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Cigarette Smoking and Secondary Smoke in Turkey: Effect on Placental Aryl Hydrocarbon Hydroxylase (AHH), Infant Birth Weight, and Size

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Polycylic aromatic hydrocarbons (PAH) are a large class of chemicals in the atmosphere, soil, waterways, oceans, and the food chain. Epidemiological studies indicate that the environment is a significant determinant in the incidence of human cancer. A causal agent in human cancer is the inhalation of cigarette smoke, resulting in a high incidence of lung cancer among smokers. Many PAHs in cigarette smoke are powerful carcinogens in experimental animals and likely cause lung cancer in humans. The PAHs may also contribute to the etiology of cancer at organ sites other than lung (Gelboin 1980). After ingestion, absorption, and transport, the initial biological receptors for the metabolism of the PAHs are the microsomal mixed-function oxidases (MFOs) containing cytochrome P450. The PAHs are oxygenated to epoxides phenols and quinones. The epoxide intermediates are hydrated to dihydrodiols and further oxygenated to carcinogenic diol epoxides or conjugated to water-soluble reduced glutathione, glucuronide or sulphate conjugates. (Yang et al. 1978, Gelboin 1980). Benzo[a]pyrene (BP) is a potent carcinogen in experimental animals inhaled during cigarette smoking and is believed to contribute, to be responsible for the carcinogenicity of cigarette smoke.The human placenta metabolizes drugs and endogenous substrates such as steroids. Many drugs and environmental chemicals metabolized in the placenta enter fetal circulation (Nebert et al.1969). AHH is present in placental microsomes and is induced to high levels in microsomes from women who smoke during pregnancy. Cigarette smoking also induces other placental enzyme activities (Nebert et al.1969).

This study investigated AHH activity in placentas of cigarette smoker women, women exposed to secondary cigarette smoke and non-smoker women. AHH activity levels were determined in the placentas of 37 women smokers, 32 women exposed to secondary cigarette smoke and a control group of eight non-smoker women.

MATERIALS AND METHODS

Chemicals: KCl, Na_2Co_3, NaOH, Sodium, Potassium tartrate, $CuSO_4$, Tris, HCl, $MgCl_2$, $CaCl_2$, Sucrose, Acetone, Hexane from Merck, BSA from Serva Feinbiochemica Heidelberg, New York, Dithiothreitol from Aldrich Chemical

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Company, MADPH and Folin's phenol reagent from Merck, 3-OHBP and B[a]P from Midwest Research, Kansas City. Normal-appearing segments of placenta were obtained from 77 women delivering at the Government Hospital, Soykan Hospital and East Clinic from Malatya and Zekai Tahir Burak Cebeci Maternity-home (Hospital) from Ankara. The placentas were stored frozen (-70°C) until assayed. All the mothers participating in this study completed a questionnaire with regard to age, ethnic origin, employment, smoking habits, diet and drug use. The data recorded included baby birth weight and length, number of previous pregnancies and medication given during pregnancy and labor.

Each placenta was thawed overnight at 4°C and then minced after removal of the membranes, umbilical cord and blood. A 5-10 g portion of the minced homogenate was removed and a sub-portion was homogenized for 2-3 minutes in ice cold 0.25 M sucrose-0.05 M Tris-chloride buffer (pH 7.7) using a PCV type homogenizer and was frozen (-70°c) and stored until assayed for AHH activity. For preparation of the microsomes, a portion of the minced tissue (10-15 g) was homogenized for 2-3 minutes in ice cold 0.25 M sucrose- 0.01 mmol DTT – 0.001M Tris-chloride buffer (PH 7.7) to yield a 25 per cent (W/V) homogenate. The homogenate was centrifuged at 5,000 rpm for 7 min in a Beckman L8-70M Ultracentrifuge. Supernatant material from the 5,000 rpm centrifugation was centrifuged at 15,000 rpm for 15 min at a final concentration of 8mM $CaCl_2$. The supernatant was centrifuged at 30,000 rpm for 30 min to separate the microsomal fraction. The pellet was re-suspended in 0.01M Tris-chloride-DTT and mixed with a vortex mixer. The pellet was re-suspended and re-centrifuged at 30,000 rpm. The pellet was re-suspended in 0.25M sucrose in Tris-chloride buffer (pH 7.7). The microsomal suspensions were distributed into 1ml aliquots and stored at –70°C until used for measuring AHH activity. Protein content was determined by Lowry method using BSA as the standard (Lowry et al.1951).

Our assay is similar to the procedure of Nebert and Gelboin (Daudel et al.1975) with some modifications. The reaction mixture in a total volume of 1.0 ml, contained 920 µl of 0.05M Tris-chloride buffer, pH 7.7, 10µl of 5.4 mmol NADPH in 1% $NaHCO_3$, 10µl 7.5 mmol $MgCl_2$, 50µl of placental homogenate (containing 1-2 mg protein / ml) or placental microsomes (containing 0.05 mg protein / ml) and 10µl of 10 mmol BP in methanol (added just prior to incubation). The mixture was incubated, with gentle shaking at 37° C for 30 min in air. The reaction was stopped by the addition of 4 ml acetone-hexane (1:3, V/V). Blank values were obtained by adding the placental homogenate after stopping the reaction. The tubes were wortex-mixed and the upper organic phase was transferred to 1M NaOH (1.0ml) and wortex-mixed for 15s. The florescence change in the alkaline phase was recorded with a F-4010 Model Hitachi Fluorescence Spectrophotometer with the following settings: slit width 10 nm; excitation, 387 nm; emission 504 nm. A standard with known of 3-Hydroxy-benzo[a]pyrene in the incubation mixture without microsomes or homogenates was used as a reference. The Fluorescence Spectrophotometer was standardized each time with rhodamine B. The quantity of BP derivatives formed was calculated by comparing the net fluorescence (sample minus blank) of the final

alkaline extract with a standard plot of 3-OHBP concentration versus fluorescence. AHH activity is expressed as picomoles product formed per milligram microsomal or homogenate protein per minute. Product refers to the alkali-extractable metabolites of BP measured spectrophotofluorometrically; relative to 3-hydroxybenzo[a]pyrene.

RESULTS AND DISCUSSION

The placental microsomes and homogenates from 8 non-smokers, 37 cigarette smokers and 32 women exposed to secondary cigarette smoke were assayed for AHH activity. AHH activity was generally increased relative to the number of cigarettes smoked per day although AHH activity varied in some individuals who smoked the same number of cigarettes per day. Table 1 and Figure 1 show that the AHH placental microsomes activity in women smoking 25 cigarettes and women smoking five cigarettes per day was 7.29 times and 3.44 times greater than to non- smokers women respectively. The AHH results are generally in agreement with other studies (Vaught et al. 1979, Huel et al.1989). A proportional relationship was found between the number of cigarettes smoked per day and the level of placental AHH activity. Moreover, AHH activity was significantly higher in pregnant women passively exposed to tobacco smoke as compare to control (Huel et al.1989). In one study the AHH activity in placentas from smoking women was 2- to 25- fold greater than the AHH in placentas from non-smokers (Nebert et al.1969). In another study, the levels of AHH activity were determined in the placentas of 52 cigarette smokers and the average level of AHH activity in smokers was 45.68 and in the non-smokers was 6.44 respectively (Hincal 1986). Placental AHH activity was determined in 207 placentas and showed that smoking during pregnancy is associated with a marked increase in placental AHH activity (Huel et al. 1989). In one study, the AHH activity was 75 times much higher in smokers than non-smokers (Vaught et al.1979). The induction of AHH activity in smoking women was considerably higher than non-smokers, compared to our results (Nebert et al.1969). It has been found that cigarette smoking increases the placental AHH activity but does not have any effect on glutathione S-transferase activity in pregnant women (Pasanen and Pelkonen 1990). The toxic effects of PAHs in tissues such as placenta have been demonstrated to be due to their metabolites, epoxides, which interact with DNA (Huel et al. 1993). In one study, They found that there was a positive linear correlation in between DNA adduct and AHH activity and the number of the adducts were 5 times higher in smokers compared to non-smokers (Bartsh et al. 1992).

The AHH activity of 32 of 40 women exposed to secondary cigarette smoke from 5 to 20 cigarettes per day and 8 women not exposed to cigarette smoke showed an increase in AHH activity parallel to the number of secondary cigarette smoked daily in comparison with non-smoking women. Table 1 and Figure 1 show that AHH activity in non-smoker microsomes was 1.52 pmole/min/mg protein compared to 3.34 pmole/min/mg protein in women exposed to secondary smoke from 20 cigarettes per day. Thus, women exposed to secondary smoke showed

Table 1. Cigarette smoking and smoke exposed effect on AHH activity in placental microsomes and homogenates in women smokers and non-smokers.

Number of Placental samples	The number of cigarettes smoked daily and exposed cigarettes smoke daily	AHH Activity[a] $\bar{X} \pm$ S .D.	Times increase againts control
8* 8** 8***	Control	*1.52 ± 0.354 **1.03 ± 0.230 ***1.52 ± 0.354	-
6* 6** 6***	5	* 3.215 ± 0.561 **1.487 ± 0.278 ***1.789 ± 0.081	*2.11 **1.43 ***1.18
10* 10** 11***	10	*4.612 ± 0.386 **2.205 ± 1.068 ***2.419 ± 0.444	*3.04 **2.14 ***1.59
6* 6*** 5***	15	*6.182 ± 0.299 **2.600 ± 0.743 ***2.788 ± 0.125	*4.07 **2.52 ***1.83
10* 10** 10***	20	*7.168 ± 0.685 **3.124 ± 0.386 ***3.345 ± 0.179	*4.71 **3.03 ***2.20
5* 5**	25	*11.089 ± 0.378 **8.254 ± 0.325	*7.29 **8.01

[a] pmole 3-OHBP formed per mg protein per min (r :* 0.97;**0.85;***0.99)

* AHH activity in placental mikrosomes

** AHH activity in placental homogenates

*** AHH activity in placental microsomes of cigarettes smoke exposed women

Table 2 . The cigarette smoking effects on birth weight and birth length in pregnant women smokers.

The number of placental samples	The number of cigarette smoking daily and exposed cigarette smoke daily	Birth weight (g)	% decrease	Birth length (cm)	% decrease
8* 8**	Control	*3988 ± 35 **3988 ± 35	- -	51.81 ± 0.53 51.81 ± 0.53	- -
6* 6**	5	*3608 ± 102 **3750 ± 197	*9.53 ** 5.97	*49.50 ± 0.55 **50.67 ± 1.03	*4.46 **2.20
10* 11**	10	*3450 ± 94 **3655 ± 192	*13.49 **8.35	*48.70 ± 0.48 **51.18 ± 0.87	* 6.00 ** 1.22
6* 5**	15	*3350 ± 74 **3560 ± 55	*15.99 **10.73	*48.16 ± 0.41 **50.40 ± 0.55	*7.04 ** 2.72
10* 10**	20	*3208 ± 38 **3440 ± 134	*19.56 **13.73	*48 **50.40 ± 1.14	*7.35 **2.72

* Birth weight, birth length and % decrease in smoker women
** Cigarette smoke exposed effect on birth weight and birth length in non-smoker women

2.20 times higher AHH activity than women not exposed to cigarette smoke. Moreover, AHH activity was significantly higher in pregnant women passively exposed to tobacco smoke compare to control (Huel et al.1989). AHH activity was also determined in placental homogenates of 37 smoker women and 8 non-smoker women. Table 1 and Figure 1 shows the AHH values in smoker and non-smoker placental homogenates. A similar increase in AHH activity appears with increasing numbers of cigarettes smoked per day in placental tissue homogenates as found with microsomal AHH activity. Some association was found between AHH activity levels from placental homogenates and the number of cigarettes smoked per day (Kaelin et al. 1983). The average weight of newborn infants in the control group was 3,988 g. Table 2 show that the weight and length of the

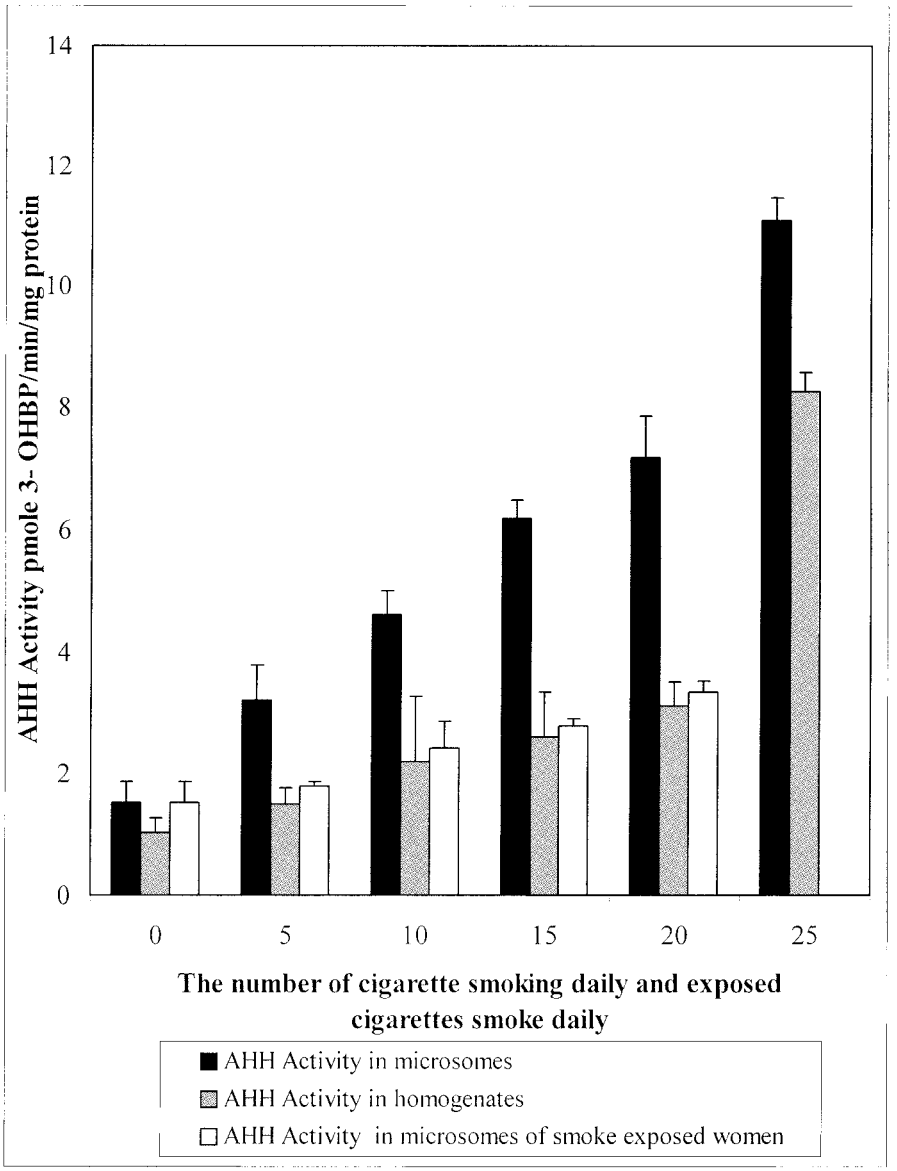

Figure 1. The distribution of AHH activity in placental microsomes, Homogenates in 37 smoker, 32 smoke exposed and 40 non-smoker women.

infants decreased with the number of cigarettes smoked per day. There is a 780 g. difference between the average weight of the infants from the 20 cigarette smoker group and the control group (Table 2). Further, there is an 3.81 cm decrease in the average infant length between the 20 cigarette and non- smoker group. The decrease of both birth weight and birth length were significant ($p<0.01$) (Table 2).

A similar but quantitatively less decrease was observed in infants from women exposed to secondary smoke. There was a 548 g weight reduction and a 1.77 cm length reduction in the 20 cigarette smoker exposed group relative to the non-smoker group (Table 2).

Statistical analysis showed that the decreases in birth weight and length (Table 2) were significant ($p< 0.01$). Reduction in both weight and length of infants from women exposed to secondary smoke were observed but quantitatively less than the decreases caused by cigarette smoking. A group of researcher found that the induction of placental P-450 $_{arom}$ during gestation is suppressed by maternal smoking, resulting in a reduction in estrogen producing ability, while placental xenobiotic P-450 is induced (Kitawaki et al. 1993). The others found that one week after smoking cessation 34.5 % of baseline 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) plus NNAL-Gluc. was detected in urine, where the corresponding values for cotinine and nicotine were 1.1 and 0.5 %, respectively (Stephen et al. 1999). In other reports, the weight of newborn infants of smoking mothers were 400 g less and size was 1 cm shorter than those from non-smoker mothers (pelkonen et al. 1979). Others showed that through pregnancy urinary estriol and serum human placental lactogen concentration were lower in pregnant women who smoked over 20 cigarettes a day than in moderate smokers and non-smokers (Mochizuki et al.1984). In a study of mice fetal weight was reduced 22% in B6 and 17% in D2 mice 18 days after a single exposure to cigarette smoke (Vahakangas et al. 1982).

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