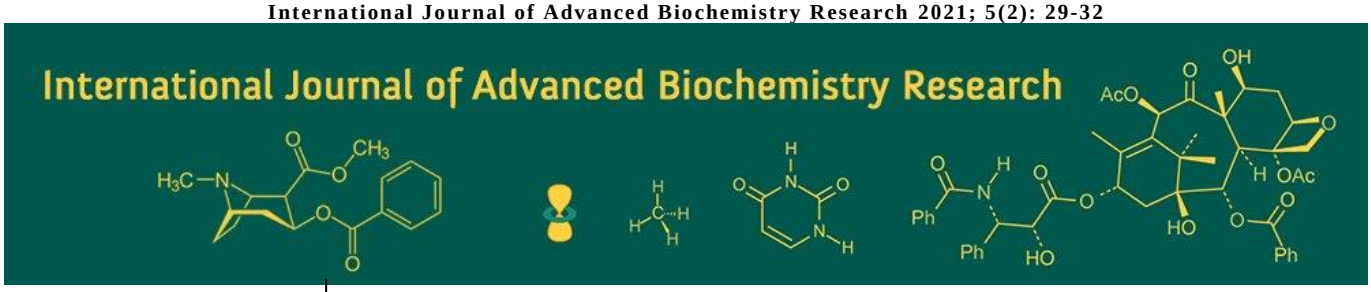

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Type II diabetes mellitus induced oxidative stress and proinflammatory cytokines in renal cells, leading to Acute Kidney Injury (AKI)

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Abstract
Introduction: Acute Kidney Injury (AKI), biochemically characterized as abnormality in kidney function test which causes accumulation of creatinine and blood urea and functionally by a rapid decline in the glomerular filtration rate (GFR). Oxidative stress plays an important role in the development of vascular complications in type 2 diabetes. The oxidant derived tissue injury occurs when the production of oxidants or reactive oxygen species (ROS) exceeds local antioxidant capacity. Inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL-6) and various growth factors in renal cells modulate the local response are responsible for AKI.
Material and Methods: 10 ml of fasting venous blood was collected from the antecubital vein in a plain, fluoride and EDTA vacutainers. The blood sample was centrifuged and stored at 4 °C for biochemical and immunological investigations. The study group consisted of n=50 healthy individuals (Group I), n=25 Type II Diabetic without AKI (Group II), n=25 Type II diabetic with AKI (Group III) of either sex aged between 50-65 years. Type II Diabetic presented with clinical signs and symptoms of Acute Kidney Injury without Nephropathy. Serum levels of inflammatory markers (IL-6 & TNF-α), antioxidants (Glutathione reductase), plasma malondialdehyde (MDA), hs-CRP were estimated.
Results: Concentration of inflammatory molecules such as TNF-α 9.32±1.08, 14.04±1.42 and 36.56±10.50; IL-6 9.24±1.20, 14.14±1.50 and 36.76±11.56; hs-CRP 0.90±1.10, 1.96±0.50 and 2.18±0.90 was significantly elevated in Group III. GSH was significantly lower in both the groups of Diabetic with and without AKI when compared to controls. 7.10±0.58, 6.90±0.70 and 5.80±0.80. Mean value of total MDA 2.32±0.98, 8.68±2.50 and 9.80±2.72 was significantly more in Group III as compared to Group I and Group II.
Conclusion: Results of the present study indicate that inflammatory markers and oxidative stress increase with decreased antioxidant defense levels in patients with AKI due to DM induced oxidative stress.

Keywords: Diabetes, proinflammatory cytokines, kidney injury

Introduction
Acute kidney injury (AKI), biochemically characterized as abnormality in kidney function test which causes accumulation of creatinine and blood urea and functionally by a rapid decline in the glomerular filtration rate (GFR). We get old because we have the bad habit of spending our whole life breathing oxygen. And what is the relationship between oxygen and tissue injury? The answer boils down to two words: Oxidative stress! Sporadically, there are O2 molecules that transform into reactive oxygen species, most of which are neutralized by our antioxidant defenses. However, there are always some reactive oxygen species that can bypass our defenses and consequently can cause minor damage to some of our biomolecules. Although these damages do not have much biological significance, when evaluated isolated, as we grow older, they accumulate, and these cumulative damages begin to translate to the loss of some functionalities (Spranger J *et al*. 2003) [1].
But not everything is bad news, because our biochemistry is full of examples where even the most dangerous situations/molecules can be converted into an advantage, at least in some contexts. This is what happens with oxidative stress. Although it is a potentially fatal situation for cells and therefore, most often, is a situation we should avoid, there is a context where oxidative stress is beneficial to our body. I'm talking about the inflammatory response (Dandona P *et al*. 2007) [2].

Therefore, everything that can accelerate our metabolic rate has the potential to make us age faster because it increases the production of reactive oxygen species. In this context, the effect of emotional stress is particularly evident! For example, people who have jobs and activities of high stress, age at a much higher rate than those who have a much more relaxed life (Piya MK *et al*. 2013) [3].

Basically, it is the oxygen that makes us live, but it is also the one that kills us little by little, that is, that makes us grow old. Oxidative stress plays an important role in the development of vascular complications in type 2 diabetes. The oxidant derived tissue injury occurs when the production of oxidants or reactive oxygen species (ROS) exceeds local antioxidant capacity (Morohoshi M *et al*. 1996) [4].

Inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL-6) and various growth factors in renal cells modulate the local response are responsible for AKI. The state of a person's health is often directly related to that person's lifestyle. India has the largest number of diabetic subjects earning the dubious distinction of being termed the "Diabetes Capital of The World". According to the Diabetes Atlas 2019 published by the International Diabetes Federation, the number of people with diabetes in India are currently around 77.0 million and expected to rise to 101.0 million by 2030. Diabetes and hypertension are the primary causes of kidney disease and blindness. AKI remains a fundamental problem in hospitalized patients worldwide. India is fast becoming world diabetes capital. The World Health Organization (WHO) has predicted that going by the current trend India will become the "diabetes capital of the world" by 2025. The cause of Acute Kidney Injury (AKI) is the rapid breakdown of renal function that occurs when high levels of uremic toxins accumulate in the blood. AKI occurs when the kidneys are unable to excrete the daily load of toxins in the urine. The types of Acute Kidney Injury are: Pre-renal AKI, Post-Renal AKI and Intrinsic renal AKI. Acute Kidney Injury does not produce a classic set of symptoms. The most common symptom is decreased urine output, which occurs in 70% of patients (Gao X *et al*. 2007) [5].

AKI is most easily diagnosed by an increase in blood levels of creatinine and blood urea nitrogen (BUN). The blood level of creatinine typically increases by 0.5 milligrams per tenth of a litter (mg/dL) every day. Nearly 90% of intrinsic AKI cases are caused by ischemia or toxins, both of which lead to acute tubular necrosis (ATN) (Rajendran K *et al*. 2012) [6].

Aims and objectives

To ascertain the role of biochemical parameters, oxidative stress and inflammatory markers in AKI caused by type II Diabetes Mellitus.

Method and Materials

The present study was carried out in Govt. Medical College, Jalaun at Department of Biochemistry. The Subjects included for the study were 50 diabetic and 50 normal individuals aged between 50-65 years. A detailed history of all the normal individuals and diabetic was taken. All the subjects were clinically examined.

The study group consisted of

- Group I Control
- Group II Diabetic without AKI
- Group III Diabetic with AKI

Inclusion criteria

Subjects with clinically proven diabetes aged more than 45 years with a history of diabetes more than 5 years, irrespective of treatment (oral drugs and/or insulin therapy) (Kwasa *et al*. 2014) [7].

Exclusion criteria

- Diabetic patients with hepatic disorders.
- Diabetic more than 70 yrs of age.
- Gestational diabetes.
- Patients already diagnosed with Diabetic nephropathy.

10 ml of blood sample was withdrawn from the antecubital vein following overnight fasting. The blood sample was collected in plain, fluoride and EDTA vacutainers. The blood sample was centrifuged for 10 min. at 3000 rpm at room temp. The serum was stored at 4 °C for biochemical and immunological investigations.

- Serum hs-CRP (Immunoturbidimetry)
- MDA (Thiobarbituric acid reactive substance TARBS)
- GSH (Ellman's method)
- TNF-α (ELISA)
- IL-6 (ELISA)
- Fasting blood sugar (GOD-POD)
- Glycated Hb (Bio Rad 10)
- Microalbumin (turbilatex)
- CYS C (ELISA)
- Urea (urease)
- Uric acid (uricase) and creatinine (jaffes) were estimated.

Statistical analysis

Results were presented as mean and standard deviation of the mean.

Statistical significance was determined at the $p<0.05$, 0.01, 0.001.

Intragroup comparisons were made for the levels of biomarkers with control and were statistically evaluated by the student's t-test.

Results

Mean value of FBS 91.60±10.44, 180.88±20.54 and 253.30±28.12. HbA1c 6.28±1.42, 8.98±2.21 and 11.00±2.06 were significantly elevated in Group III. When KFT were compared between groups, Mean values of Cys-C 2.10±1.13, 4.88±2.32 and 5.25±2.50. Urea 30.50±3.34, 45.48±4.90 and 87.32±10.29 and UA 5.00±1.00, 5.88±1.09 and 7.40±1.01. Scr 0.70±0.12, 0.90±0.30 and 2.50±0.44 was significantly higher in Group III. Microalbumin 11.28±3.22, 27.18±4.32 and 275.10±30.92 was significantly increased in Group III. Cystatin C is related to inflammation and oxidative stress, the key pathogenic components of metabolic syndrome were elevated in diabetes with or without AKI. Uric acid the final oxidation product of purine metabolism excreted by kidneys was significantly increased in Group III Concentration of inflammatory molecules such as TNF-α 9.32±1.08, 14.04±1.42 and 36.56±10.50. IL-6 9.24±1.20, 14.14±1.50 and 36.76±11.56. hs-CRP 0.90±1.10, 1.96±0.50 and 2.18±0.90 was significantly elevated in Group III. GSH were significantly lower in both the groups of Diabetic with and without AKI when compared to controls 7.10±0.58, 6.90±0.70 and 5.80±0.80. Mean value of total MDA 2.32±0.98, 8.68±2.50 and 9.80±2.72 was significantly more in Group III.

Table 1: Biochemical parameters in non-diabetic, diabetic with Aki and without Aki

Individuals	FBS (mg/dl)	HbA1c (%)	Microalbumin (mg/g)	Cystatin-C (mg/L)	Urea (mg/dl)	Uric Acid (mg/dl)	Creatinine (mg/dl)
Normal n=50 (Group I)	91.60±10.44	6.28 ± 1.42	11.28 ± 3.22	2.10±1.13	30.50 ± 3.34	5.00±1.00	0.7±0.12
Diabetes without AKI n=25 (Group II)	180.88±20.54	8.98 ± 2.21	27.18 ± 4.32	4.88±2.32	45.48 ± 4.90	5.88±1.09	0.90±0.30
Diabetes with AKI n=25 (Group III)	253.30±28.12**	11.00 ± 2.06**	275.10±30.92**	5.25±2.50**	87.32 ± 10.29**	7.40±1.01**	2.50±0.44**

The data were expressed as mean ± SD. The data was analyzed using the student's t- test. * indicates $p<0.05$ and statistically significant, **indicates $p<0.001$ and statistically highly significant.

Table-1

With respect to biochemical parameters, the mean values of fasting blood sugar 91.60±10.44, 180.88±20.54 and 253.30±28.12.; HbA1c 6.28±1.42, 8.98±2.21 and 11.00±2.06 were significantly elevated in Group III compared to Groups I and II.

When renal function tests were compared between groups, Mean values of CysC 2.10±1.13, 4.88±2.32 and 5.25±2.50; Urea 30.50±3.34, 45.48±4.90 and 87.32±10.29 and UA 5.00±1.00, 5.88±1.09 and 7.40±1.01; Scr 0.70±0.12, 0.90±0.30 and 2.50±0.44 was significantly higher in Group III compared to Group I and II.

Table 2: Inflammatory and Oxidative Stress Level in Non-Diabetic, Diabetic with Aki and Without Aki

Individuals	TNF-α (pg/ml)	IL-6 (pg/ml)	GSH (mg/dl)	MDA (μmol/L)	hs-CRP (mg/L)
Normal n=50 (Group I)	9.32±1.08	9.24±1.20	7.10±0.58	2.32±0.98	0.90±1.10
Diabetes without AKI n=25 (Group II)	14.04±1.42*	14.14±1.50*	6.90±0.70	8.68±2.50	1.96±0.50*
Diabetes with AKI n=25 (Group III)	36.56±10.50**	36.76±11.56**	5.80±0.80*	9.80±2.72**	2.18±0.90*

Microalbumin 11.28±3.22, 27.18±4.32 and 275.10±30.92 was significantly increased in Group III when Compared to Group I and Group II.

The data were expressed as mean ± SD. The data were analyzed using the student's t-test. * indicates $p<0.05$ and statistically significant, **indicates $p<0.001$ and statistically highly significant.

Table-2

Concentration of inflammatory molecules such as TNF-α 9.32±1.08, 14.04±1.42 and 36.56±10.50; IL-6 9.24±1.20, 14.14±1.50 and 36.76±11.56; hs-CRP 0.90±1.10, 1.96±0.50 and 2.18±0.90 was significantly elevated in Group III (Liguori I *et al*. 2018) [8].

GSH were significantly lower in both the groups of Diabetic with and without AKI when compared to controls. 7.10±0.58, 6.90±0.70 and 5.80±0.80 (Table II). Diabetes as a risk factor for AKI is closely associated with increased oxidative stress, and the duration of diabetes plays an important role in increasing the level of oxidative damage and reducing antioxidant defence (Girman CJ *et al*. 2012) [9]. Mean value of total MDA 2.32±0.98, 8.68±2.50 and 9.80±2.72 was more in Group III compared to Groups I & II with a significant p-value (Waikar SS *et al*. 2008) [10].

The results of the present study indicate that inflammatory markers and oxidative stress increase with decreased antioxidant defense levels in patients with diabetic nephropathy due to hyperglycemia induced oxidative stress (James MT *et al*. 2015) [11].

Discussion

As there are very few studies in India highlighting the importance and the usefulness of inflammatory markers for diagnosis of AKI and associated complications; this created an interest in studying biomarkers in early diagnosis and management, renal parameters, diabetic profile, in addition to oxidative stress markers and other variables.

In our study, we observed a significant increase in FBS and HbA1c % in Group III, which could be because of mesangial expansion is the major lesion of type 2 diabetic resulting in renal dysfunction. Glucose toxicity is a primary cause of glomerular injury. Prolonged elevations in blood glucose levels result in the formation of glycation end products which interfere with normal collagen turnover and promote vessel permeability, matrix accumulation, and formation of adhesion molecules. Our result shows a significant increase in Cys-C, B. Urea, Uric Acid and S. Creatinine level, which strengthens our statement kidney is at risk of AKI (Kim J *et al*. 2009) [12].

A significant increase in Microalbuminuria activates renal proximal tubular epithelial cells to induce tubulointerstitial inflammation. Significant increase of TNF- α, IL-6 and hs-CRP in group III is may be due to oxidative stress in diabetes, responsible for endothelial dysfunction and release of inflammatory markers such as cytokines from the damaged renal tissue. Hyperglycemia can induce oxidative stress, and thereby increases pro-inflammatory cytokines such as IL-6 via activation of NF-kB and TLR in T2DM subjects. Studies in monocytes have also shown that conditions associated high-glucose level increased the production of IL-6 and TNF-α.

This might be one of the reasons for pronounced increase in pro-inflammatory cytokines in T2DM patients with AKI. There exists a strong association between oxidative stress and inflammation. Increase in TNF-α expression induce activation of NAD(P)H oxidase and production of reactive oxidative species.

On the other hand, oxidative stress has also been shown to increase production of pro-inflammatory cytokines (TNF-α, IL-6). A number of different inflammatory cells and soluble mediators are shown to be necessary for renal damage and loss of glomerular filtration. The significant increase of hs-CRP in Group 3 indicates that acute stressful conditions during inflammatory processes. In the present study significant higher levels of MDA, a marker of oxidative stress was observed in T2DM patients with AKI when compared to T2DM patients without AKI. The produced MDA is a reactive compound, which causes toxic stress in cells and stimulates the release of inflammatory cytokines such as TNF-α and IL-6. GSH was significantly lower in both the groups of Diabetic with and without AKI when compared to controls (Navarro-Gonzalez JF *et al*. 2008) [13].

Conclusion

Arrival of new biomarkers will help define the kidney at risk rather than relying simply on creatinine. Our observations also suggest that in addition to clinical examination, baseline oxidants, antioxidants and proinflammatory cytokines may help clinicians in early management of diabetes and associated AKI. Early management of etiological factors shall prevent the risk of AKI in T2DM, as Acute Kidney Injury has good prognosis with early intervention. We believe that this study, demonstrating alterations in biochemical profile has several testable elements that if confirmed, could lead to major advances in the treatment of patients with AKI. Indeed, we believe that the time is right for clinical trials of therapeutic approaches with appropriate antioxidants, perhaps in combination with nutritional repletion and anti-inflammatory therapy simultaneously monitoring the independent risk factors.

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