DETERMINATION OF ENDOTHELIN-1 LEVEL AS A RISK FACTOR FOR COMPLICATIONS OF TYPE 2 DIABETES PATIENTS IN AL-QADISIYA GOVERNORATE-IRAQ.

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ABSTRACT:
Type 2 diabetes mellitus (DM) is a metabolic disorder identified as chronic hyperglycemia with disturbance of carbohydrate, protein and fat caused by defects in insulin action and/or secretion. This study was designed determining the effects of endothelin-1 level and its relationship to complications of type 2 diabetes patients. This study was conducted in the Department of Life Sciences - College of Science, University of Al-Qadisiya in cooperation with the laboratories of Al-Diwaniyah Teaching Hospital and some private laboratories. The study included (70) samples ranging from (40) samples of patients with type 2 diabetes and (30) samples of non-diabetics ranging in age from (25-60) years. The results of the current study showed an increase in the level of endothelin-1 in diabetic patients (190.9 ± 1.79) compared to the control (126.3 ± 1.29), and there was a significant difference between the two groups (p < 0.05).

This study aims to study the levels of ET-1 in diabetic patients and its role in the occurrence of vascular complications.

Key words: Type 2 diabetes, endothelin-1

I. INTRODUCTION
Diabetes mellitus is a chronic disorder of carbohydrates, fats and protein metabolism. A defective or deficient insulin secretory response, which translates into impaired carbohydrates (glucose) use, is a characteristic feature of diabetes mellitus, as is the resulting hyperglycemias (Singh et al., 2016). Endothelin-1 is a peptide secreted predominantly by vascular endothelial cells and is the most powerful vasoconstrictor currently known. ET-1 also has inotropic properties (Agapitov & Haynes, 2002). Some studies have indicated that elevated serum endothelin-1 measured predictive of in-hospital adverse cardiac events in patients with myocardial infarction (Setianto et al., 2016). Chowdhury et al. (2019) published in Cardiology showing that endothelin 1 (ET-1) is predictive of 1-year heart failure hospitalization in HFP EF patients, and elevated ET-1 levels were found to be associated with long-term mortality in HFP EF. (Dinarti et al., 2020) mentioned the pulmonary arterial hypertension pathomechanism involves an increased plasma level of endothelin-1 and a reduced plasma level of prostacyclin and nitric oxide. Endothelin-1 (ET-1) and arginase are both suggested to be involved in the inflammatory processes and development of endothelial dysfunction in atherosclerosis (Rafnsson et al., 2020). Schematic representation of various mechanisms by which ET-1 is involved in the causation of CKD by increased ET-1 production (Raina et al., 2020).

II. MATERIALS AND METHODS
The current study was conducted between October 2020 and March 2021 and included 40 Iraqi patients from Al-Diwaniyah Governorate suffering from type 2 diabetes and 30 healthy controls and was conducted in the laboratories of the College of Science, Al-Diwaniyah Teaching Hospital and some private.

The concentration of Endothelins-1 in serum was estimated using Elisa device according to the kit manufactured by (CUSABIO, China). The basic principle of the serum Endothelins-1 level assay kit is based on the use of Endothelins-1 purification of the pit plate layer that makes solid-phase antibodies when Endothelins-1 is added to the pits along with the anti-Endothelins-1 antibodies that with the HRP tag become Antibody anti-enzyme complex. Antigen-antibody enzyme complex, and after washing is completed, TMB is added, which turns blue.
when HRP is broken down, and the end of the reaction is by adding sulfuric acid. It is determined by comparing it to the absorbance of the sample against the standard solution curve.

**Reagents used:**

<table>
<thead>
<tr>
<th>T</th>
<th>Solution</th>
<th>Quantity</th>
<th>T</th>
<th>Solution</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>wash solution</td>
<td>20ml×1 bottle</td>
<td>7</td>
<td>Stop Solution</td>
<td>6ml×1 bottle</td>
</tr>
<tr>
<td>2</td>
<td>HRP-Conjugate reagent</td>
<td>6ml×1 bottle</td>
<td>8</td>
<td>Standard 24μg/ml</td>
<td>0.5ml×1 bottle</td>
</tr>
<tr>
<td>3</td>
<td>Microelisa stripplate</td>
<td>12well×8 strips</td>
<td>9</td>
<td>Standard diluent</td>
<td>1.5ml×1 bottle</td>
</tr>
<tr>
<td>4</td>
<td>Sample diluents</td>
<td>6ml×1 bottle</td>
<td>10</td>
<td>Instruction</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Chromogen Solution A</td>
<td>6ml×1 bottle</td>
<td>11</td>
<td>Closure plate membrane</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Chromogen Solution B</td>
<td>6ml×1 bottle</td>
<td>12</td>
<td>Sealed bags</td>
<td>1</td>
</tr>
</tbody>
</table>

**Procedure**

1- Dilute: Dilute the basic standard solution to obtain the following concentrations:

<table>
<thead>
<tr>
<th>Concentration</th>
<th>standard solution</th>
<th>How to prepare</th>
</tr>
</thead>
<tbody>
<tr>
<td>12μg/ml</td>
<td>5 Standard</td>
<td>150μl Original density Standard + 150μl Standard diluent</td>
</tr>
<tr>
<td>6μg/ml</td>
<td>4 Standard</td>
<td>150μl 5 Standard + 150μl Standard diluent</td>
</tr>
<tr>
<td>3μg/ml</td>
<td>3 Standard</td>
<td>150μl 4 Standard + 150μl Standard diluents</td>
</tr>
<tr>
<td>1.5μg/ml</td>
<td>2 Standard</td>
<td>150μl 3 Standard + 150μl Standard diluents</td>
</tr>
<tr>
<td>0.75μg/ml</td>
<td>1 Standard</td>
<td>150μl 2 Standard + 150μl Standard diluents</td>
</tr>
</tbody>
</table>

2- Sample addition: 40 microliters of the sample dilution solution and 10 microliters of the sample are added to the test sample cell, and eventually the sample is diluted five times. a step

3- Incubation: After the plate containing the cells is covered with the plate cover, the plate is incubated for 30 minutes at 37 °C.

4- Configure liquid: After revealing the cover from the plate, washing solution is added to each cell for 30 seconds each time, and it is repeated five times and then left to dry.

5- Add Enzyme: 50 microliters of HRP-Conjugate reagent were added to each well cell, except for the black cell

6- Incubate the samples again for 30 minutes at 37°C

7- Then wash again for 30 seconds each time and repeat five times and leave to dry.

8- Color: Add 50 microliters of Chromogen Solution A and Chromogen Solution B to each cell, and keep away from the light for 15 minutes at 37°C.

9- Stop reaction: Add 50 microliters of a stop solution to each cell, as stopping the reaction occurs when the blue color turns to yellow.

10- Assay: Yellowing is done against the black box well Blank, absorbance is read at 450 nm wavelength 15 minutes after addition of the stop solution.

**III. RESULTS AND DISCUSSION**

The results of the current study showed an increase in the level of endothelins-1 in patients (190.9 ± 1.79) compared to the control group (126.3 ± 1.29) and there was a statistically significant difference between the two groups (p < 0.05) (p < 0.05) (Table No. (6) and Fig. (6).
The results showed higher levels of Endothelin (ET-1) in diabetic patients compared to the control group, and these results are similar to those of Idris-Khodja et al. (2016). The results indicate that the increased secretion of ET-1 by endothelial cells in diabetic patients may be due to downregulation of CSE protein (cystathionine) expression and a decrease in H2S production (Guan et al., 2015). Increased expression of Endothelin (ET-1) amplifies diabetes-induced endothelial dysfunction and this may be due to decreased expression of eNOS Endothelial Nitric Oxide Synthase, increased vascular oxidative stress, and decreased antioxidant capacity (Idris-Khodja et al., 2016). The study of Saleh and his group (2011) confirmed that high Endothelin-1 increases glomerular permeability to albumin through loss of nephron function as well as early inflammation in hyperglycemic mice. Overexpression of ET-1 promotes the development of atherosclerosis in patients with type 1 diabetes and increases the level of oxidative stress through NADPH oxidase (Ouerd et al., 2020). Expressions of the ET-1 receptor and endothelin A (ET-RA) present in DN Diabetic nephropathy suggest a possible role for the endothelial system in diabetic nephropathy as well as in other non-diabetic glomerular diseases (Zanatta et al., 2012). It is believed that enhanced endotelin (ET-1) endothelin activity on endothelin A receptors in diabetic refractory vessels while their sensitivity to exogenous ET-1 is impaired, and this abnormality may be involved in the pathophysiology of vascular complications associated with diabetes (Cardillo et al., 2002). Endothelin-1 is associated with coronary microvascular dysfunction (CMD) in unobstructed areas in patients with coronary artery disease (CAD) indicating that endothelin-1 is a potential target for the treatment of coronary microvascular dysfunction in coronary artery disease (Naya). et al., 2021). Supplementation of phytosterols (PS), a group of natural compounds found in plant cell membranes that act as cholesterol-lowering antagonists, effectively reduces endothelin-1 independently of reductions in plasma levels of LDL-c, which contributes to the effect of plant sterols on endothelial function, and prevention of cardiovascular disease (Oliveira et al., 2020). The results show that increased ET-1 production in patients with T2D can lead to long-term increases in blood pressure (BP) that mainly reflect early changes in the blood vessels (Kostov et al., 2016). Endothelial cell inflammation is directly responsible for various cardiovascular diseases such as hypertension, atherosclerosis, aging, stroke, heart disease, diabetes, obesity, venous thrombosis and endometriosis (Zhang et al., 2019). Significantly altering the functional effects of ET-1 and its receptors during the development of cardiovascular disease and increased production of ET-1 and its receptors are believed to lead to several pathophysiological events that contribute to atherosclerosis and vascular complications in diabetes mellitus (Pernow et al., 2012).
IV. CONCLUSION

The endothelin-1 measurement is accessible and inexpensive as the serum endothelin-1 level can be used as a screening tool in hospitals to screen for complications of diabetic patients.

REFERENCE