Review Article

Nicotine hair test - A potential biomarker for smoke analysis

Authors: Suyog Chandrashekhar Savant*, Harsh G Shah**, Vasudha Sodani***, Dimpal Bajaj****

ABSTRACT

The article provides an overview of the potential biomarker which can be used to detect nicotin levels among the smokers. Cotinine, a metabolite of nicotine, has been successfully detected in the urine, blood and sweat. However, nicotine hair analysis (NHA) remained a debated issue due to its feasibility in research. Till date it faced unresolved questions of influence of hair treatment, hair colour, and growth rate on nicotine levels in hair, which need to be addressed in order to further refine this biomarker for exposure assessment. Cotinine also faced an inherent problem of short shelf life in blood, urine, sweat and saliva which could be successfully resolved with the use nicotine in hair analysis. However ambiguity surrounding the nicotinine hair analysis (NHA) has been laid to rest following its proven reliability and measure of longer term exposure that can be readily applied in dental and medical research.

Keywords : biomarker, nicotine, cotinine, hair analysis

Introduction

One-third of the cancers are caused by tobacco and tobacco products in India. The burden of tobacco usage, it is very high. Around 15 to 16 crores of males and around 7 to 8 crores female, approximately, consume tobacco products one or the other forms. According to Indian Council of Medical Research, use of smoke is the single most preventable cause of disease, disability, and death in India with an estimated 8,00,000 deaths each year. With the advancement of science and technology, medical professionals are armed with armamentarium that could suffice the need to combat the global epidemic, and biomarkers of nicotine detection might just be an answer.[1]

Nicotine

Nicotine is a highly addictive chemical substance that is derived from certain specific plants belonging to the flowering nightshade plant family named Solanaceae which acts as a stimulant in tobacco containing products such as cigarettes, chews, cigar and snuff. It may also be found in small quantities in eggplants, tomatoes, potatoes and green peppers. Nicotine is the principal identifying constituent of tobacco, and most studies assessing tobacco smoke exposure (whether active or passive) have looked for methods of measuring nicotine or one of its metabolites in the human body.[2] Levels of nicotine in hair have been suggested by several studies as a possible marker of long term smoke exposure. The relatively long term (up to several months) exposure assessment is the major advantage of this approach, and one that is particularly relevant to epidemiological studies of disease aetiology.[3] This paper reviews this biomarker and its importance as a measure of tobacco smoke exposure in research.

Nicotine metabolism

Nicotine consumed in smoke or smokeless form, reaches the bloodstream or the brain within a very short span of 5-7 seconds. The absorption of nicotine by the body varies depending upon tobacco

ADDRESS FOR CORRESSPONDENCE:
Dr. Harsh G. Shah
Senior Lecturer,
Department of Public Health Dentistry,
Ahmedabad Dental College & Hospital
Santej, Gandhinagar.
E-mail : harsh_079@yahoo.co.in

*, **** Senior Lecturer, Dept of Public Health Dentistry, Yerla Dental College and Hospital
** Senior Lecturer, Dept of Public Health Dentistry, Ahmedabad Dental College & Hospital
*** Senior Lecturer, Dept of Pedodontics & Preventive Dentistry, Ahmedabad Dental College & Hospital

inhaled or consumed, or the use of filter while smoking. However analysis proved that the amount of nicotine absorbed by the body is much more in the case of chewing, dipping or snuffing tobacco rather than smoking cigarettes. The metabolites of nicotine that gets accumulated in the body fluids are nicotine isomethonium ion, nicotine glucuronide, nornicotine, nicotine N'-oxide, 2-hydroxynicotine and cotinine. Among these cotinine is the major metabolite affecting the body by giving rise to unpleasant withdrawal symptoms which make it the hardest of addictions to get rid of. Usually nicotine persists in the body for 2-4 days but in some cases it may also last for a few months. However, in the case of passive smoking the nicotine remains in the body for a long time. The presence of nicotine is usually detected in the urine, blood, and hair follicle through medical tests.[4]

Nicotine drug test

Nicotine or its primary metabolite cotinine are most often tested to evaluate tobacco use. Nicotine and cotinine can both be measured qualitatively or quantitatively. Quantitative testing can help distinguish between active smokers, tobacco users who have recently quit, non-tobacco-users who have been exposed to significant environmental tobacco smoke, and non-users who have not been exposed. Cotinine may also be measured in saliva and in hair, although hair testing is primarily used in a research setting, such as a study of non-smokers exposure to tobacco smoke. Blood or urine nicotine may be ordered by itself or along with cotinine if a doctor suspects that someone is experiencing a nicotine overdose. When a person has reported that he or she is using nicotine replacement products but is no longer smoking, nicotine, cotinine, and urine anabasine measurements may sometimes be ordered. Anabasine is present in tobacco but not in commercial nicotine replacement products. If a sample tests positive for anabasine, then the person is still using tobacco products.[5]

Hair as a biomarker

Cigarette smoke constituents (including nicotine) enter the body by inhalation and are then absorbed into the systemic circulation. Nicotine (a tertiary amine composed of a pyridine and pyrolidine ring) is lipid soluble and therefore has a large distribution volume in the body (2–3 litres/kg) and readily permeates cell membranes. Almost 80% of nicotine is metabolised in the liver by cytochrome p450 enzyme to cotinine. Differene exists in the metabolism of nicotine between smokers and non-smokers; conversion of nicotine to cotinine and its elimination in urine can be more rapid among smokers than non-smokers.[6] However, laboratory studies measuring body clearance rates of labelled nicotine and cotinine among smokers and non-smokers have proven that the pharmacokinetics of nicotine and cotinine are similar in the two groups. Other factors such as race or ethnicity may contribute to differences in body metabolism of nicotine to cotinine and uptake by hair but have not been proved. Ethnicity or race may be an important contributor in nicotine metabolism variability, as may the type and texture of hair in relation to race be related to difference in uptake of nicotine, an area which needs further investigation. Biological variability in metabolism normally occurs for all xenobiotic agents. Nicotine inhaled into the body follows a biphasic pattern beginning with a distribution phase (5–10 minutes) and a elimination phase with high inter individual variation (70–140 minutes). The nicotine is incorporated into hair as long as it is present in the circulation; therefore, nicotine collected in hair is representative of the cumulative dose collected gradually over the period of exposure. The fact that 70–80% of nicotine is cleared by being converted to cotinine39 suggests that, over time, the metabolism rate of nicotine may affect levels of nicotine accumulation in hair.[7]

Factors influencing hair analysis

Before the hair analysis is conducted, factors influencing the exposure of hair to nicotine, growth of the hair, hair pattern, anatomical location, its colour, needs to be examined. Each hair grows approximately at the rate of 1 centimetre per month.[8] This growth continues for 2-6 years. When the hair attains full growth it resets for 2-3 months and is later shed. A new hair starts growing in its

place. Thus at any given point of time 10 percent of the total hair on our scalp is in a resting phase and 90 percent of the hair is in growth phase. The hair length and environmental smoke play a role in nicotine hair analysis.[9]

Cotinine is present in hair, but in much lower concentrations than nicotine. Nicotine in hair is always 10 times higher than cotinine even though cotinine levels in plasma are higher than nicotine. Contradictory studies lower cotinine level in hair and its inability to differentiate active and passive exposure groups. However, cotinine in hair is better correlated to history of cigarette smoking in spite of it being present in much lower concentrations. As cotinine levels in hair are much lower than nicotine levels, this may reduce detection of smoke exposure, thus explaining the relatively poor correlation to history of exposure among non-smokers in several studies. It seems that cotinine is not distributed through the body in exactly the same manner as nicotine. For example, nicotine levels are higher in the breast milk of smoking mothers than in serum, but cotinine levels in breast milk tend to be lower than in serum.[10] The only reason that could be speculated is due to the differences in shelf life. Hair colour may also influence nicotine uptake. Investigators have reported that white or fair hair has lower nicotine levels than black hair for a similar exposure level. Several participants with grey hair provided black and grey hair simultaneously, and showed lower levels in their grey hair. The reason was nicotine having a higher affinity to melanin, which is produced by melanocytes at the hair bulb and incorporated into the cortex of the hair. (This higher affinity to melanin suggests that most of the nicotine in hair is incorporated through systemic circulation by passing through the hair bulb and attaching to the melanocyte granules, which are only present in the cortex of the hair shaft). Experiments conducted on rats proved that nonpigmented rat hair had concentrations of nicotine 20 times lower than pigmented rat hair when taken through the systemic route. Hair pigmentation was also related to the levels of nicotine absorbed by cut rat hair samples directly from the external environment, but with a much lower ratio of 1.5:1 (pigmented to non-pigmented).[11]

Contradictory studies have stated there is no difference in nicotine (and cotinine) uptake in relation to hair colour. Nicotine uptake from exposure to different concentrations of nicotine did not differ in relation to the colour of hair. Some studies have also quoted the person's age, hair thickness and sex did not affect nicotine uptake rate. However, the exposure was not systemic but involved cut hair samples. Further studies are needed to investigate the relation between melanin in hair and nicotine uptake. In the meantime, including hair colour as a covariate in the analyses of hair nicotine results may minimise inter-individual variability.[12]

Effects of cosmetics on nicotine and cotinine content of hair

The ability of the hair to preserve substances incorporated into its shaft may damage the structure of the hair and affect the accurate detection of drugs in it. Use of hair dye, permanent wave, and 30% hydrogen peroxide lower nicotine and cotinine levels in hair but do not remove them altogether. Even bleaching and hair dying reduce nicotine levels were by 30%. However, normal (non-dandruff) shampoo washes for 20 minutes does not affect the levels of nicotine in the shaft of the hair.[13] Recently it was also found that adjusting for hair dying history in a multiple regression model did not significantly alter the estimated levels of nicotine in the hair of non-smoking bar and restaurant staff. Therefore, it is expected that significant cosmetic treatment of the hair (for example, bleaching) will affect hair contents of nicotine, and these variables have to be taken into account when collecting information from adults donating their hair samples for research purposes. Normal hair washing seems to wash away externally attached nicotine rather than the nicotine that is measured by analysis (nicotine incorporated into the shaft of the hair). Studies have been conducted which have proven the cotinine and nicotine presence in hair with different bio chemical techniques like gas chromatography with mass spectrophotometry, high performance

liquid chromatography with electro chemical detection and radioimmunoassay.[14]

Benefits of hair as a biomarker

Hair nicotine provides reliable information on long term smoke exposure than biomarker measures in urine, saliva, or serum because of the shorter half life of the latter biomarkers. The long term (up to several months) exposure is the usual exposure of interest in health-related studies. The absence of drug metabolism in hair and its fairly uniform growth rate for a given location in the body may provide an historical account of smoke exposure, provided that the concentrations measured in a given hair segment are related to their distance from the scalp (usually the proximal 1–2 cm). The hair nicotine biomarker can be analysed with standard laboratory methods, and some of the recently developed methods are cost effective for research. Another advantage of nicotine in hair over cotinine in urine is its increased ability to differentiate exposure status because of less variability. Hair is easily collected without the need for prior notice to the participants (as is the case when collecting urine samples). No trauma is caused to the donor when collecting the sample (as in collecting blood samples). Samples can be stored readily and inexpensively without deterioration. The nicotine levels in hair are not lowered by storage of samples for a period of up to five years. The use of a biomarker such as hair nicotine avoids problems such as recall bias, under reporting or lack of awareness of exposure that may be encountered with questionnaires. Nicotine is almost entirely specific to the smoke exposure and therefore has better validity than environmental monitors that are used for measuring non-specific pollutants such as suspended particle levels as an indicator of Environment Tobacco Smoke (ETS). Nicotine in hair can be used for assessment of intrauterine exposure to nicotine by collecting hair samples (if available) from neonates and determining the intrauterine exposure in the later months of pregnancy when foetal hair develops.[15]

Demerits of hair as a biomarker

Nicotine levels may be affected by factors such as irregular hair growth and the hair type and colour which need further research. Chemical and physical treatments such as strong hair detergents and permanent waves may influence the integrity of the hair shaft's outer cuticle layer and cause leakage of nicotine from hair.[16] There are analytical difficulties in extracting nicotine from hair in a consistent and reproducible manner in different laboratories, and there is a lack of reference material for evaluating various laboratory methodologies. It is still not understood how nicotine levels in hair relate to the biologically effective dose of smoke. Use of the hair biomarker is more costly than questionnaires.[17] There are restrictions on cutting hair in some cultures. Some infants and adults have scarce scalp hair that may not provide sufficient samples for analysis and assessment of smoke exposure.

Conclusion

Hair nicotine offers an alternative tool to measure nicotine content in the hair. Uncertainties over the mechanism by which drugs are incorporated into hair may have led to caution in the use of hair in forensic toxicology and medicine, but this may not be a serious issue. However, other issues in relation to this biomarker are still unresolved, such as how the hair colour, chemical treatments of hair, or type of hair texture can affect the results and their interpretation. Understanding and adjusting for these variables are expected to improve the precision of this measure. While this needs to be an ongoing process, it should not hamper the current use of this biomarker. This biomarker may have applications in exposure–disease association assessment. It can also be a useful tool in intervention studies to reduce tobacco consumption or exposure from the environment.

References:

1. Reddy KS, Gupta PC. Report on Tobacco Control in India. New Delhi: Ministry of Health and Family Welfare, Government of India. 2004; 5:589–94.
2. National Library of Medicine. The C. Everett Koop Papers. Tobacco, Second-Hand Smoke,

and the Campaign for a Smoke-Free America, http://profiles.nlm.gov/ps/retrieve/narrative/QQ/p-nid/85, accessed 9/21/11.

3. Etzel, RA. A Review of the Use of Saliva Cotinine as a Marker of Tobacco-Smoke Exposure. Preventive Medicine 1990; 19(2):190-97.

4. Benowitz, NL, Lessov-Schlaggar CN et al. "Female sex and oral contraceptive use accelerate nicotine metabolism." Clinical Pharmacology & Therapeutics2006; 79(5): 480-88.

5. Seiwert M, Merkel G, Krause C, et al. Determination of nicotine and cotinine in urine and hair of the general population of Germany [abstract]. Epidemiology 1995;6(suppl):S53.

6. Benowitz NL, Jacop PI. Metabolism of nicotine to cotinine studies by a dual stable isotope method. Clin Pharmacol Ther 1994;56:483–93.

7. Kyerematen GA, Damiano MD, Dvorchik BH, et al. Smoking induced changes in nicotine disposition: application of a new HPLC assay for nicotine and its metabolites. Clin Pharmacol Ther 1982;32:769–80.

8. Saitoh M, Uzuka M, Sakamoto M. Rate of hair growth. Oxford: Pergamon, 1967.

9. Zahlsen K., Nilsen OG. Gas chromatographic analysis of nicotine in hair. Environ Technol 1990;11:353–64.

10. Luck W, Nau H. Nicotine and cotinine concentrations in serum and milk of nursing smokers. Br J Clin Pharmacol 1984;18:9–15.

11. Gerstenberg B, Schepers G, Voncken P, et al. Nicotine and cotinine accumulation in pigmented and unpigmented rat hair. Drug Metab Dispos 1995;23:143–8.

12. Zahlsen K, Nilsen T, Nilsen OG. Interindividual differences in hair uptake of air nicotine and significance of cigarette counting for estimation of environmental tobacco smoke exposure. Pharmacol Toxicol 1996;79:183–90.

13. Pichini S, Altieri I, Pellegrini M, et al. Hair analysis for nicotine and cotinine: evaluation of extraction procedures, hair treatments, and development of reference material. Forensic Sci Int 1997;84:243–52.

14. Mahoney GN, Al-Delaimy WK. The measurement of nicotine in hair by reversed-phase high performance liquid chromatography with electrochemical detection. Journal of Chromatography Biomedical Applications 2001;753:179–87.

15. Al-Delaimy WK, Crane J, Woodward A. Is the hair nicotine level a more accurate biomarker of environmental tobacco smoke exposure than urine cotinine? J Epidemiol Community Health 2002;56:66–71.

16. Pichini S, Altieri I, Pellegrini M, et al. Hair analysis for nicotine and cotinine: evaluation of extraction procedures, hair treatments, and development of reference material. Forensic Sci Int 1997;84:243–52.

17. Jurado C, Kintz P, Menendez M, et al. Influence of the cosmetic treatment of hair on drug testing. Int J Legal Med 1997;110:159–63.

Source of Support :	NIL
Conflict of Interest :	NOT DECLARED
Date of Submission :	02-11-2012
Review Completed :	04-12-2012