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GENETIC POLYMORPHISM OF SOD2 AND GPX1 MODIFY OXIDATIVE STRESS BIOMARKERS IN AN IRANIAN POPULATION WITH CHRONIC KIDNEY DISEASE

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Abstract
This work aimed to investigate the association between superoxide dismutase 2 (SOD2) and glutathione peroxidase 1 (GPX1) gene single nucleotide polymorphisms (SNPs) in chronic kidney disease (CKD) patients and their influence on the serum level of oxidative biomarkers, including protein thiol and carbonyl groups (PTGs and PCGs), advanced oxidative protein products (AOPPs), nitrotyrosine, malondialdehyde (MDA), malondialdehyde adducts (MDA adducts), total oxidant status (TOS) and prooxidant-antioxidant balance (PAB). For this purpose, blood samples were obtained from 50 CKD patients with a mean age of 70.65±4.85 years. Real-time polymerase chain reaction was used for genotyping. Byproducts of oxidative stress were analyzed by ELISA and UV spectroscopic or calorimetric methods. The obtained results indicated that the risk of CKD development in subjects with the GPX1 Lue/Lue genotype increased (OR=1.67, $p<0.05$), which was considerably greater in people with the combination of SOD2 Val/Val/GPX1 Leu/Leu genotype (OR=1.92, $p<0.05$). Our results showed that the concentration of MDA, PTG, PCG, and BAP was increased in subjects carrying the SOD2 Val/Val genotype. Also, the findings revealed that individuals with the GPX1 Lue/Lue genotype showed higher levels of MDA, MDA adducts, PTG, and nitrotyrosine in the serum. Our findings supported the hypothesis that the genotypes SOD2 Val/Val and GPX1 Leu/Leu greatly enhance the risk of CKD.

Keywords: Chronic kidney disease, SOD2, GPX1, Gene polymorphism, Oxidative damage.

1. Introduction

Chronic kidney disease (CKD) refers to persistent changes in kidney structure, function, or both that have an impact on a person's health. Also, CKD is a widespread condition that affects millions of people worldwide and is a progressive and irreversible disease that requires long-term care and treatment [1]. This disease lowers the quality of life while raising mortality rates. Due to the disease's protracted length, CKD has negative effects on the body's organs and the kidney system, and it may make a patient more susceptible to acquiring chronic illnesses, including cardiovascular disease and stroke [2].

Oxidative stress, an imbalance between the body's ability to produce reactive oxygen species (ROS) and its capacity to detoxify them, has been linked to the onset and development of a number of diseases, including chronic kidney disease (CKD) [3, 4]. The human body has several important defense systems as potential modulators of oxidative stress in tissues. There are two main antioxidant groups, including antioxidant proteins that have enzymatic effects and antioxidant substances such as Vitamin C, beta-carotene and Vitamin E [6]. According to studies, in the first line of defense against ROS, the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPX) have essential roles. Individuals with CKD have experienced high levels of free radicals, which may be due to decreased kidney function in removing oxidants and toxins from the blood. SOD2 is an enzyme that converts superoxide radicals to hydrogen peroxide (H_2O_2), which can then be further detoxified by other enzymes such as GPX1 [3]. Eventually, GPX1 reduces H_2O_2 and organic peroxides using glutathione as a cofactor. Due to GPX1-dependent regulation of T helper cell proliferation and differentiation towards Th2 and Th17 phenotypes, GPX1 can also play a role in the development of ovalbumin-induced allergic asthma by promoting proinflammatory cytokine production and neutrophilia in response to endotoxins [4].

A single nucleotide in the DNA sequence that is changed into another is known as a single nucleotide polymorphism (SNP), which is a type of genetic variation. SNPs are found throughout the human genome and are considered one of the main sources of genetic diversity in different populations [5, 6]. They can be used to study inheritance patterns, identify the relationship between genetics and various diseases, assess the risk of developing certain diseases, classify different populations, and allocate drugs for patients [5, 7]. Additionally, SNPs are very useful in phylogenetic and evolutionary studies. Numerous diseases, including cancer, cardiovascular disease, and neurological disorders have been linked to GPX1 and SOD2 polymorphisms. The SOD2 gene is highly conserved across species and contains several common polymorphisms. One of these polymorphisms has been found as an amino acid substitution (Ala16Val) at position 16 of the signal peptide and nucleotide substitution (T, thymine → C, cytosine) (rs4880) in the mitochondrial localization signal (MLS), which affects the efficiency of enzyme transport into the mitochondria and, therefore, reduces its activity. As a result, this change will lead to a 30–40% reduction in the SOD2 Val allele transport efficiency in mitochondria and a reduction in the potential to neutralize superoxide anion [8, 9]. The human GPX1 gene is located on chromosome 3p21.3 and contains several common polymorphisms that have been suggested to impact gene expression and enzyme activity. A single nucleotide polymorphism at gene GPX1 at rs1050450 position was observed due to the change of proline (C**C**C codon) to leucine (C**T**C codon) at position codon 198 (Pro198Leu). Evidence from several studies suggests that the Leu allele responds less favorably to stimulation of GPX enzyme activity [10]. These genes encode enzymes that play a key role in the antioxidant defense system of the body. However, the association between SOD2 and GPX1 gene polymorphisms and oxidative stress markers in CKD patients remains unclear. In this study, we aimed to investigate the potential association between SOD2 and GPX1 gene polymorphisms and oxidative stress markers in patients with CKD in an Iranian population. Our findings could provide valuable insights into the genetic factors that contribute to oxidative stress in CKD and could potentially lead to the development of new therapeutic strategies for this condition.

2. Materials and Methods

2.1 Study design and participants

The association between SOD2 and GPX1 gene polymorphisms and oxidative stress markers in patients with CKD was investigated by designing a cross-sectional study. The trial samples were recruited from Laleh Hospital in Tehran, the capital of Iran. We recruited 50 patients with CKD and 50 healthy people (controls), and the volunteers were informed about the purpose of the study. They were of both sexes (56 men and 44 women) and different age groups (65–80). Venous blood samples (7 ml) were taken at the beginning of the investigation, placed in standard sterile polystyrene vacuum tubes with EDTA for DNA extraction, transported on ice, and kept at −20 °C.

2.2 DNA extraction and genotyping

From the whole blood of individuals that were collected, protocol was followed, DNA was extracted using a commercial DNA extraction kit using a spin column with silica membrane (Favorgen, Taiwan). In this study, TaqMan probes were utilized in real-time polymerase chain reaction (PCR) analysis to genotype two common polymorphisms associated with oxidative stress: SOD2 rs4880 and GPX1 rs1050450. Specifically, the study aimed to detect the Ala16Val polymorphism (ref. SNP ID: rs4880) and the GPX1 Pro198Leu polymorphism (ref. SNP ID: rs1050450). Genotyping assays were conducted using the 7300 Applied Biosystems instrument, a real-time system. For each reaction, 2 µL of genomic DNAs (20 ng) were added to the reaction mixtures. The PCR mixture comprised 0.5 µL of TaqMan probes and primers, 5 µL of TaqMan universal PCR master mix, and 2 µL of water, resulting in a total volume of 10 µL. The primer and probe sequences, along with the PCR genotyping conditions employed in this study, can be found in Table 1 [11-15].

Table 1. The primer and probe sequences and PCR genotyping conditions.

Gene	Primer sequence	Probe sequence	PCR protocol
rs4880	F:5'CTCCTCGTGCTTCTGCATTC 3' R:5GAGTCCGCTGTTTGGTTGT G3'	Val 16: 5'FAM-CTCGCTGCTGGCTC T-BHQ13' Ala 16: 5'VIC-CTCGCTGCTGGCCC T-BHQ13'	First step: denaturation at 95 °C for 10 min. For RT-PCR 40 cycles followed by Annealing and Extension: 60 °C for 1 min.
rs1050450	F:5'CGAGGAGCTGCCTTCTGAT T 3' R:5'GAGTCCGCTGTTTGGTTGT G 3'	Pro 198: 5'FAM-CTGCTGGGACTCTG GCCC-BHQ1 3' Leu 198: 5'VIC-CTGCTGGGACTCTG GCCG-BHQ1 3'	First step: denaturation at 95 °C for 10 min. For RT-PCR 40 cycles followed by Annealing and Extension: 62°C for 1 min.

2.2 Biochemical analyses

The serum parameters such as creatinine, urea, albumin, and hemoglobin were determined using an automated analyzer. Plasma concentrations of oxidative stress byproducts including lipid oxidation (malondialdehyde (MDA) and MDA adduct), protein oxidation like protein carbonyl groups (PCGs), nitrotyrosine, protein thiol groups (PTGs), and advanced oxidation protein products (AOPPs), as well as total oxidant status (TOS) and prooxidant-antioxidant balance (PAB), were examined according to the methods given below. According to the instructions, plasma samples were used to measure oxidative stress markers such protein carbonyl and nitrotyrosine using enzyme-linked immunosorbent assay (ELISA) Kits. Human nitro tyrosine ELISA Kit (ab210603), protein carbonyl ELISA Kit (Sigma Aldrich, MAK094A), and malondialdehyde (MDA) Adduct ELISA Kit (ARG81237) were used for measurement of nitrotyrosine, protein carbonyl, and MDA Adduct in the blood serum of two experimental groups using an ELISA reader (ELx800; BioTek microplate reader, San Francisco, CA, USA), respectively.

2.4 Impact of SOD2 and GPX1 polymorphisms on oxidative stress markers in CKD patients

Most oxidative stress parameters in serum byproducts (lipid oxidative, protein oxidative) such as MDA, PTG, and AOPP were measured spectrophotometrically (T60, PG Instruments LTD., Leicestershire, UK). The level of MDA was measured using the colorimetric technique, which was based on the reaction of thiobarbituric acid (TBA) with MDA, producing a red-colored MDA-TBA complex that had an absorbance of 530 nm (ε= 1.38 cm^{-1} mM^{-1}) [3, 16].

PTG was assayed by using a reduced 5,5′-dithiobis-2-nitrobenzoic acid reagent (DTNB) in a dark condition (light can reduce DTNB) and producing 5-thio-2-nitrobenzoic acid (TNB) with absorbance at 410 nm (ε = 8.23 cm^{-1} mM^{-1}) [3]. Other parameters such as AOPP, TOS, and PAB were measured by the methods of Witko-Sarsat [3], Erel [17], and Alamdari [18], respectively.

2.5 Statistical analyses

The results are reported as the mean±SD (standard deviation), and all data were indicative of independent experiments conducted in triplicate. For the comparison of means of the differences among two groups (patients and controls), the t-test (lack of normality), the Mann-Whitney test, and ANOVA were tested for each polymorphism, and mean ranks were compared using least-significant difference tests. P<0.05 was regarded as statistically significant in the current study's statistical analysis, which was carried out using the SPSS 20 software. To calculate the odds ratios (ORs) and 95% confidence intervals (CIs) for relationships between each polymorphism and CKD risk, logistic regression analysis was used. Using chi-square tests, the genotype and allele frequencies of the patient and control groups were compared. A subgroup analysis by age, sex, and BMI was also carried out [10, 15].

3. Results and discussion

3.1 Characteristics of patients

The demographic and some other study-relevant characteristics of the patients included in the present study are listed in Table 2. According to this table, a total of 100 subjects participated in this work, in which both patient and control groups were comprised of 22 females and 28 males. The patient and control groups' mean ages were 69.97 ± 4.08 and 70.65 ± 4.85 years, respectively. In addition, various risk factors such as hypertension, smoking status, obesity, and biochemical serum parameters were evaluated, and the findings showed significant differences in smoking and obesity between the patient and control groups (p<0.05).

Table 2. Clinical and demographic characteristics of CKD patients and controls.

Variable	Control	Patients	P value
Age (years)	69.97±4.08	70.65±4.85	0.053
Gender, *n* (%)			
Male	28 (56)	28 (56)	>0.05
Female	22 (44)	22 (44)	
Hypertension, *n* (%)			
NO	29 (58)	18 (36)	<0.05
Yes	21 (42)	32 (64)	
Smoking status, *n* (%)			
NO	32 (64)	23 (46)	<0.05
Yes	18 (36)	27 (54)	
Body Mass Index (BMI)	25.74±4.08	24.18±4.98	<0.05
Biochemical serum parameters			
Urea (mg/dl)	18.87±2.99	25.74±6.52	<0.05
Creatinine (mg/dl)	0.91±0.14	1.6±0.2	<0.05
Albumin (g/dl)	4.48±0.45	3.68±0.61	<0.05
Total cholesterol (mg/dl)	165±9.75	210±8.64	<0.05
TAG (mg/dl)	128±4.84	150±5.71	<0.05
Hemoglobin (g/dl)	14±2.14	13±1.74	<0.05

3.2 The SOD2 and GPX1 polymorphisms' relationship with CKD prevalence

This study evaluated the relationship between SOD2 and GPX1 polymorphisms and the risk of CKD, and the outcomes are presented in Table 3. According to data, in the analysis of given polymorphisms, no significant CKD risk was observed in individuals carrying the Ala/Val (OR=1.43, 95%CI=1.25–1.57, *p*=0.152) and Val/Val (OR=1.58, 95%CI=1.14–1.79, *p*=0.241) SOD2 genotypes in comparison with subjects carrying the Ala/Ala genotype. On the other hand, the analysis of GPX1 polymorphisms in patients with CKD was carried out, and only the Leu/Leu GPX1 genotype showed a statistically significant association with the risk for CKD (OR=1.67, 95%CI=1.34–1.91, *p*<0.05). However, there

was no obvious association among people with the Pro/Leu GPX1 genotype (OR=1.12, 95%CI=0.85–1.29, *p*=0.078).

In addition, SOD2 and GPX1 polymorphisms were evaluated in combination, and the results are shown in Table 3. The findings revealed that subjects with the Val/Val SOD2 genotype combined with the Leu/Leu GPX1 genotype indicated an increased risk for CKD (2-fold) when compared to individuals carrying the Val/Val SOD2 genotype and the Leu/Leu GPX1 genotype alone (OR=1.92, 95%CI=1.71–2.13, p<0.05).

Table 3. The association between *SOD2* and *GPX1* as well as combined *SOD2/GPX1* genotypes and the risk of CKD.

Genotypes	Controls, *n* (%)	Patients, *n* (%)	OR (95%CI)	*P* value
SOD2				
Ala	55 (55)	42 (42)		
Val	45 (45)	58 (58)		
Ala/Ala	16 (32)	11 (22)	1.0	
Ala/Val	23 (46)	20 (40)	1.43 (1.25-1.57)	0.152
Val/Val	11 (22)	19 (38)	1.58 (1.14-1.79)	0.241
GPX1				
Pro	61 (61)	51 (51)		
Lue	39 (39)	49 (49)		
Pro/Pro	19 (38)	13 (26)	1.0	
Pro/Lue	23 (46)	25 (50)	1.12 (0.85-1.29)	0.078
Lue/Lue	8 (16)	12 (24)	1.67 (1.34-1.91)	<0.05
SOD2* and *GPX1				
Ala/Ala+Ala/Val / Pro/Pro+Pro/Leu	24 (48)	21 (42)	1.0	
Ala/Ala+Ala/Val / Lue/Lue	9 (18)	11 (22)	1.49 (1.3-1.67)	0.205
Val/Val / Pro/Pro+Pro/Leu	14 (28)	12 (24)	0.98 (0.77-1.19)	0.396
Val/Val / Lue/Lue	3 (6)	6 (12)	1.92 (1.71-2.13)	<0.05

3.3 The relationship between GPX1 and SOD2 polymorphisms and oxidative stress biomarkers

The results indicated that SOD2 and GPX1 polymorphisms not only influence the risk of CKD development but also represent multiple relationships with biomarkers of oxidative damage. Oxidative stress has been reported in CKD due to both antioxidant depletions as well as increased reactive oxygen species (ROS) production, which can damage renal tissue and promote inflammation (due to the disturbed redox balance in the body), leading to further tissue injury and extensive damage of biological macromolecules with the accumulation of impaired biomacromolecules [19]. In the present work, the specific vulnerability of CKD patients against oxidative stress byproducts was evaluated by investigating the association between polymorphisms of SOD2 and GPX1 and plasma levels of oxidatively damaged macromolecules, including lipid peroxidation byproducts (such as MDA), protein oxidation products (such as PTG, carbonyls, AOPPs and nitrotyrosine), TOS and PAB.

3.3.1 Lipid peroxidation byproducts

In the present work, the impact of SOD2 and GPX1 polymorphisms on lipid peroxidation byproducts (produced from oxidative stress damage) was investigated, and the obtained results are summarized in Table 4. The SOD2 Ala/Ala homozygotes were considered as a reference genotype. As shown in Table 4, compared to SOD2 Ala/Ala homozygotes, the MDA level (as the main lipid peroxidation byproduct) was 11.5% higher in patients with the SOD2 Val/Val genotype. While no significant association was observed between SOD2 and GPX1 polymorphisms and MDA adducts in CKD patients. Furthermore, the impact of GPX1 polymorphisms on lipid peroxidation products was evaluated (Table 4) and the results revealed that in comparison to referent genotypes (Pro/Pro and Pro/Pro+Pro/Leu), the MDA and MDA adduct levels in CKD patients carrying Lue/Lue genotypes of these genes were slightly higher (15.45% and 16.27%, respectively).

Table 4. The association between SOD2 and GPX1 genotypes and the lipid peroxidation byproducts in CKD.

	Genotypes	MDA (mmol/L)	MDA adducts (pmol/mg)
SOD2	*Ala/Ala*	2.25±0.84 (100%)	40.23±8.61 (100%)
	Ala/Val	2.26±0.86 (100.44%)	40.39±10.43 (100.39%)
	Val/Val	2.51±0.87* (111.55%)	42.25±10.83 (105.02%)
	Ala/Ala+Ala/Val	2.2±0.85 (100%)	40.81±9.51 (100%)
	Val/Val	2.51±0.87* (114.09%)	42.25±10.83 (103.52%)
GPX1	*Pro/Pro*	2.11±0.81 (100%)	39.68±9.7 (100%)
	Pro/Leu	2.29±0.85 (108.53%)	37.59±9.52 (94.73%)
	Lue/Lue	2.54±0.89 (120.37%)	42.58±10.23 (107.3%)
	Pro/Pro+Pro/Leu	2.2±0.83 (100%)	38.62±9.61 (100%)
	Lue/Leu	2.54±0.89* (115.45%)	42.58±10.23* (110.25%)

3.3.2 Protein oxidation byproducts

However, to investigate the possible effects of SOD2 and GPX1 polymorphisms on biomarkers of oxidative stress, the protein oxidative damage byproducts were evaluated, and the obtained results are listed in Table 5. It can be seen from this table that the content of protein thiol groups (PTGs) and protein carbonyl groups (PCGs) in SOD2 Val/Val carriers was higher than that of SOD2 Ala/Ala homozygotes about 6 % and 5%, respectively. In addition, the effect of SOD2 polymorphism on the two additional markers, including nitrotyrosine and AOPP levels, was investigated and the results showed no significant association.

Moreover, the relationship between GPX1 polymorphisms and the byproducts of protein oxidative degradation was investigated, and the results showed that the concentration of PTGs was increased in individuals with the GPX1 Lue/Lue genotype when compared to the reference group. While PCGs and AOPP levels did not indicate any significant relationship. Subjects with the Lue/Lue GPX1 genotype showed an increase in nitrotyrosine concentration compared to GPX1 Pro carriers (13.4%).

Table 5. The association between SOD2 and GPX1 genotypes and the protein oxidation byproducts in CKD.

	Genotypes	PTGs (µmol/g)	PCGs (nmol/mg)	AOPPs (µmol/L)	Nitrotyrosine (nmol/L)
SOD2	*Ala/Ala*	6.5 [5.1-7.9] (100%)	2.28±0.21 (100%)	66.2 [55.1-80.4] (100%)	64.7 [45.2-95.2] (100%)
	Ala/Val	6.6 [5.8-7.6] (101.53%)	2.27±0.15 (99.56%)	62.9 [49.9-78.7] (95.01%)	64.3 [46.5-89.9] (99.38%)
	Val/Val	6.9 [5.9-8.2] (106.15%)	2.39±0.29 (104.82%)	67.3 [59.7-83.5] (101.66%)	69.8 [50.7-98.4] (107.88%)
	Ala/Ala+Ala/Val	6.5 [5.5-7.7] (100%)	2.27±0.18 (100%)	64.5 [49.7-79.5] (100%)	64.5 [45.1-90.3] (100%)
	Val/Val	6.9 [5.9-8.2] * (106.15%)	2.39±0.29* (105.28%)	67.3 [59.7-83.5] (104.34%)	69.8 [50.7-98.4] (108.21%)
GPX1	*Pro/Pro*	6.8 [5.3-8.5] (100%)	2.18±0.11 (100%)	65.8 [57.9- 80.8] (100%)	66.4 [46.7-90.8] (100%)
	Pro/Leu	6.6 [5.6-8.2] (97.05%)	2.27±0.25 (104.12%)	62.1 [49.1-80.1] (94.37%)	58.7 [45.1-86.9] (88.4%)
	Lue/Lue	7.2 [5.5-8.1]* (105.88%)	2.33±0.28 (106.88%)	65.9 [58.3-87.4] (100.15%)	75.3 [46.1-100.8]* (113.4%)
	Pro/Pro+Pro/Leu	6.7 [5.4-8.4] (100%)	2.22±0.18 (100%)	64.1 [48.7-80.8] (100%)	62.4 [45.4-90.9] (100%)
	Lue/Leu	7.2 [5.5.-8.1]* (107.46%)	2.33±0.28 (104.95%)	65.9 [58.3-87.4] (102.8%)	75.3 [46.1-100.8] (120.67%)

3.3.3 Effect of SOD2 and GPX1 polymorphisms on TOS and PAB levels

The relationship between SOD2 and GPX1 polymorphisms and TOS and PAB levels was investigated by comparing TOS and PAB concentrations in subjects with SOD2 Val/Val and GPX1 Lue/Leu genotypes. The findings are summarized in Table 6. According to the table, the two studied polymorphisms showed no significant influence on TOS concentration in CKD patients. In the case of BAP, the SOD2 Val/Val genotype caused a statistically significant increase in the BAP level by 21%, while the GPX1 polymorphism did not significantly influence the PAB concentration in CKD patients.

Table 6. The association between SOD2 and GPX1 genotypes and the TOS the PAB levels in CKD.

	Genotypes	TOS (μmol H_2O_2 equiv./L)	PAB (HK units)
SOD2	*Ala/Ala*	18.4 [13.9-42.2] (100%)	158.2 [75.9-190.2] (100%)
	Ala/Val	19.2 [14.2-50.5] (104.34%)	142.6 [67.1-195.2] (90.13%)
	Val/Val	25.2 [15.3-53.2] (136.95%)	181.9 [75.4-207.6] * (114.98%)
	Ala/Ala+Ala/Val	18.8 [14.1-48.4] (100%)	150.4 [66.3-187.7] (100%)
	Val/Val	25.2 [15.3-53.2] (134.04%)	181.9 [75.4-207.6] *(120.94%)
GPX1	*Pro/Pro*	17.9 [12.8-32.6] (100%)	147.3 [69.8-172.1] (100%)
	Pro/Leu	18.1 [12.9-35.4] (101.11%)	119.7 [61.8-192.5] (81.26%)
	Lue/Lue	23.8 [14.1-49.7] (132.96%)	162.1 [102.1-212.5] (110.04%)
	Pro/Pro+Pro/Leu	18 [12.8-32.5] (100%)	133.5 [72.1-185.4] (100%)
	Lue/Leu	23.8 [14.1-49.7] (132.22%)	162.1 [102.1-212.5] (121.42%)

4. Discussion

It has been reported that more than 800 million people suffering from chronic kidney disease worldwide [20]. Several complications of CKD, such as inflammation and cardiovascular dysfunction, as well as progressive renal dysfunction, are associated with increased levels of oxidative stress [21, 22]. Different studies have pointed to increased levels of various oxidative damage markers, including plasma F2-isoprostanes, AOPPs, lipid peroxidation byproducts, protein oxidative products, and oxidized LDL (ox-LDL) in patients with CKD [22].

Genetic background has a main role in sensitivity to oxidative stress [23]. Various studies investigated the relationship between gene polymorphisms in the key enzymes of redox regulation and the risk of CKD. In the present study, due to their main role in the antioxidant defense system, the association between SOD2 and GPX1 polymorphisms and oxidative stress damage markers in CKD patients was investigated for the first time. The obtained results revealed that the Lue/Lue genotype of GPX1 exhibited increased CKD risk. Also, Ala/Val SOD2 genotypes in combination with Lue/Lue GPX1 genotype showed an almost 2-fold increased risk for CKD. The outputs of our work indicated that there is a significant association between antioxidant gene polymorphisms (SOD2 and GPX1) and the risk of CKD. Based on the findings, the impact of SOD2 Val/Val genotype on the risk of CKD was independent. However, it was significantly more pronounced when combined with the GPX1 Leu/Leu genotype. Our findings were in good agreement with some previously reported studies concluding that the Val/Val genotype of the SOD2 gene increases the risk of diabetic nephropathy in both type 1 and type 2 diabetes mellitus [23-26]. Also, Crawford et al. [27] reported that carriers with the Val allele showed a faster progression of CKD. In agreement with the previous studies, the results of our work revealed that the risk of CKD increases with decreasing SOD2 and GPX1 antioxidant activity.

The existence of the Val allele in the SOD2 gene reduces the expression of SOD2 and produces unstable mRNA. These events inhibit the entrance of the SOD2 enzyme into the mitochondria, which results in disturbing the antioxidant defense system. Although, the polymorphism of GPX1 can impair its function [28, 29]. Mihailovic et al. investigated the effect of the GPX1 genotype on the risk of CKD and they observed no differences in the frequencies of the GPX1 genotype between the studied groups [25]. However, we found an independent impact of the GPX1 polymorphism on the risk of

CKD, and its effect was increased in combination with the Val/Val SOD2 genotype. Therefore, it can be concluded that SOD2 polymorphism can enhance the effect of GPX1 polymorphism on the risk of CKD. Evenly, Chao et al. reported a higher risk of ESRD among carriers of the combined GPX1 Leu/Leu and PPAR-γ G/G genotype [30]. These findings suggest that the antioxidant defense system involves a variety of interactions, making it difficult to determine and introduce a specific characteristic that might be used as a single disease diagnostic. This is also substantiated by the fact that, in our study, the SOD2 polymorphism showed no significant and independent impact on CKD risk. Overall, the findings of the present work confirmed that we could assume that the production of SOD2 and GPX1 enzymes with low catalytic activity can impair the antioxidant defense system and result in higher vulnerability against CKD. Fujimoto et al. [31] found that individuals carrying the Val variant were at increased risk for CAD and acute myocardial infarction. Moreover, in a large study of 776 Caucasian subjects with diabetes, Jones et al. showed an increased risk of CHD associated with the Val/Val genotype.

Moreover, in the present work, we evaluated the possible association of SOD2 and *GPX1* polymorphisms with the serum level of oxidative damage byproducts by examining the antioxidant status of the study population. The progressive oxidative stress in CKD patients could be attributed to specific genetic patterns. For example, gene polymorphisms in each component of the antioxidant enzymes can change their conformations and catalytic activities, thereby influencing the capacity of the antioxidant defense system. Therefore, it may be concluded that functional differences in genes producing antioxidant regulatory and catalytic proteins, such as SOD2 and GPX1, are responsible for the sensitivity against CKD [3, 32, 33]. Our results indicated that the concentration of some oxidative byproducts, including MDA, PTG, PCG, and BAP, was increased in subjects carrying the SOD2 Val/Val genotype. Also, the findings revealed that individuals with the GPX1 Lue/Lue genotype showed higher levels of MDA, MDA adducts, PTG, and nitrotyrosine in the serum. Cells are protected from oxidative damage by different antioxidant enzymes such as SOD and GPX, but the functional polymorphisms of the genes of these enzymes influence their localization and therefore their ability to scavenge free radicals [34, 35]. The two main enzymes that can reduce the levels of superoxide and H_2O_2 in diseases like CKD that are characterized by uremia and inflammation are GPX1 and SOD2. Therefore, malfunctioning of these enzymes can disrupt the antioxidant balance inside the cells, resulting in an increase in free radicals, which also leads to the damage of many macromolecules. Individually, the polymorphisms in the SOD2 and GPX1 genes have been investigated in the illness prognosis or susceptibility in individuals with ESRD, CKD, or diabetic nephropathy [24, 36-39]. However, in this study, we investigated the relationship between SOD2 and GPX1 polymorphisms and the risk of CKD as well as their function in the creation of oxidative stress byproducts. Möllsten et al. [39] studied the association between the Val allele and the risk of diabetic nephropathy and the development of cardiovascular disease. In addition to reporting that the MnSOD Ala16Val polymorphism is implicated in the development of diabetic nephropathy caused by type 1 diabetes and appears to predict cardiovascular disease during follow-up, they discovered a substantial correlation between carrier status of the Val allele and diabetic nephropathy.

It has been reported that decreasing plasma GPX is positively associated with declining eGFR. The plasma GPX is widely produced in the kidney. Therefore, by losing kidney function, the production of plasma GPX and its activity may decrease [24]. In this regard, some case-control investigations confirmed a direct association between reduced plasma GPX activity and declining kidney function in CKD patients [40-43].

We conclude that the Val/Val allele of the SOD2 and the Lue/Lue allele of the GPX1 polymorphisms increase the risk of CKD. Our findings support the hypothesis that impairment in the antioxidant enzymes SOD2 and GPX1, which are involved in the elimination of oxidative stress, increases the risk of CKD. These results revealed that SOD2 and GPX1 genotypes may be utilized to identify CKD

patients at risk of more rapid CKD progression. Also, some treatments, such as antioxidant therapies, might be investigated to determine whether they slow the progression of CKD.

5. Conclusion

In summary, we investigated the association between SOD2 and GPX1 polymorphisms and CKD. The findings suggested that SOD2 polymorphisms did not have a significant prognostic impact on CKD patients. However, GPX1 polymorphism (Lue/Lue genotype) either individually or in combination with SOD2 polymorphism (Val/Val genotype) showed a significant relationship with CKD, and the effects of Lue/Lue GPX1 genotype increased 2-fold when combined with Val/Val SOD2 genotype. On the other hand, the possible effects of these polymorphisms on the serum level of oxidative stress byproducts were studied, and the results showed that the concentration of MDA, PTGs, PCGs, and PAB was increased in subjects with the Val/Val SOD2 genotype. While the serum levels of MDA, MDA adducts, PTGs, and nitrotyrosine increased in individuals carrying the Lue/Lue GPX1 genotype.

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