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Scientific paper

CYTOGENETIC DAMAGE IN TURKISH COKE OVEN WORKERS EXPOSED TO POLYCYCLIC AROMATIC HYDROCARBONS: ASSOCIATION WITH *CYP1A1*, *CYP1B1*, *EPHX1*, *GSTM1*, *GSTT1*, AND *GSTP1* GENE POLYMORPHISMS

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The aim of this study was to determine the frequencies of chromosomal aberrations (CA) and cytochalasin-blocked micronuclei (CBMN) in peripheral blood lymphocytes from Turkish coke oven workers and the influence of *CYP1A1*, *CYP1B1*, *EPHX1*, *GSTM1*, *GSTT1,* and *GSTP1* gene polymorphisms on these biomarkers. Cytogenetic analysis showed that occupational exposure significantly increased the CA and CBMN frequencies. Gene polymorphisms, on the other hand, did not affect CA or CBMN in either exposed or control subjects. However, due to the limited sample size, our findings need to be verified in future studies with a larger sample.

KEY WORDS: *chromosomal aberrations, biomarkers, micronuclei, occupational exposure*

Coke oven plants are a major source of emissions that contain complex mixtures of genotoxic and carcinogenic pollutants. Although the chemical content of these mixtures changes with the technology used in coke production, all contain polycyclic aromatic hydrocarbons (PAHs), which are released into the environment when coal is pyrolysed into coke. Moreover, PAHs with four or more aromatic rings are considered to be human carcinogens (1). The International Agency for Research on Cancer (IARC) has reported an increase in cancer incidence, lung cancer in particular, in workers with high and long-term exposure to coke oven emissions (2).

Monitoring biological effects as a measure of internal effective dose can provide relevant information for the assessment of cancer risks. Cytokinesis-block micronucleus (CBMN), chromosomal aberrations (CA), and sister chromatid exchanges (SCE) have been applied as biomarkers of exposure and early effects of genotoxic carcinogens. Epidemiological studies suggest that increased frequency of CA is predictive of an increased risk of cancer (3). CBMN assay has emerged as a maturing biomarker of chromosomal damage relevant to cancer in recent years. A recent Human MicroNucleus (HUMN) group study (4) with 6718 subjects has shown that increased CBMN frequency in peripheral blood lymphocytes

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can predict cancer risk in humans. Murgia et al. (5) found that individuals with high CBMN had a significantly higher cancer death risk than individuals with low CBMN.

A recent meta-analysis (6) of chromosomal damage and occupational exposure to PAHs revealed that cytogenetic end-points such as CBMN, CA, and SCE might be indicators of early effects in workers exposed to PAHs. Various gene polymorphisms that could modulate response to genotoxicity have already been addressed in several studies during the last decade (7).

Several polymorphisms of enzymes involved in PAH metabolism, DNA repair, and/or folate-metabolism may influence CBMN formation, but this association is rather complex. In the presence of multiple external and internal exposures, and the large number of chromosomal alterations, CBMN formation is inevitable. Iarmacovai et al. (8) have shown that *EPHX1*, *GSTT1*, and *GSTM1* polymorphisms modulate chromosomal damage in individuals exposed to genotoxic agents as well as in unexposed individuals. Others (9, 10) studied the effects of polymorphisms of genes involved in the metabolism of carcinogens on biomarkers of exposure such as urinary 1-hydroxypyrene (1-OHP) or DNA and protein adducts in populations occupationally exposed to PAHs. However, there are but a few studies on the effects of PAH metabolising enzyme polymorphisms on biomarkers such as CBMN in coke oven workers (11-18). In addition, only one study (14) investigated the effects of genetic polymorphisms of PAH metabolising enzymes on CA in coke oven workers.

The aim of our study was to assess PAH exposure of Turkish coke oven workers through CA and CBMN frequencies in peripheral lymphocytes and see whether the *CYP1A1*, *CYP1B1, EPHX1, GSTM1, GSTP1,* and *GSTT1* gene polymorphisms affected these biomarkers.

SUBJECTS AND METHODS

Subjects and sampling

All study subjects were involved in our previous studies (10, 19). Participation in this research was voluntary and all subjects were informed about the aims of the study. They gave their informed consent prior to enrolment according to the Helsinki Declaration. Questionnaire data contained items about demography, work history, job description, protective measures, smoking status, dietary information (alcohol consumption, fruit, grilled meat, vitamins, etc.), and medication (past and present), but only age and smoking have been investigated as variables in this study. Persons who had worked for less than three months or had received medical or radiological treatment or vaccination within three months before sampling were excluded.

The study eventually enrolled a hundred male workers. The exposed group consisted of 50 male workers employed in a Turkish iron and steel plant in Eregli, Zonguldak. Eight were top-oven workers (tar chasers and lidmen) and 42 were side-oven workers (heaters, quenching car operators, pushers, machine operators, oven repairmen, supervision, maintenance). The control group consisted of 50 packaging and energy department workers from the same plant, occupationally unexposed to PAHs. Workers from all three shifts were included in this study. All wore protective clothing, helmets, shoes, gloves, and masks while on duty.

Post shift urine samples were collected in a PVC container without preservatives and kept at -20 °C until analysis, as described in our earlier study (10).

Coded venous blood samples were collected into heparinised tubes at the same time as the urine samples and were processed within 5 h of collection.

CBMN frequencies in lymphocytes

Blood cultures consisted of a RPMI 1640 medium (Biological Industries, Beit Ha Emek, Israel) supplemented with 20 % foetal calf serum, 2 % phytohaemagglutinine (PHA-L), L-glutamine, and 13 to 14 drops of whole blood. All experiments were carried out in duplicate. Sample cultures were incubated at 37 °C for 72 h. Binucleated cells were accumulated by adding cytochalasin B at a final concentration of 6 $\mu g\ mL^{-1}$ at 44 h following the initiation of the culture (20).

Trisodium citrate (1 %) was used for a mild hypotonic effect. Slides were stained with May-Grünwald and Giemsa. CBMN frequency was examined by microscopy in 2,000 binucleated cells with well-preserved cytoplasm using the magnification of 1000x. Micronuclei were scored according to the criteria described by Fenech (20) only in binuclear lymphocytes in which the nuclei were of equal size and of the same colour. The diameter of the micronuclei was between 1/16 and 1/3 of the main nuclei and there

was no link between the two via a nucleoplasmic bridge. CBMN frequencies were expressed in permillage (‰).

The Nuclear Division Index (NDI), a cell proliferation index, was calculated according to Eastmond and Tucker (21). Slide scorer was not aware of the exposure status of the subjects.

CA frequencies in lymphocytes

Fourteen drops of blood were added into a 5 mL RPMI 1640 medium supplemented with 20 % foetal calf serum and 2 % PHA-L on the day of sampling. The cultures were incubated in the dark at 37 °C for 48 h. Three hours before the harvest, colchicine (0.05 μg mL^{-1}) was added to the culture. The cells were collected by centrifugation, re-suspended in a hypotonic solution (0.075 mmol L^{-1} of KCl) for 20 min and fixed in acetic acid:methanol (1:3). Slides were prepared by air-drying and stained with a 5 % Giemsa solution.

The scoring of chromosomal aberrations included chromatid breaks, acentric fragments, dicentrics, and gaps. The frequencies of aberrant cells with or without gaps were statistically analysed as described below. A total of 100 well-spread metaphases with 46 chromosomes were examined per donor. Slide scorer was not aware of the exposure status of the subjects.

Genotyping

DNA was isolated from blood samples using a Promega Corporation DNA isolation kit (Madison, WI, USA). The *CYP1A1* exon 7 (Ile462Val) (rs1048943) polymorphism was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR/RFLP) method described by Cascorbi et al. (22) and the *CYP1B1* exon 3 (Asn453Ser) (rs1800440) polymorphism using the PCR/RFLP method described by Bailey et al. (23). To determine the *EPHX1* exon 3 (Tyr113His) (rs1051740) and *EPHX1* exon 4 (His139Arg) (rs2234922) polymorphisms we used the PCR/RFLP method described by Smith and Harrison (24). The *GSTM1* and the *GSTT1* gene deletions were determined using the multiplex PCR method of Abdel-Rahman et al. (25). The *GSTP1* exon 5 (Ile105Val) (rs1695) and *GSTP1* exon 6 (Ala114Val) (rs1138272) polymorphisms were determined using the PCR/RFLP method described by Park et al. (26).

For quality control, laboratory staff were blinded to the sources of DNA samples and 10 % of the samples were retested at random, showing 100 % concordance. Two authors independently reviewed all of the agarose gels and genotype data entries.

Statistical analysis

The deviation from the Hardy-Weinberg equilibrium (HWE) was tested by comparing the observed and expected genotype frequencies using the chi-square test. Data were analysed with SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, USA).

To determine the normality of distribution of continuous variables we used the Shapiro-Wilk test.

Data are shown as mean ± standard deviation (SD) or median (and range), where applicable. Between-group differences in means were compared with Student's *t*–test and in medians with the Mann-Whitney U test. Nominal data were analysed with the chi-square test. Degrees of association between continuous variables were evaluated with Spearman's correlation test, which was also used to see if there were any associations between CBMN / total CA frequency data with or without gaps and the corresponding urinary 1-OHP levels, which we published recently (10). Multiple linear regression analysis was used to see if there were significant differences in CA or CBMN between coke oven workers and controls when adjusted for age, smoking, urinary 1-hydroxypyrene (1-OHP), and polymorphisms. Log-transformed linear regression analysis was used for dependent variables that were not normally distributed. Coefficients of regression and 95 % confidence intervals were calculated for all independent variables. A p value of less than 0.05 was considered statistically significant.

RESULTS

Table 1 summarises the demographic data of the coke oven workers and controls. Subjects smoking more than 20 cigarettes per day and non-smoking subjects were more prevalent in controls and coke oven workers, respectively ($p<0.05$). Urinary 1-OHP levels as indicators of PAH exposure were about 3.5 times higher in coke oven workers than controls ($p<0.001$), as described elsewhere (10).

Table 2 shows CBMN frequencies in peripheral blood lymphocytes by age, smoking status, and duration of exposure. Median CBMN frequency in coke oven workers was significantly higher than in

Table1 *Demographic and lifestyle data for coke oven workers and controls**

Variables	Controls	Coke oven Workers	*p*
N	50	50	
Age / mean ± SD	38.7±9.5	40.4±6.6	0.301
Smoking status / n (%)			
Non-smokers	7 (14.0)	18 (36.0)	0.021
1 to 10 cigarettes per day	10 (20.0)	10 (20.0)	1.000
11 to 20 cigarettes per day	17 (34.0)	19 (38.0)	0.677
>20 cigarettes per day	16 (32.0)	3 (6.0)	<0.001
Duration of exposure / year; median (range)	-	16 (1 to 25)	-
Urinary 1-OHP levels / μmol mol^{-1} creatinine; median (range)	0.23 (0.01 to 2.69)	0.82 (0.05 to 14.99)	<0.001

** data taken from Ada et al. (10)*

Table 2 *CBMN frequencies (‰) by age, smoking, and duration of exposure in control and coke oven workers*

Variables	Controls			Coke oven workers			*p**
	n	Median (Range)	*p*†	*n*	Median (Range)	*p*†	
All	49[a]	6 (1 to 15)	-	49[a]	12 (4 to 38)	-	<0.001
Age groups / year			0.331			0.125	
≤40	20	6 (1 to 11)		19	15 (4 to 28)		<0.001
≥41	29	6 (2 to 15)		30	10.5 (4 to 38)		<0.001
Smoking status			0.291			0.248	
Nonsmokers	7	6 (2 to 10)		18	11 (4 to 23)		0.002
Smokers	42	6 (1 to 15)		31	14 (4 to 38)		<0.001
Duration of exposure / year			-			0.699	
<20	-	-		36	13 (4 to 38)		-
≥20	-	-		13	11 (7 to 21)		-

[a] CBMN data for one subject in the exposed and control group were not available
† Comparisons within both control and coke oven workers
** Comparisons between control and coke oven workers*

Table 3 *Frequencies (%) of chromosomal aberrations without gaps (CA-gap) by age, smoking and duration of exposure in controls and coke oven workers*

Variables	Controls			Coke oven workers			*p**
	n	Median (Range)	*p*†	*n*	Median (Range)	*p*†	
All	45[a]	0 (0 to 1)	-	48[a]	0.5 (0 to 7)	-	<0.001
Age groups / year			0.771			0.347	
≤40	20	0 (0 to 1)		19	1 (0 to 7)		0.016
≥41	25	0 (0 to 1)		29	0 (0 to 2)		0.008
Smoking status			0.470			0.380	
Nonsmokers	7	0 (0 to 1)		17	0 (0 to 7)		0.619
Smokers	38	0 (0 to 1)		31	1 (0 to 3)		<0.001
Duration of exposure / year			-			0.527	
<20	-	-		34	1 (0 to 7)		
≥20	-	-		14	0 (0 to 2)		

[a] Chromosomal aberration data for five subjects in the control group and two subjects in the exposed group were not available.
† Comparisons within both control and coke oven workers.
** Comparisons between control and coke oven workers.*

controls, but within-group differences were not significant in regard to age and smoking in either group or to exposure history in coke oven workers. Mean ±SD of NDI values for exposed (2.14±0.05) and control subjects (2.14±0.04) were similar ($p>0.05$).

Chromosomal aberrations mainly consisted of chromatid breaks and gaps. Table 3 shows that the overall frequencies of total aberrant cells without gaps (CA-gap) were significantly higher in coke oven workers than controls ($p<0.001$). They were also significantly higher in coke oven workers aged below 40 ($p<0.05$) and those above 41 years ($p<0.01$) compared to control subgroups. Similar was found for smoking coke oven workers compared to smoking controls ($p<0.001$). However, similar to CBMN, no significant effect on CA-gap frequencies was found for age and smoking within either group or for exposure history in coke oven workers.

Findings for aberrant cells with gaps (CA+gap) were similar to those for CA-gap (Table 4).

The two groups did not differ in the distribution of genotypes (data not shown), as it was in good agreement with the Hardy-Weinberg equilibrium. There were no homozygous mutant *CYP1A1* Val/Val and *GSTP1* Val/Val genotypes in either group and no *EPHX1* His/His genotypes in the control group. Due to a small number of *CYP1B1*, *GSTP1*, and *EPHX1* homozygous mutant genotypes in the coke oven workers (*CYP1B1* Ser/Ser $n = 2$, *GSTP1* Val/Val $n = 2$, *EPHX1* His/His $n = 2$, *EPHX1* Arg/Arg n = 3) and controls (*CYP1B1* Ser/Ser $n = 3$, *GSTP1* Val/Val $n = 2$, *EPHX1* Arg/Arg $n = 3$), heterozygous and homozygous mutant genotypes were combined for statistical analysis.

Controls did not differ in CBMN, CA-gap, or CA+gap frequencies between the genotypes (Table 5).

In coke oven workers, the only significant difference was found for CBMN frequency, which was significantly higher in the wild-type allele carriers than in mutant allele carriers of the exon7 of *CYP1A1* gene ($p=0.015$; Table 6).

We found no significant correlation between urinary 1-OHP levels and CBMN, CA-gap or CA+gap frequencies in either group (Table 7).

Multiple linear regression analyses after adjustment for age, smoking status, 1-OHP, and genotypes showed that exposure to coke oven emissions significantly increased CBMN, CA-gap, and CA+gap frequencies ($p<0.05$; Table 8). Other independent variables did not significantly affect either of the three parameters.

DISCUSSION

Epidemiological studies have shown that long-term exposure to PAHs significantly increases the risk of developing lung cancer in coke oven workers (2). Our worker population was recruited from the Turkey's largest iron and steel production plant in Erdemir. With 159 coke ovens, its coke capacity is about one million tonnes per year.

As chromosomal changes and genetic instability are the major causes of carcinogenesis, identifying reliable cytogenetic biomarkers for high cancer risk is an important task for public health services (27). Biomarkers used in our study have been demonstrated as reliable for predicting increased risk of cancer in humans (3, 4).

Previously we had established coke oven workers' exposure to PAH by determining urinary 1-OHP excretion (10, 19). Urinary 1-OHP is a good biomarker of exposure to PAHs, as it reflects all exposure routes (28). Our measurements were in line with those of industrialised western countries (15, 18, 28-30) and three to 10 times lower than in Polish, Chinese, Estonian, or Taiwanese workers (16, 17, 31, 32).

Our cytogenetic analysis shows that occupational exposure at the coke oven significantly elevated the frequencies of CA and CBMN in peripheral blood lymphocytes. These results are in line with earlier CBMN reports (12, 13, 16, 17), but in contrast with Van Delft et al. (11), who found no significant increase in CBMN frequency among coke oven workers.

Increased CA frequencies in coke oven workers have also been reported earlier (14, 33). However, Siwinska et al. (17) found no association between occupational exposure and CA, even though 1-OHP levels were about nine times higher than in our coke oven workers. Furthermore, Forni et al. (30) found no alteration in CA and CBMN frequencies in coke oven workers with 1-OHP levels similar to ours. All these contradictions could be due to different sample sizes, composition of cohorts (including age, smoking, and diet), methodology, and occupational exposure to genotoxic chemicals other than PAHs.

The only Turkish study before ours (34) demonstrated significantly higher CA frequencies in iron and steel plant workers from units other than the coke oven unit. However, the study lacks any PAH exposure data for comparison with our findings.

In this study, we found no correlation between urinary 1-OHP and CA and CBMN frequencies. This may be explained by the fact that CA and CBMN

Table 4 *Frequencies (%) of chromosomal aberrations with gaps (CA+gap) by age, smoking and duration of exposure in controls and coke oven workers*

Variables	Controls			Coke oven workers			p^*
	n	Median (Range)	$p^\dagger$	*n*	Median (Range)	$p^\dagger$	
All	45[a]	1 (0 to 5)	-	48[a]	3 (0 to 9)	-	<0.001
Age groups / year			0.652			0.577	
≤40	20	1 (0 to 4)		19	3 (0 to 9)		0.012
≥41	25	1 (0 to 5)		29	3 (0 to 7)		<0.001
Smoking status			0.331			0.677	
Non-smokers	7	2 (0 to 4)		17	2 (0 to 9)		0.383
Smokers	38	1 (0 to 5)		31	3 (0 to 8)		<0.001
Duration of exposure / year						0.809	
<20	-	-		34	3 (0 to 9)		
≥20	-	-		14	2 (1 to 5)		

[a] *Chromosomal aberration data for five subjects in control group, and two subjects in exposed group were not available.*
† Comparisons within both control and coke oven worker groups.
** Comparisons between control and coke oven worker groups.*

Table 5 *CBMN, (CA-gap), and (CA+gap) frequencies in controls by genotype*

Genotypes	CBMN / ‰			CA-gap / %			CA+gap / %		
	n	Median (Range)	*p*	*n*	Median (Range)	*p*	*n*	Median (Range)	*p*
CYP1A1 exon7			0.514			0.658			1.000
Ile/Ile	45	6 (1 to 15)		41	0 (0 to 1)		41	1 (0 to 5)	
Ile/Val+Val/Val	4	5.5 (3 to 7)		4	0 (0 to 0)		4	1 (0 to 3)	
CYP1B1 exon3			0.513			0.094			0.795
Asn/Asn	22	5.5 (3 to 11)		22	0 (0 to 1)		22	1 (0 to 3)	
Asn/Ser+Ser/Ser	23	6 (1 to 15)		23	0 (0 to 1)		23	1 (0 to 5)	
GSTM1			0.896			0.487			0.115
Null	24	6 (2 to 15)		21	0 (0 to 1)		21	1 (0 to 5)	
Positive	25	6 (1 to 13)		24	0 (0 to 1)		24	1 (0 to 4)	
GSTT1			0.889			0.493			0.298
Null	9	7 (3 to 11)		8	0 (0 to 0)		8	0.5 (0 to 3)	
Positive	40	6 (1 to 15)		37	0 (0 to 1)		37	1 (0 to 5)	
GSTP1 exon5			0.258			0.417			0.236
Ile/Ile	32	6.5 (2 to 15)		31	0 (0 to 1)		31	1 (0 to 5)	
Ile/Val+Val/Val	17	5 (1 to 12)		14	0 (0 to 1)		14	1 (0 to 3)	
GSTP1 exon6			0.645			0.658			0.234
Ala/Ala	43	6 (1 to 15)		41	0 (0 to 1)		41	1 (0 to 5)	
Ala/Val+Val/Val	6	6.5 (1 to 12)		4	0 (0 to 0)		4	0.5 (0 to 1)	
EPHX1 exon3			0.317			0.954			0.531
Tyr/Tyr	27	6 (2 to 15)		22	0 (0 to 1)		22	1 (0 to 5)	
Tyr/His+His/His	22	6 (1 to 12)		23	0 (0 to 1)		23	1 (0 to 4)	
EPHX1 exon4			0.099			0.066			0.265
His/His	35	7 (1 to 15)		30	0 (0 to 1)		30	1 (0 to 5)	
His/Arg+Arg/Arg	14	4.5 (2 to 11)		15	0 (0 to 0)		15	1 (0 to 3)	

frequencies in peripheral blood lymphocytes reflect accumulated chromosomal damage (35, 36), whereas urinary 1-OHP reflects exposure within the last 24 h.

Even though genetic polymorphisms of biomarkers of susceptibility may play a role in genetic damage involved in mutagenesis and carcinogenesis (7), only a few studies have investigated the influence of PAH

Table 6 *CBMN, (CA-gap), and (CA+gap) frequencies in coke oven workers by genotype*

Genotypes	CBMN / ‰			CA-gap / %			CA+gap / %		
	n	Median (Range)	*p*	*n*	Median (Range)	*p*	*n*	Median (Range)	*p*
CYP1A1 exon7			0.015			0.577			0.719
Ile/Ile	43	14 (4 to 38)		43	0 (0 to 7)		43	3 (0 to 9)	
Ile/Val+Val/Val	6	9 (8 to 10)		5	1 (0 to 2)		5	2 (1 to 5)	
CYP1B1 exon3			0.094			0.218			0.266
Asn/Asn	24	15 (4 to 38)		25	1 (0 to 3)		25	3 (0 to 7)	
Asn/Ser+Ser/Ser	25	11 (4 to 26)		23	0 (0 to 7)		23	2 (0 to 9)	
GSTM1			0.127			0.455			0.883
Null	25	10 (4 to 28)		24	1 (0 to 3)		24	3 (0 to 8)	
Positive	24	14 (7 to 38)		24	0 (0 to 7)		24	3 (0 to 9)	
GSTT1			0.213			0.580			0.828
Null	6	9 (4 to 23)		5	0 (0 to 1)		5	3 (0 to 5)	
Positive	42	13 (5 to 38)		42	1 (0 to 7)		42	3 (0 to 9)	
GSTP1 exon5			0.729			0.216			0.932
Ile/Ile	29	12 (4 to 38)		28	1 (0 to 7)		28	2.5 (0 to 9)	
Ile/Val+Val/Val	20	13 (5 to 26)		20	0 (0 to 2)		20	3 (0 to 8)	
GSTP1 exon6			0.967			0.249			0.775
Ala/Ala	42	12 (4 to 38)		41	1 (0 to 7)		41	3 (0 to 9)	
Ala/Val+Val/Val	7	13 (5 to 26)		7	0 (0 to 1)		7	4 (0 to 8)	
EPHX1 exon3			0.305			0.455			0.883
Tyr/Tyr	23	14 (7 to 26)		24	0 (0 to 7)		24	3 (1 to 9)	
Tyr/His+His/His	26	11 (4 to 38)		24	1 (0 to 3)		24	3 (0 to 7)	
EPHX1 exon4			0.852			0.750			0.370
His/His	37	12 (4 to 38)		36	0.5 (0 to 7)		36	2.5 (0 to 9)	
His/Arg+Arg/Arg	12	13 (7 to 26)		12	0.5 (0 to 1)		12	3 (1 to 8)	

metabolising enzyme polymorphisms (namely, *EPHX1* exon 3, *CYP1A1*, *GSTM1*, *GSTT1*, and *GSTP1* exon 5) on CBMN (11-13) and only one study (of *GSTM1* and *NAT2*) (14) on CA frequency in coke oven workers.

In this respect, our study was the first to attempt a comprehensive approach to the issue with eight PAH metabolising enzyme polymorphisms. Their distribution across the study population was similar to earlier reports in Turkish and European Caucasian populations (37-39). The only polymorphism that stands out in our study is the wild-type *CYP1A1* exon 7 which was associated with a significantly higher CBMN frequency. At this stage, the reasons behind this finding are unclear. It is possible that PAH-exposed individuals with the wild-type genotype could activate PAHs, benzo(a)pyrene (BaP) in particular, to their toxic intermediates at higher rates than individuals carrying variant genotypes. In fact, Zhang et al. (40) have reported a higher rate of BaP metabolism *in vitro* with the wild-type than mutant gene at high BaP concentrations. Alternatively, it is also possible that mutant allele carriers are so few that any (low or high) CBMN frequency finding is a product of pure chance. Moreover, our findings could have been influenced by confounding factors such as other polymorphisms, smoking, and age, since multiple linear regression analysis revealed no significant influence of this gene polymorphism on CBMN frequency, and neither have earlier studies (12, 13, 18).

This lack of association was also noted between other gene polymorphisms and CBMN frequencies, which is consistent with studies in coke oven workers in regard to *GSTM1* (11, 12, 18) and *GSTT1* polymorphisms (11-13).

In contrast to our findings, Leng et al. (12) reported a significantly higher CBMN frequencies with the *GSTP1* Val/Val genotypes and significantly lower CBMN frequencies with the *EPHX1* exon 3 mutated genotypes.

Associations between the gene polymorphisms and CA frequencies in our study were also not significant

Table 7 *Relationships between biomarkers of exposure (1-OH pyrene) and effects*

Variables	Exposed Group		Control Group	
	r	*p*	r	*p*
CBMN	0.228	0.114	-0.008	0.954
CA-gap	0.116	0.431	-0.048	0.755
CA+gap	0.207	0.158	-0.106	0.492

Table 8 *Multiple linear regression analyses of cytogenetic data*

Dependent Variables	Independent Variables	Coefficient of Regression (B)	*p* value	95 % CI for (B)	
				Lower	Upper
CBMN	*Coke oven workers*	*0.724*	*<0.001*	*0.478*	*0.971*
	Age	*0.006*	*0.372*	*-0.008*	*0.020*
	Smoking	*0.037*	*0.511*	*-0.074*	*0.148*
	1-OHP	*0.056*	*0.079*	*-0.007*	*0.119*
	CYP1A1[a]	*-0.290*	*0.086*	*-0.622*	*0.042*
	GSTT1[b]	*-0.243*	*0.100*	*-0.534*	*0.048*
CA-gap	*Coke oven workers*	*0.268*	*0.005*	*0.082*	*0.454*
	Age	*-0.006*	*0.297*	*-0.016*	*0.005*
	Smoking	*0.004*	*0.922*	*-0.081*	*0.089*
	1-OHP	*0.037*	*0.122*	*-0.010*	*0.084*
	GSTT1[b]	*-0.184*	*0.124*	*-0.418*	*0.051*
	EPHX1 exon4[a]	*-0.088*	*0.315*	*-0.260*	*0.085*
CA+gap	*Coke oven workers*	*0.440*	*0.002*	*0.167*	*0.712*
	Age	*0.000*	*0.955*	*-0.015*	*0.016*
	Smoking	*-0.053*	*0.399*	*-0.177*	*0.071*
	1-OHP	*0.052*	*0.140*	*-0.017*	*0.120*
	GSTT1[b]	*-0.308*	*0.077*	*-0.651*	*0.034*

[a] *Wild-type genotype served as reference*
[b] *Positive genotype served as reference*

in either coke oven or control workers. Kalina et al. (14) reported similar findings for *GSTM1* polymorphisms.

Multiple linear regression analysis identified work at coke oven as the single contributing factor to increased CBMN, CA+gap, and CA-gap frequencies and confirmed that occupational exposure has the major effect on CBMN and CA frequencies, as reported earlier by Qiu et al. (13).

It is well known that individual response to certain genotoxic chemicals may also be influenced by DNA repair and cell cycle control (31,41), which calls for further investigation in that direction.

In conclusion, our study has confirmed positive association between increased genetic damage and occupational exposure but not with the genetic polymorphisms of PAH metabolising enzymes in Turkish coke oven workers. However, due to the limited sample size, our findings need to be verified in further studies with a larger sample.

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Sažetak

CITOGENETIČKO OŠTEĆENJE U TURSKIH RADNIKA NA KOKSNIM PEĆIMA IZLOŽENIH POLICIKLIČKIM AROMATSKIM UGLJIKOVODICIMA: POVEZANOST S GENSKIM POLIMORFIZMIMA CYP1A1, CYP1B1, EPHX1, GSTM1, GSTT1 I GSTP1

Cilj je ovog ispitivanja bio utvrditi učestalost kromosomskih aberacija (CA) i mikronukleusa (CBMN) u limfocitima periferne krvi turskih radnika na koksnim pećima te utjecaj genskih polimorfizama CYP1A1, CYP1B1, EPHX1, GSTM1, GSTT1 i GSTP1 na te biopokazatelje. Profesionalna je izloženost ovih radnika značajno povećala učestalost CA i CBMN, ali genski polimorfizmi nisu utjecali na ove parametre bez obzira na to je li se radilo o radnicima ili o kontrolnoj skupini. Međutim, značaj je naših rezultata ograničen zbog malog uzorka te su potrebna daljnja istraživanja s većim uzorkom da ih se potvrdi.

KLJUČNE RIJEČI: *biopokazatelji, kromosomske aberacije, mikronukleusi, profesionalna izloženost*

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