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RESEARCH ARTICLE

DNA Damage and Lipid Peroxidation in Several Types of Cancer

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Summary

Reactive oxygen and nitrogen species (ROS-RNS) might cause formation of lipid peroxidation (LP), DNA damage, and destruction of membrane structure by attacking membrane lipids. DNA damage and lipid peroxidation leads to carcinogenesis related mutagenesis. In our study, we aimed to investigate 8-hydroxy-2'-deoxyguanosine (8OHdG) levels as a product of DNA damage, and malondialdehyde (MDA) levels as a product of lipid peroxidation, which cancer related oxidative stress markers. 8OHdG levels in urine were measured by a competitive ELISA kit method and normalized with creatinine, and MDA levels in serum were measured by HPLC method. Twenty one cancer patients and 21 control subjects were included in the study. Patients were classified as colon cancer (n=12), gastric cancer (n=6), and breast cancer (n=3). 8OHdG/creatinine levels were 26.78±13.56 nM/mM in total patients, and 21.60±7.12 nM/mM in healthy controls (p>0.05), and MDA levels were 3.32±0.85 µM in total patients, and 2.35±1.02 µM in healthy controls (p=0.002). Significantly increased levels of MDA were observed in colon and gastric cancer groups (p=0.048 and p=0.007, respectively). We did not find any correlation between 8OHdG and MDA. In conclusion, increased levels of MDA and, 8OHdG support the studies suggesting possible participation of oxidative stress in cancer. In addition, the results of our study support the studies which suggested that polyunsaturated fatty acids are more susceptible to reactive oxygen species than other components such as DNA or proteins in cancer.

Key Words: *DNA damage, 8-hydroxy-2'-deoxyguanosine, lipid peroxidation, malondialdehyde, cancer.*

Çeşitli Kanser Türlerinde DNA Hasarı ve Lipit Peroksidasyonu

Özet

Reaktif oksijen ve nitrojen türleri (ROT-RNT) membran lipitlerine saldırarak lipit peroksidasyonuna (LP), DNA hasarına ve membran yapısının yıkımına neden olabilmektedir. DNA hasarı ve lipit peroksidasyonu karsinogenezle ilişkili mutasyonların oluşumuna yol` açmaktadır. Çalışmamızda kanserle ilgili oksidatif stres belirteçlerinin; DNA hasar ürünü olarak 8-hidroksi-2'-deoksiguanozin (8OHdG) ve lipit peroksidasyonu ürünü olarak malondialdehit (MDA) düzeylerini araştırmayı amaçladık. İdrar 8OHdG düzeyleri yarışmalı bir ELISA kit yöntemiyle ölçüldü ve kreatinin düzeyleriyle normalize edildi, serum MDA düzeyleri ise HPLC yöntemiyle ölçüldü. Yirmi bir kanserli hasta ve 21 sağlıklı kontrol çalışmaya dahil edildi. Hastalar kolon kanseri (n=12), mide kanseri (n=6) ve meme kanseri (n=3) olarak sınıflandırıldı. 8OHdG/kreatinin düzeyleri total hasta grubunda 26.78±13.56 nM/mM ve sağlıklı kontrollerde 21.60±7.12 nM/mM (p>0.05) ve MDA düzeyleri total hasta grubunda 3.32±0.85 µM ve sağlıklı kontrollerde 2.35±1.02 µM (p=0.002) idi. Anlamlı olarak artmış MDA düzeyleri kolon ve mide kanseri gruplarında gözlendi (sırasıyla p=0.048 ve p=0.007). 8OHdG ve MDA arasında herhangi bir korelasyon bulamadık. Sonuç olarak, artmış MDA ve 8OHdG düzeyleri, kanserde oksidatif stresin olası katkısını öne süren çalışmaları desteklemektedir. Buna ek olarak çalışmamızın sonuçları kanserde çoklu doymamış yağ asitlerinin reaktif oksijen türlerine, DNA veya proteinler gibi diğer komponentlerden daha hassas olduğunu öne süren çalışmaları da desteklemektedir.

Anahtar Kelimeler: *DNA hasarı, 8-hidroksi-2'-deoksiguanozin, lipit peroksidasyonu, malondialdehit, kanser..*

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INTRODUCTION

Reactive oxygen species (ROS) are capable of creating oxidative modifications of structures such as proteins, lipids and DNA, depending on their high redox potential (1). Free radicals and other reactive species which form due to endogenous and exogenous factors in organism, have important roles in diseases such as, atherosclerosis, cardiovascular diseases, renal failure, ischemic damage, cancer, diabetes, rheumatoid arthritis, hemorrhagic shock, cystic fibrosis, AIDS, immune system decline, brain dysfunction, cataracts and events such as aging or poisoning (2,3).

Damage of DNA by oxygen free radicals, frequently leads to initiation and progression of human cancer related mutations (4-7). ROS-induced DNA damage includes modified bases, abasic sites, single and double strand breaks, DNA–protein cross-links, sister chromatid exchanges, subsequent production of clastogenic factors and alterations in the base structure (6,8,9).

Oxidation of DNA, results in damage of all four nucleic bases. Among all the purine and pyrimidine bases, guanine base tends to be the more oxidized one. In previous studies, various DNA damage products were discovered such as 8-hydroxy-2'-deoxyguanosine (8OHdG), 8-hydroxyguanine (8OHGua), 5-hydroxy-6-hydrothymine, thymine glycol, cytosine glycol, uracil glycol, 5-hydroxymethyluracil, 5-hydroxy-5-methyl hydantoin, 5-formyluracil, alloxan, 8-hydroxyadenine, oxazolone (10,11). In recent years, 8OHdG [or 8-oxo-2'-deoxyguanosine-(8-oxodG)] appears to as an oxidative stress marker. Especially the urinary 8OHdG is the most commonly measured marker to indicate the degree of endogenous oxidative DNA damage and its precursors. 8OHdG can be measured at high sensitivity, and its levels are correlated with oxidative stress and cancer incidence in target tissues. For this reason, 8OHdG is a very useful marker in oxidative damage researches (12-14).

Lipids, established mainly in the cell membranes, are very sensitive to oxidative stress. Oxidative damage of membrane lipids initiates lipid peroxidation (LP). Free radical attack especially effects the polyunsaturated fatty acid chains in cell membranes or in lipoproteins, then LP, and finally lipid hydroperoxides, thiobarbituric acid-reactive substances (TBARs) and long lived-aldehydes production occur. This reaction is a process which can cause cell injury, and can continue as a chain reaction. LP products may show free radical actvities, therefore they may cause increases in the lipid membrane injury themselves (1,5). At the end of LP reactions, products such as malondialdehyde (MDA), 4OH-nonenal, conjugated fatty acids, isoprostanes, ethane and penthane are generated (1,5,15).

MDA is produced endogenously via lipid peroxidation and prostaglandin biosynthesis and exists in biological matrices both in the free form, and bound to SH and/or NH_2 groups of various biomolecules. It is a genotoxic product of enzymatic and oxygen radical-induced lipid peroxidation (4,16). MDA has a potentially important contribution to DNA damage and mutation, and it has been shown to be mutagenic in bacterial and mammalian cell assays, and it is carcinogenic in rats (4).

MATERIAL and METHODS

Study Population

Both of patients and healthy controls were provided by Ankara Oncology Training and Research Hospital. Twelve of the patients included in this study with colon cancer, 6 patients with gastric cancer and 3 with breast cancer. Twelve out of 21 patients were female and 9 were male (age range 31-70 years). All patients were recently diagnosed by biopsy and had not yet received any clinical treatment. The patients were classified according to the TNM system American Joint Committee on Cancer criteria (17). A total of 21 healthy controls, 11 female and 10 male (age range 31-54 years), also took part in the study. Controls were selected from healthy subjects who were admitted to hospital for a routine check-up. This study was approved by the Clinical Research Ethics Committee of Mersin University. Baseline characteristics of the patients and controls are given Table 1.

Sample Collection and Storage

Blood samples were taken from patients and controls to Vacutainer® tubes. Samples were centrifuged at

Table 1. Baseline characteristics of the patients and controls.

Variables	Patients (n=21)	Controls (n=21)
Age (Standard Deviation)	56.57 (9.53)	45.14 (5.90)
Gender		
Female (%)	12 (57.1)	11 (52.4)
Male (%)	9 (42.9)	10 (47.6)
Menopausal Status		
Premenopausal (%)	3 (25.0)	8 (72.7)
Postmenopausal (%)	9 (75.0)	3 (27.3)
Cancer Type		
Colon (%)	12 (57.1)	-
Gastric (%)	6 (28.6)	-
Breast (%)	3 (14.3)	-
Cancer Stage		
Total Patients-Stage I,II,III,IV	5,4,6,5	-
Colon- Stage I,II,III,IV	4,2,3,3	-
Gastric- Stage I,III,IV	1,2,2	-
Breast- Stage II,III	2,1	-
Cancer Phase		
Total Patients		
Early Phase* (%)	9 (42.9)	-
Advanced Phase* (%)	12 (57.1)	-
Colon		
Early Phase (%)	6 (50.0)	-
Advanced Phase (%)	6 (50.0)	-
Gastric		
Early Phase (%)	1 (16.7)	-
Advanced Phase (%)	5 (83.3)	-
Breast		
Early Phase (%)	2 (66.7)	-
Advanced Phase (%)	1 (33.3)	-

* Early Phase=Stage I + Stage II, Advanced Phase=Stage III + Stage IV

3000 X g for 10 min. Serum samples were stored at -80°C until analysis. Collected spot urine samples were divided into microtubes and kept frozen at -80°C.

Chemicals and Equipments

An 8OHdG EIA kit was obtained from Cayman Chemical Company and a creatinine kit from Bio Aktif Kimya. Creatinine control solution was provided by Bio-Rad Laboratories. Tetraethoxypropane (95% TEP) was obtained from Fluka Chemika. Sulphuric acid (95-98% H_2SO_4), sodium hydroxide (NaOH), perchloric acid (70-72% PCA), hydrochloric acid (37% HCl), acetonitrile (ACN), acetic acid (96%) were obtained from Merck KGaA. In addition to methanol purchased from Sigma-Aldrich GmbH, dinitrophenylhydrazine (DNPH) was purchased from Acros Organics, and hexane (95%) from Lab-Scan Analytical Sciences.

A Molecular Devices-VersaMax plate reader was used for measurement of 8OHdG levels and a Shimadzu UV-160A spectrophometer for the creatinine. We used an ODS Hypersil HPLC analytical column (5 µm, 100 × 4.6 mm) (Hewlett-Packard) for MDA, and also used an HP Model 1050 HPLC pump and a photometric detector (Hewlett-Packard).

Measurements of Parameters

Determination of 8OHdG

The levels of 8OHdG in urine were determined using a competitive ELISA kit method. This method uses an anti-mouse IgG-coated plate and a tracer consisting of an 8OHdG-enzym conjugate, and is based on the competition between 8OHdG and an 8OHdG-acetylcholinesterase conjugate for a limited amount of 8OHdG monoclonal antibody. Experimental procedures were performed according to the manufacturer's instructions (Cayman Company).

Determination of Creatinine

The levels of creatinine in urine were determined by a spectrophotometric method. This method is based on the measurement of absorbance at 510 nm wavelength of red-coloured creatinine picrate compound formed by creatinine in urine samples and picric acid. Experimental procedures were performed according to the manufacturer's instructions (Bio Aktif Kimya).

Determination of MDA

The levels of MDA in serum were determined using an HPLC method. This method is based on the derivatization of total MDA in serum samples with 2,4-dinitrophenylhydrazine (DNPH) and measurement of peak areas of this derivative compound at 310 nm wavelenght (18). 50 µL of 6M NaOH solution was added for alkaline hydroxylation to each 250 µL serum samples. After incubation in a hot water bath at 60 °C for 30 min, samples were acidified by 125 µL of 35% perchloric acid solution and centrifuged at 14,000 X g for 10 min. Twenty five µL of 5 mM 2,4-dinitrophenylhydrazine solution was added to 250 µL supernatant for derivatization of MDA. After incubation for 10 min, the samples were extracted twice with 1.2 mL of hexane and the extract was dried by nitrogen. The extract was resolved with 100 µL of mobile phase, then the mixture was directly injected into the HPLC system.

Statistical Analysis

Data were presented as the mean (SD), and statistical analyses were performed by the Student's t-test, and Mann-Whitney U test (for groups of small numbers). Pearson correlation coefficients were calculated for determining the relationship among parameters. Statistical analyses were performed by using SPSS 11.5 Software (SPSS Inc.).

RESULTS

Serum MDA levels were significantly higher in total patients, colon and gastric cancer groups than in healthy controls (p=0.002, p=0.048 and p=0.007, respectively). Urinary 8OHdG/creatinine and serum MDA mean levels measured in patient groups and in controls, are given in Table 2.

Table 2. Mean levels of 8OHdG/creatinine and MDA in total patients and controls.

	Mean Levels (Standard Deviation)	
	8OHdG/Creatinine (nM/mM)	**MDA (µM)**
Total Patients (n=21)	26.78 (13.56)	3.32 (0.85) [a]
Colon (n=12)	27.18 (15.44)	3.19 (0.96) [b]
Gastric (n=6)	25.64 (9.67)	3.66 (0.78) [c]
Breast (n=3)	27.43 (17.00)	3.12 (0.44)
Control (n=21)	21.60 (7.12)	2.35 (1.02)

[a] p=0.002, [b] p=0.048, [c] p=0.007, when compared with controls.

When the 8OHdG/creatinine and MDA values in total patients group were compared to those in the controls with respect to the cancer phase, no significant difference was found between early and advanced phases (Table 3).

Table 3. Mean levels of 8OHdG/creatinine and MDA in total patients according to cancer phase.

	Mean Levels (Standard Deviation)	
	8OHdG/Creatinine (nM/mM)	**MDA (µM)**
Early Phase (n=9)	25.48 (10.46)	2.94 (0.51)
Advanced Phase (n=12)	27.75 (15.89)	3.60 (0.96)

When the patients and control groups were evaluated with respect to the gender, 8OHdG/creatinine and MDA mean levels did not show any significant differences between female and male groups (Table 4).

Table 4. Mean levels of 8OHdG/creatinine and MDA in total patients and controls according to gender.

	Mean Levels (Standard Deviation)	
	8OHdG/Creatinine (nM/mM)	**MDA (µM)**
Patient (n=21)		
Female (n=12)	29.00 (16.48)	3.34 (0.97)
Male (n=9)	23.82 (8.31)	3.28 (0.72)
Control (n=21)		
Female (n=11)	23.26 (7.47)	2.41 (0.94)
Male (n=10)	19.78 (6.59)	2.29 (1.14)

Also, there were no significant differences in 8OHdG/creatinine and MDA mean levels according to menopausal status of the total patients and the control groups. Urinary 8OHdG/creatinine and serum MDA mean levels measured in total patients and control groups with respect to the menopausal status, are shown in Table 5.

Table 5. Mean levels of 8OHdG/creatinine and MDA in total patients and controls according to menopausal status.

	Mean Levels (Standard Deviation)	
	8OHdG/Creatinine (nM/mM)	**MDA (µM)**
Patient (n=12)		
Premenopausal (n=3)	34.12 (14.15)	2.97 (0.60)
Postmenopausal (n=9)	27.29 (17.62)	3.47 (1.07)
Control (n=11)		
Premenopausal (n=8)	23.74 (5.80)	2.26 (0.83)
Postmenopausal (n=3)	21.97 (12.57)	2.81 (1.31)

We did not find any correlation between 8OHdG/creatinine and MDA levels in the patients and the controls. According to the gender, menopausal status, and the stages of the disease, there were also no correlation between 8OHdG/creatinine and MDA levels. There was only a negative correlation between 8OHdG/creatinine and age ($R=-0.437$, $p<0.05$) of the patients.

DISCUSSION

Reactive oxygen species are capable of creating oxidative modifications of compounds such as proteins, lipids and DNA (1). Damaging of DNA by oxygen free radicals frequently causes mutation related initiation and progression of human cancers (4-7). 8OHdG resulting from ROS-induced oxidative damage of DNA, is extensively used as a marker of oxidative DNA damage, which is important in mutagenesis and carcinogenesis processes (6,8,12). Studies have reported that urinary 8OHdG levels in patients that had lung (19,20) and prostate cancer (13), urine and serum (or plasma) 8OHdG levels in patients that had breast (13,21-23) and colon cancer (24-26), were higher than those of the healthy controls. In contrast, Dinçer et al. showed that plasma 8OHdG levels were significantly lower in gastric and colon cancer patients than in the healthy controls (27). In general, increasing of urinary 8OHdG levels in cancer patients show that this marker is very convenient in determining the cancer risk (12). Consistent with other studies which have been performed on urinary 8OHdG levels, we found in this current study higher urinary 8OHdG levels in all of colon, gastric and breast cancer and all patients than those in the controls, but the results were not statistically significant.

In a previous study with 60 breast cancer patients, Kuo et al. observed that urinary 8OHdG levels were significantly correlated to the development of breast cancer (21). Consistent with this study, we found an increased mean of urinary 8OHdG levels in cancer patients in advanced phase, but the results were not statistically significant.

An investigation performed by Loft et al. found higher levels of DNA damage products in men than in women (28). On the contrary, in this study levels of 8OHdG in urine were higher in women than in men, but not statistically significant, neither in patients nor in controls. When the women we evaluated according their menopausal status both in the patients and the controls, urinary 8OHdG levels were found to be higher in premenopausal women than in postmenopausal women.

Reactive oxygen species and the products of lipid peroxidation were found to cause mutagenesis and carcinogenesis by binding onto DNA. Increased lipid peroxidation products which are produced in various organs were released into the serum (5,29). MDA, the major product of lipid peroxidation, has potentially important contributions on DNA damage, mutation and carcinogenesis (4).

In previous studies, serum or plasma MDA levels increased in the patients with lung cancer (19,30), prostate cancer (31), endometrial cancer, ovarian cancer (32), oral cancer (33), breast cancer (34,35), colon cancer (36,37) and gastric cancer (38,39), when compared to the control group. Consistent with these studies, we observed significantly higher MDA levels in patients with colon and stomach cancers, and in all the patients, than those in the controls. In only 3 patients, the measurements of which could be taken in the breast cancer group, we observed increased, but not statistically significant, MDA levels than those of the controls.

In a previous study, Khanzode et al. reported an increase in serum MDA levels depending on the stage of the cancer in breast cancer cases (40). Ray et al. also observed a significant increase in MDA levels in stage 2 and 3 in patients with breast cancer than those in healthy controls (41). In another study with 38 gastric cancer patients, Bakan and his colleagues have reported an increase in plasma MDA levels depending on the phase of the disease (38). In this current study, we also found higher MDA levels in cancer patients in advanced-phases, than those in the early phases, but the results were not statistically significant.

In our study, females had slightly higher serum MDA levels than the males both in the patient and the control groups. In addition, serum MDA levels in females with premenopausal status were slightly higher than in those with postmenopausal status in both the patients and the control groups. Ray et al. showed significantly increased MDA levels in both premenopausal and postmenopausal study groups of breast cancer patients, when compared to their controls (41).

CONCLUSIONS

In this study, MDA levels were measured as a product of lipid peroxidation and 8OHdG levels as a product of DNA damage, which are oxidative stress markers in all the patients and in the control group, although statistically insignificant, and were found to be higher in the patients group than in the controls.

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