The Association of Antioxidant Effect of Rosuvastatin with the Superoxide Dismutase 2 Enzyme Gene Polymorphism in Coronary Artery Disease Patients at Najaf Governorate

Thuraya A. kadihm[1], **Ahmed J. Mohammed**[2]

1.B.Sc. Pharmacy in faculty of pharmacy university of kufa / Iraq , e-mail : thurayaali94@ yahoo.com .
2. Asst. Prof. Dr in Department of Clinical laboratory sciences - faculty of pharmacy university of kufa / Iraq, College of pharmacy, The Islamic University, Najaf 54001, Iraq , ahmedj.mohammed@uokufa.edu.iq .

Abstract

Background: Rosuvastatin is a cholesterol-lowering medication that also reduces the production of superoxide anion, which reduces inflammation and oxidative stress. In the mitochondria, manganese-dependent superoxide dismutase (MnSOD or SOD2) is responsible for the metabolization of superoxide anions. A gene polymorphism in humans (Ala16Val-SOD2) causes the 16th amino acid of alanine (Ala) to become valine (Val). Due to their pro-inflammatory effects and increased oxidative stress, functional polymorphisms of these antioxidant enzymes are thought to play a role in the pathogenesis of CAD. In this study, we looked at how rosuvastatin affected inflammation and oxidative stress in hypertensive and dyslipidemic patients.

Methods: We looked at the antioxidant profile of the SOD2 gene V16A polymorphism in 51 CAD patients who were taking 10 mg of rosuvastatin daily. During the study, the following variables were recorded: age, weight, BMI, blood pressure, SOD2(superoxide dismutase 2), and MDA: malondialdehyde, glutathione peroxidase (Gpx), lipoprotein lipase (LPL), and interleukin 6 (IL6) AL-Sadar and AL-Hakeem Hospitals & Research Center, as well as the university of kufa's faculty of pharmacy, were the locations of this study. Different statistical analyses were carried out.

Result: SOD2 genes were found to have significantly different distributions of alleles and genotypes in people with CAD. Rosuvastatin had a significant positive effect as an anti-inflammatory medication in CAD patients by elevated serum SOD2 antioxidant levels.

Conclusion: This study demonstrates that oxidative stress in CAD can be accelerated not only by hyperlipidemia-induced ROS production but also by a diminished antioxidant defense system, at least partially caused by SOD2 SNPs. Rosuvastatin reduced that oxidative stress and inflammation. These results show that there is an additional cardio protective effect, which could be a pleiotropic effect or a direct mechanism of action.

1. Introduction

Coronary artery disease (CAD) accounts for the deaths of a third of the world's population. According to data released by the WHO in 2023, coronary heart disease accounted for 36,594 Iraqi deaths, or 25% of all deaths. Iraq has the 23rd highest age-adjusted Death Rate per 100,000 people in the world. 17) (WHO . 2023) . The first antioxidant enzyme, superoxide dismutase (SOD), is essential for shielding cells from ROS-induced damage [4]. Superoxide radicals (O2 –) that come from outside the cell or are produced in the mitochondrial matrix as a byproduct of oxygen metabolism are broken down by the SOD family [5]. Some SNPs are silent, while others may result in altered phenotypes that affect homeostasis and protein modulation or function. Because MnSOD is the first line of defense against the production of reactive oxygen species (ROS), structural and/or functional SNPs in the MnSOD encoding gene are very important for maintaining ROS cell levels [6]. Statins like rosuvastatin were originally made to lower low-density lipoprotein (LDL) cholesterol. However, it is now thought that their antioxidant, anti-inflammatory, and antiplatelet properties improve cardiovascular morbidity and mortality through pleiotropic 7) Rosuvastatin is a cholesterol-lowering medication that also reduces the production of superoxide anion, which reduces inflammation and oxidative stress. In the mitochondria, manganese-dependent superoxide dismutase (MnSOD or SOD2) is responsible for the metabolization of superoxide anions. A gene polymorphism in humans (Ala16Val-SOD2) causes the 16th amino acid of alanine (Ala) to

become valine (Val). Multiple diseases, such as hypercholesterolemia and coronary artery disease, have been linked to the VV genotype.

2. Materials and Methods

Ethical approval

The procedure has been explained to the patients or relative for permission and the study was approved by the ethical approval committee of university of Kufa / Iraq faculty of pharmacy.

Study design

The present prospective cohort research included 51 individuals with hyper cholesterolemic coronary artery disease. with 29 males (56.86 percent) and 22 females (43.13 percent). During the course of the study, 100 patients participated; However, due to poor compliance, 49 patients were dropped cases. The study was conducted at Al Hakim Hospital and Al Sader Teaching Hospital in Najaf, Iraq, from November 2021 to May 2022. A cardiologist had diagnosed the enrollees with coronary artery disease, and they regularly went in for follow-up. The ages ranged from 23 to 66. The first blood sample was taken prior to treatment (no time), and the second blood sample was taken 60 days after receiving 10 mg of rosuvastatin. The patient's doctor determined the treatment dose in the study. The criteria that had to be met to be excluded from the study were: Rosuvastatin hypersensitivity, pregnancy, breast feeding, mental impairment, renal impairment, hepatic impairment, hemorrhagic stroke, patients receiving treatment that affects lipid profile, patients with cancer, and those taking lipid-lowering medications are all risk factors. Patients who did not adhere to their own treatment plan were excluded from the study. The BMI (body mass index), systolic and diastolic blood pressure (SBP and DBP, respectively), weight (in kilograms) and height (in meters) were measured.

Genotyping

A sterile K2EDTA tube was used to collect blood samples for DNA extraction. Using a kit (Favorgen DNA extraction kit, Taiwan), genomic DNA was extracted from whole blood in accordance with the manufacturer's instructions. Using the primers listed in Table 1, the specific primer pairs of the MnSOD gene were identified for PCR diagnosis. The Master Mix (Taq PCR PreMix), each forward and reverse primer specific at 10 picomol/l, 1.5 L of DNA template, and nuclease-free water completed the 25-L final volume of the PCR reaction tubes. According to Pournourali et al. (2016), the MnSOD gene's thermocycling conditions "were set at 95°C for 3 min followed by 40 cycles of 95°C for 45 sec, 64°C for 45 sec, and 72°C for 45 sec, and final extension at 72°C for 7 min." The presence of a 250-bp region on the electrophoresis of the PCR product on a 1.5% agarose gel indicates a positive result for the MnSOD gene.

Table 1: Primers sequences used for MnSOD gene amplification (Pournourali et al, 2016).

SNP	Primer	Sequence	Amplicon size
rs 4880	Forward	5’-CGGGCTGTGCTTTCTCGTC-3’	250 bp
	Reverse	5’-TCAGCCTGGAACCTACCCTT-3	

Table 2: Reaction condition of restriction enzyme BsawI

Protocol	**Volume (µl)**
PCR Product	10 µl
Restriction Enzyme	0.5 µl
Buffer	1.5 µl
D.W.	13 µl
Temperature Time	60 C/60 min

PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis for MnSOD gene

The restriction enzyme BsawI (Biolab, New England) was used to digest the PCR products, cutting the wild-type sequence into a 250-bp fragment. The MnSOD gene is altered by this enzyme by substituting A for T. Table 2 contains digestion reaction conditions. The digested DNA fragments were electrophoretized using ethidium bromide on a 2% agarose gel.

Laboratory analyses of phenotyping

After a 12-hour overnight fast, blood samples from volunteers were collected through venous puncture into gray and red top Vacationers (BD Diagnostics, Plymouth, UK) tubes prior to and following treatment with rosuvastatin. Interleukin 6 (IL-6) and malondialdehyde (MDA) were used as inflammatory biomarkers, and the anti-inflammatory enzymes SOD2, LPL lipoprotein lipase, and Gpx glutathione peroxidase were quantified using the ELIZA procedure in accordance with the manufacturer's instructions.

3. Results

Using a direct total blood cell sample and Primer, the Val16Ala-SOD2 genotyping was determined during the baseline examination. The Lane (1) TT homozygote that was produced as a result of this procedure still contained 243 bp bands and was not digested by the restriction enzyme. The product digested by restriction enzyme into 243 bp, 176 bp, and invisible 47 bands is the lane (2,3,4,6,7,8)CT heterozygote. The products of lane (5) CC wild were digested into bands of 47 base pairs that were invisible.

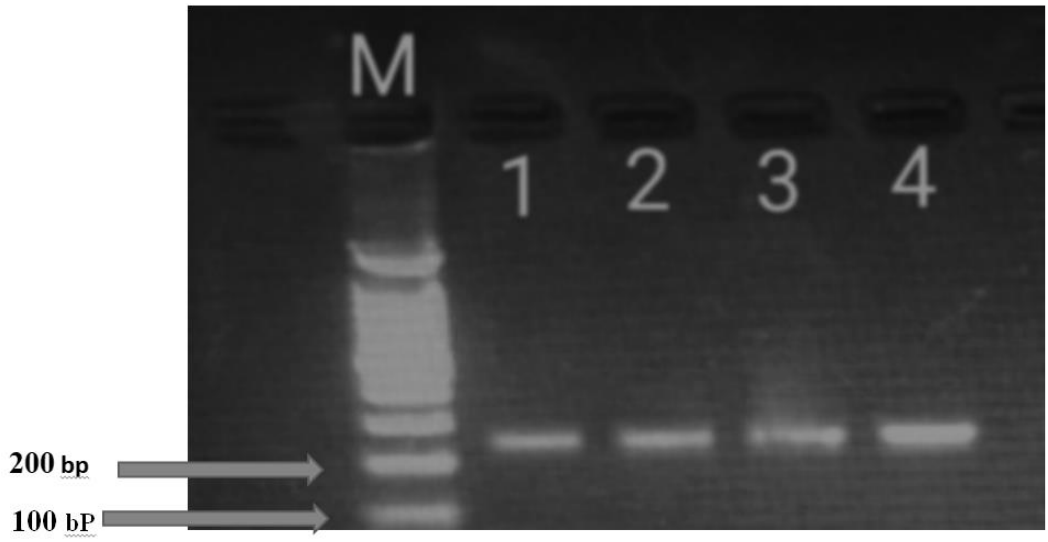

Figure 1 : Image of the SOD2 gene's PCR product analysis (rs 4880) on agarose gel electrophoresis M where: 100-bp marker DNA ladder. Some 243 bp bands of positive PCR amplification of the SOD2 gene were observed in lanes (1-4) .

Table 3: Results for digested SOD2 gene polymorphism rs4880 (C / T)

Genotyping		Number of bands	Size (bp)
Wild	CC	2	176 ,47
Heterozygous	CT	3	243, 176 ,47
Homozygous	TT	1	243

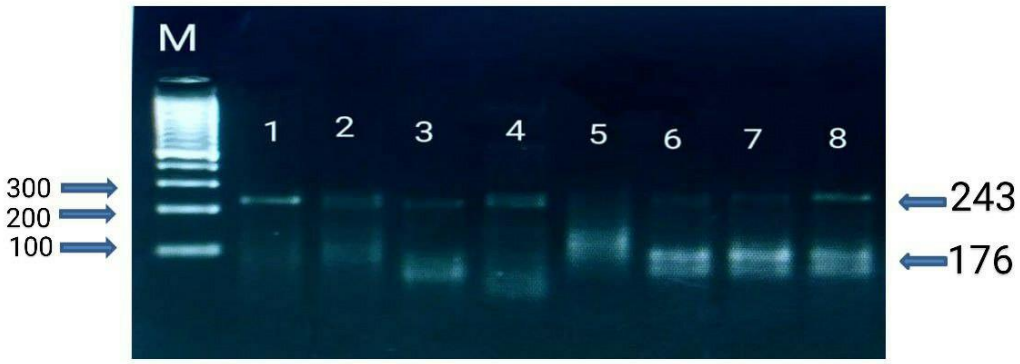

Figure 2 : The RFLP-PCR product analysis of the rs 4880 (C/T) SOD2 gene polymorphism was shown in this agarose gel electrophoresis image, which was obtained by using the BasW1 restriction enzyme in a 2.5 percent agarose gel for 45 minutes at 100 volts and ethedium bromide dye for direct UV light visualization. M where: marker with 100 bp .

The SOD2 SNP frequency distribution of CAD patients (rs4880 C > T). 14 of the patients in the sample had wild CC genotypes, representing 27.4% of the total, followed by 27 with heterozygous C/T genotypes, representing 52.9 percent, and 10 with mutant homozygous TT genotypes, representing 19.7% .

Table 4: The frequency distribution of CAD patients according to SOD2 SNP (rs4880)

SOD2 SNP (rs4880 C>T)	Frequency	Percent
CC	14	27.4%
C/T	27	52.9%
TT	10	19.7 %
TOTAL	51	100 %

Outcome

Several inflammatory biomarkers affected by the Val16Ala-SOD2 SNP were the focus of this study's interest after 60 days of daily 10 mg doses of rosuvastatin. The reductions in these biochemical variables among genotype-grouped subjects were determined to examine the pharmacogenetic influence. The difference between the last measurement before beginning the statin therapy and the first measurement after it was finished was used to calculate the reduction. The percentage of the base values of each variable was used to display the differences between these two values. Rosuvastatin's effect on serum antioxidants SOD2, IL6, and MDA, as determined by genotyping (CC, CT, and TT), is statistically not significant (p value > 0.05), with the exception of concentration Gpx and MDA, which are statistically significant (p value 0.05). SOD2 rs4880 in heterozygous (CT) LPL, Gpx, and MDA, and IL6 in homozygous (TT) but wild (CC) concentrations of SOD2 demonstrated the best response to treatment.

Table 5 shows the results of comparisons of biochemical variables between CAD subjects prior to treatment. Our results showed that genotyping the SOD2 SNP (rs4880) had the greatest positive effect on serum biomarkers like Gpx and LPL in heterozygous variants (CT), concentrations of SOD2 and MDA in wild heterozygotes (CC), and IL6 in variant heterozygotes (TT). More specifically, the results of this study indicated that variant (CC) of the SOD2 gene has the strongest effect on the effectiveness of rosuvastatin (table 6). In contrast to heterozygous (CT) individuals, homozygous (TT) individuals experienced the greatest increase in IL6, Gpx, and MDA in response to treatment, as shown in the table.

Table 5: Association between genotyping and some clinical characteristics before treatment

		Genotyping						
		CC		CT		TT		P value
		N	%	N	%	N	%	
Gender	Male	8	53.3%	17	62.9%	4	44.4%	0.030
	Female	7	46.6%	10	37.0%	5	55.5%	
Weight	BMI<25	4	26.6%	6	21.4%	3	33.3%	0.007
	BMI≥25	11	73.3%	21	78.5%	6	66.6%	
D . M	Yes	9	60%	12	44.4%	2	22.2%	0.006
	No	6	40%	15	55.5%	7	77.7%	
HTN	Yes	6	40%	11	40.7%	3	33.3%	0.015
	No	9	60%	16	59.2%	6	66.6%	

CC: wild, CT: Heterozygous , TT: Homozygous , N: number, %: percentage , BMI: body mass index, HTN: hypertension, D.M: diabetes mellitus

Table 6: Effect of SOD2 rs 4880 genotyping on serum biomarkers before treatment using ANOVA test

Characteristic	CC n=15 mean± SD	CT n=27 mean± SD	TT n=9 mean± SD	P value
SOD2	23.8 ± 18.6	13.8 ± 3.2	8.87 ± 6.16	0.012 S
LPL	51.2 ± 40.4	59.3 ± 46.7	45.2 ± 33.0	0.658 NS
IL6	49.7 ± 24.4	67.4 ± 49.2	69.1 ± 52.1	0.559 NS
GPX	66.7 ± 33.1	69.7 ± 26.2	43.8 ± 35.8	0.264 NS
MDA	18.8 ± 9.3	27.8 ± 15.9	22 ± 11.1	0.109 NS

S.d: stander deviation , SOD2 superoxide dismutase , MDA : malondialdehyde , Gpx glutathione peroxidase , LPL lipoprotein lipase and IL6 interleukin 6 , CC: wild , CT: Heterozygous, TT: Homozygous , NS: not significant , S: significant .

Table 7: Effect of Rosuvastatin on serum biomarkers according to genotyping using ANOVA test.

Characteristic	CC n=15 mean± Sd.	CT n=27 mean± Sd.	TT n=9 mean± Sd.	P value
SOD2	25.2± 20.70	23.5 ± 7.48	19.6 ± 2.2	0.561 NS
LPL	55.2 ± 24	65.2 ± 48.2	56.9 ± 51.2	0.743 NS
IL6	46.2 ±25	62.0 ± 48.1	65.5 ± 27.5	0.531 NS
Gpx	64.2 ± 25.90	90.3 ± 34	66.6 ± 26.4	0.019 S
MDA	26.2 ± 20.4	17.8 ± 3.7	16.63 ± 2.80	0.04 S

Std. deviation: stander deviation , SOD2 superoxide dismutase , MDA : malondialdehyde , Gpx glutathione peroxidase , LPL lipoprotein lipase and IL6 interleukin 6 , CC: wild, CT: Heterozygous, TT: Homozygous ,NS: not significant , S: significant .

4. Discussion

According to our findings, genotyping the SOD2 SNP (rs4880) had the greatest positive impact on serum antioxidants like Gpx and LPL in heterozygous variants (CT), wild homozygous (CC) concentrations of SOD2 and MDA, and variant homozygous (TT) concentrations of IL6. Similar to the current finding, a study found that IL-6 levels were higher in hypercholesterolemic TT genotype carriers than in A-allele (CC and CT) carriers. According to T Duarte et al. (2016), TT patients also had lower IL6 levels than CC and CT carriers.15 Statistically, the effect of SOD2 rs4880 genotyping (CC, CT, and TT) on serum antioxidants like IL6, MDA, GPX, and LPL was non-significant (p. value > 0.05), indicating that SOD2 rs4880 genotyping had no effect on serum antioxidants other than SOD2 significant

One of the essential enzymes involved in the metabolism of TG in the blood, the gene SOD2, contained the SNP rs4880. According to Yue YH et al., the SOD2 polymorphisms are linked to hypertension, coronary diseases, and ischemic stroke (19). 2016). According to the current study, variant (CC) of SOD2 has the greatest effect on the effectiveness of rosuvastatin, as shown in table 7 as an A study in (T Duarte, 2016) 15. The variant Homozygous (TT) experienced the greatest increase in SOD2 rs4880 concentrations in response to treatment, while the variant Hetrozygous (CT) experienced the greatest increase in IL6, Gpx, and MDA, as shown in table 7 .

5. Conclusion

Dyslipidemia is responsible for 80% of atherosclerosis, according to statistics (19). Additionally, the development of cardiovascular diseases is closely linked to atherosclerosis. Rosuvastatin 10 mg/day may offer a protective effect against cardiovascular disease in patients with HBP and dyslipidemia, as the current findings demonstrate a significant reduction in inflammation and oxidative stress in these patients. As a result, the current findings demonstrate some of their pleiotropic effects. We hypothesize that rosuvastatin increases body MnSOD production and that genotypes of MnSOD and oxidative stress biomarkers are associated with the risk of CAD disease.

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