ORIGINAL ARTICLE

Expression of cord blood cytochrome P450 1A1 gene according to the air pollution level of the maternal residence area

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Background: This study aimed to compare the cytochrome P450 1A1 (*CYP1A1*) gene expression in the cord blood of infants born from mothers living in low- and high-air polluted areas. **Materials and Methods:** The study was conducted in Spring 2012 in Isfahan, the second large and air-polluted city in Iran. The study comprised 60 neonates, consisting of 30 infants born from mothers residing in areas with high levels of air pollution and an equal number of infants born in areas with a lower air pollution level. The umbilical cord blood sample was taken immediately after birth. The relative gene expression levels of *CYP1A1* were examined using real time-polymerase chain reaction method. **Results:** *CYP1A1* gene expression level was 3.3-fold higher in the group living in areas with higher pollution level than in the other group ($P = 0.01$). No significant difference existed in the mean values of maternal age, gestational age, the newborns' birth weight, and the gender distribution between the two groups. **Conclusion:** This study provides confirmatory evidence of prenatal health hazards of ambient air pollution and highlights the need for pollution prevention programs to protect women of childbearing age and their children. The clinical implications of this study finding should be confirmed in future longitudinal studies.

Key words: Air pollution, *CYP1A1* gene expression, fetus, prevention

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INTRODUCTION

Pregnant women and children are of the most susceptible groups for the health hazards of air pollutants.[1-3] Human beings are in contact with synthetic and artificial in different ways, including exposure to air pollutants. Many of these artificial chemicals may be mutagenic and carcinogenic. Different assumptions have been proposed about the impact of air pollution on the fetus, one of them is the possible relationship between air pollution and cytochrome P450 1A1 (*CYP1A1*) gene expression. The cytochrome P450 proteins are monooxygenases catalyzing many reactions involved in the metabolism and synthesis of cholesterol and other lipids and xenobiotic agents. The expression is induced by some environmental factors as polycyclic aromatic hydrocarbons (PAH) found in the ambient air and tobacco smoke. *CYP1A1* is able to metabolize some PAHs to carcinogenic intermediates.[4-6] *CYP1A1* catabolizes PAH to harmful hydrophilic agent to deoxyribonucleic acid (DNA) and product DNA-adducts that they are carcinogens.

Airborne PAHs are produced essentially from combustion of fossil fuels, tobacco products, and other organic materials. Usually, emissions from motor vehicles and residential heating are the major source of PAHs in outdoor urban air, while environmental tobacco smoke is a major indoor source. A number of PAHs, of which benzo[*a*]pyrene is a representative member, are transplacental carcinogens in experimental bioassays, producing tumors in several system of the off spring (Bulay and Wattenberg 1971; Rice and Ward 1982; Vesselinovitch *et al*. 1975). It has been demonstrated that the fetus and infant are more prone to PAH-induced carcinogenesis than are adults.[7,8]

Most previous studies in this field have evaluated the association of tobacco smoke with the expression of *CYP1A1* in the fetal period, It has been shown that smokers with increased *CYP1A1* activity are more prone to developing lung cancer.[2] Furthermore, it has been demonstrated that patients who are homozygote for a specific rare allele of *CYP1A1* are at a greater risk of developing the disease.[9] Few studies exist about the

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effects of air pollutants on this gene expression.[10,11] This study aimed to compare the *CYP1A1* gene expression in the umbilical cord blood of neonates born from mothers living in low- and high-polluted areas.

MATERIALS AND METHODS

The research and Ethics Committee of Faculty of Medicine, Isfahan University of Medical Sciences approved the study. Written consent was obtained from all mothers before recruitment to the study.

Study area

Isfahan is an industrial city with a population of near 1894382, located in the center of Iranian plateau, with an average altitude of 1500 m from the sea level bounded by NW-SE mountain range of 3000 m. The air of this city is predominantly affected by industrial emissions and motor traffic, and is considered as the second large and air-polluted city of the country.[12,13]

Participants

This case-control study was conducted among 60 neonates born from March to May 2012 in Isfahan, Iran. A total number of 30 infants born from mothers residing in areas with high levels of air pollution were considered as the case group and were compared with an equal number of infants born from mothers residing in areas with lower air pollution level as controls.

Those newborns were considered eligible for the study who were born alive with a gestational age of more than 35 weeks and a birth weight of more than 1500 g, and whose mothers were living in the same area of Isfahan city for at least 1 year prior to this study.

Those mothers who had a history of tobacco use or those with a tobacco smoker in their household, and those with chronic disease or on any medication use for long time, as well as those newborns who died at birth or needed progressive resuscitation at birth were not recruited to the study.

Eligible mothers and neonates were recruited by convenient sampling. After hospitalization of the mothers before the delivery, the physician collaborating with the project was present at the mothers bedside and completed the checklist provided for this study.

Laboratory examination

At the delivery time, a 2 mL blood sample was obtained from the newborn umbilical cord immediately after birth. Total ribonucleic acids (RNAs) of blood were prepared by $TRI_{ZOL}^{®}$ Reagent (QIAGEN, CA, USA) according to the manufacturer's instructions. Isolated RNA was resuspended in 10 μL of RNase-free water. Each sample was treated twice with 2 μL of RNase-free DNase, 1 unit/μL (QIAGEN, CA, USA), for 10 min at 37°C to eliminate remaining DNA. Synthesis of complementary DNA (cDNA) was done with 2 μg total RNA using M-MLV Reverse Transcriptase (Promega, Madison, WI). Reverse-transcriptase reactions were accomplished for 1 h at 37°C in a final volume of 10 μL using 2 μg of RNA, 500 ng of oligo(dT)15 (Promega, Mannheim, Germany), 10 mM dNTPs (Fermentas Inc., Glen Burnie, MD), 8 units of RNasin (Promega), and 3 units of avian myeloblastosis virus (AMV) reverse transcriptase and 1× AMV reverse transcriptase reaction buffer (Promega).

Real-time polymerase chain reaction (PCR)

For quantitative comparison of *CYP1A1* mRNA levels real-time PCR was performed using SYBR Green fluorescence in a LightCycler System (Roche diagnostics). After optimization of PCR conditions, amplification efficiency was tested in standard curves using serial cDNA dilutions. Amplification specificity was checked using melting curves. Both negative and positive controls were included in each PCR reaction. All the assays were carried out 3 times as independent PCR runs for each cDNA sample. Gene expression was always related to expression of β-2-*microglobulin* (*B2M*) as housekeeping gene. Calculations of expression were performed with the $2^{-\Delta\Delta CT}$. The sequence of the specific primers for *CYP1A1* and *B2M* was *CYP1A1*-forward (5′-AAGAGGAGCTAGACACAGT-3′), CYP1A1-reverse (5′-GAAACCGTTCAGGTAGGA-3′), *B2M* forward (5′-CCGACATTGAAGTTGACTTAC-3′), and B2M-reverse (5′-ATCTTCAAACCTCCATGATG-3′). The PCR conditions for *CYP1A1* were as follows: Initial denaturation 15 min at 95°C, touchdown PCR two cycles of 95°C for 10 s, 67°C for 10 s, and 72°C for 25 s; 2 cycles of 95°C for 10 s, 65°C for 10 s, and 72°C for 25 s; 2 cycles of 95°C for 10 s, 63°C for 10 s, and 72°C for 25 s; and 45 cycles of 95°C for 10 s, 61°C for 10 s, and 72°C for 25 s. The PCR conditions for B2M were as follows: Initial denaturation 15 min at 95°C and PCR 55 cycles of 95°C for 10 s, 63°C for 10 s, and 72°C for 10 s.

Statistical analysis

After editing and management, the obtained data were analyzed by SPSS software (version 20:0, SPSS Inc., Chicago, IL). The Student *t*-test was used for comparing the mean quantitative levels of the *CYP1A1* gene expression and Chi-square test for comparing qualitative and nominal data between the case and control groups.

RESULTS

The case group consisted of 17 boys (56.7%) and 13 girls (43.3%), and controls were 16 boys (53.3%) and 14 girls (46.7%). There was no significant difference between the two

groups in terms of gender distribution ($P = 0.8$). Likewise, there was no significant difference between the two groups in terms of maternal age and the mean gestational age [Table 1].

As presented in [Figure 1], the cord blood level of *CYP1A1* gene expression was 3.3-fold higher in the case than in the control group ($P = 0.01$).

DISCUSSION

The aim of this study was to determine the difference of the cord blood *CYP1A1* gene expression according to the air pollution level of the maternal residence area during pregnancy.

The result of our study indicates that ambient air pollution to which the mother is exposed before and during pregnancy increased the *CYP1A1* gene expression in neonates cord blood cells.

CYP1A1 converts PAH into reactive metabolites, which may participate in the initiation of carcinogenesis through the formation of bulky PAH-DNA adducts.[3,5]

As previously mentioned, experimental and human evidence indicates that the developing fetus has increased susceptibility to certain chemical carcinogens compared with the adult.[6] Factors that may increase fetal vulnerability include higher rates of cell proliferation, the greater number of target cells at risk, lower immunologic competence, and decreased capacity to activate and detoxify carcinogens as well as to repair DNA.[7,8]

Furthermore, several studies have shown that *in utero* environmental exposures may generate effects than can be inherited transgenerationally along with epigenetic modifications.[14-16]

The induction of *CYP1A1* mRNA and resulting enzyme activity has been used as a sensitive indicator of aryl hydrocarbon receptor (AhR) activation in numerous *in vitro* and *in vivo* models to screen a variety of compounds, mixtures, and environmental matrices. As a result of the strong correlation observed between AhR binding affinity, *CYP1A1* induction, and dioxin-like toxicity of structurally related HAHs, *CYP1A1* induction has been used as a biomarker for hazard identification and risk assessment of environmental pollutants, industrial chemicals, and therapeutic compounds.[7,8,17]

Most previous studies have determined the effects of exposure of pregnant mothers to second-hand smoke on cytochrome P450 enzymes, which are the principal enzyme families involved in the reactions related to activation and detoxification of tobacco smoke components.[18,19] Indeed, Toxicants may expose the developing fetus to chemical contamination leading to possible adverse health effects. Elevated *CYP1A1* activity correlates with adverse effects in humans: High *CYP1A1* expression in lymphocytes has been related to a high lung cancer risk.[20]

CYP1A1 interferes in inactivating the carcinogenic effects of oxygen radicals which are produced during the metabolism steps. Therefore, this cytochrome has an important role in human DNA protection against the damages of gene mutations. A recent study revealed that the mentioned gene expression in a group of smoker pregnant mothers was significantly different compared with their nonsmoker counter parts.[21]

Another study assessed the cigarette smoke effect on CYP1A1 gene expression by using new born umbilical blood cord. In this study, 2Cc of newborns umbilical card blood of smoker mothers was taken and compared with new born umbilical cord blood of nonsmoker mothers. *CYP1A1* genome sequence was also compared in both groups. In this study, *CYP1A1* gene expression was determined to be significantly higher in smoker pregnant women than nonsmoker mother and meanwhile, the evidence of structural changes in *CYP1A1* gene was documented.[22]

In another study, while referring to the carcinogenic effects of aromatic hydrocarbons and the role they play in air

Table 1: Characteristics of the two groups studied

	High-air polluted area	Low-air polluted area	*P* value
Birth weight (g)	3100 (300)	3300 (600)	0.2
Gestational age (weeks)	37.4 (0.5)	38.3 (0.4)	0.2
Maternal age (years)	30.1 (2.5)	31.3 (3.5)	0.3

Data are presented as mean (standard deviation)

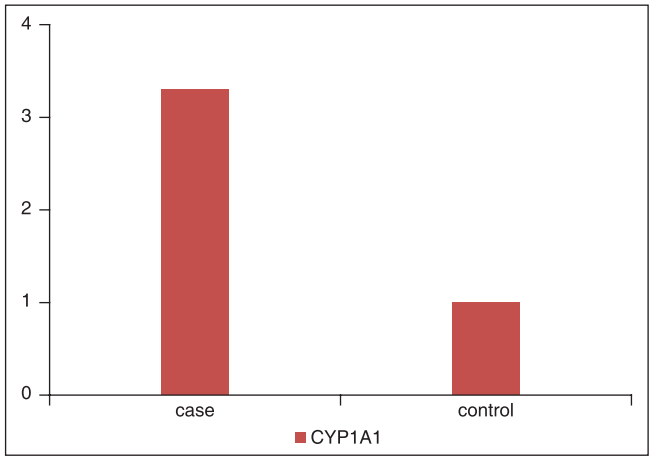

Figure 1: Expression of *CYP1A1* gene level of cord blood in high- and low-air polluted areas (case and control groups)

pollution, they investigate the relationship between PAH-DNA adduct levels (in maternal and newborn white blood cells) and polymorphism of *CYP1A1* gene. These researchers showed that the polymorphisms which lead to greater catalytic efficiency toward PAH diol epoxides in mothers who worked in the polluted areas resulted in more DNA damage from PAHs in fetal tissues.[23] As other studies revealed that gestational age and maternal age may influence the *CYP1A1* gene expression values in umbilical cord blood, we evaluated and compared this two factors, and two studied groups did not have any significant difference in terms of the mean gestational age and the mean maternal age.

Our study showed that *CYP1A1* gene level of newborns umbilical cord blood in the case group is higher than the control group, and this meant that the air pollution could elevate the *CYP1A1* gene level in the newborns cord blood. Our findings were consistent with above-mentioned studies.[23,24] Given the various health hazards of air pollution, more attention should be paid to vulnerable groups as pregnant women and their newborns.

Study limitations and strengths

Our study was also accompanied with implementation problems in performing the plan that finding suitable samples in urban areas with the highest and lowest air pollution level was among these problems, which was accomplished by the help of meteorological organization and health network. In this study, we investigated the *CYP1A1* gene expression levels, but as was said pollution can also cause mutations, we did not evaluate the altered sequences of *CYP1A1* gene, which can be considered of our study limitation. The novelty of our study was considering the association of air pollution levels with *CYP1A1* gene expression in cord blood, whereas previous studies had mainly highlighted the role of tobacco smoke.

CONCLUSION

Prenatal exposures to air pollutants may result in higher levels of ultimate carcinogens formation. Moreover, there is a higher probability of cancer incidence during the individual's life span if these effects are initiated *in utero* rather than later in life. Therefore, the results presented here have implications for risk assessment and environmental health policy and highlight the need to protect pregnant women and particularly their neonates as a sensitive group of the population.

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AUTHOR'S CONTRIBUTION

RK contributed in the conception of the work, conducting the study, writing and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. AMB contributed in the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. SHJ contributed in the conception of the work, conducting the study, writing and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. MM contributed in the conception of the work, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. PP contributed in the conception of the work, conducting the study, writing and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. MM contributed in the conception of the work, conducting the statistical analyses, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

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