

Simple colorimetric test to quantify exposure to environmental tobacco smoke: occupational health study

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Tobacco smoke is an important environmental contaminant of indoor air. Chronic exposure has been implicated in heart disease and lung cancer in adults and in upper respiratory disease in children. Subjective assessment of exposure, as noted by questionnaires, is difficult because of its emotive nature. Therefore, biochemical measurement of biomarkers may provide a more accurate determination of individual exposure. Measurement of nicotine metabolite concentration, such as cotinine, in urine, plasma or saliva is regarded as the approach of choice.¹ A variety of methods are available, such as gas chromatography-mass spectrometry and high-performance liquid chromatography, but they are restricted to the laboratory. Biofeedback about tobacco smoke intake has been shown to be helpful in reducing exposure. To facilitate this feedback we have developed a near-patient test to monitor active smoking using a colorimetric assay to measure nicotine and its metabolites incorporated into a disposable plastic enclosure.² We have used this to monitor active smoking in pregnancy and cardiovascular disease. We recently modified the reagents to improve sensitivity to detect nicotine metabolites in saliva in order to develop a chair-side test to monitor smoking in dental practice.³ Our aim was to determine whether this test could detect exposure to environmental tobacco smoke and to assess whether exposure in an occupational setting correlated with known physiological effects of passive smoking.

METHODS

The plastic testing device (SafeTube) consisted of a 2-mL sampling device and a reaction chamber. Sodium citrate (20 mg), citric acid

(18 mg), potassium cyanide (7.5 mg) (Sigma-Aldrich Co. Ltd, Poole, UK) and 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) (20 mg) (Lancaster Synthesis Ltd, Morecambe, UK) were combined as dry powders and placed in the reaction chamber. Chloramine-T (Sigma) was isolated in a washer-like annulus, with a space between two tightly fitting plastic discs which formed a seal on top of the chamber.²

In use, 2 mL of unprocessed urine were aspirated by the syringe, which was inserted into the barrel, breaking the seal and dispensing the chloramine-T into the reaction chamber. The urine was injected onto the reagents, which dissolved, thereby starting the reaction. Light absorbance at 490 nm was monitored for 10 min using a battery-operated colorimeter (Jenway) and compared with a cotinine (Sigma) standard in the range 0-1.25 mg/L, producing a cotinine equivalent concentration (CE). Correction for urine sample dilution was achieved by measuring the creatinine concentration with a picric acid colorimetric kit (Sigma). The final results were expressed as the cotinine equivalent concentration/creatinine concentration ratio (CECR) [(mg/L)/(mg/dL)].

Eight healthy non-smoking adult volunteers (five women) with a mean age of 28 years, who worked in a busy city centre public house (1200 m² trading area, 250 person capacity) for a 6-h evening shift, were studied. Atmospheric carbon monoxide (CO) levels were measured using a Bedfont Micro Smokerlyser (Sittingbourne, UK) throughout the evening. Urine samples were collected from each volunteer just before starting work and 2 h post-exposure. Passive smoke exposure has been shown to affect respiratory function and antioxidant status, so peak expiratory flow rate (PEFR) was assessed using a Mini Wright Peak Flow meter (mean of three readings) (Clement Clarke Int. Ltd, Harlow, UK). Total antioxidant capacity (TAC) was measured by enhanced chemiluminescence assay⁴ in saliva

samples collected at the same time as the urine samples.

Statistical methods used were paired *t*-tests together with, in every case, confirmatory non-parametric Wilcoxon sign rank tests and Pearson correlation coefficients. The reported *P* value is that of the *t*-test.

RESULTS

Atmospheric CO rose to 9 ppm (compared with 0 ppm outside) by the end of the shift. Post-exposure CE values ranged from 0.04 to 0.4 mg/L (median 0.14 mg/L) with a lower limit of detection of 0.04 mg/L. The CECR increased significantly from a pre-exposure concentration [mean (SD)] of 0.15 (0.44) mg/L/mg/dL to a post-exposure concentration of 2.99 (3.58) mg/L/mg/dL ($P < 0.05$). PEFR values fell in all eight individuals by an average of 5.22% ($P < 0.02$). The antioxidant capacity of saliva samples also fell significantly from a mean (SD) of 215.9 (55.1) Trolox equivalents $\mu\text{mol/L}$ to 142.8 (27.3) Trolox equivalents $\mu\text{mol/L}$ ($P < 0.04$). There was a significant Pearson correlation between CECR and PEFR values ($r = 0.71$, $P < 0.05$), but the relationship with total antioxidant capacity failed to reach significance.

CONCLUSION

A modified colorimetric smoking test based on the König reaction has been shown, for the first time, to be sensitive enough to detect exposure to environmental tobacco smoke. Previous studies have shown that this reaction is specific to nicotine metabolites, with little in the way of interfering substances. Its incorporation into a

simple-to-use, inexpensive, disposable format has allowed the test to be carried out in situations outside of the laboratory.²

The effect of passive smoking on salivary antioxidant status was unexpected. However, tobacco smoke, yielding a high concentration of free radicals, could have a local effect on the buccal cavity. Previous studies have shown salivary antioxidant capacity to be lower in smoking-related periodontitis.⁵ The significant fall in PEFR values confirms the effect of exposure to environmental tobacco smoke on respiratory function.

REFERENCES

- 1 Benowitz NL. Cotinine as a biomarker of environmental tobacco smoke exposure. *Epidemiol Rev* 1996; **18**: 188–204
- 2 Cope G, Nayyar P, Holder R, Gibbons J, Bunce R. A simple near-patient test for nicotine and its metabolites in urine to assess smoking habit. *Clin Chim Acta* 1996; **256**: 135–49
- 3 Cope G, Nayyar P, Holder R, Brock G, Chapple I. A near-patient test for nicotine and its metabolites in saliva to assess smoking habit. *Ann Clin Biochem* 2000; **37**: 666–73
- 4 Cope G, Thorpe G, Holder R, Luesley D, Jordan J. Serum and tissue antioxidant capacity in cervical intraepithelial neoplasia investigated using an enhanced chemiluminescent reaction. *Ann Clin Biochem* 1999; **36**: 86–93
- 5 Chapple IL, Mason GI, Garner I, Matthews JB, Thorpe GH, Maxwell SR, et al. Enhanced chemiluminescent assay for measuring the total antioxidant capacity of serum, saliva and crevicular fluid. *Ann Clin Biochem* 1997; **34**: 412–21

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