Serum Paraoxonase and Arylesterase Activities in Metabolic Syndrome in
Zahedan, Southeast Iran

Running title: Serum Paraoxonase and Arylesterase Activities in Metabolic Syndrome

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Abstract

Objective: Paraoxonase is associated with HDL and protect the serum lipid from oxidation. The aim of the present study was to determine serum paraoxonase, arylesterase activities and total antioxidant capacity in metabolic syndrome (MES).

Methods: This case-control study was performed on 106 patients with MES and 231 healthy subjects. Serum paraoxonase and arylesterase activities were determined spectrophotometrically. Total antioxidant capacity (TAC) was determined using ferric reducing ability of plasma (FRAP) assay.

Results: The results showed that serum paraoxonase (PON) activity was significantly lower in patients with MES (69.62±59.86 IU/L) than healthy subjects (91.64±77.45 IU/L) (p<0.05). The serum arylesterase activity in MES and normal subjects were 45.23±23.24 KU/L, and 65.69±31.10 KU/L, respectively. The arylesterase activity was significantly lower in patients with MES than normal subjects (p<0.0001). No significant differences were observed between MES and normal subjects regarding TAC.

Conclusion: The lower paraoxonase and arylesterase activities in MES may be considered an independent risk factor for cardiovascular disease which remains to be cleared.

Key words: paraoxonase, arylesterase, metabolic syndrome

Abbreviation: Metabolis syndrome, MES; paraoxonase, PON; Total antioxidant capacity, TAC; ferric reducing ability of plasma, FRAP; arylesterase, ARE; national cholesterol education program, NCEP.
1. Introduction

Metabolic syndrome (MES), a collection of cardiovascular risk factors including central obesity, hypertension, hyperglycemia, glucose intolerance, and dyslipidemia is associated with an increased risk of cardiovascular disease and diabetes (1). Human serum paraoxonase 1 (PON1) is approximately 43- to 45-kDa glycoprotein synthesized mainly by the liver which circulates in serum in association with high-density lipoprotein (HDL) and protect LDL from oxidation by the hydrolysis of biologically active lipoperoxides (2). The activity of this enzyme is measured using paraoxon or is estimated from the activity of arylesterase using phenyl acetate. It has been reported that arylesterase activity is not affected by the polymorphisms of PON1 (3, 4).

Serum PON1 activity was found to be reduced in a number of pathological conditions including coronary artery disease (5), hypercholesterolaemia (6), type 2 diabetes (6, 7), polycystic ovary syndrome (8) and renal failure (9). PON1 is recognized as an antioxidant enzyme because it hydrolyses lipid peroxides in oxidized lipoproteins.

To the best of our knowledge information regarding paraoxonase and arylesterase activities in MES is limited. The aim of the present study was to find out the levels of paraoxonase and arylesterase activities in MES.

2. Materials and Methods

This case-control study was performed on 106 individual with MES and 231 normal subjects. The demographic and biochemical characteristic of the groups are shown in table 1.

The study was approved by the local ethical committee of Zahedan University of Medical Sciences and written informed consent was obtained from all subjects. The metabolic syndrome was determined as the presence of three or more of five components according to NCEP ATP III (Table 1) (1).
Fasting blood glucose (FBG) and lipid profile (TG, total cholesterol, LDL-C, and HDL-C concentrations) were measured by automated chemistry analyzer using commercial available kits.

Paraoxonase activity assays were performed in the absence (basal activity) and presence of 1 M NaCl (salt-stimulated activity) using paraoxone (diethyl-p-nitrophenyl phosphate) as a substrate as described previously (10).

Phenylacetate was used as a substrate to determine the arylesterase activity. The rate of phenol produced was continuously monitored at 270 nm at 37 °C. Arylesterase activity was determined using molar extinction coefficient of phenol (1310 M⁻¹ cm⁻¹) and expressed as KU/L serum (10).

Serum TAC was determined by measuring their ability to reduce Fe³⁺ to Fe²⁺ which is known as FRAP assay as described previously (11).

Statistical analysis was performed by commercial software (SPSS for Windows, V17) using independent sample t-test and Pearson correlation coefficient test. A p-value less than 0.05 were considered statistically significant.

3. Results

The study consists of 106 MES (34 males and 72 females; age 43.54±14.07) and 231 normal subjects (97 males and 134 females; age 35.64±13.27). Levels of the PON1 activity in MES and healthy subjects were 69.62±59.86 IU/L and 91.64±77.45 IU/L, respectively. The activity of PON in MES was significantly lower than normal subjects (p=0.01). In addition salt stimulated paraoxonase activity was significantly lower in MES (136.23±111.80 IU/L) than normal subjects (192.24±162.68 IU/L) (p=0.02).

The serum arylesterase activity in MES and normal subjects were 45.23±23.24 KU/L, and 65.69±31.10 KU/L, respectively.
In males, there were no significant differences in the activities of PON1 between MES (64.57±58.24 U/L) and normal subjects (90.01±84.17 U/L) (p=0.106). While a significant difference was found regarding ARE activity in MES (64.68±35.08 kU/L) and normal subjects (40.97±16.23 kU/L) in males (p<0.001).

In females, the activity of PON was significantly lower in the MES (72.01±60.87 U/L) than in the controls (92.82±72.49 U/L) (p=0.039). We also found that ARE activity was significantly lower (p<0.001) in female cases (47.25±25.75 kU/L) than normal subjects (62.80±27.6 kU/L).

No significant correlation was observed between age and paraoxonase or arylesterase activities (p>0.05). A significant difference was observed among MES and normal subjects regarding the arylesterase activity (p<0.0001). No significant difference was observed among MES and normal subjects for total antioxidant capacity (TAC). In MES and normal subjects, PON activity was positively correlated with ARE activity (r=0.368, p<0.0001; r=0.594, p<0.0001). While, a positive correlation was observed between PON and HDL-c in normal subjects (r=0.168, p=0.01), the correlation among MES and HDL-c was not significant (r=0.122, p=0.21). Furthermore, there were no correlation among MES and normal subjects regarding PON and cholesterol, LDL-c, triglyceride, TAC, BMI (p>0.05).

4. Discussion

In the present study we found that paraoxonase and arylesterase activities were significantly lower in MES when compared to normal subjects. The formation of free radicals is a normal outcome of a variety of essential biochemical reactions and can occur at elevated rates under pathophysiological circumstances (12, 13). The physiological role of antioxidants is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals. PON1 is a specific anti-oxidative enzyme with both PON and arylesterase activities. There is little information regarding the levels of PON and ARE in
MES. Senti et al. had found that serum PON1 activity were significantly lower in MES than normal subjects (14). Our results are in agreement with this finding. Tabur et al. (15) have found that the levels of PON and ARE activities were not significantly different among non-diabetic MES, non-MES obese patients and healthy subjects, while total antioxidant status (TAS) was low in both MES and obese groups compared to controls.

A variety of PON1 gene polymorphisms has been recognized (16, 17). It has been well documented that two common coding region polymorphisms of the gene PON1 (L55M and Q192R), leads to changes of both the level and activity of the enzyme (18, 19). Moreover, it has been found that promoter polymorphisms of PON1, especially -107T/C, affects the PON1 expression and serum concentration (20). On the other hands, acquired factors such as diseases, diet and life style can also affect the PON1 activity. It has been proposed that consumption of red wine or flavonoid containing drinks (21) as well as moderate alcohol intake (22) increased serum PON1 activity. The exact mechanisms affecting low PON1 and ARE activities in MES yet to be clear.

It is well known that in the general population MES is associated with increased cardiovascular morbidity and mortality (23, 24) and high prevalence of type 2 diabetes mellitus (25). Human PON1, a HDL-associated enzyme, is capable to prevent LDL oxidation. According to this and previous results (14) reduced paraoxonase and arylesterase activities in MES might be an independent risk factor for cardiovascular disease in these patients. However, more studies in diverse populations are needed for clear confirmation.

Acknowledgment

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The authors declare that they have no conflict of interest
References


Table 1. Biochemical parameters in MES and normal subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MES</th>
<th>Normal subjects</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mg/dL)</td>
<td>118.50±58.71</td>
<td>85.42±12.33</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>222.89±190.66</td>
<td>113.33± 48.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total Chol (mg/dL)</td>
<td>212.71±48.80</td>
<td>173.71± 40.25</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL-c (mg/dL)</td>
<td>41.58±8.00</td>
<td>45.14±6.89</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL-c (mg/dL)</td>
<td>125.9±45.17</td>
<td>102.73±34.23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.71±9.70</td>
<td>163.46±10.17</td>
<td>0.02</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.39±14.02</td>
<td>63.72±13.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>29.15±4.68</td>
<td>23.83±4.79</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>99.32±12.41</td>
<td>82.39±15.77</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>125.89±19.78</td>
<td>113.81±13.75</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>80.84±12.25</td>
<td>73.85±10.63</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 2. Criteria for diagnosis of the metabolic syndrome according to NCEP ATP III

<table>
<thead>
<tr>
<th>Criterion</th>
<th>NCEP ATP III *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist (cm)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>≥102</td>
</tr>
<tr>
<td>Female</td>
<td>≥88</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>&lt;40</td>
</tr>
<tr>
<td>Female</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>≥150</td>
</tr>
<tr>
<td>Fasting Glucose (mg/dL)</td>
<td>≥100</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>≥130/85</td>
</tr>
</tbody>
</table>

*Three of five required.
Table 3. The levels of paraoxonase, salt-stimulated paraoxonase, arylesterase activities and total antioxidant capacity (TAC) in MES and healthy subjects.

<table>
<thead>
<tr>
<th></th>
<th>MES</th>
<th>Normal subjects</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1 activity (IU/L)</td>
<td>69.62±59.86</td>
<td>91.64±77.45</td>
<td>0.01</td>
</tr>
<tr>
<td>Salt stimulated PON (IU/L)</td>
<td>136.23±111.80</td>
<td>192.24±162.68</td>
<td>0.02</td>
</tr>
<tr>
<td>ARE (KU/L)</td>
<td>45.23±23.24</td>
<td>65.69 ± 31.10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TAC (µmol/L)</td>
<td>972.46±374.13</td>
<td>966.97 ± 380.60</td>
<td>0.912</td>
</tr>
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