, 50 and 100 ng/ml in mean levels of salivary cotinine between two populations, with 90% power and an  $\alpha$  of 0.05. Assuming a mean of 553.8 ng/ml and a standard deviation of 228.6, based on men in the study by Coultas et al. (1993), the following sample sizes were derived:

### **Difference**

#### **to be detected**

in ng/ml	N needed
20	3405
50	545
100	136

In addition, sample size calculations were performed for comparing the slopes of the relation between cigarettes smoked daily and salivary cotinine concentration. The estimate of slope for this calculation was also from Coultas et al. (1993). To detect a difference of 5 ng/ml in cotinine concentration per cigarette between two groups, with 80% power and an  $\alpha$  of 0.05, 344 subjects are needed in each group.

Based on these calculations, we will include 550 smokers of bidi and 550 smokers of cigarette. This size of population allows detection of a difference of 50 ng/ml in mean cotinine levels and provides enough statistical power to study modification of the

relations between salivary cotinine and its determinants, e.g. number of cigarettes or bidi smoked.

## **Measurement methods**

### *Salivary cotinine*

Since salivary concentrations of cotinine seem to give the same information about cotinine disposition in the body as do plasma concentrations (Curvall et al. 1990), we have chosen to collect saliva samples as a less invasive and more feasible method for use in these multinational studies.

The subjects will be asked to first rinse their mouth and then chew a lemon candy. They are asked to first spit out a small amount of saliva, and then to spit approximately 6 ml in a test tube (Schneider et al 1997). The specimen is frozen to -20 C. The cotinine concentration will be determined using gas chromatography technique (Jacob et al 1981). The samples will be analyzed in the laboratory of Dr. Neal L. Benowitz, University of California, San Francisco. Duplicate analyses will be carried out for 5 % of samples.

### *Height and weight*

Height will be measured using a tape mounted on the wall. Participants will be asked to remove their shoes. Weight will be measured with a portable scale.

### *Collection of cigarettes*

Samples of the most commonly smoked cigarette and bidi brands will be collected. These will be analyzed for nicotine content.

## **Data Analysis**

The distribution of salivary cotinine levels in different ethnic/national groups will be described. In bivariate analyses, the mean salivary cotinine level will be studied in different ethnic categories as well as in categories of other determinants of interest (see aim # 2). Determinants of interest are classified as dichotomous variables or as variables with several categories or levels (e.g. number of cigarettes/ bidis smoked, duration of

smoking, cigarette brand, frequency of inhalation, age, BMI). The outcome-determinant relations will be evaluated by t-test and simple linear regression.

Multivariate linear regression will be used to evaluate the relations between salivary cotinine concentration and determinants of interest taking into account other factors as potential confounders. Dose-response relations will be evaluated by including the determinant of interest (e.g. number of cigarettes smoked) as dummy variables corresponding to different levels of the determinant or as a continuous variable. Potential modification of the relations by ethnic or national group will be addressed by introducing ethnicity-determinant of interest product terms one by one in the model and by retaining them according to statistical significance of the regression coefficients.

### **Consent procedures**

Potential study subjects will be approached by field personnel from the Tata Memorial Centre. The interviewers will give information about the study orally and show the informed consent to the subject. The text in the informed consent will be read to the subject and he/she has an opportunity to ask questions and express concerns. The potential respondent can refuse at this point.

### **Ethical issues**

The protocol will be submitted for approval to the Committees on Human Research of the Johns Hopkins University, the National Institute of Public Health, Mexico, and the Ethical Committee of the Tata Memorial Centre.

It is not anticipated that there would be any risks to the study subjects from this study. They will not receive any personal benefits, apart from the opportunity to receive education concerning the health risks of active smoking provided by the interviewers. In general, study results are important for development of smoking cessation programs in Mexico.

### **Confidentiality**

All survey information will be kept confidential and saliva specimens anonymous, using assigned unique identifier codes.. The saliva specimens will be shipped to Dr. Benowitz at the University of California, San Francisco.

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