การตรวจวิเคราะห์ Lipid hydroperoxides, thiobarbituric acid-reactive substance และ Total antioxidant capacity ประกาศภาวะ oxidative damage ในผู้ป่วยเบาหวานชนิดที่ 2

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บทคัดย่อ

Lipid hydroperoxides (LOOH) และ thiobarbituric acid reactive substance (TBARS) เป็นผลลัพธ์ของกระบวนการ lipid peroxidation ที่เกิดขึ้นภายในเซลล์และทำให้เกิด cell adhesion การเพิ่มจำนวนของเซลล์ การตอบสนองต่อการอักเสบ การเก็บและการตายของเซลล์ กระบวนการ lipid peroxidation มีบทบาทสำคัญในการพัฒนาของ atherosclerosis การศึกษาการตรวจวิเคราะห์ LOOH, TBARS และ total antioxidant capacity (TAC) ซึ่งเป็น markers ของการเกิด oxidative damage ในผู้ป่วยเบาหวานชนิดที่ 2

โดยศึกษาในเอกสารที่มีข่าวสารการตรวจสุขภาพผู้ป่วยเบาหวาน 206 ราย แบ่งเป็นกลุ่มผู้ป่วยเบาหวาน 123 ราย (เพศชาย 37 รายและเพศหญิง 86 ราย) และกลุ่มสุขภาพ 83 ราย (เพศชาย 18 รายและเพศหญิง 65 ราย) และทำการตรวจวิเคราะห์ LOOH, TBARS และ TAC ในกลุ่มเบาหวานและสุขภาพ

ผลการศึกษาพบว่า ระดับของ LOOH และ TBARS ในกลุ่มผู้ป่วยเบาหวานสูงกว่ากลุ่มสุขภาพอย่างมีนัยสำคัญทางสถิติ (p<0.05) และระดับ TAC ในกลุ่มผู้ป่วยเบาหวานต่ำกว่ากลุ่มสุขภาพอย่างมีนัยสำคัญทางสถิติ (p<0.05) กลุ่มผู้ป่วยเบาหวานแสดงถึงการเกิด oxidative stress เพิ่มขึ้น

การตรวจวิเคราะห์ LOOH, TBARS และ TAC สามารถวัดภาวะ oxidative stress ในผู้ป่วยโรคเบาหวาน

คำที่สำคัญ: Lipid peoxidation, Lipid hydroperoxide, Thiobarbituric acid reactive substance, Total antioxidant capacity, บทความนี้ที่ 2

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Lipid hydroperoxide, thiobarbituric acid–reactive substances and total antioxidant capacity as assays for oxidative damage in type 2 diabetes patients

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Abstract

Lipid hydroperoxide (LOOH) and thiobarbituric acid–reactive substances (TBARs), products of lipid peroxidation, are produced in cellular processes and involved in cell adhesion, proliferation, inflammatory responses, cell aging, and death. Lipid peroxidation plays an important role in the premature development of atherosclerosis. We aimed to use LOOH, TBARs, and total antioxidant capacity (TAC) as the markers of oxidative damage in type 2 diabetes patients (T2D).

The study included 206 participants, which were randomized from Phitsanulok residents stratified as 123 T2D (37 males and 86 females) and 83 healthy controls (18 males and 65 females). LOOH, TBARs and TAC levels were measured in these participants.

LOOH and TBARs were significantly higher in T2D than healthy controls (p<0.05), while TAC was significantly lower (p<0.05) in T2D. LOOH, TBARs, hypertriglyceridemia, and abdominal obesity in T2D were 12.86, 8.54, 3.96, and 4.05 fold higher than healthy controls (p<0.05). Increased LOOH and TBARs as well as decreased TAC levels indicated oxidative stress in T2D.

LOOH, TBARs and TAC assay were useful to assess the overall oxidative stress in T2D.

Keywords: Lipid peoxidation, Lipid hydroperoxide, Thiobarbituric acid reactive substance, Total antioxidant capacity, Type 2 diabetes

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Introduction

Lipid peroxidation is one of the common cellular processes and is involved in cell adhesion, proliferation, inflammatory responses, cell aging and death. In addition, it has been shown to be associated with disturbances of fine structure and function loss of biological membranes.\(^1\) Lipid peroxidation may contribute to initiation, promotion, and/or progression of many human diseases, including diabetes, atherosclerosis, rheumatoid arthritis, and liver diseases.\(^23\) There are various plasma markers of lipid peroxidation, including lipid hydroperoxides (LOOH), malondialdehyde (MDA), conjugated dienes, oxidation resistance assay, oxyysterols and \(\text{F}_2\alpha\)-isoprostanes. The peroxidation of lipoproteins is believed to play an important role in atherosclerosis.\(^45\) (Oxidation modified) lipoproteins in serum are involved in the activation of the series of cellular events, ultimately resulting in atherosclerosis.\(^6\) Several studies have reported increased oxidative stress\(^78\) and decreased antioxidative defense mechanisms\(^911\) in diabetic patients with angiographically documented cardiovascular diseases (CAD).

Type 2 diabetic patients (T2D) have been described to be under enhanced oxidative stress.\(^12\) Glycation of proteins can lead to oxidative stress by direct release of superoxide and hydrogen peroxide and activation of phagocytes through a specialized receptor for advanced glycation end products.\(^13\) The most important source of reactive oxygen species (ROS) in the vessel wall includes nicotinamide adenine dinucleotide phosphate (NADPH) oxidases,\(^14\) xanthine oxidase,\(^15\) uncoupled endothelial nitric oxide synthase (eNOS),\(^16\) enzymes involved in arachidonic acid metabolism, and mitochondrial sources.\(^17\) Increased oxidative stress is involved in the development of myocardial ischemia, stroke, reperfusion injury, and restenosis after bypass surgery or angioplasty, as well as other conditions associated to high cardiovascular disease (CVD) risks, such as hypertension and diabetes. This brought to the focus issue of understanding the source of the LOOH and MDA in plasma lipoproteins, while the biological effect of oxidative modified lipoproteins on vascular cells became a major issue in cardiovascular disease study. It has been demonstrated in previous studies that levels of MDA often measured as thiobarbituric acid reactive substances (TBARs). We aim to use LOOH, TBARs, and TAC assays as markers of oxidative damage in T2D.

Materials and methods

Subjects: 206 participants in this study were stratified as 123 T2D (mean age = 59.45, SD = 10.03 yr; 37 males and 86 females) and 83 healthy control subjects (mean age = 58.69, SD = 8.27 yr; 18 males and 65 females). They were randomized
from Phitsanulok residents who came for their health check up in our service project during July 2008 to March 2009. The inclusion criteria of T2D in the present study included (i) history of hyperglycemia more than 5 years (ii) no acute illness and clinical signs of ischemia, myocardial infarction, unstable angina or stroke and (iii) no changes in treatments over 30 days. Healthy controls were from general population in the same district. Inclusion criteria for healthy controls included: absence of any history of CVD, hypertension, any condition limiting mobility, life-threatening diseases, or any other disease or condition that would impair compliance. Exclusion criteria were smoking and intake of antioxidant supplementation in 2 months before their inclusion in the study. All T2D patients were regularly treated with glycemic lowering drugs (glipizide, pioglitazone, daonil (glibenclamide), diamicron (Gliclazide), rosiglitazone, voglibose, and metformin), lipid lowering drugs (hydrochlorothiazide, simvastatin, mevalotin, gemfibrozil, lopid), and anti-hypertensive drugs (β-blocker: atenolol, dilatrend (carvedilol), metoprolol, ACE inhibitor: coversyl, enalapil, angiotensin II- antagonist: cozaar (losartan potassium), diovan (valsartan), irbesartan, calcium channel blockers: diltiazem, felodipine, manidipine, nifedipine, amlopidine). The Ethics Committee of the Naresuan University approved the study protocol. All participants gave written informed consent.

**Anthropometric measurement**

Waist circumference (WC) of these volunteers was measured at the midpoint between the rib cage and the top of the lateral border of the iliac crest during minimal respiration. The blood pressure measurements were obtained in seated position for 5 min after resting. Measurements were made twice on all participants at 5 min intervals with a digital blood pressure monitor, ES-P 110 (Terumo cooperation, Japan). The average of the two measurements was used for data analysis.

**Blood sampling**

Venous blood was taken in the morning after an overnight fast for at least 8-12 h. The serum was separated and kept frozen at -7°C until assayed.

**Glucose and lipid profile assays**

Fasting plasma glucose (Glu), serum total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL-C) were measured by using enzymatic method with a Hitachi 912 autoanalyzer (Roche Diagnostic, Switzerland). We calculated low density lipoprotein cholesterol (LDL-C) using Friedewald’s formula in specimens with TG levels <400 mg/dl.
Chemicals

Ammonium ferrous sulphate, $\text{H}_2\text{SO}_4$, butylated hydroxyxylene (BHT), triphenyl-phosphine (TPP), thiobarbituric acid (TBA), and xylenol orange were purchased from Sigma Aldrich. All general chemicals and reagents were of the highest purity available.

Preparation of FOX2-reagent

FOX2-reagent was prepared by dissolving xylenol orange and ammonium sulphate in 250 mM $\text{H}_2\text{SO}_4$ to yield final concentration of 1 mM and 2.5 mM, respectively. One volume of this concentrated reagent was added to 9 volumes of HPLC-grade methanol containing 4.4 mM BHT to make the working reagent, which comprised 250 μM ammonium sulphate, 100 μM xylenol Orange; 25 mM $\text{H}_2\text{SO}_4$ and 4 mM BHT in 90% (V/V) methanol. The working reagent was routinely calibrated against solutions of $\text{H}_2\text{O}_2$ of known concentrations. Reagent was also obtained as the commercially available material from Pierce (Peroxoquant; methanol-compatible formulation).

Lipid hydroperoxide (LOOH) assay

Aliquots (90 μL) of serum samples were transferred into 1.5 mL microcentrifuge vials together with 10 μL of methanol (in duplicate) or 10 μL of TPP (10 M) in methanol (in duplicate). This generated duplicate blank and test samples, respectively, as described previously.$^{15,10}$ The samples were then vortexed and subsequently incubated for 30 min at room temperature. FOX2-reagent (900 μL) was then added and the samples were vortexed and incubated for 30 min. The samples were then centrifuged at 12000 g for 10 min prior to determination of the absorbance of the supernatants at 560 nm. Levels of hydroperoxide in the serum sample were then determined using the difference between the mean absorbance of samples with and without TPP pretreatment. In the case of lipoprotein suspensions or liposomes, samples (90 μL) were mixed with 10 μL of methanol or with 10 μL of TPP (10 mM) in methanol, incubated at room temperature for 30 min and then mixed with FOX2-reagent (900 μL) prior to incubation for 30 min at room temperature. After centrifugation at 12000 g for 10 min, the absorbance of the supernatants was monitored at 560 nm. Hydroperoxide content was determined using an molar absorption coefficient of $4.3 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ or by reference to a cumene hydroperoxide standard curve. We also confirmed the serum signal authenticity as a ferric-xylenol Orange complex can be established by scanning from 500 nm to 600 nm and identifying an absorbance peak at 560 nm.
**TBARs assay**

The TBARs was determined by the method of Tangvarasittichai et al.\(^2\) The method is based on the formation of red (pink) chromophore following the reaction of TBA with MDA and the other breakdown products of peroxidized lipids called TBARs. One molecule of MDA reacts with 2 molecules of TBA to yield a pink pigment with absorption maximum at 532 nm.

**Total antioxidants capacity assay (TAC)**

The assay is based on the reaction of metmyoglobin with hydrogen peroxide to form ferryl myoglobin, a free radical species. A chromogen 2, 2′-amino-di-[3-ethylbenzthiazole sulphonate] is incubated with ferryl myoglobin to produce radical cation which has a relatively stable blue-green color that can be measured at 600 nm. Antioxidants in serum can suppress this color production to a degree proportional to their concentration. The assay was calibrated using 6-hydroxy-2, 5, 8-tetramethylchroman-2-carboxylic acid (Trolox), and results were expressed as mmol/L trolox equivalent.\(^{30}\) The within-run coefficient of variation for the TAC assay in control material assay was 4.8% (n=10).

**Statistical analysis**

The values were expressed as mean and standard deviation. Differences in the mean of each variable were calculated using t-test. Logistic regression analyses were performed to test the mediating effect of T2D on the associations of elevated LOOH, TBARs, hypertriglyceridemia, and abdominal obesity, respectively. Correlations between LOOH and TBARs were done using Pearson correlation analysis. The receiver operating characteristic (ROC) curve is a plot between sensitivity (Y-axis) versus false positive (X-axis), obtained for different cutoff points. Areas under the curve (AUC) of the ROC curve and their 95 percent confidence intervals (CI) were evaluated as a measure of diagnostic accuracy. In general, an AC of less than 0.5 suggested no discrimination. P-values less than 0.05 were considered statistically significant. All analyses were carried out using SPSS computer program version 13.0 (SPSS Inc., Chicago, IL).

**Results**

Clinical characteristics of the T2D patients and healthy controls are shown in Table 1. Our data showed that both LOOH and TBARs in T2D patients were significantly higher \((p<0.001)\) than healthy controls. The total antioxidant capacity in T2D was also significantly lower \((p<0.001)\) than healthy controls. All the conventional cardiovascular risk factors in T2D patients (blood pressure, WC, Glu, TC, and TG) were also significantly higher \((p<0.05)\) than healthy controls (Table 1). HDL-C levels in T2D patients were significantly lower \((p<0.001)\). Excepted LDL-C was not significantly different.
Table 1  General characteristics of healthy control subjects and type 2 diabetes mellitus patients

<table>
<thead>
<tr>
<th></th>
<th>Healthy control (n=83)</th>
<th>Type 2 diabetic patients (n=123)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>55.93±12.01*</td>
<td>59.45±10.03*</td>
<td>0.560</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>122.88±13.84</td>
<td>136.80±19.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic PB (mmHg)</td>
<td>74.02±9.08</td>
<td>80.45±11.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.86±3.94</td>
<td>25.54±3.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>80.72±9.64</td>
<td>96.31±20.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>95.46±9.10</td>
<td>124.44±38.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>201.81±28.93</td>
<td>227.47±57.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>103.81±46.33</td>
<td>191.56±152.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>71.84±14.79</td>
<td>60.87±16.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>108.25±27.85</td>
<td>119.30±50.21</td>
<td>0.07</td>
</tr>
<tr>
<td>Lipid Hydroperoxide</td>
<td>4.01±2.03</td>
<td>8.50±2.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TBARs (mmol/l)</td>
<td>2.87±0.96</td>
<td>5.94±3.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TAC (Trolox unit)</td>
<td>0.534±0.018</td>
<td>0.393±0.018</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Mean ± SD

Logistic regression analysis

Table 2 shows the results of the logistic regression analyses, performed to evaluate the adjusted odds ratio (OR) of T2D patients associated with elevated lipid hydroperoxide was 12.86 fold greater (95% CI, 5.23–31.63), elevated TBARs of T2D was 8.54 fold greater (95% CI, 3.15–23.18), hypertriglyceridemia was 3.96 fold greater (95% CI, 1.49–10.48), and abdominal obesity was 4.05 fold greater (95% CI, 1.58–10.35) compared with healthy controls in the present study (Table 2).
Table 2  Adjusted odds ratios and 95% confidence interval (CI) of T2D patients for elevated lipid hydroperoxide, TBARs, hypertriglyceridemia, and abdominal obesity model.

<table>
<thead>
<tr>
<th>Test variable(s)</th>
<th>OR</th>
<th>95% confidence interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower bound</td>
<td>Higher bound</td>
</tr>
<tr>
<td>Elevated LOOH</td>
<td>12.86</td>
<td>5.23</td>
<td>31.63</td>
</tr>
<tr>
<td>Elevated TBARs</td>
<td>8.54</td>
<td>3.15</td>
<td>23.18</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>3.96</td>
<td>1.49</td>
<td>10.48</td>
</tr>
<tr>
<td>Abdominal obesity</td>
<td>4.05</td>
<td>1.58</td>
<td>10.35</td>
</tr>
</tbody>
</table>

Univariate correlation between LOOH, TBARs, TAC and other variables

Significant positive correlations between LOOH with WC and Glu (r = 0.370 and 0.236, p<0.05) and negatively with HDL-C (r = -0.244, P=0.007) were observed in T2D. And the significant negative correlations between TBARs with HDLc (r=-0.241, p<0.05) was observed in T2D. TAC was not correlated with any variables that may act as independent marker (Table 3).

Table 3  Bivariate correlation between LOOH, TBARs, TAC and other variables

<table>
<thead>
<tr>
<th>LOOH (U/mL) with</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC (cm)</td>
<td>0.370</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glu (mg/dl)</td>
<td>0.236</td>
<td>0.009</td>
</tr>
<tr>
<td>HDLc (mg/dl)</td>
<td>-0.244</td>
<td>0.007</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TBARs (μmol/L) with</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDLc (mg/dl)</td>
<td>-0.241</td>
<td>0.007</td>
</tr>
</tbody>
</table>
ROC curve

ROC curve (Fig. 1) analysis showed that LOOH, TBARs, and TAC assays were significantly discriminator and cutoff values of these markers for diabetes with oxidative stress. The AUC of the ROC curves were greater than 0.5, used as the better prediction markers for oxidative stress in diabetes patients. The AUC of these markers (LOOH, TBARs, and TAC) were greater 0.5 indicating that these markers superior for estimating the oxidative stress in T2D in this study (Table 4). LOOH, MDA, and TAC cutoff levels for predictions of oxidative stress in the present study were 6.01 U/mL with sensitivity and specificity of 85.4 and 84.3 percent, 3.50 μmol/L with sensitivity and specificity of 74.0 and 83.1 percent, 0.503 Trolox units with sensitivity and specificity of 71.1 and 99.9 percent, respectively.

Table 4  Area under the curve of LOOH, TBARs and TAC in T2D patients

<table>
<thead>
<tr>
<th>Test Result Variable(s)</th>
<th>Area</th>
<th>Std. Error(a)</th>
<th>Asymptotic Sig.(b)</th>
<th>Asymptotic 95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOOH</td>
<td>.909</td>
<td>.024</td>
<td>.000</td>
<td>.863 to .955</td>
</tr>
<tr>
<td>TBARs</td>
<td>.805</td>
<td>.031</td>
<td>.000</td>
<td>.745 to .865</td>
</tr>
<tr>
<td>TAC</td>
<td>.999</td>
<td>.001</td>
<td>.000</td>
<td>.997 to 1.001</td>
</tr>
</tbody>
</table>

The test result variable(s): LOOH, and TBARs had at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a  Under the nonparametric assumption

b  Null hypothesis: true area = 0.5
Fig. 1 ROC curves for (A) lipid hydroperoxide (LOOH), TBARs (MDA) and (B) total antioxidant of T2D in the type 2 diabetes patients

Discussion

The precise pathogenesis of diabetic dyslipidemia is not known; however, evidence suggests that insulin resistance has a central role in the development of this condition.\textsuperscript{(21, 22, 23)} The associations with increased triglyceride levels, decreased the level of HDL-C and normal or slightly elevated LDL-C levels (increased the concentration of small dense LDL-C
particles). Our results showed that T2D patients had significantly elevated triglyceride, low HDL-C and no significantly difference in LDL-C levels, as well as as elevated lipid peroxidation markers. In general, it is assumed that increased lipid peroxidation is the result of increased oxidative stress in diabetes mellitus. Therefore, measurement of LOOH and TBARs, the products of lipid peroxidation, is used to estimate in vivo or in vitro of oxidative damage.

The elevated coronary heart disease risks in patients with T2D may be attributed to a combined dyslipidemia. That is characterized by increased serum triglyceride and decreased serum HDL-C concentrations and usually accompanied by the presence of small, dense LDL particles and together comprise the “atherogenic lipoprotein phenotype”. This phenotype prevalence and concomitance with the elevation of oxidative stress may suggest a higher overall burden of atherosclerotic disease. There is increasing evidence that oxidative stress plays an important role in the premature development of atherosclerosis. Our results showed that T2D had increased TG levels and decreased HDL-C levels, abdominal obesity, and also increased LOOH and TBARs levels. Nourooz-zadeh et al. showed that the majority of hydroperoxides generated in plasma were recovered in the LDL fraction. Furthermore, when isolated lipoproteins oxidized by 2, 2′-azo-bis-(2-amidino-propane) hydrochloride (AAPH), very-low-density lipoprotein and LDL showed the greatest propensity for hydroperoxide accumulation, whereas HDL seemed relatively resistance. Yoshida et al. reported that the oxidative susceptibility of LDL in diabetic patients was increased as compared with that in healthy control subjects. Moreover, proinflammatory activities relevant to early events in atherogenesis appear to be manifested, at least by mean of lipid peroxide- (and oxidized LDL)-mediated expression of vascular adhesion molecules in endothelial cells and other genes regulating inflammation. Several studies have shown that oxidative modification of LDL may promote fatty-streak formation and early lesion of atherosclerosis. When elevated oxidative stress occurs, concomitance with decreased TAC, accelerated atherosclerosis may be present. Thus, antioxidant might potentially be useful in preventing or delaying development of atherosclerosis as shown in rabbits, and epidemiologic studies have shown a favorable effects of high plasma vitamin E levels on cardiovascular morbidity and mortality in human subjects. As in our data were showed that TAC in T2D patients were significantly decreased (p<0.001) than healthy controls. These raise questions whether antioxidants treatments can be
effective to prevent or delay the onset of diabetic complications. Antioxidants such as vitamin C and E, lipoic acid, antioxidative enzymes, deacetylcysteine, and others have been reported to prevent hyperglycemia-induced biological changes such as cytokine induction, matrix synthesis, and cellular growth and turnover.\(^{(34-36)}\)

A number of limitations of the studies discussed here need to be highlighted. All of the methods used to assess oxidative stress in these studies are indirect and there is limited evidence that these methods reflect oxidative stress \textit{in vivo}. Furthermore, the clinical utility of these assays is questionable due to cost and the time required for sample preparation and analyses. There is also a lack of standardization of these methods potentially explaining different outcomes on sample population groups. For example, sample handle is extremely important for measures of oxidative stress. If samples are not immediately centrifuged and store at \(-70^{\circ}C\) lipids have been shown to auto-oxidized. Furthermore, samples which have been freeze thawed can also undergo lipid auto-oxidation. Oxidative modification of lipid associated with LDL and cellular constituents contribute to endothelial dysfunction and inflammatory pathway is associated with atherosclerosis.\(^{(37, 38)}\)

In conclusion, the LOOH and TBARs assays measure products of oxidative damage in T2D. These are useful to assess the overall impact of oxidative stress. Therefore, LOOH, TBARs, and TAC assay were good markers for predicting or monitoring oxidative damage in T2D.

**Acknowledgement**

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