The association of therapeutic responses of rosuvastatin with the superoxide dismutase 2 enzyme gene polymorphism in coronary artery disease patients at najaf governorate

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Abstract

Background: One of the most consistent hypotheses to explain atherogenesis postulates and reported to be involved in the pathogenesis of CAD is triggered by LDL oxidation caused by Superoxide anions. Rosuvastatin is a drug that lowers cholesterol, but it is now thought to improve cardiovascular morbidity and mortality through pleiotropic effects arising from their antioxidant, anti-inflammatory, and antiplatelet properties. In the mitochondria, manganese-dependent superoxide dismutase (MnSOD or SOD2) metabolizes the superoxide anions. A gene polymorphism in humans (Ala16Val-SOD2) causes the 16th amino acid of alanine (Ala) to become valine (Val).

Methods: We examined the lipid profile and SOD2 gene V16A polymorphism in 51 CAD patients who were on rosuvastatin 10 mg/day. During the investigation, the following factors were noted: Age, weight, BMI, blood pressure, TG (triglyceride), LDL (low density lipoprotein), HDL (high density lipoprotein), VLDL (very low density lipoprotein), and SOD2 (superoxide dismutase) are some of the variables that are taken into consideration. The AL- Sadar and AL-Hakeem Hospitals & Research Centre, the university of Kufa's pharmacy faculty, and other locations participated in this investigation. Several statistical analyses were used.

Result: SOD2 genes showed significant changes in allele and genotype distribution across CAD patients. After using rosuvastatin, serum ldl levels in CAD participants considerably fell.

Conclusion: The findings of this study show that Rosuvastatin decreased inflammation and oxidative stress in CAD and increased the capability of the antioxidant defense system, which was at least partially triggered by SNPs of SOD2. This improved the lipid profile in hyperlipidemia. These findings show the existence of an extra cardioprotective effect, which may be a pleiotropic effect or arise from a direct mechanism of action.

Keywords: coronary artery disease, oxidative stress, SOD2 gene V16A polymorphism, PCR- RFLP, lipoproteins, cholesterol

Introduction

The cause of death for one in three people worldwide is coronary artery disease (CAD). According to WHO data published in 2023, coronary heart disease caused 36,594 deaths in Iraq, or 25% of all fatalities. Iraq is ranked 23 in the world by age-adjusted Death Rate, which is 227.26 per 100,000 of the population [17]. (WHO 2023). The first antioxidant enzyme to begin working is called superoxide dismutase (SOD), and it is essential for shielding cells from ROS-caused damage [4]. Some SNPs are silent, while others may lead to altered phenotypes in protein regulation or function and may even have an impact on homeostasis. Given the significance of MnSOD as the first line of defense against the formation of reactive oxygen species (ROS), structural and/or functional SNPs of the MnSOD encoding gene are crucial for the preservation of within the maintenance of ROS cell levels [6]. As a free radical scavenger in the body, manganese superoxide dismutase (Mn-SOD) can protect against free radicals attacking unsaturated fatty acids in biofilms and prevent lipid peroxidation. Statins, like rosuvastatin, were first created to lower low-density lipoprotein (LDL) cholesterol, but they are now believed to have pleiotropic benefits due to their antioxidant, anti-inflammatory, and antiplatelet activities, which reduce cardiovascular morbidity and mortality [7] by lowering the formation of superoxide anion. There is a gene polymorphism in humans that causes the 16th amino acid to shift from alanine (Ala) to valine (Val) (Ala16Val-SOD2). The VV genotype has been linked to an increased risk of acquiring a number of illnesses, including hypercholesterolemia and coronary artery disease.

Materials and methods

Ethical approval

The study was approved by the ethical approval committee of the faculty of pharmacy at the University of Kufa, Iraq, after the procedure was explained to the patients or their relatives in order to obtain permission.

Study design

51 patients with hypercholesterolemia and coronary artery disease are included in the current prospective cohort research, 29 of whom are men (56.86%) and 22 of whom are women (43.13%). 100 individuals were enrolled in the trial over the research period, however 49 cases were discontinued due to low compliance. The study was conducted at Al Hakim Hospital and Al Sader Teaching Hospital in Najaf, Iraq, between November 2021 and 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. The research excluded patients who did not follow their own treatment plan. We examined the subjects' body mass index (BMI), systolic and diastolic blood pressures (SBP and DBP, respectively), weight (in kilograms), and height (in meters).

Genotyping

DNA extraction in a clean K2EDTA tube, blood samples were taken. According to the manufacturer's instructions, a kit (Favorgen DNA extraction kit, Taiwan) was used to separate genomic DNA from whole blood. PCR detection of the MnSOD gene Using the primers listed in Table 1, the specific primer pairs for the MnSOD gene were found. The final volume of the PCR reaction tubes is 25 L, which is made up of 1.5 L of DNA template, 10 picomols/L of each forward and reverse primer, 5 L of Taq PCR PreMix, and the remaining volume was created by adding nuclease-free water. The MnSOD gene's thermocycling settings "were 95°C for 3 min, then 40 cycles of 95°C for 45 sec, 64°C for 45 sec, and and final extension at 72°C for 7 min" (Pournourali *et al*, 2016). The electrophoresis of the PCR product was done on a 1.5% agarose gel, and the presence of a 250 bp band indicates that the MnSOD gene was successfully detected.

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 B saw I

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 PCR products were digested with the restriction enzyme BsawI (Biolab, New England), which separated the wild-type sequence into a 250-bp fragment. This enzyme alters the MnSOD gene by substituting A for T. The conditions of the digestion reaction are listed in Table 2. On a 2% agarose gel, the digested DNA fragments were electrophoretized with ethidium bromide.

Laboratory analyses of phenotyping

Blood samples from volunteers drawn before and after treatment with rosuvastatin were collected by venipuncture into gray and red festive tubes (BD Diagnostics, Plymouth, UK) after a 12 hour overnight fast. Plasma was used to measure fasting lipid profile levels of "total cholesterol and triglycerides"; Concentrations and serum were measured using standard enzymatic methods using orthoclinic diagnostic reagents on a fully automatic analyzer (Vitros 950 Dry Chemistry System; Johnson and Johnson, Rochester, NY, USA). High density lipoprotein cholesterol was measured in plasma supernatant after precipitation of apolipoprotein B-containing lipoproteins with dextran sulfate and magnesium chloride as previously described. LDL cholesterol was estimated using the Friedewald equation. Plasma citrate was collected for further analysis of coagulation parameters.

Results

The Val16Ala-SOD2 genotype was established by polymerase chain reaction utilizing a direct total blood cell sample and Primer at the baseline test. This technique produced the Lane [1] TT homozygote, which showed restriction enzyme undigested and still 243bp bands. The product of the lane (2,3,4,6,7,8) CT heterozygote was digested by restriction enzyme into 243bp, 176bp, and invisible 47 bands. Table 3 shows the digested 176bp and invisible 47bp bands from lane [5] CC wild products.

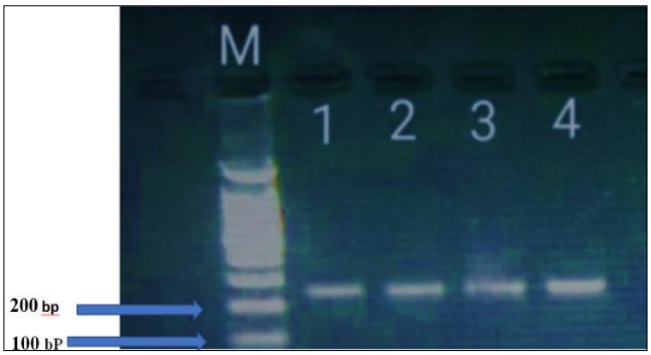

Fig 1: SOD2 gene PCR product analysis (rs 4880) on agarose gel electrophoresis M, with 100-bp marker DNA ladder. In lanes (1-4), 243 bp bands of positive PCR amplification of the SOD2 gene were detected

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

Genotype		Bands Number	Size (bp)
Wild	CC	2	176,47
Heterozygous	CT	3	243, 176,47
Homozygous	TT	1	243

The CAD patient SOD2 SNP frequency distribution (rs4880 C > T). 14 of the patients in the sample had wild CC genotypes, which made up 27.4 percent of the total. The remaining 27 patients had heterozygous C/T genotypes, which made up 52.9 percent, and 10 of the patients had mutant homozygous TT genotypes, which made up 19.7 percent of the total.

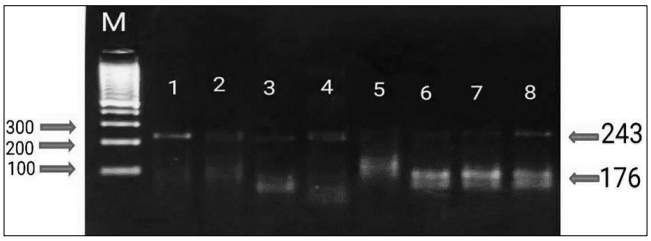

Fig 2: Agarose gel electrophoresis image that showed the RFLP - PCR product analysis of rs 4880 (C/T) SOD2 gene polymorphism by using BasW1 restriction enzyme in 2.5 % agarose gel (100 volt and 45 minutes) and directly visualized under UV light using ethedium bromide dye. Where, M: marker (100bp)

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

In this study, the outcome of interest was the effect of a 60-day therapy consisting of daily 10 mg doses of rosuvastatin on patients' lipid profile (mainly total and LDL cholesterol) response, as well as on the modulation of other lipid molecules (HDL cholesterol, VLDL and triglycerides), influenced by Val16Ala-SOD2 SNP. To analyze the pharmacogenetic influence, the reductions in these biochemical variables among subjects grouped by genotype were determined. The reduction was calculated as the difference between the last measurement before starting the statin therapy and the first measurement after its completion. The differences between these two values were presented as a percentage of the basal values of each variable. The effect of Rosuvastatin on serum lipid profile like cholesterol, Triglyceride, LDL, HDL and VLDL according to genotyping (CC, CT, TT) statistically consider not significant (p. value > 0.05). Best response to treatment were seen in SOD2 rs4880 in Heterozygous (CT) in LDL and Cholesterol while Triglyceride, VLDL and HDL in Homozygous (TT).

Comparisons of biochemical variables among hypercholesterolemia subjects before treatment were performed, and the results are presented in Table 5. Our finding demonstrated the best positive effects of SOD2 SNP (Rs4880) genotyping on serum biomarkers like cholesterol, TG, LDL, HDL, VLD were seen in heterozygous variant (CT). The negative effect of SOD2 rs4880 genotyping on serum biomarkers like cholesterol, Triglyceride, LDL, HDL and VLD was seen in wild homozygous (CC). Rosuvastatin lowered the lipid levels after 60 days of treatment. However, the intensity of the response was significantly influenced by the Val16Ala-SOD2 polymorphism (Table 6). More specifically, The present study indicated that the polymorphisms of SOD2 have positive effect on the effectiveness of rosuvastatin and variant (CC) have best effect (table 6) Best reduction in response to treatment were seen in SOD2 rs4880 in Cholesterol and LDL in wild Homozygous (CC) but Triglyceride and VLDL in variant Homozygous TT. The best increment in response to treatment in SOD2 rs4880 in HDL was seen in variant Homozygous (TT).

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%	
DM	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: ANOVA test results for the effect of SOD2 rs 4880 genotyping on serum biomarkers prior to therapy

Characteristic	CC n=15 mean± SD	CT n=27 mean± SD	TT n=9 mean± SD	*P* value
TC	243.3±64.5	182.8±56.1	222.8±47.1	0.004 S
T.G	238.8 ± 87.8	190.1 ± 85.10	229.4 ± 95.5	0.180 NS
LDL	184.5 ± 52.3	161.50 ± 45.09	156.6±31.30	0.214 NS
HDL	43 ±7.5	49.6 ± 7.9	39 ± 9.2	0.002 S
VLDL	47.6±17.2	37.8 ± 17.4	46.1 ± 19.0	0.180 NS

SD: Stander Deviation, TG: Triglyceride, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein and VLDL: Very Low Density Lipoprotein, CC: wild, CT: Heterozygous, TT: Homozygous, NS: not significant, S: significant

Table 7: ANOVA test results for the effect of Rosuvastatin on serum biomarkers

Characteristic	CC n=15 mean± Sd.	CT n=27 mean± Sd	TT n=9 mean± Sd	*P* value
TC	186.8±49.0	183.4±55.1	183.2±50.21	0.978NS
TG	193.1±77.1	190.5±83.47	177.2±70.6	0.884NS
LDL	156.8±49.05	135.74±38.5	139.3±27.5	0.270 NS
HDL	47.5 ±6.31	49.4±7.9	50.1±12.4	0.713 NS
VLDL	38.8±15.2	37.9±17.1	35.5±14.0	0.886 NS

Std Deviation: Stander Deviation, TG: Triglyceride, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein and VLDL: Very Low Density Lipoprotein, CC: wild, CT: Heterozygous, TT: Homozygous,NS: not significant, S: significant

Statistical analysis

The statistical analysis was performed using SPSS (Version 26.0). Initially, the lipid, inflammatory and antioxidant variables were compared among hypercholesterolemic carriers of different Val16Ala-SOD2 genotypes (CC, TT and CT) using analysis of variance, followed by paired t-test. A second analysis was performed to evaluate whether rosuvastatin response was influenced by body mass index and gender the investigated here using independent sample t-test analysis of variance, Spearman Correlation coefficient analysis was used to verify the relationship between studied variables the effect of SOD2 rs 4880 genotyping on serum biomarkers before and after treatment in this study was also performed by using ANOVA test and chi-square.

Discussion

Our discovery demonstrated the heterozygous SOD2 SNP (rs4880) genotyping had the most favorable influence on serum biomarkers such as cholesterol, TG, LDL, HDL, and VLD (CT). In wild homozygous individuals, SOD2 rs4880 genotyping had a deleterious influence on blood biomarkers such as cholesterol, triglycerides, LDL, HDL, and VLD (CC). A study demonstrated that hypercholesterolemic carriers of the TT genotype had greater levels of total cholesterol and LDL when compared to carriers of the A-allele (CC and CT). However, TT patients had lower HDL levels when compared to CC and CT carriers (T Duarte *et al*, 2016) [1]. The effect of SOD2 rs4880 genotyping (CC, CT, TT) on blood biomarkers such as Triglyceride, LDL, and VLDL is statistically insignificant (p value > 0.05) Table 6 shows that SOD2 rs 4880 genotyping had no influence on serum biomarkers except cholesterol and HDL, which are very significant (p. value 0.005). Another study found an increase in reported serum TG and total cholesterol values between TT genotyping variations (Ali IIKLI *et al*, 2018) [16].

Our study found that the TT genotype was associated with significantly increased high-density lipoprotein cholesterol (HDL-C), whereas other studies found that HDL levels were significantly elevated and triglyceride levels were significantly reduced in the Ala 16 Val (TT) genotype in comparison to the Ala 16 Val (TT) genotype (CC). Bresciani *et al*. (2013) [17]. In contrast to our findings that HDL levels were lower and triglyceride levels were higher in the (TT) genotype (CT).

SNP rs4880 was located in the gene SOD2, which is one of key enzymes involved into blood TG metabolism. It has been showed that the SOD2 polymorphisms are associated with hypertension, coronary diseases and ischemic stroke 19 (Yue YH *et al*., 2016). The present study indicated that the polymorphisms of SOD2 have positive effect on the effectiveness of rosuvastatin and variant (CC) have best effect table 7 as A study in (T Duarte, 2016) 15 Best reduction in response to treatment were seen in SOD2 rs4880 in Cholesterol and LDL in wild Homozygous (CC) but Triglyceride and VLDL in variant Homozygous TT. The best increment in response to treatment in SOD2 rs4880 in HDL Concentrations was seen in variant Homozygous (TT) as show in table 7.

Conclusion

According to statistics, 80% of atherosclerosis is caused by dyslipidemia [19]. And atherosclerosis is closely related to the development of cardiovascular diseases. The present results show that patients with HBP and dyslipidemia who are treated with rosuvastatin 10 mg/day experience a significant reduction in their serum TC, LDL-C, and TG concentrations, plus a moderate increase in their HDL-C concentration therefore offer a protective effect against cardiovascular disease. We suggest an association between the genotypes of MnSOD, hypercholesterolemia with the danger CAD disease in people and rosuvastatin increase production MnSOD inside body.

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