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Validation of Self-Reported Smoking Behavior: Biochemical Analyses of Cotinine and Thiocyanate

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Abstract: Biochemical determinations of plasma and salivary cotinine and thiocyanate were used to differentiate smokers from non-smokers and to follow daily smoking patterns in smokers. Results indicate that cotinine is better suited than thiocyanate to determine smoking status in large scale epidemiologic studies and to follow alterations in smoking behavior over periods of time. Salivary cotinine is a reliable alternative to plasma for validation of smoking status and for following changes in daily smoking patterns. [*Am J Public Health* 1983; 73:1204–1207.]

Introduction

Smoking control research has generally relied upon self-report for information concerning smoking status, but the validity of this measure is severely limited. Denial and minimizing the extent of cigarette smoking are common practices among youth and announced quitters.[1-3] Therefore, investigators[4-7] recognize the need for biochemical validation of smoking behavior. There is controversy, however, concerning the most appropriate measuring devices for smoking status. Salivary sampling has been suggested as an alternative to invasive venipuncture, but the relation of salivary levels to plasma levels of cigarette smoke metabolites remain imprecise. Thiocyanate measurement has been used in large scale epidemiologic studies.[8-11]

We investigated the question of whether cotinine or thiocyanate measurements should be used to separate smokers from non-smokers, to follow changes in daily cigarette smoke absorption, and to investigate smoker compensation. We also attempted to validate analysis of these components in saliva.

Methods

Experiment 1

Thirty individuals were asked to volunteer both blood and saliva samples. The participants were 12 smokers and 18 nonsmokers. Blood was collected into vacutainers containing EDTA as the anticoagulant and resulting plasma was frozen.

Saliva was collected at the same time. Participants were instructed to deposit saliva directly into a vial marked at the one ml level. Control studies on this method showed routine recovery of more than 95 per cent of both exogenous thiocyanate and ^{3}H-cotinine.

Participants then completed a questionnaire on smoking behavior as well as a 24-hour dietary recall.

Experiment 2

Two smokers* and two non-smokers were asked for a saliva sample each morning for two or four weeks. Samples were collected directly into premarked vials and then frozen. Dietary recalls were requested from both smokers and non-smokers.

Analytical Techniques

Cotinine was quantitated by a modification of the radioimmunoassay (RIA) as developed by Langone, *et al.*[9] This method uses a specific antiserum produced by injection of trans 4 carboxycotinine bound to albumin into rabbits. The inter- and intra-assay variations are less than 6 per cent. Approximately 60 samples plus standards and controls can be analyzed per day with this RIA methodology. Results compare well with those obtained by GLC.

Plasma thiocyanate was determined by an automated procedure following the method of Butts, *et al.*[8] Saliva was analyzed for thiocyanate content following suitable dilution. Samples were run in duplicate with excellent reproducibility (±1.5 per cent).

Results

Table 1 displays the mean thiocyanate and cotinine values for plasma and saliva. No cotinine was detected in non-smokers. Plasma thiocyanate was increased in smokers compared to non-smokers, reinforcing the consensus of other researchers that plasma thiocyanate levels greater than 100 μM/l can serve as indicators of regular smoking behavior.[8-11]

Salivary analysis for cotinine showed high levels present in smokers (161 ng/ml) with no cotinine being detected in non-smokers. Comparisons of salivary thiocyanate in these groups showed a difference in mean values, but a loss of resolution in the standard deviations. This is better illustrated in Figures 1A and 1B where individual values can be compared. In both plasma and saliva, cotinine analysis could distinguish between smokers and non-smokers with a high degree of accuracy, while thiocyanate determinations provide a less clear cut answer. The "grey area" resulted from smokers reporting 10 or less cigarettes smoked per day as well as from 20 per cent of the non-smoking group. Cotinine in plasma and saliva is highly correlated (0.98) while the correlation for thiocyanate in plasma and saliva is less than 0.40.

Thiocyanate levels are influenced by daily food consumption, but dietary recalls for the collection periods showed no abnormal consumption of vegetables or products known to influence the dietary background for thiocyanate.

Daily salivary analyses of cotinine and thiocyanate in nonsmokers gave the results for thiocyanate shown in Figure

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*Smokers were instructed to collect the sample prior to the first cigarette of the day.