Clinical and Experimental Health Sciences

Investigation of Gpx1 Gene Expression, Serum Gpx1 and Selenium Levels on Colorectal Cancer

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ABSTRACT

Objective: Colorectal cancer is one of the first lines when examined the death cause of cancer. It is well known that family history, and racial and ethnic background and daily life are related with colorectal cancer and its treatment options are surgery, chemotherapeutic agents, and radiotherapy. One of the factors that play a critical role in the development of colorectal cancer is the oxidative stress. In this research, we aimed to investigate the association of level of glutathione peroxidase 1 (GPX1) and selenium element with colon cancer.

Methods: In this study, we aimed to determine expression levels of GPX1 genes and selenium that maintain protection against oxidative stress, with 35 colon cancer patients and their normal and tumor tissues associated with clinical and prognostic aspects by using qRT-PCR and atomic absorption methods.

Results: The results of our study showed that GPX1 gene expression were 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 selenium levels and increase in expression of GPX1 gene and serum GPX1 levels, there was no statistical significance (p>0,05).

Conclusion: Colorectal cancer, that many factors are involved in its etiology, we have found that our preliminary studies results might show the potential role of association between GPX1 gene expressions and selenium 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.

1. INTRODUCTION

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

Cancer is a disease that possibly ends with death and characterized by uncontrolled cell proliferation and invasion of surrounding tissue and/or cells from the center of the damaged cells. The characteristics of cancer cells are such as escape from the apoptosis, cell proliferation, angiogenesis and metastasis (4,5). Due to the statistical result of the world, the number of cancer patients in the world in 2020 will be more than 15 million (6).

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

The body spends its energy to make homeostasis work with full capacity with oxidant and antioxidant enzyme systems for protection of the reactive oxygen species (ROS) products. In case of any corruption of the protective capacity of oxidant and antioxidant enzyme system, DNA damage might initiate and it might cause carcinogenesis and cell death (7-10). Some of these antioxidant enzyme systems are superoxide dismutase (SOD), glutathione peroxidase (GPX), myeloperoxidase (MPO) and nicotinamide adenine dinucleotide (phosphate) (NQO1), glutathione-S-transferase (GST) and non-enzymes antioxidants such as vitamin C, vitamin E, vitamin A, flavonoids etc.

GPX is an enzyme that isolated from mammalian red blood cells and has two subgroups; selenium-dependent and selenium-independent GPX. Selenium (Se)-dependent GPX is located in

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the cell mostly in cytoplasm and mitochondria and it needs to selenium to be active. Even though GPX is active almost all tissues, most GPX activity occurs in liver and erythrocytes. GPX is responsible to protect the cells from oxidative damage by the reduction to lipid peroxides and H_2O_2 (11).

Se-dependent GPX has four subgroups; cytosolic/mitochondrial GPX (GPX1), gastrointestinal (GPX2), plasma/extracellular (GPX3) and phospholipid-hydro peroxide (GPX4). GPX1 is an important antioxidant enzyme that plays a protective role from hydrogen peroxide and reactive oxygen species. However, GPX1 metabolized cholesterol and hydro peroxides such as fatty acid peroxides, the enzyme doesn't metabolized the fatty acid peroxides absence of phospholipase A2. Se is an element that intake the body between 71µg and 152µg per day by daily products from varies resources such as, mainly grains, wheat, milk products, meat, fish, drinking water (12). Se is a trace element with a long history as a preservative for cancer (13). The hypotized for Se and carcinogenesis relation is focused on its action on apoptosis, arresting the cell cycle and supporting the DNA repair system (14). In addition, the researches are showed that low Se level was responsible and related for the risk of several cancers such as lung, esophagus, stomach, liver, breast, prostate, bladder and colorectal cancer (14-18).

According to this, in our study, it was aimed to investigate of GPX1 gene expression, serum GPX1 level and Se level to understand their role on the disease pathogenesis with colorectal cancer patients.

2. METHODS

2.1. Patients 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 XXX Hospital. The tissues were collected after obtaining written informed consent from the participants and approval from XXX Ethics Committee based on World Medical Association Declaration of Helsinki. Patient's medical records, and pathological reports were received to confirm the diagnosis and cancer status.

2.2. Isolation of RNA and determination of RNA purification

First of all, tumor tissues were homogenized. To homogenization procedure were completed according to kit procedure (Purelink(TM) RNA Mini Kit). MagNa Lyser Green Beads were used to start homogenization including an incubation period 60 seconds 6500 rpm. The tubes were included 25 mg tissue sample and 500 µL lysis buffer. After homogenization, RNA were isolated by using Purelink(TM) RNA Mini Kit due to the instruction. Quality and quantity of RNA was measured by using Nanodrop (Thermo Scientific, USA). The concentration maintained at the optical density (OD) of 260 nm and the purification level was detected at OD ratio of 260 nm/280 nm.

2.3. Synthesis of cDNA

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

2.4. Gene expressions by qRT-PCR

Glutathione peroxidase-1 (TaqMan ® Gene Expression Assay,Assay ID: Hs00829989_gH) gene expressions were determined by using Real-Time PCR with Agilent Tecnologies Stratagene Mx3005p. Amplifications were completed with 20 µL reaction volume including cDNA, gene expresion probe of TaqMan, suitable primers and TaqMan master mix. . β-aktin (TaqMan ® Gene Expression Assay, Assay ID: Hs99999903_m1) was used to normalized GPX1 gene expession. The results were analyzed by using "Relative Quantification" method. Due to the method, targeted gene from samples CT value and endogenous control HGPRT genes CT value were used to calculate the DCt.

Calculation procedure: $DDCT=[(C_{target})-(C_{housekeeping})] - [(C_{control\ target})-(C_{control\ housekeeping})]$

The results were examined by 2 – DDCT (19). GPX1 gene amplification for all tissues and their CT results were given in Figure 1.

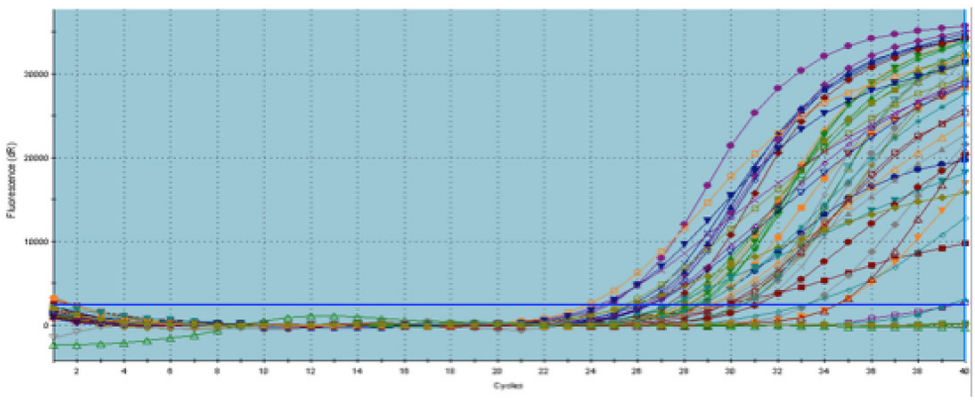

Figure 1. GPX1 gene amplification result

2.5. Level of Selenium with Atomic Absorption

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

2.6. Level of serum GPX1

Serum GPX1 level was determined by ELISA due to the kit instructions (USCN Life Science). Serum samples were isolated from patient blood. All patients and their serum were studied duplicate.

2.7. Statistical analysis

The statistical analyses were performed using the SPSS 21.0 statistical software package (SPSS, Chicago, IL). P values lower than 0.05 were assumed 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 tissues samples were analyzed using the Mann–Whitney U test.

3. RESULTS

The characteristics of the patients with colorectal cancer and their demographic data are given in Table 1. The main age were 63,57±10,74 for the patients.

Table 1. *Demographic and clinical data of the patients*

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
³4 cm	78,1
Diferentiation (%)	
Advanced	20,7
Middle	51,7
Week	27,6

GPX1: Glutathione peroxidase 1

It was statistically found that GPX1 gene expression were found in 2 times high level on tumor tissues in contrast to surrounding tissues (p=0,045) (Table 2).

Table 2. *GPX1 gene expression on tumor tissues in contrast to surroundind tissues and their fold change*

Gene	95% CI	P Value	Fold Change
GPX1	(0,19-0,80)	0,045*	-2,015

GPX1: Glutathione peroxidase 1

CI: Confidence interval

In addition, the relationship between serum GPX1 and selenium 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 could not find statistical significance (p>0,05).

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

Level of Serum	GPX1 gene expession		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

The relationship between clinical parameters and serum GPX1, selenium levels were shown in Table 4. There was no statistical significance between the relation of expression and levels with clinical parameters.

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 (Erken)	69,09±9,75	212,50±81,37
3-4 (İleri)	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

5. DISCUSSION

Individual differences in oxidant and antioxidant enzyme systems are thought to have a role in many cancer types. The system is responsible to remove of harmful compounds

that caused by reactive oxygen species (10). If the balance doesn't work properly between reactive oxygen species and antioxidant level, the pathological process begins even it ends up with cell damaged.

Understanding the antioxidant capacity and its mechanism for the organism might block the pathological conditions. The most effective antioxidants are SOD, CAT and GPX. SOD, CAT and GPX1 enzymes involved the primer endogenous antioxidant members and they work together to remove the impact of free radicals. While SOD is responsible especially to detoxification of ROS to hydrogen peroxide, CAT and GPX1 work for detoxification of hydrogen peroxide to H_2O and oxygen (20). The other antioxidants that non-enzymatic ones are vitamin C, vitamin E, carotenoids, thiol antioxidants (glutathione, thioredoxin ve lipoic acid), flavonoids, selenium and others (20).

There are a variety of studies on antioxidants and their role and the subject is still ongoing. Not only the level of antioxidant systems is important for all disease but also system is important in especially cancer patients. There are several contradictory results about specific antioxidant and the diseases. It might be related of the disease specific pathway. Hoffman et al. have studied with colorectal cancer patients and they resulted that the patients had not showed any change of GPX activities (21). Despite that Hasegawa et al. have worked with anaplastic and papillary thyroid tumors. Their results have shown that patient's GPX levels were found statistically low on mRNA expressions based (22). Malinowska et al. have studied the relation of colorectal cancer and antioxidants and the results have come up statistically increased level of glutathione peroxidase and superoxide dismutase (23).

In our study, we have found the similar results with the literature about GPX1 gene. Our results demonstrated that GPX1 gene expression was two times higher in normal tissue in contrast to tumor tissue (p=0,04). In addition to this, GPX1 have found 1,9 times less on tissue with lymphatic invasion in contrast to tissue without lymphatic invasion (p=0,04). Moreover, we have compared the GPX1 gene expression with tissues with metastasis. The results showed that GPX1 gene was found 1,34 less expressed on tissue with metastasis. In addition to the increase in GPX1 gene expression, we have observed that serum GPX1 levels also 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).

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

In many studies, selenium 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 selenium level and different cancer types although Se element has been shown to inhibit some type of cancer (29,30). In a study of 169 colon cancer patients and 169 controls in Poland and Estonia, selenium levels in patients were reported to be at the lowest level. These results demonstrated that low level of selenium might be related high level of malignancy (31). 451 colorectal patients were enrolled the study and the results were ended up selenium protective capacity such as high selenium level has the positive effect on cancer (31). Nevertheless, serum selenium level has the protective role for cancer; the results are been thought to the mean level of selenium in the serum has the ability of protection. Other than these studies, in a Phase III cohort study with 35535 participants from 427 centers in the United States, Canada and Puerto Rico, it was suggested that selenium supplementation could not reduce the risk of prostate or colorectal cancer (32-34).

In our study, we could not have found any statistical result about serum selenium 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 selenium levels (31,35). Similarly, in our study has the results that there could not observe any correlation between serum selenium level or selenium and GPX1 levels. Another study suggests that there is an inverse link between dietary selenium levels and cancer-related deaths, including colon and rectum cancers (36). Other than that the study demonstrated that statistically high level of selenium related to decreased level of colorectal cancer (36). In addition to selenium protective capacity, it is also thought to that selenium is effective on cancer progression and metastasis (37,38). The mechanism might work on during the progression of cancer by blocking the carcinogenesis process on affecting the individual tumor like tumor specific effect (37,38).

It is believed that the tumor specific effect of selenium 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 potential might promotes more selenium uptake into the cell. The other hypothesis is about the selenium roles on metastasis. It is believed that selenium inhibits metastasis by reducing the expression of genes such as osteoporosis and collagen (39).

5. CONCLUSION

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

According to literature, there are several contradictory results about specific antioxidant and the disease. In our study has the preliminary result about gene expression, serum GPX1 and selenium levels on colorectal cancer that could be related personal characteristics such as gender, epidemiological differences and dietary habits on Turkish patients. The area

needs to be lighten with large scale patient group and further studies to understand underlying pathological role.

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