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SMALL ANTIOXIDANT MOLECULES AND THEIR IMPORTANCE IN ACUTE KIDNEY INJURY

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ABSTRACT

Background: Diabetes is a risk factor for developing Acute Kidney Injury (AKI) in which oxidative stress and inflammation are characteristic features. **Methods**: Case control prospective study. Subjects were categorized into two groups; Group I: Type 2 Diabetes Mellitus (T2DM) without AKI and Group II: T2DM with AKI. Group II further sub classified into five groups based on **R**isk, **I**njury, **F**ailure, **L**oss and **E**nd stage (RIFLE) criteria. Parameters estimated were serum NGAL (Neutrophil Gelatinase Associated Lipocalin) by ELISA (Biovendor, USA), Serum creatinine (Scr), Uric Acid (UA), high sensitive C - reactive protein (hs-CRP), Nitric oxide (NO), Malondialdehyde (MDA), vitamin C and Glutathione peroxidase (GPx) by Spectrophotometric assays. eGFR calculated by Modification of Diet in Renal Disease (MDRD) equation. **Results:** In Group II NGAL, Scr, MDA, NO, hs-CRP and UA showed a significant elevation while eGFR, Vitamin C, and GPx concentrations were statistically significant in Group I. In Group II as per RIFLE classification NGAL, Scr and NO were significantly elevated in all the subgroups. **Conclusion:** An imbalance between oxidants and antioxidants were seen in both Groups I and II. So estimation of these parameters in early stages may reduce morbidity rate and prevent risk of AKI in T2DM. In addition to a clinical examination, estimation of MDA and Vitamin C can serve as supportive biochemical markers for early diagnosis and therapeutic intervention.

KEYWORDS: Acute kidney Injury, RIFLE, Malondialdehyde, Nitric oxide, hs-CRP.

Impact statement

Morbidity and mortality due to diabetes and its complications pose significant health care burden on families and society in developing countries.

This study was conducted in rural population mainly engaged in agriculture. Most are illiterate and their occupation exposes them to pesticides which can affect insulin levels and brings about insulin resistance. This in turn may alter oxidants, antioxidants, inflammatory molecules and damage tubulointerstitium. Large number of patients with diabetes and its complications are observed in young age groups.

The observations of this study suggest estimation of baseline oxidants (MDA) and antioxidant (Vitamin C) as supportive biochemical markers in diabetes and AKI could be of use in early diagnosis.

INTRODUCTION

Diabetes is a major risk factor for developing acute kidney injury (AKI) and this clinical complication affects about 1-7% of hospitalized patients and 1-25% of patients in the intensive care unit (ICU).[1] AKI is a rapid reduction in kidney function causing failure to maintain fluid, electrolyte and acid base homeostasis. The RIFLE classification proposed by the Acute Dialysis Quality Initiative (ADQI) group classify AKI into five stages (1) Risk [R] (2) Injury [I] (3) Failure [F] (4) Loss [L] and (5) End-stage Kidney [E].[2]

The prevalence and the factors which lead to AKI in diabetes are not clear. Three main hypotheses put forward for an explanation are genetic, metabolic, and hemodynamic. The Genetic and Hemodynamic mechanism has been very well explained by the mathematical model for glucose regulation in the whole body system.[3] The Metabolic theory assumes that nephropathy is observed more frequently in patients with poorly controlled diabetes, as evidenced by increased glycated hemoglobin (HbA1c).[4,5] Entry of glucose into kidney cells is driven mainly by its concentration gradient, even in insulin deficiency.[5] Hyperglycemia at

the cellular level may involve functional and structural changes characteristic of diabetic nephropathy.

Oxidative stress occurs when production of oxidants or reactive oxygen species (ROS) exceeds local antioxidant capacity. Residues of Reactive Nitrogen Species (RNS) which are by-products of nitric oxide (NO) can also contribute to several pathological processes at high levels. RNS comprises nitrite ($NO2^-$), nitrate ($NO3^-$) and peroxy nitrite (ONOO). Studies have suggested that oxidative stress is a common pathogenic factor for the dysfunction of beta and endothelial cells.[6,7]

Antioxidants are biomolecules which can inhibit the oxidation of oxidative substrates. Antioxidants are of two types depending on mechanism of action, either chain breaking antioxidants or preventive antioxidants.[8,9] Preventive antioxidants reduce the rate of chain initiation by deactivating metals, quenching singlet oxygen and removing hydroperoxides, including transferrin, Ferritin, Ethylenediamine Tetra Acetic acid (EDTA), ceruloplasmin, Catalase, SOD and GPx. Chain breaking antioxidants are the molecules that have the ability to receive or donate an electron from a radical with the formation of stable byproducts including α-tocopherol, ascorbic acid, uric acid and β-carotene.[10]

Oxidative stress is an important factor associated with pathogenesis in AKI and diabetes. The pathogenesis of diabetic nephropathy remains far from clear. Studies conducted by Shestakova MV and Jarek IR etal., showed that there is a close relationship between endothelial dysfunction and the development and progression of kidney disease in patients with Type 2 Diabetes Mellitus (T2DM).[11] We hypothesized that, oxidants and inflammatory markers are associated with T2DM and Acute Kidney Injury. To test this hypothesis, estimation and comparison of oxidants, antioxidants and inflammatory markers in T2DM patients with and without AKI were performed.

MATERIALS AND METHODS

Single centered prospective case control study, conducted at RL Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medical College a Constituent of Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India. Study was approved by the Institutional Ethical Committee. Informed consent was obtained from all the study subjects. Total 150 subjects in the age group 45-75 years of either gender were included and grouped into two categories; Group I: T2DM without AKI; Group II: T2DM with AKI. Group II further sub classified into five groups based on **R**isk, **I**njury, **F**ailure, **L**oss, and **E**nd-stage (RIFLE) classification (Figure: 1).[5]

Inclusion criteria: clinically proven cases of diabetes of more than 5 years duration irrespective of treatment (oral drugs and/ or insulin therapy).

Exclusion criteria: subjects with inability to give informed consent, patients with diabetes predisposed to radiocontrast induced nephrotoxicity, DM with type IV renal tubular acidosis (hyperreninemic hypo aldosteronism, patients on drugs known to cause proteinuria/ albuminuria), patients with hepatobilary disorders leading to proteinuria/ albuminuria, gestational diabetes mellitus, patients already diagnosed with diabetic nephropathy, patients with Chronic kidney disease who underwent dialysis.

Blood sample was collected in plain tubes after overnight fasting from diabetic subjects and for the Group II patients, blood sample was collected from Intensive Care Unit (ICU) and Medical Intensive Care Unit (MICU). Method characteristics are represented in table1.

Statistical analysis

Comparison of one time data between the two study groups was done by Student ‘t’ test and Analysis of Variance (ANOVA) test was done within the Group-II for the variables which are normally distributed and non parametric statistics “Mann-Whitney U test” was performed for non- normally distributed variables. Correlation among continuous data was performed by the Pearsons correlation coefficients. Categorical data was done by Fischer’s exact test. Serum NGAL concentration was expressed as median and interquartile range (IQR). All tests were two-tailed, and a $p<0.05$ considered as statistical significance. The SPSS statistical software program (licensed version 16.0, SPSS, Chicago, IL) was used for all analysis.

RESULTS

T2DM with AKI (Group II) are associated with other complications. A total of 75 subjects n= 38 (50.66%) had hypertension, cardio pulmonary bypass was seen in n=12 (16%), sepsis n=8 (10.66%), snake bite in n= 9 (12%), gastroenteritis n= 6 (8%) and malaria n=2 (2.66%).

Patients were in the age group of 45-75 years with a mean age of 58±6.45 and 58±6.31 in groups I and II respectively. Among the anthropometric measurements, Waist Circumference (WC) was significantly higher in Group II compared to Group I.

There was a significant difference in serum NGAL between Group I (median 359 ng/mL, IQR 236 ng/mL) and Group II (median 612.41 ng/mL, IQR 540.88 ng/mL; $p<0.05$) reflecting the greater severity of illness in Group II (Figure 2).

With respect to biochemical parameters, Fasting Blood Glucose, Post Prandial Blood Glucose HbA1c, Scr, MDA, NO, hs-CRP and UA were higher and statistically significant in Group II compared to Group I. Antioxidant parameters such as Vitamin C and GPx status was decreased in Group II compared with Group I. The mean and p values of these variables are depicted in table 2.

Among the 75 T2DM patients with AKI (Group II); 48 (64%) had RIFLE-R, 15 (20%) RIFLE-I, 7 (9.33%) RIFLE- F, 3 (4%) RIFLE- L and 2 (2.66%) had RIFLE-E. This data was obtained from patients on the day of admission as baseline values and second estimation was done on the day of discharge (on an average 14 days) in ICU and MICU admissions.

As shown in figure 3, serum NGAL concentration was significantly elevated between Risk (median 343.69 ng/mL, IQR 314.24ng/mL) Injury (median 510.64ng/mL, IQR 415.94 ng/mL), Failure (median 813.83 ng/mL, IQR 311.00 ng/mL), Loss (median 963.00 ng/mL) and End stage (median 1486.73 ng/mL; $p<0.05$) sub-groups within Group II.

Table 3, shows the mean and p values of the data analyzed at the time of admission ('0' day) and at the day of discharge (on an average 14th day). Scr and NO were significantly elevated in all the groups, eGFR was statistically significant between R vs L groups and R vs E groups, UA was significant between R vs L groups. Serum MDA and hs-CRP concentrations were slightly elevated in Loss, Failure and End stage groups respectively compared to Risk and Injury groups.

Table: 4 make clear that Scr was significant and positively related with eGFR and Uric acid. However, Vitamin C was negatively correlated in T2DM without AKI group. In T2DM with AKI group, eGFR, uric acid, NO, hs-CRP and MDA were positively correlated with serum creatinine with an exception of Vitamin C ($r=0.049$; $p=0.563$) and GPx ($r=0.038$; $p= 0.481$). Table: 4 depicts that eGFR was positively correlated with Scr and negative correlation with Vitamin C in T2DM without AKI group. However, in T2DM with AKI group, eGFR was positively related to Scr and shown significant weak negative correlation with UA, NO, and hs-CRP.

Table 1: Method Characteristics.

Sl.No	Parameter	Detection limit	Analytical range	Assay imprecision (%)	Instrumentation/Method
1	Blood Glucose (mg/dL)	20	20-625	1.4-1.7	Vitros 250; OCD United States (Newyork) GOD-POD Method[1]
2	HbA1c (%)	3.8	3.8-18.5	1.16-1.22	BIO RAD D10; USA HPLC Method
3	NGAL (ng/mL)	10		Intra assay: 7.03-8.38 Inter Assay: 9.73-9.77	ELISA, Meril Germany
4	Serum Creatinine (mg/dL)	0.05	0.05-17	1.6-2.6	Vitros 250; OCD United States (Newyork) Modified Jaffes method[1]
5	Malondialdehyde (nmol/mL)	0.8	0.8-15	3.28-4.77	PerkinElmer UV/VIS Spectrophotometer Lambda 35, United Kingdom Modified TBARS method[1]
6	Nitric oxide (µM/mL)	10	10-90	15.01-24.38	PerkinElmer UV/VIS Spectrophotometer Lambda 35, United Kingdom Modified Griess Method[1]
7	hs-CRP (mg/dL)	0.010	0.01-1.5	1.8-5.0	PerkinElmer UV/VIS Spectrophotometer Lambda 35, United Kingdom Turbidimetric Immunoassay[1]
8	Uric acid (mg/dL)	0.50	0.50-17	1.0-1.3	Vitros 250; OCD United States (Newyork) Uricase method[1]
9	Vitamin C (mg/dL)	0.2	0.2-12	0.4-0.8	PerkinElmer UV/VIS Spectrophotometer Lambda 35, United Kingdom 2,4-DNPH method[1]
10	GPx (mU/mL)	0.5			PerkinElmer UV/VIS Spectrophotometer Lambda 35, United Kingdom Glutathione Peroxidase Activity Colorimetric assay; Biovision, USA

Table 2: Comparison of anthropometric and biochemical parameters between T2DM patients with and without AKI.

Variables	Group I Mean ± SD	Group II Mean ± SD	p-value
Age (years)	58±6.45	58±6.31	0.896
Height (cm)	161.56±8.48	160.81±6.51	0.195
Weight (Kg)	70.68±10.16	75.71±8.96	0.517
Waist Circumference (cm)	96.35±5.01	101.40±0.78	0.001*
Hip Circumference (cm)	94.47±5.08	95.11±5.09	0.371
Waist Hip Ratio	1.02±0.04	1.00±0.06	0.138
Body Mass Index (BMI) (Kg/m^2)	27.13±3.53	26.8±3.45	0.213
Fasting Blood Glucose (mg/dL)	195.99±25.61	250.91±32.18	0.001*
Post Prandial blood glucose (mg/dL)	215.09±33.41	296.47±38.12	0.02*
HbA1c (%)	9.06±3.35	11.85±2.08	0.004*
NGAL (ng/ml)	160±13.35	210±25.42	0.007*
∞Serum Creatinine mg/dL)	0.94±0.31	4..45±1.45	0.012*
eGFR ($mL/min/1.73m^2$)	84.67±16.55	50.78±7.05	0.005*
MDA (nmol/mL)	6.48±2.50	8.90±2.41	0.017*
∞NO (µM/mL)	34.48±7.44	54.06±18.59	0.015*
hs-CRP (mg/dL)	1.69±0.57	1.99±0.45	0.012*
Uric acid (mg/dL)	4.92±1.17	6.09±1.02	0.022*
Vitamin C (mg/dL)	0.56±0.15	0.44±0.23	0.031*
∞GPx (mU/mL)	1.91±0.90	0.41±0.12	0.015*

∞ Mann-Whitney U Test

*p<0.05 considered as significant; Group I: T2DM without AKI, Group II: T2DM with AKI

Table 3: Comparison of oxidants and antioxidants within Group II (T2DM with AKI) based on RIFLE criteria.

Parameters	Risk (64%) Mean ± SD	Injury (20%) Mean ± SD	Failure (9.33%) Mean ± SD	Loss (4%) Mean ± SD	End stage (2.66%) Mean ± SD	p value
NGAL (ng/mL)	168±12.12ǂ	175±19.49ǂ	190±23.14ǂ	210±24.15ǂ	280±25.18ǂ	0.003*
	125.02±12.52˜	142.25±15.10˜	160.56±20.14˜	190.96±23.56˜	250.85±22.15˜	
∞ Serum Creatinine (mg/dl)	2.40±0.48ǂ	2.9±0.45ǂ	3.2±0.49ǂ	3.9±0.77ǂ	5.4±1.14ǂ	0.004*
	1.9±0.21˜	2.5±1.25˜	2.8±1.96˜	3.1±1.99˜	4.8±2.53˜	
eGFR ($mL/min/1.73m^2$)	51.77±7.35ǂ	47.73±4.54ǂ	40.28±9.37ǂ	35.33±12.7ǂ	25.45±15.1ǂ	0.02*
	68.14±5.22˜	57.44±6.54˜	55.14±12˜	50.14±14.52˜	42.56±13.36˜	
∞ MDA (nmol/mL)	9.08±2.47ǂ	8.30±2.24ǂ	9.60±2.55ǂ	10.66±1.75ǂ	9.75±2.85ǂ	0.03*
	7.52±3.75˜	6.45±2.89˜	6.80±4.12˜	6.45±3.25˜	7.91±2.42˜	
NO (µM/mL)	58.69±18.80ǂ	54.08±9.72ǂ	64.07±22.60ǂ	56.73±14.16ǂ	66.17±21.15ǂ	0.02*
	49.10±12.52˜	51.21±15.15˜	60.54±11.25˜	53.02±12.47˜	58.74±14.85˜	
hs-CRP (mg/dL)	1.99±0.49ǂ	1.97±0.38ǂ	2.12±0.31ǂ	1.66±0.15ǂ	2.90±0.95ǂ	0.04*
	0.85±0.12˜	1.32±0.96˜	1.91±0.19˜	1.31±1.12˜	2.35±1.25˜	
Uric acid (mg/dL)	7.28±1.04ǂ	5.54±0.86ǂ	5.83±0.94ǂ	6.13±0.66ǂ	5.80±0.14ǂ	0.01*
	7.19±2.03˜	5.48±1.78˜	5.81±2.45˜	6.11±1.48˜	5.80±1.25˜	
Vitamin C (mg/dL)	0.45±0.42ǂ	0.38±0.25ǂ	0.45±0.21ǂ	0.69±0.32ǂ	0.55±0.06ǂ	0.481
	0.56±1.23˜	0.51±0.12˜	0.54±0.99˜	0.92±0.29˜	0.56±1.02˜	
GPx (mU/mL)	0.54±0.12ǂ	0.51±0.22ǂ	0.39±0.21ǂ	0.14±0.09ǂ	0.06±0.03ǂ	0.613
	0.71±1.12˜	0.55±0.85˜	0.42±0.14˜	0.35±0.72˜	0.23±0.11˜	

∞ Mann-Whitney U Test

ǂ on the day of admission ('0'-Day); ˜day of Discharge (on an average 14th day);

* p<0.05 statistical significant

Table 4: Correlation of Scr and eGFR with oxidants, antioxidants and inflammatory markers in T2DM patients with and without AKI Groups respectively

Variables	T2DM without AKI		T2DM with AKI		T2DM without AKI		T2DM with AKI	
	r value	p value	r value	p value	r value	p value	r value	p value
Scr	-	-	-	-	0.353	0.025*	0.541	0.014*
eGFR	0.353	0.025	0.541	0.014	-	-	-	-
Uric acid	0.260	0.015*	0.487	0.001*	0.075	0.371	-0.316	0.001*
NO	-0.130	0.431	0.411	0.002*	-0.099	0.482	-0.424	0.001*
hs-CRP	0.062	0.612	0.241	0.004*	-0.016	0.612	-0.226	0.007*
MDA	0.005	0.965	0.106	0.031*	-0.078	0.553	-0.055	0.517
Vitamin C	-0.215	0.044*	0.049	0.563	-0.189	0.041*	-0.072	0.396
GPx	0.011	0.925	0.038	0.481	0.091	0.09	0.059	0.441

* p<0.05 statistical significant

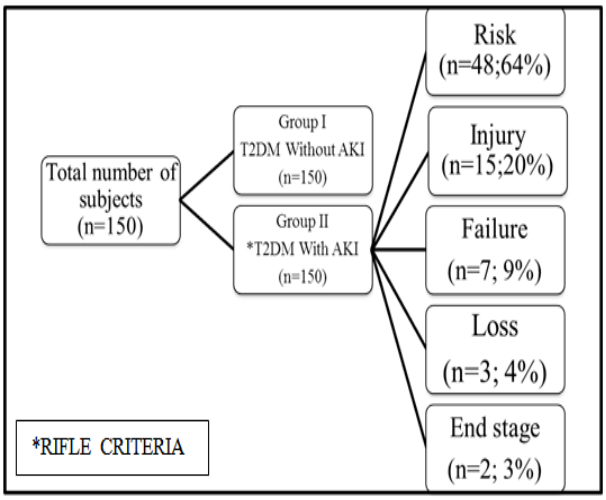

Figure 1: Classification of subjects.

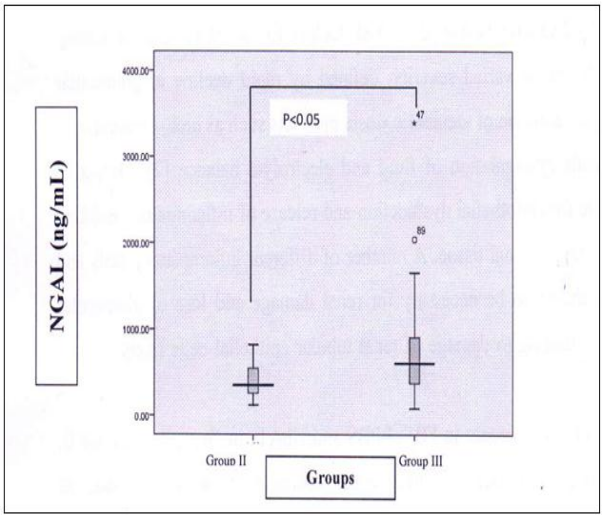

Figure 2: Box and whisker plot of serum NGAL concentration measured between T2DM without AKI (Group I) and T2DM with AKI (Group II) (The vertical box represents the 25th percentile (bottom line), median (middle line), and 75th percentile values. The dots represent values outside the 10th and 90th percentile respectively).

Source: plotted graph using SPSS statistical software program (licensed version 16.0, SPSS, Chicago, IL)

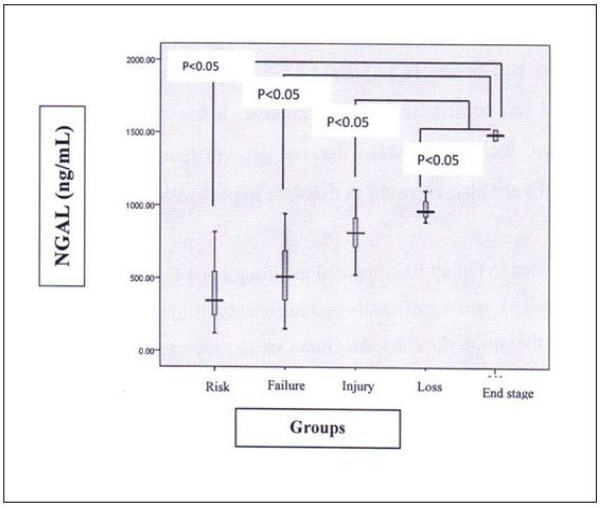

Figure 3: Box and whisker plot of serum NGAL concentration measured in T2DM with AKI (RIFLE classification) (The vertical box represents the 25th percentile (bottom line), median (middle line), and 75th percentile values. The dots represent values outside the 10th and 90th percentile respectively).

Source: plotted graph using SPSS statistical software program (licensed version 16.0, SPSS, Chicago, IL)

DISCUSSION

In our study, on comparison between Group I (T2DM without AKI) and Group II (T2DM with AKI), WC, fasting and post Prandial blood glucose, HbA1c, NGAL Scr, MDA, NO, hs-CRP and UA were significantly increased and concentration of eGFR, Vitamin C and GPx were significantly decreased in Group II. Within Group II (T2DM with AKI) based on RIFLE criteria, Concentration of NGAL, Scr, MDA, NO, hs-CRP, UA were exponentially increased from Risk to End stage groups whereas, eGFR, vitamin C and GPx concentrations were gradually decreased from risk to End stage groups.

Studies conducted by Barnett AH et al., and Mogensen CE et al., have shown that diabetes leads to increased glomerular hyperfiltration and the resultant increased glomerular pressure leads to damage of glomerular cells and development of focal and segmental

glomerulosclerosis.[4,5] Ryu et al. found that UA decrease the expression of E cadherin in epithelial cells resulting in loss of cell-to-cell contact in the renal tubular cells. This loss of cell-to-cell contact results in epithelial cells unable to coordinate efforts to secrete substances needed to increase renal blood flow such as NO. An increased acute phase molecules such as MDA, NO, hs-CRP and UA induced NAPDH dependent oxidative changes may promote apoptosis with varying degree of renal function.[12,13]

In this study, serum NGAL was significantly elevated in Group II compared to Group I and in Group II sub classes, concentration of NGAL was significantly and exponentially elevated in all the groups. Concentration of NGAL can be elevated after activation of neutrophils, suggesting influence of systemic inflammation and infections.[14] Systemic inflammation triggered by conditions such as sepsis or procedures like CPB was strongly associated with AKI development. At the same time, such inflammatory triggers will activate circulating neutrophils to release their granular contents including NGAL. Similar conclusions can be drawn from general ICU population where sepsis is associated with elevated plasma NGAL independent of degree of renal impairment. Under normal conditions, filtered NGAL is almost completely reabsorbed by the proximal tubules via megalin-cubulin receptor- mediated endocytosis, resulting in minimal urinary NGAL levels.[14,15] Our results were consistent with the study done by de Guess et al., who had reported the expression pattern of NGAL prior to the rise in Scr and that its predictive power also is closer to the AKI presentation time and whom had suggested that the time to injury relationship is important and should be obtained for a correct interpretation of its AKI predictive value. NGAL is a protein with a molecular weight of 25 KDa expressed at low concentrations in different tissues and upregulated especially in injured epithelial cells in AKI. NGAL is gaining momentum as a biomarker for early diagnosis. Although a number of markers have been investigated for diagnosing AKI, NGAL appears to be the most promising biomarker.

In this study Scr was significantly elevated in Group II versus Group I. The loss of kidney function that defines AKI is most easily detected by measurement of Scr, which is used to estimate GFR.

Our study has shown MDA concentration was increased in Group II compared to Group I. This was in agreement with results of the studies done by Oberley L.W et al., and Vecchi et al., who reported diabetes is a state of oxidative stress, characterized by the strong prevalence of oxidant factors over antioxidant mechanisms, and the activities of antioxidant enzymes which depend on duration and extent of compensation of diabetes.[16,17] According to Himmelfarb et.al., Malondialdehyde a terminal compound of lipid peroxidation is commonly used as an index of oxidative stress; in biological matrices, MDA exists as both free (fMDA) and bound (bMDA) to -SH and / or -NH_2 groups of proteins, nucleic acids and lipoproteins.[18] Veechi AP et.al., documented that chemically reactive fMDA is an index of recent and potential damage, while bMDA, excreted by the kidney, is a marker of an older injury.[17] Oberley LW et.al., also stated that, under conditions of increased glucose concentration in diabetes, augmented glucose autoxidation may contribute to the enhanced free radical production and facilitate lipid peroxidation.[16]

In this study, concentration of NO was elevated in Group II compared to Group I. Within Group II, NO was highly significant in Risk, Failure, Loss and End stage groups compared to Injury group. Study done by Saulo K et al., observed similar results and reported that increased release of NO into the circulation, may lead to high blood pressure, up-regulation of NO production and also affects of renal blood flow, shear stress and other related mechanical stimuli. These factors may account for the increased production of NO and also increase expression of endothelial Nitric Oxide Synthase (eNOS).[19] Our results are inconsistent with the study done by Brodsky et al., who stated that vascular endothelium undergoes structural and functional changes in early ischemic renal failure and alters NO production and /or decreased bioavailability of NO which may comprise the endothelial dysfunction in acute renal failure.[20]

Our study included hs-CRP as a marker of inflammation which showed elevated concentrations in Group II. Failure and End stage groups also showed elevated hs-CRP concentration compared to Risk, Injury and Loss groups in Group II. Similar findings were also observed by Howaida Attia et.al., indicating that acute stressful condition may lead to increased, concentration of hs-CRP during inflammatory processes.[21] Pegues MA et. al., reported that CRP might play an active role in AKI; worsening the damage resulting in renal ischemia and reperfusion injury (IRI).[22]

We have observed in our study that serum UA concentration was significantly increased in Group II. Within Group II, Risk and Loss groups has shown significant UA increase compared to Injury, Failure and End-stage groups. Our findings are consistent with the studies conducted by Kanbay et al., that elevated UA concentration can be a consequence of impaired renal function and the study documented that patients with higher serum uric acid concentration have associated high systolic blood pressure, increased hs-CRP concentration, decreased eGFR, and low flow-mediated dilatation.[23] An in house study done by Shashidhar KN et.al., observed that elevated UA could induce renin expression from the juxtaglomerular cells and inhibit nitrous oxide system (NOS) expression in the macula densa.[24] They also reported UA is known to impair endothelial function and stimulates the production of cytokines from leukocytes and chemokines from vascular smooth muscle cells.[24]

We could observe in our study that concentration of serum Vitamin C and GPx were decreased in Group II versus Group I and also within the Group II, these antioxidants gradually decreased from Risk group to End-stage groups. Our results are consistent with the study done by Yao-Bin Zhu et.al., where they have stated that Vitamin C as an antioxidant, plays a major role in the acute phase conditions and in a natural water soluble form, has a potential to intervene in the development of kidney disease by modulating redox steps.[25]

Vitamin C is known to influence NO and decrease glutathione concentration. The current results of our study may suggest that the effect of vitamin C is related to the elevations in NO. Yao BZ et.al., and Ceballos P et.al., have explained that generated superoxide is first converted into hydrogen peroxide and then hydrogen peroxide is metabolized into water and oxygen by Catalase and / or GPx. The free radical $O^{\cdot}_2$ at high concentration will react with NO to produce peroxynitrite and there is a possibility that Vitamin C restores NO level by reducing $O^{\cdot}_{2..}$ [25,26]

In our study GPx concentration was decreased in Group II Vs Group I and within Group II also GPx was exponentially decreased from Risk to End Stage group. Our results are on par with the study done by EL-Far MA et.al and Zagrodzki et al., that GPx is an important antioxidant, the plasma form of which is synthesized mainly in the kidney.[27,28] Utilization of glutathione dependent antioxidant process through generation of ROS will usually accompany AKI. Decreased GPx activity in AKI may be because of an increased rate of lipid peroxidation, decrease in functional renal mass, interference of uremic toxins, plasma GPx consumption and inactivation of GPx by biochemical modification and/ or an abnormality in the hexose mono phosphate pathway.[27]

In our study there was a significant correlation between Scr, eGFR with inflammatory / oxidant markers. However, concentration of Vitamin C and GPx were not correlated with Scr and eGFR in T2DM with AKI group.

This is a prospective case control study conducted in rural tertiary care referral hospital. Most of the people in this area are agriculturists who depend on scanty rainfall for earning a livelihood. Moreover, majority of this population are illiterates and their occupation exposes them to pesticides and fertilizers which can affect the formation of reactive oxygen species (ROS) or reactive nitrogen species (RNS), and insulin resistance in the human body, can cause oxidative damage to biological macromolecules, especially in the plasma membrane which may contribute to the development of diabetes, cancer, cardiovascular diseases and other oxidative stress mediated dysfunctions.

This has prompted us to find if there is any association between small antioxidants/ inflammatory molecules and tubulointerstitial renal damage in T2DM. Our hospital statistics show that majority of the patients addressed by the physicians either have diabetes or diabetes related issues and / or associated comorbidties.

Strengths of our study include study subjects who also had other associated complications such as hypertension, Cardio pulmonary Bypass, Snake bite, Gastroenteritis, Sepsis and Malaria in addition to diabetes with altered renal function. Estimated upregulated protein biomarker NGAL for positive prediction of developing AKI might be an interesting option for clinicians to decide in future as a routine parameter in the management of diabetes mellitus. Vitamin C play a defensive role in the acute phase, administration of chronic vitamin C has beneficial effects on glucose and lipid metabolism in T2DM patients.

Limitations

Small sample size, where sub set classification based on RIFLE criteria was relatively small and did not allow for sufficient data analyses (IQR for L and E groups in T2DM with AKI). We believe detection of both free and total MDA concentrations helps to explain the different MDA patterns in patients with renal insufficiency. However, values observed here were estimated with strict consideration of inclusion and exclusion criteria, pre-examination, examination and post-examination errors.

CONCLUSION

An imbalance between oxidants and antioxidants was seen in diabetes and AKI. Oxidative stress is one of the important factors contributing to AKI by increasing production of oxidants, particularly ROS and RNS or a deficiency of glutathione peroxidase. Ascorbic acid a water soluble antioxidant helps in reducing oxidized tocopherol in membrane and may inhibit the formation of nitrosamines. Vitamin C may help in detection of risk factors in the early stage of disease which may help in decreasing the morbidity rate.

Thus, with the observations of this study we would suggest that along with clinical examination, estimation of base line oxidants (MDA) and antioxidants (Vitamin C) as an add on parameter may serve as supportive biochemical markers for early management of diabetes, AKI and associated comorbidities. Early management of the etiological factors can prevent the risk of AKI in T2DM, as acute kidney injury has good prognosis with early intervention.

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