The effect of hyperglycemic peak induced by oral glucose tolerance test on the oxidant and antioxidant levels

Abstract

Background/aim: Possibility of adverse effects of oral glucose tolerance test (OGTT), carried out for the screening of gestational diabetes, on the pregnant women and fetus is a frequently discussed topic. The purpose of this study was to investigate the effect of the hyperglycemia peak during OGTT on the levels of oxidants and antioxidants in the body.

Materials and methods: Eighty individuals who had applied to the Outpatient Clinic with suspected diabetes and OGTT indication were included in the study. Glucose, total oxidant capacity (status) (TOS), total antioxidant capacity (TAS), superoxide dismutase (SOD), and lipid hydroperoxide (LOOH) levels were tested on blood samples collected from these individuals at minutes 0, 60 and 120 during the OGTT carried out with 75 g glucose. Oxidative stress index (OSI) was calculated as the ratio of TOS to TAS.

Results: While oxidative parameters TOS, LOOH were significantly increased in the 60th minute of OGTT, only LOOH was significantly increased in the 120th minute of OGTT. A significant decrease in antioxidative parameters (TAS, SOD) were observed at 60th minutes of the OGTT and at 120th minutes of the OGTT. Oxidative stress index was significantly increased at the 60th minutes of the OGTT and 120th minutes of the OGTT.

Conclusion: Oxidative stress parameters were increased and antioxidative parameters were decreased during OGTT. However, more extended studies are required to enlighten the effect of the increased oxidative stress on pregnant women and the fetus.
**Key words:** Oral glucose tolerance test, oxidative parameters, antioxidative parameters, oxidative stress

### 1. Introduction

Oxidative stress has an important role in the pathogenesis of diabetes and late complications of diabetes. As a result of having one or more unshared electron, free radicals are so reactive and they tend to grab electrons from other atoms or molecules to fill their outer energy levels [1]. In diabetes mellitus, oxidative stress can occur because of production of reactive oxygen species (ROS) including superoxide (O$_2^-$) radical, hydroxide radical (HO$^-$), hydrogen peroxide (H$_2$O$_2$) in high levels and/or inadequacy of antioxidant defense systems [2]. Increase in ROS production is related to protein glycosylation and/or autooxidation of glucose under hyperglycemic conditions. The reason for the inadequacy of neutralization of free radicals is related to the inadequacy of enzymatic and non-enzymatic radical scavengers (antioxidants) [3].

Glucose oxidation, non-enzymatic protein glycation and following oxidative degradation of glycosylated proteins in diabetes result in the formation of free radicals in excessive amounts. Free radicals form in high amounts formed in general simultaneously with the reduction in the efficiency of antioxidant defense mechanisms damage the cellular organelles and enzymes and cause lipid peroxidation and increase in insulin resistance. Hence, oxidative stress may lead to the occurrence of diabetic complications [4].

Furthermore, a high concentration of glucose in diabetes cause sorbitol production through the polyol pathway. Since NADPH is used for glucose reductase enzyme activity in this pathway, intracellular NADPH will be consumed in diabetes. NADPH is required to transform oxidized glutathione to the reduced form and for the synthesis of
nitric oxide (NO). Therefore, the active sorbitol pathway and consequently, the absence of NADPH will mean the limitation of the antioxidant capacity [4,5]

Diabetes mellitus is a prevalent disease group in the population, and sometimes OGTT is used for diagnostic purposes. Possibility of adverse effects of OGTT, carried out for the screening of gestational diabetes, on the pregnant women and fetus is a frequently discussed topic. The purpose of this study was to investigate the effect of the hyperglycemia peak during OGTT on the levels of oxidants and antioxidants in the body and the balance between oxidants and antioxidants in the body.

2. Materials and methods

This prospective, cross-sectional study was conducted between January 1, 2018, and August 29, 2018, at the outpatient clinics of the Internal Medicine and Endocrinology departments in a tertiary hospital.

2.1. Subjects

Eighty individuals who had applied to Internal Diseases and Endocrinology and Metabolism Diseases Outpatient Clinic with suspected diabetes and OGTT indication were included in the study. These individuals were 18-65 years old and had no previous disease. Glucose, total oxidant capacity (status) (TOS), total antioxidant capacity (TAS), superoxide dismutase (SOD), and lipid hydroperoxide (LOOH) levels were tested on blood samples collected from these individuals at minutes 0, 60 and 120 during the OGTT carried out with 75 g glucose. Oxidative stress index (OSI) was calculated as the ratio of TOS to TAS (OSI = TOS / TAS). Changes on these parameters obtained at minutes 0, 60, and 120 of OGTT and oxidant stress index (OSI) were statistically evaluated.
In addition, patients were divided into three groups based on OGTT results: Group 1: Normal (baseline glucose level < 110 mg/dl and glucose level at minute 120 of OGTT < 140 mg/dl); Group 2: Individuals with impaired fasting glucose and/or impaired glucose tolerance (glucose level between 100 mg/dl and 126 mg/dl at the baseline and/or glucose level between 140 mg/dl and 200mg/dl at minute 120); Group 3: Diabetic individuals (baseline glucose level ≥ 126 mg/dl or glucose level ≥ 200mg/dl at minute 120 of OGTT) and compared in these groups in terms of oxidative and antioxidative parameters.

2.2. Blood collection and preparation
Venous blood samples were collected from an antecubital vein of each patient after a 12-hour overnight fast, and a 10 ml sample of venous blood was placed into a biochemistry tube. Blood samples were withdrawn, their sera were separated with centrifugation at 3000 rpm for 10 min. All material was stored at –80 °C until analysis.

2.3. Analysis of blood samples
Serum TOS, TAS, LOOH, and SOD were determined with Rel Assay Diagnostics kit (Mega Tip, Gaziantep, Turkey) developed by the Erel and Oxidative Stress Index (OSI) values were calculated.

2.4. Determination of total antioxidant status (TAS)
Total antioxidant status (TAS) was measured in serum by the generation of 2,2′-azino-di-(3-ethylbenzthiazoline sulphonate) (ATBS) radical cation using the commercial kit according to the manufacturer’s manual.

2.5. Determination of total oxidant status (TOS)
Total oxidant status (TOS) was measured as described by the manufacturer’s protocol. In this method, the oxidants present in the sample oxidized the ferrous ion-o-dianisidine
complex to ferric ion. Ferric ion produces a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of μmol H₂O₂ equivalent/L of serum.

2.6. Measurement of LOOH levels

Serum LOOH levels were measured with ferrous ion oxidation-xylenol orange (FOX-2) assay, which involves the oxidation of ferrous ion to ferric ion by various oxidants. The ferric ion is then measured with xylenol orange. The LOOH levels are reduced by the application of triphenylphosphine (TPP), which is a specific reductant for lipids. LOOH levels can be estimated as the difference in values in the absence or presence of TPP.

2.7. Calculation of oxidative stress index (OSI)

The TOS: TAS ratio was used as the oxidative stress index (OSI), and was calculated as follows: OSI (arbitrary units) = [(TOS, μmol H₂O₂/L) / (TAS, mmol Trolox equiv./L)].

2.8. Determination of superoxide dismutase (SOD) activity

The total SOD activity was determined in serum using the SOD Activity Assay kit, according to the manufacturer's instructions. SOD was measured by utilizing a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. Measurements of TOS, TAS, LOOH, and SOD were performed on microplate reader Multiscan GO (Thermo Scientific, USA).

2.9. Statistical analysis

All analyzes were performed using SPSS version 23.0 (IBM Co., NY, USA). The suitability of the data for normal distribution was evaluated by Kolmogorov - Smirnov and Shapiro-Wilk tests. Quantitative data showing normal distribution are given as
mean and standard deviation. In comparison between groups; One Way Anova was
used, and Repeated Measures Anova was used for comparison of repeated measures. In
case of difference, post hoc Bonferroni test was used to find out which group(s) were
caused by the difference. The relationships between the variables were evaluated by
Pearson Correlation test. Statistical significance level was taken as p <0.05.

3. Results

OGTT was performed to 80 individuals with OGTT indication in this study. We found
that glucose, TOS, LOOH, and OSI values reached the highest values at minute 60, and
the values at minute 120 were lower than those at minute 60 while they remained high
as compared to the baseline. We also found that TAS and SOD values reached the
lowest levels at minute 60, and increased at minute 120, while they remained lower as
compared to the baseline (Table 1).

There was no statistically significant difference between the TOS levels at the baseline
and minute 120 (p = 0.06), while there were statistically significant differences between
the levels of all the other parameters at time points (p < 0.05) (Table 2).

We compared all the parameters (glucose, TOS, LOOH, TAS, SOD, and OSI) at the
baseline, 60th minute and 120th minute of OGTT in three groups. We found a
statistically significant difference between the glucose levels (p < 0.05), while we found
no statistically significant differences between the other parameters (p > 0.05).

When we evaluated the relationship between glucose and TOS, LOOH, TAS, SOD, and
OSI, we found a statistically significant positive correlation between glucose and TOS,
LOOH and OSI (p < 0.001, r = 0.572; p < 0.001, r = 0.501; p < 0.001, r = 0.470,
respectively) and statistically significant negative correlation between glucose and TAS
and SOD (p < 0.001, r = -0.243 and p = 0.012, r = -0.162, respectively).
However, we were unable to carry out intra-group evaluations because of the small numbers of patients in groups (25 patients in group 2 and 13 patients in group 3).

4. Discussion

We aimed to evaluate the effect of the hyperglycemic peak during OGTT on oxidative stress markers and we found that oxidative stress parameter was increased and antioxidative parameters were decreased during OGTT in our study. Güntaş Korkmaz G et al. [6] investigated total antioxidant status and markers of oxidative stress in subjects with normal or impaired glucose regulation in patients with diabetes. They found that the hyperglycemia was related to increased ischemia-modified albumin, advanced oxidation protein products, and prooxidants-antioxidants balance concentrations and an increase in glucose concentrations during glucose loading could cause tissue damage by increasing oxidative stress. Serin O et al. [7] showed post-challenge 2 hours' serum thiobarbituric acid reactive substances and oxidized low-density lipoprotein levels in subjects with impaired glucose tolerance and diabetic glucose tolerance groups were found to be higher than their baseline levels, which might suggest oxidative stress occurs at an early stage in diabetes. In our study, oxidative stress markers increased significantly in all the groups and antioxidant markers decreased significantly. The difference between the results of studies could be explained by using different antioxidative and oxidative stress markers in determining the oxidative stress status in the body.

Choi JH et al. [8] investigated 16 plasma markers as indicators of inflammation and oxidative stress during OGTT in 54 individuals. Leptin, retinol-binding protein-4, CRP, osteopontin, angiogenin, macrophage-derived chemokine, and macrophage colony stimulating factor concentrations significantly decreased during OGTT, while IL6, IL8,
and monocyte chemoattractant protein-3 concentrations significantly increased during OGTT, which might support that glucose ingestion have to impact on systemic inflammation and oxidative stress. Manning PJ et al. [9] measured the levels of inflammatory cytokines (IL 6, TNF α) and peroxides as oxidative stress markers during OGTT performed in 33 overweight or obese individuals at minutes 0, 30, 60, 90 and 120. They found that IL 6 decreased at minutes 30 and 60, while peroxides decreased significantly at minute 60 as compared to the baseline. In contrary to the results of this study, we found that oxidative stress markers (TOS, LOOH) significantly increased.

Andreeva-Gateva P et al. [10] investigated SOD, glutathione and total antioxidant status levels as antioxidant parameters in 36 healthy volunteers and 36 patients with metabolic syndrome at 0, 60. and 120. minutes of OGTT. They found significant decreases in SOD and glutathione peroxidase activity at minute 120 in both groups while total antioxidant status level increased significantly at minute 120. In our study, the total antioxidant status value was significantly lower at minutes both 60 and 120.

Ceriello A, et al. [11] demonstrated that antioxidant defenses were reduced during the oral glucose tolerance test both in normal and non-insulin-dependent diabetic subjects. Similarly, we also found that OGTT resulted in a significant decrease in antioxidant defense in both diabetic and nondiabetic patients in our study. Nakanishi S et al. [12] examined serum glucose and urine isoprostane as the oxidative stress marker at the baseline, hour 1 and hour 2 of the 75 g oral glucose tolerance test (OGTT) in 775 Japanese-American individuals who had normal glucose tolerance (NGT), impaired glucose tolerance, or diabetes. They found glucose excursion might lead to oxidative stress which is similar to the results of our study.
Muratoğlu C et al. evaluated the effect of 50 g glucose challenge test (GCT) on thiol/disulfide balance in 100 women at 24 - 28 weeks of gestation [13]. In GCT positive pregnant individuals, the glucose load increased oxidative stress by changing the thiol/disulfide homeostasis in GCT positive pregnant individuals while not in healthy pregnancies. Gelaelti RB et al demonstrated increased oxidative DNA damage was present in patients with gestational, overt diabetes and mild gestational hyperglycemia [14]. On the other hand, Rueangdetnarong H et al. [15] compared the levels of oxidative stress biomarkers between pregnancies with gestational diabetes mellitus (GDM) or without GDM. They reported that pregnant with GDM had increased oxidative stress and apoptosis markers levels which were not correlated with the pregnancy outcomes. A better glycemic control did not prevent the increase in oxidative stress. Biomarker levels in cord blood of pregnant with GDM were not altered which suggest the placenta could be the barrier for the oxidative stress and cytokines.

Although there are many studies carried out on many patient groups using many biomarkers to evaluate the relationship between OGTT and oxidative stress and their effects on the human body, diverse results have been reported as well as some of which are contrary to each other. In our opinion, such difference results mainly attributed to the use of many diverse antioxidative and oxidative stress markers to determine the oxidative stress status of the body. To avoid this, reaching a consensus on which antioxidative and oxidative stress markers should be used to determine the oxidative stress status of the body and measuring these determined markers will be a good approach.

We aimed to enlighten the issue of whether to perform or not OGTT in pregnant women with screening purposes. When planning the study, we were hoping that we would find
that OGTT screening would not result in any significant increase in oxidative stress in
humans, and therefore to conclude that OGTT could safely be carried out pregnant
women also. However, we found that OGTT increased the oxidative parameters and
decreased antioxidative parameters in humans, and consequently, caused an increase in
oxidative stress. However, since we did not evaluate whether these biomarkers are
present in cord blood of pregnant women we don't know its effect on the fetus. This will
require further studies on the effects of OGTT on humans and particularly on pregnant
women and if the oxidative stress in the pregnant woman affects the fetus or not, and if
it does, what are these effects. Furthermore, studies are also required to enlighten
whether the hyperglycemia peak caused by OGTT in pregnant women will result in a
hyperglycemia peak in the fetus, and if it does, will this result in oxidative stress and
possible related damage.

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Table 1. TOS, LOOH, TAS, SOD and OSI levels at the baseline and 60 and 120 minutes in OGTT.

<table>
<thead>
<tr>
<th></th>
<th>0 (n: 80)</th>
<th>60 (n: 80)</th>
<th>120 (n: 80)</th>
<th>P*</th>
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</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>103.33 ± 19.02</td>
<td>188.70 ± 54.93</td>
<td>145.85 ± 50.67</td>
<td>&lt;0.001</td>
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<tr>
<td>TOS</td>
<td>1.77 ± 0.22</td>
<td>2.64 ± 0.39</td>
<td>1.89 ± 0.23</td>
<td>&lt;0.001</td>
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<tr>
<td>LOOH</td>
<td>16.40 ± 2.56</td>
<td>26.97 ± 3.66</td>
<td>19.16 ± 4.07</td>
<td>&lt;0.001</td>
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<tr>
<td>TAS</td>
<td>2.20 ± 0.86</td>
<td>1.61 ± 0.43</td>
<td>1.85 ± 0.47</td>
<td>&lt;0.001</td>
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<tr>
<td>SOD</td>
<td>1.81 ± 0.23</td>
<td>1.50 ± 0.18</td>
<td>1.68 ± 0.25</td>
<td>&lt;0.001</td>
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<tr>
<td>OSI</td>
<td>0.89 ± 0.29</td>
<td>1.80 ± 0.64</td>
<td>1.09 ± 0.33</td>
<td>&lt;0.001</td>
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</tbody>
</table>

* Repeated Measures ANOVA

TOS (μmol H₂O₂/L): Total antioxidant capacity; LOOH (μmol/L): Lipid hydroperoxide; TAS (mmol Trolox equiv./L): Total antioxidant capacity; SOD (U/mL): Superoxide dismutase; OSI: Oxidative stress index; OGTT: Oral glucose tolerance test.
Table 2. Comparison of glucose, TOS, LOOH, TAS, SOD and OSI at the baseline and 60 and 120 minutes in OGTT.

<table>
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<tr>
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<th>J (minute)</th>
<th>Mean Difference (I-J)</th>
<th>P*</th>
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<tr>
<td>Glucose (mg/dl)</td>
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<td>-85.38</td>
<td>&lt;0.001</td>
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<td></td>
<td></td>
<td>120</td>
<td>-42.53</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>60</td>
<td>120</td>
<td>42.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TOS</td>
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<td>60</td>
<td>-0.87</td>
<td>&lt;0.001</td>
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<td></td>
<td></td>
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<td>-0.13</td>
<td>0.060</td>
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<tr>
<td></td>
<td>60</td>
<td>120</td>
<td>0.74</td>
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<tr>
<td>LOOH</td>
<td>0</td>
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<td>-10.57</td>
<td>&lt;0.001</td>
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<td></td>
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<td>120</td>
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<td></td>
<td>60</td>
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<td>7.80</td>
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<td>TAS</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
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<td>120</td>
<td>0.35</td>
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<td></td>
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<td>120</td>
<td>-0.18</td>
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<td>&lt;0.001</td>
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<td></td>
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<td>120</td>
<td>0.70</td>
<td>&lt;0.001</td>
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</table>

* Bonferroni Post-Hoc Test

TOS (μmol H₂O₂/L): Total antioxidant capacity; LOOH (μmol/L): Lipid hydroperoxide; TAS (mmol Trolox equiv./L): Total antioxidant capacity; SOD (U/mL): Superoxide dismutase; OSI: Oxidative stress index; OGTT: Oral glucose tolerance test.