Hair as a biomarker for exposure to tobacco smoke

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This article provides an overview of the hair nicotine biomarker for assessment of exposure to tobacco smoke, with emphasis on environmental tobacco smoke (ETS). Measurement of nicotine in hair can be an informative tool for research looking at ETS and related illnesses. There are still unresolved issues in relation to this biomarker such as 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. Nevertheless, hair nicotine promises to be a valid and reliable measure of longer term exposure that can be readily applied in epidemiological studies of exposure to tobacco smoke, and more specifically ETS, and its risk to health.

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. 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. This paper reviews this biomarker and its importance as a measure of environmental tobacco smoke (ETS) exposure in the epidemiological setting, examining issues ranging from the physiology of hair to the possible disadvantages of this approach. The definition of ETS in this review is: the tobacco smoke produced by an active smoker (from the exhalation of smoked tobacco; known as mainstream smoke, and that produced by the burning end of the cigarette; known as side-stream smoke) that is inhaled by non-smokers. The Medline database was searched from 1966 to May 2001 using the keywords “nicotine” and “hair”. The overview is divided into four parts: the first describes the physiology of human hair; the second part reviews published literature on the topic (mainly summarised in table 1), the third part describes in detail aspects related to hair as a biomarker of tobacco smoke exposure, and the fourth part outlines the advantages and disadvantages of this biomarker.

PHYSIOLOGY OF HUMAN HAIR

Hair physiology is rather complex and not very well understood. However, there seems to be agreement that the mean (SD) rate of hair growth of the scalp is generally 1 (0.3) cm per month. Hair growth rate has been calculated as 1.1 cm/month using drug markers incorporated into the hair through the systemic circulation. The anatomical location of the hair is the most important factor in hair growth rate. Scalp hair grows more quickly than pubic or axillary hair, and 85–90% of it is continuously found in the growing stage. The back of the scalp is the area with most uniform growth pattern; it is therefore the recommended site for sample collection. This is because the continuous growth provides updated information on exposure, and the limited percentage of non-growing hair minimises variability of results. Further details on hair growth stages can be found in references 10 and 11. In general, scalp hair grows faster in women than in men, which may be related to female hormones. Other factors such as race and age may also affect hair growth rate, but there is no strong evidence in the literature.

Bearing in mind these sources of variability, each cm of scalp hair reflects approximately one month of past exposure. The cm-by-cm distribution of nicotine has been found to approximately match the self report of the month-by-month mean number of cigarettes smoked daily (fig 1). Findings from other studies are similar. Therefore, when using hair samples for analysing “time related exposure” to nicotine or other external substances, it is important to measure the length of the analysed hair segment from the scalp. However, if the aim is to determine the concentration of nicotine per weight of hair as a measure of usual exposure, regardless of the time of exposure, or if history of past exposure was constant, then the length of hair sample becomes irrelevant.

STUDIES OF HAIR NICOTINE AS A BIOMARKER OF TOBacco EXPOSURE

The first recorded use of hair as a biomarker of exposure was in the mid 19th century when arsenic was detected in the hair of a deceased person. However, it was Baumgartner et al who in 1979 laid the foundation for hair analysis in modern times, applied first to testing for opiates with the radioimmunoassay method. Ishiyama et al in 1983 were the first to report the presence of nicotine in hair of humans. Since then a number of studies from different countries have investigated the use of hair for measuring exposure to nicotine. Table 1 summarises these studies.

The level of active smoking can be simply assessed by calculating the self reported number of cigarettes smoked by an individual in a given period. Several studies have compared the self reported number of cigarettes smoked per day with nicotine levels in hair and found significant
corresponding neonates. Mothers and their hair nicotine levels, or that of their newborns in Toronto, Canada, no relation was found between the number of cigarettes smoked by the mothers and their newborn hair nicotine levels than neonates of smoking mothers (p < 0.001). Similar findings were reported in another study by the same group (fig 2). However, they found a strong correlation between maternal and neonatal hair nicotine levels (whether active or passive).

The first study to compare urine cotinine levels to hair nicotine levels looked at hair nicotine and urine cotinine as biomarkers of ETS in a population of 94 children aged 1–3 years from Norway. They reported that cotinine in children’s hair correlated more closely with parental smoking history than that of their corresponding neonates. These studies were concerned with intrauterine exposure to maternal tobacco smoke exposure (whether active or passive). However, they found a strong correlation between maternal and neonatal hair nicotine levels (r = 0.78, p < 0.01). Neonates from non-smoking mothers had significantly lower hair nicotine levels than neonates of smoking mothers (p < 0.001). Similar findings were reported in another study by the same group (fig 2) and in other studies (table 1).

The results from these studies show promise of a reliable and valid new method of ETS exposure measurement. The exposure–dose relation with reported history of exposure indicate appropriateness of the use of hair nicotine for collecting objective results from studies investigating the health effects of ETS. Questionnaires still have advantages of low cost and availability for researchers and should be considered complementary to the hair nicotine measures in epidemiological studies that use this biomarker.

Hair as a biomarker for tobacco smoke exposure

Metabolism of nicotine

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 pyrrolidine 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.

It has been suggested that there is a difference 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. However, based on laboratory studies measuring body clearance rates of labelled nicotine and cotinine among smokers and non-smokers, it has been argued 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. In Toronto, 48 “Western and Eastern Indian” children had higher levels of cotinine in their hair than 112 European children with similar reports of exposures to ETS. The colour of hair was not related to a difference in cotinine levels. The authors attributed this racial difference in cotinine hair levels to slower metabolism of nicotine by Indian children, leading to a longer duration of circulating nicotine and cotinine serum levels which contributed to higher hair levels of these compounds. Three papers have reported similar findings for active smoking among African American adults compared with Americans of European origin. Therefore, ethnicity or race may be an important contributor in nicotine metabolism variability, as may the type and texture of hair in relation to race and phenotype 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 short distribution phase (5–10 minutes) and a longer elimination phase with high interindividual 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
Table 1  Main studies published on hair nicotine and cotinine levels in relation to reported exposure

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Number of subjects (smoking status)</th>
<th>Corresponding nicotine levels (ng/mg hair)</th>
<th>Corresponding cotinine levels</th>
<th>Mean age (range)</th>
<th>Analytical method and sensitivity (ng/mg hair)</th>
<th>Other results</th>
<th>Type of study and subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zaltsen et al&lt;sup&gt;11&lt;/sup&gt;</td>
<td>94</td>
<td>Norway</td>
<td>7 (non-smokers)</td>
<td>Mean: 0.87</td>
<td></td>
<td>4 years</td>
<td>GC/MS, 0.05 nicotine</td>
<td>n=0.83</td>
<td>Volunteers</td>
</tr>
<tr>
<td>Eliopoulus et al&lt;sup&gt;13&lt;/sup&gt;</td>
<td>94</td>
<td>Canada</td>
<td>35 (non-exposed, mother, newborn)</td>
<td>Mean: 1.2, 0.4 (mother, newborn)</td>
<td>0.3, 0.26</td>
<td>1–3 day</td>
<td>RIA, 0.25 nicotine; 0.1 cotinine</td>
<td></td>
<td>Newborn nurseries</td>
</tr>
<tr>
<td>Nafstad et al&lt;sup&gt;12&lt;/sup&gt;</td>
<td>95</td>
<td>Norway</td>
<td>19 (non-exposed)</td>
<td>Median: 0.7</td>
<td></td>
<td>12–36 months</td>
<td>GC/MS, 0.05 nicotine</td>
<td>n=0.64</td>
<td>Child health centre</td>
</tr>
<tr>
<td>Pichini et al&lt;sup&gt;14&lt;/sup&gt;</td>
<td>97</td>
<td>Italy</td>
<td>10 (non-exposed)</td>
<td>Mean: 1.3</td>
<td></td>
<td>Adults</td>
<td>RIA, 0.5 nicotine, 0.25 cotinine</td>
<td>n=0.48</td>
<td>Volunteers</td>
</tr>
<tr>
<td>Nafstad et al&lt;sup&gt;15&lt;/sup&gt;</td>
<td>97</td>
<td>Norway</td>
<td>24 (non-smoker, mother, child)</td>
<td>Median: 2.0 (child)</td>
<td>&lt;0.01</td>
<td>1–3 year</td>
<td>GC/MS, 0.05 nicotine</td>
<td>n=0.69</td>
<td>Child health centre</td>
</tr>
<tr>
<td>Dimich-Ward et al&lt;sup&gt;16&lt;/sup&gt;</td>
<td>97</td>
<td>Canada</td>
<td>8 (non-exposed)</td>
<td>G mean: 0.1</td>
<td>&lt;0.01</td>
<td>35 years</td>
<td>GC/MS, 0.03 nicotine; 0.01 cotinine</td>
<td>p=0.019</td>
<td>Selected hospitality workers</td>
</tr>
<tr>
<td>Sovik et al&lt;sup&gt;17&lt;/sup&gt;</td>
<td>99</td>
<td>Norway</td>
<td>16 (non-exposed)</td>
<td>Median: 0.7</td>
<td></td>
<td>Newborn</td>
<td>GC/MS, 0.01 nicotine</td>
<td>At 10 weeks of age; n=0.63 nicotine to cig of mother (at 10 weeks: median 2.15 non-exposed; median 8.66 exposed)</td>
<td>Case control study of oxygen sensitivity</td>
</tr>
<tr>
<td>Al-Delaimy et al&lt;sup&gt;18&lt;/sup&gt;</td>
<td>00</td>
<td>New Zealand</td>
<td>23 (non-exposed)</td>
<td>Median: ND</td>
<td>0.025</td>
<td>Children</td>
<td>RIA, 0.1 nicotine; 0.05 cotinine</td>
<td>Median nicotine; 0.49 (child), 3.02 mother. Median cotinine: 0.07 (child), 0.17 (mother)</td>
<td>Cross sectional survey of hospital children</td>
</tr>
<tr>
<td>Al-Delaimy et al&lt;sup&gt;19&lt;/sup&gt;</td>
<td>01</td>
<td>New Zealand</td>
<td>13 (no exposure)</td>
<td>Median: 0.62</td>
<td></td>
<td>24 years</td>
<td>HPLC-ECD, 0.1 nicotine</td>
<td>n=0.48</td>
<td>Cross sectional bars and restaurant staff</td>
</tr>
</tbody>
</table>
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Table 1 Continued  Main studies published on hair nicotine and cotinine levels in relation to reported exposure

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<thead>
<tr>
<th>Study</th>
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<th>Analytical method and sensitivity (ng/mg hair)</th>
<th>Other results</th>
<th>Type of study and subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Delaimy et al*</td>
<td>01</td>
<td>New Zealand</td>
<td>101 (no exposure)</td>
<td>G mean: 0.58</td>
<td>3-27 months</td>
<td>HPLC-ECD, 0.1 nicotine</td>
<td>Cross sectional hospital children</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>69 (smoking out)</td>
<td>G mean: 2.63</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>127 (smoking in)</td>
<td>G mean: 5.62</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GC/MS, gas chromatography with mass spectrophotometry; G mean, geometric mean; HPLC-ECD, high performance liquid chromatography with electrochemical detection; ND, no detection; RIA, radioimmunoassay.

Cumulative dose collected gradually over the period of exposure. The fact that 70–80% of nicotine is cleared by being converted to cotinine suggests that, over time, the metabolism rate of nicotine may affect levels of nicotine accumulation in hair. This is not expected to cause wide interindividual variability because of the slow growth rate of hair, a concept indirectly supported by findings from Eliopoulos et al who reported that levels of nicotine in hair were not correlated to (the more variable) serum nicotine levels of 36 adult smokers ($r = 0.24, p = 0.19$) at a single point of time. The poor correlation between hair nicotine and serum nicotine levels can be expected because the serum levels depend on the dose inhaled or ingested. Knowing the rate at which hair grows, and the distance of the analysed hair section from the scalp, levels in hair may then be related to exposure levels (whether active or passive) in a time dependent manner.

In support of this theory, a constant nicotine concentration has been found along the hair shaft for smokers ($n = 36$) and a decreasing concentration towards the proximal end of the hair shaft for individuals who had quit smoking in recent months ($n = 14$). Similar findings are explained by the uptake of nicotine being related to the inhalation of nicotine, where active smokers who quit or decreased smoking had decreasing hair nicotine levels in their newly grown hair. Nilsen and colleagues suggested that the uptake of nicotine being related to the inhalation of nicotine, where the concentration of nicotine in hair is calculated by dividing the amount of nicotine detected by the weight of the hair sample. It was also reported that short term (72 hours) nicotine exposure, while a linear uptake of nicotine seems to exist in the case of long term (eight weeks) exposure to nicotine at low concentration ($1.5 \mu g/m^2$) from controlled air chambers.

Hair uptake of nicotine and other substances

The mode of uptake of drugs by hair has been strongly debated in the literature. Most argue that hair mainly takes up external substances like nicotine or its metabolites from the systemic circulation through the hair bulb blood supply. It is believed that nicotine moves by passive diffusion from the bloodstream into the growing hair cells at the base of the follicle and then becomes tightly bound in the interior of the hair shaft during subsequent keratogenesis. Therefore, nicotine incorporation in hair is dependent on the average concentration in blood over time, which in turn depends on the dose inhaled or ingested. Knowing the rate at which hair grows, and the distance of the analysed hair section from the scalp, levels in hair may then be related to exposure levels (whether active or passive) in a time dependent manner.

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If adsorption occurs directly from the ambient environment, it is clearly not the only route of entry of nicotine into hair. Gerstenberg et al. found that 71–90% of the nicotine from hair samples of laboratory animals that were directly exposed to tobacco smoke was removed by washing, while Haley and Hoffman found up to 28-fold higher nicotine levels in non-washed hair samples compared to washed hair samples from the same individuals indicating limited, if any, adsorption from external contamination. These findings have also been reported for drugs such as morphine and cocaine. Hence, in the laboratory, all hair samples are washed by cleansing solutions before being digested and analysed to avoid contamination from nicotine externally attached to the hair shaft.

It may be that hair does adsorb nicotine directly from the environment after a lengthy contact period, but this route is probably secondary to the main route of systemic circulation nourishing the hair because of the above mentioned observations in human and animal hair studies. Judging from hair washing studies, it seems external adsorption happens for a small fraction from the total external exposure—that is, high sudden external exposure has a limited effect on variability of nicotine levels incorporated into hair shaft if hair samples are washed before analyses. In a recently published study we found hair nicotine levels to be correlated with hair cotinine levels. Since cotinine is only produced endogenously, this close correlation supports systemic absorption of nicotine into hair. More experimental studies will be required to reach consensus on the percentage of environmental adsorption of nicotine and other chemicals into the hair shaft.

This debate does not directly affect the use of hair nicotine as a biomarker of ETS exposure. Regardless of whether the uptake is systemic or external, nicotine seems to be consistently incorporated in the hair shaft and is available for analysis, and has been found to be well correlated with reports of exposure (table 1). The hair and the face are in close proximity to each other and are therefore expected to be exposed to the same level. Several participants with grey hair provided black and grey hair simultaneously, and showed lower levels in their grey hair. This difference was explained by 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). Gerstenberg et al. recognised that non-pigmented 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).

Others have argued that there is no difference in nicotine (and cotinine) uptake in relation to hair colour. Zahlsen et al. found that nicotine uptake from exposure to different concentrations of nicotine did not differ in relation to the colour of hair. They quantitatively measured melanin levels in hair in order to assess this relation. They also found that the person's age, hair thickness, and sex did not affect nicotine uptake rate. However, their exposure was not systemic but involved cut hair samples. Knight et al. reported that among 112 white children in Toronto, dark and fair hair had similar cotinine levels for similar reports of exposure, but their findings on cotinine levels do not necessarily apply to nicotine. 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.

**Cosmetics and other treatments and their effect on hair**

The ability of the hair to preserve substances incorporated into its shaft may be compromised by externally damaging factors. Several researchers have suggested that the chemical treatment of hair, such as dyeing and permanent waving, can damage the structure of the hair and affect the accurate detection of drugs in it. Others have argued that there is no difference in nicotine and cotinine incorporation rate into hair. Li and Cheng found that the cuticle of the hair was not damaged after bleaching or dying and nicotine levels were reduced by 30%.

On the other hand, normal (non-dandruff) shampoo washing does not seem to affect the levels of nicotine in the shaft of the hair. We recently found that adjusting for hair dyeing 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).

**ADVANTAGES AND DISADVANTAGES OF HAIR AS A BIOMARKER OF ETS**

**Advantages**

- Hair nicotine provides better information on long term ETS exposure than biomarker measures in urine, saliva, or serum than the shorter half life of the latter biomarkers. The long term (up to several months) ETS exposure is the usual exposure of interest in health-related studies.
Hair nicotine provides the advantage of longer term exposure assessment than other available biomarkers. In addition, the time dependent ETS exposure assessment is unique to hair nicotine. 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 for ETS studies. 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. It is important to extend the published literature with new studies from different parts of the world, using different study designs, bearing in mind the concerns mentioned above, and attempting to adjust for the contribution of these factors to the variability of results.

In particular, what are most needed are studies that apply this measure of exposure to assessment of ETS health effects. 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. Hair nicotine measure of ETS exposure can be a valuable tool in research and clinical settings. With the advancement of molecular epidemiology and the need for more precise methods of exposure measurement, the hair nicotine biomarker will likely become a standard measure of ETS exposure.

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