Research Article

Investigation of SOD2 gene polymorphism in patients with chronic kidney disease in Babylon province

Dhifaf Zeki Aziz[1]*, Samah Amer Hammood[1], Naser Jawad Kadhim[2]

ABSTRACT

Background: Oxidative stress has been connected to the progressingof many diseases, like chronic kidney disease (CKD). Single-nucleotide polymorphisms (SNPs) of antioxidant enzymes may participate to some diseases that can be associated with oxidative stress. **Methodology:** The blood sampled from chronic kidney disease -hemodialysis patients- (thirty patients) who visit the Dialysis center in Marjan teaching hospital /Babylon /Iraq in addition to the thirty control samples. **Results:** PCR-SSCP results for SOD2 gene representedthe presence of two different haplotypes due to the numbers of bands in the SOD2gene including 5 and 6 bands, these haplotypes were detected between renal failure patient groups and control, sothisindicatethe association between 5 and 6bands in patients when compared with the control group. **Conclusion:** The current study conclude that, SSCP technique could be a good way to detect SOD2SNPs in a patient with chronic renal failure.

KEY WORDS: Antioxidant, CKD, GFR, Oxidative, PCR, SNP, SOD2

INTRODUCTION

The factor ofreactive oxygen species (ROS) have an importantrolein a wide variety of renal diseases [1]. Increased concentrations of (ROS) can lead to oxidative stress and/or a reduction in antioxidants.[2]. The oxidative stress evidences in chronic renal failure disease, speciallyin patients that submitted to the hemodialysis therapy, thisoxidative stress may beresulted from production of toxic substances of oxidative metabolism which likeoxygenderived agents that produced by the leucocytes, metal compounds, other toxins as well as decreases in antioxidant defenses. Many physiological and pathological pathways can generateoxidative stress and harms the cellular components as membranes lipid, proteins, and DNA[3]. Oxidative stress damage responsible to the progressionof uremic patients and can be a strong factor in the pathogenesis ofCKD[4].

The antioxidant enzymes like the superoxide dismutase (SOD), glutathione peroxidase, and catalase are a primary defense system against reactive species. oxygen and hydrogen peroxide produced by SOD, this enzyme have three isoforms, SOD1 that present in red blood cells, SOD2 that is mitochondrial enzyme, and SOD3 which is extracellular [5-6].

Many reports mentioned that the antioxidant enzymes Single-nucleotide polymorphisms (SNPs) may be contributed to many diseases whichlinked with oxidative stress like SOD2 SNP, rs4880 Ala16Val genotype, which lead to substitute a C>T at position 2734 and changingalanine position 16 from

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Website: jprsolutions.info | ISSN: 0975-7619

[1]Department of Laboratory Investigation, College of Science, University of Kufa, Iraq, [2]Department of Biology, College of Science, University of Kufa, Kufa, Iraq

***Corresponding author:** Dhifaf Zeki Aziz, Department of Laboratory Investigation, College of Science, University of Kufa, Iraq. E-mail: dhifafz.darwish@uokufa.edu.iq

Received on: 15-06-2019; Revised on: 19-07-2019; Accepted on: 22-08-2019

to valine[7]. The SNPs withthealteration ofrespective enzymes activities have been associated with abnormal diseases, like many types of cancer, diabetic neuropathy and acute kidney injury [8]. This study aimed to assess an important genetic factor that may affect the oxidative and antioxidant balance in hemodialysis patients.

MATERIALS AND METHODS

Sampling

The blood sampled from chronic kidney disease - hemodialysis patients- (thirty patients) who visit the Dialysis centerin Marjan teaching hospital /Babylon / Iraq in addition tothe thirtycontrol samples.

DNA Extraction

The kit from Favergen company (Taiwan) used to Extract and purify DNAfrom blood.

Amplification of DNA (PCR)

The SOD2 (rs4880)targeted siteswere amplified using specificprimer (BioneerUSA).forward sequenceis (5-GCTGTGCTTTCTCGTCTTCAG-3), while the reverse sequence is(5-TGGTACTTCTCCTCGGTGACG-3).

Final quantities of the reaction tubes was 20µl containing 1.5 µL (primersµ), 3 UL (genomic DNA),12.5 µL (Green Master Mix), and 5 µI (nuclease free water). Amplification was also performed via a 5-minute programmed heat cycle at 94 C°; for 35 cycles:1 min. at 94 C°, 1 min. at 60 C° and 1 min at 72 C°; finally the 5-min.extension at 72 C° . Electrophoresisdone by 2% agarose gel, and ethidium bromide (EtBr) for staining.

PCR-SSCP

The Single Strand Conformation Polymorphism (SSCP) was done for analysis of amplified gene fragments which contain theamplification of interesting gene sequences with PCR, denaturation of double -stranded DNA to by heating in loading buffer; then cooling of the single-stranded DNA to lowering the possibilities of self-annealing; finally analyzing of differences in the mobility of single -stranded DNAs by electrophoresis. The gel sandwich glasses were, fitted well with correct alignment, then thebottom side was sealed using 10 milliliters of (12-tone) system gel mix Which mixed with 50 µl APS.Twentymicroliter ofTEMED was injected by using syringe, allowed to polymerize. Whenpolymerization finished, the sandwich of gel was placed in the casting stand. The 12% native PAGE gel mix (25 ml) is prepared through adding (APS 100 µl+ TEMED 40 µl) with mixing. and this combine waspouredinupper side of gel. This gel was kept for a of 45 min , then sample loaded for 3 hours.Finally, thegel staining by (EtBr).

RESULTS

A comparison between dialysis patients (HD) and healthy volunteers was performed according to general characteristics as age, body mass index and sex distribution as shown in Table 1.

Genotyping Study

The result of SOD2 gene amplification product is shown in figure 1, the figure 2 represent the polyacrylamide gel electrophoresis for SSCP-PCR of SOD2 gene (208) bp. amplified product.

The outcome of SOD2 gene analysis showeda two different haplotypes presentationdepending on numbers of bands which were(5 and 6) bands, these haplotypes were detected in the two groups: hemodialysis patients (HD)group whom suffering from renal failure and healthy controls group; in HD patient groups. The activity of antioxidase may be affected by the possible Mutations (Ala16Val polymorphism), It leads to an increase in oxidative stress [9],The effect of SOD against renal failure has also been approved by

numerous investigations such as detection of the genotype "CC" (Ala / Ala) reported in cases of renal failure and control subjects using A16V polymorphism (rs4880) [10].The most important feedback werelower accelerated growth factor (eGFR) in samples with SOD Ala/Val or Val/ Val genotypes, so the relationship between this polymorphism and chronic kidney failure is unclear.It was previously confirmed that changing the amino acids from Ala to Val may have an effect on SOD structure, and modify alpha helix structure to beta sheet[11]. One study suggested that Ala allele is associated with mitochondrial SOD activity that increase the protective effect against oxidative stress, and the development of some diseases, such as CKD [12].

All SSCP gels that obtained were aligned with each other to analysis and detecthow many haplotypes can be found,the two SSCP band patterns typeshad been observed in gels ofSSCP. The ssDNA bands appeared at the top of the gel whereas the double stranded (dsDNA) appeared at the bottom. The variation ofssDNAin SSCP gels was relied to detect the genetic pattern of each amplified sample, and the suitable condition for SSCP -PCR.The ssDNA variation in SSCP gels is supported to detect the genotype of each amplified sample.

DISCUSSION

The results showed a significant differences in the age, BMI and GFR in HD group when compared with the controlgroup,also showed asignificantdifference in agemean and BMI in both genders of the study groups with high incidence of disease infemales.This is result consistent with [4] study which report that the chronic kidney disease with percent about(15.1%) in females and (12.1%)in males.The female had a high incidence of high urinary creatinine ratio (8.1%) and a high incidence of decreased GFR (7.6% versus 5.4% in male; decreased GFR is definitely as eGFR<60 ml/ min/ 1.73 m2). It is essential to reveal that numerous earlier studies have reported opposite information concerning this issue [7].While,another study confirmed similar CKD occurrence among men and women. And that may be resulted from geographical variation in in addition to the effect of gender on the occurrence of chronic kidney disease[8].

Table 1: The demographic and clinical characteristics in HD patients and controls

Parameters	HD Patients	Healthy controls	*P*
Number of patients	30	30	
Sex (Male/Female)	12/18	15/15	
Age (year)	49.03±2.45	34.53±3.33	0.01
BMI (kg/m^2)	82.65±2.75	69.43±2.23	0.0001
GFR	7.70±0.62	92.65±4.56	0.001

Data are means±SE. HD: Hemodialysis

Finally, the data indicate oxidative stress in patients suffering from(chronic renal failure), especially in patients undergoing dialysis treatment. [4]. This may be due to multiple factors such as high levels of toxic factors resulting from oxidative metabolism with reduced antioxidant defenses as well as uremic toxicity, malnutrition, progressive deterioration of clinical status and certain [13,14]. Besides, the use of low biocompatible membranes can be responsible for the deterioration of the balance between the mechanisms of oxidation and antioxidants in HD patients [15-17].

CONCLUSION

Our examination proved that SSCP technique could be a good way to detect SOD2 SNPs in a patient with chronic renal failure.

GenotypeSOD2	Patients	Control	*P*	OR=(95%CI)
5 band[a]	7111 (36.66%)	4 (13.33%)		
6 band	219 (63.33%)	26 (86.66%)	0.032*	3.76 (1.03–13.04)
Total	30	30		

*$P \leq 0.05$, [a]0.032. SE: Standard error

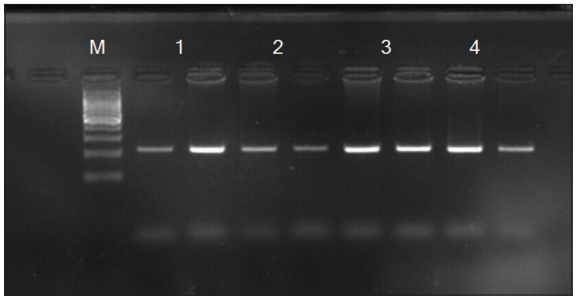

Figure 1: Agarose gel electrophoresis of amplified products of SOD2 (rs4880) M; refers to DNA size marker lane 1–8 lane refers to the patterns of amplified products of SOD2 (208 bp)

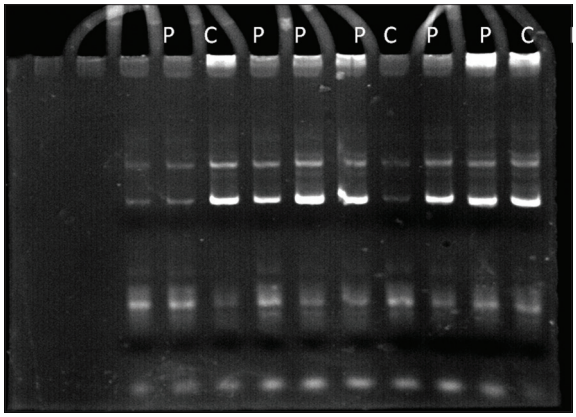

Figure 2: DNA polymorphisms of SOD2 gene of syphilis patients

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Source of support: Nil; Conflict of interest: None Declared