Increased Plasma Levels of Thioredoxin in Patients with Glucose Intolerance

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Abstract

Objective The aim of the present study was to determine the effects of glucose intolerance on oxidative stress in patients with coronary artery disease (CAD).

Methods The patients were divided into 3 groups, diabetes mellitus (DM), IGT or normal glucose tolerance (NGT) according to the criteria of the American Diabetes Association.

Patients The present study consisted of 178 consecutive patients who underwent diagnostic coronary arteriography and a 75-g glucose tolerance test.

Results The level of plasma thioredoxin, a marker of oxidative stress was measured in every patient during the fasting state. The levels of plasma thioredoxin were significantly higher in the DM and IGT groups than the NGT group. Furthermore, we found that there was a positive association between thioredoxin levels and glycated hemoglobin ($\alpha=0.225$, $p=0.018$). In multivariate logistic regression analysis, glucose intolerance (DM or IGT) was only independently associated with the high levels of thioredoxin. The levels of plasma thioredoxin were significantly higher in the CAD group compared to the non-CAD group. In multivariate logistic regression analysis, high levels of thioredoxin, male, age and hypertension were independently associated with the presence of CAD.

Conclusion Glucose intolerance was associated with the high levels of thioredoxin. High levels of thioredoxin were related to the presence of CAD. The measurement of thioredoxin as the marker of oxidative stress may be useful for monitoring the development of the cardiovascular diseases.

Key words: oxidative stress, impaired glucose tolerance, diabetes mellitus, coronary disease

Introduction

Glucose intolerance including impaired glucose tolerance (IGT) is an important risk factor for coronary artery disease (CAD) (1, 2). There is a hypothesis that high glucose levels per sé directly accelerates atherosclerosis with the de novo synthesis of diacylglycerol and subsequent stimulation of protein kinase C (3), increased production of advanced glycosylation end-products (4), and activation of the polyol pathway (5) thought to have major roles in atherogenesis. We and other investigators have reported that increased oxidative stress not only contributes to the development of CAD (6–8), but also influences the prognosis (9).

Recently a sandwich enzyme-linked immunosorbent assay has been developed for measuring the levels of thioredoxin in human plasma (10, 11). Thioredoxin is a small multifunctional protein that contains a redox-active dithiol/disulfide in the active site and shows a variety of biological functions, including cytoprotection against oxidative stress (12). Thioredoxin provides a sensitive marker of the host response to oxidative stress with plasma and serum levels of thioredoxin being elevated in patients with disorders associated with oxidative stress such as viral infections and ischemia-reperfusion (10). The aim of the present study was to determine the effects of glucose intolerance on oxidative stress.
Methods

Study population
The subjects in the study were 178 consecutive patients (120 males and 58 females; mean age 64.8±8.9 year, range 36–83 year) who had chest pain and underwent diagnostic cardiac catheterization and a 75-g glucose tolerance test. The patients were divided into 2 groups, a CAD group and a non-CAD group according to the results of the cardiac catheterization. The CAD group consisted of 102 patients who had ≥50% narrowing of at least one of the major coronary arteries. All medication with the exception of sublingual nitroglycerin was withdrawn at least 3 days before the study investigations. The non-CAD group consisted of 76 patients without coronary arterial stenosis (<50% of luminal diameter), or evidence of coronary spasm following intracoronary injection of acetylcholine. Smoking history was defined as smoking ≥10 cigarettes/day for ≥10 years. None of the patients had taken pharmacological doses of antioxidants at least 1 month before the study. The study complied with the Declaration of Helsinki and was approved by the local ethics committee and informed consent was obtained from each patient and his or her family.

Coronary arteriography
Diagnostic coronary arteriography was performed on all patients. Patients in the non-CAD group were also given an intracoronary acetylcholine injection with the aim of provoking coronary spasm in the manner described previously (13). After intracoronary injection of isosorbide dinitrate, coronary arteriography was carried out with the aim of detecting organic coronary lesions.

Glucose tolerance test
Glucose tolerance in the patients was assessed by a 75-g glucose tolerance test according to the criteria of the American Diabetes Association (14).

Blood sampling
Fasting blood samples were obtained from all patients after admission using venipuncture, with 2.7 ml of blood being collected into a tube containing sodium citrate for the thioredoxin assay. The samples for the thioredoxin assay were centrifuged immediately at 3,000 rpm at 4°C for 15 minutes and stored at –80°C until analyzed.

Plasma thioredoxin assay
A sandwich enzyme-linked immunosorbent assay kit for human thioredoxin and 2 different anti-human thioredoxin murine monoclonal antibodies were obtained from Redox Bioscience, Inc. (Kyoto, Japan) (10, 11). Serial dilutions of 5–320 ng/ml of human thioredoxin were used as standards. Data were analyzed using SOFT max Version 2.3 software by fitting a 4 parameter logit-log transformation of human thioredoxin standards.

Statistical analysis
All data are expressed as mean±SD. Comparison of continuous variables among the groups was performed using one-way analysis of variance (ANOVA). Results that were statistically significant were then analyzed using Fisher’s test. Because the distribution in levels of thioredoxin was abnormal, nonparametric analyses (Mann-Whitney U test) were compared between CAD and non-CAD groups. Spearman’s rank correlation test was used to determine the relationship between plasma thioredoxin and glycosylated hemoglobin (HbA1c). To examine an independent risk factor for the high levels of thioredoxin (>30.0 ng/ml, which was 75th percentile of the distribution of the plasma thioredoxin levels in all patients), multivariate logistic regression procedure was conducted using the following factors: age (≥70), sex (% male), smoking, hypertension (>140/90 mmHg or requiring antihypertensive medication), glucose intolerance (DM or IGT), high low-density lipoprotein cholesterol (>130 mg/dl), low high-density lipoprotein cholesterol (<35 mg/dl) and hypertriglyceridemia (>150 mg/dl). To examine an independent risk factor for the presence of CAD, multivariate logistic regression procedure was conducted using the following factors: age (≥70), sex (% male), smoking, hypertension (>140/90 mmHg or requiring antihypertensive medication), glucose intolerance (DM or IGT), high low-density lipoprotein cholesterol (>130 mg/dl), low high-density lipoprotein cholesterol (<35 mg/dl), hypertriglyceridemia (>150 mg/dl) and high levels of thioredoxin (>30.0 ng/ml, which was 75th percentile of the distribution of the plasma thioredoxin levels in all patients). Probability levels of <0.05 were considered statistically significant.

Results

Characteristics of the study population
The clinical characteristics of the study subjects are detailed in Table 1. There were no significant differences among the 3 groups for the serum levels of total cholesterol, low-density lipoprotein cholesterol or triglyceride. However the prevalence of hypertension and the percentage of male were significantly higher in the DM group than in the IGT or normal glucose tolerance (NGT) group. The rate of obesity was significantly lower in the NGT group than in the DM or IGT group. Furthermore, serum high-density lipoprotein cholesterol levels were lowest in the DM group. Mean age was significantly lower in the IGT group compared to the NGT group.

Plasma levels of thioredoxin
According to glycemic status, the levels of plasma thioredoxin were higher in the DM and IGT groups than the NGT group (Fig. 1). However, the difference in levels of plasma thioredoxin between DM and IGT groups was not significant. In order to investigate the relationship between oxidative stress and control of blood glucose, we determined the correlation between thioredoxin levels and HbA1c.
found there was a positive association between these two variables (\(\sigma = 0.225, p = 0.018, n = 110\) [DM: \(n = 67\), IGT: \(n = 18\), NGT: \(n = 25\)]) (Fig. 2). In univariate logistic regression analysis, smoking, hypertension and glucose intolerance was independently associated with the high levels of thioredoxin. In multivariate logistic regression analysis performed with a forward stepwise method, glucose intolerance was only independently associated with the high levels of thioredoxin (Table 2).

**Table 1. Clinical Characteristics of the Study Groups**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Diabetes mellitus ((n=70))</th>
<th>Impaired glucose tolerance ((n=41))</th>
<th>Normal glucose tolerance ((n=67))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>67±8*</td>
<td>60±11</td>
<td>66±8*</td>
</tr>
<tr>
<td>Range</td>
<td>49–83</td>
<td>36–77</td>
<td>41–79</td>
</tr>
<tr>
<td>Sex (n) males/females (males %)</td>
<td>53/17 (76)**</td>
<td>28/13 (68)**</td>
<td>39/28 (58)</td>
</tr>
<tr>
<td>Coronary risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension (BP ≥140/90 mmHg, n, %)</td>
<td>35 (50)*</td>
<td>19 (46)</td>
<td>20 (30)</td>
</tr>
<tr>
<td>Obesity (BMI ≥25 kg/m², n, %)</td>
<td>23 (33)**</td>
<td>14 (34)**</td>
<td>7 (10)</td>
</tr>
<tr>
<td>Smoking (n, %)</td>
<td>47 (67)**</td>
<td>28 (69)**</td>
<td>27 (40)</td>
</tr>
<tr>
<td>Serum total-cholesterol (mg/dl)</td>
<td>191±39</td>
<td>195±34</td>
<td>190±33</td>
</tr>
<tr>
<td>Serum LDL-cholesterol (mg/dl)</td>
<td>119±33</td>
<td>120±33</td>
<td>116±30</td>
</tr>
<tr>
<td>Serum HDL-cholesterol (mg/dl)</td>
<td>48±14</td>
<td>52±18**</td>
<td>53±16**</td>
</tr>
<tr>
<td>Serum triglyceride (mg/dl)</td>
<td>129±57</td>
<td>136±58</td>
<td>114±66</td>
</tr>
<tr>
<td>Coronary artery disease (n, %)</td>
<td>52 (74)**</td>
<td>25 (61)*</td>
<td>35 (52)</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD. *p<0.05 vs. normal glucose tolerance, **p<0.01 vs. normal glucose tolerance, *p<0.05 vs. impaired glucose tolerance, **p<0.05 vs. diabetes mellitus, BMI: body mass index, BP: blood pressure, LDL: low-density lipoprotein, HDL: high-density lipoprotein.

**Figure 2. Correlation between plasma thioredoxin and glycosylated hemoglobin (HbA1c) levels.**

The levels of plasma thioredoxin were significantly higher in the CAD group than in the non-CAD group (27.0±13.3 vs. 20.1±8.1 ng/ml, p<0.001) (Fig. 3). In univariate logistic regression analysis, smoking, glucose intolerance, high levels of thioredoxin, male, age and hypertension were independently associated with the presence of CAD. In multivariate logistic regression analysis, high levels of thioredoxin, male, age and hypertension were independently associated with the presence of CAD (Table 3).
In the present study, we showed that the levels of plasma thioredoxin were higher in the DM and IGT groups than in the NGT group, and in multivariate logistic regression analysis, glucose intolerance was only independently associated with the high levels of thioredoxin. However, the difference in levels of plasma thioredoxin between DM and IGT groups was not significant. These findings may suggest that not only DM but also IGT is an important risk factor for CAD because oxidative stress in patients with IGT was as high as that in patients with DM. These findings are agreement with the previous reports that postprandial hyperglycemia is one of the risk factors for CAD through an increase in oxidative stress (6, 15). Furthermore, in multivariate logistic regression analysis, high levels of thioredoxin were independently associated with the presence of CAD. These findings suggested that there is a close relation between CAD and oxidative stress. In addition, the results are in agreement with previous studies (28, 29) and demonstrate that coronary atherosclerosis may be associated with increased oxidative stress. The mechanisms responsible for the association between CAD and oxidative stress are unknown. It is possible that one of the mechanisms is related to impaired endothelial function. In the experimental model or patients with CAD, the relations between oxidative stress and impaired endothelial function have been reported (30–32). Previous reports have shown that increased superoxide production of human blood vessels is associated with endothelial vasomotor dysfunction and with risk factors (33, 34). Previous studies have reported that an increase in oxidative stress is associated with the prediction of cardiovascular events in patients with CAD (9, 35). Thus, it is important to monitor these oxidative stress markers in patients with CAD. The measurement of thioredoxin as one of the markers of oxidative stress may be useful for monitoring the development of cardiovascular diseases.

Table 2. Multivariate Logistic Regression Analysis: Variables between High Thioredoxin and Low Thioredoxin Groups

<table>
<thead>
<tr>
<th>Relative risk</th>
<th>95% confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose intolerance</td>
<td>5.72</td>
<td>2.07–15.8</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.62</td>
<td>0.78–3.35</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.62</td>
<td>0.74–3.54</td>
</tr>
</tbody>
</table>

Discussion

In the present study, we showed that the levels of plasma thioredoxin were higher in the DM and IGT groups than in the NGT group, and in multivariate logistic regression analysis, glucose intolerance was only independently associated with the high levels of thioredoxin. However, the difference in levels of plasma thioredoxin between DM and IGT groups was not significant. These findings may suggest that not only DM but also IGT is an important risk factor for CAD because oxidative stress in patients with IGT was as high as that in patients with DM. These findings are agreement with the previous reports that postprandial hyperglycemia is one of the risk factors for CAD through an increase in oxidative stress (6, 15). Furthermore, it has been reported that hyperglycemia induces oxidative stress in animal model and the level of thioredoxin is higher in patients with multiple coronary risk factors (16, 17). The majority of patients with IGT will become DM (18, 19) that in turn is associated with further increases in oxidative stress and the development of atherosclerosis (20, 21). Several large randomized studies (22, 23) have shown that an improvement in postprandial hyperglycemia may lead to a reduced occurrence of cardiovascular events. Epidemiological studies have reported that of patients with acute myocardial infarction (AMI), 31% have DM while 35% have IGT (24). Other studies have shown that IGT is also closely associated with the occurrence of future cardiovascular events (1, 2). Taken together these findings indicate that a large proportion of patients with AMI have disturbances in glucose regulation, and that prognosis following AMI may be improved by stabilizing glucose metabolism. In the present study, we observed a positive correlation between thioredoxin levels and HbA1c, a marker of long-term blood glucose control, a finding that supports the rationale that improving blood glucose control provides a means of decreasing oxidative stress. It is also possible that control of glucose intolerance is required in order to prevent CAD or improve prognosis in patients with CAD. Our study suggests that measuring plasma thioredoxin levels may be useful for monitoring the effect of the treatment during clinical follow-up of patients with CAD.

We have previously reported that the levels of thioredoxin were elevated in patients with acute coronary syndrome including AMI or unstable angina (25–27). In the present study we showed that the levels of thioredoxin were significantly higher in patients with CAD than those without CAD. Furthermore, in multivariate logistic regression analysis, high levels of thioredoxin were independently associated with the presence of CAD. These findings suggested that there is a close relation between CAD and oxidative stress. In addition, the results are in agreement with previous studies (28, 29) and demonstrate that coronary atherosclerosis may be associated with increased oxidative stress. The mechanisms responsible for the association between CAD and oxidative stress are unknown. It is possible that one of the mechanisms is related to impaired endothelial function. In the experimental model or patients with CAD, the relations between oxidative stress and impaired endothelial function have been reported (30–32). Previous reports have shown that increased superoxide production of human blood vessels is associated with endothelial vasomotor dysfunction and with risk factors (33, 34). Previous studies have reported that an increase in oxidative stress is associated with the prediction of cardiovascular events in patients with CAD (9, 35). Thus, it is important to monitor these oxidative stress markers in patients with CAD. The measurement of thioredoxin as one of the markers of oxidative stress may be useful for monitoring the development of cardiovascular diseases.
Thioredoxin and Glucose Intolerance

Table 3. Multivariate Logistic Regression Analysis: Variables between CAD and Non-CAD Groups

<table>
<thead>
<tr>
<th></th>
<th>Relative risk</th>
<th>95% confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>High thioredoxin level</td>
<td>3.36</td>
<td>1.30–8.72</td>
<td>0.012</td>
</tr>
<tr>
<td>Age</td>
<td>4.68</td>
<td>2.15–10.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>Male</td>
<td>3.00</td>
<td>1.10–8.22</td>
<td>0.032</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.73</td>
<td>1.25–5.99</td>
<td>0.012</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.97</td>
<td>0.39–2.44</td>
<td>0.948</td>
</tr>
<tr>
<td>Glucose intolerance</td>
<td>2.16</td>
<td>0.98–4.75</td>
<td>0.056</td>
</tr>
</tbody>
</table>

Conclusions

Glucose intolerance was associated with high levels of thioredoxin. High levels of thioredoxin were related to the presence of CAD. The measurement of thioredoxin may be useful for monitoring the development of cardiovascular diseases.

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References


