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DNA damage in children with scoliosis following X-ray exposure

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Aim. **It has been suggested that cancer incidence is high in subjects with scoliosis who are relatively more often exposed to X-ray for diagnosis and follow-up. X-ray is a kind of ionizing radiation and leads to formation of oxygen free radicals which are capable of damage to DNA, thus altered gen expression and mutation. p53 tumor suppressor gene plays a crucial role in the damage response. It controls the checkpoint of cell cycle and redirects the cell metabolism to either repair of damaged DNA or apoptosis as response to DNA damage. The aim of the present study was to examine serum levels of 8-Hydroxydeoxyguanosine (8-OHdG), a strongly mutagenic product of oxidative DNA damage, p53, superoxide dismutase (SOD) and glutathione peroxidase (G-Px), as antioxidant activity, in children with scoliosis who had got whole spine radiograph two times during the last year.**

Methods. **A total of 31 children with adolescent idiopathic scoliosis and age-matched 21 healthy children were included in the study. Serum levels of 8-OHdG and p53 were measured with ELISA kits. SOD and G-Px activities were determined with spectrophotometric assays.**

Results. **Serum levels of 8-OHdG and p53 were found to be higher (P<0.001 and P<0.01, respectively), SOD activity was found to be lower (P<0.001) in the children with scoliosis as compared to age-matched controls. There was no significant difference between the groups for G-Px activity.**

Conclusion. **Our data show that X-ray exposure causes increased 8-OHdG level, and decreased SOD activity, which both may reflect a tumor promoting condition. Increased p53 level may be interpreted as a compensatory effort of cell to X-ray mediated DNA damage.**

KEY WORDS: X-rays - Diagnosis - Glutathione peroxidase - 8-Hydroxydeoxyguanosine - Scoliosis - Superoxide dismutase.

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Reactive oxygen species (ROS)-mediated deoxyribonucleic acid (DNA) damage plays an important role in the initiation of carcinogenesis.[1, 2] ROS are known to interact with genomic DNA, cause the damage on specific genes which control cell growth and differentiation and stimulate growth of malignant cells.[3] ROS attack to DNA causes base oxidation. Among the oxidized bases, 8-hydroxydeoxyguanosine (8-OHdG) is the most mutagenic lesion which is strongly implicated in the initiation stage of carcinogenesis.[4-6] 8-OHdG residues on DNA are excised by constitutive enzymatic DNA re-

pair systems, appear in the blood and subsequently excreated in the urine. 8-OHdG level is commonly measured as a reliable marker of ROS-induced DNA damage.[4, 5, 7]

Ionizing radiation such as X-rays and γ-rays is a well-known carcinogen that induces oxidative DNA damage via ROS production.[8, 9] It is well established that ionizing radiation induces a wide spectrum of DNA damage lesions, including single strand breaks, oxidized bases, abasic sites.[10] Ionizing radiation can induce genomic instability in cells by enhancing the rate of mutations and other genetic alterations. Response to X-ray induced DNA damage at the cellular level requires coordination of cell-cycle checkpoint control, induction of DNA repair or apoptosis.[11] p53 tumor suppressor gene plays a crucial role in the cellular response to DNA damage. X-ray-induced DNA strand breaks represents a signal for activation of p53 gene and up-regulation of p53.[12] p53 protein acts to induce cell cycle arrest in response to DNAdamage. This p53-mediated cell cycle arrest allow the repair of damaged DNA or the elimination of highly damaged cells by apoptosis.[13] Apoptosis plays a major role in preventing the survival of genetically modified cells that may constitute a cancer risk. Another defense against ROS is provided by antioxidant system that is capable of preventing excess radical production and neutralizing ROS. Superoxide dismutase (SOD) and glutathione peroxidase (G-Px) are major antioxidant enzymes.[14, 15]

Recently, potential role of medical exposure to X-ray in carcinogenesis has received considerable interest.[16] It has been suggested that cancer incidence is high in subjects with scoliosis who are relatively more often exposed to X-ray for diagnosis and follow-up, later in their life.[17] The present study has purposed to evaluate serum levels of 8-OHdG, p53, SOD and G-Px in children with adolescent idiopathic scoliosis.

Materials and methods

A total of 31 (mean age: 14±3 years) children who diagnosed adolescent idiopathic scoliosis and followed-up in Istanbul University, Cerrahpasa Medical Faculty, Department of Orthopaedics and Traumatology were included in the study. All of the children had got PA and lateral standing whole spine radiographs two times during the last year. The dose that body is exposed in every radiographic examination for follow-up of scoliosis was 0.72±0.02 mGy. The control group was constituted by age-matched 21 healthy children (mean age:13±2 years). Children with acute and/or chronic inflammatory disease, autoimmune disorder and infectious disease; and had taken any drug, vitamin or iron supplements within the previous three months were excluded from the study. All of the control cases had two radiographs for various reasons during the last year. Approval of Cerrahpasa Medical Faculty Ethics Committee was taken in accordance with the principles of the Declaration of Helsinki and informed consent was obtained from subjects.

Five mL of venous blood samples were collected within 3h after the whole spine radiography. Following the centrifugation at 2000 X g for 10 minutes serum was removed and kept at -80 °C until the time of analysis. Serum level of 8-OHdG and p53 were measured with a competitive ELISA kit purchased from Northwest Life Science Specialties, LLC (catolog no: NWK-8OHDG02) and Bender MedSystems (catolog no: BMS256), respectively. Activity of SOD and G-Px in serum were measured by spectrophotometric kits purchased from Randox (catolog no: SD125 and RS 504, respectively). All parameters were assayed as respect with the instructions of the producing company.

Statistical analysis

Data were expressed as mean and range. Since data were not normally distributed, statistical analysis of measured parameters were performed by non-parametric Mann-Whitney U test. Differences between groups were considered significant at $P<0.05$. Spearman correlation coefficient was used for correlation analysis.

TABLE I.—*Measured parameters in the study groups.*

	Control group (N.=21)	Scoliosis group (N.=31)
Mean age (year)	13 (7-9)	14 (9-20)
8-OHdG (ng/mL)	1.01 (0.50-1.50)	2.51 (0.30-12.00)*
p53 (U/mL)	0.63 (0.53-0.93)	1.10 (0.54-11.84)**
SOD (U/mL)	3.71 (1.80-6.90)	1.62 (1.00-2.47)*
G-Px (U/L)	10.08 (5.46-12.80)	11.64 (5.46-17.36)

Statistically significant at*P<0.001 and **P<0.01 *versus* control group.

Results

Data are shown in Table I. Serum levels of p53 and 8-OHdG were found to be significantly higher (P<0.01 and P<0.001, respectively), SOD activity was found to be significantly lower (P<0.001) in the children with scoliosis as compared to age-matched controls. There was no significant difference between the groups for G-Px activity. Serum level of p53 was found to be low correlated with age (r:0.37, P<0.05) and with 8-OHdG (r:-0.31, P<0.05) in the scoliosis group. No significant correlation was determined between the variables in the control group.

Discussion

About 15% of the ionizing radiation exposure to the general population is due to medical radiation for dental and diagnostic procedures.[16] Exposure of the body to X-rays produces ROS that damage biomolecules in the body. Carcinogenic effect of X-rays, especially in the case of chronically exposure at young ages has been shown by various studies.[18-20] It has been shown that exposure of the breast to ionizing radiation increases the relative risk of breast cancer, especially for younger women.[18] Risk of breast cancer development among scoliosis patients who had frequent diagnostic X rays during late childhood and adolescence has taken considerably interest. In the 1980s, it has been suggested that the cumulative additional risk for scoliosis patients varies from 0.2% for breast carcinoma as compared with the natural incidence of cancer in the general population, and this risk is considerably smaller than had been estimated.[21] In 1989, Hoffman *et al.*,[22] reported increased breast cancer risk among the women with scoliosis who had followed more than 30 years. In their investigation, risk increased with follow-up time, with number of X-ray exposure and with the estimated radiation dose to the breast. Afterwards, an excess risk of breast cancer has been reported among scoliosis patients by Dutkovsky *et al.*[23] and Doody *et al.*[17] All of these cohort or retrospective studies indicated an increased breast cancer risk in subjects with scoliosis; and suggested that breast cancer risk does not vary appreciably by type of scoliosis or age at diagnosis but rises significantly with indicators of increasing radiation exposure, including spinal curve magnitude and total number of X-rays involving the breast. X-ray mediated 8-OHdG formation in rat mammary gland has been shown by Haegele *et al.*[24] in 1998. Later, Manda *et al.*[25] have reported augmented 8-OHdG in serum obtained from X-irradiated C57BL mice. To date, as far as we know, acute changes in oxidative DNA damage and antioxidant activity has not been determined; cellular markers children with scoliosis. In the present study we determined increased 8-OHdG level in serum after X-irradiation. Considering the fact that the only mechanism for the appearance of 8-OHdG in serum is repair of 8-OHdG residues by DNA repair systems, increased serum 8-OHdG level may reflect not only increased oxidative DNA damage but also induced DNA repair.

p53 suppresses tumorigenesis by promoting cell cycle arrest, influencing DNA repair and inducing apoptosis. More than 50% of all human tumors carry inactivating mutations in the p53 gene.[26] X-ray-induced DNA

damage may represent a signal for p53-dependent apoptosis in the majority of mammalian cells.[27] p53 protein has a short half-life under physiological conditions [28] but after the X-ray exposure, DNA strand breaks occur which in turn leads to phosphorylation of p53. The phosphorylation increases half-life of p53.[13] Therefore, after the X-ray exposure p53 level is upregulated, p53 level increases dramatically in the cells as well in peripheral blood. After the X-ray exposure, p53-dependent regulation has been found to be tissue-specific in mice.[29] Bystander effects are frequent consequences of radiation exposure. It has been reported that cranial X-ray exposure led to increased DNA damage and p53 expression and also altered levels of cellular proliferation and apoptosis in bystander spleen tissue of mouse.[30] For a whole spine radiography, it is easy to estimate that all organs in the body may be influenced and all cells exhibit response to X-ray. In the present study, high p53 level determined in the serum may indicate response to X-radiation. Negative correlation determined between p53 and 8-OHdG may bring to mind that p53 activation had to directed the cell to apoptosis rather than DNA repair due to extensive damage. However correlation coefficient is weak (r:-0.31, P<0.05), and as a limitation of this study, no apoptosis parameter was determined.

The susceptibility to X-ray mediated oxidative damage depends on cellular antioxidant defence. X-rays can produce ROS such as superoxide anion radical, hydrogen peroxide and hydroxyl radical due to decomposition of cellular water. SOD and G-Px are enzymatic antioxidants which catalyze the inactivation of superoxide anion radical and hydrogen peroxide, respectively. Protection from X-ray mediated DNA damage by G-Px has been shown by Baliga *et al*.[31] They have determined that when transgenic mice that express reduced levels of glutathione peroxidase-1 were exposed to X-rays, DNA damage was higher compared to the same cells obtained from irradiated wild-type controls. The first data for G-Px activity in patients with scoliosis were reported by Dastych *et al*.[32] They determined that no significant difference is present between patients with idiopathic scoliosis and controls for erythrocyte G-Px activity. After that, no study was published about this subject so far. Furthermore, as far as we know, SOD activity in patients with scoliosis has not been investigated. In the present study, decreased SOD activity may result from exhaustion of SOD due to increased ROS production. Alternatively, considering the fact that oxidative stress leads to damage to antioxidant enzymes.[33, 34] increased ROS production during the repeated X-ray exposure may lead to down regulation of SOD gene. Since product of the reaction catalyzed by SOD is the substrate for G-Px reaction, low G-Px activity may be expected when SOD activity is low. However, antioxidant activity is complex and multifactorial, and that they may not exhibit harmonious alteration. As a matter of fact, G-Px activity in children with scoliosis was found to be similar to those in healthy children in the present study.

Taken together, as a preliminary study, our data is the first evidence for the acute increase in serum 8-OHdG and p53 levels and decrease in SOD activity immediately after whole spine radiograph in children with scoliosis. Children with scoliosis receive inevitable repeated X-ray examinations until maturity. This may be a risk for cancer development in future. Although the spine is the prime target for exposure in scoliosis radiography, several radiosensitive organs are also exposed during the procedure. Optimal positioning, dose adjustments and protection of breasts and gonads with lead filtration systems are useful to decrease the radiation risks in these patients.

As limitation; if 8-OHdG, p53, SOD and G-Px levels before and after the radiography had been assessed, and time dependent changes in the levels of measured parameters had been evaluated, more reliable data would have obtained. In future studies, in order to clarify the carcinogenic effect of repeated X-ray exposure in children with scoliosis, frequency 8-OHdG adducts in DNA of leukocyte should be examined at least six months after the radiography.

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