A pilot study of the association of manganese superoxide dismutase and glutathione peroxidase 1 single gene polymorphisms with prostate cancer and serum prostate specific antigen levels

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Abstract

Introduction: The aim of the study was to evaluate the potential association of single gene polymorphisms of the antioxidant enzymes manganese superoxide dismutase (MnSOD) and glutathione peroxidase (GPX1) with prostate cancer (PCa).

Material and methods: Manganese superoxide dismutase and glutathione peroxidase 1 genotypes and allele frequencies in 49 prostate cancer cases (PCa group) and 98 control subjects were determined. Analysis of genotypes in control group individuals were performed in two subgroups according to serum prostate-specific antigen levels: the control group (n = 49), with prostate specific antigen (PSA) level < 4 ng/ml; and the nonPCa-high PSA control group (n = 49), with serum PSA > 4 ng/ml. Determination of MnSOD Ala-9Val and GPX1 Pro198Leu polymorphisms was performed using real-time polymerase chain reaction amplification.

Results: No association was found between GPX1 polymorphisms and PCa in all groups (p > 0.05). In the PCa group, the frequency of homozygote Val allele carriers was significantly higher in comparison to nonPCa-high PSA control cases. Therefore, Val/Val genotype was found significantly suspicious for PCa risk (OR = 2.48; 95% CI: 1.37–4.48; p = 0.002). Furthermore, an overall protective effect of the Ala allele of the MnSOD polymorphism on PCa risk was detected. These findings in this small Turkish population suggested that individual risk of PCa may be modulated by MnSOD polymorphism especially in patients with high PSA, but GPX1 polymorphism seemed to have no effect on PCa risk.

Conclusions: The presence of genetic variants of antioxidant enzymes could have a potential influence on genesis of prostatic malignancy.

Key words: genetic polymorphism, antioxidant enzymes, manganese superoxide dismutase, glutathione peroxidase 1, prostate cancer.

Introduction

Prostate cancer (PCa) is the most frequently diagnosed cancer in men and it is still the second leading cause of cancer-related mortality in the aged male population despite decreasing rates in recent years [1, 2]. During
the last decade, great efforts have been made to discover the etiological and contributing factors in tumorigenesis of the prostatic tissue [3–5]. The possible role of oxidative stress (OS), along with the formation of free radicals or reactive oxygen species (ROS) and the hypothesis of anticancer potentials of endogenous antioxidant enzymes, such as manganese superoxide dismutase (MnSOD) and glutathione peroxidase 1 (GPX1), has been reported in recent studies about the etiology and genetics of PCa [6, 7]. In the context of the endogenous antioxidant pathway, MnSOD converts ROS to $\text{H}_2\text{O}_2$, which is converted into water and oxygen by GPX and catalase (CAT) enzymes. At the end of these enzymatic chain reactions most of the ROS in the cells are scavenged and potential oxidative damage is reduced [1, 4, 7, 8].

According to their metal content SOD enzymes are divided into three subtypes, and MnSOD is the single free radical scavenger acting in the mitochondria [9]. The MnSOD enzyme is encoded by the gene which is located in the long arm of chromosome 6 (6q25). The type of polymorphism which is described as the substitution of a single nucleotide at codon 16 of the mitochondrial targeting sequence of the human MnSOD gene, resulting in either valine or alanine alterations (Ala-9-Val), is the most widely studied single nucleotide polymorphism (SNP) for MnSOD and cancer risk [1–6, 9–11]. GPX1 is a selenium-dependent enzyme which protects the cells from oxidative injury by reducing $\text{H}_2\text{O}_2$ to water [7, 8, 12]. The GPX1 gene is located on chromosome 3p21, and the genetic polymorphism which is associated with a substitution encoding for either proline or leucine at codon 198 of the human GPX1 gene has been identified previously as Pro198Leu [7, 8, 12, 13].

Considering the active role of MnSOD and GPX1 enzymes in the antioxidant ROS scavenging pathway, we hypothesized that the allelic variations in MnSOD and GPX1 could be related to PCA development risk due to the alterations in antioxidant ROS scavenging capacities of the variants of these enzymes. We further hypothesized that the allelic variations of the antioxidant enzymes and PCa may also have an association with serum prostate specific antigen (PSA) levels.

Therefore, in the present study, we aimed to evaluate the relationship between PCa, PSA levels and MnSOD (Ala-9Val) and GPX1 (Pro198Leu) polymorphisms in a small Turkish population.

Material and methods

Study design and study population

This study was executed in the Urology Department of Gaziosmanpasa University Training Hospital with 49 PCa patients and 98 control subjects. The study was approved by the local ethical committee and written informed consent was taken from all participants of the study. All of the patients were recruited from the outpatient clinics of the Urology Department. In the PCA group ($n = 49$) all of the prostatic cancers were diagnosed histologically with specimens obtained by transrectal ultrasound (TRUS) guided biopsy. Age-matched male subjects ($n = 98$) who admitted to the same department served as the control group. The control group was further divided into two subgroups: one control subgroup included the individuals with PSA levels < 4 ng/dl ($n = 49$), and no further evaluations were performed for these control cases. The other subgroup was the nonPCA-high PSA control group, which included the patients who had PSA levels > 4 ng/dl and had a TRUS biopsy of the prostate, but pathologic evaluation yielded non-neoplastic prostatic tissues. None of the participants gave a history of histologically proven neoplastic disease in other parts of their body and none of them were taking an antioxidant or vitamins including selenium. None of them had consumed alcohol within 48 h prior to blood collection, either. The control subjects were eligible if they were healthy males with or without benign prostatic hyperplasia and not diagnosed positive for prostatic or any other malignancy. Serum total PSA determinations were performed with the chemiluminescent enzyme immunoassay method using a reference range of 0–4 ng/ml (Immuliite 2000 PSA, Diagnostic Products Corporation, LA, CA, USA).

DNA isolation

Blood specimens were drawn into ethylene-diaminetetraacetic acid (EDTA) containing tubes and genomic DNA samples were extracted from the peripheral leukocytes of the collected venous blood by the High Pure PCR Template Preparation Kit (Roche Molecular Biochemicals, Mannheim, Germany) according to the instructions of the manufacturer.

MnSOD Ala-9Val and GPx Pro198Leu genotyping

To identify MnSOD Ala-9Val and GPX1 Pro198Leu SNPs, genotyping was performed using polymerase chain reaction (PCR) amplification and polymorphisms were detected with hybridization probes labeled with fluorescent dyes (LightCycler 480 II Real-Time PCR System, Roche Diagnostics, Mannheim, Germany). Target fragments of the human MnSOD and GPX1 genes were amplified with specific primers. To detect the MnSOD Ala-9Val polymorphism, we applied 10 pmol of the forward primer 5’-CAGCCTGCTAGACGGTCCC-3’ and
the reverse primer 5'-CGTGGTGCTTGCTGTGGTGC-3', and 3 pmol of the sensor probe 5'-CTCCGGTATTCTGGTGC-3' and the anchor probe 5'-LC Red 640-CGACAGCTCATCGA-fluorescein-3' and 3 pmol of the sensor probe 5'-CTCCGAAGTCTTGTTG-3' and the reverse primer 5'-TTCTGACCTGATAGTGGTAG-3', and 3 pmol of the sensor probe 5'-CAGACATTGACATCGA-fuorescein-3'. The LC FastStart Master Hybridization Probes buffer (Roche Diagnostics Inc.) was used as a reaction buffer. All primers and hybridization probes were designed and synthesized by TIB MOLBIOL (Berlin, Germany). The genotypes were identified by running a melting curve with specific Tm. Wild type MnSOD Ala exhibits a Tm of 65 ±0.5°C, while wild type GPX1 Pro yields a Tm of 66 ±0.5°C. The allele variant MnSOD Val exhibits a Tm of 56 ±0.5°C, and the allele variant GPX1 Leu exhibits a Tm of 57 ±0.5°C. The PCR reaction was as follows: initial denaturation at 95°C for 10 min, followed by 20 cycles at 95°C for 10 s, annealing at 60°C (MnSOD) and 50°C (GPX1) for 20 s, extension at 72°C for 20 s. Then a melting curve was recorded by an initial increase in temperature to 95°C, cooling the reaction mixture to 40°C at 20°C/s, holding for 30 s and then slowly heating it to 85°C at 0.1°C/s with continuous acquisition. Finally, the fluorescence signal was plotted against temperature in real time to produce melting curves for each sample.

Results

The characteristics of the groups are presented in Table I. There was no statistically significant difference between groups in terms of mean age (p > 0.05). In the PCa group, PSA levels were significantly higher in comparison to the control group (p < 0.001).

The distributions of GPX1 Pro198Leu and Mn-SOD Ala-9Val polymorphisms in all groups are presented in Tables II–IV. The distributions of the genotypes were consistent with the Hardy-Weinberg equilibrium in all groups. In terms of GPX Pro198Leu polymorphism and allele frequencies (Pro/Leu), there were no statistically significant differences between groups (p = 0.75 and p = 0.44, respectively). However, there was a significant difference in the MnSOD genotype distributions between the PCa and nonPCa-high PSA groups in terms of Val/Val genotypes. The Val/Val genotype frequency was found to be significantly higher in the PCa group in comparison to the nonPCa-high PSA group (p = 0.006). The presence of significantly high ratio of the Val allele in PCa patients was accepted as suspicious for PCa genesis in patients with high PSA with an odds ratio of 2.48 (95% CI: 1.37–4.48, p = 0.002).

Discussion

The molecular mechanisms in the initiation of PCa and other malignancies are not clearly defined yet. However, the particular role of oxidative damage and polymorphisms of the antioxidant enzyme genes in the process of carcinogenesis has been attracting great attention in recent studies [1, 6, 8, 11, 14, 15]. One well-known type of these enzyme polymorphisms is the

Table I. Distribution of patient characteristics

| Parameter         | Control (n = 49) | NonPCa-high PSA control (n = 49) | PCa (n = 49) | Value of p |
|-------------------|-----------------|----------------------------------|-------------|------------|
| Age               | 63.27 ±8.08     | 64.22 ±7.77                      | 64.81 ±6.77 | > 0.05     |
| PSA [ng/ml]       | 1.16 (0.59–1.84) | 6.63 (4.66–8.88)                 | 10.9 (7.39–14.37) | < 0.001    |
| Gleason score     | < 7             | ≥ 7                              |             |            |
| N                 | 49              | 49                               | 49          |            |

| N (%)            | 24 (49.97%)      | 25 (50.3%)                       |             |            |

Data are shown as mean ± standard deviation and median (IQR). *There were statistically significant differences from both control and non-PCa control groups.
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Mitochondrial MnSOD has a key role in the antioxidant defense mechanism, because oxidative metabolism leading to production of ROS takes place in the mitochondria. As a result of polymorphism, the secondary structure of the protein is destroyed and active transport of MnSOD into the mitochondria may be affected. This situation may hamper the localization of MnSOD in the mitochondrial matrix and deteriorate the antioxidant function, which is essential for protection of cells from oxidation [10, 11].

**Table II.** Distribution of GPX1 and MnSOD polymorphisms in patients with prostate cancer and controls

| Polymorphism | Control (N = 49) n (%) | PCa group (N = 49) n (%) | Value of p | OR (95% CI) |
|--------------|------------------------|--------------------------|------------|-------------|
| GPX1 Pro198Leu: | Pro/Pro 24 (49) 27 (55) 0.75 | | | |
| | Pro/Leu 17 (35) 16 (33) | | | |
| | Leu/Leu 8 (16) 6 (12) | | | |
| Allele frequency: | Allele Pro 65 (66) 70 (71) 0.44 0.79 (0.43–1.45) | | | |
| | Allele Leu 33 (34) 28 (29) | | | |
| MnSOD Ala-9Val: | Ala/Ala 5 (10) 3 (6) 0.65 | | | |
| | Ala/Val 20 (41) 23 (47) | | | |
| | Val/Val 24 (49) 23 (47) | | | |
| Allele frequency: | Allele Ala 30 (31) 29 (30) 0.87 1.05 (0.57–1.94) | | | |
| | Allele Val 68 (69) 69 (70) | | | |

PCa – Prostate cancer, OR – odds ratio, GPX1 – glutathione peroxidase 1, MnSOD – manganese superoxide dismutase.

**Table III.** Distribution of GPX1 and MnSOD polymorphisms in patients with prostate cancer and nonPCa-high PSA controls

| Polymorphism | NonPCa-high PSA control (N = 49) n (%) | PCa (N = 49) n (%) | Value of p | OR (95% CI) |
|--------------|----------------------------------------|-------------------|------------|-------------|
| GPX1 Pro198Leu: | Pro/Pro 22 (45) 27 (55) 0.24 | | | |
| | Pro/Leu 24 (49) 16 (33) | | | |
| | Leu/Leu 3 (6) 6 (12) | | | |
| Allele frequency: | Allele Pro 68 (69) 70 (71) 0.75 0.91 (0.49–1.68) | | | |
| | Allele Leu 30 (31) 28 (29) | | | |
| MnSOD Ala-9Val: | Ala/Ala 11 (23) 3 (6) 0.006 | | | |
| | Ala/Val 28 (57) 23 (47) | | | |
| | Val/Val 10 (20) 23 (47) | | | |
| Allele frequency: | Allele Ala 50 (51) 29 (30) 0.002 2.48 (1.37–4.48) | | | |
| | Allele Val 48 (49) 69 (70) | | | |

PCa – Prostate cancer, OR – odds ratio, GPX1 – glutathione peroxidase 1, MnSOD – manganese superoxide dismutase.

MnSOD polymorphism.
The results of the previous studies about the association of PCa with genetic polymorphisms in the antioxidant enzyme genes are generally inconclusive and contradictory [16, 17]. In a meta-analysis which included ten studies from different ethnic groups with 4608 PCa cases and 5861 controls, Wei et al. concluded that Ala/Ala and Ala/Val genotype carriers had an increased risk of PCa in comparison to Val/Val allele carriers [6]. In some other related publications, the association of the Ala allele with the increased risk of early onset, high grade and aggressive PCa risk has been reported [18, 19]. Additionally, in some reports, a stronger association between the Ala allele and the development of PCa in men with low antioxidant status or heavy smokers has been stressed [20]. In these studies the increased incidence of PCa in Ala allele carriers has been attributed to elevated levels of H$_2$O$_2$ in PCa cells due to the overexpression of SOD [18–20]. It was also noted that expression of antioxidant enzymes was at lower levels in patients with PCa than men with benign disease or healthy controls. Therefore the higher level of hydroxyl radicals eventually caused DNA damage in prostatic cells to induce cancer [2, 13, 19]. As in the study of Choi et al., some investigations have detected no significant association between PCa risk and allelic variants [1]. The effects of chronic OS in cells and implications of the damaging effects on cellular DNA, proteins and lipids to produce neoplastic transformation and promotion of tumorigenesis in steroid-dependant tumors such as PCa and breast cancer in males have also been studied previously [21]. Bica et al. reported that genetic polymorphism of MnSOD enzyme and OS may be associated with prostate cancer and rarely seen breast cancer in males [21]. Regarding the association of MnSOD Val-9 Ala polymorphism and cancer, 34 published case control studies were included in the meta-analysis by Wang et al. According to the results, genetic polymorphism of MnSOD may be associated with cancer development in various organ cancers including breast, lung, skin, ovary, pancreas and prostate through disturbed balance in the antioxidant enzyme system [22].

In the present study, there was no significant difference in PCa risk between controls with lower levels of PSA and other groups in spite of the MnSOD Ala-9Val polymorphism. However, the presence of homozygous Val/Val genotype in the PCa group seemed to be an increased risk factor for detection of PCa ($p = 0.006, OR = 2.48, 95\% CI: 1.37–4.48$) in comparison to high PSA with benign biopsy results (high PSA-nonPCa group). It was thought that, in patients with increased PSA levels, presence of the Val allele at a high frequency may be a suspicious genetic finding for detection of PCa.

Some studies concerning the potential relationship between GPX1 genetic polymorphism and PCa have been found in the literature [7–9].

### Table IV. Distribution of GPX1 and MnSOD polymorphisms in patients with prostate cancer and controls (nonPCa-high PSA controls + controls)

| Polymorphisms     | NonPCa-high PSA control + control (N = 98), n (%) | PCa (N = 49) n (%) | Value of $p$ | OR (95% CI) |
|-------------------|-----------------------------------------------|-------------------|--------------|-------------|
| **GPX1 Pro198Leu:** |                                              |                   |              |             |
| Pro/Pro           | 46 (47)                                       | 27 (55)           | 0.53         |             |
| Pro/Leu           | 41 (42)                                       | 16 (33)           |              |             |
| Leu/Leu           | 11 (11)                                       | 6 (12)            |              |             |
| **Allele frequency:** |                                              |                   |              |             |
| Allele Pro        | 133 (68)                                      | 70 (71)           | 0.54         | 0.84 (0.50–1.43) |
| Allele Leu        | 63 (32)                                       | 28 (29)           |              |             |
| **MnSOD Ala-9Val:** |                                              |                   |              |             |
| Ala/Ala           | 16 (16)                                       | 3 (6)             | 0.13         |             |
| Ala/Val           | 48 (49)                                       | 23 (47)           |              |             |
| Val/Val           | 34 (35)                                       | 23 (47)           |              |             |
| **Allele frequency:** |                                              |                   |              |             |
| Allele Ala        | 80 (41)                                       | 29 (30)           | 0.06         | 1.64 (0.98–2.78) |
| Allele Val        | 116 (59)                                      | 69 (70)           |              |             |

PCa – Prostate cancer, OR – odds ratio, GPX1 – glutathione peroxidase 1, MnSOD – manganese superoxide dismutase.
As it was revealed in the meta-analysis by Liwei et al., most of the investigators could not find an association between GPX1 Pro198Leu polymorphism and prostatic malignancy [8]. In this study, there was no association between GPX1 Pro198Leu polymorphism and PCa detection rates, either. The results about the association of GPX1 polymorphism and PCa are also inconsistent. For example, Kucukgergin et al. reported that Leu/Leu genotype was correlated with lower GPX1 activity in both control and PCa patients, whereas Erdem et al. did not find any relationship between GPX1 polymorphism and PCa [13, 23].

This is the first study in the literature to investigate the relationship between MnSOD and GPX1 polymorphism and PSA levels in control and PCa subjects. The association between PCa detection rate and Val genotype dominance in PCa patients compared to nonPCa-High PSA control patients was the most critical preliminary result of this study. In the clinical setting, keeping this knowledge in mind would increase the prediction rate and probability of PCa detection prior to TRUS biopsy by investigation of genetic allelic variants and polymorphisms of the suspected patients for PCa, especially in patients with higher PSA levels necessitating biopsy.

With the relatively small sample size of this investigative study, these results might have limited power of suggestive value and power of analysis. This study would be regarded as a hypothesis-generating study, upon which grounded further, larger population studies. Another limitation of this study was the absence of data about the antioxidant status of the patients. The study harbors one more limitation, which is the small number of PCa cases. Therefore, analyzing the association between gene polymorphisms and Gleason scores of the cancer and fully investigating the relationship between gene polymorphisms and allelic variations and Gleason score rankings was omitted.

In conclusion, the results of this preliminary study indicate that genetic polymorphisms of the antioxidant enzyme genes may play a role in the development of PCa, particularly among PCa patients with high PSA values, and men having high ratios of the Val allele may be at risk for PCa detection. However, well-designed epidemiological studies including larger sample sizes are needed to further explore the influence of genetic polymorphisms of the antioxidant enzymes and other environmental factors in different ethnicities.

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