Assessment of Interleukin 6, High Sensitive C-reactive Protein and Lipid Profile Levels among Sudanese with Type2 Diabetes Mellitus

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Abstract:
A comparative cross sectional study was conducted during the period from 2013 to 2015 to assess the plasma levels of interleukin 6, high sensitive C-reactive protein and lipid profile among Sudanese with type2 diabetes mellitus. Two hundred patients with type2 diabetes mellitus were selected as a test group compared with a control

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group which included 100 healthy volunteers. Blood specimens were collected from both groups and glycated hemoglobin, interleukin 6, high sensitive C-reactive protein and lipid profile were estimated. Age and gender of the test group were matched with the control group. Spectrophotometric methods were used for measurement of lipid profile. Glycated hemoglobin was measured by using fluorescence immunooassay technology (sandwich immunodetection method) and the high sensitive C-reactive protein was measured by using immunoturbidmetric method. Furthermore, Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) was used to estimate interleukin 6. Statistical package for social science (SPSS version 16) computer software was used for data analysis. The results of this study indicated a significant increase in mean of the plasma interleukin 6, high sensitive C-reactive protein and lipid profile of the test group when compared with the healthy control group, and a significant elevation in mean of the interleukin 6, high sensitive C-reactive protein and triacylglycerol in uncontrolled type2 diabetic patients when compared with the mean of those controlled type2 diabetic but there was no significant differences in total cholesterol, high density lipoprotein, low density lipoprotein. Also there was a significant elevation in mean of plasma interleukin 6, high sensitive C-reactive protein and lipid profile in obese with type2 diabetes mellitus when compared with the mean of non obese type2 diabetic patients. Furthermore, the result indicated a significant positive correlation between high sensitive C-reactive protein in type2 diabetic patients to level of interleukin 6, total cholesterol, low density lipoprotein and triacylglycerol; however, there was a negative correlation between high sensitive C-reactive protein to high density lipoprotein cholesterol. Moreover, there was no significant correlation between interleukin 6 and lipid profile.

**Key words:** Inflammatory cytokines, Cholesterol, Triacylglycerol, High density lipoprotein cholesterol, Low density lipoprotein cholesterol, Hyperglycemia, glycated hemoglobin, body mass index.
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INTRODUCTION:

Diabetes is a metabolic disorder with inappropriate hyperglycemia either due to an absolute or relative deficiency of insulin secretion or reduction in the biologic effectiveness of insulin or both. It is also associated with disturbances concerned with protein, carbohydrate and lipid metabolism. The decreased uptake of glucose into muscle and adipose tissue leads to chronic extra cellular hyperglycemia which results in tissue damage and chronic vascular complications in both type I and II Diabetes Mellitus (1, 2)

Among several markers of inflammation, interleukin 6 (IL-6) and C-reactive protein (CRP), are associated with hyperglycemia, insulin resistance, and overt type 2 DM. (3) Interleukin 6 (IL-6) is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine (4), secreted by T cells and macrophages to stimulate immune response, e.g. during infection and after trauma, especially burns or other tissue damage leading to inflammation. (5) IL-6 is produced in a variety of tissues including adipocytes and is thought to be a reason why obese individuals have higher endogeneous levels of CRP. IL-6 is responsible for stimulating acute phase protein synthesis, as well as the production of neutrophils in the bone marrow. (6) hs–CRP is found to be significant in people with diabetes. CRP, a pentameric protein produced by the liver has emerged as the golden marker for inflammation. It is a non-immunoglobulin protein having five identical subunits. It is a member of pentraxin family proteins; it is primarily derived via IL-6–dependent hepatic biosynthesis. It is an acute phase response protein markedly increased in both inflammatory and infectious diseases. It plays an important role in innate immunity. It assists in complement binding to foreign and damaged cells and enhances phagocytosis. (7) People with Type 2 diabetes, regardless of blood sugar control, tend to have
increased cholesterol, decreased HDL, and increased LDL. This lipid profile tends to persist even if blood sugar levels are under control pointing to an even higher likelihood of developing plaques. These LDL molecules are oxidized, taken up by macrophages, which become engorged and form foam cells. These cells often become trapped in the walls of blood vessels and contribute to atherosclerotic plaque formation. In fact, plaques formed in the arteries of people with Type 2 diabetes tend to be more fatty and less fibrous than in people with Type 1 diabetes, leading to an even higher risk of a plaque dislodging to cause a heart attack or stroke.\(^8\)

Diabetes is now one of the major health problems in Sudan resulting in hospital admissions and mortality. The actual number of people with diabetes in Sudan is not known. One study showed that people with diabetes constituted 7\% of all hospital admissions, a value higher than that reported for other African countries. Another study indicated that 10\% of adult patient deaths in hospitals were caused by diabetes. Acute complications, especially DKA, are the commonest cause of diabetes-related mortality in Sudan.\(^9\)

This study aims to assess the plasma levels of interleukin 6, high sensitive C-reactive protein, total cholesterol, triacylglycerol, high density lipoprotein cholesterol and low density lipoprotein cholesterol among Sudanese with type2 diabetes mellitus for a lot of reasons some of these reasons are;

Rates of diabetes have increased markedly over the last 50 years in parallel with obesity. As of 2011 there are approximately 285 million people with the disease compared to around 30 million in 1985. Long-term complications from high blood sugar can include heart disease, strokes, diabetic retinopathy where eyesight is affected, kidney failure which may require dialysis, and poor circulation of limbs leading to amputations. Type2 diabetes makes up about 90\% of cases of
diabetes with the other 10% due primarily to diabetes mellitus type 1 and gestational diabetes.

C-reactive protein is the principal downstream mediator of the acute phase response and is primarily derived via IL-6–dependent hepatic biosynthesis. In rodent models of glucose metabolism, the in vivo infusion of human recombinant IL-6 has been shown to induce gluconeogenesis, subsequent hyperglycemia, and compensatory hyperinsulinemia. Similar metabolic responses have been observed in humans after administration of subcutaneous recombinant IL-6. Cross-sectional investigations further support a role for inflammation in the etiology of diabetes. Several studies have demonstrated elevated levels of IL-6 and CRP among individuals both with features of the insulin resistance syndrome and clinically overt type 2 DM.

CRP level is an independent risk factor for atherosclerotic disease. Patients with high CRP concentrations are more likely to develop stroke, myocardial infarction, and severe peripheral vascular disease.

Total cholesterol is important and necessary for human health but high levels of cholesterol in the blood have been linked to damage to arteries and cardiovascular disease.

To be honest, I have to mention that, this is not obviously all the reasons which make me focuses in these phenomena which present or stand as an obstacle in front of the patient affected by this disease.

The objectives of this research were;
- To assess the plasma levels of interleukin 6, high sensitive C-reactive protein and lipid profile among Sudanese with type2 Diabetes Mellitus in comparison with healthy Sudanese volunteers.
- To measure the plasma levels of IL6, hsCRP, HbA1c, triacylglycerol, total cholesterol, HDLc and LDLc in Sudanese with Type 2 Diabetes Mellitus.
- To assess the relationship between plasma levels of interleukin 6 and hsCRP, triacylglycerol, total cholesterol, HDLc, LDLc in Sudanese with Type 2 Diabetes Mellitus.
- To assess the plasma levels of interleukin 6, high sensitive C-reactive protein and lipid profile among uncontrolled diabetic patients in comparison with controlled diabetic patients.
- To assess the plasma levels of interleukin 6, high sensitive C-reactive protein and lipid profile among obese diabetic patients in comparison with non-obese diabetic patients.

MATERIALS AND METHODS:

This is a quantitative, descriptive, analytic, comparative cross-sectional and hospital-based study. It was conducted in Khartoum state in different hospitals and Diabetes centers during the period from 2013 to 2015. A total of two hundred patients with type 2 diabetes (Test group) were enrolled in this study compared with hundred healthy volunteers. Age and sex of the test group were matched with the control group. Patients with type 1 diabetes mellitus and those with any inflammatory disorders were excluded from this study.

The study was approved by the research board of the Faculty of Medical Laboratory, University of Science and Technology, and full permission was obtained from local health authorities in the area of the study. All participants provided a written consent, for each participant an interview with a questionnaire was used to obtain the clinical data.

After a written consent and used alcohol swab (70% ethanol) as antiseptic for skin, a sample of venous blood (6ml) was collected from each participant in this study, using disposable plastic syringe. The blood was collected from the arm.
directly and then was divided into 2 containers; lithium heparin container for lipid profile and the other container is EDTA container for Glycosylated Haemoglobin (HbA1c), interleukin 6 and high sensitive C - reactive protein. The containers were then centrifuged at 3000 rpm for 3 minutes and obtained plasma. The plasma prepared was stored at -80°C until used.

Spectrophotometric methods were used for measurement of triacylglycerol, total cholesterol, HDLc, and LDLc levels. Glycated hemoglobin was measured by using fluorescence immunoassay technology (sandwich immunodetection method) and the high sensitive C-reactive protein was measured by using immune-turbidmetric method. Furthermore, Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) was used to estimate interleukin 6.

The precision and accuracy of all methods used in this study were checked each time; a batch was analyzed by including commercially prepared control sera. Statistical Package for Social Science (SPSS version 16) computer software was used for data analysis. (Significance levels were set at P≤0.05). Independent t-test, Pearson’s correlation and linear regression were used to compare between means and to assess the correlation between different variables.

RESULTS:

This study was conducted on 200 patients with type 2 diabetes as a test group and 100 healthy volunteers as a control group. Age and gender of the test group were matched with control group.

In this study the test group was composed of 109 males (54.5%) and 91 females (45.5%), where as the control group was composed of 56 males (56%) and 44 females (44%). 24% (n=48) of patients were described as obese according to Body Mass
Index calculation and 65% (130) of patients were described as uncontrolled diabetic.

Table (1) shows a significant difference between the means of interleukin 6, high sensitive c-reactive protein, body mass index, total cholesterol, triacylglycerol, HDLc and LDLc in test group and control group.

Table (2) shows a significant difference between the means of interleukin 6, high sensitive c-reactive protein, triacylglycerol, and an insignificant difference between the means body mass index, total cholesterol, HDLc and LDLc in control diabetic patients and uncontrolled diabetic group.

Table (3) shows a significant difference between the means of interleukin 6, high sensitive c-reactive protein, triacylglycerol, total cholesterol, HDLc and LDLc in obese and non-obese diabetic group.

Figure (1) shows a significant positive correlation between the hsCRP (mg/L) and Interleukin 6 (pg/mL) (r= 0.263, P=0.001).

Figure (2) shows a significant positive correlation between the hsCRP (mg/L) and total cholesterol (mg/dl) (r= 0.293, P=0.000).

Figure (3) shows a significant negative correlation between the hsCRP (mg/L) and HDLc (mg/dl) (r= -0.326, P=0.000).

Figure (4) shows a significant positive correlation between the hsCRP (mg/L) and LDLc (mg/dl) (r= 0.328, P=0.000).

Figure (5) shows a significant positive correlation between the hsCRP (mg/L) and triacylglycerol (mg/dl) (r= 0.158, P=0.042).

Figure (6) shows an insignificant correlation between the interleukin 6 (pg/ml) and total cholesterol (mg/dl) (r= 0.016, P=0.847).
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Figure (7) shows an insignificant correlation between the interleukin 6 (pg/ml) and HDLc (mg/dl) (r= 0.010, P=0.903).

Figure (8) shows an insignificant correlation between the interleukin 6 (pg/ml) and LDLc (mg/dl) (r= 0.021, P=0.801).

Figure (9) shows an insignificant correlation between the interleukin 6 (pg/ml) and triacylglycerol (mg/dl) (r= 0.030, P=0.714).

Table (1) Comparison between means of interleukin 6, high sensitive C-reactive protein, body mass index, total cholesterol, triacylglycerol, HDLc and LDLc in the test group and control group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test group (n=200)</th>
<th>Control group (n=100)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin 6 (pg/ml)</td>
<td>233 ± 211</td>
<td>10.8 ± 8.7</td>
<td>0.000</td>
</tr>
<tr>
<td>High sensitive c-reactive protein (mg/L)</td>
<td>1.9 ± 1.0</td>
<td>0.3 ± 0.18</td>
<td>0.000</td>
</tr>
<tr>
<td>Body mass index (kg/m2)</td>
<td>26.8 ±4.4</td>
<td>23.8 ± 3.5</td>
<td>0.000</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>163 ± 43</td>
<td>148 ± 32</td>
<td>0.006</td>
</tr>
<tr>
<td>Triacylglycerol (mg/dl)</td>
<td>115 ± 50</td>
<td>90 ± 42</td>
<td>0.000</td>
</tr>
<tr>
<td>HDLc (mg/dl)</td>
<td>42 ± 16</td>
<td>65 ± 10</td>
<td>0.000</td>
</tr>
<tr>
<td>LDLc (mg/dl)</td>
<td>98 ± 39</td>
<td>63 ± 28</td>
<td>0.000</td>
</tr>
</tbody>
</table>

-The table shows the mean ± Std. deviation and probability value (P-value).
-Independent t-test was used for comparison.
-P-value ≤ 0.05 is considered significant.

Table (2) Comparison between means of interleukin 6, high sensitive C-reactive protein, body mass index, total cholesterol, triacylglycerol, HDLc and LDLc in the control diabetic and uncontrolled diabetic patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control diabetic (n=70)</th>
<th>Uncontrolled diabetic (n=130)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin 6 (pg/ml)</td>
<td>97 ± 72</td>
<td>233 ± 219</td>
<td>0.000</td>
</tr>
<tr>
<td>High sensitive c-reactive protein (mg/L)</td>
<td>1.4± 0.59</td>
<td>2.1 ± 1.3</td>
<td>0.018</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>160 ± 46</td>
<td>168 ± 43</td>
<td>0.217</td>
</tr>
<tr>
<td>Triacylglycerol (mg/dl)</td>
<td>104 ± 52</td>
<td>120 ± 48</td>
<td>0.026</td>
</tr>
<tr>
<td>HDLc (mg/dl)</td>
<td>43 ± 15</td>
<td>42 ± 17</td>
<td>0.480</td>
</tr>
<tr>
<td>LDLc (mg/dl)</td>
<td>100 ± 39</td>
<td>104 ± 42</td>
<td>0.568</td>
</tr>
</tbody>
</table>
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The table shows the mean ± Std. deviation and probability value (P-value).
-Independent t-test was used for comparison.
-P-value ≤ 0.05 is considered significant.

Table (3) Comparison between means of interleukin 6, high sensitive C-reactive protein, body mass index, total cholesterol, triacylglycerol, HDLc and LDLc in the obese and non obese diabetic patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal BMI (Non obese) (n=86)</th>
<th>Obese (n=48)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin 6 (pg/ml)</td>
<td>116 ± 99</td>
<td>215 ± 210</td>
<td>0.029</td>
</tr>
<tr>
<td>High sensitive c-reactive protein (mg/L)</td>
<td>1.6 ± 0.91</td>
<td>2.0 ± 1.18</td>
<td>0.032</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>139 ± 27</td>
<td>210 ± 45</td>
<td>0.000</td>
</tr>
<tr>
<td>Triacylglycerol (mg/dl)</td>
<td>96 ± 34</td>
<td>143 ± 66</td>
<td>0.000</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>42 ± 16</td>
<td>49 ± 17</td>
<td>0.017</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>79 ± 26</td>
<td>135 ± 43</td>
<td>0.000</td>
</tr>
</tbody>
</table>

The table shows the mean ± Std. deviation and probability value (P-value).
-Independent t-test was used for comparison.
-P-value ≤ 0.05 is considered significant.

Fig. (1): A scatter plot shows the relationship between hsCRP (mg/L) and Interleukin 6 (pg/mL) (r= 0.263, P=0.001).
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Fig. (2): A scatter plot shows the relationship between hsCRP (mg/L) and Total cholesterol (mg/dl) (r= 0.293, P=0.000).

Fig. (3): A scatter plot shows the relationship between hsCRP (mg/L) and HDLc (mg/dl) (r= -0.326, P=0.000).

Fig. (4): A scatter plot shows the relationship between hsCRP (mg/L) and LDLc (mg/dl) (r= 0.328, P=0.000).
Fig. (5): A scatter plot shows the relationship between hsCRP (mg/L) and triacylglycerol (mg/dl) \((r= 0.158, P=0.042)\).

Fig. (6): A scatter plot shows the relationship between interleukin 6 (pg/ml) and total cholesterol (mg/dl) \((r= 0.016, P=0.847)\).

Fig. (7): A scatter plot shows the relationship between interleukin 6 (pg/ml) and HDLc (mg/dl) \((r= 0.010, P=0.903)\).
Fig. (8): A scatter plot shows the relationship between interleukin 6 (pg/ml) and LDLc (mg/dl) ($r=0.021$, $P=0.801$).

Fig. (9): A scatter plot shows the relationship between interleukin 6 (pg/ml) and triacylglycerol (mg/dl) ($r=0.030$, $P=0.714$).

DISCUSSION:

Diabetes mellitus type 2(formally non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes) is characterized by hyperglycemia as a result of an individual’s resistance to insulin with an insulin secretory defect. This resistance results in a relative, not an absolute, insulin deficiency. Type2 constitutes the majority of the diabetes cases. However, these patients are more likely to go into a hyperosmolar coma and are at an increased risk of developing macrovascular and microvascular complications.\(^{10}\)
The elevated cardiovascular risk of diabetic patients is only partially explained by the presence of conventional cardiovascular risk factors, such as glycemic control, lipid abnormalities, hypertension and visceral obesity. This has suggested that additional risk factors, such as genetic risk factors, may favor the increased cardiovascular morbidity and mortality observed in diabetic patients.\(^{(11)}\)

This study was conducted on 200 patients with type 2 diabetes as a test group and 100 healthy volunteers as a control group. Age and gender of the test group were matched with control group.

In this study the test group was composed of 109 males (54.5%) and 91 females (45.5%), whereas the control group was composed of 56 males (56%) and 44 females (44%). 24% (n=48) of patients were described as obese according to Body Mass Index calculation and 65% (130) of patients were described as uncontrolled diabetic.

In this study patients with type 2 diabetes mellitus have a significant increase in the mean of plasma levels of interleukin 6 compared with the control subjects (p=0.000). This agrees with a study done by Pradhan, et al. 2001\(^{(12)}\) and Das. 2004\(^{(13)}\) reporting that there was a significant elevation of the mean of the interleukin 6 in diabetic patients compared to the non-diabetic control.

Furthermore, patients with type 2 diabetes mellitus have a significant increase in the mean of plasma levels of hs-CRP compared with the control subjects (p=0.000). This agrees with a study done by Pradhan, et al. 2001\(^{(12)}\); Haffiner, 2003\(^{(14)}\) and Safiullah Amanulla, et al. 2010\(^{(15)}\). They reported that there was a significant elevation of the mean of the hs-CRP in diabetic patients compared to the non-diabetic control. Moreover, patients with type 2 diabetes mellitus have a significant increase in the mean of plasma levels of total cholesterol, triacylglycerol, LDLc compared with the control
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subjects (p=0.006), (p=0.000), (p=0.000) respectively, and there was significant decrease in the mean of plasma levels of HDLc compared with the control subjects (p=0.000). This conformity with the results reported by Gavey, et al. 2003 (16) and Haffner, 1998 (17). Also there was a significant elevation of the plasma levels of interleukin 6, hsCRP and triacylglycerol in uncontrolled diabetics when compared with the mean of those controlled diabetics (p=0.000), (p=0.018), (p=0.026) respectively. This finding is consistent with recent performed study Choudhary, 2008 (18) which showed that there was a positive correlation between interleukin 6, hsCRP and HbA1c. Also in our study there was no significant difference in the plasma level of total cholesterol, HDLc, LDLc, in uncontrolled diabetics comparing with controlled diabetic (p=0.217), (p=0.480), (p=0.568) respectively. Our findings seem to be in contrast with findings from some, but not all, VinodMahato, 2011 (19) studies which showed that uncontrolled diabetic patients had significantly higher value of triacylglycerol, total cholesterol and LDL-C, as compared to the controlled diabetic patients but there was no significant difference in value of HDL-C between the two groups. This might be attributed to differences in the ethnic background of the volunteers in the different studies, which shows large differences in adipose tissue mass distribution and/or the level of insulin resistance.

The current study showed that there is a significant increase in the plasma interleukin 6, hsCRP, total cholesterol, HDLc, LDLc and triacylglycerol in obese diabetic patients compared with those non obese diabetics, (p=0.029), (p=0.032), (p=0.000), (p=0.017), (p=0.000), (p=0.000) respectively. Hansen, 2010 (20) study showed high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), and triacylglycerol levels were elevated in obese type 2 diabetes patients. However, Samir, 2006 (21) Study showed total cholesterol, HDLc, LDLc levels
were elevated in obese type 2 diabetes patients, which agrees with our study.

In the present study there was a significant positive correlation between hsCRP in type2 diabetic patients to interleukin 6, total cholesterol, triacylglycerol and LDLc. \( r= 0.263^{**}, P=0.001 \), \( r= 0.293^{**}, P=0.000 \), \( r= 0.158^{**}, P=0.042 \) and \( r= 0.328^{**}, P=0.000 \) respectively. Also there was a significant negative correlation between hsCRP to HDLc. \( r= -0.326^{**}, P=0.000 \). These findings seem to agree with Choudhary N 2008\(^{18}\) which showed a positive correlation between interleukin6, hsCRP and HbA1c. Moreover, levels of hsCRP correlated positively with triacylglycerides, total cholesterol and LDL cholesterol but negatively with HDL cholesterol as previously reported by Rajarajeswari, 2011\(^{22}\).

In the current study there was an insignificant correlation between interleukin 6 in type2 diabetic patients to total cholesterol, HDLc, LDLc and triacylglycerol. \( r= 0.016, P=0.847 \), \( r= 0.010, P=0.905 \), \( r= 0.021, P=0.801 \), and \( r= 0.030, P=0.714 \) respectively. This finding is consistent with a recent performed study in type 2 diabetic patients done by Ghadiri-Anari1, 2011\(^{23}\) where IL-6 was not correlated with LDLc. Nonetheless, our study does not support the hypothesis that IL-6 levels positively correlated with triacylglycerols, and negatively correlated with HDL which was proposed by Cardellini, 2007\(^{24}\). Further studies are necessary to confirm this hypothesis or find other possible mechanisms.

**CONCLUSIONS:**

In conclusion, levels of interleukin 6, high sensitive C - reactive protein and lipid profiles were invariably higher in type2 diabetic patients compared with healthy controls and consistently higher in obese type2 diabetic patients than non
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obese diabetics, except the high density lipoprotein which decreased in patients with type2 diabetes compared with healthy controls. Nonetheless, there was increased in levels of interleukin 6, hsCRP and triacylglycerol in uncontrolled type2 diabetic patients when compared with of those controlled type2 diabetic but there was no differences in levels of total cholesterol, HDLc, LDLc. Furthermore, there was positive correlation between hs-CRP in type2 diabetic patients to the level of interleukin 6, total cholesterol, LDLc and triacylglycerol; however, there was a negative correlation between hsCRP to HDLc. Moreover, there was no significant correlation between interleukin 6 and lipid profile.

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