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The Role of Glutathione in Determining Chromosome Radiosensitivity in Lung and Prostate Cancer Patients

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ABSTRACT

One of the major problems in the radiation treatment of cancer is that they have different sensitivities to radiation. Although these different sensitivities originate from many factors such as cell repair ability, repair rate, and increased damage tolerance, the initial damage levels caused by radiation are important in human tumor cells. There is a role of non-protein thiols in the observation of the first damage levels in the DNA molecule. In particular, glutathione (GSH) can reduce the radiosensitivity of cells by reducing the amount of free radicals generated by radiation due to its effect on free radical detoxification caused by radiation.

In this study, chromosome radiosensitivity determined by micronucleus formation in peripheral blood lymphocytes of patients who were diagnosed with lung and prostate cancer and who had not started radiation therapy and GSH levels and were measured and the role of GSH in radiosensitivity was studied.

MN formation frequencies measured as a result of irradiation of patients with lung cancer were not different from the MN frequencies of prostate cancer patients. This shows that lung and prostate patients have a similar radiosensitivity spectrum. There was no significant difference in GSH levels between the two groups in the patients, although decreasing GSH levels with age in prostate cancer group and increasing GSH levels with age in the lung cancer group was observed. When the data of both groups were combined, no correlation was observed between the micronucleus frequencies and GSH levels measured in lymphocytes of patients (r=-0.34). The GSH molecule did not have a role in determining the radiosensitivity of cells.

Key Words: *Micronucleus, GSH, Radiosensitivity, Lymphocytes, Lung cancer, Prostate cancer.*

1. INTRODUCTION

The factors affecting the prognosis in the radiation treatment of the tumor are histology of tumor, size, growth rate and tumor sensitivity to radiation [1-3]. Radiosensitivity differences of tumors are due to different DNA damages or different repair of these damages. Many biological markers identifying DNA damage have been shown. These include DNA strand breaks, chromosome aberrations, micronucleus incidence, aneuploidy, shortening of telomeres, DNA oxidation, DNA methylation, nuclear p53 levels, point mutations, mitochondrial point mutations [4,5]. Studies on human peripheral blood lymphocytes have shown the predictive nature of chromosome aberrations and micrunucleus formation in terms of cancer risk [6,7,8].

Free radicals occur in radiation therapy of tumors, damage many biological materials such as protein, lipid, DNA and nucleotide coenzymes [9]. In particular, damage to the DNA molecule causes the death of cells if they are not repaired. The glutathione

(GSH) molecule is an important element in antioxidant mechanisms that prevent damage caused by free radicals. Glutathione serves as the substrate of many enzymes, such as peroxides, which inhibit or reduce the destructive effects of free radicals. [9,10,11]. It is a tripeptide containing l-cysteine, L-glutamic acid and glycine amino acids. Glutathione, a water-soluble thiol and found in very high concentrations in many cells, protects biological membranes against lipid peroxidation [10,11].

The total glutathione redox cycle has a protective effect against the cellular degradation of the radiation-induced reactant oxygen species and their endogenous products to sustain the structural and functional integrity of the cell. GSH is able to detoxify the main source of damage in the irradiated biological systems by saturating the highly reactive radical $OH^{\cdot}$. In addition, GSH can saturate carbon radicals to repair excess radiation damage on nucleic acids and can prevent free radical attacks elsewhere. In studies with low and high GSH-containing cells, GSH has been shown to protect cells against radiation [12,13], although the literature on this subject is not sufficient. Therefore, in this study, the relationship between radiosensitivity which is one of the factors affecting the prognosis in the treatment of cancer patients and GSH which may have a role in determining this radio sensitivity was studied.

2. MATERIALS AND METHODS

In this study, peripheral blood from 22 patients who were diagnosed with lung and prostate cancer and who had not started radiation therapy were used. This work was approved by the ethical committee of the Istanbul Medical Faculty of the Istanbul University. Volunteers signed informed consent documents. Chromosome radiosensitivity was measured by micronucleus method and glutathione (GSH) levels were measured by Tietze enzyme technique in lymphocytes.

2.1 Micronucleus method

In order to determine chromosome radiosensitivity, blood was taken into the vacuum and sterile 4.5 ml 2 tubes coated with lithium-heparin from the patients intravenously. One of the tubes was left as control and the other was given 2 Gy of radiation dose. Chromosome damage was measured by micronucleus (MN) technique [4]. For this technique, 0.5 ml of the blood was incubated in culture medium containing lymphocyte medium (RPMI-1640), serum (Sigma, newborn calf serum) and phytohemaglutinin (PHA, Sigma). Cell division was blocked after adding Cytochalasin-B (Sigma) at 44 hours of incubation and cultures were left to complete 72 hours in total. Cells washed with hypotonic salt (0.075M KCl) solution and then were fixed with methanol: acetic acid mixture. Micronucleus frequencies were calculated by counting the binucleate cells with and without micronucleus in cell preparations stained with Giemsa. Chromosome radiosensitivity of each patient was calculated by calculating the absolute MN values derived by subtracting the spontaneous MN values from the irradiated MN values in the binucleated cells.

2.2 Tietze enzyme technique

Intracellular GSH levels were measured using Tietze enzyme technique [14]. According to the procedure, GSH is sequentially oxidized to glutathione disulfide (GSSG) by 5,5'-dithiobis- (2-nitrobenzoic acid (DTNB)) and glutathione disulfide (GSSG) is reduced by NADPH in the presence of GSH reductase (GR). The formation of nitro-5-thiobenzoic acid (TNB) ratio was monitored using a spectrophotometer and the measured values were calculated with reference to the standard curve.

The stock buffer was prepared using 143 mM sodium phosphate and 6.3 mM Na2 EDTA (pH 7.5) in distilled water and used to prepare 0.3 mM NADPH, 6 mM DTNB and 50 units / ml GSH Reductase (made from Sigma, E.coli) solutions. Each lysate was made in 700 µl NADPH solution, 100 µl DTNB, 100 µl GSH standard or sample and 100 µl dH_2O containing tubes. This mixture was heated at 30 C^0 for 10 min before transferring to a cuvette containing 10 µl GSH Reductase and was measured with a Spectrophotometer (Mecasys Optizen Pop) at 412 nm absorbance. The known standard GSH was treated with 0.6% 5-sulfosalicylic acid (5-SA) series and reference curves were made for samples.

The Spearman correlation test was used to observe the relationship between age and glutathione levels, and MN frequencies.

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3. RESULTS

3.1 Results of Lung Patient Group

Table 3.1 provides information on 14 lung cancer patients aged between 45-85 years who were diagnosed with lung cancer and who had not been previously treated with radiotherapy.

Table 3.1: Spontaneous MN frequencies, MN frequencies after 2 Gy and absolute MN frequencies obtained from lymphocytes of lung cancer patients, and GSH values.

Patient no	Age (years)	MN frequencies			GSH (nmol/ng)
		Spontaneous	2 Gy	Absolute	
1	65	0,007	0,088	0,081	1,3
2	63	0,017	0,094	0,077	2,4
3	63	0,027	0,036	0,009	39,9
4	70	0,011	0,027	0,016	10,7
5	45	0,015	0,024	0,009	4,4
6	77	0,018	0,029	0,011	54,2
7	56	0,013	0,024	0,011	7,6
8	65	0,072	0,086	0,014	54,2
9	72	0,013	0,022	0,009	25
10	70	0,026	0,027	0,001	15
11	60	0,024	0,041	0,017	2,1
12	65	0,02	0,025	0,005	0,3
13	71	0,02	0,07	0,05	6,4
14	85	0,002	0,061	0,059	21,5

Absolute MN frequencies were calculated by subtracting the spontaneous MN frequencies from the MN frequencies obtained after 2 Gy irradiation and used as the indicator of radiosensitivity. The absolute MN frequency of lung cancer patients was found to be 0.026±0.015 (mean±standard deviation) with a mean distributed between 0.001 and 0.081 (Table 3.1). There was no relationship between MN frequency and either an increasing age of the patients ($r=0.09$; $p=0.76$) or GSH levels ($r=-0.24$; $p=0.40$).

3.2 Results of Prostate Patient Group

Table 3.2 shows the results of 8 patients diagnosed with prostate cancer aged between 50 and 69 years.

Table 3.2: Spontaneous MN frequencies, MN frequencies after 2 Gy and absolute MN frequencies obtained from lymphocytes of prostate cancer patients, and GSH values.

Patient no	Age (years)	MN frequencies			GSH (nmol/ng)
		Spontaneous	2 Gy	Absolute	
1	64	0,013	0,017	0,004	-
2	50	0,011	0,013	0,002	9,5
3	50	0,02	0,024	0,004	64,2
4	69	0,018	0,025	0,007	15
5	50	0,023	0,034	0,011	11
6	56	0,014	0,029	0,015	7,6
7	55	0,024	0,026	0,002	28,9
8	64	0,017	0,03	0,013	54,2

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The number of prostate cancer patients is less than the number of lung cancer patients. GSH measurement results of patient 1 were not included in the Table 3.2 because of experimental errors. Absolute MN frequency was found to be 0.007±0.005 (mean±standard deviation) with a mean distributed between 0.002 and 0.015 (Table 3.2). There was no correlation between MN frequency and either an increasing age (r=0.35; p=0.39) or GSH levels (r=-0.23; p=0.59).

The data of two patient groups were combined and the relationship between MN frequencies and GSH was examined by Spearman correlation test and given in Figure 3.1.

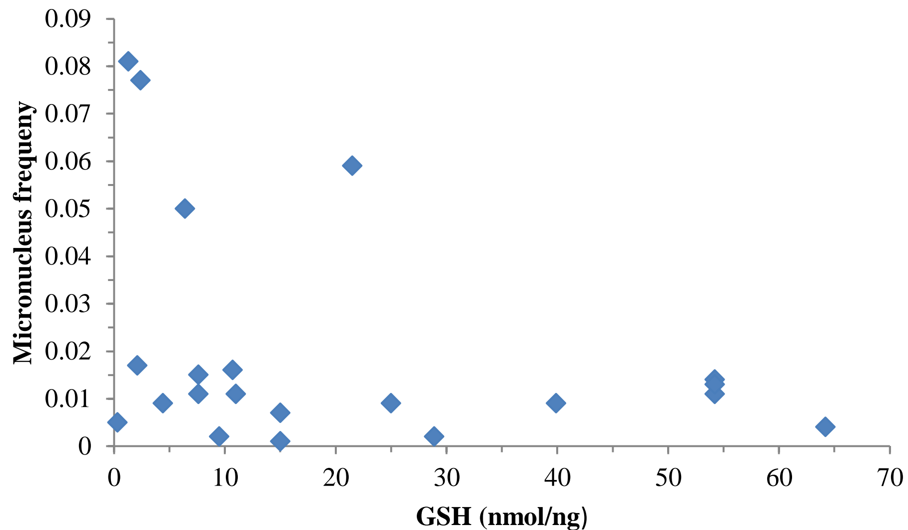

Figure 3.1 The relationship between the MN frequencies and the GSH of the two patient groups

As seen in Figure 3.1, the frequencies of MN occurring in cells with low (10 nmol/ng around) and high (60 nmol/ng) GSH levels are at the same levels. There was no correlation between MN frequency and GSH levels of the patients (r=-0.34; p=0.13).

In Figure 3.2, the Spearman correlation test was applied to the relationship between MN frequencies and the age of the patients.

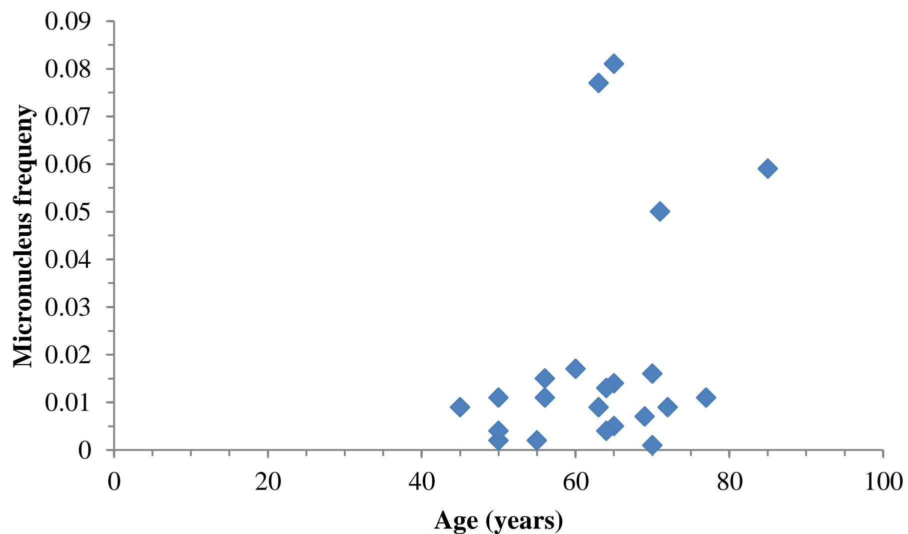

Figure 3.2 Relationship between MN frequency and age of two patient groups.

There was no relationship between the MN frequency and the increasing age of the patients (r=0.28; p=0.19).

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4.DISCUSSION

Patients with the same type of cancer can receive optimal treatment by applying radiation therapy, according to the radiosensitivity of individuals. Widespread differences in radiation damage in both the tumor and normal tissues of patients are thought to be influenced by variations in personal cellular radiosensitivity and are generally determined by genetic factors. In particular, individualization of radiotherapy can be applied after showing the relationship between radiosensitivity of the normal tissues and late tissue damage. The total dose given to patients with high radiosensitivity may be reduced to decrease the risk of late effects, and on the contrary, for normal and resistant patients, the radiation dose and the possible treatment course can be increased. This type of individualization in radiotherapy increases the overall success rate.

The cellular sensitivity of the cell to radiation can vary depending on the DNA repair activity, chromatin structure, endogenous modulators, cell water content, ability to repair potential lethal and subletal damages, and membrane structure. The antioxidant molecule GSH involved in the elimination of radiation-induced free radicals is an important component of cellular radiosensitivity. Studies showing the effect of cellular GSH levels on aerobic radiosensitivity, which is a determinant factor in radiolocation under hypoxic conditions, are insufficient.

Micronucleus test is a cytological test commonly used to determine the genotoxic effect of physical and chemical agents in cells. MNs appear during the mitotic division of the cell and are not involved in the nucleus. MNs are all chromosomal or asymmetric chromosome fragments. It is widely used because of its advantages such as simplicity, reliability, validity and applicability to different cell types. This technique can be used as a biomarker because it can show the morphological disorder, chromosome fractures, premalignant changes and cancer of the cells and can be used to show increased cancer risk in individuals exposed to carcinogens [5,7,15].

In this study, chromosome damage was observed as MN formation by using the MN method as a radiosensitivity marker. MN formation frequencies measured as a result of irradiation of patients with lung cancer were not different from the MN frequencies of prostate cancer patients. This shows that lung and prostate patients have a similar radiosensitivity spectrum. The radiosensitivity spectrum of patients with lung and prostate cancer was found to be in the range of MN frequencies of 0.001 and 0.081. This range represents a possible spectrum of susceptibility of different cells to radiation. There was no correlation between the radiosensitivity in such a broad spectrum and the increasing age of patients (r=0.28).

There was no significant difference in GSH levels between the two groups, although the patients in the prostate cancer group have decreasing and the patients in the lung cancer group have increasıng GSH levels with age. When the data of both groups were combined, no correlation was observed between the micronucleus frequencies and GSH levels measured in lymphocytes of patients (r=-0.34). The GSH molecule did not have a role in determining the radiosensitivity of cells.

5. CONCLUSION

In order to study the role of the GSH molecule, which is an important element in antioxidant mechanisms, in eliminating radiation-induced free radicals, it is necessary to identify GSH or other sulfhydryl groups in the vicinity of the DNA molecule, not cellular.

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