Increased Plasma Levels of 8-Hydroxydeoxyguanosine Are Associated with Development of Colorectal Tumors

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Summary **Increased oxidative stress is generally thought to be associated with tumorigenesis. In this cross-sectional study, we evaluated plasma 8-hydroxydeoxyguanosine (8-OHdG) levels in patients with colorectal adenoma and cancer, as a surrogate marker of oxidative damage to deoxyribonucleic acid (DNA). We collected blood samples from 58 patients with adenoma, 32 with early cancer, 25 with advanced cancer, and 36 without polyps or cancer (as controls), and measured plasma levels of 8-OHdG by enzyme-linked immunosorbent assay. Univariate analysis by logistic regression showed that an increased level of 8-OHdG was a significant risk for adenoma [odds ratio (OR) 1.393, 95% confidence interval (CI) 1.008–1.926, $p = 0.045$]. In patients with early cancer, univariate analysis revealed significant differences for age, body mass index (BMI), systolic blood pressure, and 8-OHdG level. Subsequent multivariate analysis revealed that 8-OHdG [OR 1.627, 95% CI 1.079–2.453, $p = 0.020$] and BMI [OR 1.283, 95% CI 1.038–1.585, $p = 0.021$] were significant risk factors for early cancer. However, 8-OHdG was not a significant risk factor for advanced cancer. Our results suggest that an increased plasma level of 8-OHdG is associated with development of colorectal adenoma and cancer.**

***Key Words*: oxidative stress, tumorigenesis, reactive oxygen species, colonoscopy, early cancer**

Introduction

Reactive oxygen species (ROS) are generally produced endogenously through metabolic processes in living cells. Excessive energy intake causes excessive production of nicotinamide adenine dinucleotide and superoxide anions in mitochondria. These ROS can easily attack proteins, lipids, sugars, and deoxyribonucleic acid (DNA) [1]. It has been suggested that DNA damage due to oxidation leads to tumorigenesis, although the precise mechanisms are still being investigated [1–4].

Urinary level of 8-Hydroxydeoxyguanosine (8-OHdG) has been well studied as a marker of oxidized DNA, and urinary 8-OHdG was also expected to be prospective biomarker for early prediction of lifestyle related-disease [5–7]. In addition, so far, urinary levels of 8-OHdG in patients with various cancers have been reported [8]. Measurement of 8-OHdG *in vitro* has been performed by high-performance liquid chromatography (HPLC), but the procedure is rather complicated. Recently, enzyme-linked immunosorbent assay (ELISA) kits for 8-OHdG, which are applicable for not only urine but also blood samples, have become commercially available [1]. Comparing to urinary samples, studies of colorectal tumors using blood samples are not so many. Chang *et al.* have reported that serum levels

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of 8-OHdG in patients with colorectal cancer are approximately 4 times higher than those in controls [9]. In contrast, Dincer *et al.* reported that plasma levels of 8-OHdG in patients with colon cancer were decreased [10].

In the present study, to investigate the association of oxidative stress with colorectal tumorigenesis, we collected plasma samples from patients with adenoma, early cancer, and advanced cancer, and evaluated the link between 8-OHdG level and the risk of colorectal tumors.

Patients and Methods

Patients

This investigation was a cross-sectional study of hospital-based patients. From 2004 until 2006, patients who visited Yamagata University Hospital for colonoscopic examination were consecutively screened. Patients were enrolled on the basis of the following inclusion criteria: 1) age of at least 31 years; 2) successful completion of colonoscopic observation through to the cecum; 3a) histopathological confirmation by hospital pathologists (for patients with adenoma or cancer) and 3b) confirmation of lack of obvious polyps upon endoscopic examination of the entire colon and rectum (for controls). The following exclusion criteria were also applied: 1) previous surgery of the gastrointestinal tract; 2) presence of inflammatory bowel disease; 3) current or previous cancer in any organ; and 4) insulin injection therapy. This study was approved by the Ethics Committee of Yamagata University Faculty of Medicine. When we had obtained informed consent from the patients that met our criteria, fasting blood samples were collected. If the patients

had suspected cancer lesions or advanced cancer lesions, blood was collected before endoscopic or surgical resection. Adenomas or cancerous lesions were treated by polypectomy, endoscopic mucosal resection, laparoscopic surgery, or open surgery, as appropriate, after which the histopathological diagnosis was confirmed. Cancer lesions with a depth of up to T1 were designated as early cancer, according to the pathologic T factor of the TNM staging system [11].

Measurement

Using plasma samples, we measured the levels of fasting glucose, triglyceride (TG), high-density lipoprotein cholesterol (HDL), and 8-OHdG. Before measurement of 8-OHdG, plasma samples were filtered using membrane filters (Microcorn YM-10, Nihon Millipore Co Ltd, Tokyo, Japan), then 8-OHdG was measured by ELISA (high-sensitivity ELSA kit for 8-OHdG, Nikken Seil Co. Ltd, Shizuoka, Japan).

We first compared the mean values for controls, adenoma, early cancer, and advanced cancer. Then, we estimated the risk of colorectal tumors by logistic regression analysis (each tumor group versus the control).

Statistics

For all statistical analyses we used the SPSS statistical software package for Windows version 11.0J (SPSS Inc. Tokyo, Japan). Distributions were tested by chi-squared test. Comparisons among groups were tested by ANOVA, and Dunnett's test was additionally used if ANOVA revealed significance ($p<0.05$). Odds ratios were tested by logistic regression analysis.

Table 1. Backgrounds of the patients with adenoma, early cancer, and advanced cancer

	Controls	Adenoma	Early Cancer	Advanced Cancer	p value
Number	36	58	32	25	
Male/ Female	19/17	40/18	23/9	14/11	NS
Age [years]	61 ± 13	65.1 ± 12.1	67.5 ± 11	67.8 ± 9.6[f]	$p = 0.046$
C. Smokers (%)	5 (13.9%)	18 (31.0%)	9 (28.1%)	8 (32.0%)	NS
BMI [kg/m^2]	23 ± 2.7	22.7 ± 3	24.8 ± 3.2	22.7 ± 3.8	NS
BP-S [mmHg]	123 ± 13	127 ± 14	130 ± 13	128 ± 18	NS
BP-D [mmHg]	73 ± 9	74 ± 10	77 ± 7	76 ± 10	NS
FPG [g/dl]	109 ± 32	106 ± 22	102 ± 15	113 ± 42	NS
TG [mg/dl]	105 ± 78	136 ± 81	128 ± 70	116 ± 53	NS
HDL [mg/dl]	58 ± 18	54 ± 21	48 ± 14	61 ± 83	NS
8-OHdG [ng/10 ml]	3.11 ± 1.3	3.92 ± 1.88	4.17 ± 1.62[§]	3.47 ± 1.8	$p = 0.046$

C. Smokers, current smokers; BMI, body mass index; BP-S, systolic blood pressure; BP-D, diastolic blood pressure; FPG, fasting plasma glucose; TG, triglyceride; HDL, high-density lipoprotein; 8-OHdG, 8-hydroxydeoxyguanosine. Values were expressed as mean ± SD. In p values, Gender and smokers were evaluated by chi-squared test, and Age, BMI, BP, FPG, TG, HDL, and 8-OHdG were evaluated by ANOVA. Additional Dunnett's test revealed a significant difference in age for advanced cancer ([f], $p<0.033$) and in 8-OHdG of early cancer ([§], $p<0.041$).

Results

Backgrounds of patients and mean values of 8-OHdG

We collected blood samples from 58 patients with adenoma, 32 with early cancer, 25 with advanced cancer and 36 without polyps or cancer (as controls).

Clinical backgrounds of patients in the adenoma, cancer, and control groups are shown in Table 1. There were no significant differences in the distributions of genders and current smokers. Mean values of body mass index (BMI), blood pressure (BP), fasting plasma glucose, TG, or HDL were not significantly different among the groups. However, mean age in patients with advanced cancer was significantly higher than that in the controls (Dunnett's test, $p = 0.033$). Controls had the lowest mean level of 8-OhdG, and patients with early cancer had the highest, being significantly higher than in the controls (Dunnett's test, $p = 0.041$).

Risks of 8-OHdG for colorectal tumors

In the patients with adenoma, only the 8-OHdG level [odds ratio (OR) 1.393, 95% confidence interval (CI) 1.008–1.926, $p = 0.045$] revealed a significant difference by univariate analysis with logistic regression (Table 2). Current smoking, BMI, systolic BP, glucose, and TG were not recognized as significant risk factors in patients with adenoma.

In the patients with early cancer, age [OR 1.408, 1.003–1.096, $p = 0.037$], BMI [OR 1.236, 95% CI 1.034–1.478, $p = 0.020$], systolic BP [OR 1.047, 95% CI 1.004–1.092, $p = 0.03$], and 8-OhdG level [OR 1.647, 95% CI 1.143–2.378, $p = 0.007$] showed significant differences by univariate analysis (Table 3). Multivariate analysis (adjusted by age, BMI, systolic BP, and 8-OHdG) showed that 8-OHdG level [OR 1.627, 95% CI 1.079–2.453, $p = 0.020$] and BMI [OR 1.283, 95% CI 1.038–1.585, $p = 0.021$] were significant risk factors for early cancer.

In the patients with advanced cancer, only age [OR 1.064, 95% CI 1.010–1.121, $p = 0.020$] revealed a significant odds ratio by univariate analysis (Table 4). Unlike adenoma and early cancer, 8-OHdG level was not recognized as a significant risk factor for advanced cancer.

Table 2. Logistic regression analysis of factors in colorectal adenoma

	Univariate analysis		
	OR	(95% CI)	*p* value
Gender	1.988	(0.842–4.693)	0.117
Age [years]	1.026	(0.992–1.062)	0.131
C. Smokers	2.790	(0.932–8.350)	0.067
BMI [kg/m^2]	0.980	(0.839–1.144)	0.794
BP-S [mmHg]	1.026	(0.992–1.061)	0.133
FPG [g/dl]	0.996	(0.981–1.012)	0.645
TG [mg/dl]	1.006	(0.999–1.013)	0.083
8-OHdG [ng/10 ml]	1.393	(1.008–1.926)	0.045*

Odds ratios of adenoma vs controls were evaluated by univariate logistic regression analysis. *$p<0.05$.

Table 4. Logistic regression analysis in colorectal advanced cancer

	Univariate analysis		
	OR	(95% CI)	*p* value
Gender	1.139	(0.408–3.176)	0.804
Age [years]	1.064	(1.010–1.121)	0.020*
Smoker	2.918	(0.824–10.330)	0.097
BMI [kg/m^2]	0.97	(0.821–1.146)	0.72
BP-S [mmHg]	1.025	(0.989–1.063)	0.182
FPG [g/dl]	1.004	(0.990–10.18)	0.601
TG [mg/dl]	1.003	(0.995–1.010)	0.511
8-OHdG [ng/10 ml]	1.168	(0.834–1.636)	0.367

Odds ratios of advanced cancer vs controls were evaluated by univariate logistic regression analysis. *$p<0.05$.

Table 3. Logistic regression analysis of factors in colorectal early cancer

	Univariate analysis			Multivariate analysis		
	OR	(95% CI)	*p* value	OR	(95% CI)	*p* value
Gender	2.287	(0.832–6.284)	0.109			
Age [years]	1.048	(1.003–1.096)	0.037*	1.039	(0.986–1.094)	0.156
Smoker	2.426	(0.717–8.210)	0.154			
BMI [kg/m^2]	1.236	(1.034–1.478)	0.020*	1.283	(1.038–1.585)	0.021*
BP-S [mmHg]	1.047	(1.004–1.092)	0.033*	1.028	(0.979–1.080)	0.270
FPG [g/dl]	0.989	(0.967–1.011)	0.313			
TG [mg/dl]	1.004	(0.998–1.011)	0.209			
8-OHdG [ng/10 ml]	1.649	(1.143–2.378)	0.007**	1.627	(1.079–2.453)	0.020*

Odds ratios of early cancer vs controls were evaluated by univariate and multivariate logistic regression analysis. *$p<0.05$, **$p<0.01$.

Discussion

Previously, Chang *et al.* reported that the serum 8-OHdG level was about 4-fold higher in colorectal cancer patients than in controls [9]. Our present data differed in several respects. Mean levels of 8-OHdG in all three groups (adenoma, early cancer, and advanced cancer) tended to be increased from the mean level in the controls. However, the mean 8-OHdG level was significantly increased only in early cancer, and was merely 1.3-fold higher than in the controls. Of course, the studied cohorts in the two investigations were different. In addition to the mean level, we examined whether the plasma 8-OHdG level was a risk factor for colorectal tumors, and found that it was a significant risk factor for colorectal adenoma and early cancer. Previous reports have not calculated the odds ratios for plasma 8-OHdG in patients with adenoma, early cancer, or advanced cancer.

Two potential sources of the increased plasma 8-OHdG in patients with colorectal tumors have been proposed. One is extra production from tumor tissues. Kondo *et al.* [12] and Park *et al.* [13] reported high levels of 8-OHdG in colorectal tumor cells obtained by surgical resection. Using immunohistochemistry, they also demonstrated strong reactivity for 8-OHdG in colorectal cancer tissues, but not in adenoma tissues [14]. However, in our present study, the plasma 8-OHdG level was not so high in patients with advanced cancer in comparison to patients with early cancer. Thus, based on our data, it appears that cancer tissues themselves are not always the major source of increased 8-OHdG levels in plasma. Of course, we were unable to draw any firm conclusion from our preliminary data, and a further more detailed study will be needed. The other potential source of 8-OHdG is general oxidative stress brought about by excessive production of ROS. In this connection, it has been demonstrated that obesity or metabolic syndrome is strongly associated with the incidence of colorectal tumors [15]. In obesity or metabolic syndrome, oxidative stress and 8-OHdG levels (in blood and urine) are certainly increased [16–18]. DNA damage caused by oxidative stress probably plays a role in tumorigenesis caused by obesity or metabolic syndrome [18]. Our present finding that plasma levels of 8-OHdG were increased in patients with adenoma and early cancer would appear to support this hypothesis of tumorigenesis in relation to obesity or metabolic syndrome. By the way, oxidative stress can occur not only through excessive ROS production but also reduction of antioxidative agents. Chang *et al.* have reported that antioxidative agents such as superoxide dismutase are slightly decreased in patients with colorectal cancer [9]. Future studies of cancer incidence will therefore also need to focus on antioxidative agents.

In the present study, 8-OHdG was not detected at high levels in patients with advanced cancer. Possible reasons could have been decreased energy intake and nutritional disorders in patients with advanced cancer. In the present study, age was recognized as a risk factor for advanced cancer, which seems to be reasonable. Our patients with advanced cancer had a significantly higher mean age, and generally 8-OHdG levels increase with age [19]. Therefore, in the present study, our results must be interpreted carefully for patients with advanced cancer.

We must discuss about smoking. The plasma 8-OHdG level is usually increased by smoking [20]. However, in our study, a current smoking habit was not recognized as a risk factor for colorectal tumors. A recent meta-analysis has suggested that smoking increases the risk of colorectal cancer [21]. Hereafter, therefore, the effects of smoking must be investigated in more detail.

In conclusion, we have revealed that increased plasma levels of 8-OHdG are associated with an increased risk of colorectal adenoma and early cancer. Our data support the hypothesis that colorectal tumorigenesis is linked to excessive oxidative stress, and suggest that further investigations of factors such as ROS production are warranted in patients with obesity and metabolic syndrome.

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