

Determining mitotic index in peripheral lymphocytes of welders exposed to metal arc welding fumes

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Abstract: Gas metal arc welding is a widely used method in a lot of industrial areas as it is cheap and produces high quality results. In the present study, peripheral blood lymphocytes of 23 welders and 25 non-exposed subjects were monitored for cytotoxicity. Information regarding the subjects' ages, smoking habits, alcohol consumption, duration of exposure, and medicine usage was recorded. According to the results, mitotic index (2.278 ± 1.325) of the welders was higher than that of those non-exposed (0.956 ± 0.616) ($P < 0.01$). There was no significant difference between mitotic index rates of smoking and non-smoking subjects. In contrast, there were significant differences between non-exposed subjects and welders for both smoking and non-smoking ($P < 0.05$). It was found that as the time of exposure to welding fumes increased so did the mitotic index rates ($P < 0.05$). Both a positive correlation in welders and a negative correlation in non-exposed subjects were observed between mitotic index frequency and age. In the light of the results obtained, it is advised that welders who work with gas metal arc welding be instructed and necessary protective measures be taken because of the risk caused by metal arc welding fumes.

Key words: Gas metal arc welding, mitotic index, welder

Gazaltı kaynağı gazlarına maruz kalan kaynakçıların periferik lenfositlerinde mitotik indeksin belirlenmesi

Özet: Gazaltı kaynağı ucuz ve kaliteli kaynak yapabilmeyi sağlaması nedeniyle birçok endüstriyel alanda yaygın bir şekilde kullanılan metotlardan biridir. Bu çalışmada, 23 kaynakçı ve 25 kontrol deneğin periferik kan lenfositleri sitotoksiste için izlendi. Deneklerin yaşları, sigara ve alkol alışkanlıkları, gazaltı kaynağına maruz kalma süreleri ve ilaç kullanımları ile ilgili bilgiler kaydedildi. Araştırma sonuçlarına göre kaynakçıların mitotik indeks oranı ($2,278 \pm 1,325$) maruz kalmayan deneklerin oranına ($0,956 \pm 0,616$) göre oldukça yüksek bulundu ($P < 0,01$). Sigara içen ve içmeyen kişilerin mitotik indeks oranları arasında istatistiki olarak önemli bir fark bulunmadı. Aksine kaynağına maruz kalmayan denekler ve kaynakçılar arasında hem sigara içenler hem de içmeyenler açısından istatistiki olarak önemli farklılık tespit edildi ($P < 0,05$). Gazaltı kaynağı gazlarına maruz kalma süresinin artmasıyla mitotik indeks oranlarının arttığı belirlendi ($P < 0,05$). Mitotik indeks sıklığı ve yaş arasında kaynakçılarda pozitif korelasyon, maruz kalmayan deneklerde ise bu iki değer açısından negatif korelasyon gözlemlendi. Elde edilen sonuçlara göre gazaltı kaynağı gazlarının oluşturduğu risk sebebiyle bu işle uğraşan kaynakçılar bilgilendirilmeli ve koruyucu önlemler alınmalıdır.

Anahtar sözcükler: Gazaltı kaynağı, mitotik indeks, kaynakçı

Introduction

The International Agency for Research on Cancer (IARC) classified welding fumes as possibly carcinogenic to humans (group 2B) (1). Welding processes produce gases and aerosols that are composed of a complex mixture of metal oxides (2). Welders are exposed to different gases and particles generally smaller than 1 µm and which are thus respirable, depending on the welded material and the welding process used. The biological effects associated with welding fumes exposure are diverse and depend upon metal interactions and speciation. Respiratory effects observed in full-time welders have included pneumoconiosis (3), bronchitis and airway irritation (4), fibrosis (5,6), a possible increase in the incidence of lung cancer (1), metal fume fever (7), and nasal septum perforation (8).

Gas metal arc welding generates fumes that contain oxides of chromium (Cr) and nickel (Ni), together with a number of other metal oxides. The cytotoxic effects of Cr, Ni, and their compounds have been tested in different populations exposed to Cr and Ni compounds. In the literature, there are a lot of positive and negative toxic results for Cr (9,10) and Ni (11,12). Cr and Ni compounds are designated as a confirmed human carcinogen by IARC (1). However, the literature data indicated that only hexavalent-chromium (Cr^{VI}), formed in the welding process, may pose a carcinogenic risk, and only when inhaled at very high doses as indicated by IARC (1).

The mitotic index (MI) or the percentage of metaphases among harvested, fixed lymphocytes, is a cytogenetic test that is used both in vivo and in vitro. This assay requires the addition of colchicine or colcemid to arrest the progression of cells from metaphase to anaphase ensuring sufficient number of metaphases for cytogenetic analysis. The MI was used to characterize proliferating cells and identify compounds that inhibit or induce mitotic progression (13). The MI depends on 2 factors: first the proportion of the cell population that participates in the whole cycle of interphase leading to division, and second the relative lengths of interphase and recognizable mitotic stages (14).

We report a study of welders, evaluating rates of MI in their peripheral blood lymphocytes, compared to a non-exposed group.

Materials and methods

Subjects

The study involved 48 male subjects divided into 2 groups (Table 1). The first group consisted of 23 welders employed in welding in Yozgat, Turkey. The welders had varying durations of exposure (1-20 years) and they were in the age group 16-42 years. All the welders were engaged in shielded manual metal arc welding. Welders were working with consumable stainless steel electrodes usually containing ~20% Cr with 10% Ni. The second group, comprising 25 subjects, was the non-exposed group. It was selected from the general population with no history of

Table 1. Questions of the questionnaire and general information of non-exposed subjects and welders.

	Non-exposed subjects (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 time (years ± SD)	-	8.39 ± 6.86
Alcohol consumption	-	-
Medicine usage	-	-

occupational exposure to welding fumes or any known physical or chemical agent in the workplace, but belonged to the same socio-economic status as the welders. They were in the age group 14-60 years. The selection criteria for the subjects were based on a questionnaire. The questionnaire was intended to elicit information on the subjects' age, smoking habits, alcohol consumption, duration of exposure, and medicine usage. We ensured that the welders and the non-exposed subjects did not markedly differ from each other except for occupational exposure. We also ensured that all the subjects had not been taking any medicines nor had they been exposed to any kind of radiation for 12 months before sampling. The subjects had not been drinking alcohol. The subjects who smoked >5 cigarettes/day at least for 1 year were considered smokers in both groups. All subjects were informed of the objective of the study and gave their consent. The institutional ethical committee approved the research procedures used in this study.

Chemicals

Peripheral blood (PB) karyotyping medium (Biological Industries, Israel), colcemid (Sigma, Germany) and Giemsa stain (Merck, Germany) were used in peripheral blood cultures. PB karyotyping medium is based on RPMI-1640 basal medium supplemented with L-glutamine, fetal bovine serum, antibiotics (gentamicin) and phytohemagglutinin.

In vitro mitotic index assay

The heparinized blood samples (0.4 mL), obtained from the subjects, were placed in sterile culture tubes containing 5 mL of PB karyotyping 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. After 70 h of incubation, 0.1 mL of colcemid solution (10 µg/mL) was added to each culture tube and mixed by shaking gently. After 72 h of incubation, 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 (0.075M 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: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. Then 3 or 4 drops of the cell suspension were dropped on to a cold wet glass slide. Slides were air dried and were stained with 5% Giemsa. MI was calculated as the proportion of metaphase for 1000 cells for each donor and concentration.

Statistical analysis

The subjects were coded at the time of preparation and scoring. Mean and standard deviation (SD) were calculated for each subject. The significance ($P < 0.01$) of the differences between non-exposed and welder end-point means was analyzed using Student's t-test. All calculations were performed using the computer software program SPSS 10.0. Mean values and standard deviations were computed for the scores and the statistical significance ($P < 0.05$) of effects (exposure, smoking, and age) was determined using analysis of variance (ANOVA). Differences between effects were determined by the Tukey-Kramer test.

Results and discussion

Welding fumes are a complex mixture of potentially toxic fume and noxious gases. Welders were investigated for in vitro cytotoxic effects in the current study using the MI assay in peripheral blood lymphocytes. In literature, there are a lot of in vitro studies regarding peripheral lymphocytes of welders. Knudsen et al. (15) reported the measurements of chromosomal aberrations (CA) and sister-chromatid exchanges (SCE) in peripheral lymphocytes and showed a higher frequency of CA. It was reported the cytogenetic and chromosomal damages in lymphocytes of welders related to manual metal arc welding fumes (16,17). Iarmarcovai et al. (18) suggested that the combined analysis of genetic polymorphisms and centromeres in micronucleus may improve the sensitivity of the MN assay in detecting genotoxic effects. The current study has added to the in vitro additional results regarding peripheral lymphocytes of welders.

The frequency of MI was studied in 23 welders and in 25 non-exposed subjects. Welders revealed a significant induction of MI when compared with non-exposed subjects ($P < 0.01$). MI rate ($2.278 \pm$

1.325) of the welders was higher than that of non-exposed subjects (0.956 ± 0.616) (Table 2). It may be thought that the increasing MI rate is a risk for carcinogenicity in welders. The Cr and Ni from welding fumes may be considered as leading to carcinogenicity for the welding fumes and gases have been classified by IARC as possibly carcinogenic to humans (1) and the carcinogenicity of Cr has been well established through studies of Cr induced lung cancer (1,19). The literature data indicated that only Cr^{VI}, which is formed in the welding process, may pose a carcinogenic risk, and only when inhaled at very high doses as indicated by IARC (1). However, it is not possible to measure the concentration of Cr^{VI} in biological material because its oxidizing properties mean that it readily reacts with a number of substances present in the human body. In this situation the observation that only Cr^{VI} is able to pass cell membranes is of great value. In this manner chromate ions also enter erythrocytes, where they are reduced and bound to constituents of the cell. In contrast, trivalent chromium ions do not succeed in passing cell membranes (20). These differences between tri and hexavalent Cr ions offer the possibility of obtaining a specific measure of internal chromate exposure by determining the Cr concentration in blood (21,22). This property of chromate ions is especially valuable for the biological monitoring of exposed workers.

The dispersion graphic of MI values of non-exposed subjects and welders is given in the Figure. MI values of non-exposed subjects were between 0.2 and 2.5. However, MI values of welders had a wider spectrum (0.1-4.2) than did those of the non-exposed subjects. The most MI values for non-exposed were 2.5, 2.0, and 1.9. These values were obtained from smokers. The highest MI values for welders were 4.2,

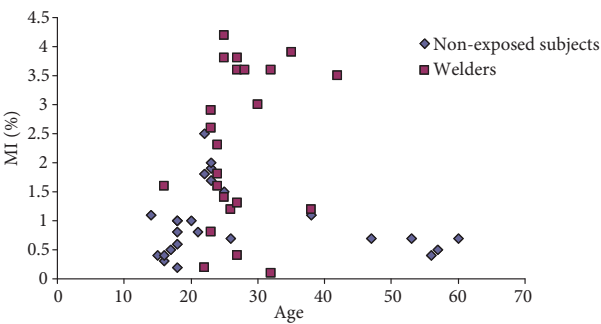


Figure. The dispersion graphic of mitotic index values of non-exposed subjects and welders.

The MI values of 11 welders (47.82%) are higher than the highest MI value (2.5) of non-exposed subjects. In spite of 17 non-exposed subjects (68%), the MI values of only 4 welders (17.39%) are between 0 and 1.

3.9 and 3.8. These values were obtained from people both smoking and exposed to welding fumes for a long time (≥ 8). According to the Figure, MI rates were increased by smoking and exposure to fumes. It was reported that there was a positive correlation between MI and smoking (23).

Table 3 shows MI frequency with respect to smoking habit, work duration, and age in non-exposed subjects and welders. There was not a significant difference between smokers and non-smokers. However, there were significant differences between non-exposed subjects and welders with both a smoking and non-smoking history ($P < 0.05$). However, the highest MI values were detected among smokers; these values were not statistically significant. When the MI frequencies in lymphocyte cultures of the welders were analyzed, a significant difference was found for time exposed to welding fumes ($P < 0.05$). MI rate (2.792 ± 1.239) of 13 welders (years of exposure ≥ 8) was higher than MI rate (1.610 ± 1.110)

Table 2. Inter-group comparison of mean mitotic index in non-exposed subjects and welders.

	Total counted cells	Total number: dividing cells	Mean MI $\pm$ SD (%)
Non-exposed subjects	25,000	239	0.956 ± 0.616
Welders	23,000	524	2.278 ± 1.325 *
All subjects	48,000	763	1.589 ± 1.209

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

Table 3. Mitotic index frequency with respect to smoking habit, work duration and age in non-exposed subjects and welders.

Parameter	Non-exposed subjects (n = 25) Mean MI $\pm$ SD	Welders (n = 23) Mean MI $\pm$ SD
Smoking		
Yes	1.062 $\pm$ 0.677 (16) ^a	2.171 $\pm$ 1.442 (14) ^b
No	0.766 $\pm$ 0.466 (9) ^a	2.444 $\pm$ 1.183 (9) ^b
Years of exposure		
≥ 8		2.792 $\pm$ 1.239 (13) ^a
<8		1.610 $\pm$ 1.110 (10) ^b
Age (years)		
≥ 30	0.616 $\pm$ 0.285 (6) ^a	2.550 $\pm$ 1.539 (6) ^b
<30	1.063 $\pm$ 0.658 (19) ^a	2.182 $\pm$ 1.280 (17) ^b

^{a,b} ANOVA: P < 0.05

F values = 7.114

of the other 10 welders (years of exposure <8). The MI frequency was not statistically affecting with the age for both non-exposed subjects and welders (P > 0.05). A negative correlation for the non-exposed subjects was observed between MI frequency and age; the higher the MI frequency was detected, the lower the age. On the other hand, there was a positive correlation between MI frequency and age among welders. Although MI frequency decreased with age, it increased with welding fumes. It was reported that a rising mitotic index will cause a more rapid decrease with increasing age, and the opposite will occur with a falling index (14).

In conclusion, the welders showed significantly higher levels of MI compared to the non-exposed subjects. The results of the present study are fundamental and provide direction for future investigations. According to both the present report and previous ones, metal arc welding fumes are a

potential risk for welders. Welders using gas metal arc welding in occupational settings are exposed to certain toxic agents. These welders may not be aware that they have been exposed to toxic agents nor may they know the type and amount of agent to which they have been exposed. Therefore, there is a need to educate those who work with gas metal arc welding about the potential hazard of occupational exposure and the importance of using protective measures.

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