

# The Total Antioxidant Capacity and its Relationship with Atherosclerosis Risk Factors in a Sample of Iraqi Individuals with Type 2 Diabetes Mellitus

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## Abstract

**Background:** Diabetes mellitus is significantly related to cardiovascular disease, such as atherosclerosis. Antioxidants are essential in the prevention of atherosclerosis by a variety of mechanisms, which encompass the suppression of free radical production, inhibition of low-density lipoprotein oxidation, and prevention of atherosclerotic plaque formation.

**Objectives:** This study aims to evaluate the levels of total antioxidant capacity and malondialdehyde as oxidative stress indicators in Iraqi type 2 diabetes mellitus (T2DM) patients and investigate their relationship with atherosclerotic risk factors.

**Methods:** This case-control study took place between October 2023 to January 2024 at Al-Karkh General Hospital in Baghdad. The study included a total of 130 participants: 70 individuals diagnosed with T2DM and 60 healthy controls were recruited from relatives of patients attending the hospital and hospital employees who did not have T2DM. The two study groups were age-matched. Blood samples from both groups were analyzed to determine the following parameters: Lipid profile, total antioxidant capacity (TAC), Malondialdehyde (MDA) as an oxidative stress marker, glycated hemoglobin (HbA1c), and atherogenic indices (e.g. atherogenic index of plasma).

**Results:** The mean serum TAC in the T2DM group was significantly lower than the control group ( $46.9 \pm 5.05$  U/mL vs.  $70.8 \pm 4.71$  U/mL). This indicates a highly significant difference between the groups. Additionally, in the T2DM group, statistically significant inverse correlations were observed between TAC and most of the measured lipid profile parameters, HbA1c, and atherogenic indices.

**Conclusions:** Low TAC may be a potential predictor of atherosclerosis in T2DM patients and highlight the relationship between oxidative stress, lipid metabolism, and thermogenesis.

**Keywords:** Antioxidant; Diabetes Mellitus Type 2; Glycated Hemoglobin; Malondialdehyde; Oxidative stress.

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## Introduction:

Diabetes mellitus is a persistent metabolic condition that impacts the processing of carbohydrates, fats, and proteins, due to insufficient insulin production, diminished insulin effectiveness, or a mix of both (1). It is identified by elevated blood glucose levels (2). The persistently elevated levels of glucose in the blood cause long-term damage and dysfunction in several organs, including the heart and blood vessels (3). Over the past decade in Iraq, diabetes has increased by 115%, from 19.6 cases per 1000 individuals in 2000 to 42.3 cases per 1000 individuals in 2015, marking a significant epidemic (4). Atherosclerosis is the predominant type of cardiovascular disease (CVD), characterized by the buildup of lipids and inflammation in the major arteries (5). Oxidative stress (OS) arises from an imbalance between free radical generation and antioxidant defense mechanisms (6). OS has been associated with various disorders, such as

atherosclerosis, revealing the several routes via which oxidants cause cellular damage (7). Antioxidants are compounds that prevent the oxidation of various molecules through the scavenging of oxidants or the reduction of free radical production (8). The development and advancement of atherosclerotic plaques is promoted by elevated levels of OS (9). Total antioxidant capacity (TAC) is an important indicator of the body's total antioxidant defense system, which is vital in the fight against OS (10), and is the capacity of every antioxidant in plasma to scavenge free radicals (11). The existing epidemiological studies show that CVDs have associations with lower levels of antioxidants and higher levels of oxidants (12). Malondialdehyde (MDA), a result of the cellular oxidation of polyunsaturated fatty acids, is widely used as an oxidative stress biomarker. In T2DM, there has been a significant increase in MDA (13, 14).

The current study aims to evaluate the levels of total antioxidant capacity and malondialdehyde as oxidative stress indicators in Iraqi T2DM patients and investigate their relationship with atherosclerotic risk factors.

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**Patients and Methods**

This case-control study took place between October 2023 to January 2024 at Al-Karkh General Hospital in Baghdad. After the provision of informed written permission, 130 Iraqi individuals were grouped into: Group 1 (70 individuals with a confirmed diagnosis of T2DM), and Group 2 (60 healthy individuals as controls) were recruited from relatives of patients attending the hospital and hospital employees who did not have T2DM. The two study groups were age-matched. The study was approved by the College of Medicine/ University of Baghdad.

Exclusion criteria were: Type 1 DM, alcoholism, smoking, pregnancy, antioxidant supplement use, CVDs, renal disease, liver diseases, or other medical disorders that might affect the results, in addition to recent surgery.

Serum samples were analyzed for the following biochemical markers: Total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides (TG). These parameters were assayed enzymatically on a fully automated Monarch 240 analyzer using Biorex Instruments, United Kingdom. Glycated hemoglobin (HbA1c) was measured by an automated instrument (Cobass C111, Germany). TG/5, the Friedewald formula, was used to determine very low-density lipoprotein (VLDL). Additionally, the atherogenic indices (AIs) were estimated as follows: The atherogenic coefficient (AC) and the atherogenic index of plasma (AIP) were calculated as Log (TG/HDL-C), and TG/HDL, respectively. Total antioxidant capacity (TAC) and MDA were estimated using enzymatic colorimetric and enzyme-linked immunosorbent assay (ELISA) methods, respectively. (Elabscience, USA, and Human, USA).

**Statistical analysis:**

The study employed SPSS® software version 26.0 on Microsoft Windows for statistical analysis. When comparing the difference between means of variables in the study groups, the student’s t-test was used. Pearson's correlation coefficient was calculated to test the correlation between continuous variables. The scatter diagrams were used to show these correlations. Statistical significance was indicated by a P-value of less than 0.05.

**Results**

The mean age was 57.9 ± 5.85 years in G1, and 53.3 ± 6.16 years in G2. There were 40 and 30 males in G1, 28 females, 32 males and females in G2. G1 had a lower mean TAC level than the controls (G2). Significant differences were found between the mean levels of AIs of the two groups, with G1 showing higher levels than G2. The mean MDA was higher in G1 than G2. The mean values of the lipid profile were significantly higher in G1 (TG, LDL, HDL, and VLDL) than G2. There was no statistically significant difference in the mean TC between the two groups, table (1).

**Table (1): Mean± SD values for biochemical parameters in the two study groups**

Parameter (Mean ± SD)	Study Groups		P-Value
	T2DM -G1 (n = 70)	Controls - G2 (n = 60)	
HbA1c (%)	8.9 ± 1.55	5.3 ± 0.22	<0.001*
TAC (U/ml)	46.9 ± 5.15	70.8 ± 4.71	<0.001*
MDA (ng/ml)	40.0 ± 9.22	11.2 ± 3.36	<0.001*
TC (mmol/l)	4.8 ± 1.13	4.5 ± 0.93	0.67
TG (mmol /l)	2.8 ± 0.98	1.2 ± 0.52	<0.001*
HDL (mmol /l)	1.2 ± 0.21	1.6 ± 0.30	<0.001*
LDL (mmol /l)	2.9 ± 0.76	2.1 ± 0.45	<0.001*
VLDL (mmol /l)	39.7 ± 10.19	20.5 ± 9.59	<0.001*
AIP	0.3 ± 0.17	0.1 ± 0.18	<0.001*
AC	3.0 ± 0.65	1.8 ± 0.45	<0.001*

The Pearson correlations (r) between TAC and biochemical markers in G1 and G2 are shown in Table (2) and Figures (1 and 2). TAC shows significant negative correlations with HbA1c, AIs, and the majority of lipid profiles components (P < 0.01). As for HDL, the correlation was positive. TC was not significantly correlated.

**Table (2): Pearson’s correlation coefficient between TAC and biochemical markers in the study groups**

Parameters	TAC (U/ml)	
	r	P
HbA1c (%)	- 0.75	<0.01*
MDA (ng/ml)	- 0.63	<0.01*
TG (mmol/L)	- 0.68	<0.01*
TC (mmol /L)	- 0.15	0.099
HDL (mmol /L)	0.59	<0.01*
LDL (mmol /L)	- 0.47	<0.01*
VLDL (mmol /L)	- 0.60	<0.01*
AIP	- 0.69	<0.01*
AC	- 0.64	<0.01*

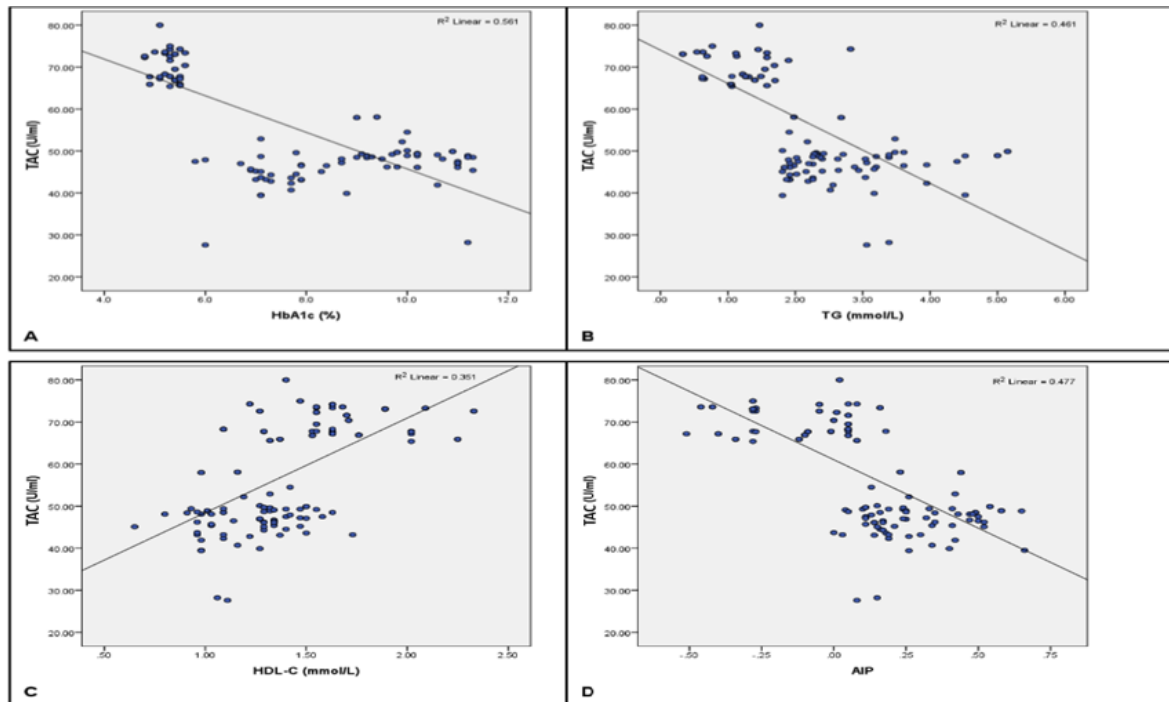


Figure 1: TAC correlations with HDL ( $r = 0.59$ ), TG ( $r = -0.68$ ), HbA1c ( $r = -0.75$ ), and AIP ( $r = -0.69$ )

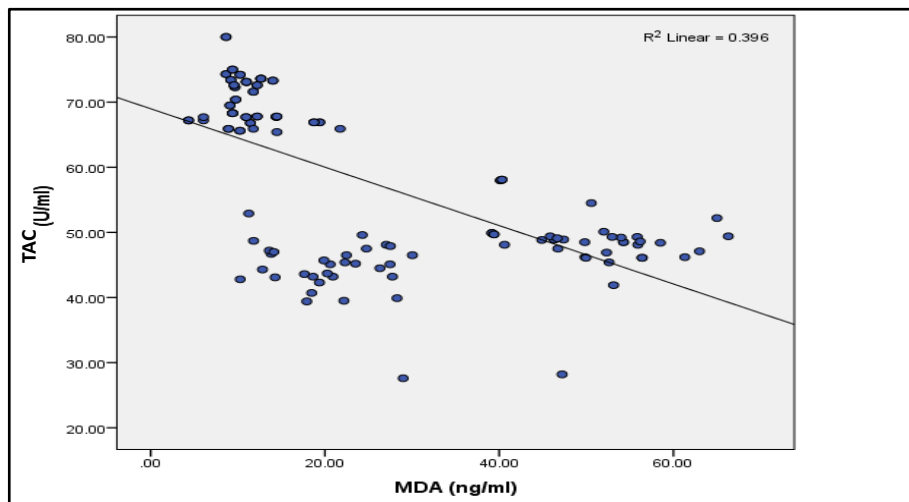


Figure 2: Negative correlation between TAC and MDA ( $r = -0.63$ )

### Discussion

The lower TAC and higher MDA in T2DM than the controls in the current study corroborate those of earlier studies by Mousa et al. (15) and Yarube et al. (16) which demonstrated increased OS and reduced antioxidant capacity in individuals with T2DM. Elevated levels of MDA indicate heightened OS among individuals with T2DM, possibly contributing to the development and advancement of atherosclerosis. According to a study by Mehri et al. (17), the TAC in individuals with CVDs was significantly lower than in healthy people. Additional studies have indicated the possibility of a correlation between reduced overall antioxidant capacity and CVDs (18). In addition, Kumar et al., indicated that patients with T2DM have substantially elevated MDA levels (19). Mahreen et al. (20), provided

evidence that MDA may serve as a predictive indicator for diabetic complications, as it was found to be substantially elevated in T2DM patients who suffered complications as opposed to those without. These studies collectively support the potential link between reduced TAC, elevated MDA, and atherosclerosis.

The findings of this study align with the common dyslipidemia pattern seen in individuals with T2DM as reported by Mazzone et al. (21). Previous studies have linked metabolic dyslipidemia in T2DM to an increased risk of CVDs and an accelerated development of atherosclerosis (22, 23). The current study corroborates the connection between increased levels of serum TG and the development of T2DM. This finding is consistent with earlier studies that

have shown this relationship in other populations (24, 25). High TG levels are acknowledged as a significant risk factor for CVDs (26).

Patients with T2DM have reduced HDL-C values, which may be attributed to obesity and hypertriglyceridemia (27). The mechanism by which HDL particles obtain free cholesterol from cells has been recognized as the basis for the atheroprotective effects associated with HDL (28, 29).

LDL is especially exposed to oxidation to form oxidized LDL (oxLDL), which plays a role in atherosclerosis development (30). Previously established correlations between elevated LDL-C levels and diabetes align with the results obtained in the current investigation (31). T2DM dysregulates lipoprotein metabolism, leading to elevated hepatic VLDL production and impaired clearance of intestinal chylomicrons and VLDL (32).

The findings of the current study of a significantly higher mean AI value in T2DM than controls can be related to an elevated risk of CVDs by greater atherogenicity in T2DM patients. The AIs marker, which is derived from lipid profiles, may enhance clinical risk assessment for CVDs, according to Acar et al. (33). Our results corroborate those by Fu et al., showing that T2DM patients have higher than normal levels of AIP (34). In addition, HbA1c levels were affirmed to be positively related to AIP, which may suggest that AIP can play a part in evaluating diabetes (35). Moreover, as compared with conventional lipid variables, AIP and AC were found to have a more useful effect on the likelihood of CVDs (36). These results provide evidence for the role that AIs may have in the development of atherosclerosis in T2DM.

#### Limitation

This case-control study with a moderate sample size (n=130) establishes associations but not causation. The short study duration (October 2023- January 2024) limits assessment of longer-term trends. The study population from one Baghdad hospital may not be generalizable to the entire Iraqi T2DM population or other populations. Additionally, selection bias may be present as the study compared T2DM patients to healthy controls, ideally a group with confirmed atherosclerosis would have been included for a more robust comparison.

#### Conclusions

The study concluded that individuals with T2DM have lower levels of TAC than healthy controls. This indicates increased oxidative stress, which is linked to atherosclerosis. The study also showed a relationship between low TAC and various risk factors for atherosclerosis, suggesting that low TAC could be a predictor of the atherosclerosis.

#### Authors' declaration:

We certify that every figure and table in the manuscript is a part of the present study. Additionally, permission has been obtained for the Figures and photos to be republished in conjunction with the text even though they are not related to the

current study. Signing the permission based on ethical Considerations-Ethical Approval: According to code number 56, the project was given approval by the local ethics committee at the College of Medicine/University of Baghdad on (26/ 2/ 2024).

**Conflicts of Interest:** None

**Funding:** None

#### Authors' contributions:

Study conception & design: (Halla Gh. Mohamoud). Literature search: (Hasan H. Idan ). Data acquisition: (Hasan H. Idan). Data analysis & interpretation:(Hasan H. Idan). Manuscript preparation: (Hasan H. Idan & Halla Gh. Mohamoud ). Manuscript editing & review: (Marwa M. Talib).

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## القدرة المضادة للأكسدة الكلية وعلاقتها بعوامل خطر تصلب الشرايين لدى الأفراد العراقيين المصابين بداء السكري من النوع الثاني

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### الخلاصة:

**خلفية البحث:** يرتبط مرض السكري النوع الثاني بشكل كبير بأمراض القلب والأوعية الدموية، مثل تصلب الشرايين. تعتبر مضادات الأكسدة ضرورية في الوقاية من تصلب الشرايين من خلال مجموعة متنوعة من الآليات، والتي تشمل منع إنتاج الجذور الحرة، وتثبيط أكسدة الدهون البروتينية منخفضة الكثافة، ومنع تكوين الخثرة الدموية داخل الشرايين.

**الأهداف:** تهدف هذه الدراسة إلى تقييم مستويات إجمالي القدرة المضادة للأكسدة والمالونديالدهيد كمؤشرات للإجهاد التأكسدي لدى مرضى السكري من النوع الثاني العراقيين، وكذلك التحقيق في علاقتهما بعوامل خطر تصلب الشرايين.

**طرائق العمل:** هذه الدراسة هي دراسة للحالات والعينة الضابطة أجريت في الفترة من أكتوبر 2023 إلى ديسمبر 2023 في مستشفى الكرخ العام بمدينة بغداد. شملت الدراسة 130 مشاركاً، منهم 70 مصاباً بالسكري من النوع الثاني و60 من الأصحاء. تم إجراء تحاليل لعينات الدم لتحديد المعايير التالية: فحص نسبة الدهون، إجمالي القدرة المضادة للأكسدة والمالونديالدهيد كعلامة للإجهاد التأكسدي، فحص مستوى السكر في الدم حيث تشمل (HbA1c)، ومؤشرات تصلب الشرايين (على سبيل المثال، مؤشر تصلب الشرايين في البلازما).

**النتائج:** أظهر مرضى السكري من النوع الثاني في العراق انخفاضاً في مستويات إجمالي القدرة المضادة للأكسدة في مصل الدم إلى جانب ارتفاع مستوى المالونديالدهيد والعديد من عوامل خطر تصلب الشرايين، بما في ذلك الدهون الثلاثية والكوليسترول البروتيني الدهني منخفض الكثافة ومؤشرات تصلب الشرايين. وتجدر الإشارة إلى وجود ارتباطات عكسية إحصائية داخل مجموعة مرضى السكري من النوع الثاني بين إجمالي القدرة المضادة للأكسدة ومعظم نسبة الدهون في الدم، HbA1c، ومؤشرات تصلب الشرايين، باستثناء الدهون البروتينية عالية الكثافة حيث لوحظ وجود ارتباط موجب.

**الاستنتاجات:** قد يكون انخفاض إجمالي القدرة المضادة للأكسدة مؤشراً محتملاً لتصلب الشرايين لدى مرضى داء السكري من النوع الثاني ويسلط الضوء على العلاقة بين الإجهاد التأكسدي واستقلاب الدهون وتصلب الشرايين.

**الكلمات المفتاحية:** مرضى السكري من النوع الثاني، تصلب الشرايين، مرضى القلب، القدرة المضادة للأكسدة الكلية، الجهد التأكسدي، انزيم مالونديالدهيد، نسبة الدهون في الدم