Association between Serum Glutathione Peroxidases and Superoxide Dismutases mRNA Level with Coronary Artery Disease

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Abstract

Background: Oxidative stress plays a crucial role in the pathogenesis and progression of many diseases, including cardiovascular disease (CVD) and diabetes mellitus. Oxidative stress results from an imbalance between free radical formation and the protective antioxidant mechanisms. The latter mechanisms include superoxide dismutases (SODs) and glutathione peroxidases (GPx) that scavenge excessive ROS and protect cells against excess ROS production. The aim of current study was to determine the serum levels of SOD and serum GPx mRNA as well as the serum prooxidant-antioxidant balance in CVD patients. Method: A total of 103 subjects were recruited, with ≥50% stenosis (Angio+) or <50% stenosis of one or more coronary arteries by angiography (Angio−). The expression levels of SOD and GPx in serum were measured using real-time PCR. Biochemical-analyses (e.g., triglycerides; high-density lipoprotein cholesterol; low-density lipoprotein cholesterol; fasting-blood-glucose) were determined in all the subjects. Associations of SOD and GPx levels with biochemical and anthropometric characteristics were assessed together with evaluation of the serum pro-oxidant-antioxidant balance (PAB). Results: CVD subjects had a significantly higher level of fasting blood glucose (FBG), TC, LDL-C, TG and hs-CRP levels, as compared to control subjects. The level of serum PAB was significantly higher in the CVD group, 117.92 ± 35.51 and 110.65 ± 27.65 μg/dl in the angio+ and angio− groups, respectively compared to

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the control group (54.26 ± 23.25). Additionally we observed that the SOD-3 level was higher in angio+ group versus control subjects. **Conclusion:** We have found that patients with CVD had a significantly higher prooxidant-antioxidant and SOD-3 levels. Further studies in larger multi-center setting are warranted to explore the value of emerging biomarker in CVD patients.

**Keywords**

Coronary Artery Disease, Glutathione Peroxidases, Superoxide Dismutases, Real Time PCR, Angiography

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1. **Introduction**

Coronary artery disease (CVD) is the leading causes of death globally [1]. It is estimated that 17.3 million people died by CVD [2]. A growing body of studies have shown an association between oxidative stress and the development of CAD [3] [4]. The prooxidative/antioxidative cellular imbalance can induce excess oxidative stress [5] [6]. The human body defence against the cytotoxic effects of stress oxidative by biologic system provided by enzymatic and non-enzymatic mechanisms to recompense oxidative damage, including antioxidant enzymes, such as superoxide dismutases (SODs) and glutathione peroxidases (GPx) [7].

There are three isoforms of the SOD, cytosolic copper/zinc SOD (Cu/ZnSOD or SOD1), mitochondrial manganese SOD (MnSOD or SOD2) and extracellular SOD (EC-SOD or SOD3). EC-SOD is responsible for 70% of the total SOD activity and found abundantly in the arterial wall that produced by vascular smooth muscle cells in normal physiologic conditions [8].

Among the members of the GPx family just GPx-3 has extracellular isoform. Plasma GPx-3, including antioxidant seleno-cysteine protein, catalyzes the reduction of hydrogen peroxide and lipid peroxides by glutathione. GPx-3 is a major defender of ROS produced during normal metabolism and even after stress [9].

Recently, a number of studies have pointed to the importance of the genetic determinants of CVD risk [10] [11]. Previous studies in our laboratory have revealed a relationship between decreased serum levels of extracellular isoforms of GPx and SOD activity in CAD patients [12]. Both enzymes are involved in the redox status, and over-expression of these ROS eliminating enzymes leads to diminished oxidative stress; thus they have been the subject of many clinical investigations. These enzymes decrease in complicated atherosclerotic plaques; thereby, GPx and SOD could be considered as a potential diagnostic marker. Thus we designed a study to evaluate the association of serum GPx3, SOD1 and SOD3 mRNA in coronary artery disease.

2. **Methods and Subjects**

2.1. **Population**

62 patients (30 males and 32 females) and 41 age and sex adjusted healthy vo-
Volunteers (20 males and 21 females) were recruited from Ghaem Medical Education Hospital Mashhad, Iran. The presence of stenoses ≥ 50% in at least one major coronary artery was assessed using angiographic assessment. Coronary angiograms were performed using routine procedures [13]. Anthropometric parameters were measured. The study was reviewed and approved by the Ethic Committee of Tarbyat Modares University and informed consent was obtained from all participants.

2.2. Collection of Serum Samples and Measurement of Lipids and Lipoproteins

Biochemical analysis including fasting triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) was determined.

2.3. Oxidative Stress Assays

Prooxidant-antioxidant balance (PAB) assay was used as described previously [14].

2.4. RNA Extraction, cDNA Synthesis and Real-Time RT-PCR

RNAs was extracted by QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) according to the manufacturer’s Instructions. The RNA concentration was measured by measuring UV light absorbance at 260 nm with nanodrop. cDNA synthesis was carried out by RT (reverse transcription) using Quanti Tect Reverse Transcription Kit (Qiagen, Germany).

cDNA was amplified with real-time PCR using the RotorGene system (Qiagen, Hilden, Germany), with SYBR green master mixed (Takara, Japan). The primers for GAPDH, GPx3, and SOD1 were designed by Gene Runner (Table 1).

Real-time PCR reactions consisted of denaturation at 95°C for 4 min, annealing/extension at 60°C for 35 s, by 40 cycles. The final extension was at 72°C for

**Table 1.** Primers used for amplification.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward/primers</th>
<th>Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>F</td>
<td>5'-GACAACAGCCTCAAGATCATCAG-3'</td>
</tr>
<tr>
<td>GAPDH</td>
<td>R</td>
<td>5'-ATGGCATGGACTGTGGTCATGAG-3'</td>
</tr>
<tr>
<td>GPx3</td>
<td>F</td>
<td>5'-AACAGGAGCCAGGCAAGAATT-3'</td>
</tr>
<tr>
<td>GPx3</td>
<td>R</td>
<td>5'-CCCGTTCACATCTCTTCTTACTAAA-3'</td>
</tr>
<tr>
<td>SOD3</td>
<td>F</td>
<td>5'-GTGTCACCAAGACACT-3'</td>
</tr>
<tr>
<td>SOD3</td>
<td>R</td>
<td>5'-GTGCTATGGGACAGG-3'</td>
</tr>
<tr>
<td>SOD1</td>
<td>F</td>
<td>5'-CCACTCTAGGTSTCACCCT-3'</td>
</tr>
<tr>
<td>SOD1</td>
<td>R</td>
<td>5'-ATGGTGCTCAGGTGAAGT-3'</td>
</tr>
</tbody>
</table>

GAPDH: glyceraldehyde-3-phosphate dehydrogenase, GPx: glutathione peroxidases, SOD: superoxide dismutases, F: forward primer, R: reverse primer.
5 min. The $2^{-\Delta\Delta\text{Ct}}$ formula for GPX and SOD: $2^{(\text{CT target-CTGAPDH})\text{patients}-(\text{CT target-CTGAPDH})\text{controls}}$ was used to determine differences in GPX and SOD genes expression [15].

2.5. Statistical Analysis

All statistical analyses were performed using the SPSS 16 (SPSS Inc., Chicago, IL, USA). Descriptive statistics including mean ± standard deviation (SD) were determined for variables with normally distribution or data were expressed as median ± Inter Quartile Range (IQR) for not normally distributed variables. For normally distributed variables, t-student test was used, while Bonferroni correction was considered for multiple comparisons. The Mann-Whitney U test was used for continuous variables. For categorical parameters, Chi-square or Fisher exact tests were used. A two-sided $P$ value < 0.05 was considered statistically significant.

3. Results

As shown in Table 2, we found a significant difference for waist circumference, Table 2. Demographic and clinical characteristics of population.

<table>
<thead>
<tr>
<th></th>
<th>Angio+</th>
<th>Angio−</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>36</td>
<td>26</td>
<td>41</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>19/17</td>
<td>13/13</td>
<td>21/20</td>
</tr>
<tr>
<td>Age (year)</td>
<td>60.6 ± 10.7</td>
<td>58.8 ± 9.9</td>
<td>59.6 ± 10.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.25 ± 9.28</td>
<td>162.04 ± 8.78</td>
<td>161.68 ± 7.94</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.19 ± 11.48</td>
<td>69.73 ± 13</td>
<td>67.97 ± 11.97</td>
</tr>
<tr>
<td>BMI</td>
<td>27.85 ± 4.77</td>
<td>26.53 ± 4.66</td>
<td>25.96 ± 3.8</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>93.5 ± 11.48</td>
<td>92.77 ± 13.53</td>
<td>86.05 ± 12.17</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>93.5 ± 9.7</td>
<td>93.50 ± 13.53</td>
<td>91.61 ± 10.5</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.996 ± 0.05</td>
<td>0.992 ± 0.06</td>
<td>0.938 ± 0.08</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>138.47 ± 16.25</td>
<td>131.92 ± 17.27</td>
<td>125.53 ± 14.08</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79.31 ± 9.79</td>
<td>78.27 ± 11.04</td>
<td>71.05 ± 7.98</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>135.92 ± 74.68</td>
<td>115.04 ± 64.46</td>
<td>96.79 ± 14.96</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>182.56 ± 38.12</td>
<td>157.99 ± 37.11</td>
<td>158.74 ± 35.91</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>113.08 ± 31.27</td>
<td>90.19 ± 31.54</td>
<td>92.13 ± 28.28</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>42.83 ± 6.96</td>
<td>41.65 ± 16.67</td>
<td>39.37 ± 8.08</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>132.50 ± 54.02</td>
<td>141.81 ± 68.29</td>
<td>102.32 ± 34.95</td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
<td>10.34 ± 17.70</td>
<td>8.98 ± 12.44</td>
<td>4.68 ± 7.17</td>
</tr>
<tr>
<td>PAB</td>
<td>117.92 ± 35.51</td>
<td>110.65 ± 27.65</td>
<td>54.26 ± 23.25</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD. BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglycerides, hs-CRP: high-sensitivity C-reactive protein, PAB: pro-oxidant anti-oxidant balance. Compared with the control group: $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$; compared with the Angio+ group: $^dP < 0.05$, $^eP < 0.01$, $^fP < 0.001$; comparison between all groups: $^gP < 0.05$, $^hP < 0.01$, $^iP < 0.001$. 
waist/hip ratio, systolic blood pressure and diastolic blood pressure \((p < 0.05)\) between the groups. Not surprisingly no significant differences were detected in FBS, TC, TG and LDL-C between the groups \((p < 0.05)\). The Angio\(^+\) patients had a significantly higher serum levels of TC and LDL-C compared with Angio\(^-\) \((p < 0.05)\) and control groups \((p < 0.05)\). Finally, no significant difference was observed in HDL-C level among the three groups \((p > 0.05)\). Demographic and clinical characteristics of the subjects are summarized in (Table 2).

The intra-assay coefficient of variation (CV) for the quantitation of GAPDH, GPX3, SOD1 and SOD3 mRNA expression were 3.17\%, 3.94\%, 3.11\% and 3.81\% respectively. The inter-assay coefficient of variation (CV) for GAPDH was 6.3\%, 9.2\%, 5.6\% and 9.3\% respectively. There was no significant difference in serum PAB between the Angio\(^+\) and Angio\(^-\) groups \((p > 0.05)\), while there was a significant difference in serum PAB between two patient groups with normal group \((p < 0.001)\) (Figure 1 and Figure 2). Gene expression analyses showed that the expression levels of GPx3, SOD1 and SOD3 genes were increased in patient groups.

4. Discussion

We have found that the serum levels of GPx and SOD were elevated in CVD patients with angiographic evidence of coronary artery stenosis.

Excess oxidative stress is an important step in the development of atherosclerosis. Hydrogen peroxide, superoxide and hydroxyl radicals have direct cytotoxic effects, induce endothelial dysfunction and apoptosis of vascular smooth muscle

![Figure 1](image-url). Serum pro-oxidant anti-oxidant balance (PAB) in relation to the CAD. Angio\(^+\): the presence of one or more stenoses \(\geq 50\%\) in diameter of at least one major coronary, Angio\(^-\): the presence of one or no stenoses \(\geq 50\%\) in diameter of at least one major coronary artery PAB: pro-oxidant anti-oxidant balance.
cells and lead to the destabilization of atherosclerotic plaques [14]. GPx and SOD form essential cellular mechanisms to scavenge ROS in the vessel wall [16]. Several studies have been revealed association between decreased serum activity of ROS-inactivating enzymes; SOD and GPx in CAD patients. Emerging evidence has focused on SOD and GPx due to their fundamental role in control of cellular homeostasis and redox condition in response to internal and external stimulants [17]. A number of lines of evidence suggest that an altered oxidant-antioxidant imbalance is associated with CAD [15]. Other studies have also revealed an elevated oxidative stress and reduce of plasma SOD and GPx in CAD patients [18] [19]. Moreover, several studies have demonstrated that GPx-3 deficiency has been associated with cardiovascular disease [20] [21], although its role in transcriptional level remained unknown [10]. Of note the role of oxidative stress and variations in the intracellular redox states as modulators of the transcription of numerous genes [22], such as GPx-3 and SOD [23]. The association between decreased GPx-3 activity and the development of ischemic stroke [24] and CAD [25] indicates the importance of this enzyme for homeostasis of vascular functions. However, a number of controversies have developed regarding the role of plasma GPx-3 as an antioxidant enzyme in plasma due to the relatively low serum GSH.

Thioredoxin and glutaredoxin have been recognized as electron donors for GPx-3 [26]. It is appeared that the selenium (Se) in selenoproteins such as in GPx, is a key component required for normal health. Ghayour et al. have reported the higher risk of cardiovascular disease in individuals with low serum selenium [12]. Moreover regulation of selenoproteins is special that their translation machinery enables antermination codon (UGA) to be coded for seleno-
cysteine. It would be appeared that several translational cofactors have been necessary for biosynthesis of these proteins [27]. We have found that serum GPx and SOD mRNA is not associate with known coronary risk factors, while serum GPx and SOD mRNA level was elevated in individuals with established coronary stenosis.

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**Conflict of Interest**

The authors have no conflict of interest to disclose.

**References**


