Relationship between Total Antioxidants Capacity, Total Oxidant Status and Oxidative Stress Index among Type-2 Diabetes Mellitus patients in Anbar Governorate

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Abstract
Background: The human body is continually exposed to diverse chemicals, resulting in reactive species known as free radicals (reactive oxygen species/reactive nitrogen species), which damage cellular machinery by transferring their free unpaired electron. The body possesses endogenous antioxidant systems or obtains exogenous antioxidants from the diet to fight the adverse effects of such species, which neutralize the species and maintain biological homeostasis. Any imbalance between free radicals and antioxidants results in a situation known as "oxidative stress," which can contribute to the development of pathological illnesses like diabetes. Patients and methods: This study showed the link between total antioxidants capacity, total oxidant status and oxidative stress Index among (60) patients with type 2 diabetes mellitus and (30) healthy people as control.The parameters of the cases and controls using standard spectrophotometric methods were assessed. Results: highly significant ($p<0.01$) increase of OSI in T2DM (5.24 ± 1.17) compared to healthy control (0.85 ± 0.06) and significantly elevated ($p < 0.05$) of TOS concentrated in T2DM (3.55 ± 0.8 µmol H2O2 Eq./l) compared to healthy control (1.29 ± 0.059). also, there was decreased in TAC concentration in T2DM patients (0.65± 0.087µmol Vit C. Eq. /L) compared to healthy control (1.51 ± 0.084µmol Vit C. Eq. /L) ($p < 0.05$). Conclusion: T2DM is a disorder characterized by elevated oxidative stress, which necessitates the use of antioxidants to counteract the oxidants.
Keywords: Hyperglycemia, TAC, TOS, OSI.

1. Introduction

Several experimental, epidemiologic, and clinical research support the idea believe there is a role for oxidative stress in T2DM progression. The progression of organ injuries is defined by diabetes complications, often known as late or chronic diabetes illnesses. One of the key causes of microvascular and macrovascular diabetic problems is oxidative stress [(1)]. Some of the mechanisms implicated in oxidative stress in diabetes patients include glycosylation-induced oxygen free radicals, auto-oxidation of glycation products, alterations in tissue material, and/or activation of antioxidant defense systems [(2)]. Several investigations in T2DM patients have discovered evidence of oxidative damage to numerous macromolecules, as well as their impact on antioxidant/pro-oxidant systems. Antioxidants operate as enzymatic (glutathione peroxidase, superoxide dismutase, and catalase) or non-enzymatic (glutathione and uric) scavengers of TOS in cells, protecting biological systems against oxidative damage [(3,4)]. In this context, it appears that determining the level of oxidative stress measurements in blood as biomarkers for recognizing and avoiding diabetic complications is critical. Until date, there has been a scarcity of methods for identifying the presence of oxidative stress in patients at the clinical level. While there are various ways for evaluating the oxidative profile, none of them are appropriate for clinical diagnosis. On the other hand, some of these strategies can be put to better use for explain the emergence of problems that takes place throughout the evolution of metabolic disorders like diabetes. When assessing antioxidant ability, the combined activity of TAC present in serum and bodily fluids is taken into account. As a result, an integrated parameter is obtained rather than a basic amount of the observable antioxidants. As a result, the antioxidant potential of known and undiscovered antioxidants is assessed, as well as their synergistic connection, revealing the precise balance between oxidants and antioxidants in the body.

Subjects' collection and sample preparation

There are two groups in this study (control and patients' group). All of the samples were taken in the governorate of AL-Anbar. The research was carried out at the laboratories of the Fallujah maternity and children's hospital. Questionnaires were created to collect data from the control and patient groups. Thirty seemingly healthy male subjects made up the control group to get homogeneous group. The age of this group ranged from 32 to 55 years, with a mean of 48.4± 5.04 and the patients group is about

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60 T2DM male patients. The patients' ages ranged from 31 to 55 years, with a mean of 48.36± 6.04. The subjects inquired to come for blood sampling in fasting status.

Ethical consent

The study protocol was assessed and approved by the Ethics Committee of our institution, the research protocol did not interfere with any medical recommendations or prescriptions. Informed consent was taken from the patient with keeping the patients` records confidential in all stages of the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

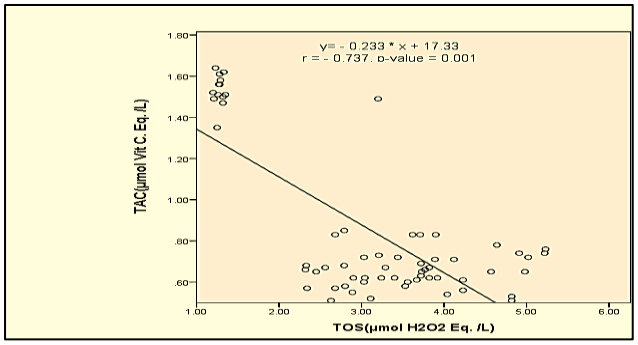
2. Methods

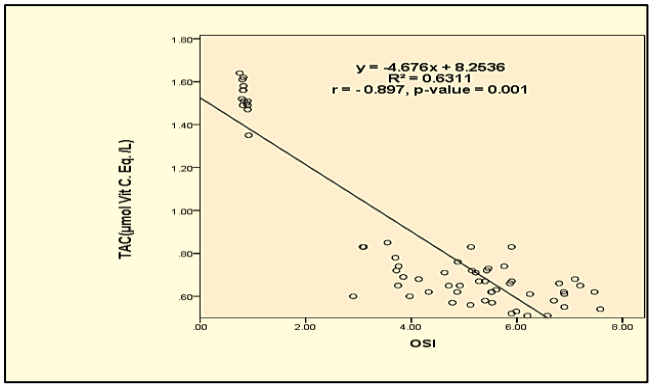
Total antioxidant capacity (TAC) and Total oxidant status (TOS) were determined according to the method described by Erel (5,6). The ratio of TOS to TAC represents the oxidative stress index (OSI) which is an indicator of the degree of oxidative stress (7).

3. Statistical Analyses

The statistical operations on the acquired data were run using the Statistical Package for the Social Sciences (SPSS) version 23.0. Pearson's correlation coefficient was used to determine the correlation between variables in the serum T2DM. The results of the statistical study are presented as mean ± standard deviation. Significant is defined as a *p*-value of less than 0.05.

4. Results





The results of the present study showed strong positive correlation $p < 0.001$, r = (0.638) of OSI levels with TOS. These correlations shown in and table 3.

There is a highly significant ($p<0.01$) increase of oxidative stress index (OSI) in T2DM (5.24 ± 1.17) compared to healthy control (0.85 ± 0.06) as showed in table 1.

Level of TAC has decreased significantly ($p < 0.05$) in T2DM (0.65± 0.087µmol Vit C. Eq. /L) compared to healthy control (1.51 ± 0.084µmol Vit C. Eq. /L) as showed in table 1.

The level of total oxidant status (TOS) was significantly elevated ($p < 0.05$) in T2DM (3.55 ± 0.8 µmol H2O2 Eq./l) compared to healthy control (1.29 ± 0.059) as showed in table 1.

Table 1: Mean± SD of TAC, TOS and OSI levels in T2DM Patients and Control Group

Parameter	Control, N=30 Mean± SD	T2DM, N=60 Mean ± SD	p-value*
TOS (µmol H2O2 Eq./L)	1.29±0.059	3.55±0.8	< 0.05
OSI	0.85±0.06	5.24±1.17	< 0.01
TAC (µmol Vit.C.Eq./L)	1.51±0.084	0.65±0.087	< 0.05
* Correlation is strong at p < 0.05			

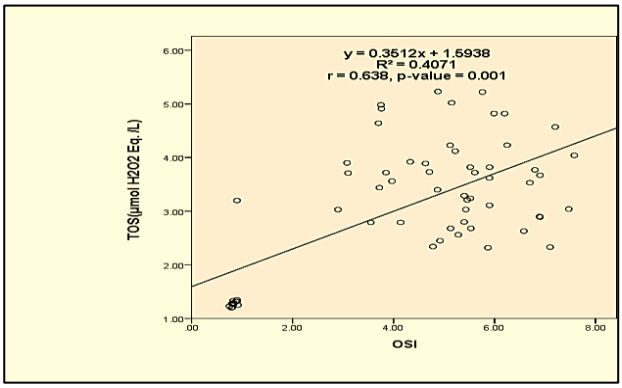
The results of linear regression analysis showed strong negative association $p < 0.001$ r = (-0.737, -0.897) of TOS and OSI levels with TAC in T2DM patients group. This correlations is shown in table 2.

Table 2: Correlation between TAC, TOS and OSI in T2DM group

Parameters	Correlation coefficient r	p-value*
TOS	-0.737	0.001
OSI	-0.897	0.001
Correlation is strong at p < 0.05		

Table 3: Correlation between TOS and OSI in T2DM group

Parameters	Correlation coefficient r	p-value*
OSI	0.638	0.001
* Correlation is strong at p < 0.05		



5. Discussion

There is a high free radical (FR) load in T2DM, which is linked to hyperglycemia. The increased glucose binds to proteins and glycates them, resulting in the

development of advanced glycation end-products (AGEs) and a large number of FRs (8). Tissue damage and aging have been linked to AGEs (9). Glycation of proteins raises the rate of FR synthesis by roughly fifty times when compared to control levels in diabetes. Once generated, AGEs bind to their receptors advanced glycation end-products RAGE cell surface receptors in endothelial cells and macrophages, triggering post receptor signaling, the production of intracellular ROS.

Also, by the generation of ROS its effects on the vasculature of tissues, Low physiologic ROS levels may have a role in insulin's activation of glucose consumption (10). Inflammatory ROS overproduction, on the other hand, appears to block insulin metabolic activities, leading to insulin resistance (11).

Nina et al (12) showed that OSI, and TOS were higher in patients with diabetic nephropathy compared to healthy control group.

A rise in TOS is accompanied by a rise in ROS production. Antioxidants react with or neutralize the majority of ROS produced by macrophages and neutrophils. Tissue injury happens either before or simultaneously with the formation of ROS as a result of an inflammatory response (13). As a result, oxidative stress indicators such as advanced oxidation protein products, lipid peroxidation products MDA and thiobarbituric acid-reacting substances, and oxidative DNA damage product 8-hydroxydeoxyguanosine (14), as well as advanced oxidation protein products, are widely utilized (15,16).

Azmi et al (17) found that the TOS and OSI in patient groups were considerably greater than the control group. Furthermore, they claimed that insulin resistance is linked to an increase in oxidative stress. This agree with the results of the present study which showed strong positive association $p < 0.05$, $r = (0.639)$ of serum OSI level with TOS.

An increase in TOS in patients with diabetic, as well as neurotransmitter alterations that occur as a result of Long-term exposure to high blood glucose levels has been linked to the development of diabetes which is a sign of neuronal damage (18).

Pieme, (19) mentioned that the levels of reduced GSH and TAC potentially offer data on the likelihood of acquiring diabetic complications given that the modification of these biomarkers levels was correlated to oxidative stress.The findings of this study support those of Ganjifrockwala (20), who found significant reduction in TAC when compared to controls.

Reactive oxygen metabolites and their products are increased in hyperglycemia (21). TAC depletion causes increase in oxidative stress. Papachristoforou and others (22) According to the findings, hyperglycemia increased oxidative stress and a decrease in TAC levels in the vascular straight muscles. In this study, linear regression analysis results demonstrate a strong negative association of levels of TOS and OSI with TAC ($p < 0.05$) in T2DM patients.

There is a highly significant $p < 0.01$ increase of oxidative stress index OSI in T2DM compared to healthy control.

Another study showed, OSI were considerably greater in T2DM group than the control group. OSI was found to be positively linked with blood glucose and HbA1c (23).

Ihsan Boyac [24] discovered that the OSI was significantly decrease in those of control group than T2DM, and he also indicated that insulin resistance is the primary pathophysiologic mechanism in T2DM. Insulin resistance causes hyperinsulinemia as a secondary effect. Hyperinsulinemia causes oxidative stress, which results in cell death and insulin shortage. According to these findings, breaking and preventing insulin resistance in T2DM should be an aim of our treatment techniques.

Akin et al (25) found that patients' TOS levels were much higher than healthy controls. Sögüt et al (26) found a significant rise in the level of TOS in the patients. As previously stated, each of these investigations measured the level of oxidants individually. Existing methods allow for the detection of blood each oxidant/antioxidant level independently, but They take a long time and need a lot of effort, and for two reasons, they may not be correct: (1) Unknown oxidants/antioxidants it's possible that it's still present in the serum, and (2) multiple types of oxidants/antioxidants in the same system may combine and provide an additive or synergistic effect. To demonstrate a definitive relationship between oxidative stress and T2DM, measuring merely one or a few particular oxidants/antioxidants in the blood is insufficient. As a result, TOS is commonly used to evaluate the body's total oxidation state (27).

6. Conclusions

TAC is a measurement of the body's overall antioxidant status. Because it is a full measurement of TAC and TOS, The OSI is defined as the ratio of TOS to TAC, is a more precise indicator of oxidative stress in the body. The OS measures TOS, TAC, and OSI are used to assess the body's total oxidative stress level. The levels of TOS, TAC and OSI in T2DM patients were compared to a control group for the purpose of investigate the relationship between T2DM and oxidative stress factors, as well as the clinical importance of this index in T2DM. In T2DM patients, a higher FBS altered the levels of TAC, indicating a reduction in scavenging capability. This result of Hyperglycemia (high blood sugar) could explain the causes of production of free radicals, as well as an increase in TOS and OSI.

Conflicts of interest

There are no conflicts to declare.

7. Acknowledgments

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References

Gawlik K, Naskalski J, Fedak D (2016): Markers of Antioxidant Defense in Patients with Type 2 Diabetes. Oxidative Medicine and Cellular Longevity. doi: 10.1155/2016/2352361.
Almulathanon A, Mohammad J, Fathi F (2021): Comparative effects of metformin and glibenclamide on the redox balance in type 2 diabetic patients. Pharmacia, 68(2):327–332.
Ragy M, Ahmed S (2019): Protective effects of either C-peptide or l-arginine on pancreatic beta-cell function, proliferation, and oxidative stress in streptozotocin-induced diabetic rats. Journal of Cellular Physiology, 234:11500–11510.
Idris A, Etet P, Saeed A et al., (2020): Evaluation of metabolic, antioxidant and anti-inflammatory effects of Garcinia kola on diabetic rats. Saudi Journal of Biological Sciences, 27(12):3641–3646.
Erel O (2004): A novel automated method to measure total antioxidant response against potent free radical reactions. Clinical Biochemistry, 37(2):112–119.
Erel O (2005): A new automated colorimetric method for measuring total oxidant status. Clinical Biochemistry, 38(12):1103–1111.
Lagemaat E, Groot L, Heuvel E (2019): Vitamin B12 in Relation to Oxidative Stress: A Systematic Review. Nutrients, 11(2):482.
Buege J, Aust S (1987): Microsomal lipid peroxidation. Methods Enzymol, 52:302-10.
Ellman L (1959): Tissue sulfhydryl groups. Arch Biochem Biophys, 82(1):70-7.
Wang M, Li Y, Li S (2022): Endothelial Dysfunction and Diabetic Cardiomyopathy. Frontiers in Endocrinology, 13: 851941. doi: 10.3389/fendo.2022.851941.
Rao P, Gan S (2020): A Review on Oxidative Stress, Diabetic Complications, and the Roles of Honey Polyphenols. Oxidative Medicine and Cellular Longevity, (9):1-16.
Hojs N, Bevc S, Ekart R et al., (2020): Oxidative Stress Markers in Chronic Kidney Disease with Emphasis on Diabetic Nephropathy. Antioxidants, 9(10):925.
Navasardyan I, Bonavida, B (2021): Regulation of T Cells in Cancer by Nitric Oxide. Cells. 10, 2655. https://doi.org/10.3390/cells10102655
Martínez-García M, Hernández-Lemus E (2021): Periodontal Inflammation and Systemic Diseases: An Overview. Frontiers in Physiology. https://www.frontiersin.org/article/10.3389/fphys.2021.709438.
Meñaca-Guerrero L, SuarezCausado A (2020): Reactive species of oxygen, oxidative stress and its relationship with tissular destruction in periodontitis. Reveiw CES Odontologia, 33(2):112-127.
Tóthová L, Kamodyová N (2015): Salivary markers of oxidative stress in oral diseases. Front Cell Infect Microbiol., 5:73. doi: 10.3389/fcimb.2015.00073.
Azami M, Baradaran H, Dehghanbanadaki H et al., (2021): Association of Helicobacter pylori infection with the risk of metabolic syndrome and insulin resistance: an updated systematic review and meta-analysis. Diabetol Metab Syndr, 13:145. https://doi.org/10.1186/s13098-021-00765-x
Cemil K, Ali A (2015): Evaluation of Pre-Treatment and Post-Treatment S100B, Oxidant and Antioxidant Capacity in Children with Diabetic Ketoacidosis Journal of Clinical Research in Pediatric Endocrinology, 7(2):109-113.
Pieme C, Tatangmo J (2017): Relationship between hyperglycemia, antioxidant capacity and some enzymatic and non-enzymatic antioxidants in African patients with type 2 diabetes. BMC research notes, 10(1):141.
Ganjifrockwala F, Joseph J (2017): Decreased total antioxidant levels and increased oxidative stress in South African type 2 diabetes mellitus patients. Journal of Endocrinology, Metabolism and Diabetes of South Africa, 22(2):21-25.
Ceriello A, Giugliano D (2001): Oxidative stress and Diabetic complications. In: Alberti KGMM, Zimmet P, Fronzo RA, editors. International Textbook of Diabetes Mellitus. Chichester, UK: John Wiley., 1453–1461.
Papachristoforou E, Lambadiari V (2020): Association of Glycemic Indices (Hyperglycemia, Glucose Variability, and Hypoglycemia) with Oxidative Stress and Diabetic Complications. Journal Diabetes Research. 2020;2020: 7489795. doi:10.1155/2020/7489795.
Gautam S, Alam F (2021): Role of ferritin and oxidative stress index in gestational diabetes mellitus. Journal Diabetes Metabolic Disorders, 20(2):1615-1619.
Boyacı I, Yiğitbaşı T (2021): Is Oxidative Stress a Consequence of Hyperglycemia? Or is Hyperglycemia the Consequence of Oxidative Stress? Or are Both Caused by Insulin Resistance? International Archives of Endocrinology Clinical Research, 7:023. DOI: 10.23937/2572-407X.1510023
Akin S, et al (2018): Comparison of gender difference in terms of oxidant/antioxidant status in chronic renal failure patients before hemodialysis. Journal of Cellular Neuroscience & Oxidative Stress. 10(2).
Söğüt İ (2021): Evaluation of Thiol/Disulfide Homeostasis and Other Oxidative Stress Markers in Patients Undergoing Hemodialysis. Turkish Journal of Nephrology, 30:17-24.
Wang, J, Schipper M (2015): Salivary biomarkers of oxidative stress: a critical review. Free radical biology & medicine, 85:95–104.