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Validity of Hair Cadmium in Detecting Chronic Cadmium Exposure in General Populations

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Human exposure to cadmium can not only occur by occupational exposure, but also by environmental exposure including contamination of food supplies, air, and tobacco smoke which is a major source of respirable cadmium in general population (Chmielnicka and Cherian 1986; Kjellström 1979; Markard 1983). Autopsy studies have shown that smokers in general have about twice the Cd content of non-smokers in most of their tissues (Drasch et al. 1985). Its toxicity at high doses is well known (Chilsom 1980, Carmichael et al. 1982), but remains controversial at environmental doses, particularly in pregnant women and children, most sensitive to toxic insult.

One difficulty in assessing effects of cadmium for past or long term exposure is to find a suitable indicator. Cadmium in blood is widely used as biological indicator of current exposures. Cadmium in urine more suitable for past exposure is not very practical for epidemiology of newborns. Because of its lifetime of several months, hair should better reflect an average of integrated environmental exposure, than does blood or urine. For assessment of past or continuous exposure levels, hair seems to be more adequate, since it is easy to obtain and it bioconcentrates metallic trace element. Moreover, cadmium remains in the blood only briefly and at very low levels (Fischbein 1984). The possibility of exogenous contamination has led to substantial controversy concerning the reliability of hair analysis as measure of absorbed dose. Use of hair from newborns effectively eliminated most concerns about exogenous contamination. Recent comparisons of Cd levels in scalp hair and pubic hair demonstrated that analysis in samples close to the scalp is not seriously invalidated by sources of external contamination (Wilhelm et al. 1990). Several studies investigating the clinical relevance of the hair cadmium content found significant relationships with the diagnosis of mental retardation or pregnancy outcome (Jiang et al. 1990; Huel et al. 1981, 1984). This suggests that systemic cadmium exposure can be quantified by hair analysis.

The purpose of this study was to assess the reliability of hair cadmium in regard to past or continuous cadmium exposure to smoke in two populations without known occupational exposure to Cd:

1) a male population with a high expected exposure to tobacco smoke, in order to evaluate the possible dose-response relationship and,

2) a pregnant woman population and their newborns with a mild expected exposure to tobacco smoke, in order to estimate the sensitivity of cadmium hair as an indicator of cadmium exposure via smoking habit.

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MATERIALS AND METHOD

For population I, the sample study was composed of 123 caucasian men recruited among active employees at the Paris Police Administration, with no occupational cadmium exposure. They were invited to participate on the occasion of their routine professional physical examination. They ranged in age from 25 to 55 years. A standardized questionnaire was proposed to each subject by the same observer to collect information including detailed tobacco consumption, among others. Subjects were categorized according to their smoking habits: **non-smokers**, if they had never smoked (n=45); **ex-smokers**, if they had stopped smoking at least one month before the visit (n=24); and **current smokers** (n=54). For each current smoker, the amount of tobacco smoked per day was converted into grams (1 cigarette=1g, 1 cigarillo=2g, 1 cigar=4g; for the pipe smokers, the weight of tobacco was used). Two groups of current smokers were formed according to the quantity of tobacco smoked daily: less than 20 grams (light smokers) or more (heavy smokers). Two groups of ex-smokers were defined according to the duration elapsed since the habit was dropped: less than 10 years (recent ex-smokers), or 10 years and more (late ex-smokers). For both current and ex-smokers, cigarettes x years correspond to the total amount of tobacco smoked during life divided by 365.
Hair samples were taken from the occipital region and were analysed for the determination of cadmium.

In population II, biopsy materials were obtained from 117 mothers and their newborns in the Baudelocque maternity Hospital (Paris) during a five month period. They ranged in age from 19 to 43 years and were not occupationally exposed to cadmium. Information available on the mother included some items about dietary habits (frequency and amount of consumption of bread, milk, meat among others by day and by week) and smoking habits (number of cigarettes smoked per day, duration, passive exposure). We defined four groups concerning smoking habits: **non-smokers** and non passive (n=54), if they did not smoke during pregnancy and the trimester before and if they were not environmentaly exposed to tobacco smoke; non-smoker but **passive** (n=37), if they did not smoke during the same period and if more than two cigarettes daily were smoked in their presence during the entire length of pregnancy; **quitters** (n=10), if they smoked at least one cigarette a day only during the first or second trimester of pregnancy; and **smokers** throughout the pregnancy (n=16), if they smoked at least one cigarette a day from the begining to the end of pregnancy. The definition of quitters was chosen according to well known results (Butler et al. 1972; Rush et al. 1983) showing that the third trimester is the most important for the growth of the fetus with regard to tobacco smoke.
At birth, hair samples were taken from the occipital region of the head of both the mother and her neonate, and placed in polystyrene tubes. Hair weighing at least 3 mg was used in the analysis, to have reliable samples. Only the first 8 cm of mother's hair were retained because 1) it is the length representing the record of the pregnancy (less than 1cm/month, Pecoraro et al. 1967) and 2) this removes a great part of the exogenous contamination.

Samples of both populations were analyzed in the same laboratory. Hair samples were washed, dried, weighed, mineralized and analyzed. The method for heavy metal assay and procedures for quality control have been described previously by Huel et al. (1981). This procedure includes a careful washing technique; measurement of heavy metal concentration is performed by electrothermal atomic absorption spectrometry using a stabilised temperature platform furnace and

Deuterium background correction. Trace element levels are expressed as parts per million (ppm).
As previously observed (Huel et al. 1981), cadmium in hair is far from normally distributed, so that we used median and percentiles to describe variables and non parametric statistical techniques were performed: Wilcoxon test for two samples comparisons and Spearman coefficient of correlation (SAS software v6). The ratio Cd newborn hair / Cd maternal hair was compared to 1 by using the Student's t-test on the Log transformed ratio.

RESULTS AND DISCUSSION

In population I, mean consumption of cigarettes per day was 9.1 (sd: 5.1) for light smokers and 27.8 (5.3) for heavy smokers. All ex-smokers had in fact stopped smoking at least one year before the visit.

Table 1. Hair cadmium distribution according to the smoking status in the male population (I)

	Non-smoker (n=45)	Ex-smoker late (n=12)	Ex-smoker recent (n=12)	Current smoker <20 g (n=27)	Current smoker ≥20 g(n=27)
Median of hair Cd	13 [4-76]	25.5 [3-188]	24.5* [8-121]	12 [4-56]	39** [6-210]
Mean of nb cig x years	0	272 ± 277§	488 ± 317	178 ± 115	370 ± 169

cadmium concentrations are expressed in ppm x 100.
5th,and 95th percentiles are between brackets.
§: mean ± standard deviation.
*: p<0.05, **: p<0.01, compared to the non smoker group (Wilcoxon test).

Table 1 gives the medians of cadmium (Cd) according to the classes: non-smokers, ex-smokers (late and recent) and current smokers (<20g/day and ≥20g/day). There was a significant increase of Cd in the groups of heavy smoker and recent ex-smoker compared to the non-smoker group (Wilcoxon test: p<0.01 and p<0.05 respectively). Although the median of Cd for the late ex-smoker group was similar to that of recent ex-smoker group, its Cd concentrations did not significantly differ from those of the non-smoker group (Wilcoxon test: p=0.22). For light smokers, the median of Cd was as low as that of the non-smokers, and their Cd concentrations were significantly lower than those of the heavy smokers (Wilcoxon: p<0.01). We can note in this group the lowest mean for the number of cigarettes x years (except for non-smokers, see table 1).

Table 2. Hair cadmium distribution according to the number of cigarettes x years in the male population (I)

Nb cig x years	0	1-100	100-200	200-300	300-400	>400
Median Cd	13	16	13	16.5	35*	52**
[5th-95th]	[4-76]	[4-188]	[4-56]	[7-153]	[3-154]	[6-210]
(n)	(45)	(12)	(15)	(18)	(12)	(22)

Nb cig x years x 365: total consumption of cigarettes during life.
cadmium concentrations are expressed in ppm x 100.
5th, 95th are percentiles.
*: p<0.05, **: p<0.01, compared to the non smoker group (Nb cigxyears=0), Wilcoxon test

We did not find any correlation between Cd levels and age, but we observed a positive correlation between the number of cigarettes x years and Cd values for ex-smokers and smokers combined (Spearman correlation coefficient: $r=0.30$, $p<0.01$, $n=78$). The relationship between cigarettes x years and Cd is illustrated in table 2 where six classes for the number of cigarettes x years were formed. For each class, the median and 5th, 95th percentiles of Cd are shown. No significant variations of Cd were noticed until a threshold of about 300 cigarettes x years, followed by a marked increase of Cd.

In population II, no statistical difference was observed for dietary habit items between non smokers (passive or not) and smokers (quitters or not). Age was not found correlated to Cd level; however the range of mothers 's age was not wide. The women who smoked until the last trimester of pregnancy (n=16) had an average of 8.4 (sd: 5.7) cigarettes per day. Table 3 lists the medians, the 5th and 95th percentiles relative to cadmium hair levels for mothers and newborns according to the smoking habits. It does not show significant variations for cadmium concentrations in maternal hair among the different groups, though we can note a perceptible decrease in the smoker group (IV). However, cadmium values in newborn's hair were significantly higher in the smoker group (IV) than in the different groups (each Wilcoxon test with $p<0.05$). This discrepancy between Cd concentrations in maternal hair and newborn hair in the smoker group was also reflected in the ratios Cd newborn hair/ Cd maternal hair shown in table 3: it can be seen that this ratio was inferior or equal to 1 in groups I, II, III; it was significantly greater than 1 in group IV (t-test=2.31, $p<0.05$). Similar results were obtained by using the quantitative consumption of cigarettes over the pregnancy period (for active smokers, quitters and for passive using a rough equivalence), *i.e.* an increase of Cd in newborn hair and no particular tendancy for maternal hair Cd except a decrease for the highest cigarettes consumption.

Table 3. Median of hair cadmium according to the smoking status in the female population (II).

Cadmium indicators	**Non-smoker** (and non-passive, n=54) I	**Passive** (and non smoker, n=37) II	**Quitters** (during the 1st or 2nd trimester, n=10) III	**Smoker** (throughout the pregnancy, n=16) IV
Maternal hair Cd (1)	15 [6-87]	18 [4-67]	17 [8-64]	10.5 [5-63]
Newborn hair Cd (2)	14.5 [4-45]	14 [3-48]	10 [5-85]	28.5 [a] [7-44]
(2) / (1)	0.7 [0.15-4.8]	1 [0.1-4.4]	0.6 [0.15-3.1]	1.7 [b] [0.3-7.8]

(1), (2) are expressed in ppm x100.
5th and 95th percentiles are between brackets
a: each comparison of group IV with groups I, II,III: $p<0.05$ with Wilcoxon test.
b: paired t-test comparison with $\log(2/1)=2.31$, $p<0.05$.

Human hair is an attractive indicator to assess long term or past exposure to cadmium, because it is easy to obtain and store, and it provides a measure of chronic exposure through its ability to bioconcentrate metals. In this study, the chronic or past exposure to Cd was determined by the smoking status, since cigarettes are known to be a major non occupational source of Cd (1-2 µg Cd/cig. and 10% inhaled: Elinder et al. 1983).
Because Cd from foods may widely vary among individuals, we checked that diet was similarly distributed in the smokers and the non-smokers. Our dietary

questionnaire did not express difference between the two groups, however it was not detailed enough to know which sort of cereals or vegetables was included in the diet. We can think that the major determinant which differentiates smokers from non-smokers about the cadmium exposure is the tobacco smoke.
Our cadmium values were in agreement with those found in the literature. Generally, authors express their results with arithmetic mean ± standard deviation, though not adequate, since distribution of cadmium in hair is not normal. If the medians of Cd mentioned above could seem low, in fact means ± sd (0.33 ppm ± 0.39 for males, 0.22 ± 0.19 for mothers, 0.21 ± 0.16 for newborns) were similar to the average levels obtained for hair by Azhari et al. (1990), Tagaki et al. (1986) and Gross et al. (1976) in different countries. They were lower than values reported by Chattopadhyay et al. (1990) in India, by Durak et al. (1990) in Turkey, maybe because techniques of measurement are not standardized and because of geographical variations of exposure.

Results of this study suggest that Cd hair is essentially a reliable indicator for subjects with highest environmental Cd exposure, *i. e.*, smokers with a high tobacco consumption. Thus, highest Cd concentrations were observed among the male heavy smokers, whereas the Cd values of light smokers were as low as non-smokers. Female smokers, who can be considered as light smokers since they smoked on average less than a half pack a day, also exhibited low Cd values. Actually, the total consumption of tobacco during life seems a major determinant of the Cd concentration in hair, since we observed in population I increasing values of Cd over 300 cigarettes x years. This is not surprising because the half-life of Cd in the body is many years and may be as long as 30 years. The tobacco effect observed with a threshold in adult hair could explain why light smokers had so low Cd values. This notion of threshold seems applicable to ex-smokers, since only those with a mean of cigarettes x years superior to 300 differed significantly from non-smokers. Anyway, the current exposure also seems important; although the mean of tobacco x years was higher among recent ex-smokers than among heavy smokers, Cd concentrations were greater in this last group. The tobacco effect on Cd increase in the body was already reported in blood and urine. In blood, the increase in Cd with tobacco use is no longer controversial, either in a general population (Grasmick et al. 1985; Elinder et al. 1983) or among pregnant women (Kühnert et al. 1982), nevertheless Cd decreases more rapidly after exposure than in hair. In urine, Suna et al. (1991), Cresta et al. (1989) observed a Cd level in smokers slightly higher than that in non-smokers. However, results expressed by authors are not homogeneous and it is not quite clear which is the best way to express them, in µg Cd/24h or µg Cd/g of creatinine. Moreover, Araki and Aono (1989) have recently shown variation of urinary excretion of Cd according to water restriction and loading, which suggests that glomerular filtration is the major factor determining renal excretory mechanism of Cd.
This study tends to support the use of hair analysis as a measurement of systemically distributed cadmium, since we did not find significant difference between passive and non-smokers. It can be thought that the above-mentioned precautions (retaining only the first 8 cm from the scalp and the use of a careful washing technique) allowed reduction of exogenous contamination or that this contamination due to environmental smoke was negligible. Moreover, Wilhelm et al. (1990) recently showed, comparing Cd levels in scalp hair and pubic hair, that hair metal analysis in samples close to the scalp is not seriously invalidated by sources of external contamination. Most concerns about contamination can be eliminated concerning newborn hair samples since it reflects only fetal exposure (Huel et al. 1984).

The slight decrease of Cd values observed among female smokers contrary to male smokers could be attributed to pregnancy, a particular state which may modify the metallic metabolism. In mothers, Cd could be displaced to other biological compartments, such as the fetoplacental unit. Thus, it seems that Cd was accumulating preferentially during pregnancy in the newborn of exposed mothers against a concentration gradient. The same observation was obtained in a study we realized among hypertensive mothers (Huel et al. 1981); hair Cd levels expected to be high in hypertensive mothers (Perry et al. 1974; Chisolm and Handorf 1985), was in fact lower than in normotensive mothers and the converse was observed in newborns.
Pharmacokinetic studies of Cd in blood and placenta (Levin et al. 1987) demonstrated the existence of a partial placental barrier to Cd. Nevertheless, Cd can cross the placenta, as shown in several studies in which the fetus was exposed through maternal exposure (Schramel et al. 1988; Huel et al. 1984; Siegers et al. 1983). And hair Cd which reflects integrated environmental exposure during several months showed that this exposure is not negligible, since hair Cd concentrations were twice as great in newborns of smokers than of non-smokers (table 3). So, women even lightly exposed to Cd, gave substantially more Cd to their offspring as did women not exposed. Thus, the use of newborn hair could be considered as a suitable indicator for at least mild exposure to Cd of mother. Moreover, in newborns, hair is easier to obtain than urine or blood and is not affected by exogenous contamination.

Human head hair has a propensity to be an attractive tissue useful in epidemiological studies. Based on these two populations, with those already cited, hair seems a reliable biochemical indicator for assessing cadmium exposure in the human being. When high tobacco consumption occurred, we observed a dose-response relationship above a threshold value. However the determination of the concentration of cadmium in adult head hair has limitations since somewhat non sensitive enough to detect low levels of exposure. In the current investigation applied to pregnancy, a situation where low tobacco exposure occurs, newborn's hair seems more sensitive and therefore more adequate than mother's hair. Nevertheless we were not able to verify if this finding is attributable to the physiological changes or to the lower reliability of the mother's head hair.

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