Quantitative analysis of DNA strand break and oxidative stress enzymes in Saudi with non-insulin dependent diabetes mellitus patients

Abstract

Background: Non-insulin dependent diabetes mellitus (NIDDM) is a chronically progressive metabolic disorder, which strongly associated with high risk for cardiovascular disorders and mortality.

Objective: This study aims to quantitative analysis of DNA strand break and oxidative stress enzymes in Saudi NIDDM patients.

Materials and Methods: Twenty healthy subjects (group 1, mean age was 32.2±5.13), thirty patients with NIDDM had no diabetic complications (group 2, mean age was 40.1±5.78), and twenty patients with NIDDM had diabetic complications (group 3, mean age was 41.5±5.24) participated in this study. Glucose profile, oxidative stress markers and DNA damage that may be induced by oxidative stress was measured in this study.

Results: There were significant differences in the mean values of the oxidative stress markers (MDAP, MDAE, SOD and GSH) between the healthy subjects (group 1) and patients with NIDDM had no diabetic complications (group 2), patients with NIDDM had diabetic complications (group 3). However, DNA single strand break was linear increase activity from group 1 to group 3. Moreover, there was significant correlation between the values of oxidative stress markers and the DNA single strand break (P<0.05).

Conclusion: There was an association between DNA strand break and poor antioxidant defense among patients with NIDDM patients.

Keywords: DNA quantitative analysis, oxidative stress, non-insulin dependent diabetes mellitus

Introduction

Over the previous 2 decades, non-insulin dependent diabetes (NIDDM) becomes a common health problem that lead to morbidity and mortality worldwide.1-3 However, both non-insulin dependent diabetes and obesity is progressively increased leads to high risk of dyslipidemia, coagulation disorders, insulin hormone resistance and cardiovascular complications.4,5 About 6% of populations are affected with diabetes worldwide.6,7

Diabetic complications are extensive and associated with multiple system dysfunction.8 Abnormal level of oxidative stress usually related to vascular diabetic complications.8 Obesity and diabetes are characterized with high levels of oxidative stress in comparison to the control group.9

Oxidative phosphorylation produces highly reactive molecules that induce tissue damage.10 DNA is the target site for the harmful effects of the oxidative markers, as oxidative markers induce breaks in the DNA strand.11 Shortened DNA telomeres has been reported in some NIDDM patients which was suggested to be related to abnormal levels of oxidative stress markers.12

The aim of this study was to measure the degree of the association between quantitative analysis of DNA strand break and oxidative stress markers among NIDDM patients.

Materials and methods

Subjects

Twenty healthy subjects (group 1, mean age was 32.2±5.13 year), thirty patients with NIDDM had no diabetic complications (group 2, mean age was 40.1±5.78 year), and twenty patients with NIDDM had diabetic complications (group 3, mean age was 41.5±5.24 year) participated in this study.

Laboratory analysis

A. Measurement of oxidative stress markers and anti-oxidant status: Few millimeters of venous blood and plasma in EDTA vial were separated and were stored at -80°C until analysis of markers of lipid peroxidation to include determining plasma levels of malondialdehyde-P (MDAP) and malondialdehyde-E (MDAE) were expressed as mmol/L. studied oxidative stress However, anti-oxidant status were studied by glutathione (GSH) and superoxide dismutase (SOD).

B. Measurement of glycosylated hemoglobin and serum glucose: Colorimetric method was used for estimation of glycosylated hemoglobin (HBA1c). However, Hitachi 912 Chemistry Analyzer using the hexokinase reagent from Boehringer Mannheim (Indianapolis, IN 46256) was used to measure serum glucose.
C. Oxidative DNA damage measurement: The quantitative analysis of DNA strand break: was conducted in accordance with Tice et al. using the comet assay. However, the percentage of DNA in the comet tail and the tail moment are the comet parameters that was detected with Tritek Comet Score TM Free ware Version 1.5.

Statistical analysis

All variables were presented as mean ± SD. However, Mann Whitney’s U-test measured comparison between the mean values of the three groups. Moreover, Pearson’s correlation coefficient (r) was used to detect the degree of correlation between the oxidative stress markers and the DNA single strand break.

Results

Serum glucose and HbA1c (%) levels of the three groups were presented in Table 1, there were significant differences between the healthy subjects (group 1) and patients with NIDDM had no diabetic complications (group 2), patients with NIDDM had diabetic complications (group 3).

Table 1 Serum Glucose and HbA1c level in control healthy group, patients with controlled T2DM and patients with uncontrolled T2DM

<table>
<thead>
<tr>
<th></th>
<th>Group (1)</th>
<th>Group (2)</th>
<th>Group (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>84±7</td>
<td>151±10*</td>
<td>207±14*</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>5±0.53</td>
<td>6.7±0.85*</td>
<td>8.03±0.41*</td>
</tr>
</tbody>
</table>

* p<0.001 as compared with control.

Concerning the results of oxidative stress markers (MDAP, MDAE, SOD and GSH), there were significant differences between the healthy subjects (group 1) and patients with NIDDM had no diabetic complications (group 2), patients with NIDDM had diabetic complications (group 3). Moreover, there was significant degree of correlation between the values of oxidative stress markers and the DNA single strand break as shown in Table 3.

Table 2 The oxidative stress, anti-oxidant enzyme and DNA single strand break in the three groups

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDAP (nmol/ml)</td>
<td>0.33±0.04</td>
<td>0.42±0.1*</td>
<td>0.47±0.05*</td>
<td>0.001</td>
</tr>
<tr>
<td>MDAE (nmol/ml)</td>
<td>0.71±0.12</td>
<td>0.81±0.15*</td>
<td>0.88±0.18*</td>
<td>0.014</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>4.62±1.66</td>
<td>4.25±1.44*</td>
<td>3.05±1.12*</td>
<td>0.009</td>
</tr>
<tr>
<td>GSH (mU/mg protein)</td>
<td>75.15±14.27</td>
<td>64.3±13.65*</td>
<td>57.8±15.23*</td>
<td>0.001</td>
</tr>
<tr>
<td>Strand Break (Tail moment)</td>
<td>2.5±0.73</td>
<td>5.4±1.03*</td>
<td>8.7±1.55*</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* p<0.005 as compared with control.

Table 3 Correlation coefficient (r) of DNA single strand break and oxidative stress markers in the three groups

<table>
<thead>
<tr>
<th></th>
<th>Group 1 Strand Break (Tail moment)</th>
<th>Group 2 Strand Break (Tail moment)</th>
<th>Group 3 Strand Break (Tail moment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDAP (nmol/ml)</td>
<td>0.6310*</td>
<td>0.5821*</td>
<td>0.5765*</td>
</tr>
<tr>
<td>MDAE (nmol/ml)</td>
<td>0.8112 **</td>
<td>0.6136*</td>
<td>0.5519***</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>-0.6321 *</td>
<td>-0.7143**</td>
<td>-0.6225*</td>
</tr>
<tr>
<td>GSH (mU/mg protein)</td>
<td>-0.7643**</td>
<td>-0.6924**</td>
<td>-0.7139**</td>
</tr>
</tbody>
</table>

Spearman’s correlation was used **; p < 0.01 *; P < 0.05

There are several serious complications associated with NIDDM as cardiovascular complications which are related to increased oxidative stress, inflammatory cytokines and insulin resistance that are linked with DNA strand break.37,38 Moreover, abnormal levels oxidative stress induce many diabetic complications19-22 as antioxidant defense is poor that is induced by the metabolic disturbances among patients with NIDDM.22

Concerning oxidative stress markers, the mean values of MDAP and MDAE were significantly higher and the mean values of SOD and GSH were significantly lower among NIDDM patients in comparison to normal control subjects. Our results agreed with Kumawat and colleagues23 reported that GSH significantly reduced and MDA significantly elevated in diabetic patients. Similarly, Kavitha and colleagues stated that diabetic patients had elevated MDA levels.24 Moreover, many studies found an elevation in the MDA levels in patients with NIDDM compared with non-diabetics.25-27 Similarly, several studies found significant higher level of MDA and fasting plasma glucose among poorly controlled NIDDM.28-30 In the other hand, reduced GSH levels were reported among patients with NIDDM.31-35 Similarly, Moussa proved an association between hyperglycemia and GSH depletion.36

Regarding DNA single strand break, there was linear increase activity from group 1 to group 3 in DNA single strand break. Moreover, there was significant correlation between the values of oxidative stress markers and the DNA single strand break. Previous studies reported that prolonged exposure to hyperglycemia induces abnormal levels of oxidative stress markers in NIDDM patients that causes DNA damage.37-40
Conclusion

There was an association between DNA strand break and poor antioxidative defense among patients with NIDDM patients.

Acknowledgments

None.

Conflict of interest

The author declares there is no conflict of interest.

References

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