

Investigation of GPX1 Gene Expression, Serum GPX1 and Selenium Levels on Colorectal Cancer

Kolon Kanseriinde GPX1 Gen Anlatımı, Serum GPX1 ve Selenyum Düzeylerinin İncelenmesi

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Abstract

Objective: Colorectal cancer is one of the first diseases to consider when determining the cause of cancer death. It is well known that family history, racial and ethnic background, and lifestyle play roles in colorectal cancer. Its treatment options include surgery, chemotherapy, and radiotherapy. Oxidative stress is one of the factors that play a critical role in the development of colorectal cancer. The aim of the present study was to investigate the association of the level of glutathione peroxidase 1 (GPX1) and selenium (SE) with colon cancer.

Methods: In the current study, we aimed to determine the expression levels of GPX1 genes and SE to maintain protection against oxidative stress in 35 patients with colon cancer and to investigate their normal and tumor tissues in association with clinical and prognostic aspects by using real-time polymerase chain reaction and atomic absorption spectroscopy.

Results: The results of our study showed that GPX1 gene expression was found to be statistically significantly different (two-fold greater in normal tissue; $p<0.05$) between normal and tumor tissue. Although there was a positive correlation between serum GPX1 and SE levels and increase in expression of GPX1 gene and serum GPX1 levels, there was no statistical significance ($p>0.05$).

Conclusion: Many factors are involved in the etiology of colorectal cancer. We have found that our preliminary results might show the potential role of the association between GPX1 gene expressions and SE levels. For this reason, it has been suggested that the subject should be supported by a large-scale group of patients.

Keywords: Colorectal cancer, Glutathione peroxidase 1, Selenium.

Öz

Amaç: Kolon kanseri, kanser nedeni ile meydana gelen ölümler incelendiğinde ilk sıralarda yer almaktadır. Kolon kanserinin kişinin aile ve etnik alt yapısı ile günlük yaşam tarzı ile ilişkili olduğu bilinmekte ve tedavi seçeneklerine bakıldığında cerrahi, kemoterapötik ilaçlar ve radyoterapiden oluştuğu görülmektedir. Kolon kanserinin meydana gelişinde kritik rol oynayan etkenlerden biri de oksidatif strestir. Bu bağlamda çalışmamızda, antioksidan kapasitesi çeşitli kanser türlerinde bilinen GPX1 ve selenyum elementinin kolon kanseri ile ilişkisinin araştırması amaçlanmıştır.

Yöntemler: Bu çalışmada, Kolon kanseri tanısı konmuş, 35 hastanın tümör ve çevre dokuları kullanılarak, oksidatif strese karşı savunmada kritik öneme sahip olan GPX1 gen ifade seviyelerinin, serum GPX1 düzeyi ve selenyum düzeyleri, qRT-PCR ve Atomik Absorbsiyon tekniği ile tümörlü ve normal dokular arasındaki ilişkinin klinik ve prognostik değerlendirmeler ile birlikte karşılaştırılması amaçlanmıştır.

Bulgular: Çalışmamızda elde ettiğimiz sonuçlarda, kolon kanseri hastalarında GPX1 geninin normal dokuda tümör dokusuna göre yaklaşık 2 kat daha fazla ifade edildiği ve istatistiksel olarak anlamlılık seviyesine ulaştığı tespit edilmiştir ($p=0,045$). Tümörlü dokularda GPX1 gen anlatımındaki artış ve azalmaya göre serum GPX1 ve selenyum düzeyleri ve GPX1 gen anlatımındaki artışıyla serum GPX1 serum düzeyleri arasında pozitif bir ilişki gözlemlenmekle beraber istatistiksel bir anlamlılık saptanmamıştır ($p>0,05$).

Sonuç: Etiyolojisinde birçok faktörün yer aldığı kolon kanserinde GPX1 ve selenyumun gen ifadelerinin arasındaki olası ilişkinin araştırıldığı ön çalışma sonuçlarımızın kritik rol oynayabileceği izlenimi elde etmemize neden olmuştur. Bu sebeple, büyük ölçekli hasta grubu ile desteklenmesi gerektiği sonucuna ulaşılmıştır.

Anahtar Kelimeler: Kolon kanseri, glutatyon peksidaz 1, selenyum

INTRODUCTION

Colorectal cancer is one of the first cancer types to consider when deaths are examined caused by cancer (1). It is treated like other cancer diseases with multiple and combine treatment options including surgery, radiotherapy, and chemotherapy. It is well known that colon cancer is related to the patient's family history, ethnicity, environmental factors, and lifestyle (2, 3).

Cancer is a disease that possibly ends with death and is characterized by uncontrolled cell proliferation and invasion of the surrounding tissue and/or cells from the center of the damaged cells. Cancer cells are characterized by escaping apoptosis, cell proliferation, and angiogenesis and metastasis (4, 5). The number of patients with cancer in 2020 is estimated to increase to more than 15 million worldwide (6).

It is important to develop new agents with low toxicity and high specificity because the developed chemotherapeutic agents induce drug resistance mechanisms in tumor cells, and the toxicity rates are high. Free radicals and hypoxia both trigger the inflammation processes. These cause the change in the formation of cellular micro-macrosenvironment in oxidative damage. In addition to this oxidative damage mechanism, deformation of normal cell respiration and metabolism in xenobiotics could end up causing the cancer.

The body spends its energy making homeostasis work with full capacity with oxidant and antioxidant enzyme systems for protection from reactive oxygen species (ROS) products. In case of any corruption of the protective capacity of oxidant and antioxidant enzyme systems, DNA damage might initiate and cause carcinogenesis and cell death (7-10). Some of these antioxidant enzyme systems are superoxide dismutase (SOD), glutathione peroxidase (GPX), myeloperoxidase, nicotinamide adenine dinucleotide (phosphate), and glutathione S-transferase and non-enzyme antioxidants such as vitamin C, vitamin E, vitamin A, and flavonoids.

GPX is an enzyme that is isolated from mammalian red blood cells and is divided into two subgroups: selenium (SE)-dependent and SE-independent GPX. SE-dependent GPX is located in the cell, mostly in the cytoplasm and mitochondria and needs SE to be activated. Even though GPX is active in almost all tissues, most GPX activity occurs in the liver and erythrocytes. GPX is responsible in protecting the cells from oxidative damage by reducing lipid peroxides and H₂O₂ (11).

SE-dependent GPX has four subgroups: cytosolic/mitochondrial GPX (GPX1), gastrointestinal (GPX2), plasma/extracellular (GPX3), and phospholipid- hydroperoxide (GPX4). GPX1 is an important antioxidant enzyme that plays a protective role from hydrogen peroxide and ROS. However, GPX1 metabolized cholesterol and hydroperoxides such as fatty acid peroxides. The enzyme does not metabolize fatty acid peroxides in the absence of phospholipase A2. SE is an element that the body intakes ranging between 71 µg and 152 µg per day through daily products from various resources such as mainly grains, wheat, milk products, meat, fish, and drinking water (12). Se is a trace element with a long history as a preservative for cancer (13). We hypothesized that the relationship of Se and carcinogenesis is focused on its action on apoptosis, arresting the cell cycle and supporting the DNA repair system (14). In addition, several studies showed that low Se level is responsible and related to the risk of several cancers such as lung, esophagus, stomach, liver, breast, prostate, bladder, and colorectal cancer (14-18).

The aim of the present study was to investigate GPX1 gene expression, serum GPX1 level, and Se level to understand their role on the pathogenesis of disease in patients with colorectal cancer.

METHODS

Patient selection

35 tumor tissue (25 male and 10 female) that diagnosed by colorectal cancer and their surrounding tissues were enrolled the study which received from Surgery Clinic of Istanbul Research and Education Hospital. The tissues were collected after obtaining written informed consent from the participants and approval from Ethical Committee of Istanbul University, The Istanbul Faculty of Medicine based on World Medical Association Declaration of Helsinki. Patient's medical records, and pathological reports were received to confirm the diagnosis and cancer status.

Isolation of RNA and determination of RNA purification

Tumor tissues were homogenized. The homogenization procedure was completed using PureLink™ RNA Mini Kit. MagNa Lyser Green Beads were used to start homogenization including an incubation period of 60 s at 6500 rpm. The tubes had 25 mg tissue sample and 500 µl lysis buffer. After homogenization, RNA was isolated by using PureLink™ RNA Mini Kit according to the manufacturer's instruction. The quality and quantity of RNA were measured by using NanoDrop (Thermo Scientific, USA). The concentration was maintained at an optical density (OD) of 260 nm, and the purification level was detected at an OD ratio of 260 nm/280 nm.

Synthesis of cDNA

After isolation of RNA, cDNA was synthesized by using suitable oligo (dT) primers and High Capacity RNA-to-cDNA™ Kit. Samples were amplified at 37 °C for 1 min and 95 °C for 5 min.

Gene expressions by real-time polymerase chain reaction (qRT-PCR)

GPX1 (TaqMan® Gene Expression Assay, ID: Hs00829989_gH) gene expressions were determined by using qRT-PCR with Agilent Technologies Stratagene Mx3005p. Amplifications were completed with 20 µl reaction volume including cDNA, gene expression probe of TaqMan, suitable primers, and TaqMan master mix. β-Actin (TaqMan® Gene Expression Assay, ID: Hs99999903_m1) was used to normalize the GPX1 gene expression.

Evaluation of qRT-PCR results

The results were analyzed by using the "Relative Quantification" method. The CT values of targeted gene from samples and endogenous control HGPRT genes were used to calculate ΔCT.

$$\Delta\Delta CT = [(C_{\text{target}}) - (C_{\text{housekeeping}})] - [(C_{\text{control target}}) - (C_{\text{control housekeeping}})]$$

The results were examined by 2^{-ΔΔCT} (19). Figure 1 shows the GPX1 gene amplification for all tissues and their CT results.

Level of Se with atomic absorption spectroscopy

Thermo Scientific Atomic Absorption Spectrophotometer was used to determine SE level by using specific Se lamb. The calibration of the measurements was based on the copper standard (3.5 mg/l (ppm) copper standard measurement: 0.4 A).

Level of serum GPX1

The level of GPX1 serum was determined by enzyme-linked immunosorbent assay (USCN Life Science) according to the manufacturer's instruction. Serum samples were isolated from the patient's blood. All patients and their serum were studied in duplicate.

Statistical analysis

Statistical analyzes were performed using SPSS version 21.0 statistical software package (SPSS, Chicago, IL, USA). A p-value <0.05 was con-

Parameters	Patients (n=35)
Age	63,57±10,74
Sex (Female/Male)	25/10
Level of Selenium	65,57± 21,93
Level of GPX1 (pg/ml)	155,14 ±19,81
Metastasis (%)	57,1
Stage (%)	
Early Stage (T1-T2)	6,7
Advanced (T3-T4)	93,3
Lymph nodes metastasis (%)	
N0	35,7
N1	42,9
N2	21,4
Tumor Size (%)	
<4cm	21,9
≥4 cm	78,1
Differentiation (%)	
Advanced	20,7
Middle	51,7
Week	27,6
GPX1: Glutathione peroxidase 1	

Gene	95% CI	P Value	Fold Change
GPX1	(0,19-0,80)	0,045*	-2,015
GPX1: Glutathione peroxidase 1			
CI: Confidence interval			

sidered to be statistically significant. Analysis of relative expression data was performed according to the threshold cycle (CT) method. Differences in the fold changes of the tissue samples were analyzed using the Mann–Whitney U test.

RESULTS

Table 1 shows the characteristics of the patients with colorectal cancer and their demographic data. The mean age was 63.57±10.74 years for patients. It was statistically found that the GPX1 gene expression level is two times higher in tumor tissues than in surrounding tissues (p=0.045) (Table 2).

In addition, the relationship between serum GPX1 and SE levels in contrast to the increase and decrease in the expression of GPX1 gene in the tumor tissue is shown in Table 3. Although there was a positive correlation between serum GPX1 levels and the increase in GPX1 gene expression, there was no statistical significance (p>0.05).

Table 4 shows the relationship between clinical parameters and serum GPX1 and SE levels. There was no statistical significance between the relationship of expression and levels of clinical parameters.

DISCUSSION

Individual differences in oxidant and antioxidant enzyme systems are thought to play a role in many types of cancer. The system is responsible for removing harmful compounds caused by ROS (10). If the balance does not work properly between ROS and antioxidant level, the pathological process begins even if it ends up with cell damage.

Understanding the antioxidant capacity and its mechanism for the organism might block the pathological conditions. The most effective antioxidants are SOD, catalase (CAT), and GPX. SOD, CAT, and GPX1 enzymes are involved in the primer endogenous antioxidant members, and they work together to remove the impact of free radicals. While SOD is responsible especially for detoxification of ROS to hydrogen peroxide, CAT and GPX1 work for detoxification of hydrogen peroxide to H₂O and oxygen (20). The other antioxidants that are non-enzymatic are vitamin C, vitamin E, carotenoids, thiols (glutathione, thioredoxin, and lipoic acid), flavonoids, and SE, among others (20).

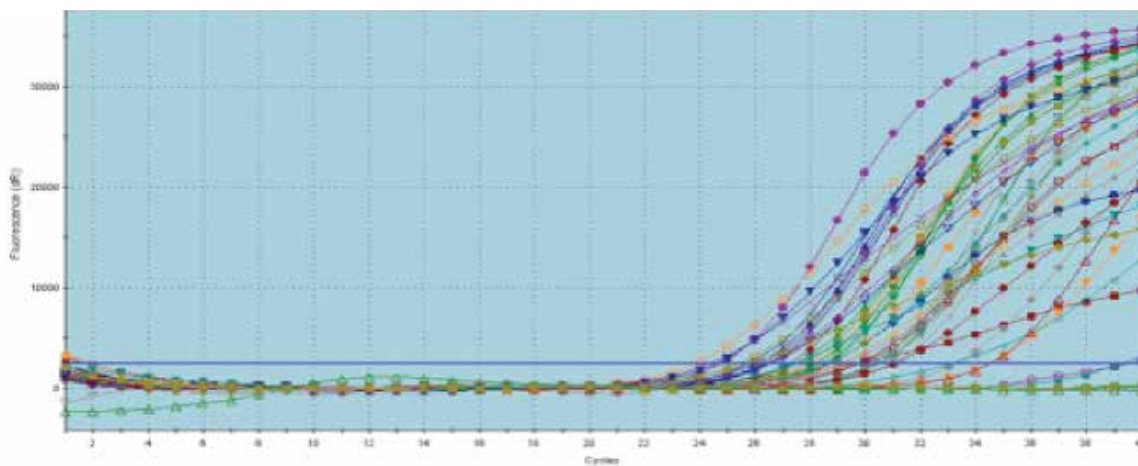


Figure 1. GPX1 gene amplification results

Table 3. The relation of level of Selenium, serum GPX1 level and GPX1 gene expression

Level of Serum	GPX1 gene expression		P Value
	Decrease Level (n:12)	Increased Level (n:23)	
Selenium	65.75±13.84	65.47±25.45	0.972
GPX1 (pg/ml)	129.17±40.11	168.70±140.92	0.351

GPX1: Glutathione peroxidase 1

Table 4. Clinical parameters and patients level of serum selenium and serum GPX1

Clinical Parameters	Level of Selenium	Level of GPX1 (pg/ml)
Tumor Size		
>4cm	64,84±23,15	140,20±62,87
<4cm	61,45±18,46	129,29±72,30
Stage		
1-2 (Early)	69,09±9,75	212,50±81,37
3-4 (Advanced)	64,37±23,14	128,57±60,22
Sex		
Male	63, 74±17,27	167,20±135,63
Female	70,13±31,45	125,00±38,22
Metastasis		
Yes	63,14±23,95	131,50±34,41
No	68,80±19,23	186,67±172,95

GPX1: Glutathione peroxidase 1

There are a variety of ongoing studies on antioxidants and their roles. Not only the level of antioxidant systems is important for all diseases but also the system is important especially in patients with cancer. There are several contradictory results about the specific antioxidant and diseases. It might be related to the disease-specific pathway. Hoffman et al. found that patients with colorectal cancer have not shown any change in GPX activities (21). Despite that finding, Hasegawa et al. conducted a study about anaplastic and papillary thyroid tumors. Their results showed that patient's GPX levels are found to be statistically low on mRNA expressions (22). Malinowska et al. studied the relationship of colorectal cancer and antioxidants and found a statistically increased level of GPX and SOD (23).

In our study, we have found similar results with the literature about GPX1 gene. Our results demonstrated that GPX1 gene expression was two times higher in normal tissue than in tumor tissue ($p=0.04$). In addition, GPX1 was 1.9 times less in tissue with lymphatic invasion than in tissue without lymphatic invasion ($p=0.04$). Moreover, we have compared the GPX1 gene expression with tissues with metastasis. The results showed that the GPX1 gene was 1.34 times less expressed in tissue with metastasis. In addition to the increase in GPX1 gene expression, we have observed that serum GPX1 levels increased in a positive manner. In contrast to these results, there are some studies that indicate an increase in GPX activity related with tumor presence (24, 25).

SE has a complex cellular biochemical system that contains the gene expression of a large number of SE-dependent proteins such as GPX1. GPX1 is an SE-dependent protein in its active form (26).

In many studies, SE has been found to play a role in the activation and expression of GPX1 (27, 28). In different epidemiological studies, it has been shown that there is an inverse proportional relationship between serum SE level and different cancer types although Se element has been shown to inhibit some type of cancer (29, 30). In a study of 169 patients with colon cancer and 169 controls in Poland and Estonia, SE levels in patients were reported to be at the lowest level. These results demonstrated that the low level of SE may be related to the high level of malignancy (31). Four hundred fifty-one patients with colorectal cancer were enrolled in the study, and the results showed the protective capacity of SE; a high SE level has a positive effect on cancer (31). Nevertheless, serum SE level has a protective role against cancer; the results showed that the mean level of SE in the serum has the ability to protect. Other than these studies, in a Phase III cohort study with 35,535 participants from 427 centers in the United States, Canada, and Puerto Rico, it was suggested that SE supplementation cannot reduce the risk of prostate or colorectal cancer (32-34).

In our study, we could not find any statistical result about serum SE level on tumor tissues and normal tissues. In many studies, characteristics such as gender, epidemiological differences, and dietary habits have led to conflicting results between colorectal cancer risk and SE levels (35, 36). Similarly, in our study, results showed that there was no any correlation between serum SE level or SE and GPX1 levels. Another study suggested that there is an inverse relationship between dietary SE levels and cancer-related deaths, including colon and rectum cancers (37). On the other hand, the study demonstrated that a statistically high level of SE is related to a decreased level of colorectal cancer (37). In addition to the protective capacity of SE, it was thought that SE is effective on cancer progression and metastasis (38, 39). The mechanism might work during the progression of cancer by blocking the carcinogenesis process on affecting the individual tumor like tumor-specific effect (38, 39).

It is believed that the tumor-specific effect of SE is caused by the fact that the extracellular area of the cancer cell is more reductive than the intracellular area. Furthermore, this differentiation of cells might potentially promote more SE uptake into the cell. The other hypothesis is about the roles of SE on metastasis. It is believed that SE inhibits metastasis by reducing the expression of genes such as osteoporosis and collagen (40).

CONCLUSION

In conclusion, we have found a statistically decreased level of GPX1 gene expression on tumor tissues. However, in serum GPX1 level that has similar changes with gene expression results, we could not find a statistically important result when comparing clinical parameters.

According to the literature, there are several contradictory results about the specific antioxidant and disease. In our study on the preliminary result of gene expression, serum GPX1 and SE levels on colorectal cancer could be related to personal characteristics such as gender, epidemiological differences, and dietary habits of Turkish patients. Further studies are needed with a large-scale patient group to understand the underlying pathological role.

Ethics Committee Approval: Ethics committee approval was received for this study from Ethical Committee of Istanbul University, Istanbul School of Medicine.

Informed Consent: Yazılı hasta onamı bu çalışmaya katılan hastalardan alınmıştır.

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