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Assessment of Individual Susceptibility to Baseline DNA and Cytogenetic Damage in a Healthy Turkish Population: Evaluation with Lifestyle Factors

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Background: Cytogenetic biomarkers are most frequently used well-established endpoints in human population studies with their sensitivity for measuring exposure to genotoxic agents. They have an important role as early predictors of cancer risk. Identification of individual genotypes of metabolic gene polymorphisms helps to understand the modulation of cancer susceptibility by environmental exposures, such as cigarette smoking and other lifestyle factors. *Aim:* To evaluate individual susceptibility to chemicals, we determined individual DNA damage related to glutathione S-transferase (GST) genotypes (*GSTM1*, *GSTT1*, and *GSTP1*) in a Turkish population. *Methods:* Peripheral blood lymphocytes (PBL) and DNA samples of 127 subjects were analyzed for the presence of DNA damage, using single-cell gel electrophoresis (the Comet assay), and for cytogenetic parameters (chromosomal aberrations [CAs], bleomycin-induced CA, and a cytokinesis-blocked micronucleus assay), and the polymerase chain reaction/restriction fragment length polymorphism method, respectively. *Results:* Individuals carrying a *GSTT1*-null allele showed higher frequencies of CA and micronucleus (MN) ($p = 0.026$, $p = 0.003$, respectively), whereas the *GSTM1*-null and *GSTP1* mutant genotypes did not show any differences in cytogenetic parameters. Our findings demonstrated that none of the lifestyle factors (smoking, alcohol drinking, dietary habits, vitamin intake, and physical activity), except for vitamin intake ($p = 0.002$), were significantly associated with the studied cytogenetic parameters. *Conclusion:* Our results suggest that the *GSTT1* gene polymorphism may influence the baseline cytogenetic frequency in a healthy population.

Introduction

The role of genetic factors (early predictors) in determining human susceptibility to the carcinogenic effects of chemical agents has become a major research area in molecular epidemiology (Au, 2007). Biomarkers of early biologic effect include cytogenetic assays, such as chromosomal aberrations (CAs), micronucleus (MN), and the comet assay, which are the most frequently used endpoints in human population studies. Their sensitivity for measuring exposure to genotoxic agents and their role as early predictors of cancer risk have contributed to this success (Bonassi *et al.*, 2005). CAs and cytokinesis-blocked micronucleus assay (CBMN) have value as biological dosimeters, and more recently, as predictors of cancer risk in epidemiological studies (Bonassi *et al.*, 2004, 2011). Mechanistic evidence linking CAs to early stages of cancer has been supported by the results of cohort studies that associated the CA frequency in peripheral lymphocytes of healthy individuals to future cancer incidence (Bonassi *et al.*, 2008). Furthermore, it has been found that an increased MN frequency in peripheral blood lymphocytes predicts the risk of cancer in humans (Bonassi *et al.*, 2007). The alkaline comet assay is also one of the most promising biomarkers to detect single-strand breaks (SSBs) as well as alkaline-labile sites under alkaline pH conditions. It has been modified by using lesion-specific enzymes (endonuclease III [Endo III] and formamidopyrimidine glycosylase [Fpg]) to be a more specific and a reliable marker for oxidative DNA damage (Collins *et al.*, 1993). Besides the intensive use of these cytogenetic biomarkers in occupational settings (Karahalil *et al.*, 1998; Burgaz *et al.*, 2002; Karakaya *et al.*, 2005; Cakmak *et al.*, 2010), clinical studies (Sardas *et al.*, 2001; Karahalil *et al.*, 2005; Kadioglu *et al.*, 2009; Burgaz *et al.*, 2011), and environmental exposures (Rossner *et al.*, 2011), they are also used in healthy population studies to determine background cytogenetic damage levels (Barale *et al.*, 1998).

The glutathione S-transferase (GST) gene family, which encodes phase II metabolizing enzymes, has an important role

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in the biotransformation and detoxification of different xenobiotics and endogenous compounds. Two polymorphic genes of this family, *GSTM1* and *GSTT1*, present null alleles that consequently do not produce the respective enzyme when the genotype is homozygous. These polymorphisms are also interesting for population dynamics studies, because they have great frequency variations among different ethnic groups and have been reported worldwide (Hiragi *et al.*, 2007). *GSTP1*, encoded by a single locus (GSTP1) on chromosome 11, is also subject to polymorphic variation. Ile–Val substitution at codon 105 is associated with susceptibility to various nonmalignant and malignant human diseases (Allan *et al.*, 2001).

Numerous studies using case–control approaches have examined the association of one or more polymorphisms with cancer risk. There are also some studies assessing individual susceptibility in defined healthy populations. Such data could be of use to investigators for quality control purposes if control allele frequencies are observed to be significantly different from those reported; in such cases, investigators might consider increasing their sample size or checking for methodological errors (Garte *et al.*, 2001). There is also a need to evaluate genotoxic damage among healthy populations to determine the baseline damage to obtain valuable data for future biomonitoring of these individuals.

In the view of this approach, the present study aimed to evaluate the relationship between biomarkers of effect and susceptibility in a healthy Turkish population. For this purpose, the most promising biomarkers of effect (CA, CBMN, and modified comet assay) and a few susceptibility genes (*GSTM1*, *GSTT1*, and *GSTP1*), as well as mutagen sensitivity (bleomycin [BLM]-induced CA) were determined in healthy volunteers. Moreover, individual information on DNA damage was analyzed in relation to genotypes of metabolic genes to assess individual susceptibility to mutagens/carcinogens; the association between lifestyle factors (such as diet and cigarette smoking) and susceptibility of individuals was also investigated.

Materials and Methods

Subjects

A total of 186 unrelated healthy individuals were recruited from 2003 to 2004. However, only 127 of them (63 men and 64 women) were consecutively included in the present study to evaluate all genotoxic data together. All individuals were volunteers, and written informed consent was obtained from all of them. The participants were disease-free individuals and not taking any regular medication at time of blood sampling.

Blood sampling

Lymphocytes were obtained from venous blood samples supplemented with the anticoagulant heparin for the CAs, BLM-induced CAs, CBMN, and modified comet assays. Blood for genotyping was collected in tubes coated with ethylenediaminetetraacetic acid. After collection and during transport, the samples were kept at 4°C and protected from light. CAs and CBMN tests were assessed immediately, and the comet assay was performed no later than 24 h after sampling. Blood samples for genotyping were stored at −20°C until use.

CA test

Heparinized peripheral blood samples were cultured in an RPMI-1640 medium supplemented with 20% fetal calf serum, phytohaemagglutinin (PHA-L) 2%, and L-glutamine for 48 h at 37°C. Colcemid (0.05 μg/mL; Biochrom) was added at 45 h. After hypotonic treatment with 0.075 M KCl, the cells were fixed in methanol/acetic acid (3:1), spread on wet slides, and stained with 5% Giemsa for 5 min. A total of 100 well-spread metaphases with 46 chromosomes were examined for each subject blindly by one reader. Chromatid and chromosome breaks, dicentric fragments, and gaps were scored. %CA with and without gaps was demonstrated.

BLM-induced CA test

The experimental protocol of Hsu *et al.* (1989) was followed by using the RPMI-1640 medium supplemented with 20% fetal calf serum, PHA-L 2%, and L-glutamine. The ratio of blood to medium was 0.5:4.5. The cultures were incubated at 37°C for 72 h and treated with BLM (30 μg/mL) for 5 h at 67 h of incubation. Colcemid (0.05 μg/mL; Biochrom) was added to the cultures at 70 h. The conventional cell harvest procedure was followed as described above for the CA test. Slides were randomly coded, and according to Hsu *et al.* (1989), more than 12 breaks per metaphase were described as 12, and scoring of gaps did not influence the outcome of the mutagen sensitivity analysis, and thus were omitted in further investigations. A total of 50 well-spread metaphases with 46 chromosomes were examined for each individual blindly by one reader. The mean number of chromatid breaks per cell (b/c) was used as the measure of mutagen sensitivity.

Cytokinesis-blocked micronucleus assay

The CBMN test was performed as described previously by Fenech (2000) with minor modifications. The induction of MN in peripheral blood lymphocytes was evaluated by scoring a total of 1000 binucleated cells per subject at ×400. Scoring was performed blindly by a single reader on coded slides according to the scoring and identification criteria of Fenech *et al.* (2003). MN frequencies were expressed as MN per thousand binucleated cells.

Modified comet assay

The basic alkaline technique was followed with modifications for the detection of oxidized bases by using bacterial repair endonucleases as described previously by Collins *et al.* (1993). The percentage of DNA in the tail (TD%) was taken as a measure of the DNA-break frequency. TD% was assessed in 50 cells by using a Comet Assay III image analysis system (Perceptive Instruments) blindly by one slide reader.

DNA isolation and genotyping procedures

Genomic DNA was isolated from frozen peripheral blood samples by using the sodium perchlorate/chloroform extraction method (Johns and Paulus-Thomas, 1989) and used as a template in polymerase chain reaction (PCR)-based genotyping analysis.

The genetic polymorphism analysis for the *GSTM1* and *GSTT1* genes was determined by using the PCR as described previously (Bringuier *et al.*, 1998; Kadioglu *et al.*, 2010).

Genotyping of the GSTP1 *Ile/Val* polymorphism was performed by using the PCR/restriction fragment length polymorphism (RFLP) method as described by Ada *et al.* (2007).

Statistics

Data analysis was performed using SPSS for Windows, version 11.5. Whether the continuous variables were distributed normally or not, they were determined using the Shapiro–Wilk test. The Levene test was applied for the evaluation of the homogeneity of variances. Data were shown as mean ± standard deviation (SD) or median (minimum–maximum) for continuous variables, where appropriate. While means were compared by Student's *t*-test or one-way analysis of variance (ANOVA), regarding the number of independent groups, in comparisons of medians, the Mann–Whitney U or Kruskal–Wallis test was applied, where applicable. When the *p*-value from the one-way ANOVA or Kruskal–Wallis test statistics was statistically significant, *post hoc* Tukey or nonparametric multiple comparison test was used to determine which groups differ from which others. Nominal data were evaluated by Pearson's Chi-square or Fisher's exact test, where applicable. Degrees of association between continuous variables were evaluated by either Pearson's product–moment or Spearman's rank correlation test. Coefficients of regression and 95% confidence intervals for all independent variables were calculated. Logarithmic transformations were applied for all dependent variables in linear regression analyses, because the variables were not normally distributed. A p value < 0.05 was considered statistically significant.

Results

Demographic characteristics of the study subjects are demonstrated in Table 1. The mean age (±SD) of the participants was 32.04 (±8.02), and 62.2% of them were under 35 years of age. The male/female ratio, which is 63/64, was balanced. The percentages of nonsmokers and smokers were similar (55.9% and 44.1%, respectively), and the alcohol intake was low among the studied subjects. About 9.4% of the participants were taking vitamins regularly, and most of them (67.7%) had a balanced diet. More than half of the subjects (52.8%) were having regular physical activity at least once a week, and 47.2% of the subjects were not having any physical activity regularly.

The frequencies of the *GSTM1*-null and *GSTT1*-null genotypes were 59.8% and 21.3%, respectively. Genotype frequencies of GSTP1 exon 5 were *Ile105Ile:* 52.1%, *Ile105Val:* 42.1%, and *Val105Val:* 5.8% (data not shown). Tables 2 and 3 summarize the distributions of %CA, ‰MN, b/c, SSB, Endo III sites, and Fpg sites between variables in terms of mean ± SD. The MN frequency was only significantly higher in female subjects than male subjects, when we stratified our results relevant to gender ($p = 0.003$). We did not observe any difference in comparison to subjects stratified based on life styles as indicated in Tables 2 and 3. Moreover, there were no significant differences in both CA and MN frequencies of smokers compared to nonsmokers and light smokers (1–10 cigs/day) (Table 2). Only, vitamin users had significantly higher Fpg sites than nonusers ($p = 0.002$) in the comet assay (Table 3). The influences of *GSTM1*, *GSTT1*, and *GSTP1* genotypes on cytogenetic parameters are presented in Table 4. Individuals carrying a *GSTT1*-null allele showed higher frequencies of CA (with gaps) and MN ($p = 0.026$, $p = 0.003$, respectively), whereas *GSTM1*-null and *GSTP1* mutant genotypes did not result in any differences in cytogenetic parameters (Table 4).

Table 1. Frequency of Selected Demographic Variables for the Studied Population

Variables	*Population (n = 127)*
Mean age (±SD) (minimum–maximum)	32.04 (±8.02) (20–50)
<35	79 (62.2%)
≥35	48 (37.8%)
Gender (male/female)	63 (49.6%)/64 (50.4%)
BMI (kg/m^2) (mean ± SD)	24.15 ± 3.94
Cigarette smoking (cigarettes/day)	
Nonsmokers	71 (55.9%)
Smokers (1–10)	27 (21.3%)
Smokers (11–20)	15 (11.8%)
Smokers (>20)	14 (11.0%)
Alcohol drinking	
Nondrinker/occasional drinker	109 (85.8%)
Once a week	13 (10.2%)
2–3 times a week	5 (3.9%)
Vitamin intake	
Yes	12 (9.4%)
No	115 (90.6%)
Dietary habits	
Vegetable based	16 (12.6%)
Meat based	25 (19.7%)
Balanced	86 (67.7%)
Physical activity	
No	60 (47.2%)
Once a week	32 (25.2%)
2–3 times a week	26 (20.5%)
More than three times a week	9 (7.1%)

SD, standard deviation; BMI, body mass index.

There was a significant correlation between MN and CA frequencies ($r = 0.495$, $p < 0.0001$). Similarly, b/c showed a significant correlation with Endo III and Fpg sites ($r = 0.215$, $p = 0.045$; $r = 0.303$, $p = 0.004$, respectively). There was also a significant correlation between age and MN frequencies ($r = 0.182$; $p < 0.05$). Since significant results have been observed between the MN frequency and selected variables (gender and *GSTT1* genotype), further multiple linear regression analysis has been performed (data not shown). Age, gender, smoking status, alcohol drinking, vitamin intake, dietary habits, physical activity, and *GSTM1*, *GSTT1*, and *GSTP1* genotypes were considered as independent variables. Regression analysis of data indicated that the *GSTT1* genotype ($B = -0.545$; $p = 0.007$) and gender ($B = 0.430$; $p = 0.012$) were the most effective parameters for the MN frequency of the study group.

Discussion

The present study highlighted an influence of the *GSTT1* polymorphism on baseline levels of CA and MN with significantly elevated CA and MN frequencies in null alleles with respect to the active allele. Genotype analysis did not reveal a

Table 2. Distributions of Chromosomal Aberrations, Micronucleus, and Breaks/Cell Between Selected Variables

Variables	*% CA without gaps*	*% CA with gaps*	*‰ MN*	*b/c*[a]
Gender				
Male	0.16 ± 0.37 (*n* = 56)	0.7 ± 0.93 (*n* = 56)	6.45 ± 4.83 (*n* = 60)	0.53 ± 0.19 (*n* = 54)
Female	0.21 ± 0.56 (*n* = 56)	1.11 ± 1.6 (*n* = 56)	9.58 ± 6.48 (*n* = 55)[b]	0.52 ± 0.15 (*n* = 49)
Total	0.19 ± 0.47 (*n* = 112)	0.9 ± 1.32 (*n* = 112)	7.95 ± 5.87 (*n* = 115)	0.53 ± 0.17 (*n* = 103)
Age				
< 35	0.17 ± 0.48 (*n* = 69)	1.01 ± 1.54 (*n* = 69)	7.21 ± 5.27 (*n* = 72)	0.50 ± 1.16 (*n* = 64)
≥ 35	0.21 ± 0.47 (*n* = 43)	0.72 ± 0.82 (*n* = 43)	9.19 ± 6.64 (*n* = 43)	0.56 ± 1.19 (*n* = 39)
Cigarette smoking				
Nonsmokers	0.19 ± 0.50 (*n* = 62)	0.94 ± 1.38 (*n* = 62)	7.90 ± 5.07 (*n* = 63)	0.53 ± 0.17 (n = 57)
Smokers (1–10 cigarettes/day)	0.04 ± 0.20 (*n* = 23)	0.57 ± 0.66 (*n* = 23)	7.44 ± 6.53 (*n* = 25)	0.55 ± 0.15 (n = 21)
Smokers (11–20 cigarettes/day)	0.43 ± 0.65 (*n* = 14)	1.29 ± 1.94 (*n* = 14)	9.67 ± 7.99 (*n* = 15)	0.50 ± 0.23 (n = 13)
Smokers (> 20 cigarettes/day)	0.15 ± 0.38 (*n* = 13)	0.92 ± 1.11 (*n* = 13)	7.08 ± 5.70 (*n* = 12)	0.49 ± 0.17 (*n* = 12)
Smokers (total)	0.18 ± 0.44 (*n* = 50)	0.86 ± 1.27 (*n* = 50)	7.95 ± 5.87 (*n* = 52)	0.52 ± 0.18 (*n* = 46)
Alcohol drinking				
Nondrinker/occasional drinker	0.17 ± 0.45 (*n* = 95)	0.84 ± 1.32 (*n* = 95)	8.05 ± 5.67 (*n* = 97)	0.53 ± 0.18 (*n* = 87)
Once a week	0.42 ± 0.67 (*n* = 12)	1.42 ± 1.38 (*n* = 12)	8.23 ± 8.08 (*n* = 13)	0.53 ± 0.15 (*n* = 11)
2–3 times a week	0 (*n* = 5)	0.80 ± 1.38 (*n* = 5)	5.2 ± 2.05 (*n* = 5)	0.43 ± 0.08 (*n* = 5)
Drinker (total)	0.29 ± 0.59 (*n* = 17)	1.24 ± 1.35 (*n* = 17)	7.39 ± 7.0 (*n* = 18)	0.50 ± 0.14 (*n* = 16)
Vitamin intake				
Yes	0.27 ± 0.47 (*n* = 11)	0.82 ± 0.87 (*n* = 11)	8.58 ± 4.6 (*n* = 12)	0.54 ± 0.13 (*n* = 11)
No	0.18 ± 0.48 (*n* = 101)	0.91 ± 1.36 (*n* = 101)	7.87 ± 6.01 (*n* = 103)	0.52 ± 0.18 (*n* = 92)
Dietary habits				
Vegetable based	0.20 ± 0.41 (*n* = 15)	0.80 ± 0.86 (*n* = 15)	6.50 ± 4.16 (*n* = 16)	0.53 ± 0.10 (*n* = 14)
Meat based	0.24 ± 0.24 (*n* = 21)	1.38 ± 1.60 (*n* = 21)	8.65 ± 7.41 (*n* = 23)	0.48 ± 0.16 (*n* = 19)
Balanced	0.17 ± 0.47 (*n* = 76)	0.79 ± 1.30 (*n* = 76)	8.04 ± 5.67 (*n* = 76)	0.54 ± 0.19 (*n* = 70)
Physical activity				
No	0.24 ± 0.58 (*n* = 54)	0.80 ± 1.20 (*n* = 54)	8.43 ± 6.17 (*n* = 53)	0.50 ± 0.15 (*n* = 47)
Once a week	0.14 ± 0.36 (*n* = 28)	0.96 ± 1.17 (*n* = 28)	7.43 ± 5.63 (*n* = 30)	0.55 ± 0.16 (*n* = 26)
2–3 times a week	0.14 ± 0.36 (*n* = 21)	1.19 ± 1.89 (*n* = 21)	7.95 ± 6.15 (*n* = 23)	0.57 ± 0.22 (*n* = 21)
More than three times a week	0.11 ± 0.33 (*n* = 9)	0.67 ± 0.87 (*n* = 9)	6.78 ± 4.52 (*n* = 9)	0.49 ± 0.16 (*n* = 9)

The values are in mean ± SD.
[a]Chromatid b/c in BLM-induced CA test.
[b]Significantly different from male (age and smoking adjusted $p = 0.003$) (Mann–Whitney U).
CA, chromosomal aberrations; MN, micronucleus; b/c, breaks/cell; BLM, bleomycin.

clear association between other studied SNPs (*GSTM1* and *GSTP1*) and cytogenetic biomarkers.

Similarly, some studies suggested increased baseline levels of sister chromatid exchange (SCE), CA, and MN in GSTT1-null individuals. Schröder *et al.* (1995) have demonstrated lower SCE rates in a group of GSTT1-positive subjects than GSTT1-negative individuals, and suggested that GSTT1 is protective against background genotoxic damage. An association of the GSTT1 deletion polymorphism with increases in background SCEs has been reported by Wiencke *et al.* (1995), and they hypothesized that substrates for this isozyme are encountered commonly in the environment or are endogenous in nature. A modifying effect of the genetic polymorphism in GSTT1 on the mean frequencies of the different types of CAs has been demonstrated previously (Skjelbred *et al.*, 2011). In a study of Tuimala *et al.* (2004), GSTT1-null subjects showed a statistically significant increase in the frequency of baseline CAs in an unexposed Caucasian population. An altered MN frequency has also been associated with occupationally or environmentally exposed GSTT1-null individuals previously (Laffon *et al.*, 2006; Chen *et al.*, 2010; Kumar *et al.*, 2011).

Our results regarding the frequencies of the studied GST polymorphisms are consistent with those in previous Turkish studies (Kocabas *et al.*, 2000; Ada *et al.*, 2004, 2007). The *GSTM1*-null genotype is very common among Caucasians, being found in roughly 50% of the population (Hirvonen, 1999). A homozygous deletion of the *GSTT1* gene (null genotype) is found in 10–20% of Caucasians, making them unable to perform GSTT1-mediated detoxification reactions (Norppa, 2001). GSTP1 exon 5 *Ile105Val* and *Val105Val* mutations are 27.3–47.8% and 4.1–46.0%, respectively, in European Caucasians (Ada *et al.*, 2007).

Our study revealed that the MN frequency is positively correlated with age, although the MN frequency between subjects aged below and above 35 was not statistically different. This result might be a consequence of an unbalanced distribution of age, since 62.2% of our study subjects were under 35 years of age. The first results of the HUman MicroNucleus project international collaborative study on CBMN assay suggested that the MN frequencies were increased by age (Bonassi *et al.*, 2001). Additionally, according to the pooled analysis of nearly 7000 subjects of the mentioned collaborative study, female subjects were found to have higher MN frequencies when compared to the male subjects (Bonassi *et al.*, 2001). Confirming these findings, female subjects showed a higher MN frequency compared to males in our

Table 3. Distribution of Single-Strand Breaks, Endo III Sites, and Fpg Sites Between Selected Variables

Variables	*SSBs*	*Endo III sites*	*Fpg sites*
Gender			
Male	7.12 ± 1.67 (n = 63)	2.51 ± 2.76 (n = 56)	2.54 ± 2.21 (n = 55)
Female	7.29 ± 1.74 (n = 63)	2.28 ± 2.95 (n = 54)	3.14 ± 2.69 (n = 57)
Total	7.20 ± 1.70 (n = 126)	2.40 ± 2.85 (n = 110)	2.85 ± 2.47 (n = 112)
Age			
< 35	7.24 ± 1.74 (n = 78)	2.35 ± 2.98 (n = 68)	2.75 ± 2.62 (n = 69)
≥ 35	7.14 ± 1.65 (n = 48)	2.47 ± 2.64 (n = 42)	3.00 ± 2.23 (n = 43)
Cigarette smoking			
Nonsmokers	7.19 ± 1.62 (n = 70)	2.57 ± 2.99 (n = 60)	3.04 ± 2.35 (n = 61)
Smokers (1–10 cigarettes/day)	7.58 ± 1.86 (n = 27)	2.16 ± 2.51 (n = 25)	2.87 ± 2.99 (n = 24)
Smokers (11–20 cigarettes/day)	6.50 ± 1.86 (n = 15)	3.20 ± 3.55 (n = 12)	2.31 ± 2.43 (n = 14)
Smokers (> 20 cigarettes/day)	7.26 ± 1.57 (n = 14)	1.33 ± 1.80 (n = 13)	2.45 ± 2.16 (n = 13)
Smokers (total)	7.21 ± 1.82 (n = 56)	2.19 ± 2.68 (n = 50)	2.61 ± 2.62 (n = 51)
Alcohol drinking			
Nondrinker/occasional drinker	7.23 ± 1.69 (n = 108)	2.50 ± 2.95 (n = 95)	2.71 ± 2.32 (n = 96)
Once a week	6.96 ± 1.75 (n = 13)	1.96 ± 1.97 (n = 10)	3.99 ± 3.42 (n = 12)
2–3 times a week	7.16 ± 2.12 (n = 5)	1.38 ± 2.22 (n = 5)	2.78 ± 2.44 (n = 4)
Drinker (total)	7.01 ± 79 (n = 18)	1.77 ± 1.99 (n = 15)	3.69 ± 3.17 (n = 16)
Vitamin intake			
Yes	6.46 ± 1.99 (n = 11)	2.89 ± 3.44 (n = 11)	5.37 ± 2.63 (n = 10)[a]
No	7.27 ± 1.66 (n = 115)	2.34 ± 2.79 (n = 99)	2.60 ± 2.33 (n = 102)
Dietary habits			
Vegetable based	6.55 ± 1.50 (n = 16)	2.90 ± 2.49 (n = 14)	3.74 ± 3.00 (n = 15)
Meat based	7.39 ± 1.81 (n = 25)	1.81 ± 2.60 (n = 23)	2.02 ± 1.91 (n = 24)
Balanced	7.27 ± 1.70 (n = 85)	2.48 ± 2.99 (n = 73)	2.94 ± 2.47 (n = 73)
Physical activity			
No	7.35 ± 1.76 (n = 60)	2.31 ± 3.18 (n = 53)	2.85 ± 2.52 (n = 54)
Once a week	6.96 ± 1.47 (n = 32)	2.27 ± 1.89 (n = 28)	3.13 ± 2.44 (n = 31)
2–3 times a week	6.94 ± 1.87 (n = 25)	2.71 ± 3.21(n = 22)	2.76 ± 2.68 (n = 19)
More than three times a week	7.76 ± 1.58 (n = 9)	2.53 ± 2.61 (n = 7)	1.93 ± 1.85 (n = 8)

The values are in mean ± SD.

[a]Significantly different from nonusers (p = 0.002) (age-, sex-, and smoking-adjusted) (Mann-Whitney U). The values are in %DNA in tail (TD%).

Endo III, endonuclease III; Fpg, formamidopyrimidine glycosylase; SSBs, single-strand breaks.

study. Some authors suggested that the greater tendency of the X chromosome, which exists as two copies in females, to be lost as an MN relative to other chromosomes is responsible for higher MN frequencies in females (Tucker *et al.*, 1996; Norppa and Falck, 2003). Furthermore, the significant correlation between the MN and CA frequency in our study could be assumed as valuable data for the background cytogenetic effects of a healthy population.

In our study, we have also assessed the mutagen sensitivity in lymphocytes of the subjects with BLM-induced CA assay and could not find any association with the GST polymorphisms. In our previous study with small-sized healthy individuals, no significant differences between GSTM1-positive and null individuals were observed for BLM-induced CAs (Kocabas *et al.*, 2000). However, with the same group, we observed statistical significance in the average values of CAs between GSTM1-positive and GSTM1-null individuals when lymphocytes were induced with a dose of 1 Gy (Karahalil *et al.*, 2002). In the present study, despite a larger sample size compared to the previous one, we could not observe a statistical significance between any of the GST genotypes and BLM-induced CA frequencies. On the other hand, the correlation found between the purine and pyrimidine DNA damage and the BLM-induced CA frequency in the present study indicates basal oxidative DNA damage, although the mechanisms underlying the genotoxic effect detected by the two assays are different.

Genotype analysis revealed a clear association between GSTT1-null and both CA and MN frequencies, whereas the effect of the studied GST polymorphisms was not significant with regard to modified comet assay parameters in our study. Although more oxidative DNA damage was expected in GSTM1-null, GSTT1-null, and GSTP1-variant allele carriers, basal SSBs and Endo III or Fpg sites did not elevate alleles with a potential risk. As reviewed by Pavanello and Clonfero (2000), the number of studies demonstrating the positive influences of the *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms on comet assay parameters is limited. After Collins *et al.* (1993) have modified the comet assay with specific endonucleases, we conducted a small-sized study to investigate the role of some polymorphic genes and oxidative DNA damage (detected with a modified comet assay) in Barrett's esophagus patients and found no association for the *GSTM1* gene (Kadioglu *et al.*, 2010). However, some recent studies on environmental exposures or cigarette smoking found an influence of the *GST* genotypes on the comet assay (Abhishek *et al.*, 2010; Lee *et al.*, 2010).

Table 4. Distribution of Genotypes Between Cytogenetic Parameters

Genotypes	*%CA without gaps*	*%CA with gaps*	*‰MN*	*b/c*	*SSBs*[a]	*Endo III sites*[a]	*Fpg sites*[a]
GSTM1							
Null	0.17 ± 0.49 (*n* = 65)	1.05 ± 1.52 (*n* = 65)	7.07 ± 5.19 (*n* = 68)	0.52 ± 0.18 (*n* = 59)	7.11 ± 1.61 (*n* = 72)	2.21 ± 2.98 (*n* = 64)	3.13 ± 2.87 (*n* = 63)
Active	0.23 ± 0.48 (*n* = 43)	0.72 ± 0.96 (*n* = 43)	9.29 ± 6.72 (*n* = 45)	0.52 ± 0.17 (*n* = 40)	7.29 ± 1.87 (*n* = 49)	2.84 ± 2.69 (*n* = 42)	2.46 ± 1.84 (*n* = 44)
GSTT1							
Null	0.37 ± 0.60 (*n* = 19)	1.58 ± 1.92 (*n* = 19)[b]	12.28 ± 8.29 (*n* = 21)[b]	0.57 ± 0.14 (*n* = 18)	7.45 ± 1.58 (*n* = 26)	2.60 ± 1.72 (*n* = 22)	3.15 ± 2.72 (*n* = 25)
Active	0.16 ± 0.45 (*n* = 89)	0.78 ± 1.14 (*n* = 89)	6.97 ± 4.76 (*n* = 92)	0.51 ± 0.18 (*n* = 81)	7.11 ± 1.75 (*n* = 95)	2.42 ± 3.11 (*n* = 84)	2.76 ± 2.45 (*n* = 82)
GSTP1							
Ile/Ile	0.19 ± 0.51 (*n* = 57)	0.84 ± 1.25 (*n* = 57)	8.59 ± 6.03 (*n* = 59)	0.54 ± 0.19 (*n* = 54)	7.41 ± 1.57 (*n* = 62)	2.09 ± 3.06 (*n* = 56)	3.18 ± 2.36 (*n* = 52)
Ile/Val	0.23 ± 0.47 (*n* = 44)	1.11 ± 1.50 (*n* = 44)	7.41 ± 5.89 (*n* = 46)	0.51 ± 0.14 (*n* = 41)	6.95 ± 1.88 (*n* = 51)	2.93 ± 2.78 (*n* = 42)	2.71 ± 2.63 (*n* = 47)
Val/Val	0 (*n* = 6)	0.33 ± 0.52 (*n* = 6)	7.14 ± 5.18 (*n* = 7)	0.35 ± 0.18 (*n* = 3)	7.00 ± 1.79 (*n* = 7)	2.59 ± 1.61 (n = 7)	2.02 ± 2.62 (*n* = 7)

[a]The values are in %DNA in tail (TD%).
[b]Significantly different from active genotype ($p = 0.026$; $p = 0.003$, respectively) (age-, sex-, and smoking-adjusted) (Mann-Whitney U).
GST, glutathione S-transferase.

Contrary to our expectations, no correlation between lifestyle factors and MN, CAs, or BLM-induced CA frequencies was observed in the present study. However, Fpg sites were shown to be higher among regular vitamin users as compared to nonusers. Similar to the other biomarkers, the comet assay did not reveal any significant results for the rest of the lifestyle factors examined. Since we do not have clear information on the type and dose of the vitamin taken by the individuals, it is difficult to discuss this result obtained by the comet assay. In an article of Cemeli *et al.* (2009), several studies on vitamin intake and altered DNA damage levels as measured with the comet assay were reviewed, and those studies indicate that the effect on DNA damage depends on the dose and the type of the vitamin used.

In conclusion, the results of this study indicate that GSTT1-null subjects had higher CA and MN frequencies than their positive counterparts in the total population. The main limitations of our study were its sample size and its consideration of only SNPs of *GST* genes and no other susceptibility SNPs of drug-metabolizing genes or DNA repair genes. Moreover, lifestyle factors might have been considered in a more detailed questionnaire. Data presented here have to be considered as a starting point for the inherited basis of DNA damage in a Turkish population and should encourage further investigation with a larger sample size and including more SNPs for primary prevention and early detection of disease-associated genes by identifying high-risk individuals.

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Author Disclosure Statement

No competing financial interests exist.

References

Abhishek S, Kaur N, Kaur S, *et al.* (2010) Association of GSTM1 and GSTT1 gene deletions with susceptibility to DNA damage in the pesticide-exposed workers of Punjab. Rejuvenation Res 13:281–284.

Ada AO, Süzen SH, Iscan M (2004) Polymorphisms of cytochrome P450 1A1, glutathione S-transferases M1 and T1 in a Turkish population. Toxicol Lett 151:311–315.

Ada AO, Suzen HS, Iscan M (2007) Polymorphisms of microsomal epoxide hydrolase and glutathione S-transferase P1 in a male Turkish population. Int J Toxicol 26:41–46.

Allan JM, Wild CP, Rollinson S, *et al.* (2001) Polymorphism in glutathione S-transferase P1 is associated with susceptibility to chemotherapy induced leukemia. PNAS 98:11592–11597.

Au WW (2007) Usefulness of biomarkers in population studies: from exposure to susceptibility and to prediction of cancer. Int J Hyg Environ Health 210:239–246.

Barale R, Chelotti L, Davini T, *et al.* (1998) Sister chromatid exchange and micronucleus frequency in human lymphocytes of 1,650 subjects in an Italian population: II. Contribution of sex, age, and lifestyle. Environ Mol Mutagen 31:228–242.

Bonassi S, El-Zein R, Bolognesi C, *et al.* (2011) Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. Mutagenesis 26:93–100.

Bonassi S, Norppa H, Ceppi M, *et al.* (2008) Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22 358 subjects in 11 countries. Carcinogenesis 29:1178–1183.

Bonassi S, Ugolini D, Kirsch-Volders M, *et al.* (2005) Human population studies with cytogenetic biomarkers: review of the literature and future prospectives. Environ Mol Mutagen 45:258–270.

Bonassi S, Vral A, Zijno A, *et al.* (2001) HUman MicroNucleus Project: International database comparison for results with the cytokinesis-block micronucleus assay in human lymphocytes: I. Effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei. Environ Mol Mutagen 37:31–45.

Bonassi S, Znaor A, Ceppi M, *et al.* (2007) An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. Carcinogenesis 28:625–631.

Bonassi S, Znaor A, Norppa H, *et al.* (2004) Chromosomal aberrations and risk of cancer in humans: an epidemiologic perspective. Cytogenet Genome Res 104:376–382.

Bringuier PP, McCredie M, Sauter G, *et al.* (1998) Carcinomas of the renal pelvis associated with smoking and phenacetin abuse: p53 mutations and polymorphism of carcinogen-metabolising enzymes. Int J Cancer 79:531–536.

Burgaz S, Coskun E, Demircigil GC, *et al.* (2011) Micronucleus frequencies in lymphocytes and buccal epithelial cells from patients having head and neck cancer and their first-degree relatives. Mutagenesis 26:351–356.

Burgaz S, Demircigil GC, Karahalil B, *et al.* (2002) Chromosomal damage in peripheral blood lymphocytes of traffic policemen and taxi drivers exposed to urban air pollution. Chemosphere 47:57–64.

Cakmak GD, Coskun E, Vidinli N, *et al.* (2010) Increased micronucleus frequencies in surrogate and target cells from workers exposed to crystalline silica-containing dust. Mutagenesis 25:163–169.

Cemeli E, Baumgartner A, Anderson D (2009) Antioxidants and the Comet assay. Mutat Res 681:51–67.

Chen L, Guo H, Yuan J, *et al.* (2010) Polymorphisms of GSTT1 and GSTM1 and increased micronucleus frequencies in peripheral blood lymphocytes in residents at an e-waste dismantling site in China. J Environ Sci Health A Tox Hazard Subst Environ Eng 45:490–497.

Collins AR, Duthie SJ, Dobson VL (1993) Direct enzymic detection of endogenous oxidative base damage in human lymphocyte DNA. Carcinogenesis 14:1733–1735.

Fenech M. (2000) The *in vitro* micronucleus technique. Mutat Res 455:81–95.

Fenech M, Chang WP, Kirsch-Volders M, *et al.* (2003) HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. Mutat Res 534:65–75.

Garte S, Gaspari L, Alexandrie AK, *et al.* (2001). Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol Biomarkers Prev 10:1239–1248.

Hiragi Cde O, de Oliveira SF, Hatagima A, *et al.* (2007) Glutathione S-transferase M1 and T1 polymorphisms in Brazilian African descendants. Hum Biol 79:131–140.

Hirvonen A (1999) Polymorphisms of xenobiotic-metabolizing enzymes and susceptibility to cancer, Environ Health Perspect 107:37–47.

Hsu TC, Johnston DA, Cherry LM (1989) Sensitivity to genotoxic effects of BLM in humans: possible relationship to environmental carcinogenesis, Int J Cancer 43:403–409.

Johns MB, Paulus-Thomas JE (1989) Purification of human genomic DNA from whole blood using sodium perchlorate in place of phenol. Anal Biochem 80:276–278.

Kadioglu E, Sardas S, Ergun M, *et al.* (2010) The role of oxidative DNA damage, DNA repair, GSTM1, SOD2 and OGG1 polymorphisms in individual susceptibility to Barrett's esophagus. Toxicol Ind Health 26:67–79.

Kadioglu E, Sardas S, Erturk S, *et al.* (2009) Determination of DNA damage by alkaline halo and comet assay in patients under sevoflurane anesthesia. Toxicol Ind Health 25: 205–212.

Karahalil B, Burgaz, S, Fişek G, *et al.* (1998) Biological monitoring of young workers exposed to polycyclic aromatic hydrocarbons in engine repair workshops. Mutat Res 412:261–269.

Karahalil B, Sardas S, Kocabas NA, *et al.* (2002) Chromosomal aberrations under basal conditions and after treatment with X-ray in human lymphocytes as related to the GSTM1 genotype. Mutat Res 515:135–140.

Karahalil B, Yağar S, Bahadir G, *et al.* (2005) Diazepam and propofol used as anesthetics during open-heart surgery do not cause chromosomal aberrations in peripheral blood lymphocytes. Mutat Res 581:181–186.

Karakaya AE, Ozcagli E, Ertas N, *et al.* (2005) Assessment of abnormal DNA repair responses and genotoxic effects in lead exposed workers. Am J Ind Med 47:358–363.

Kocabas NA, Karahalil B, Karakaya AE, *et al.* (2000) Influence of GSTM1 genotype on comet assay and chromosome aberrations after induction by bleomycin in cultured human lymphocytes. Mutat Res 469:199–205.

Kumar A, Yadav A, Giri SK, *et al.* (2011) Influence of GSTM1 and GSTT1 genotypes and confounding factors on the frequency of sister chromatid exchange and micronucleus among road construction workers. Chemosphere 84:564–570.

Laffon B, Teixeira JP, Silva S, *et al.* (2006) Assessment of occupational genotoxic risk in the production of rubber tyres. Ann Occup Hyg 50:583–592.

Lee SH, Park E, Park YK (2010) Glutathione S-transferase M1 and T1 polymorphisms and susceptibility to oxidative damage in healthy Korean smokers. Ann Nutr Metab 56:52–58.

Norppa H (2001) Genetic polymorphisms and chromosome damage. Int J Hyg Environ Health 204:31–38.

Norppa H, Falck GC (2003) What do human micronuclei contain? Mutagenesis 18:221–233.

Pavanello S, Clonfero E (2000) Biological indicators of genotoxic risk and metabolic polymorphisms. Mutat Res 463:285–308.

Rossner P Jr., Rossnerova A, Sram RJ (2011) Oxidative stress and chromosomal aberrations in an environmentally exposed population. Mutat Res 707:34–41.

Sardas S, Yilmaz M, Oztok U, *et al.* (2001) Assessment of DNA strand breakage by comet assay in diabetic patients and the role of antioxidant supplementation. Mutat Res 490:123–129.

Schröder KR, Wiebel FA, Reich S, *et al.* (1995) Glutathione-S-transferase (GST) theta polymorphism influences background SCE rate. Arch Toxicol 69:505–507.

Skjelbred C, Svendsen M, Haugan V, *et al.* (2011) Influence of GSTM1, GSTT1, GSTP1, NAT1, NAT2, EPHX1, MTR and MTHFR polymorphism on chromosomal aberration frequencies in human lymphocytes. Carcinogenesis 32:399–405.

Tucker JD, Nath J, Hando JC (1996) Activation status of the X chromosome in human micronucleated lymphocytes. Hum Genet 97:471–475.

Tuimala J, Szekely G, Wikman H, *et al.* (2004) Genetic polymorphisms of DNA repair and xenobiotic-metabolizing enzymes: effects on levels of sister chromatid exchanges and chromosomal aberrations. Mutat Res 554:319–333.

Wiencke JK, Pemble S, Ketterer B, *et al.* (1995) Gene deletion of glutathione S-transferase theta: correlation with induced genetic damage and potential role in endogenous mutagenesis. Cancer Epidemiol Biomarkers Prev 4:253–259.

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