

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(7): 543-547
www.biochemjournal.com
 Received: 05-05-2026
 Accepted: 08-06-2026

Thulfeqar A Hamza
 Medical Laboratory
 Department, Al-Mustaqbal
 University, Hilla City,
 Babylon Governorate, Iraq

Zahraa A Taha
 Medical Laboratory
 Department, Al-Mustaqbal
 University, Hilla City,
 Babylon Governorate, Iraq

Blood urea, creatinine, and oxidant–antioxidant status in type 2 diabetic patients with nephropathy

Thulfeqar A Hamza and Zahraa A Taha

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i7h.9076>

Abstract

Often, diabetic nephropathy is associated with disturbances of renal biochemistry and imbalance of oxidants and antioxidants. Serum blood urea, creatinine, total antioxidant capacity (TAC) and total oxidant status (TOS) were measured in 70 patients with type 2 diabetic nephropathy and 50 apparently healthy volunteers to characterize these changes. Patients had significantly elevated blood urea and creatinine levels, and significantly reduced TAC levels, compared to the control group, while also having significantly increased TOS levels. These findings contribute to demonstrating that renal function in T2D is accompanied by a pro-oxidant state and therefore propose that oxidative-stress markers should be monitored in addition to the traditional renal-function markers in patients with T2D.

Keywords: Diabetic nephropathy; blood urea; serum creatinine; total antioxidant capacity; total oxidant status

Introduction

Diabetes mellitus

Diabetes mellitus is a common chronic condition of endocrine metabolism in which there is a chronic lack of adequate insulin activity. Polyuria, polydipsia, unexplained weight loss, and visual disturbance are common presenting features of patients, either as a result of impaired insulin production from pancreatic β -cells or as a result of peripheral tissues' reduced sensitivity to insulin or a combination of both. Uncontrolled disease continues to claim more than 1.5 million lives per year globally (Christopher *et al.* 2002) ^[1] and is known to affect the eyes, kidneys and nervous system (Susman & Helseth, 1997) ^[2]; in severe cases, it can lead to cardiovascular problems or acute ketoacidosis (Burtis & Ashwood, 1994) ^[20].

Diabetes can be subdivided clinically into two broad categories. Type 1 disease is characterized by very little insulin production (requiring exogenous insulin injections for life), and type 2 disease is more of a mixture of insulin resistance and relative insulin deficiency, usually controlled by diet and exercise and sometimes by drugs like metformin or semaglutide (Kataoka *et al.*, 1983; Shewade *et al.*, 2001) ^[3]. A third is a gestational type that occurs during the pregnancy and typically resolves after delivery. Oxidative stress, a mechanism suggested by a great deal of observational evidence, has been shown to play a role in the development and progression of diabetes (Baynes, 1991; Cerillo, 2000) ^[7], and free-radical generation is generally increased in diabetic patients compared with non-diabetic patients (Young *et al.*, 1995; Baynes & Thorpe, 1999) ^[6, 7].

Clinical and biochemical changes

Diabetes increases cardiovascular risk by about 2-fold; the majority of diabetes-related deaths (~3/4) occur due to coronary artery disease (CAD), and peripheral arterial disease and cerebrovascular events contribute to this macrovascular burden as well. The long-term effects of injury to the blood vessels (macrovascular and microvascular) predominate the clinical picture.

The damage of the small vessels primarily affects the retina, kidneys, and peripheral nerves. Due to DR being the leading cause of blindness in young working adults, regular eye examinations are recommended, especially considering the increased risk for cataract and glaucoma.

Corresponding Author:
Thulfeqar A Hamza
 Medical Laboratory
 Department, Al-Mustaqbal
 University, Hilla City,
 Babylon Governorate, Iraq

Diabetic nephropathy is the most common renal complication, accounting for over 50% of cases of dialysis in the United States. Peripheral neuropathy, on the other hand, is characterized by sensory loss, neuropathic pain, and autonomic symptoms such as postural hypotension, bowel dysfunction, and erectile dysfunction. Lower-limb amputation (non traumatic) is the main cause of diabetic foot ulcer, in turn the main cause of reduced protective sensation due to neuropathy (Anderson, 1980).

In the absence of glucose utilization (for extended periods of fasting, uncontrolled diabetes, and high-fat feeding), body fat supplies energy in increasing amounts. This change decreases the supply of oxaloacetate (which is formed from the pyruvate by carboxylation); if it is not adequate, there will not be enough oxaloacetate to combine with the acetyl-CoA that is formed from the fatty acids broken down (Ouellet, 1984). When the acetyl-CoA units of the liver are unable to enter the citric acid cycle, they condense with one another to give acetoacetyl-CoA and free coenzyme A, which is released by a Claisen-type condensation (Ouellet, 1984).

Glucose must be maintained within a narrow range in normal physiology, typically 65 – 110 mg/dl: some glucose assays give slightly higher values because of cross reactivity with similar molecules (Christopher *et al.*, 2002) ^[1]. Values outside this range are considered hypoglycaemic (below) and hyperglycaemic (above). Normally, the concentration of ketone bodies is about 3 mg/dL, and when the concentration is greater than 20 mg/dL, it is considered ketonemia and occurs significantly in diabetes.

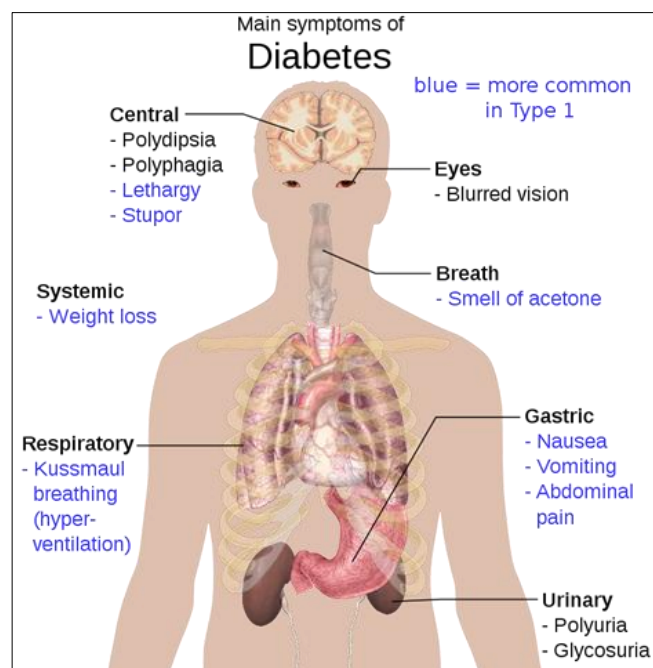


Fig 1: Main clinical symptoms of diabetes mellitus.

Oxidative stress

Oxidative stress means when there is an imbalance between the production of ROS and the body's ability to detoxify or repair the damage. This is not a bad thing by itself; in fact, ROS are used as weapons in an immune system's arsenal to combat pathogens, and brief exposures to ROS can elicit protective responses that actually increase lifespan (Gems & Partridge, 2008) ^[9]. Oxidative stress has also been linked to the pathophysiology of attention-deficit hyperactivity

disorder, in addition to metabolic disease, by Joseph *et al.* (2015) ^[8].

These electrons can escape the respiratory chain in normal aerobic metabolism and react with oxygen, yielding reactive species (superoxide, singlet oxygen, hydroxyl radicals, and hydrogen peroxide) whose effect can be either beneficial or detrimental depending on their concentration, length of time in existence, and distance from cellular targets (Auten & Davis, 2009) ^[10]. While most of the mitochondrial oxygen consumption is converted to water, a portion of it generates the principal reactive species, superoxide, which then becomes hydrogen peroxide, hydroxyl radicals and peroxynitrite (Moussa, 2008) ^[11].

Mechanisms of ROS production

Aerobic cells are constantly exposed to oxidants that result from normal metabolism, and these are generally classified as endogenous or exogenous, and are lifestyle-dependent.

The main endogenous sources for the generation of ROS are mitochondria, peroxisomes, and the endoplasmic reticulum which have high oxygen turnover.

External factors: Exposure to heavy metals, environmental pollution, cigarette smoking, some medications, ionizing radiation, and other xenobiotic agents (Nachiappan & Muthukumar, 2010) ^[12].

Classification of antioxidants

The substances that inhibit oxidation and thus free-radical chain reactions that could harm cells are called antioxidants (Abner *et al.*, 2011) ^[13]. There are enzymes such as superoxide dismutase and catalase which break oxidative chain reactions at various sites, the intracellular tripeptide glutathione and dietary antioxidants (vitamins E and C), which each stop a different step in an oxidative chain reaction. These defenses can be grouped under enzymatic and non-enzymatic defenses as shown in Figure 2 (Misra *et al.* 2014) ^[14].

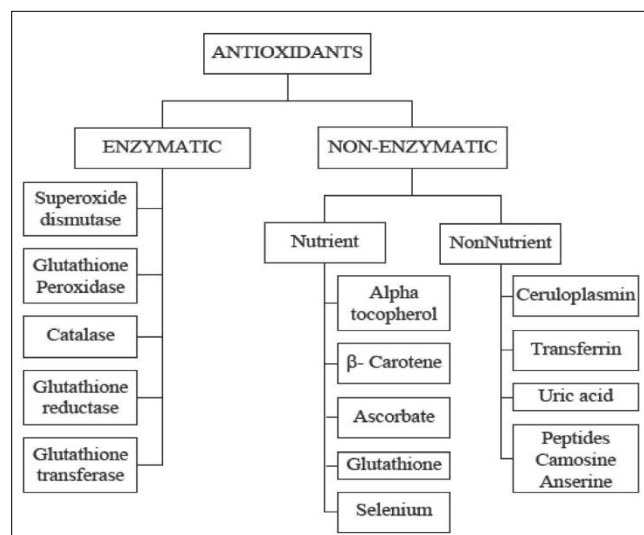


Fig 2: Classification of enzymatic and non-enzymatic antioxidants (adapted from Misra *et al.*, 2014) ^[14]

Enzymatic antioxidants: There are two levels of human enzymatic defence. The primary enzymes (Glutathione peroxidase, Superoxide dismutase, Catalase) directly combat the reactive species while the secondary enzymes (Glutathione reductase, Glucose-6-phosphate dehydrogenase) maintain the defense indirectly by replenishing the NADPH for the regeneration of reduced glutathione (Misra *et al.*, 2014) ^[14].

Non-enzymatic antioxidants: These are antioxidants that are not synthesized endogenously but are essential for metabolism; such as carotenoids, vitamins, trace minerals, and polyphenols (Figure 2; Misra *et al.*, 2014) ^[14].

Chronic kidney disease

Chronic kidney disease is defined by Klahr *et al.* (1998) ^[16] as a result of an irreversible acute event, or as an independent progressive process, which usually progresses without any early warning symptoms.

Acute on chronic renal failure: If chronic kidney disease (CKD) is complicated by a new insult, such as an infection or bleeding, it is termed acute on chronic renal failure. As in isolated acute kidney injury, the management objective is to restore renal function (usually measured by serum creatinine) to the individual's "baseline" and the acute phase of the disease may pass. But distinguishing between acute-on-chronic renal failure and chronic kidney disease alone can be difficult if laboratory values were not obtained in the past and careful clinical monitoring is not done (Katzung, 2007) ^[15].

Aim of the study

The objectives of this study were to:

1. Quantify serum blood urea.
2. Quantify serum creatinine.
3. Evaluate the impact of oxidative stress on antioxidant activity.
4. Investigate the association between diabetic nephropathy and markers of oxidative stress.

2. Materials and Methods

Sample collection

Using disposable syringes and needles, six milliliters of venous blood were drawn from every participant in the patient and control groups alike. After allowing the samples to clot, they were centrifuged for 10 minutes at 3000 rpm, and the separated sera were aliquoted and kept frozen at -70 °C pending analysis.

Determination of blood urea

Principle: Urea concentration was determined spectrophotometrically from the intensity of a colorimetric reaction.

Standard	Sample	Blank	Reagent
10µl	—	—	Standard
—	10µl	—	Sample
1.0 ml	1.0 ml	1.0 ml	Reagent 1

The tubes were mixed and incubated for 5 minutes at 37 °C, or alternatively for 10 minutes at room temperature.

Standard	Sample	Blank	Reagent
1.0 ml	1.0 ml	1.0 ml	Reagent 2

A second incubation of 5 minutes at 37 °C (or 10 minutes at room temperature) followed. Absorbance (A) of the sample and standard was then read at 600 nm against the blank, and urea concentration was derived by comparing sample absorbance to standard absorbance.

Determination of creatinine

Principle: A kinetic colorimetric reaction, read spectrophotometrically at 490 nm using a 1 cm cuvette at 37 °C, was used to determine serum creatinine.

Standard	Sample	Blank	Reagent
100µl	—	—	Standard
—	100µl	—	Sample
1.0 ml	1.0 ml	1.0 ml	Reagent 1 + Reagent 2

The reaction mixture was thoroughly mixed and allowed to stand for 10 minutes at room temperature. After addition of samples, absorbance readings were obtained 30 seconds (A1) and 90 seconds (A2) later and the concentration of creatinine was calculated by the difference in absorbance (A2 – A1) compared to that in the standard (ΔA).

Total Antioxidant Capacity (TAC) assay

Principle: This method utilizes a Fenton reaction in which the hydrogen peroxide reacts with a known Fe-EDTA complex to produce hydroxyl radicals which would degrade benzoate and release TBARS. The more antioxidants present in the serum the more TBARS will be suppressed (spectrophotometrically measured) thus determining the activity of the antioxidants, AOA (Gutteridge *et al.*, 1990; Yamazaki & Piette, 1990) ^[17, 18].

Reagent	A1	A0	K1	K0	UA1	UA0
Serum	10µl	10µl	—	—	—	—
Uric acid	—	—	—	—	10µl	10µl
Buffer	490µl	490µl	500µl	500µl	490µl	490µl
Na-benzoate	500µl	500µl	500µl	500µl	500µl	500µl
Acetic acid	—	1000µl	—	1000µl	—	1000µl
Fe-EDTA	200µl	200µl	200µl	200µl	200µl	200µl
H ₂ O ₂	200µl	200µl	200µl	200µl	200µl	200µl

Tubes were then incubated for 60 minutes at 37 °C, after which:

Reagent	A1	A0	K1	K0	UA1	UA0
Acetic acid	1000µl	—	1000µl	—	1000µl	—
TBA	1000µl	1000µl	1000µl	1000µl	1000µl	1000µl

Antioxidant activity (AOA) was derived using the following equation:

$$\text{AOA (mmol/L)} = C_{\text{UA}} \times (K - A) / (K - UA)$$

in which K denotes the absorbance of the control (K1 – K0); A, the absorbance of the sample (A1 – A0); UA, the absorbance of the uric acid solution (UA1 – UA0); and CUA, the concentration of uric acid (mmol/L).

Total Oxidant Status (TOS) (Erel, 2005) ^[19]

Principle: Serum TOS was assessed by the method of Erel (2005) ^[19]. The oxidants in the sample oxidize a ferrous ion-o-dianisidine complex to its ferric form; the presence of the glycerol in large excess in the reagent mixture speeds the reaction. In acidic solution, the ferric ions produced form a coloured complex with xylenol orange, whose colour is proportional to the amount of oxidant detected at 570 nm. Results were reported as micromolar equivalents of hydrogen peroxide (µmol H₂O₂ Eq/L) using a calibration curve of hydrogen peroxide.

	Blank	Standard	Sample
Distilled water	50µl	—	—
Serum	—	—	50µl
Hydrogen peroxide	—	50µl	—
R1	2 ml	2 ml	2 ml

Each tube was vortex-mixed, after which:

	Blank	Standard	Sample
R2	2 ml	2 ml	2 ml

Tubes were mixed gently, incubated for 3 minutes at room temperature, and read spectrophotometrically at 560 nm.

Total oxidant status = (A test / A standard) × concentration of standard

3. Results and Discussion

Blood urea

Although the blood urea level was significantly higher in the nephropathy group than in the control group ($p \leq 0.05$; Table 1), this finding is similar to those previously reported for prerenal and renal diseases (Marsh *et al.*, 2005) [21].

Table 1: Blood urea (mg/dL) in nephropathy patients compared with controls

Group	N	Mean ± SD (mg/dL)	SE	95% CI Lower	95% CI Upper	P-value
Control	50	36.10 ± 7.100	0.500	25.02	30.07	0.013**
Nephropathy patients (G)	70	52.93 ± 7.801	0.112	33.55	62.22	0.001*

Creatinine

Serum creatinine increased significantly ($p \leq 0.05$) in the nephropathy group compared to the control group (Table 2), which was similar to previous findings of high creatinine level in patients with prerenal and renal impairment (Shemesh *et al.*, 1985) [25].

Table 2: Creatinine (mg/dL) in nephropathy patients compared with controls

Group	N	Mean ± SD (mg/dL)	SE	95% CI Lower	95% CI Upper	P-value
Control	50	0.887 ± 0.378	0.132	0.555	1.232	0.000**
Nephropathy patients (G)	70	2.123 ± 0.426	0.165	0.926	3.423	0.002*

Although the most frequently used renal marker, due to its simplicity, creatinine is only abnormal after a significant amount of nephron loss, making it an inadequate marker for early renal disease (Taylor, 1989) [26].

Total antioxidant capacity

TAC was significantly lower ($p \leq 0.05$) in nephropathy patients when compared to control (Table 3) which is similar to what has been reported by Jamuna Rani and Mythili (2014) [28].

Table 3: Total antioxidant capacity (μmol/L) in nephropathy patients compared with controls

Group	N	Mean ± SD (U/L)	SE	95% CI Lower	95% CI Upper
Control	50	996 ± 387	56.3	676.1	1023
Nephropathy patients (G)	70	546 ± 148	82.1	398.3	701.1

This decline likely reflects heightened oxidative stress — evident from elevated lipid peroxidation — coupled with faster antioxidant turnover as these molecules are consumed while neutralizing excess free radicals.

Total oxidant status

Total oxidant levels were significantly elevated ($p \leq 0.05$) among nephropathy patients relative to controls (Table 4).

Table 4: Total oxidant status (μmol/L) in nephropathy patients compared with controls

Group	N	Mean ± SD (U/L)	SE	95% CI Lower	95% CI Upper
Control	50	0.453 ± 0.224	0.0232	0.223	0.509
Nephropathy patients (G)	70	0.957 ± 0.396	0.101	0.623	1.012

Because oxidative stress reflects free-radical production exceeding the system's neutralizing capacity, this excess ROS generation is considered a shared mechanism behind the biochemical abnormalities driving β-cell impairment and insulin resistance.

Diabetic patients additionally face heightened cardiovascular risk, the foremost contributor to diabetes-related death and illness (Orasanu & Plutzky, 2009) [27]; oxidative stress is thought to fuel much of this risk through its role in the systemic inflammation underlying both macro- and microvascular disease.

4. Conclusion

The findings of this study support the following conclusions:

1. Both blood urea and serum creatinine were significantly higher among diabetic nephropathy patients than among healthy controls.
 2. Total oxidant status rose significantly in nephropathy patients relative to controls.
 3. Total antioxidant capacity declined significantly among nephropathy patients compared with controls.
- Collectively, these findings suggest that diabetic nephropathy involves a measurable shift toward a pro-oxidant state, reinforcing the proposed role of oxidative stress in the progression of diabetic kidney disease.

References

1. Christopher H, Chilvers ER, Bean NA, Colledge NR, Davidson AA. *Davidson's principles and practice of medicine*. 19th ed. Edinburgh: Churchill Livingstone; 2002. p. 644-647.
2. Susman JL, Helseth LD. Reducing the complication of type II diabetes: a patient-centered approach. *Am Fam Physician*. 1997;56:471-478.
3. Kataoka S, Satoh J, Fujiya H, Toyota T, Suzuki R, Itoh K, Kumagai K. Immunologic aspects of the non-obese diabetic (NOD) mouse. *Abnormalities of cellular immunity*. *Diabetes*. 1983;32(3):247-253.
4. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care*. 1997;20:1183-1197.
5. Chen APF, Chen B, Pletsky SA. *In vitro* diagnostics in diabetes: meeting the challenge. *Clin Chem*. 1999;45(9):1596-1601.
6. Young IS, Tate S, Lightbody JH, McMaster D, Trimble ER. The effects of desferrioxamine and ascorbate on oxidative stress in the streptozotocin diabetic rat. *Free Radic Biol Med*. 1995;18(5):833-840.

7. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes*. 1999;48(1):1-9.
8. Joseph N, Zhang-James Y, Perl A, Faraone SV. Oxidative stress and ADHD: a meta-analysis. *J Atten Disord*. 2015;19(11):915-924.
9. Gems D, Partridge L. Stress-response hormesis and aging: "that which does not kill us makes us stronger". *Cell Metab*. 2008;7(3):200-203.
10. Auten RL, Davis JM. Oxygen toxicity and reactive oxygen species: the devil is in the details. *Pediatr Res*. 2009;66(2):121-127.
11. Moussa SA. Oxidative stress in diabetes mellitus. *Rom J Biophys*. 2008;18(3):225-236.
12. Nachiappan V, Muthukumar K. Cadmium-induced oxidative stress in *Saccharomyces cerevisiae*. *Indian J Biochem Biophys*. 2010;47(6):351-356.
13. Abner EL, Schmitt FA, Mendiondo MS, Marcum JL, Kryscio RJ. Vitamin E and all-cause mortality: a meta-analysis. *Curr Aging Sci*. 2011;4(2):158-170.
14. Misra K, Dhillon GS, Brar SK, Verma M. Antioxidants. In: Brar SK, Dhillon GS, editors. *Biotransformation of waste biomass into high value biochemicals*. New York: Springer; 2014. p. 117-138.
15. Katzung BG. *Basic and clinical pharmacology*. 10th ed. New York: McGraw-Hill Medical; 2007. p. 733.
16. Klahr S, Miller SB. Acute oliguria. *N Engl J Med*. 1998;338(10):671-675.
17. Gutteridge JMC, Maidt L, Poyer L. Superoxide dismutase and Fenton chemistry. *Biochem J*. 1990;269(1):169-174.
18. Yamazaki I, Piette LH. ESR spin-trapping studies on the reaction of Fe^{2+} ions with H_2O_2 : reactive species in oxygen toxicity in biology. *J Biol Chem*. 1990;265:13589-13594.
19. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem*. 2005;38(12):1103-1111.
20. Burtis CA, Ashwood ER. *Tietz textbook of clinical chemistry*. 3rd ed. Philadelphia: W.B. Saunders Company; 1999. p. 1034-1054.
21. Marsh KL, Sims GK, Mulvaney RL. Availability of urea to autotrophic ammonia-oxidizing bacteria as related to the fate of ^{14}C - and ^{15}N -labeled urea added to soil. *Biol Fertil Soils*. 2005;42(2):137-145.
22. Moore EM, Bellomo R, Nichol AD. The meaning of acute kidney injury and its relevance to intensive care and anaesthesia. *Anaesth Intensive Care*. 2012;40(6):929-948.
23. Nelson DL, Cox MM. *Lehninger principles of biochemistry*. 4th ed. New York: W.H. Freeman and Company; 2005.
24. Ricci Z, Ronco C. New insights in acute kidney failure in the critically ill. *Swiss Med Wkly*. 2012;142:w13662.
25. Shemesh O, Golbetz H, Kriss JP, Myers BD. Limitations of creatinine as a filtration marker in glomerulopathic patients. *Kidney Int*. 1985;28(5):830-838.
26. Taylor EH. *Clinical chemistry*. New York: John Wiley & Sons; 1989. p. 4, 58-62.
27. Orasanu G, Plutzky J. The pathologic continuum of diabetic vascular disease. *J Am Coll Cardiol*. 2009;53(Suppl 5):S35-S42.
28. Jamuna Rani A, Mythili SV. Study on total antioxidant status in relation to oxidative stress in type 2 diabetes mellitus. *J Clin Diagn Res*. 2014;8(3):108-110.