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*RESEARCH ARTICLE*

# Paternal Smoking and the Increased Frequency of Chromosome Aberrations in Umbilical Cord Blood Samples

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***Paternal Smoking and the Increased Frequency of Chromosome Aberrations in Umbilical Cord Blood Samples***

***Summary***

*In the present study, we investigated the effects of paternal smoking on the frequency of Chromosomal Aberrations (CAs) in newborns. Newborns and their mothers who participated in this study were from Ankara Hospital, Gynecology and Obstetrics Clinics, comprised of 31 newborns of smoking fathers and 31 newborns of non-smoking fathers. In order to quantify the degree of maternal exposure, we detected the levels of urinary cotinine in the mothers. (CAs) were analyzed in umbilical cord blood samples and the levels of urinary cotinine were detected in The mothers' urine samples by gas chromatography. We found a significant increase in the frequency of CAs in the newborns exposed to paternal smoking when compared to paternal non-smokers (1.45±1.52 and 1.19±1.30, respectively, p=0.05). Our results indicated that paternal smoking increases the frequency of CAs and may make a substantial contribution to newborn exposure. We also found that urinary cotinine: creatinine ratios were the highest in women whose husbands smoked more than 20 cigarettes per day.*

***Key Words:*** *Paternal Smoking, Chromosomal Aberrations, Newborn Cotinine, Gas Chromotography*

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***Paternal olarak Sigaraya Maruziyette Kromozom Aberasyon Sıklığı***

***Özet***

*Sunulan çalışmada, paternal (baba kaynaklı) sigara dumanı maruziyetinin yeni doğanlarda Kromozom Aberasyon (KA) sıklığına olan etkisi incelenmiştir. Ankara Hastanesi Kadın Hastalıkları ve Doğum Kliniğinde doğum yapmış bireyler çalışma grubumuzu oluşturmaktadır, sigara içen ve içmeyen babaların bebekleri çalışmaya dahil edilmiştir. Anne rahminde bebeğin maruziyetini yansıtması açısından annelerin idrarındaki kotinin miktarları ölçülmüştür. Kordon kan örneklerinde KA sıklıkları tespit edilmiş, annenin idrar örneklerinde ise kotinin miktarları gaz kromatografisi yöntemi kullanılarak ölçülmüştür. Babası sigara kullanan yenidoğanların KA sıklıkları kullanmayanlarla karşılaştırıldığında istatistiksel olarak anlamlı bir fark bulunmuştur.(1.45±1.52 ve 1.19±1.30, p=0.05). Sonuçlarımız, sigara kullananlarda KA sıklıklarının arttığı ve bu durumun sigaraya bağlı olabileceği sonucunu ortaya koymaktadır. Ayrıca, en yüksek idrar kotinin:kreatinin oranları da, günde 20'den fazla sigara içen babaların eşlerinde gözlenmiştir.*

***Anahtar Kelimeler:*** *Paternal Sigara maruziyeti, Kromozom Aberasyonları, Yenidoğan Kotinin Sevileri, Gaz Kromatografisi.*

## INTRODUCTION

Smoking is important factor causing of lung cancer and 87% of lung cancer cases are caused by smoking. Cigarette smoke is a highly complex mixture of various chemicals (1). Some of them are generally considered to be responsible for a substantial number of human health problems and include well-known

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carcinogenic agents (2). Many carcinogens, co-carcinogens and mutagens are direct DNA damaging agents and cause changes in DNA. These changes are an integral part of neoplastic development (2).

Exposure to cigarette smoking has been linked to numerous adverse health outcomes in young children, ranging from prematurity and increased infant mortality to higher rates of asthma and sudden infant death syndrome (3). Up to 20% of pregnant women smoke and there is indirect evidence that certain tobacco-specific metabolites can cross the placental barrier and are genotoxic to the fetus (4). Newborns of smoking mothers have elevated frequencies of HPRT mutants, translocations, and DNA strand breaks. Paternal exposure may also affect the fetus in terms of chromosomal damage. Sperm of smokers have elevated frequencies of aneuploidy, chromosomal changes, DNA adducts, strand breaks, and oxidative damage (5). Chromosomal Aberrations (CAs) are generated by breakages and exchanges of chromosomal material that have been fixed in the genome (6). The study of the frequency of CAs in peripheral blood lymphocytes is a sensitive assay for detecting exposure to mutagens and carcinogens and is frequently applied in the situations of suspected genotoxic exposure. Consistent CAs is observed not only in rare tumor types but also in the relatively common lung, colon, and breast cancers(7). El-Zein et al. evaluated 22 lung cancer patients and 35 controls to determine whether the frequency of CAs was significantly associated with specific clinical variables such as the histological type, grade and stage of the tumors. Their results indicated a significant linear increase ($p = 0.01$) in the level of breaks with respect to the grade of the carcinoma (8). Some studies were found that the increased frequency of CAs in peripheral blood lymphocytes is a predictor of cancer, but further data are needed to better characterize CA as a marker of cancer risk (2,9).

In this study we used cord blood samples from newborns to investigate whether there are any effects of paternal smoking on the frequency of CAs of the offspring. In order to quantify the maternal exposure to secondhand smoking from their husbands we collected urine samples from mothers and analyzed the levels of cotinine in mother's urine. Cotinine, the major metabolite of nicotine, can be measured in saliva or urine and is a commonly used biochemical indicator of smoking status. The urinary cotinine level in newborns was lower than that of their mothers (10).

## MATERIAL AND METHODS

### Subjects

Newborns and their mothers who participated in this study were from Ankara Hospital, Gynecology and Obstetrics, comprised of 31 newborns of smoking fathers and 31 newborns of non-smoking fathers from January to August 2005. This study was approved by the Local Ethics Committee. Mothers were informed and their consent was obtained before enrollment. The mothers were interviewed before delivery, using a standardized questionnaire to obtain their husbands' smoking history including the number of cigarettes smoked per day and the duration of smoking and to cross-examine environmental tobacco smoke exposure (other people's tobacco smoke except for parental smoking) of mothers.

### Lymphocyte cultures and chromosomal aberration scoring

Umbilical cord blood samples were taken from the funiculus at the end of delivery and processed immediately. One ml of heparinized umbilical cord blood was collected from each newborn. Cultures were established for 48h at 37°C using TC 199 Medium (Biological Industries, Cat. No.01-106-1B) plus 25% Fetal Calf Serum (FCS, Biological Industries, Cat. No.04-001-1B). The cells were stimulated with 2% phytohaemaglutinin (PHA-L Biological Industries Cat. No. 12-006 1H) and colcemid (Biological Industries Cat. No. 12-004-1B) was added at a final concentration of 1µg/ml for the last 3h. Cells were harvested, lysed by exposure to a hypotonic solution of 75 mM potassium chloride (KCl) (Merck), and fixed with methanol and acetic acid in the ratio 3:1 (11) . A total of 100 cells were analyzed from each newborn using x100 (magnification) oil objective on a Zeiss Axioskop microscope.

The following types of aberrations were observed during the analysis: (1) chromosome break; (2)

chromosome gap; (3) chromatid break; (4)chromatid gap; (5)acentric fragment (a pair of chromatids without a centromere); (6) minute, (7) dicentric and (8) ring.

**Measurement of urinary cotinine levels by gas chromatography (GC)**

Spot urine samples were collected from mothers because some parents refused to allow sample collection from the newborns and most of the newborns failed to produce urine for analysis. It was so difficult to collect urine samples from newborns. Cotinine in urine samples was extracted by Beckett and Triggs method with slight modifications (12). Urine samples were stored at –20°C until GC analysis was conducted. The stored urine samples were left to defrost, homogenized and kept 30 min at room temperature. After adding quinoline as an internal standard (50 μg/ml), 5 ml of urine was mixed with 0.5 ml 5 N ammonium hydroxide in a glass tube. The solution was extracted with 4x5 ml of dichloromethane. The combined dichloromethane extracts were passed through anhydrous sodium sulfate to remove the moisture. After centrifugation in 1000 rpm for 10 min, the organic phase was concentrated to 100 μl and 1 μl was injected into the gas chromatograph.

Gas Chromatography (GC) analysis was carried out in a Hewlett-Packard Model 5890 gas chromatograph equipped with a Flame Ionization Detector (FID) and a HP 3396 integrator. Chromatographic determination of cotinine was carried out using a 25 m x 0.2 mm fused silica capillary column HP-Ultra 1 from Hewlett-Packard. The operating conditions were as follows: injector temperature 250 °C; detector 250 °C; column 110 °C hold 13 °C/min to 220 °C; 1/30 split ratio. Peak areas were used as the basis for quantification. A spiked quality control sample was used. Spiked samples were analyzed to provide a quality control for analysis (13).

Creatinine was measured by a spectrophotometre on all urine samples to correct for the dilution of the urine. Cotinine levels were expressed in micrograms/ml (14).

**Statistical Analysis**

Data were shown as mean± standard deviation. Statistical analyses were performed through Student's t-test. Correlation was assessed by Spearman's rank test (p=-0.04). A p value less than 0.05 was considered statistically significant

**RESULTS**

In the present study, all mothers stopped smoking during their pregnancy. The mean age of the mothers was 28.4±4.9 years (range 20–38). There was no significant difference between the mothers' ages (p>0.05). 45.2 % of the fathers always smoked in the presence of mothers (in the same room) and most of the fathers smoked more than 10 cigarettes per day. Of the two important factors of the research, the number of the cigarettes smoked by the fathers is one, and the mothers' and fathers' being in the same room during the fathers' smoking period was another. To detect the mother's exposure (excluding secondhand smoking from their husband), mothers were asked to be exposed to environmental tobacco smoke. If there was exposure, they were marked as 'passive smoker'.

Table 1 demonstrates age, smoking status, the types of CAs, the frequencies of CAs and the levels of urinary cotinine of mothers exposed to paternal smoking and the types and frequencies of CAs of newborns that were not exposed to paternal smoking were also shown in Table 2. The mean frequency of CAs (mean±SD) for 31 newborns of smoking fathers and 31 newborns of nonsmoking fathers are shown Table 3. The mean frequency of CAs (mean±SD) in the newborns of the fathers who smoked during the wife's pregnancy were significantly higher than those of the newborns whose fathers did not smoke (1.45±1.52 and 1.19±1.30, respectively, $p$=0.05). Our results demonstrated that paternal smoking induces the frequency of CAs.

We also measured the levels of urinary cotinine in the mother's urine to confirm their exposure, and therefore the fetus' exposure, to the paternal smoking. We found that the average cotinine: creatinine ratio for all mothers enrolled in this study was 40.2 μg/ml. Spearman's rank correlation was calculated to

**Table 1.** Smoking status, types and frequencies of CAs of newborns and levels of urinary cotinine of mothers exposed to paternal smoking

| No | Mother's Age | Smoker Status for Fathers Cigarettes/day | Duration of smoking (year) | Passive Smoking Y N | Levels of urinary cotinine of mothers (µg/ml) | %CA | G | CB | AF | R | M | DC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 23 | 20- > | 5 | Y | 20.5 | 0 | 0 | 0 | | | | |
| 2 | 24 | 11-20 | 5 < | Y | 8.0 | 2 | 2 | 0 | | | | |
| 3 | 29 | 11-20 | 5 < | Y | 32.6 | 1 | 0 | 1 | | | | |
| 4 | 25 | 11-20 | 5 < | Y | 25.9 | 1 | 1 | 0 | | | | |
| 5 | 38 | 11-20 | 5 < | Y | 11.5 | 3 | 3 | 0 | | | | |
| 6 | 27 | 20- > | 5 < | N | 17.7 | 7 | 4 | 3 | | | | |
| 7 | 19 | 1-10 | 2-5 | N | 10.9 | 4 | 3 | 1 | | | | |
| 8 | 39 | 11-20 | 5 < | Y | 4.6 | 2 | 1 | 1 | | | | |
| 9 | 20 | 11-20 | 5 < | Y | 22.6 | 2 | 1 | 1 | | | | |
| 10 | 40 | 1-10 | 5 < | N | 22.5 | 4 | 1* | 1 | | 1 | 1 | |
| 11 | 20 | 11-20 | 5 < | Y | 34.2 | 1 | 1 | 0 | | | | |
| 12 | 23 | 1-10 | 5 < | Y | 38.2 | 1 | 0 | 0 | | | 1 | |
| 13 | 26 | 11-20 | 5 < | N | 7.2 | 1 | 0 | 0 | 1 | | | |
| 14 | 39 | 20- > | 5 < | Y | 22.6 | 1 | 0 | 1 | | | | |
| 15 | 24 | 20- > | 5 < | N | 55.3 | 1 | 1 | 0 | | | | |
| 16 | 21 | 11-20 | 5 < | Y | 18.0 | 0 | 0 | 0 | | | | |
| 17 | 34 | 20- > | 5 < | Y | 17.3 | 1 | 1 | 0 | | | | |
| 18 | 24 | 11-20 | 5 < | N | 28.7 | 2 | 1 | 0 | | | | 1 |
| 19 | 25 | 20- > | 5 < | N | 15.7 | 1 | 1 | 0 | | | | |
| 20 | 35 | 20- > | 5 < | Y | 14.4 | 0 | 0 | 0 | | | | |
| 21 | 20 | 11-20 | 5 < | N | 18.7 | 0 | 0 | 0 | | | | |
| 22 | 29 | 11-20 | 5 < | N | 23.9 | 2 | 0 | 2 | | | | |
| 23 | 34 | 11-20 | 5 | N | 27.1 | 0 | 0 | 0 | | | | |
| 24 | 35 | 11-20 | 5 < | N | 14.9 | 0 | 0 | 0 | | | | |
| 25 | 37 | 20- > | 5 < | N | 23.3 | 1 | 0 | 1 | | | | |
| 26 | 35 | 20- > | 5 < | N | 17.1 | 0 | 0 | 0 | | | | |
| 27 | 33 | 3-4 | 1 | N | 26.9 | 0 | 0 | 0 | | | | |
| 28 | 28 | 20- > | 5 < | N | 18.9 | 2 | 0 | 2 | | | | |
| 29 | 27 | 20- > | 5 < | Y | 13.0 | 3 | 1 | 2 | | | | |
| 30 | 31 | 11-20 | 5 < | N | ND | 1 | 0 | 1 | | | | |
| 31 | 24 | 11-20 | 5 < | N | ND | 1 | 1 | 0 | | | | |

CG; Chromatid Gap (**Chromosome Gap*), CB; Chromatid Break, AF; Acentric Fragment, R; Ring, M; Minute, DC; Dicentric ChromoCG; Chromatid Gap (**Chromosome Gap*), CB; Chromatid Break, AF; Acentric Fragment, R; Ring, M; Minute, DC; Dicentric Chromosome

determine the associations between the frequency of CAs in the newborns and urine cotinine-to-creatinine concentration ratios in the mothers. Urinary cotinine: creatinine ratios were the highest in women whose husbands smoked more than 20 cigarettes per day, but we could not find any correlation between the levels of urinary cotinine and the number of cigarettes per day (r=-0.04, Spearman's rank test). Furthermore, we observed that there was a moderate linearity in subjects who smoked more than 20 cigarettes per day (25±13 µg/ml; min-max;17.1-55.3). Lastly, we also did not find any correlation between the maternal levels of urinary cotinine and the frequency of CAs in the newborns.

In this study we used cord blood samples from newborns to investigate whether there are any effects of paternal smoking on the frequency of CAs in the offspring. In order to quantify the maternal exposure to secondhand smoking from their husbands, we

**Table 2.** Types and frequencies of CAs of newborns that were not exposed to paternal smoking

| No | Age | %CA | G | CB | AF | R | M | DC |
|---|---|---|---|---|---|---|---|---|
| 1 | 28 | 1 | 1 * | 0 | | | | |
| 2 | 23 | 4 | 2 * | 2 | | | | |
| 3 | 38 | 2 | 0 | 1 | 1 | | | |
| 4 | 35 | 2 | 1 | 0 | 1 | | | |
| 5 | 22 | 0 | 0 | 0 | | | | |
| 6 | 20 | 3 | 2 | 0 | | | 1 | |
| 7 | 22 | 0 | 0 | 0 | | | | |
| 8 | 30 | 4 | 2 (+1*) | 1 | | | | |
| 9 | 30 | 1 | 1 | 0 | | | | |
| 10 | 28 | 1 | 1 | 0 | | | | |
| 11 | 27 | 4 | 2 | 1 | | | | 1 |
| 12 | 25 | 0 | 0 | 0 | | | | |
| 13 | 31 | 1 | 1 * | 0 | | | | |
| 14 | 23 | 1 | 1 * | 0 | | | | |
| 15 | 36 | 0 | 0 | 0 | | | | |
| 16 | 38 | 2 | 1* | 1 | | | | |
| 17 | 31 | 1 | 0 | 1 | | | | |
| 18 | 29 | 1 | 1 | 0 | | | | |
| 19 | 28 | 0 | 0 | 0 | | | | |
| 20 | 30 | 3 | 2 | 1 | | | | |
| 21 | 24 | 0 | 0 | 0 | | | | |
| 22 | 30 | 1 | 1 | 0 | | | | |
| 23 | 26 | 0 | 0 | 0 | | | | |
| 24 | 25 | 0 | 0 | 0 | | | | |
| 25 | 24 | 0 | 0 | 0 | | | | |
| 26 | 26 | 1 | 1 | 0 | | | | |
| 27 | 23 | 2 | 1 | 0 | | | 1 | |
| 28 | 27 | 0 | 0 | 0 | | | | |
| 29 | 23 | 0 | 0 | 0 | | | | |
| 30 | 36 | 2 | 2* | 0 | | | | |
| 31 | 32 | 0 | 0 | 0 | | | | |

CG; Chromatid Gap (**Chromosome Gap*), CB; Chromatid Break, AF; Acentric Fragment, R; Ring, M; Minute, DC; Dicentric Chromosome -Only No 11 passively exposed to smoking.

collected urine samples from mothers and analyzed the levels of cotinine. Cotinine, the major metabolite of nicotine, can be detected in saliva or urine and is a commonly used biochemical indicator of smoking status. Several studies have reported a significant correlation between the number of cigarettes per day and urinary cotinine concentrations in the smoker. Our study shows that urinary cotinine: creatinine ratios were the highest in women whose husbands smoked more than 20 cigarettes per day, but we could not find a statistically significant correlation between the levels of urinary cotinine and the number of cigarettes per day. However, we observed that there was a moderate linearity in subjects smoked more than 20 cigarettes per day (in heavy smokers).

## DISCUSSION

Cigarette smoke contains several well-known carcinogens and DNA damaging agents. A substantial number of human health problems have been directly

**Table 3.** Mean frequency of CAs in newborns

| Paternal | N | Frequency of CA % (mean±SD) | Min-Max | Frequency of CA % (excluding gap) (mean±SD) | Min-Max |
|---|---|---|---|---|---|
| Smoker | 31 | 1.45±1.52* | 0-7 | 0.71±0.9 | 0-3 |
| Non-smoker | 31 | 1.19±1.30 | 0-4 | 0.42±0.67 | 0-2 |

*p=0.05, Student's t-test

attributed to smoking. Studies of large populations using cytogenetic banding techniques for CAs have given mixed results, with one study finding that CAs frequencies were not increased by smoking (15) and another finding that smoking caused a 10–20% increase (16). Smaller studies have also given mixed results; however, several have found significant increased frequencies of CAs in lymphocytes from smokers relative to non-smokers (17-19). Exposure to secondhand smoking, both in utero and in infancy has also been linked to numerous adverse health outcomes in young children (3). It is well established that newborns of women who smoke during pregnancy have twice the risk of intrauterine growth retardation or low birth weight compared to infants of non-smokers (20). On the other hand, very little is known about the contribution of paternal smoking to cigarette associated health effects in children. There was no significant linear trend between the maternal age at the time of delivery and the frequency of CAs in newborns of smoking and nonsmoking fathers, respectively (r=−0.013, $p$=0.9 and r=−0.02, $p$=0.9, respectively).

We also compared the frequency of CAs in the newborns of smoking fathers to non-smoking fathers regarding environmental tobacco smoke (Not shown). We could not find any significant difference between the frequencies of CAs of the newborns of smoking fathers and those of the non-smoking fathers regarding environmental tobacco smoke. We observed that environmental tobacco smoke did not contribute to newborn exposure and induce paternal smoking.

Burquet et al. measured cotinine with high-performance liquid chromatography from mothers' urine samples. The average cotinine level for non-smoking mothers, for those who smoked one to nine cigarettes a day and heavy smokers (ten or more cigarettes per day) were 0.21, 2.17 and 4.28 mol/l respectively (p<0.001) (10) and found correlation in heavy smokers. Sorsa et al. investigated cytogenetic effects of tobacco smoke among involuntary smokers. They could not find any induction of chromosomal damage (21). The few studies on this subject suggested that paternal smoking is as important as maternal smoking on newborn development and childhood health prognostics. Thus, reducing exposure to parental tobacco smoke in infancy is likely to bring about significant improvements in infant health.

Most studies have addressed paternal smoking and their effects on pulmonary functions, intellectual capacity, respiratory system etc. We investigated the effects of paternal smoking on the frequency of CAs in newborns and analyzed the levels of cotinine in mother's urine samples to confirm their exposure to father's smoking habit. Furthermore, we investigated whether there was any correlation between the levels of urinary cotinine and the number of cigarettes per day in mothers exposure to father's smoking.

Several studies indicated that maternal and paternal smoking increased genotoxicity. Zalacain et al. and Pluth et al. described an increase in micronuclei and translocations in the newborns of smoking mothers (4, 22). Perera et al. reported that adults exposed to air pollution in Poland had increased levels of aromatic-DNA adducts and CAs in peripheral blood samples (p<0.01), and that aromatic adducts were significantly higher in Polish newborns than in mothers (p=0.002), indicating greater susceptibility of the fetus to DNA damage (23). In our previous study, the frequencies of Sister Chromatid Exchange (SCE)

in lymphocytes of 21 smoking mothers and their 21 newborns were compared to those of 10 infants whose mothers had never smoked and to those of 8 infants whose mothers were passive smokers, and reported high exposure to environmental tobacco smoke. A significant difference was found between the mothers and newborns in both smoking and non-smoking subjects (7.31±1.40 and 5.06±0.62; 5.20±1.14 and 3.83±0.45, respectively), however, the levels of cotinine were not measured to confirm the mothers' statements regarding their smoking habits (23). In the present study, we found statistically significant increase in the frequencies of CA in the newborns exposed to paternal smoking compared to those who were not paternally exposed. Our data is consistent with Zalacain and Pluit et al.'s results. SCE frequencies in peripheral lymphocytes are generally elevated among smokers compared to non-smokers. Numerous studies of SCE frequencies in peripheral lymphocytes in environmentally or occupationally exposed or unexposed populations have found that cigarette smoking induces SCEs and can be a confounding factor in occupational studies (5). Ermis et al. investigated the serum and milk in active smoking and non-smoking mothers, and their infants' insulin-like growth factor-I (IGF-I) and insulin-like growth factor binding protein-3 (IGFBP-3) levels. They did not find any difference in either IGF-I, IGFBP-3 or IGF-I/IGFBP-3 ratios in the serum and milk of mothers, and their infants' serum samples relative to maternal smoking (3).

On the other hand, there are many controversial studies about the effect of paternal smoking on newborns. Venners et al. investigated exposure-response relationship between paternal smoking and children's pulmonary function. Their analysis did not reveal a significant association between exposure to paternal smoking and a decrease in pulmonary functions (25). Mascola et al. investigated urine cotinine levels from breast-fed and bottle-fed infants of smoking mothers and they reported that breast fed infants of smoking mothers had 10-fold higher urine cotinine levels than bottle fed infants whose mother's smoked (26). In our study, we could not find any correlation between the levels of cotinine and the frequencies of CA, but drastically an increase in the levels of cotinine was observed in mothers who smoked more than 20 cigarettes per day. Mitchell et al. assessed the effect of maternal smoking and environmental tobacco smoke on the risk of small for gestational age infants (SGA) and showed that maternal smoking in pregnancy was associated with an increased risk of SGA, but they could not detect an increased risk of SGA with paternal smoking (20).

In the present study, we found that urinary cotinine: creatinine ratios were the highest in women whose husbands smoked more than 20 cigarettes per day. Also, the study of Köhler et al. (27) supported our study. They assessed prenatal smoke exposure by determining nicotine and its metabolites in maternal and neonatal urine. They found that the newborns of active smokers exhibited significantly higher nicotine metabolite concentrations than did those of either non-exposed women or passive smokers (27). Groner et al. compared tobacco smoke exposure in mothers and their healthy children less than 3 years old using hair cotinine (HC) levels as an objective long-term measure of exposure. They obtained that children of nonsmokers had higher HC levels than their mothers (0.77 ng/mg vs. 0.35 ng/mg), while HC levels of smokers and their children were not different (1.91 ng /mg vs. 1.92 ng/mg, p=0.978) (28).

While complementary studies are necessary to define the nature of the relationship between the observed effects and smoking, this study underlines the fact that the reduction of prevalence of paternal smoking and environmental tobacco smoke exposure in pregnant women is a major health issue. Scoring CAs, besides assessing exposure to smoking, may be useful for early diagnosis of some heritable chromosome instability syndromes and predict some adverse health effects in the future. In conclusion, couples planning to have children would be well advised to stop smoking before pregnancy and to refrain from exposure to active and passive smoking during pregnancy.

Tobacco smoke is genotoxic, and the increase of CA s frequencies in active smokers is nowadays a generally accepted fact, as shown by a number of studies. However, no absolute determinations can be

concluded regarding the effect of paternal smoking or maternal smoking on the fetus, either from our study or others, but there is evidence which points out that smoking is genotoxic to the fetus. Our results presented in this paper will hopefully prompt further research in this direction.

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