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Serum levels of testosterone and gonadotrophins with respect to smoking status and genetic polymorphism of *GSTT1*

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Abstract *Objectives* It is reported that parental exposure to toxicants can influence offspring sex ratio at birth. Studies have reported that several chemicals found in cigarette smoke are substrates of glutathione S-transferase T1 (GSTT1, a member of GSTθ). To determine the effect of cigarette smoke on serum levels of testosterone and gonadotrophins of smokers and possible association of these hormones levels with *GSTT1* polymorphism, the present study was done. *Methods* Our study was conducted on 181 (40 smokers, 141 non-smokers) male subjects. Genomic DNA was extracted from peripheral blood. The *GSTT1* genotyping was performed using PCR-based method. All measurements for testosterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH) were done in one laboratory. *Results* In smoker subjects the mean ± sd of serum testosterone, FSH, and LH were 4.64 ± 1.63 ng/ml, 2.72 ± 1.17 IU/l, and 3.03 ± 1.04 IU/l, respectively. In non-smoker subjects the mean ± sd of serum testosterone, FSH, and LH were 4.49 ± 1.24 ng/ml, 2.89 ± 1.26 IU/l, and 3.07 ± 1.28 IU/l, respectively. There was no significant difference between smokers and non-smokers for serum testosterone ($t = 0.622$, df = 179, $P = 0.535$), FSH ($t = -0.757$, df = 179, $P = 0.450$), and LH ($t = -0.179$, df = 179, $P = 0.858$). Also there was no significant difference between smokers and non-smokers in either *GSTT1* null or positive genotypes for levels of testosterone, FSH, and LH. *Conclusion* Based on present data, it might be concluded that serum levels of testosterone and gonadotrophins were not significantly different between smoker and non-smoker males in both null and present *GSTT1* genotypes.

Keywords FSH · *GSTT1* · LH · Polymorphism · Smoking · Testosterone

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Introduction

It is reported that parental exposure to toxicants can influence the offspring sex ratio at birth [1]. Fukuda and co-workers found that both maternal and paternal smoking were associated with a diminution of offspring sex ratio, and the effect seemed dose related [2]. Their findings, however, are inconsistent with the results of Mills et al. [3] and Parazzini et al. [4] which reported that maternal smoking or environmental exposure to tobacco smoke had no effect on their offspring sex ratio at birth.

Cytosolic glutathione S-transferases (GSTs; EC:2.5.1.18) constitute a family of dimeric proteins consisting of two identical or closely related subunits belonging to the same class. GSTs play a role in the detoxification of electrophilic xenobiotics or electrophilic metabolites of xenobiotics. Also, GST proteins control the uptake and transport of numerous hydrophobic endogenous compounds, including bilirubine, glucocorticoids, steroids, and thyroid hormones. In addition, these enzymes are intimately involved in the biosynthesis of prostaglandin, testosterone, and progesterone [5–7].

The human GSTs have number of subclasses, such as α, μ, π, θ, etc. *GSTT1* (McKusick no. 600436, Ref Seq = NM000853.1; position 22704740, spanning from 22700695 to 22708785) is a member of class GSTθ. There

is well-defined genetic polymorphism in the *GSTT1*. Polymorphic deletion variant surrounding *GSTT1* gene produces a non-functional null allele (*GSTT1-0*). Homozygosity for this allele (null genotype) results complete absence of the protein [8].

Cigarette smoke is a rich source of several chemicals which can produce DNA bulky adducts that may lead to DNA damage. Studies have reported that several chemicals found in cigarette smoke are substrates of GSTT1. Previously, we have hypothesized that genetic polymorphisms in xenobiotic metabolizing genes (such as *GSTT1*) that are responsible for individuals' differences in detoxification reaction may profoundly influence the offspring sex ratio when the parents are exposed to toxins [9–11].

According to the James' hypothesis, mammalian offspring sex ratio at birth is partially controlled by maternal and/or paternal levels of sex hormones around the time of conception [1]. If parental exposure to cigarette smoke can influence sex ratio, and if the GSTT1 activity is important in xenobiotic detoxification, and if the James' hypothesis is correct, we speculate that genotypes of *GSTT1* may be associated with serum concentrations of testosterone and gonadotrophins among smokers. Therefore the present cross sectional study was performed in Shiraz, southern Iran.

Materials and methods

Subjects

Our study was conducted on 181 (40 smokers, 141 non-smokers) male subjects. The mean age of smokers (34.5 ± 10.4 years) and non-smokers (33.2 ± 9.9 years) was statistically similar ($t = 0.724$, df = 179, $P = 0.469$). Both groups were unrelated Iranian Muslims. Informed consent was obtained from all participants. At the time of blood donation participants completed a brief questionnaire that ascertained smoking status, age, history of cancers, asthma and current illness. Because it was reported that cancers [12–14], asthma [15, 16] and schizophrenia [17] associated with the GSTs polymorphisms and in order to minimize the possible confounding factors, our subjects had negative history of cancers, asthma, schizophrenia and current illness.

Measurements

Blood specimens were collected during June to September 2006 at work places of participants between 9.00 AM and 11.00 AM. A single measurement of total serum testosterone, folliclestimulating hormone (FSH), and luteinizing hormone (LH) was obtained from the morning blood draw. The reference ranges of testosterone (Spectria; Orion Diagnostica Oy, Espoo, Finland), LH (Kavoshyar Co., Tehran, Iran), and FSH (Kavoshyar), according to manufacturer's directions, were 2.36–9.97 ng/ml, 0.63–7.89 IU/l, and 1.4–10.9 IU/l, respectively. All measurements were done in same laboratory.

DNA extraction and genotyping analysis

Genomic DNA was isolated from whole blood. Evaluating the *GSTT1* genotypes and laboratory quality control were the same as that reported previously [8, 13].

Statistical analysis

The levels of the serum hormones represents as mean ± sd. Comparisons of the mean value were done using two tailed un-paired Student's *t*-test. The comparison of *GSTT1* genotypes between smokers and non-smokers was done by χ^2 test. Statistical analysis was performed using SPSS statistical software package (version 11.5). A probability of $P < 0.05$ was considered significant difference.

Results and discussion

The mean levels of serum hormones were in normal range in both smoker and non-smoker persons (Table 1). In smoker subjects the mean ± sd of serum testosterone, FSH, and LH were 4.64 ± 1.63 ng/ml, 2.72 ± 1.17 IU/l, and 3.03 ± 1.04 IU/l, respectively. In non-smoker subjects the mean ± sd of serum testosterone, FSH, and LH were 4.49 ± 1.24 ng/ml, 2.89 ± 1.26 IU/l, and 3.07 ± 1.28 IU/l, respectively. There was no significant difference between smokers and non-smokers for serum testosterone ($t = 0.622$, df = 179, $P = 0.535$), FSH ($t = -0.757$, df = 179, $P = 0.450$), and LH ($t = -0.179$, df = 179, $P = 0.858$).

In order to investigating whether *GSTT1* polymorphism modulate the serum concentrations of sex hormones, we compared the concentration of testosterone, FSH, and LH between smoker and non-smoker subjects, with respect of their genotypes. It should be noted that there is no significant difference between smoker and non-smoker subjects for prevalence of *GSTT1* null genotype ($\chi^2 = 1.105$, df = 1, $P = 0.293$), which is in accordance with our previous study [18]. There was no association between *GSTT1* polymorphism and concentrations of serum testosterone and gonadotrophins in smokers and non-smokers (Table 1).

Previously we showed that the *GSTT1* and *GSTM1* polymorphisms were not associated with offspring sex ratio at birth in the general population of Shiraz [19]. However,

Table 1 Concentrations of testosterone (ng/ml), FSH (IU/l), and LH (IU/l) among smoker and non-smoker subjects

	Smoker group			Non-smoker group			Test for equality of means		
	N	Mean	SD	*N*	Mean	SD	*t*	df	*P*-value
Testosterone									
All participants	40	4.64	1.63	141	4.49	1.24	0.622	179	0.535
GSTT1 positive genotype	28	4.77	1.73	110	4.36	1.22	1.471	136	0.144
GSTT1 null genotype	12	4.34	1.37	31	4.98	1.22	−1.491	41	0.144
FSH									
All participants	40	2.72	1.17	141	2.89	1.26	−0.757	179	0.450
GSTT1 positive genotype	28	2.50	1.07	110	2.80	1.17	−1.225	136	0.223
GSTT1 null genotype	12	3.24	1.26	31	3.22	1.52	0.046	41	0.964
LH									
All participants	40	3.03	1.04	141	3.07	1.28	−0.179	179	0.858
GSTT1 positive genotype	28	2.81	0.96	110	3.06	1.26	−0.999	136	0.319
GSTT1 null genotype	12	3.55	1.08	31	3.08	1.36	1.047	41	0.301

we showed that the *GSTT1* polymorphism was associated with sex ratio at birth when at least one of the parents exposed to petrochemical compounds [9–11]. Very recently we showed that there was significant association between *GSTT1* polymorphism and serum testosterone level in filling station workers [20]. The present data did not support our previous report [20] and the James' hypothesis [1]. To the best of our knowledge, this study is the first to evaluate the alteration of serum sex hormones between smokers and non-smokers in respect to the *GSTT1* genotype of subjects.

Additional researches are needed to determine association between smoking, *GSTT1* polymorphism and male sex hormones.

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