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Effects of X-rays and cigarette smoking on leukocyte, lymphocyte and mitotic index values and SCE rates: the relationship between mitotic index and lymphocyte count

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Many previous studies revealed that smoking increases leukocyte and lymphocyte counts while exposure to X-rays decreases these counts. However the relationships between lymphocyte life span and smoking as well as X-rays were not well documented. The primary aim of this study was to determine relationships between smoking X-rays (in combination and individually) and life span of lymphocytes. Blood samples from 200 healthy individuals, half of which were X-ray exposed individuals, were collected. Half of X-ray exposed and of non-X-ray exposed individuals were smokers. There were equal numbers of male and female participants. Two lymphocyte cultures, one for the sister chromatid exchange (SCE) analysis and the other for the determination of mitotic index values were prepared using one part of the blood samples collected from the individuals. From the other part of the blood sample leukocyte and lymphocyte counts were determined with a haemogram device. Evaluation of the findings suggested that leukocyte count, lymphocyte count, mitotic index were relatively lower for the X-ray exposed individuals. In addition these values were higher for smokers than nonsmokers in general. The highest SCE rates were recorded for smoking radiology technicians. The most important finding is that lymphocyte life span is relatively low in smokers and in X-ray exposed males. *Toxicology and Industrial Health* 2003; **19:** 81–91.

Key words: *leukocyte; lymphocyte life span; mitotic index; SCE; X-rays*

Introduction

It has been demonstrated in various studies that smoking increases the risk of primarily lung cancer and larynx cancer, heart and vascular diseases and respiratory system infectious diseases. Four thousand different chemical agents enter the body by means of cigarette tar. These agents deform the structure of various molecules and cause disruptions in biochemical mechanisms. Of these agents benzopryn, 2-toludyn, 2-naphtylamine, acrilonitryl, 4-aminobiophenil, benzene, hydrazine, arsenic, chromium, and cadmium are carcinogenic (Sorsa *et al*., 1984; Report of the Surgeon General, 1992a,b). Since some of the others are toxic for the body, they should be neutralized by our immune system. In this case, as the task of our immune system cells, namely lymphocytes, will be greater, more cells will be needed (Anderson *et al*., 1988; Heimdal *et al*., 2000). Considering the fact

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that smoking shortens human life span, we expect that lymphocytes will be degenerated and short-lived due to the increased workload of immune system cells because of smoking.

Many of the previous studies indicate that production of lymphocyte in the lymphoid tissue increases when the need for leukocytes and lymphocytes increases due to the toxic substances in the body. For example, Schwartz and Weiss (1991), after analyzing blood samples from 8635 donors, Aghaji *et al*. (1990), after comparing the leukocyte counts of 176 nonsmoker with that of 176 smokers, and Ogova *et al*. (1998), using blood samples of 1384 factory workers, reported that leukocyte counts were higher for smokers than for nonsmokers. Hansen *et al*. (1990) found that the leukocyte count was higher in smokers and ex-smokers who stopped smoking less than one year before than in ex-smokers who stopped more than one year before and those who never smoked. Similarly, Freedman *et al*. (1996), after collecting blood samples from 3467 men aged 31 to 45 years, reported a higher leukocyte count among current cigarette smokers than among men who never smoked. Boscarino and Chang (1999) investigated the values of the lymphocytes and leukocytes in soldiers and found that the number of leukocytes and T-cells were higher in smokers than nonsmokers.

Several sources of rays are increasingly being used for diagnostic and therapeutic purposes. The most commonly used ray type in radiology units of hospitals are X-rays. Because X-rays are ionizing rays, they spoil the structure of vitally important molecules such as DNA, RNA, proteins and enzymes or hamper the operation of biochemical activities (Rowley *et al*., 1999). From this standpoint, many studies have clearly shown the fact that radiation exhibits an effect that is mutagenic and limits DNA synthesis. Radiation causes a delay in the life cycle of the cell; in G1, S and G2 phases of the interphase. The tumor suppressor gene P53 arranges the blockage of G1. This event develops, in connection with the increase of P53 expression, as the inhibition of the CDK and the inhibition of the WAF1/Cip 1 transformation. The delay in phase S is thought to be taking place through sensitive and radiation resistant components being affected. Currently, the mechanism has not yet been elucidated. The delay of G2, on the other hand, is caused by the decrease in the expression of the gene that codes the protein cyclin B1 and p34cd2, which arranges the transition from G2 to mitosis (Bernhard *et al*., 1995). With cellular level studies, the effect of radiation causing changes in stem cells and retarding reproduction has been demonstrated (Yi *et al*., 1993; Gupta *et al*., 1996). Ionizing radiation causes cell death in two different ways; mitotic or clonogenic death and death through apoptosis (Ross, 1999). In studies carried out on victims of the nuclear disaster in two cities in Japan during the Second World War and those affected by the leakage from the Chernobyl nuclear plant, long-term and permanent anemia and leucopenia have been determined, as a result of a serious decrease in the multipotent stem-cells which are in charge of forming blood cells (Suvorova *et al*., 1991; Taradii *et al*., 1997). Based on the knowledge that radiation has a restrictive effect on cell mitosis, a certain dose of radiation (Sezium 137 isotope 2500–3000 cGy) is, in a controlled manner, applied to the blood samples prior to blood transfusion and reproduction of lymphocytes in the blood through mitosis is prevented (in order to prevent 'Graft Versus Host' disease) (Bayık, 2000).

Radiology technicians are exposed to various adverse effects of radiation on a daily basis as a consequence of their professional life. Because of these risks, governments have provided those working in radiology units with certain compensations in work conditions with legal guaranties (e.g., shorter work hours, longer leaves, special clothes, and special food expenses). Nevertheless, this does not change the fact that these people are under serious risk due to probable insensitivity in the implementation of those measures and the obligation for them to stay in this field for a considerably long period of time. The fact that nearly half of the radiology technicians are smokers increases these risks.

In previous studies, it has been demonstrated that the immune system produces leukocytes and lymphocytes in greater quantities in order to offset the effect of toxic materials that enter the body through cigarette tar. More leukocytes and lymphocytes mean that the mechanism of mitosis in forming these cells works faster. It is understood from the studies mentioned above that radiation

stops the mitosis mechanism at various phases and causes cell death. In this study, the effects of smoking, which quickens the mitosis mechanism, and of X-rays, which slow the mechanism, on leukocyte and lymphocyte counts and number of lymphocytes entering mitosis (mitotic index) were determined. Mitotic index provide indirect information on the rate of proliferation of lymphocyte (e.g., Winger *et al*., 1977; Sivikova and Dianovsky, 2000). In the present study we predicted that leukocyte and lymphocyte counts and mitotic indices should be higher for smokers than nonsmokers and they should be lower for X-ray exposed individuals than non-X-ray exposed individuals. In addition we predicted that lymphocyte life span should be lower for smokers than nonsmokers and for X-ray exposed individuals than non-X-ray exposed individuals. In order to determine lymphocyte life span, slopes of regression lines for predicting lymphocyte counts on the basis of mitotic index values were compared between smokers and nonsmokers as well as X-ray exposed and non-X-ray exposed individuals. We reasoned that if X-ray exposure and smoking shortens lymphocyte life span, amount of increase in number of lymphocytes for any given increment in mitotic index should be lower for smokers and X-ray exposed groups than nonsmokers and non-X-ray exposed individuals. Finally possible genotoxic effects of both smoking and X-ray, individually and combined, were investigated on the basis of sister chromatid exchange (SCE) ratios.

Materials and methods

Subjects

The sample comprised from 200 healthy individuals who did not have any history of alcohol or narcotic substance abuse, and reported to have no sign of viral or bacterial infection prior to the present study. None of the participants were under medication. Participants were chosen from individuals with a body mass index (BMI) between 18 and 30. Participants were also asked whether they had been receiving radiotherapy prior to the study. None of the participants reported to receive radiotherapy. All 200 individuals were living in Mersin and Adana, Turkey. Non-X-ray exposed nonsmokers were chosen among the individuals who reported to be nonsmokers prior to the study. Non X-ray exposed smokers were chosen among the individuals who reported to smoke approximately one packet of cigarettes per day (15–25 cigarettes) for at least 5 years.

X-ray exposed individuals (X-ray exposed smokers and non-X-ray exposed nonsmokers) were selected among those who had been working as radiology technicians for at least five years in the radiology units of any one of the 11 hospitals in Adana or Mersin, Turkey. The largest radiology unit contained seven X-ray machines and the smallest unit contained two X-ray machines. The average number of film badges was 43 (min 34, max 47) per X-ray machine per day. The amount of X-ray applied to a patient varied between 20 and 60 millirem (40 on average) per film badge. The average amount of X-ray applied to patients per day from an X-ray machine was 1.5 rem. Standards and calibration of the radiology units and X-ray machines were in accordance with the standards of the International Atomic Energy Agency (IAEA). The working conditions and hours of the radiology technicians were also in accordance with the standards of the IAEA (extra support in clothing, diet, longer breaks and working duration per day, etc.). The radiology units have been inspected regularly by the Turkish Atomic Energy Intuition and the Turkish Ministry of Health.

There were an equal number of female and male participants in each of the four groups (1. non-X-ray exposed nonsmokers: 25 males, 25 females; 2. non-X-ray exposed smokers: 25 males, 25 females; 3. X-ray exposed nonsmokers: 25 males, 25 females; 4. X-ray exposed smokers: 25 males, 25 males). In addition the age range was between 23 and 37 for each of the four groups (the mean age was 27.8 for the non-X-ray exposed nonsmokers; 27.9 for the non-X-ray exposed smokers; 31 for the X-ray exposed nonsmokers; and 32.1 for the X-ray exposed smokers). All blood samples were collected between March and February of 2001 in order to control any variations in blood parameters due to seasonal changes. In order to control the variations due to circadian rhythms of the participants the blood samples were collected between 10 and 12 a.m.

Preparation of blood samples

A 3 mL heparinized peripheral blood sample was obtained from each of the 200 donors and each sample was given a protocol number so that the researchers analyzing the samples were not aware of the identities of the samples. Blood sample from each donor was divided into two parts. Using 0.3 mL of the blood sample from each donor, a lymphocyte culture was prepared for each donor by adopting the chromosome analysis procedure that was very similar to the one used previously by Brown and Lawce (1997). A 0.3 mL blood sample was cultured for 72 hours at 37°C in 15 mL centrifuge tubes with 5 mL medium containing 79% RPMI 1640 (biochrom)+18% fetal bovine serum (biochrom)+3 mL phytohamegglutinin (biochrom)+1.5% L-gluthamine (biochrom)+ 0.2% penicillin+0.1% streptomycin. Phytohaemagglutinin M was used to provoke mitosis for lymphocytes. After 71 hours incubation, three drops of 0.04 μg/mL colcemid solution was added to the culture using an insulin injector in order to block mitosis of lymphocytes at metaphase. After 72 hours of the incubation, the cells were then treated with a hypotonic 0.075 M KCl solution for 12 min and fixed in methanol:acetic acid (3:1). The fixation was repeated five times. The fixed lymphocytes were dropped onto clean slides. Air-dried slides were then stained with 5% giemsa solution for 10 minutes. The number of lymphocytes in mitosis stages per 1000 lymphocytes was then counted at $400\times$ magnification using a light microscope. Mitotic index is normally determined by counting the number of cells found in mitosis stage per 100 cells. However, for the present research, a more sensitive criterion was adopted for the mitotic index of lymphocytes and the number of lymphocytes at mitosis stages in every 1000 lymphocytes was counted. This index, by itself, provides an indirect information for the rate of proliferation of lymphocytes (Marsh and Haeney, 1983; Winger *et al*., 1977; Darbelley *et al*., 1989; Driss-Ecole *et al*., 1994; Sivikova and Dianovsky, 2000). It was reasoned that by assessing the relationship between the mitotic index values and the lymphocyte counts for X-ray exposed groups with the degree of relationship between the mitotic index values and the lymphocyte counts for X-ray nonexposed groups, some information about the effects of X-ray and smoking onto the life span of lymphocytes could be obtained. The slopes of regression lines for predicting lymphocyte counts on the basis of mitotic index for nonexposed X-ray groups and exposed X-ray groups were compared one by one. If smoking increases the rate of proliferation of lymphocytes while it decreases life span of lymphocytes, the slope of the regression line for nonsmokers should be steeper than the slope of the regression line for smokers.

Analysis of SCE was made in a medium containing 10 mM BUdR and consisting of 16% fetal calf serum+80% medium 199+4% phytohemaglutinin, in accordance with IAEA protocol. SCE is used to determine the effect of mutagenic and clastogenic agents at chromosomal level on the wrong exchange rate of sister chromatids on chromosomes, based on the principle of DNA combination in the presence of BUdR, which is the timin nucleotide analogue. From the preparations for SCE, 50 metaphase plates for each individual were evaluated. The second culture was made for mitotic index in a medium, which does not contain BUdR, unlike the SCE culture medium. A blockage of the cells at mitosis stage was obtained via application of colchicine to the cultures for an hour and a half before the preparation time (Brown and Lawce, 1997). Preparations for each individual were scanned with the research microscope under $40\times10=400$ magnification, 1000 cells were counted and the ones in the mitosis phase were determined. This value was converted to a percentage in order to form the mitotic index. The mitotic index value was interpreted as the speed of cells entering mitosis. The second section of the blood sample was evaluated in the haemogram device type CELLOYN 3500 and lymphocytes per unit volume were determined. As mitotic index shows the speed of cells entering mitosis and lymphocyte count shows the current situation, these two values, evaluated together, were used in the calculation of lymphocyte life span.

The results from each of the four measures of blood cells were then analysed using the appropriate statistical techniques. First a three-way analysis of variance was conducted separately for each of the four measures of blood cells in order to explore the effect of gender and of group

(nonsmokers, smokers, nonsmokers radiological technicians, smokers radiological technicians). In addition Pearson's correlation coefficient between the mitotic index values and the number of lymphocytes per unit volume is calculated for each of the eight subgroups (1. non-X-ray exposed non-smoker males, 2. non-X-ray exposed non-smoker females, 3. non-X-ray exposed smoker males, 4. non-X-ray exposed smoker females, 5. X-ray exposed non-smoker males, 6. X-ray exposed non-smoker females, 7. X-ray exposed smoker males, 8. X-ray exposed smoker females). Furthermore, we predicted that since smoking and X-rays should shorten the life span of lymphocytes, the value of the increase in the number of lymphocytes for any given increment in mitotic index values should be lower for the groups exposed to smoking and X-rays than for the groups which were neither smokers nor X-ray exposed. In order to explore this prediction we first draw regression lines for predicting lymphocyte counts on the basis of mitotic index values for each of the eight subgroups. Regression slopes (nonstandardized regression coefficients) were then compared using *t*-tests (Zar, 1999) for the various subgroups.

Findings

The means of leukocyte count, lymphocyte count, lymphocyte/leukocyte ratio, mitotic index and of SCE ratio for each subgroup are shown in Table 1. As can be seen in Table 1, leukocyte count, lymphocyte count, mitotic index and SCE ratio were generally higher for smokers than nonsmokers and all these parameters except SCE ratio were lower for the radiology technicians than the non-X-ray exposed individuals. In order to confirm these observations a three-way ANOVA with the factors of X-ray, smoking and gender was conducted separately for leukocyte count, lymphocyte count, mitotic index, SCE ratio and lymphocyte/leukocyte ratio. These analyses revealed that leukocyte count, lymphocyte count, mitotic index and SCE ratio were significantly higher for smokers than nonsmokers, $Fs(1, 192) > 4.4$, $Ps < 0.05$. The analysis also revealed significant effects of X-ray for each of the parameters apart from lymphocyte/leukocyte ratio, leukocyte count, lymphocyte count and mitotic index are found to be significantly lower in the radiology technicians than in non-X-ray exposed individuals, $Fs(1, 192) > 6.34$, $Ps < 0.05$, but SCE ratios were significantly higher for the radiology technicians than non-X-ray exposed individuals, $F(1, 192) = 17.389$, $Ps < 0.05$. The main effects of gender were only significant for the lymphocyte/leukocyte ratio, $F(1, 192) = 4.00$, $P = 0.05$. In addition smoking $\times$ gender interaction for the lymphocyte/leukocyte ratio, $F(1, 192) = 5.5$, $P < 0.05$ and X-ray $\times$ gender interaction for the leukocyte count, $F(1, 192) = 4.5$, $P < 0.05$ were significant. The significant interactions were further explored using a least significant difference (LSD) test (Zar, 1999). Exploration of smoking $\times$ gender interactions for the lymphocyte/leukocyte ratio revealed that the ratio was significantly higher for the non-smoking males than the non-smoking females and that none of the remaining comparisons for this ratio was significant. Exploration of X-ray $\times$ gender interaction for the leukocyte count revealed that leukocyte count was significantly lower for X-ray exposed males than non-X-ray exposed males. None of the remaining three comparisons involving this interaction was significant.

Table 1 also shows correlation coefficients for each of the subgroups. There was a strong correlation between the mitotic index values and lymphocyte counts for each groups ($P < 0.001$). This relationship was explored in more detail through regression analysis. For this purpose, against one unit increase in the mitotic index value the increase in lymphocyte count for each subgroup was determined and these non-standardized regression coefficients (B) are shown in Table 1 and in the form of regression curves in Figure 1. The angle difference between any of the two regression curves is evidence for the difference in lymphocyte life span. In order to find out whether the angle difference between two regression curves was statistically reliable, separate *t*-tests were conducted to compare various subgroups. The outcome of these analyses indicated that lymphocyte life span was significantly shorter for the non-X-ray exposed smoking males and for the X-ray exposed smoking males than for the non-X-ray exposed non-smoking males and X-ray exposed non-smoking males, respectively, $ts(48) > 2.03$. Contrary to the results from the X-ray exposed males, life span of

Table 1. Means (X), standard deviations (SD) and coefficients for leukocytes counts, mitotic index values, lymphocytes counts and lymphocyte/leukocyte ratio of, non-X-ray exposed groups (nonsmokers, smokers), X-ray exposed groups (nonsmokers and smokers). The lower two rows show Pearson's correlation coefficients between mitotic index and lymphocytes count and regression coefficients for the regression lines predicting lymphocyte count on the basis of mitotic index

	Non-X-ray exposed groups				X-ray exposed groups			
	Nonsmokers		Smokers		Nonsmokers		Smokers	
	Male (I)	Female (II)	Male (III)	Female (IV)	Male (V)	Female (VI)	Male (VII)	Female (VIII)
Leukocyte counts (/mm^3) X ± SD	7328 ± 360	7434 ± 401	8617 ± 473	7722 ± 396	5865 ± 299	6764 ± 309	6522 ± 328	7147 ± 449
	(a) 7381 ± 267		(b) 8169 ± 311		(c) 6314 ± 274		(d) 6834 ± 286	
Lymphocyte counts (/mm^3) X ± SD	2341 ± 123	2044 ± 127	2578 ± 124	2489 ± 155	1901 ± 90	1884 ± 107	2013 ± 80	2146 ± 137
	(a) 2193 ± 91		(b) 2533 ± 99		(c) 1892 ± 82		(d) 2079 ± 88	
Mitotic index (%) X ± SD	2.23 ± 0.10	1.93 ± 0.12	2.70 ± 0.13	2.59 ± 0.15	2 ± 0.10	1.82 ± 0.12	2.4 ± 0.12	2.44 ± 0.11
	(a) 2.08 ± 0.8		(b) 2.64 ± 0.10		(c) 1.91 ± 0.8		(d) 2.42 ± 0.10	
Lymphocyte/leukocyte	32.53 ± 1.34	28.08 ± 1.3	31.34 ± 1.8	33.44 ± 2.1	33.24 ± 1.5	27.7 ± 1.3	32.1 ± 1.4	30.7 ± 1.3
Correlation between mitotic index and lymphocyte counts (R)	0.935***	0.971***	0.953***	0.946***	0.941***	0.894***	0.881***	0.909***
Degrees of regression coefficients B ± SD	1163 ± 94	1012 ± 49	909 ± 60	1008 ± 50	871 ± 63	917 ± 78	750 ± 78	1124 ± 107
SCE/cell X ± SD	5.95 ± 0.33	5.72 ± 0.32	6.8 ± 0.34	6.68 ± 0.36	7.44 ± 0.35	7.08 ± 0.31	7.48 ± 0.45	7.32 ± 0.35
Age X ± SD	27.7 ± 4.54	27.9 ± 4.70	27.8 ± 5.2	28.1 ± 5.09	32.4 ± 4.84	29.7 ± 4.86	31.7 ± 4.96	32.5 ± 4.78

*** $P < 0.001$.

[a] Non-X-ray exposed nonsmokers; [b] non-X-ray exposed smokers; [c] radiological technicians, nonsmokers; [d] radiological technicians, smokers.

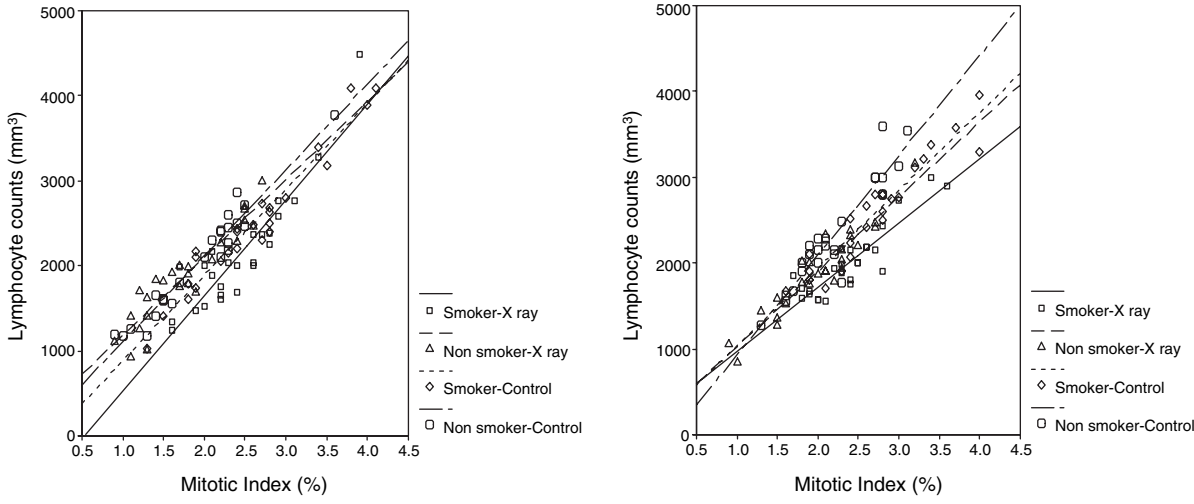

Figure 1. Regression lines between mitotic index–lymphocyte counts for the X-ray exposed smokers (smoker X-ray), the X-ray exposed non-smokers (nonsmoker X-ray), the non X-ray exposed smokers (smokers control) and the non X-ray exposed non-smokers (non-smoker control). The left-hand panel shows the data from males and the other shows the data from females.

lymphocytes for the X-ray exposed smoking females was significantly longer than that of the X-ray exposed non-smoking females, $t(48) = 2.74$. Thus, these results suggest that lymphocyte life span is shorter for smoking males than non-smoking males and that it is somehow longer for the X-ray exposed smoking females than the X-ray exposed non-smoking females. When the slopes of regression curves were compared between the X-ray exposed and non-X-ray exposed subgroups, t-tests indicated that lymphocyte life span was significantly shorter for the X-ray exposed smoking and non-smoking males than the non-X-ray exposed smoking and non-smoking males, respectively, $ts(48) > 2.01$. However, the slopes of regression lines did not differ significantly between the X-ray exposed females and the non-X-ray exposed females, $ts(48) < 1.93$.

Discussion and conclusion

In this investigation mitotic index values, leukocyte and lymphocyte counts and SCE rates were found to be higher for smokers than nonsmokers in general. For the X-ray exposed individuals, the mitotic index values, leukocyte counts, and lymphocyte counts were lower but SCE rates were higher than for the non-X-ray exposed individuals. The regression curves for the eight subgroups are presented in Figure 1. The most important findings in this study are the angle differences between the two regression curves of different groups. As we argued before, we suggested that the differences between the groups is evidence for the difference in lymphocyte life span. As Figure 1 indicates, lymphocyte life span is shorter for the smokers than the nonsmokers in general and for the X-ray exposed individuals than for the non-X-ray exposed individuals.

As far as the outcomes for mitotic index values, leukocyte and lymphocyte counts and SCE rates are concerned, our findings are consistent with previous findings: Aghaji *et al*. (1990) reported, after studying smoking and non-smoking individuals of ages ranging from 18 to 52, that leukocyte count was higher in smokers than nonsmokers. Boscarino and Chang (1999) reported that, among soldiers who had been under certain stress for 20 years, lymphocyte, leukocyte and T-lymphocyte values were higher for smokers than nonsmokers. Ghosh and Ghosh (1987) reported that while the SCE rate was 5.48 in the control group, it rose to 8.15 in cigarette smokers and to 10.12 in pipe smokers. Hansen *et al*. (1990) reported that leukocyte count in 1 mm^3 blood was 634 higher in cigarette smokers and 877 higher in pipe smokers compared to nonsmokers. Ogova *et al*. (1998), in their study on leukocyte and neutrophil values in cigarette addicted factory workers with heart disease, reported that leukocyte and lymphocyte counts were higher in smokers than nonsmokers.

Freedman *et al*. (1996) investigated leukocyte and lymphocyte counts among 3467 blacks and Caucasians and reported that average leukocyte count in smoking blacks was 15% less than that in Caucasians and the lymphocyte value was 10% lower. Pendzich *et al*. (1997) investigated the effects of smoking and environmental pollutants on seasonal SCE rates and reported that while SCE rates in nonsmokers are 6.80 in the summer and 7.18 in the winter, the rates in smokers rose to 7.10 in the summer and 7.90 in the winter. Schwartz and Weiss (1991) reported that leukocyte count was higher in smokers compared to nonsmokers, using 8635 male individuals aged 30–74.

We previously reported that combined effects of trimethoprim, cigarettes and X-rays increased the mitotic index value (Akbaş and Budak, 1996). Bogen (1993) tried to assess the effects of ionizing radiation on lymphocyte life span by taking into account its cytogenetic and cytotoxic effects and determined the life span of T-lymphocytes, normally averaging 1.5–10 years. He reported that the life span was 1.1 years (0.76–1.9) for T1 cells and 6.3 years (5.8–6.9) for T2 cells in patients with ankylosing spondylitis who received X-rays for 20 years for the treatment purpose. Rozgaj *et al*. (1999), by conducting a cytogenetic and haemotologic study on hospital employees, determined that leukocyte, lymphocyte and thrombocyte values were lower for those working at the radiology unit. Additionally, they found that the chromosomal instability rates were relatively higher in these individuals working at the radiology unit compared to the control group. Rowley *et al*. (1999) investigated the effects of ionizing radiation on the DNA synthesis in eukaryotic cells and found that ionizing rays retard mitosis through S-phase onset checkpoint affecting the S-phase damage sensing. Salone *et al*. (1996) investigated effects of low-dose radiation on cell cycle kinetics and mitotic delay, and reported that the blockage increased at the G2 and S phases of lymphocytes exposed to 2cGy dose of X-rays for 4–6 weeks. Suvorova *et al*. (1991) observed a 50% drop in lymphocyte count in Chernobyl victims who were exposed to radiation for 3–6 days and were experiencing nausea and vomiting problems. Taradii *et al*. (1997) observed a group of Chernobyl victims with low T-lymphocyte value who were affected by radiation for 3–15 years and reported a rise in lymphocyte count of the victims after rehabilitating them at altitude for 24 days.

We have previously investigated the effects of trimethoprim, cigarettes and X-rays on chromosomal instability and found that structural chromosomal instability rate, which was 1.72% in the control group, rose to 3.73% in radiology technicians (Akbaş and Budak, 1996). Bellorin and Fernandez (2002) reported that chromosomal alterations induced by acute exposure to X-rays increased linearly while irradiation doses increased. Holmberg *et al*. (1995) investigated the effects of ionizing radiation on chromosomal instability in T-lymphocytes, and concluded that 3-Gy X-rays cause chromosomal breaks which are not subclonal or clonal. Lazutka and Dedonyte (1995) observed the SCE frequency in outdoor workers and found that SCE frequency, being 7.45 in the control group, rose to 10.3 in the study group. Pleskach *et al*. (1990) investigated SCE and the inhibition of DNA synthesis and reported that X-rays retarded DNA synthesis and increased SCE rates. Sabti *et al*. (1992) investigated chromosomal damages in workers affected by occupational clastogens and found that SCE rates, being 4.87 in office workers, rose to 9.47 in reactor workers, and to 9.8 in radiology unit workers.

Our finding that smoking and leukocyte count are positively correlated was supported by literature (Aghaji *et al*., 1990; Hansen *et al*., 1990; Schwartz and Weiss, 1991; Freedman *et al*., 1996; Ogova *et al*., 1998; Boscarino and Chang, 1999). Similarly, our findings that smoking increases lymphocyte count are in agreement with previous findings (Freedman *et al*., 1996; Ogova *et al*., 1998; Boscarino and Chang, 1999). No literature has been found to compare with our finding about the mitotic index values. Nevertheless the previous findings that the lymphocyte count is higher in smokers than nonsmokers (Freedman *et al*., 1996; Ogova *et al*., 1998; Boscarino and Chang, 1999) can be perceived to indirectly support our finding that mitotic index is higher in smokers than nonsmokers. Finally our finding that smoking increases SCE rates accords with literature (Ghosh and Ghosh, 1987; Pendzich *et al*., 1997).

Similar to the results of the present study, the previous studies also revealed a lower leukocyte

count for radiology technicians (Suvorova *et al*., 1991; Taradii *et al*., 1997; Rozgaj *et al*., 1999). The present study suggests that mitotic index value, that is the proliferation rate of cells entering mitosis, decreases with the effect of X-rays and this suggestion is in agreement with previous findings: Pleskach *et al*. (1990) and Rowley *et al*. (1999) demonstrated radiation's retarding effect on DNA synthesis and (Salone *et al*., 1996) reported radiation's retarding effect on mitosis. Our finding that lymphocyte life span is shorter in male radiology technicians is supported by a previous study in spite of the use of a different method (Bogen, 1993). The present study indicates that X-rays increase SCE rates and this indication is consistent with the previous findings (Aghaji *et al*., 1990; Pleskach *et al*., 1990; Sabti *et al*., 1992; Lazutka and Dedonyte, 1995). Additionally, Akbaş and Budak (1996), Bellorin and Fernandez (2002) and Holmberg *et al*. (1995) reported that X-rays cause chromosomal aberrations and increase the instability rates and these results are indirectly in agreement with our finding that SCE rates were relatively higher in the X-ray exposed individuals. The most important conclusions in our study are those related to lymphocyte life span. We have found only a single previous study on this subject (Bogen, 1993). Although Bogen (1993) studied a different type of population and employed a different method, his conclusion was similar to ours in that smoking and X-rays shorten lymphocyte life span for males. Thus, male radiology technicians, who are under the effect of X-rays as a consequence of their professional life, can be characterized as the highest-risk group.

Finally the results imply a gender difference in terms of X-ray exposure effect. The present study revealed that leukocyte counts were lower and that lymphocyte life span was shorter for the X-ray exposed males than for females. While we had been collecting the blood samples from the laboratory technicians, we had observed the technicians do not always take films but they shift from task to task, such as developing filming and pursuing some secretarial duties. In addition we had observed that female technicians rather than male technicians are more likely to be asked to do secretarial duties. Thus, it very likely that duration of X-ray exposure is longer for the male technicians than the female technicians because of an informal discrimination between genders for the laboratory duties.

In conclusion, smoking increases the formation and the number of immune system cells. One reason for this may be the increasing workload of our immune system cells. The increase in the number of immune system cells does not mean that the immune system was strengthened but it may be a consequence of life span of lymphocytes getting shorter. X-rays demonstrate a contrasting effect with smoking and decrease the formation speed and number of immune system cells additionally shortening lymphocyte life span. Thus X-rays decrease the formation speed and number of immune system cells that are increased by smoking. Once again, this effect of X-rays does not mean the strengthening of the immune system as the shortening in lymphocyte life span seem to reach its highest level for the X-ray exposed smoking males of the present study. While lymphoid organs in charge of the formation of immune system cells in smoking radiology technicians are, on the one hand, under pressure for the preparation of mitosis to form more cells, on the other hand, they are also face to face with the effect of X-rays retarding mitosis. The bidirectional pressure on the mechanism of mitosis, which should be in prime balance as a necessity of homeostasis, can be an explanation to the well-known relationship of smoking–cancer and radiation–cancer. This also explains why smokers and those under the effect of X-rays are more susceptible to infectious diseases.

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