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# The impact of oxidative stress on the activity of adenosine deaminase and its isoenzymes in nephropathy patients from Wasit-Iraq

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***Abstract*---**Diabetes mellitus is a group of metabolic disorders that share with symptoms of hyperglycemia led to elevated free radical activity. Hyperglycemia is linked to a higher level of (ADA), which is one of the factors that cause oxidative stress by creating reactive oxygen species (ROS), that leads to insulin resistance. This study aimed to determine the relationship between serum total adenosine deaminase(TADA) and its isoenzyme (ADA1and ADA2) with the progression of nephropathy in patients with type 2 diabetes using a case-control study from Wasit-Iraq. In addition, we aimed to find if there is a link between oxidative stress with TADA and its isoenzyme. The One-way ANOVA was used to compare the mean values of fasting plasma sugar (FPS), total oxidant status (TOS), total antioxidant status (TAS), malondialdehyde (MDA), microalbumin in urine, HbA1c, and TADA, ADA1, ADA2 between the three studied groups (Control(C), diabetic patients(DM), and diabetic nephropathy patient(DN). The results of our study revealed that there was a highly significant increase in TADA and its isoenzyme activities of DM and DN groups, as compared to the C group. The mean values of TAS and TOS showed significant differences between the studied groups while serum MDA showed a highly significant increase in the DN group only as compared to the C group. The results indicate a correlation between ADA and its isoenzymes with oxidative stress. The results found from our study were statistically significant in all studied parameters. A significant links was found between oxidative stress, TADA, and its isoenzyme with the glycemic state.

***Keywords*-**diabetic nephropathy, ADA, ADA isoenzyme, oxidative stress.

**Introduction**

Diabetes Mellitus (DM) is one of the primary causes of death and disability in the world. In the following decade, the prevalence of diabetes will almost certainly raised from 6% to over 10%. The World Health Organization (WHO) estimated that 366 million (4.4 percent of the global population) suffer from diabetes by 2030(Matough et al. 2012; Nagarajrao and Alharbi 2018). Type 2 diabetes mellites (T2DM) is a group of metabolic disorders characterized by a high level of glucose in the blood (hyperglycemia) resulting from insulin resistance, insulin deficiency, or both (Al-Musawi, Al-Lami, and Al-Saadi 2021; Association 2014). An insulin insufficiency causes chronic hyperglycemia, resulting in problems with carbohydrate, lipid, and protein metabolism(Domecq et al. 2019). As the condition advances, tissue or vascular damage occurs, resulting in serious diabetes consequences including retinopathy, neuropathy, nephropathy, cardiovascular problems, and ulceration. As a result, diabetes encompasses a wide range of disorders(Ahmad and Hoda 2020; Rao et al. 2019). Glycated hemoglobin (HbA1c) is currently the greatest indicator of diabetes management(Aktas et al. 2020). HbA1C >6.5% and FBS >126 mg/dl, as well as symptoms of diabetes with random blood glucose readings,>200 mg/dl or two-hour plasma glucose >200 mg/dl during an oral glucose tolerance test, are considered criteria for diagnosis of T2DM by the American Diabetic Association(Pragada et al. 2019).

Diabetic nephropathy (DN) is a serious microvascular consequence of diabetes that is widely acknowledged as the leading cause of end-stage renal disease (ESRD) in clinical practice(Tao et al. 2019). About 30% of type 2 diabetes individuals are complicated to diabetic nephropathy which is defined as abnormal albumin excretion in urine, glomerular lesions, and a reduction in glomerular filtration rate (GFR). The severity of hyperglycemia correlates with the development of vascular problems in diabetics(Hamid, Allawi, and Ghudhaib 2021; Tao et al. 2019). Oxidative stress occurs when there is a mismatch between the systemic expression of reactive oxygen (ROS) and reactive nitrogen species (RNS) and the ability of the human biological system to quickly detoxify the reactive intermediates or repair the harm they cause. Pathological implications of redox imbalance produced by increased ROS concentration and/or diminished antioxidant reserve include damage to proteins, lipids, and deoxyribonucleic acid (DNA). Several studies on DM patients revealed that oxidative stress was linked to a rise in oxidizing species formation or a considerable reduction in antioxidant and antioxidant enzyme efficacy(Ganjifrockwala, Joseph, and George 2017; Kumawat et al. 2013; Nagarajrao and Alharbi 2018).

Adenosine deaminase(ADA) (EC 3.5.4.4) is a purine metabolism enzyme that is linked to severe combined immunodeficiency illness and is thought to regulate insulin bioactivity(Nwankwo, Osim, and Bisong 2013). Its contributory role in patients with metabolic syndrome (having features such as obesity, insulin resistance, fasting hyperglycemia, lipid abnormalities, and hypertension). ADA widely expressed enzyme found in the cytosol that catalyzes the irreversible deamination of adenosine (deoxyadenosine) to inosine (deoxy inosine)(Gonçalves and Weide 2021). It is well recognized that ADA is involved in the differentiation and proliferation of lymphocytes, monocytes, and macrophage systems as well as that it is utilized to monitor immune system illnesses. Lokendra et, al and

Larijani et, al found that serum ADA activity was significantly higher in type 2 diabetic patients compared with controls having a significant positive correlation with glycemic parameters(Larijani et al. 2016; Sapkota, Thapa, and Subedi 2017). The primary isoenzymes of TADA are ADA1 and ADA2. A recent study revealed that serum ADA levels are independently associated with islet beta-cell function in patients with T2D. To the best of our knowledge, there is no report regarding the activity of ADA and its isoenzymes ADA1 and ADA2 in Iraqi subjects with diabetes. The present study aims to determine the activity of serum total ADA and its isoenzymes ADA1 and ADA2; and correlate these parameters with glycemic control, oxidative stress, and biochemical characteristics of type 2 diabetic individuals from Wasit-Iraq.

**Design and methods of research**

This is a hospital-based cross-sectional study conducted in the department of biochemistry, in Al Karama Educational Hospital (Wasit- Iraq), from December 2020 to April 2021.

**Study population**

This study comprised a total of 94 samples, including 32 patients with type 2 diabetes (DM, age= 37-65 years), 32 patients with type 2 diabetic nephropathy (DN, age 43-65 years), and 30 healthy individuals served as controls (C, age =35-65 year). All the participants in this study were attending Al Karama Educational Hospital for their normal medical examination. The WHO criteria were used to diagnose patients with type 2 diabetes and its complications (DN). Other systemic disorders such as diabetic patients with other complications (Cardiovascular disease, neuropathy, retinopathy), chronic liver disease, chronic kidney disease, TB, rheumatoid arthritis, systemic lupus erythematosus, infectious mononucleosis, Bechet's disease, and any malignancies were excluded from the research. All data was gathered through personal interviews utilizing a series of pre-made questionnaires.

**Sample collection and biochemical analysis**

A sample of blood (5 ml) at fasting state was obtained from the venous blood of each participant in this study using a sterile 5 mL syringe. The blood sample was divided into two aliquots, 2 ml in EDTA tube to calculate HbA1c using an automatic biochemistry analyzer and 3 ml in gel tube to obtained serum for measuring fasting blood sugar (FBS) (mg/dl) (Giesse / Italy), Total Oxidant (TOS)(Eril method)(Erel 2005). Total antioxidant (TAS) (Eril method) (Erel 2004). Malondialdehyde (MDA) (Hunter method)(Hunter, Nlemadim, and Davidson 1985). Total protein (GIESSE / Italy ). In addition, 3 ml of urine were collected from all of the participants to measure microalbumin in urine (BIOLABS / France). Reagents of (Spinreact, Italy) kits were used to determine the activity of total ADA and its isoenzyme forms (ADA1 and ADA2 ) in serum. Erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) purchased from sigma Aldrich was utilized as an inhibitor of ADA1, and the difference between total ADA and ADA2 activities was used to calculate ADA1 activity. All parameters were investigated using a spectrophotometer (Biotech Engineering) but semiautomated chemical analyzer

(HumaLyzer-3500) was used to determine HbA1C. BMI was calculated as weight (kg)/ height ($m^2$).

**Statistical analyses**

SPSS software was used to analyze the data (SPSS 26.0; IBM Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to determine the distribution of variables among research groups. The mean and standard deviation of homogeneously distributed variables were calculated and compared using the one-way ANOVA test. Statistical significance was determined by P values less than $P< 0.05$.

**Results and Discussion**

T2DM is a multifactorial illness marked by an abnormal protein, lipid, and glucose metabolism as a result of insulin resistance. Insulin resistance can be detected early, which helps to prevent later issues. It is not routinely occurred for all individuals due to the difficulties of the procedures for detecting insulin resistance(Khemka et al. 2013). Adenosine plays a role in insulin-mediated glucose uptake in skeletal muscle, and increased ADA activity reduces glucose absorption into cells, contributing to insulin resistance. The distribution of ADA varies per tissue, however, the lymphoid and fatty tissues have the largest concentration(Sohn et al. 2021), also it has been demonstrated to promote gluconeogenesis and glycogenolysis, as well as accelerate glucose production, in several in vivo and in vitro investigations. ADA modulates myocardial activities by interacting with A1 and A2 adenosine receptors (Khemka et al. 2013; Lu et al. 2021). Many studies have reported increased activity of ADA in type 2 diabetic patients compared with healthy controls (Gowda et al. 2012; Nagarajrao and Alharbi 2018; Sapkota et al. 2017).

Table1(1) illustrates the results of FBS, HbA1C, BMI, and the duration of the disease in all the studied groups. A highly significant increase in FBS was shown between DM and DN($P< 0.001$) when compared with the C group, and a highly significant increase in DN when compared with the DM group ($P<0.001$). The percentage of HbA1c in DM and DN showed a highly significant increase($P <0.001$) as compared to the C group as well as highly significant ($P<0.001$) was found between DM and DN. The results of our study indicate that there is poor control of diabetes associated with the increasing glycosylation of hemoglobin since a highly significant increase was obtained in HbA1c and FBS in DM and DN as compared to the C group. The results of our study were conducted with Kharroubi et.al (Kharroubi et al. 2015), Hemalatha et.al , and Bottini et.al who found highly significant differences in FBS and HbA1c when compared control group with diabetic patients.

The values of BMI listed in Table (1) indicate no differences between DM and DN as compared to the C group, while a significant increase was found between DM and DN groups ($P <0.037$). The process of obesity-related kidney injury has been described by glomerular hyperfiltration, which is aided by increased extracellular volume and salt(sodium) reabsorption in the proximal tubule, as well as altered tubuloglomerular feedback(Murlidharan et al. 2020). Obesity-induced

hyperfiltration causes the glomerular basement membrane to thicken over time, contributing to mesangial sclerosis and, eventually, renal failure(Garofalo et al. 2017). The highly significant increase in FBS indicates the severity of hyperglycemia which correlates with vascular damage as reported in a previous study(Tao et al. 2019). To minimize the occurrence of diabetic microangiopathy, the HbA1c test should be less than 7% and the BMI needs to be less than 25 kg/m2(Nakanishi et al. 2019).

Table(1): Mean value ± SD for FBS, HbA1c, BMI, and duration of the disease among different groups (n=94)

| Parameter | C (n=30) (Male=17 ,Female=13) Mean ± SD | DM (n=32) (Male=17 ,Female=15) Mean± SD | DN (n=32) (Male=17 ,Female=15) Mean± SD | Groups | P Value |
|---|---|---|---|---|---|
| FBS(mg/dl) | 92.32±7.90 | 177.53±43.64 | 222.19±49.11 | C*DM | 0.001** |
| | | | | C*DN | 0.001** |
| | | | | DM*DN | 0.001** |
| HbA1c% | 5.04±.42 | 8.47±1.02 | 10.76±1.27 | C*DM | 0.001** |
| | | | | C*DN | 0.001** |
| | | | | DM*DN | 0.001** |
| BMI(Kg/$m^2$) | 24.73±2.81 | 24.96 ± 3.60 | 22.74±3.82 | C*DM | 0.962 |
| | | | | C*DN | 0.065 |
| | | | | DM*DN | 0.031* |
| Duration(Year) | --------- | 6.83 ± 2.88 | 12.63± 4.34 | | |

*p<0.05 is significant.**p<0.001 is highly significant.
C=Control , DM=DiabeticTyp2, DN=Diabetic nephropathy.

Patients with DM who were underweight had a higher risk of developing ESRD than those who were overweight. Furthermore, in patients with DM, a weight loss of more than 10% was linked to the quickest decline in renal function. As a result, patients with diabetes must lose at least 5% of their body weight to avoid the development of ESRD(Bae et al. 2022). The results listed in Table (2) illustrate that microalbumin in the urine of DM showed no significant difference as compared to the C group (P>0.05) while a highly significant increase (P<0.001) was found between DN and C group. Also a highly significant increase (P<0.001) between DM and DN.

Table(2): Mean value± SD of microalbumin in urine among different groups (n=94)

| Parameter | C (Male=17 ,Female= 13 ) Mean ± SD | DM (Male=17 ,Female=15 ) Mean ± SD | DN (Male=17 ,Female=15 ) Mean ± SD | P value | |
|---|---|---|---|---|---|
| Microalbumin ( mg/l ) | 8.51 ±1.46 | 15.30±4.01 | 111.06±51.22 | C*DM | 0.647 |
| | | | | C*DN | 0.001** |
| | | | | DM*DN | 0.001** |

*p<0.05 is significant.**p<0.001 is highly significant.
C=Control , DM=Diabetic Typ2, DN=Diabetic nephropathy.

The most common early indication of chronic vasculopathy is microalbuminuria, which is linked with the onset of damage to the nephrons of the kidneys. Microalbuminuria (30-300 mg/day) is the first clinical sign of diabetic nephropathy. Diabetic nephropathy is a serious health problem that affects people with diabetes and is responsible for around 30% of chronic renal failure, we found in other research high levels of microalbuminuria in the early stages of diabetic nephropathy (Lemley et al. 2000; Warjukar et al. 2020). The activity of total adenosine deaminase (TADA) showed highly significant increases in DM and DN groups when compared to the C group (P<0.001) as listed in Table 3.

Table 3 also revealed that the activity of ADA1 showed no significant difference when compared to DM with the C group (P= 0.951**),** while a highly significant increase was found between DN and C group (P<0.001)**.** In contrast, ADA2 activity showed **a** highly significant increase(P<0.001) in both DM and DN groups as compared to the C group. The elevated ADA activity found in this study could be related to alterations in T-lymphocyte responses or proliferation, which could point to a mechanism for releasing ADA into circulation. Insulin is a well-known player in T-cell intermediary metabolism. Insulin is thought to improve lymphocyte function, differentiation, and proliferation while also keeping T-lymphocytes engaged by boosting energy expenditure and supplementing protein synthesis required for optimal lymphocyte function(Ramani et al. 2012). Increasing serum ADA in patients with DM can indicate the start and progression of DKD by promoting macrophage infiltration and the production of macrophage-derived cytokines. Furthermore, multiple investigations have indicated that adenosine, the substrate of ADA, has renal and cardiovascular protective benefits. Adenosine can modulate renin release, renal vascular tension, and glomerular filtration rate in the kidney(Yap and Lee 2012). ADA may block adenosine's renal protective actions, exacerbating renal impairment in people with T2DM. As a result, elevated serum ADA may be a risk factor for DKD in T2DM patients(Lu et al. 2021).

Table 3: Mean value ± SD for Total adenosine deaminase (TADA) and isoenzyme (ADA1, ADA2)activities among different groups (n=94)

| parameter | C (n=30) (Male=17 ,Female=13) Mean ± SD | DM (n=32) (Male=17 ,Female=15) Mean± SD | DN (n=32) (Male=17 ,Female=15) Mean± SD | P value | |
|---|---|---|---|---|---|
| TADA (U/L) | 12.30 ± 1.79 | 23.63 ± 3.71 | 34.56 ± 2.99 | C*DM | 0.001** |
| | | | | C*DN | 0.001** |
| | | | | DM*DN | 0.001** |
| ADA1 (U/L) | 3.67±0.95 | 3.78±1.13 | 7.94±1.77 | C*DM | 0.951 |
| | | | | C*DN | 0.001** |
| | | | | DM*DN | 0.001** |
| ADA2 ( U/L ) | 8.63±1.52 | 19.86±2.77 | 26.61±1.58 | C*DM | 0.001** |
| | | | | C*DN | 0.001** |
| | | | | DM*DN | 0.001** |

*p<0.05 is significant.**p<0.001 is highly significant.
C=Control , DM=Diabetic Typ2, DN=Diabetic nephropathy.

Table (4) showed the results of oxidative stress status obtained from our study. Total oxidants (TOS) value showed a highly significant increase(p<0.001) in DM and DN when compared to the C group, while no significant difference was found

between DM and DN (P>0.05). Total antioxidants (TAS) values showed highly significant decreases (P<0.001) when compared DM and DN with the C group but no significant difference(P>0.05) was found between DM and DN. Oxidative stress (OSI) resulting from dividing TOS value on TAS value showed highly significant differences (P<0.001) when compared DM and DN with C group, while no significant difference(P>0.05) was found between DM and DN. A significant increase (P<0.05) of MDA was found in DM and a highly significant increase (P<0.001) in DN as compared to the C group was also a highly significant increase (P<0.001) in DN as compared to DM (Table 4).

Table ( 4): Mean value ± SD of TOS, TAS, OSI, and MDA among different groups (n=94)

| Parameter | C(Mean ± SD) (Male=17 ,Female=13) | DM(Mean ± SD) (Male= 17 ,Female=15) | DN(Mean ± SD) (Male=17 ,Female=15) | P value | |
|---|---|---|---|---|---|
| TOS (µmol/L) | 26.38±7.97 | 60.90±10.26 | 64.02±13.19 | C*DM | 0.001** |
| | | | | C*DN | 0.001** |
| | | | | DM*DN | 0.480 |
| TAS (mmol/L) | 1.67±0.55 | 1.04±0.326 | 0.89±0.12 | C*DM | 0.001** |
| | | | | C*DN | 0.001** |
| | | | | DM*DN | 0.248 |
| OSI | 0.02±0.01 | 0.07±0.032 | 0.076±.029 | C*DM | 0.001** |
| | | | | C*DN | 0.001** |
| | | | | DM*DN | 0.439 |
| MDA (nmol/ml) | 5.20±0.83 | 7.53±1.94 | 10.49±1.98 | C*DM | 0.001** |
| | | | | C*DN | 0.001** |
| | | | | DM*DN | 0.001** |

*p<0.05 is significant.**p<0.001 is highly significant.
C=Control , DM=Diabetic Typ2, DN=Diabetic nephropathy.

Oxidative stress may have a role in chronic T2DM and has been linked to the evolution of insulin resistance (IR), cell dysfunction, mitochondrial malfunction, and diabetic complications(AL-Fayyadh 2022; Newsholme et al. 2019). Oxidative stress is described as an imbalance amongst oxidant species synthesis, such as reactive oxygen and nitrogen species (ROS and RNS), and antioxidant system protection capabilities, such as enzymatic and nonenzymatic antioxidant systems. Oxidative stress damages tissues and cells causing molecular changes in cell components. It is generally known that increased oxidative stress is linked to cardiovascular, metabolic, inflammatory, and neurological diseases(Incalza et al. 2018). Malondialdehyde (MDA) is also a measure of oxidative stress since it is a byproduct of lipid peroxidation, studies found high levels of MDA when compared to control with T2DM(Marrocco, Altieri, and Peluso 2017; Najafi, Pourfarzam, and Zadhoush 2021).

Tables (5),(6), and(7) showed the correlations of serum TADA, ADA1, and ADA2 respectively with other biochemical parameters in DM and DN groups. The results showed that TADA, ADA1, and ADA2 were positively correlated in DM and DN with FBS, HbA1C, and BMI. The strong positive correlation between the serum

ADA and its isoenzymes with HbA1c in all the studied groups include in our study assists the role of ADA in diabetic control(Pinnelli et al. 2016). Our results were conducted with Ramani NS et al(Ramani et al. 2012). and Mohd(Anon 2020). who found a positive correlation between serum ADA and HbA1c. Moreover, past research has shown that lowering or normalizing HbA1c levels is associated with reduced ADA activity(Kurtul et al. 2004). In addition, a negative correlation was found between TADA and ADA2 with TAS (P<0.05) in DM (Table 5). The scientists discovered that total antioxidant status is substantially lowered in all diabetes patients, and it is even lower in diabetic nephropathy patients. Antioxidant supplementation therapy has the potential to prevent the start and progression of diabetes problems, particularly diabetic nephropathy(Singla et al. 2019).

Table 5:The Pearson correlation of Total Adenosine deaminase activity

| Parameters | TADA Activity (U/L) | | | | | |
|---|---|---|---|---|---|---|
| | DM group (n= 32) | | | DN group (n=32) | | |
| | *r* | *p* | *Sig.* | *r* | *p* | *Sig.* |
| FBS(mg/dl) | 0.725** | 0.001 | HS | 0.683** | 0.001 | HS |
| HbA1c (%) | 0.987** | 0.001 | HS | 0.968** | 0.001 | HS |
| BMI (Kg/m$^2$) | 0.638** | 0.001 | HS | 0.492** | 0.004 | HS |
| TOS (µmol/L) | -0.052 | 0.776 | NS | 0.058 | 0.752 | NS |
| TAS (mmol/L) | -0.362* | 0.042 | S | 0.087 | 0.635 | NS |
| OSI | 0.233 | 0.200 | NS | -0.053 | 0.773 | NS |
| MDA (nmol/ml) | 0.310 | 0.085 | NS | -0.134 | 0.466 | NS |

S=significant, NS= No significant. DM=Diabetic Typ2, DN=Diabetic nephropathy.

Table (6) also showed a positive correlation between ADA1 and MDA in the DM group. In the late complications of diabetes mellitus, the intermolecular cross-linking of collagen by MDA is critical. It plays a role in the hardening of cardiovascular tissue. It could also be a relationship between glycation and further lipid peroxidation(Slatter, Bolton, and Bailey 2000).

Table 6: The Pearson correlation of isoenzyme (ADA1)

| Parameters For male and female | ADA1 Activity (U/L) | | | | | |
|---|---|---|---|---|---|---|
| | DM group (n= 32) | | | DN group (n=32) | | |
| | *r* | *p* | *Sig.* | *r* | *p* | *Sig.* |
| FBS (mg/dl) | 0.699** | 0.001 | HS | 0.636** | 0.001 | HS |
| HbA1c (%) | 0.860** | 0.001 | HS | 0.876** | 0.001 | HS |
| BMI (Kg/m2) | 0.659** | 0.001 | HS | 0.491** | 0.004 | S |
| TOS (µmol/L) | -0.123 | 0.504 | NS | 0.023 | 0.902 | NS |
| TAS (mmol/L) | -0.327 | 0.068 | NS | 0.119 | 0.517 | NS |
| OSI | 0.186 | 0.308 | NS | -0.100 | 0.588 | NS |
| MDA (nmol/ml) | 0.386* | 0.029 | S | -0.208 | 0.253 | NS |

S=significant, NS= No significant. DM=Diabetic Typ2, DN=Diabetic nephropathy.

Table 7: The Pearson correlation of isoenzyme (ADA2)

| Parameters For male and female | ADA2 Activity (U/L) | | | | | |
|---|---|---|---|---|---|---|
| | DM group (n= 32) | | | DN group (n=32) | | |
| | *r* | *p* | *Sig.* | *r* | *p* | *Sig.* |
| FBS(mg/dl) | 0.686** | 0.001 | HS | 0.584** | 0.001 | HS |
| HbA1c (%) | 0.972** | 0.001 | HS | 0.856** | 0.001 | HS |
| BMI (Kg/m2) | 0.586** | 0.001 | HS | 0.384* | 0.030 | S |
| TOS (µmol/L) | -0.020 | 0.913 | NS | 0.085 | 0.643 | NS |
| TAS (mmol/L) | -0.352* | 0.048 | S | 0.032 | 0.860 | NS |
| OSI | 0.236 | 0.194 | NS | 0.011 | 0.954 | NS |
| MDA (nmol/ml) | 0.257 | 0.156 | NS | -0.021 | 0.910 | NS |

Pearson coefficient (r).
*Statistically significant at $p \leqslant 0.05$, **Statistically highly significant at $p \leqslant 0.001$.

Based on this, we hypothesized that higher serum ADA levels could lead to a higher risk of DKD in T2DM patients. Our research indicates that serum ADA1 levels were significantly positively connected with MDA and ADA2 levels were negatively correlated with TAS in DM patients, these results supporting this theory. Inflammation is a key factor in the progression of DKD, and macrophages are the ones who start it(Alicic, Johnson, and Tuttle 2018).

## Conclusion

The results of this study reveal that the higher activities of adenosine deaminase (TADA) and isoenzyme (ADA1, ADA2) in DM and DN are associated with the increase of oxidative stress. The negative correlation of TADA and ADA2 with total antioxidants and the positive correlation of ADA1 with MDA imply that ADA could be utilized as a type 2 diabetes predictor. Because ADA is a major enzyme in changing insulin bioactivity and plays a significant role in insulin action and glycemic management, it could be utilized to assess the severity of oxidative stress linked to nephropathy problems.

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