Prognostic significances of oxidative DNA damage evaluated by 8-hydroxy-deoxyguanosine and antioxidant enzymes in patients undergoing resection of gastric and colon carcinoma

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Oxidant/ antioxidant balance has been suggested as an important factor for initiation and progression of cancer. In order to determine whether the degree of oxidative DNA damage and antioxidant enzyme activities in plasma obtained from patients with gastric and colon cancer who undergo resection can be used as a useful prognostic predictor, plasma level of 8-hydroxydeoxyguanosine (8-OHdG), activities of glutathione peroxidase (G-Px) and superoxide dismutase (SOD) were examined. 19 patients with gastric cancer and 26 patients with colon cancer who were undergoing resection of tumor were included by the study. Venous blood samples were taken just before the surgery. Plasma level of 8-OHdG was determined with ELISA , SOD and G-Px activities in plasma were measured by spectrophotometric kits. 8-OHdG level and activitiy of G-Px were found to be decreased, SOD activity was found to be increased in both gastric and colon cancer groups as compared to control group. Alpha fetoprotein was found to be correlated with G-Px in the gastric cancer group and correlated with 8-OHdG in the colon cancer group. SOD activity was correlated with CA-15-3 in the gastric cancer group. Low plasma level of 8-OHdG and altered antioxidant activity may implicate the deficient repair of oxidative DNA damage in patients with gastric and colon cancer. Those measured parameters were not found to be related with histopathological data but correlated with some tumor markers.

Key words: 8-hydroxydeoxyguanosine, glutathione peroxidase, superoxide dismutase, gastric cancer, colon cancer

Recent studies have demonstrated the role of oxygen free radicals (OFR) in carcinogenesis. OFR caused by extrinsic and intrinsic factors are very small and reactive molecules and they can readily react with cellular components. OFR are known to interact with genomic DNA, damage specific genes which control cell growth and differentiation [1], increase the activity of carcinogenic xenobiotics [2], and stimulate faster growth of malignant cells [3]. Epidemiological studies have demonstrated a close association between chronic inflammation and certain types of cancer such as ulcerative colitis-colon cancer, atrophic gastritis-gastric cancer, schistosomiasis-urinary bladder cancer and hepatitis B-hepatocellular carcinoma. Increased oxidative stress via inflammatory response has been suggested as a responsible factor for development of these types of cancer [4]. Enormous amount of OFR is released during the respiratory burst. Those oxidant molecules induce DNA strand breakage, point mutations and chromosome abnormalities. Major DNA oxidation product, 8-hydroxydeoxyguanosine (8-OHdG), formed by the reaction of the hydroxyl radical at the C-8 position of the guanine on DNA. 8-OHdG has a pro-mutagenic potential by mispairing with A residues, leading to an increased frequency of spontaneous G:C®T:A transversion. This mutation is generally observed in mutated protooncogenes and tumor suppressor genes [5]. 8-OHdG residues on DNA are excised by constitutive enzymatic repair systems, appear in the blood and subsequently excreated in the urine [6]. 8-OHdG level in blood and/or urine is measured as a marker of oxidative DNA damage [7].

The gastrointestinal tract is particularly susceptible to OFR attacks which lead to carcinogenesis. An important role in defence strategy against OFR is played by antioxidants. The first step in the defense against free radicals is executed by

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superoxide dismutase (SOD). SOD catalyses the conversion of superoxide anion to hydrogen peroxide and O_2. Hydrogen peroxide is then turned into H_2O and O_2 by glutathione peroxidase (G-Px) [8]. Although SOD and G-Px are cellular enzymes they are available in the plasma at a detectable level [9,10]. A balance is available between oxidant and antioxidant systems under physiological conditions, but it is impaired in the pathological circumstances. Oxidant/ antioxidant balance has been suggested as an important factor for initiation and progression of cancer [11]. The present study has proposed determination of alterations in DNA oxidantion and antioxidant enzyme activities in patients with gastric and colon cancer; evaluation of the role of those alterations in carcinogenesis; and determination of the relations between measured parameters and tumor markers, clinical stage, histological grade and metastase.

Materials and Methods

Our study comprises 19 patients with gastric cancer and 26 patients with colon cancer who admitted to Istanbul University, Cerrahpasa Medical Faculty, Department of General Surgery. None of the patients had undergone any previous treatment. The control group was constituted by twenty-seven healthy volunteers (mean age 58±12 years; 15 men and 12 women). Both patients and controls were euthyroid, had normal liver and renal functions and had a similar life style and eating habits. None of the included subjects were on dietary limitation of any kind. Cases who had a smoking habit or were taking drugs capable of interfering with oxidant system and vitamin supplements in previous 6 months were excluded from the study. Cerrahpasa Medical Faculty Ethical Committee approval was taken in accordance with the principles of Declaration of Helsinki and informed consent was obtained from the cases. Patient and tumor characteristics are shown in the Table 1.

Table 1. Patient and tumor characteristics

	Gastric cancer (n=19)	Colon cancer (n=26)
Mean age (year)	65±10	62±10
Male	12	14
Female	7	12
Tumor types		
Adenocarcinoma	15	23
Signet ring carcinoma	2	–
Diffused type B cell non-Hodgkin lymphoma	1	–
Neuroendocrin carcinoma	–	2
Adenosquamous carcinoma	–	1
Undifferentiated carcinoma	1	–
Grade		
Grade (I+II)	8	24
Grade (III+IV)	11	2
Stage		
Stage (I+II)	8	12
Stage (III+IV)	11	14
Metastases	13	14
Tumor markers		
CEA	16.8 (0.5 – 118)	9.7 (0.5 – 70)
AFP	2.7 (0.9 – 3.5)	1.6 (0.9 – 2.5)
CA 12-5	13.4 (4.1 – 31.8)	15.0 (1.6 – 84.5)
CA 15-3	35.3 (5.6 – 195)	19.6 (8.2 – 34.0)
CA 19-9	20.0 (2.5 – 81.6)	20.3 (1.1 – 81.7)

10 ml of venous blood samples were collected into heparinised tubes. Following the centrifugation at 2000 X g for 10 minutes plasma was removed and kept at the –80 ^{0}C until the time of analysis. Plasma level of 8-OHdG was measured with a competitive ELISA kit obtained from Japan Institute for the Control of Aging, Fukuroi, Japan. Briefly, 8-OHdG monoclonal antibody and the samples (both patients and controls) were added to a microtiter plate well which has been precoated with 8-OHdG. The 8-OHdG in the sample or standard competes with the 8-OHdG bound on the plate for the 8-OHdG monoclonal antibody binding sites. Therefore, higher concentrations of 8-OHdG in the sample caused to a reducing binding of the antibody to the 8-OHdG on the plate. The antibodies that are bound to the 8-OHdG in the sample were washed out the well, while those that have bound to the 8-OHdG coated on the plate were remain. The enzyme-labeled secondary antibody was added and binds to the monoclonal antibody that remains on the plate. Unbound enzyme-labeled secondary antibody was removed by a wash step. By the addition of a chromogen a color developed in proportion to the amount of antibody bound to the plate. The color reaction was terminated and than the absorbance was measured. Determination range of the kit was 0,125–10 ng/ml.

Activity of SOD and G-Px were measured by spectrophotometric kits from Randox, UK (catolog no: SD125 and RS 504, respectively).

CEA, AFP, CA12-5, CA15-3, CA19–9 levels were measured by chemiluminescent Microparticle Immunassays.

Statistical Analysis

Mesured parameters are expressed as means ± SD (standard deviation of the mean). The data were examined using ANOVA with Tukey test. When patient groups were divided into subgroups as respect with the grade, stage of tumor and metastase, comparisons between those groups were made by Wilcoxon test. Differences between groups were considered significant at $P<0.05$. Pearson correlation coefficient was used for correlation analysis.

Results

8-OHdG level and SOD, G-Px activities in the plasma of the patients and controls are shown in Table 2. 8-OHdG level and activitiy of G-Px were found to be decreased, SOD activity was found to be increased in both gastric and colon cancer groups as compared to control group. No significant differ-

ences were found between gastric and colon cancer groups for any of measured parameters. When the cancer groups were divided into subgroups, that is, grade (I + II) group and grade (III + IV) group; that is stage (I + II) group and stage (III + IV) group; that is patients with metastases and patients without metastases, no significant diffrerence for any parameter was determined between those groups in both gastric cancer and colon cancer patients (Table 3). As an exception, patients with colon cancer who has a tumor at grade (III+IV) were just two cases therefore statistical evaluation for any parameter did not performed as respect with the tumor grade in colon cancer patients. Alpha fetoprotein (AFP) was found to be correlated with G-Px (r:0.63, P<0.05) in the gastric cancer group; and correlated with 8-OHdG (r:0.52, P<0.05) in the colon cancer group. SOD activity was correlated with CA-15-3 (r:-0.73, P<0.05) in the gastric cancer group. When patient groups were combined (gastric cancer+colon cancer) those correlations were determined: 8-OHdG – grade (r:0.45, P<0.05); G-Px – grade (r:0.41, P<0.05); G-Px – AFP (r:0.55, P<0.01) and SOD – grade (r:0.35, P<0.05).

Table 2. 8-OHdG level and SOD, G-Px activities in the plasma of the patients and controls

	Control (n=27)	Gastric cancer (n=19)	Colon cancer (n=26)
8-0H dG (ng/ml)	3.39±0.75	1.00±0.34*	1.30±0.55*
G Px (U/L)	1.13±0.22	0.46±0.21*	0.48±0.34*
SOD (U/mL)	2.61±0.43	3.30±0.91*	3.76±0.76*

* P<0.001 versus control

Table3:Measured parameters in plasma of the patients as respect with the tumor grade, stage and metastase.

	8-OH dG (ng/ml)	G-Px (U/L)	SOD (U/ml)
Gastric cancer (n=19)			
Grade (I+II) group (n=8)	0.99±0.31	0.36±0.14	3.40±0.49
Grade (III+IV) group (n=11)	1.18±0.37	0.43±0.21	3.14±0.95
Stage (I+II) group (n=8)	0.99±0.31	0.36±0.14	3.40±0.49
Stage (III+IV) group (n=11)	1.18±0.37	0.43±0.21	3.14±0.95
Patients with metastases (n=13)	1.07±0.34	0.48±0.24	3.15±0.95
Patients without metastases (n=6)	0.82±0.31	0.40±0.16	3.61±0.81
Colon cancer (n=26)			
Grade (I+II) group (n=26)	1.24±0.53	0.50±0.38	3.83±0.74
Grade (III+IV) group (n=2)	1.36±0.38	0.37±0.18	3.60±0.80
Stage (I+II) group (n=12)	1.23±0.61	0.55±0.47	3.60±0.78
Stage (III+IV) group (n=14)	1.34±0.53	0.42±0.17	3.89±0.65
Patients with metastases (n=14)	1.37±0.52	0.41±0.18	3.90±0.65
Patients without metastases (n=12)	1.20±0.61	0.55±0.47	3.60±0.78

Discussion

There is strong evidence that oxidative damage may be involved in the initiation and promotion of colon carcinogenesis [12]. According to Ames et al. [13] 10^4 hits per cell may occur every day. Nearly 99% of this damage is repaired by repair systems, 1% leads to irreparable damage that accumulates during life time and contributes to development of cancer. The colon may be susceptible to oxidants by the route of the gut lumen [14]. This contains bacteria that can generate free radicals, hydrogen peroxide and genotoxins. Furthermore, Ehardt et. al. [15] have shown that diets rich in fat and poor in fiber increase the in vitro formation of OFR in the human feces. Among the factors involved in oxidative events leading to DNA damage in colonocytes, inflammation has got special interest. It has been reported that even a mild acute inflammation can double the amount of 8-OHdG in the DNA of rat colonic mucosa within 48 h [16]. Chronic colitis has found to be associated with an increased frequency of colon cancer in animal and humans [17,18]. In agreement with those data, non-steroidal anti-inflammatory drugs have been shown to protect man against colon cancer by ~ 2 fold [19,20].

Chronic inflammation mediated-DNA damage has been implicated as a contributory factor also for gastric carcinogenesis [21–24]. Epidemiological studies have demonstrated a close association between helicobacter pylori infection and the subsequent development of gastric adenocarcinoma in humans [25–26]. The damage could be caused directly by helicobacter pylori, through the release of cytotoxins, lipase, or phospholipase or the urease mediated release of toxic-ammonia [27]. Alternatively, the damage could be due to the inflammatory reaction elicited by the microorganisms. In the latter case DNA damage by OFR released from polymorphonuclear leukocytes might be involved in carcinogenesis [28]. Mutagenity of the gastric fluid and 8-OHdG level in the gastric mucosa has been found to be higher in helicobacter pylori infected subjects than those in helicobacter pylori (-) subjects by various investigators [22,28,29,30]. According to Farinati et al. [28] the presence of mild dysplastic changes did not correlate with any further significant increase in 8-OHdG tissue concentrations. Patients with no or mild intestinal metaplasia had significantly lower concentrations of 8-OHdG than patients with moderate or or severe changes. The highest levels of 8-OHdG were observed in patients having chronic atrophic gastritis associated with high grade intestinal metaplasia, and 8-OHdG level in the gastric biopsy specimens were moderately correlated with helicobacter pylori infection. As another reliable evidence, significantly decreased mutagenicity of gastric fluid and 8-OHdG levels in the human gastric mucosa after the eradication of helicobacter pylori have been shown [23,29].

Although lots of data indicating the role of 8-OHdG in the carcinogenesis, findings show the relation of 8-OHdG with tumor stage, grade and metastases is highly limited and controversial. It has been reported that the level of 8-OHdG in tumor speciments from patients with helicobacter pylori-infected non-cardiac gastric adenocarcinoma were significantly higher in stage 3 and 4 patients than in stage 1 and 2 patients [31]. 8-OHdG in renal cell carcinoma was also found to be

correlated with histological grade and clinical stage [32]. However, 8-OHdG in tumor tissue was not found to be associated with grade and stage in breast [33] and prostate cancer [34]; and was not found to be associated with clinical stage in colorectal cancer [35]. As far as we know, tissue and peripheral leukocyte level of 8-OHdG has been measured in all studies carried on so far. This is the first study investigating 8-OHdG level in plasma. The aim of the present study was to investigate whether plasma level of 8-OHdG has a predictive value in patients who undergo resection for colon and gastric carcinoma. At the beginning of the study we had expected greater 8-OHdG level in the plasma. Increased 8-OHdG level in cancer was reported by many investigators so far [28,31,35]. We supposed that if 8-OHdG level at a high level in tumor tissue it may also increase in plasma and plasma level of 8-OHdG may reflect the oxidative stress on DNA, and may be in a relation with tumor grade or clinical stage. However, when we determined lower level of 8-OHdG in plasma we realized that this mechanism is not so simply, repair mechanism may be responsible also for the level of 8-OHdG in plasma. It is possible to measure 8-OHdG level in tissue, urine, peripheral leukocytes and plasma samples. However methods of determination are different. In order to determine the 8-OHdG levels in tissue and peripheral leukocytes, cells are isolated at once and then 8-OHdG remain on DNA obtained from cells are released by various methods. Released 8-OHdG is then measured by HPLC. In the case of plasma, 8-OHdG level can be measured in a short time and easily by ELISA. The unique mechanism for appearance of 8-OHdG in plasma is repair of 8-OHdG residues on DNA by excision repair system. When 8-OHdG remain on DNA at a high level, high level of 8-OHdG can be detected in plasma at the maintanence of an effective DNA repair. Otherwise, 8-OHdG level in plasma can be found at a low level, although it is high on the DNA of tissues or leukocytes. 8-OHdG level in tissue and peripheral leukocytes directly shows degree of oxidative damage on DNA. However plasma level of 8-OHdG shows not only oxidative DNA damage but also effective DNA repair. Since plasma level of 8-OHdG derived from repaired 8-OHdG residues on DNA, our finding may indicate a defective repair mechanism in those patients. Various defects in DNA repair results in different forms of cancer. Indeed mismatch repair defects have been determined in some kinds of gastric and colorectal cancer [36–38]. Defective repair of 8-OHdG residues may leads to determination of high level of 8-OHdG in tissue and peripheral leukocytes but may cause the low level of 8-OHdG in plasma. Kondo et al. [35] have examined the relation between 8-OHdG level in colon tumors (not in the plasma) and expression of hOGG1 which is the enzyme responsible for repair of 8-OHdG residues on DNA. They have found that hOGG1 expression was significantly up-regulated in carcinoma, and there is a proportional association between 8-OHdG levels in tumor tissue and hOGG1 expression. However, they have detected no significant difference in the 8-OHdG level between early and advanced-stage cancer, although hOG1 expression was 1.6-fold increased in advanced-stage of cancer. Their findings clearly show that 8-OHdG level increases and repair activity is induced in colon tumors but although hOGG1 expression is increased 1.6-fold in advanced-stage of cancer, no significant difference of 8-OHdG level in tissue is could be proved early and advanced-stage of colon cancer. According to our mind, this contradiction may derived from a defect in posttranslasyonel modifications of the hOGG1. However, findings of Kondo et al. [35] has not provide satisfactory data for plasma level of 8-OHdG.

We could not find any relation between metastases and plasma level of 8-OHdG. Indeed, we also could not see any article examined the relation between metastases and plasma level of 8-OHdG in patients with neither gastric nor colon cancer. We determined a correlation between 8-OHdG and histological grade when we combined the patient groups ($r:0,45$; $p<0.001$). However, this is a highly poor evidence in order to suggest that plasma 8-OHdG level is a reliable marker for tumor grade in patients with gastric and colon cancer. A large number of plasma samples must be analyzed to validate this hypothesis. Determined correlation between 8-OHdG and AFP is moderate only but may have gain interest. However, to the best of our knowledge, this is the unique finding in literature. More detailed studies are needed to reveal the association between plasma 8-OHdG level and AFP.

In the present study, plasma SOD activity was found to be increased in both patient groups as compared to control group whereas G-Px decreased. Antioxidant system is highly complex and multifactorial. It includes various enzymes and small molecules and they may not exhibit harmonious change. Changes in the activity of G-Px and SOD may derived from changed energy metabolism and free radical formation via disordered mitochondrial function in carcinoma [39], or enzyme inactivation during the tumorigenesis. It is well known that expression of certain genes may be altered during the malignat transformation [40]. The genes coding antioxidant enzymes may be one of a number of genes whose expression is down or up regulated during the carcinogenic process. In agreement with our finding decreased G-Px activity in plasma as well in serum obtained from patients with gastric and colorectal cancer has been determined previously [41,42] Increased SOD activity in gastric tumors has been reported recently [43,44]. It has been reported that the activity of SOD in the gastric mucosa decreased significantly following the successful eradication of helicobacter pylori, whereas in the corpus activity did not change significantly in biopsy specimens obtained from helicobacter pylori infected patients [45]. Data showing plasma SOD activity in gastric and/or colon cancer are highly poor. In agreement with our finding, plasma SOD activity in the patients with gastric cancer were measured higher than the value obtained in healthy donors [46]. We could not find any association between metastases and neither SOD nor G-Px activity. Indeed, as far as we know, there is no study examined the relation between metastases and plasma G-Px activity in patients with gastric or colon cancer. Toh et al.[47] determined a positive

correlation between the Mn-SOD expression level and venous invasion in both gastric and colorectal carcinomas and suggested that the colorectal carcinoma with lymph node metastases showed significantly higher Mn-SOD expression than those without it. In a study investigating the activity of various antioxidant enzymes in mitochondrial fraction of colorectal tumor tissues, no relationship was observed between the histopathological results and the mitochondrial G-Px and SOD activities [48]. In the present study, we determined correlations between histological grade and both G-Px and SOD in the gastric+colon cancer group. Those correlations were so weak that may not have a clinical significance. However, determined correlations between antioxidant enzymes and tumor markers in the gastric cancer group (AFP and G-Px ,r:0.63, P<0.05; CA15-3 and SOD, r:-0.73, P<0.05) are strong and should be taken into consideration by clinicans

In conclusion, low plasma level of 8-OHdG determined in the present study may implicate deficient repair of oxidative DNA damage in patients with gastric and colon cancer. Neither 8-OHdG nor G-Px and SOD were in relation with histopathological data and metastases but associated with some tumor markers. We hope our data will inspire to further investigations.

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