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Original Article

Paraoxonase-1 and arylesterase activities in patients with colorectal cancer

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Abstract: Background: The aim of this study was to evaluate paraoxonase-1 (PON1) and arylesterase (ARE) activities and oxidative stress status in patients with colorectal carcinomas (CRC). Materials and methods: Thirty-three patients (20 male, 13 female) with CRC and 30 healthy controls were enrolled in the study. Blood samples were obtained from the CRC patients before adjuvant therapy. Serum samples from CRC patients and healthy controls were analyzed for PON1 and ARE activities. Results: The PON1 and ARE activities of the patients with CRC were significantly higher compared to those of the control group (PON1 activity is 125.35±20.07 U/L for CRC patients and 1.22±0.48 U/L for control group, P<0.001; ARE activity is 160.76±10.79 U/L for CRC patients). ARE levels showed a positive correlation with smoking status (P=0.04). PON1 activity was higher in colon carcinoma patients (135.95±19.3 U/L) rather than rectal carcinoma patients (97.08±5.24 U/L) but it was not statistically significant (P=0.72). Conclusion: Serum PON1 activity is increased in patients with CRC, and serum ARE levels showed a positive correlation with smoking status. PON1 activity was higher in colon carcinoma patients. There is no other study in literature investigating these activities for CRC patients. It should be reevaluated by larger clinical trials.

Keywords: Colorectal cancer, paraoxonase, arylesterase

Introduction

Colorectal cancer (CRC) is the leading cause of cancer-related death worldwide [1]. The risk of developing CRC is influenced by both environmental and genetic factors. CRC mortality rates are substantially higher in males than in females. Although inherited susceptibility results in the most striking increases in risk, the majority of CRCs are sporadic rather than familial [2].

There is a well documented association between chronic ulcerative colitis and colonic neoplasia, with the extent, duration, and activity of disease being the primary determinants [3]. Although there are much less data, it appears that pancolitis due to Crohn's disease is associated with a similar relative risk of colon malignancy although the data are less consistent. Increasing evidence suggests that diabetes mellitus is associated with an elevated risk of CRC [4, 5]. Two large prospective cohort studies have shown that being obese confers an approximately 1.5-fold increased risk of developing CRC relative to being normal weight [6, 7] while a third concluded that the increased risk associated with obesity was limited to men [8]. Cigarette smoking has been associated with increased incidence and mortality from CRC [9]. Physical activity, diet high in fruits and vegetables, fiber, resistant starch, folic acid and folate, vitamin B6 (pyridoxine), calcium and dairy products, vitamin D, magnesium, garlic, fish consumption and drugs (aspirin and NSAIDs) are protective factors against CRC [10-20].

Oxidative stress plays an important role in carcinogenesis. PON1 is an antiatherosclerotic enzyme located on high-density lipoprotein

Table 1. Mean age, PON1 and ARE activities of the patients' and control group

	CRC patients group (n=33)	Control group (n=30)	*P* value
	Mean ± SD[1]	Mean ± SD	
Age (years)	62.28±14.77	60.77±6.43	0.600
PON1 (U/L)	125.35±20.07	1.22±0.48	0.001**
ARE (U/L)	160.76±10.79	0.98±0.01	0.001**

**P<0.01. [1]SD: standart deviation.

(HDL) [21]. Oxidative DNA damage may contribute to cancer risk and the antioxidant PON1 is one endogenous free radical scavenger in the human body which could therefore exert an influence [22]. In literature, serum levels of PON and ARE were found to be lower in the metabolic syndrome group compared to the control group [23]. Decreased activities were associated with worser prognosis in multiple myeloma patients [24]. ARE activity is higher in patients with ovarian cancer than in patients with benign ovarian tumors; however, the serum activity of ARE is similar between patients with cancer and healthy individuals [25]. Serum PON1 enzyme activities may play a role in the progression and/or development of esophageal squamous cell carcinoma [26]. ARE enzyme activity was significantly lower in patients with bladder compared to controls, whereas PON1 enzyme activity did not show significant differences [27]. PON1 enzyme activity was found higher in prostate cancer patients [28]. In small studies, PON1 and ARE activities were found significantly lower in patients with CRC compared to controls [22, 29]. A decrease in serum activities of ARE as well as lactonase activity of PON were found in the patients with pancreatic cancer (PC) and those with chronic pancreatitis. The lowest PON1 activities were observed in the patients with PC [30].

In a study done in our country, PON1 activity was found to be lower in patients with breast cancer than in patients with lung and colorectal cancer and there were positive correlations between the serum PON1 and ARE activities in patients with colorectal cancer but they didn't mention how many colon and rectal carcinoma patients were included in the study [31].

Our aim was to investigate the PON1 and ARE activities in CRC patients and their relationship with clinical parameters.

Materials and methods

The study population

This prospective clinical trial was conducted in our community hospital at department of medical oncology. A total of thirty-three newly diagnosed operated CRC patients and thirty age and sex-matched healthy controls were included in the study. The patients with known lipid disorders, cardiovascular diseases, diabetes mellitus, chronic infection and inflammation, renal failure, those who used antilipidemic and antioxidant drugs were excluded from the study. The protocol for sample collection was approved by the Dr Sadi Konuk Research and Education Hospital Ethics Committee and written informed consent was provided prior to the assessment. The pathological tumor stage was defined according to the 7th edition of the tumor-node-metastasis (TNM) classification of American Joint Committee on Cancer (AJCC).

Serum preparation

Venous blood samples were collected in tubes from the antecubital vein, after an overnight fastening. The tubes were centrifuged at 2000 g (10 min) to remove the plasma and serum. Then plasma and serum samples were kept at -80°C until the analysis of PON1 and ARE activities.

Determination of PON1 and ARE activities

PON1 activity was measured by adding 20 µL of serum to Tris buffer (100 mmol/L, pH 8.0) containing 2 mmol/l $CaCl_2$ and 1 mmol/L paraoxon (O,O-diethyl-Onitrophenylphosphate (Sigma). ARE activity was measured using phenylacetate as a substrate and the reaction mixture contained 750 µL of 0.1 mol/L Tris- HCl (pH 8.5), 1 mmol/L $CaCl_2$, 125 µL of 12 mmol/L phenylacetate and 125 µL of diluted serum with water (1:10). Initial rates of hydrolysis were determined by following the increase of phenol concentration at 270 nm at 37°C. Enzyme activities were expressed in international units per 1 liter of serum (U/L). An international unit is the amount of hydrolyzed substrate in mmol/minute.

Statistical analysis

Statistical analysis was done by NCSS (Number Cruncher Statistical System) 2007&PASS

Table 2. PON1 and ARE activities according to patients' clinical parameters

n=33		n	%	Paraoksonase Mean ± SD (Median)	*p* value	Arylesterase Mean ± SD (Median)	*p* value
Gender	Female	13	39.4	172.43±275.31 (99.80)	*0.579*	161.23±13.12 (161.30)	*0.855*
	Male	20	60.6	94.74±8.48 (94)		160.45±9.33 (161.30)	
Living Status	Exitus	2	6.1	93.40±10.18 (93.40)	*0.697*	149.94±7.13 (149.94)	*0.146*
	Alive	31	93.9	127.41±178.53 (96.40)		161.46±10.68 (160.40)	
Performance Status	1	5	15.2	291.76±444.97 (91.50)	*1*	160.82±14.89 (163.20)	*0.989*
	0	28	84.8	95.63±11.05 (97.80)		160.75±10.25 (159.58)	
Smoking	Yes	14	42.4	97.25±11.28 (97.80)	*0.781*	165.26±8 (165.95)	*0.037**
	No	19	57.6	146.05±228.24 (94.30)		157.44±11.55 (159.55)	
Comorbidity	Yes	18	54.5	95.02±13.43 (91.85)	*0.48*	160.74±11.30 (160.76)	*0.99*
	No	15	45.5	161.73±256.21 (99.80)		160.79±10.54 (159.60)	
Lymphovascular invasion	(+)			134.04±198.97 (93.70)	*0.451*	159.06±10.63 (159.30)	*0.11*
	(-)			98.19±8.72 (101.20)		166.08±10.10 (167.20)	
Perineural invasion	(+)			134.66±198.84 (94.30)	*0.861*	160.39±10.88 (159.55)	*0.737*
	(-)			96.24±9.51 (100.20)		161.90±11.16 (162.60)	
Liver metastasis	(+)			205.66±330.80 (94.30)	*0.819*	158.49±12.13 (159.30)	*0.467*
	(-)			95.23±11.91 (97.80)		161.61±10.39 (160.80)	
Metastasis	(+)	8	24.2	219.58±350.81 (95.95)	*0.781*	158.35±12.96 (159.20)	*0.522*
	(-)	25	75.8	95.19±11.66 (96.40)		161.53±10.18 (160.40)	
Grade	1	9	27.3	207.72±329.80 (100.60)	*0.289*	164.47±10.32 (161.20)	*0.264*
	2	22	66.7	95.06±14.27 (95.35)		160.03±10.87 (159.58)	
	3	2	6.1	87.85±2.33 (87.85)		152.10±10.18 (152.10)	
Deep tumoral invasion[a]	1	1	3.7	101.90±--	*0.648*	177.20±--	*0.191*
	2	3	11.1	87.50±12.78 (83.50)		167.64±11.73 (169.50)	
	3	20	74.1	94.50±11.45 (95.35)		157.60±9.79 (159.58)	
	4	3	11.1	95.17±19.37 (86.20)		162.63±17.46 (163.20)	
Lymph node metastasis[a]	0	10	37	96.16±9.94 (100.20)	0.819	165.61±8.10 (164.30)	0.163
	1	4	14.8	95.78±13.59 (95.35)		156.80±6.23 (159.15)	
	2	12	44.4	92.41±14.06 (91.35)		157.65±13.34 (159.11)	
	3	1	3.7	86.20±--		144.90±--	
Stage	1	1	3	101.9±--	*0.915*	177.2±--	*0.348*
	2	10	30.3	96.22±11.97 (100.2)		163.03±8.34 (162.25)	
	3	12	36.4	93.98±13.14 (93.95)		159.30±11.78 (159.51)	
	4	10	30.3	194.46±313.88 (94)		158.59±11.44 (159.43)	
Localisation	Rectum	9	27.2	97.08±5.24 (96.4)	*0.728*	161.53±6.44 (159.6)	*0.748*
	Colon	24	72.8	135.95±203.06 (96.45)		160.47±12.13 (160.85)	

[a]Patients with unknown data concerning the variables are not included in the analysis. *P<0.05.

(Power Analysis and Sample Size) 2008 Statistical Software (Utah, USA). During the evaluation of study variables; descriptive statistical methods (mean, standard error, median, rate and ratio); Student t test for intergroup comparison of normal distribution curved variables; Kruskal Wallis test and Mann Whitney U test for non-normally distributed variables (because of limited number of cases) were used. Linear regression analysis was used for multivariate analysis. Statistical significance was accepted as P<0.05 in all tests.

Results

Mean age, PON 1 and ARE activities of the patients' and control group were shown in **Table 1**. The PON1 and ARE activities of the patients with CRC were significantly higher compared to those of the control group (PON1 activity is 125.35±20.07 U/L for CRC patients and 1.22±0.48 U/L for control group (P<0.001); ARE activity is 160.76±10.79 U/L for CRC patients). ARE levels showed a positive correlation with smoking status (P=0.04) (**Table 2**). PON1 activity was higher in colon carcinoma patients (135.95±19.3 U/L) rather than rectal carcinoma patients (97.08±5.24 U/L) but it was not statistically significant (P=0.72) (**Table 2**). Patients with metastasis showed higher levels of PON1 rather than the group with no metastasis but it was not statistically significant (P=0.781). Female patients with CRC had high-

er levels of PON1 but it was not significant (P=0.579).

Discussion

Research on lipid peroxidation increased in recent years. The gastrointestinal tract, especially the colon, is constantly exposed to reactive oxygen species (ROS) which originates from endogenous and exogenous sources [32]. The low antioxidant capacity in the colon may facilitate ROS-mediated injury and lead to inflammatory diseases such as ulcerative colitis, specifically in the colon [32]. Human serum PON1 binds to HDL and contributes to the elimination of carcinogenic lipid-soluble radical from lipid peroxidation [31].

In literature, PON1 levels were generally lower in various types of cancers [24, 29-31], but in prostat carcinoma PON1 activity was found to be higher [28]. ARE levels were found to be higher in ovarian carcinoma [25]. Possible involvement of oxidative stress influences the levels of PON1 and ARE and their activities in different types of cancers. ARE levels showed a positive correlation with smoking status in our study. This finding also suggests the possible role of antioxidant stress.

PON1 levels were higher in female CRC patients but ARE levels did not change according to gender but this finding was not statistically significant. There are only few reports which emphasizes the differences of PON1 and ARE activities according to sex [33]. For example, for prostat carcinoma patients (all men), PON1 levels were found to be higher [28]. For colon and rectal carcinoma patients, it is not known if PON1 and ARE activities differ. In our study, there was a trend for higher PON1 levels in favor of colon carcinoma but the same correlation was not true for ARE levels.

PON1 serum levels are modulated by disease state, dietary, lifestyle and environmental factors and therefore, may vary up to 13-fold between individuals [34, 35]. The importance of PON1 as a predictive risk factor and its role in prognosis must be investigated. To our knowledge, this is the first study from Turkey which demonstrated higher PON1 and ARE levels in CRC patients.

The results of this study were derived from a small number of subjects, but might represent an important working hypothesis for further research.

Disclosure of conflict of interest

None.

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