Serum ADAM -17 and Interleukin-6 Levels as a Predictors in Type 2 Diabetic Patients with Myocardial Infarction Patients

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Abstract
The association of heart diseases with type 2 diabetes, especially myocardial infarction, calls for a search for biomarkers that have a relationship between the two. Which facilitates the process of reducing the development of myocardial infarction in patients with type 2 diabetes. The most prominent of these associations is long-term inflammation and its first and largest factor is interleukin 6, and its close association with A disintegrin and metalloprotease 17 (ADAM-17) and its inverse relationship with type 2 diabetic patients with and without myocardial infarction. This study aims to investigate the role of ADAM-17 in the pathogenesis of diabetic type 2 Iraqi men patients with and without MI by comparing them with a apparently healthy as control group and to see their association with interleukin-6 levels and other biomarkers. The current study was conducted on 90 Iraqi men between Ja., 2023 and Aug. 2023, 60 samples with T2DM (with or without myocardial infarction) with or without MI and the remaining 30 as apparently healthy control. The patients were selected from the visitors of the coronary care unit (CCU) and Al-Hassan Center for Diabetes and Endocrinology in Kerbala, and they were diagnosed clinically and by laboratory investigations. Various biomarkers such as ADAM-17 and IL-6 have been determined by different biochemical techniques. As a result The highest ADAM-17 and IL-6 level in type 2 diabetes without myocardial infarction was seen as comparison serum levels of IL-6, and ADAM-17 for T2DM (with or without myocardial infarction) with the control group. The correlation between IL-6 and ADAM-17 is strong in type-2 diabetes without MI and between IL-6 and electrocardiogram represented by the two types STEM and NSTEMI in type-2 diabetes with MI. In conclusion, The current study found that ADAM-17 and IL-6 have a negative effect on chronic inflammation as in T2DM without MI is more severe than acute inflammation as T2DM with MI , due to elevation of ADAM-17 and IL-6 levels in type 2 diabetic patients without MI than type 2 diabetic patient with MI.
مستويات ADAM و Interleukin-

و 17-6

ومرضى احتشاء عضلة القلب

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الملخص

إن ارتباط أمراض القلب بمرض السكري من النوع الثاني، وخاصة احتشاء عضلة القلب، يدعو إلى البحث عن المؤشرات الحيوية التي لها علاقة بين الاثنين. مما يسهل عملية الحد من تطور احتشاء عضلة القلب لدى مرضى السكري من النوع الثاني. وأبرز هذه الارتباطات هو الالتهاب طويل الأمد وعامله الأول والأكبر هو الإنترلوكين 6، وارتباطه الوثيق مع البروتين المحلل 17 (ADAM-17) وعلاقته العكسية مع مرضى السكري من النوع الثاني المصابين باحتشاء عضلة القلب وبدونه. تهدف هذه الدراسة إلى التحقق من دور ADAM-17 في التسبب في مرض السكري من النوع الثاني لدى المرضى الرجال العراقيين الذين يعانون من احتشاء عضلة القلب أو بدونه من خلال مقارنتهم مع مجموعة الأصحاء كمجموعة ضابطة ورؤية ارتباطه بمستويات الإنترلوكين 6 والمؤشرات الحيوية الأخرى. أجريت الدراسة في الفترة ما بين يناير 2023 وأغسطس 2023، ستين عينة من مرضى T2DM مع أو بدون MI والثلاثون المتبقية كمجموعة مراقبة صحية. تم اختيار المرضى من زوار وحدة العناية التاجية ومركز السكريات والعيادات السكرية في كربلاء، وتم تشخيصهم سريرياً وفحوصات مخبرية. تم تحديد المؤشرات الحيوية المختلفة بواسطة تقنيات كيميائية مختلفة. ونتيجة لذلك، فإن أعلى مستوى لـ ADAM-17 و IL-6 كمصدر تفاعلي بين مرضى السكري من النوع الثاني دون احتشاء عضلة القلب كان قد وجد عند المقارنة مع المجموعة الضابطة. العلاقة بين IL-6 و ADAM-17 قوية في مرض السكري من النوع الثاني دون احتشاء عضلة القلب ويبين IL-6 والكورية القلب المتماثل في النوع II وSTEM 2 بدون احتشاء عضلة القلب ويبين IL-6 ومخطط كهربية القلب المتماثل في النوع II للدكتور الحالة أن 17 ADAM-17 لليوم الأول سليم على الالتهاب المزمن حيث أن T2DM دون احتشاء عضلة القلب في الختام وجدت الدراسة الحالية أن 17 ADAM-17 و IL-6 مستويات في مرضى السكري من النوع 2 أكثر من مرضى السكري من النوع 2 مع MI.
1. Introduction
Type 2 diabetes is a disease that comes primarily from insulin resistance (Wondmunk, 2020), caused by long-term inflammation under the auspices of the factors released by ADAM-17, the most important of which is interleukin-6 (Iemmolo, Ghersi and Bivona, 2023). A disintegrin and metalloprotease 17 (ADAM-17) has been identified as the sheddase for many membrane-bound proteins present in various cell types. It significantly impacts the release of chemokines and cytokines, cell signaling, proliferation, and growth (Iemmolo, Ghersi and Bivona, 2023). There are both positive and negative impacts of ADAM-17. Although it supports healthy liver function, adipocyte differentiation, and embryonic development, it is also linked to the pathophysiology of numerous illnesses, including but not limited to heart disease (MI) and diabetes (Maekawa et al., 2019).

The ADAM-17 activation pathway depends on protein kinase for phosphorylation to switch from the inactive phase to the secretory active phase, which exercises its capabilities in everyday situations, including releasing crucial cytokines and growth hormones (Chen et al., 2023). However, when exposed to cellular activators such as the ROS-dependent p38 membrane-associated protein kinase pathway, ADAM-17 sheds its cells at a higher pace. Reactive oxygen species produced by oxidative stress contribute to the development of atherosclerosis, abnormal blood flow, and arterial wall remodeling (He and Zuo, 2015). Local and systemic inflammation causes an increase in ADAM-17 activation when reactive oxygen species are produced. Additionally, nitric oxide has been shown to activate Adam-17 (Sisto, Ribatti and Lisi, 2021). Nitric oxide (NO) is involved in the physiologic control of circulation and serves a pathogenic function in CAD. The synthesis of oLDLR1, essential for the initiation and progression of atherosclerosis, is known to be triggered by the activation of Adam-17 by C-reactive protein (Zhao et al., 2011). As a result, ADAM-17 is a crucial enzyme rather than just one of numerous inflammatory factors. Important mediators at the start and progression of T2DM and CAD are its activators and subsequently shed proteins. ADAM-17 is found on the cell surface as dimers and binds to TIMP3, its inhibitor (Liao et al., 2023). ADAM-17 is released from TIMP3 and changes from a dimeric form to a monomeric structure when the ERK or p38 MAPK pathway is activated (Adu-Amankwaah et al., 2021). ADAM-17 has been linked to the beginning of CAD and its development into acute coronary syndrome (ACS), according to several studies. According to clinical research, patients with AMI had higher plasma levels of IL-6 and TNF than healthy individuals. This suggests that IL-6 and TNF maturation, which depends on ADAM-17, may trigger systemic inflammation and cause plaque rupture (De Queiroz, Lakkappa and Lazartigues, 2020). The white adipocytes' activation of ADAM-17 causes the release of inflammatory chemicals, including IL-6. Once a low-grade inflammatory state is created due to this expression, the macrophages are forced to go into (migrate into) adipose tissue, where they cause increased insulin resistance (Ni et al., 2020).

A pleiotropic cytokine called IL-6 is secreted in response to disturbances of homeostasis. When activated, this cytokine exhibits evident pro- and anti-inflammatory characteristics. It's interesting that ADAM-17 can cleave its receptor, IL-6R. The processes involved in IL-6's signaling and stimulation influence its characteristics. As a result, whereas IL-6 is generally beneficial when stimulated acutely, its chronic response results in long-term signaling that induces inflammation and autoimmune infarction. Which are crucial in the development of type 2 diabetes and heart attacks (Scheller et al., 2011), (Adu-Amankwaah et al., 2021). As mentioned above, IL-6 and TNFα maturation, which relies on ADAM-17, may activate systemic inflammation and contribute to plaque rupture.

2. Materials and Methods
This case-control study includes ninety participants, sixty of whom are type 2 diabetes (T2DM) with and without myocardial infarction, thirty patients per group, and another thirty participants are nondiabetic persons as a control group. The study was conducted in the coronary care unit (CCU) at Kerbala Centre for Cardiac Diseases and Surgery and Al-Hassan Center for Endocrinology and Diabetes / Kerbala Health Directorate from Jan. 2023 to Aug. 2023. Thirty patients presented with typical chest pain to the coronary care unit (CCU); the diagnosis was based on clinical history, physical examination, ECG, and investigation of a cardiac biomarker. Thirty patients with T2DM without MI attended the Al-Hassan Center for Endocrinology and Diabetes. They were diagnosed according to the analysis of RBS and HbA1c. Thirty nondiabetic persons were selected as a healthy control group. Exclusion criteria involve subjects who are suffering from acute kidney injury (AKI), cancer, infections, or other inflammatory conditions.

The current study analyzed several clinical parameters, including serum or blood tests (cTnI, RBS, HbA1c, lipid profile, and (ADAM-17 and IL-6), which were measured by the following methods (immune-chromatography with a unique two-site sandwich immunoassay. Hexokinase (HK), colorimetric enzymatic, and Sandwich-ELISA methods, respectively. They include a number that identifies the participants (sample No.), sex, age, smoking status, sedentary
lifestyle, family history regarding MI, and the duration of MI (in the case of a patient). Furthermore, the collected data are also registered. As a mandatory step, this study was approved by the following ethical committees: the University of Kerbala, the College of Medicine, and the Kerbala Centre for Cardiac Diseases and Surgery committee, Al-Hassan center for endocrinology and diabetes, and Kerbala Health Directorate / Kerbala-Iraq.

The Statistical Program for Social Scientists program, version 28.0 (IBM, SPSS, Chicago, Illinois, USA), and the Real Statistical Resources Pack software for Mac (Release 7.2) of the resources pack for Excel 2016 were used to create the data analysis for this project. 2013 until 2020 for copyright. Scale-related variables were displayed as mean ± SD. Analytical, statistical tests confirmed significant variations in categorical variables between the parameters. The results of all hypothesis tests were deemed statistically significant when their p-values were 0.05 (two-sided). Using Fisher's LSD method, the simultaneous confidence level for each of the confidence intervals was computed. This accompanying confidence level represents the likelihood that the genuine difference is contained within each confidence interval. ANOVA used Fisher's LSD method to generate confidence intervals.

3. Results

Figure 1 compares the serum levels of ADAM-17 and IL-6 between the T2DM and the health control groups. The level of ADAM-17 and IL-6 increased in type 2 diabetic patients, and there was a statistically significant difference between type 2 diabetic patients (with and without MI) compared with the healthy control. The mean ± SD of each ADAM-17 and IL-6 level in type 2 diabetic patients was (3256.4 ± 883.03 pg/dl; 30.04 ± 1.75 pg/dl) respectively, while their mean ± SD level in the healthy control group was (1645.33 ± 41 pg/dl), (23.44 ± 1.46 pg/dl) Table 1.

Table 1: Difference Between Mean Levels for ADAM-17 and IL-6 for Between T2DP with or without MI and the Nondiabetic Control Group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Type 2 Diabetic Patients, N=60</th>
<th>Healthy Control N=30</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>IL-6, pg/dl</td>
<td>30.04 ± 1.75</td>
<td>23.44 ± 1.46</td>
<td>&lt;0.001[S]</td>
</tr>
<tr>
<td>ADA1M-17, pg/dl</td>
<td>3256.4 ± 883.03</td>
<td>1645.33 ± 41</td>
<td>&lt;0.001[S]</td>
</tr>
</tbody>
</table>

T-test was used *: significant at p ≤ 0.05
SD: standard deviation; S: significant; NS= Non-significant.

Figure 1: Difference Between Mean ± SD Levels of IL-6, ADAM-17 in Each T2DM with or Without MI Compared to Healthy Control. (T-Test was used S= Significant at p ≤ 0.05, NS= Nonsignificant)
In Figure 2, a comparison of serum levels of ADAM-17 and IL-6 among type 2 diabetic patients with MI, type 2 diabetic patients without MI, and the control group was performed. A statistically significant difference in the mean ± SD levels of ADAM-17 and IL-6 was found between DM+MI, DM without MI, and the control group. The highest mean ± SD level was observed in diabetic patients without MI. Their values for ADAM-17 and IL-6 were 3371.05 ± 995.97 pg/ml, 30.20 ± 1.67 pg/dl, while their values for the non-diabetic control group were 1645.33 ± 413.91, 23.43 ± 1.46 pg/dl respectively as shown in Table 2.

### Table 2: Difference in ADAM-17 and IL-6 Mean Levels Between T2DM with or without MI and the Nondiabetic Control Group

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>MI+T2DM N=30 Mean ± SD</th>
<th>Control N=30 Mean ± SD</th>
<th>T2DM N=30 Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6, pg/dl</td>
<td>29.89±1.85</td>
<td>23.44±1.46</td>
<td>30.20±1.67</td>
<td>&lt;0.001[S]</td>
</tr>
<tr>
<td>ADAM-17, pg/dl</td>
<td>3141.75±753.16</td>
<td>1645.33±413.91</td>
<td>3371.05±995.97</td>
<td>0.002[S]</td>
</tr>
</tbody>
</table>

Two Way ANOVA Test was Used *: Significant at p ≤ 0.05  
N: Number of Cases; SD: Standard Deviation; S: Significant; NS= Non-Significant

**Figure 2:** Difference in IL-6 and ADAM-17 mean ± SD levels between T2DM with and without MI and the nondiabetic control group (Two way ANOVA test was used S= significant at p ≤ 0.05, NS= Nonsignificant)

The results showed that there was a very strong relationship and a significant correlation between IL-6 and ADAM-17 in type 2 diabetic patients without MI (p = 0.001, r=0.9), as shown in Table 3

### Table 3: The Correlation Coefficient between IL-6 and ADAM-17 in T2DM without MI

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>ADAM-17, pg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient (r)</td>
<td>P value</td>
</tr>
<tr>
<td>IL-6, pg/dl</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Figure 3 and 4 shows a comparison in the serum levels of ADAM-17 and IL-6 between the type 2 diabetic patients groups and the control group according to the BMI. The mean ± SD level of ADAM-17 and IL-6 was increased and a
statistically significant difference in type 2 diabetic patients as compared to control within the BMI ranges (normal weight), (overweight), and (obesity) respectively. Mean ± SD values of ADAM-17 and IL-6 in the BMI ranges mentioned above was (3130.30 ± 819.98, 3328.19 ± 888.57, 3274.62 ± 940.77), (30.07 ± 1.73, 30.30 ± 2.19, 29.82 ± 1.39 pg/ml) in patients group and mean value (1762.75 ± 382.20, 1629.53 ± 433.44, 1652.58 ± 419.48), (22.92 ± 0.14, 23.66 ± 1.81, 23.08 ± 0.20) pg/dl in the control group respectively as shown in table 4.

Table 4: The effect of BMI on serum level of ADAM-17 and IL-6 as compared between Type 2 Diabetic Patients with or without Myocardial Infraction and the control group, BMI ranges were used :((normal weight), (overweight), and (obesity))

<table>
<thead>
<tr>
<th>IL-6, pg/dl</th>
<th>Patient N=60</th>
<th>Control N=30</th>
<th>N=30</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal weight</td>
<td>30.07±1.73</td>
<td>22.92±0.14</td>
<td>2</td>
<td>&lt;0.001[S]</td>
</tr>
<tr>
<td>Overweight</td>
<td>30.30±2.19</td>
<td>23.66±1.81</td>
<td>18</td>
<td>&lt;0.001[S]</td>
</tr>
<tr>
<td>Obesity</td>
<td>29.82±1.39</td>
<td>23.08±0.20</td>
<td>10</td>
<td>&lt;0.001[S]</td>
</tr>
<tr>
<td>ADAM-17, pg/dl</td>
<td>3130.30±819.98</td>
<td>1762.75±382.20</td>
<td>2</td>
<td>0.038[S]</td>
</tr>
<tr>
<td>Normal weight</td>
<td>3328.19±888.57</td>
<td>1629.53±433.44</td>
<td>18</td>
<td>&lt;0.001[S]</td>
</tr>
<tr>
<td>Overweight</td>
<td>3274.62±940.77</td>
<td>1652.58±419.48</td>
<td>10</td>
<td>&lt;0.001[S]</td>
</tr>
</tbody>
</table>

T-test was used *: significant at p ≤ 0.05
SD: standard deviation; S: significant; NS= Non-significant.

Figure 3: The effect of BMI on serum level of ADAM-17 as compared between T2DP with or without MI and the nondiabetic control group, BMI ranges were used :((normal weight), (overweight) and (obesity), (T-test was used, S= significant at p ≤ 0.05, NS= Nonsignificant).
Figure 4: The effect of BMI on serum level of IL-6 as compared between T2DP with or without MI and the nondiabetic control group, BMI ranges were used: (normal weight), (overweight) and (obesity) (T-test was used, S= significant at p ≤ 0.05, NS= Nonsignificant).

4. Discussion

Several studies explain the elevation of the biomarkers mentioned above for several reasons. ADAM-17 sheds more frequently when exposed to cellular activators such as lipopolysaccharides, which depend on reactive oxygen species (ROS) and the p38 mitogen-activated protein kinases (MAPK) pathway (Chemaly et al., 2017). ADAM-17 exists as dimers on the cell surface and interacts with TIMP3, its inhibitor. ADAM-17 is released from TIMP3 metallopeptidase inhibitor three and changes from a dimeric to a monomeric structure when the ERK or p38 MAPK pathway is activated. The balance between ADAM-17 dimers and monomers can change with activation of the p38 mitogen-activated protein kinase pathway. This change is accompanied by increased ADAM-17 cell surface presentation and decreased TIMP3 interaction (Sikora et al., 2023). Due to the cleavage and activation of several pro-inflammatory cytokines and their corresponding receptors, ADAM-17 has become a key regulatory hub in regulating inflammation. The most prominent examples are TNF, IL-6R, and tumor necrosis receptors 1 and 2 ([Ferencova et al., 2023], (Finneran et al., 2023]). As membrane-bound proteins, these cytokines are abundantly expressed in the heart (Kunnathattil, Rahul and Skaria, 2024). Following the cleavage of ADAM-17, excessive elevations in their soluble forms can set off cascades that lead to acute cardiac inflammation, myocardial lipotoxicity, and poor energy generation (Defer et al., 2007).

Increased plasma levels of IL-6 and TNF were found in patients with AMI in clinical studies by Latini et al. and Suzanne et al., suggesting that TNF and IL-6 maturation, which depends on ADAM-17, may activate systemic inflammation and contribute to plaque rupture ([Latini et al., 1994], (Engelen et al., 2022)).

It has been suggested that high glucose levels in people with type 2 diabetes may lead to protein misfolding and aggregation in the endoplasmic reticulum, which in turn results in a reduction in the ADAM-17 inhibitor TIMP3 and an increase in ADAM-17 activity (Lorenzon et al., 2021).

An increase in IL-6 levels was observed in MI patients. This increase may be related to macrophages, the predominant cells in vascular atherosclerotic lesions, which abundantly produce and secrete IL-6, along with other cytokines, growth factors, and chemokines. Additionally, co-stimulating1 endothelial cells leads to the stimulation of protein kinase and thus the recruitment of many macrophages, leading to endothelial dysfunction (Souza et al., 2008). IL-6 promotes many mechanisms that contribute to the inflammatory process of atherosclerosis (Zhang et al., 2023). The buildup of atherosclerotic plaques in the coronary artery is one of the causes of MI (Iuchi, Harada and Tanaka, 2018). The development and advancement of plaque and the rupture of the fibrous cap, which would result in local thrombosis and hypoxia-related myocardial damage, are primarily influenced by vascular inflammation. (Xu, Yuan and Wang, 2023). IL-6 signaling is connected to the adverse effects of acute ischemia as well as the development and
destabilization of plaque. Therefore, the data obtained hypothesizes that IL-6 and MI etiology are related (Huang et al., 2015).

The results showed a strong relationship and a significant correlation between IL-6 and ADAM-17 (p = 0.001, r=0.9) for T2DM without MI. ADAM-17, is responsible for sIL-6R shedding in humans (Mahmud-Al-Rafat, 2023). Based on what was previously mentioned about the participation of the two biomarkers in insulin resistance and vascular inflammation, evidence for that is treatment with the ADAM-17 inhibitor improves insulin sensitivity, corrects hyperglycemia, and inhibits vascular inflammation. As a result, ADAM-17 overactivity likely causes the balance of IL-6 signaling to shift toward trans-signaling, resulting in vascular inflammation and diabetes (Federici et al., 2005). Another study highlighted that when ADAM-17 is activated within the white adipocytes, it leads to the expression of inflammatory molecules such as IL-6. This expression then leads to a low-grade inflammatory state that forces the macrophages to migrate into adipose tissue, mediating enhanced insulin resistance and T2DM (Menghini et al., 2013).

The current study noted a significant increase in levels of ADAM17 and IL-6 in both groups under study, T2DM with or without MI, and the reasons for this increase in both are the same, but when compared between the two groups with the control, T2DM without MI showed the most significant rise in the two biomarker levels. These data are consistent with other studies in which high glucose causes stimulation of ADAM17. This condition is chronic, so the higher the sugar, the more protein misfolding is generated in the endothelial cell, thus reducing the ADAM17 inhibitor and thus increasing the activity of ADAM17, which in turn increases the 1activity of IL-6 by ectodomain shedding, causing a decrease in the insulin receptor alpha subunit (IRα) in all three layers of the vascular wall (Lorenzon et al., 2021).

As mentioned previously, activation of ADAM17 within white adipocytes causes the expression of interleukin 6 (IL-6). A low-grade inflamed condition is then brought on by this process, forcing macrophages to quickly move into inflamed adipose tissue, where they drive increasing insulin -resistance, and T2DM.

5. Conclusion

ADAM 17 and its substrate IL-6 hurt chronic inflammation, as in T2DM, which is more severe than acute inflammation in AMI. This result was supported by what was shown of a higher ADAM 17 and IL-6 level in type 2 diabetic patients without myocardial infarction than in type 2 diabetic patients with MI.
References


