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The in vitro determination of genotoxicity in peripheral lymphocytes of welders exposed to fumes from metal arc welding

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Aim: Gas metal arc welding is a widely used method in many industrial areas. In this study, the peripheral blood lymphocytes of 23 welders and 25 nonexposed subjects were monitored for genotoxicity.

Materials and methods: The blood samples obtained from the subjects were incubated with a culture medium at 37 °C for 72 h. Cytochalasin-B and BrdU were added for micronucleus and replication index. After incubation, the cultures were harvested, stained, and examined.

Results: The micronucleus rate (2.165 ± 1.645) of the welders was quite higher than that of those not exposed (1.352 ± 0.978) ($P < 0.01$). There was a significant difference between micronucleus rates of smoking and nonsmoking subjects. It was found that as the time of exposure to welding fumes increased, so did the micronucleus rates (2.938 ± 1.723) ($P < 0.05$). A positive correlation was observed between micronucleus frequency and age. The replication index rate (1.026 ± 0.020) of the welders was higher than that of those not exposed (1.019 ± 0.016) ($P = 0.01$).

Conclusion: It is advised that welders who work with gas metal arc welding be instructed and that necessary protective measures be taken because of the risk caused by metal arc welding fumes.

Key words: Gas metal arc welding, micronucleus, replication index, welder

1. Introduction

Humans have been affected by a large number of environmental contaminants via respiration, ingestion, and absorption (1). The fumes from welding are one of the most important environmental contaminants. They are classified by the International Agency for Research on Cancer (IARC) as Group 2B (2) as being hazardous to human health and consisting of carcinogenic properties. Various gaseous and aerosol particles are produced with welding. Respiratory effects observed in full-time welders have included pneumoconiosis (3), bronchitis and airway irritation (4), fibrosis (5), lung cancer (2), metal fume fever (6), and nasal septum perforation (7).

Gas metal arc welding generates fumes containing oxides of chromium and nickel, together with a number of other metal oxides. Cr and Ni compounds are also classified by the IARC as Group 2B (2). However, the welding process produces only hexavalent-chromium (Cr^{VI}), a carcinogenic at high concentrations.

The micronucleus (MN) test is used in genotoxicity studies to provide sensitive and rapid monitoring of induced genetic damages such as primary DNA damage in human peripheral blood cultures (8). MNs can be formed when either the intact chromosome or acentric chromosomal fragments become separated from the nucleus during mitosis or meiosis and are seen within a binucleate cell after cytokinesis block (9). The MN is shaped in the initial step of human carcinogenic processes. The replication index (RI) was used to characterize proliferating cells and determine compounds decreasing mitotic division.

We report a study of welders by evaluating rates of MNs and the RI in their peripheral blood lymphocytes and we compare the results with observations of the nonexposed subjects.

2. Materials and methods

2.1. Subjects

In this study, blood was drawn and studied from 48 male donors. The donors included 23 welders exposed to welding fumes and 25 controls not exposed to welding

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fumes. The welders were engaged in shielded manual metal arc welding with consumable stainless steel electrodes usually containing approximately 20% Cr with 10% Ni. The general information of the welders and nonexposed subjects is given in Table 1. The peripheral blood samples were obtained from donors who did not drink alcohol and had no viral infection. No subjects had been taking any medication and they had not been exposed to any kind of radiation for 12 months before sampling. They were also informed about the objective of the study.

2.2. Chemicals

The culture medium (CAS No. 01-201-1, Biological Industries), colcemid (CAS No. D1925, Sigma), BrdU (B5002, Sigma), cytochalasin-B (CAS No. 14930-96-2, Sigma), and Giemsa stain (CAS No. 5400512, Merck) were used in lymphocyte cultures. The culture medium included 1% L-glutamine, 20% fetal bovine serum, 1% antibiotic, and 2% phytohemagglutinin.

2.3. Human lymphocyte cultures and cell harvesting

The heparinized blood samples (0.4 mL) were obtained from the subjects with the permission of the local ethics committee and were placed in sterile culture tubes containing 5 mL of culture medium. After mixing the contents of each culture tube by gently shaking, the culture tubes were incubated in a slanted position at 37 °C for 72 h. Cytochalasin-B and BrdU were added for MNs and RI. After 72 h of incubation the cultures were harvested, stained, and examined according to the methods of İkbal et al. (10) and Gülçe İz et al. (11).

The tubes were centrifuged at 2000 rpm for 4 min and the supernatant was discarded. The pellet was resuspended using 10 mL of hypotonic solution (0.075 M KCl) and the tubes were incubated at 37 °C for a further 4 min. After the tubes were centrifuged at 2000 rpm for 4 min and the supernatant discarded, the pellet was resuspended using 10 mL of fresh fixative solution (methanol and acetic acid, 3:1). The tubes were centrifuged at 2000 rpm for 4 min and the supernatant was discarded. This procedure was repeated 3 times. The pellet was resuspended and 0.5–1 mL of fresh, cold fixative solution was added to the tubes. Three or 4 drops of the cell suspension were then dropped onto a cold, wet glass slide. Slides were air-dried and were stained with 5% Giemsa.

2.4. Examination of micronuclei

The slides were randomized and scored by a single observer. About 500 cells were examined at 600× magnification from each slide, and when MN cells were located, they were examined under 1000× magnification. Dead or degenerating cells were excluded from evaluation. Nuclear blebbing (MN-like structures connected with the main nucleus by a bridge) were not considered. Only MNs equal to or smaller than one-fifth of the main nucleus were considered. Multimicronucleated cells were also scored but were not included in the evaluation of the MN rate.

2.5. Examination of replication index

The RI was defined by counting the percentage of cells containing 1, 2, 3, or more MNs per individual. The RI was calculated according to the following formula: $RI = (1 \times M1) + (2 \times M2) + (3 \times M3) / 500$.

2.6. Statistical analysis

SPSS 10.0 was used to analyze the data. The mean and standard deviation (SD) of each subject were calculated. Student's t-test was used to determine the significance ($P < 0.01$) of the differences between nonexposed subjects and exposed welders. Analysis of variance (ANOVA) was used to determine the statistical significance of the effects of exposure to welding fumes, smoking, and age. The differences between the groups were determined by the Tukey–Kramer test with $P < 0.05$. The correlation and regression coefficients between 2 parameters, including MN–exposed to fumes, MN–age, RI–age, and RI–exposed to fumes, were calculated.

3. Results

The rate of MNs was studied in 23 welders and in 25 nonexposed subjects. Welders revealed a significant induction of MNs when compared with the nonexposed subjects ($P < 0.01$). The MN rate (2.165 ± 1.645) of the welders was quite higher than that of those nonexposed subjects (1.352 ± 0.978) (Table 2).

Table 1. The general information of nonexposed subjects and welders.

	Nonexposed (n = 25)	Welders (n = 23)
Age (mean ± SD)	27.36 ± 14.82	27.17 ± 5.65
Smoking: n (%)		
Yes	16 (64%)	14 (60.87%)
No	9 (36%)	9 (39.13%)
Welding fumes exposure duration (years ± SD)	-	8.39 ± 6.86
Alcohol consumption	No	No
Medicine usage	No	No
Exposed to radiation	No	No

Table 2. Intergroup comparison of mean MNs (%) in nonexposed subjects and welders.

	Total counted cells	Total MNs	Mean MNs ± SD
Nonexposed	12,500	169	1.352 ± 0.978
Welders	11,500	249	2.165 ± 1.645*
All subjects	24,000	418	1.741 ± 1.387

*Student's t-test: $P < 0.01$ (different from nonexposed subjects).

The dispersion graphic of MN values in nonexposed subjects and welders is given in Figure 1. MN values of nonexposed subjects were between 0.2 and 4.4. However, MN values of welders had a wider range (0.4–5.8) than did those of the nonexposed subjects.

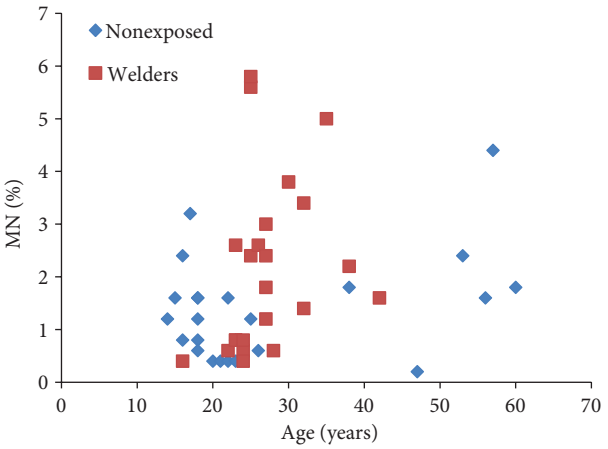

Figure 1. The dispersion graphic of MN values of nonexposed subjects and welders. MN values of 11 welders (47.82%) are higher than the MN value of 2.0. On the contrary, the MN values of only 4 nonexposed subjects (16.00%) are higher than this value. The 8 subjects (16.66%) with MNs values higher than 3.0 are smokers with long-term exposure to welding fumes (≥8 years).

Table 3 shows MN rate with respect to smoking habit, work duration, and age of nonexposed subjects and welders. There was not a significant difference between smokers and nonsmokers among nonexposed subjects ($P = 0.05$). However, there were significant differences between smokers and nonsmokers among welders ($P < 0.05$). There were also differences between nonexposed subjects and welders with both a smoking and nonsmoking history ($P < 0.05$). When the MN frequencies in lymphocyte cultures of the welders were analyzed, a significant difference was found for time exposed to welding fumes ($P < 0.05$). The MN rate (2.938 ± 1.723) of 13 welders (years of exposure ≥ 8) was quite higher than the MN rate (1.160 ± 0.820) of the other 10 welders (years of exposure < 8). The MN frequency was not statistically affected by age for both nonexposed subjects and welders ($P = 0.05$).

The RI values were studied in 23 welders and in 25 nonexposed subjects. Welders revealed an induction of RI when compared with nonexposed subjects ($P = 0.01$). The RI rate (1.026 ± 0.020) of the welders was higher than that of nonexposed subjects (1.019 ± 0.016) (Table 4).

The dispersion graphic of RI values of nonexposed subjects and welders is given in Figure 2. RI values of nonexposed subjects were between 1.002 and 1.060. The RI values of welders had a wider range (1.002–1.068) than did those of the nonexposed subjects.

Table 3. Micronucleus rate (%) with respect to smoking habit, work duration, and age in nonexposed subjects and welders.

Parameter	Nonexposed (n = 25) Mean MNs (%) ± SD	Welders (n = 23) Mean MNs (%) ± SD
Smoking		
Yes	1.125 ± 0.822 (16) [ac]	2.685 ± 1.842 (14) [b]
No	1.755 ± 1.147 (9) [abc]	1.355 ± 0.847 (9) [c]
Years of exposure		
≥8		2.938 ± 1.723 (13) [a]
<8		1.160 ± 0.820 (10) [b]
Age (years)		
≥30	2.033 ± 1.370 (6) [ab]	2.900 ± 1.407 (6) [b]
<30	1.136 ± 0.742 (19) [a]	1.905 ± 1.682 (17) [ab]

[a, b, c] ANOVA: Values with differing superscripts are significant at $P < 0.05$.

Table 4. Intergroup comparison of mean replication index in nonexposed subjects and welders.

	Total counted cells	M1	M2	M3	Mean RI ± SD
Nonexposed	12,500	12,292	176	32	1.019 ± 0.016
Welders	11,500	11,245	210	45	1.026 ± 0.020
All subjects	24,000	23,537	386	77	1.022 ± 0.018

Student's t-test: P > 0.01 (not different from nonexposed subjects).

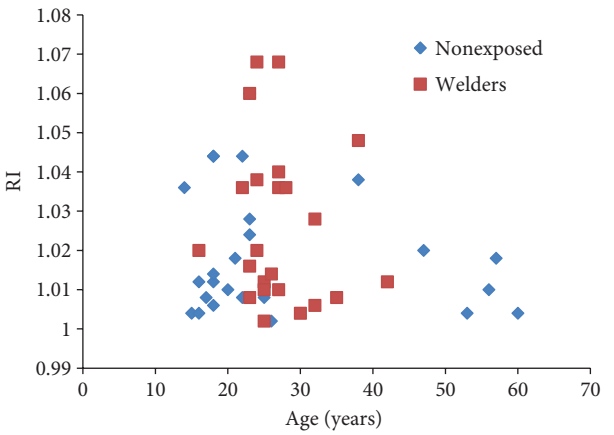

Figure 2. The dispersion graphic of RI values of nonexposed subjects and welders. The RI values of 9 welders (39.13%) are higher than the RI value of 1.030. On the contrary, the RI values of 6 nonexposed subjects (24.00%) are higher than this value. Compared to 11 nonexposed subjects (44.00%), the RI values of 7 welders (30.43%) are between 0 and 0.010.

Table 5 shows the RI frequency with respect to smoking habit, work duration, and age in nonexposed subjects and welders. There was not a significant difference between smokers and nonsmokers and between nonexposed subjects and welders (P = 0.05); however, the highest RI values were detected among smokers. When the RI frequencies in lymphocyte cultures of the welders were analyzed, a significant difference was not found for time exposed to welding fumes (P = 0.05). The RI rate (1.016 ± 0.013) of 13 welders (years of exposure ≥ 8) was lower than the RI rate (1.039 ± 0.021) of the other 10 welders (years of exposure < 8). The RI frequency was not statistically affected by age for both nonexposed subjects and welders (P = 0.05).

4. Discussion

Welders were investigated for in vitro cytotoxic and genotoxic effects in this study by using the MN and RI assays in human peripheral blood cultures. In the literature, there are few in vitro studies regarding peripheral lymphocytes

Table 5. Replication index with respect to smoking habit, work duration, and age in nonexposed subjects and welders.

Parameter	Nonexposed (n = 25) Mean RI ± SD	Welders (n = 23) Mean RI ± SD
Smoking		
Yes	1.021 ± 0.016 (16) [a]	1.026 ± 0.020 (14) [a]
No	1.016 ± 0.016 (9) [a]	1.026 ± 0.020 (9) [a]
Years of exposure		
≥8		1.016 ± 0.013 (13) [a]
<8		1.039 ± 0.021 (10) [a]
Age (years)		
≥30	1.015 ± 0.012 (6) [a]	1.017 ± 0.017 (6) [a]
<30	1.020 ± 0.017 (19) [a]	1.029 ± 0.021 (17) [a]

[a] ANOVA: P > 0.05, not different among all parameters.

of welders (12–14), and this study will contribute to the literature. Because the genotoxicity was reported with the parameters of the sister chromatid exchange, the chromosome aberration, and the polymorphisms in these studies, this study is the first report for genotoxicity with the parameters of MN and RI in welders.

According to the MN results, it may be thought that the increasing MN rate reveals a risk of genotoxicity and carcinogenicity for welders. The Cr and Ni may cause genotoxic and carcinogenic effects. Chromate ions are especially valuable for the biological monitoring of exposed workers. The highest MN values for nonexposed control subjects were 4.4, 3.2, and 2.4. These values were obtained from older subjects or smokers. The highest MN values for welders were 5.8, 5.6, and 5.0. These values were obtained from people both smoking and exposed to welding fumes for a long time (≥8 years). According to Figure 1 and Figure 3A, MN rates were increased by smoking and exposure to welding fumes. It was reported that there was a positive correlation between MN values and smoking (15), and the harmful effects of cigarette smoke were also reported (16,17). A positive correlation for both nonexposed subjects and welders was observed between MN rate and age (Figure 3B); namely, the higher the detected MN rate, the higher the age. It was reported that there was a positive correlation between MN and age (18).

According to the RI results, although there is not a statistically significant difference between nonexposed subjects and welders, it may be thought that the increasing RI rate is evidence of cytotoxicity. The highest RI values for nonexposed subjects were 1.060, 1.044, and 1.038. These values were generally obtained from smokers. The highest RI values for welders were 1.068, 1.060, and 1.048. These values were obtained from smokers or people exposed to welding fumes for a long time (≥8 years).

A negative correlation was observed between RI frequency and age (Figure 3C); namely, the lower the detected RI frequency, the higher the age. A negative correlation was also observed between RI frequency and welding fumes (Figure 3D). Pastor et al. (19) determined an inverse negative relationship between RI frequency and age. It was reported that a rising mitotic index and cell proliferation will cause a more rapid decrease with increasing age, and the opposite will occur with a falling index (20).

In conclusion, the welders showed significantly higher levels of MNs compared to the nonexposed subjects. According to the MN and RI rates, the welding fumes are a potential risk for welders. The welders should be informed about the toxic potential of welding fumes and the importance of taking protective measures. Here it is important to note that the best remedy for occupational exposure is prevention.

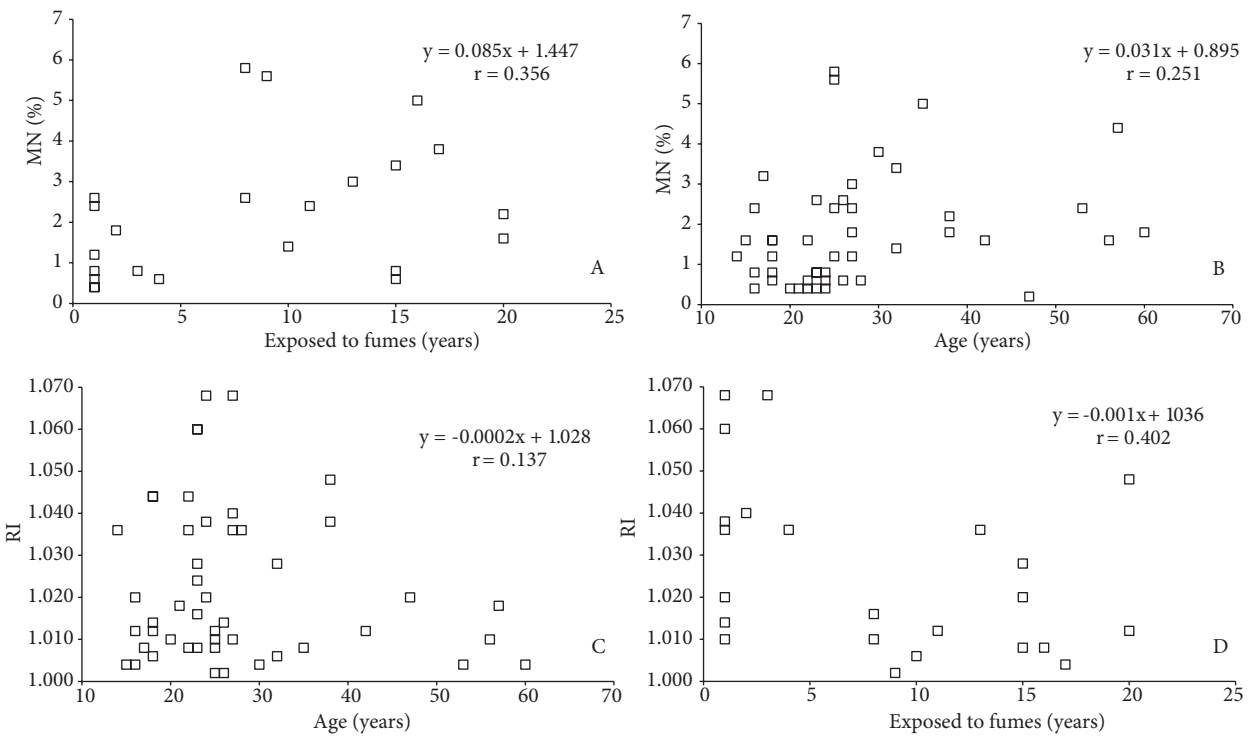

Figure 3. The scatter diagrams. A) MN–exposed to fumes, B) MN–age, C) RI–age, D) RI–exposed to fumes.

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