COMPARISON OF GLUCOSE STATUS AND OXIDATIVE STRESS AMONG DIFFERENT STAGES OF CHRONIC KIDNEY DISEASE

Manpreet Saini [1*], Vijay Kumar[2], Arunima Deep[3]

Abstract-

Introduction-One of the most crucial trigger factors involved in developing chronic kidney disease (CKD) is diabetes, indicated by hyperglycemia or glycated haemoglobin (HbA1c). Hyperglycemia causes increased generation of reactive oxygen species (ROS). CKD is considered a similar condition that leads to an excess generation of free radicals. The resultant lipid peroxidation releases malondialdehyde (MDA) as its product, which indicates the degree of oxidative stress (OS). This study aimed to evaluate and compare HbA1c and oxidative stress (MDA)) among different stages of CKD, which can benefit to end or slow the advancement of the disease.

Material and method-This study was conducted in Lucknow, India from November 2020 to April 2021. A total of 88 CKD patients were taken after applying exclusion criteria. HbA1c, MDA and eGFR was estimated and patients were classified into different stages of CKD. Association of CKD stages with age and gender was seen by Chi-square test. ANOVA and Post-hoc Tukey test were used to find out the difference in mean of HbA1c and MDA among CKD patients and the control group.

Result-In this study, more than half of the CKD patients belonged to age of 40 to 59 years with CKD stage 5 being commonest. The association of CKD stage with age was seen to be non-significant, but with gender it was statistically significant. In our study mean difference of HbA1c was found to be significant among CKD stages and control. Level of MDA was found to be more in CKD patients and it increased progressively with CKD stages.

Conclusion- Our study proves the association of hyperglycemia and oxidative stress with advancement of CKD. So, routine evaluation of these parameters and taking appropriate steps to reduce oxidative stress and glucose level may be of value in improving the survival of CKD Patients.

Keywords:- Chronic kidney disease, diabetes, oxidative stress, HbA1c, CKD Stage

[1*]Assistant Professor,Department of Biochemistry, Mayo Institute of Medical Sciences, Barabanki, Uttar Pradesh, India;
[2]Associate Professor,Department of Community Medicine, PhuloJhano Medical College, Dumka, Jharkhand, India;
[3]Assistant Professor, Department of Physiology, Gautam Buddha Chikitsa Mahavidyalaya, Dehradun, Uttarakhand, India;

***Corresponding Author**: Manpreet Saini,
*Assistant Professor, Department of Biochemistry, Mayo Institute of Medical Sciences, Barabanki, Uttar Pradesh, India; radhasoami46@yahoo.com

Introduction-

CKD or chronic kidney disease is the sure loss of many actively functional nephrons in the kidneys. Over time this causes improper functioning of the kidneys and paves the path to decline of glomerular filtration rate (GFR). GFR offers an idea of the numeral of total efficient nephrons. So GFR can be indicator of functional renal mass.(1)

In India, inclination towards CKD has taken the form of a silent epidemic. A systematic review and meta-analysis revealed that CKD prevalence in general population in India is 16%.(2) The overall prevalence of CKD in the SEEK-India cohort was also found to be 17.2%.(3)

One of the most crucial trigger factors involved in CKD is diabetes mellitus (DM). Approximately, 40% of patients with diabetes develop CKD.(4) In clinical setting, HbA1c is presently known as finest index of hyperglycemia in diabetic patients.(5) Hyperglycemia is the principle molecular pattern that is alleged as accountable for pathogenesis of diabetes driven CKD.(6) It causes changes in renal circulation leading to diabetic kidney disease (DKD).(7) DKD has a complicated pathophysiology that has not been wholly explained. This is yet again induced by non-enzymatic glycation involving proteins. This helps to generate advanced levels of end products of glycation. It further clues to genesis of PKC

(protein kinase C) & increases index of polyol pathway. This is the main reason for occurrence of oxidative stress (OS).[8] This increased glucose leading to OS is one of the foremost cause that leads to genesis of DKD.[9]

Most patients suffering from CKD face adverse conditions as their body is exposed to more significant oxidative stress due to decrease of antioxidants in body.[10] The free radicals present in body are liable for OS as they prime the oxidation of polyunsaturated fatty acids of bio-membranes, leading to formation of Malondialdehyde or MDA, altering the structure of many biomolecules.[11] As the end product of this lipid peroxidation is MDA, it acts as a marker for assessing lipid peroxidation. OS along with inflammation is present in several ailing conditions like diabetes, cancer, arthritis, kidney problems etc.[12] CKD once established, inclines to evolve to end stage renal disease (ESRD). The struggles to stop or even slow the advancement of CKD have been unsuccessful. Moreover, there may be a link between blood glucose status and oxidative stress with progression of CKD. So in our study, we have attempted to evaluate and compare HbA1c and oxidative stress among different stages of CKD, which can benefit to end or slow the advancement of the disease.

Material and Method-

This cross-sectional, observational study was hospital based done in Lucknow, India from November 2020 to April 2021. This study was approved by the Ethics Committee of Malwanchal University, India (MU/Research/EC/Ph.D/ 2020/04). Participants were enrolled from 2 major kidney hospitals of Lucknow. A total of 88 CKD patients could be taken in the current study after applying exclusion criteria. People not willing to participate, having age less than 18 years, sepsis and critically ill patients or having pregnancy were excluded from the study. Healthy controls were taken from the same hospitals and nearby area for comparison. All the participants were informed and consent was taken before conduction of the study.

Blood sample collected through venipuncture under aseptic precautions from patients as well as control group and were analysed for HbA1c and MDA. eGFR was estimated by using online GFR calculator grounded on CKD-EPI equation. The patients were classified into different stages of CKD based on novel guidelines of National Kidney Foundation/Kidney Dialysis Outcomes Quality Initiative (KDOQI) according to eGFR as follows:

Stage 0 =>90 ml/min/1.73m^2 (GFR with CKD risk factors)
Stage 1 =>90 ml/min/1.73m^2 (GFR with established kidney damage)
Stage 2 = 60-89 ml/min/1.73m^2
Stage 3 =30-59 ml/min/1.73m^2
Stage 4 =15-29 ml/min/1.73m^2
Stage 5 = <15 ml/min/1.73m^2

Statistical analysis: Analysis was done by using SPSS software version 15. Association of age and gender with CKD stages was seen by Chi-square test. ANOVA and Post-hoc Tukey test were used to find out the difference in mean level of HbA1c and MDA among different stages of CKD patients and the control group. For these tests, p-value less than 0.05 was considered statistically significant.

Result-

As depicted in table 1, more than half of the CKD patients (57.95%) were in stage 5. Stage 4 comprised of 28 (31.81%) patients and stage 3 was having 9 (10.22%) patients only. Among total 88 CKD patients, 13 were in age category of 20-39 years, 50 were in 40-59 years and remaining 25 were ≥60 years of age.

Distribution of CKD stage 3, 4 and 5 among patients of 20-39 years age group was 15.38%, 38.46% and 46.15% respectively. Distribution among 40-59 years and ≥60 years age group was almost equal (10.00% versus 8.00%, 30.00% versus 32.00% and 60.00% versus 60.00% respectively for stage 3, 4 and 5). Association of CKD stages and age was found to be non-significant.

Table 1: Distribution of stages of CKD according to age

		Age				
CKD stage	**Total n (%)**	**20-39yrs**	**40-59yrs**	**≥60yrs**	**χ2**	**p-value**
3	9 (10.22)	2 (15.38)	5 (10.00)	2 (8.00)	1.061	0.900
4	28 (31.81)	5 (38.46)	15 (30.00)	8 (32.00)		
5	51 (57.95)	6 (46.15)	30 (60.00)	15 (60.00)		
Total	**88 (100)**	13 (100)	50 (100)	25 (100)		

*p-value by Chi-square test

Table 2 shows that, among total patients, more than 2/3rd i.e. 60 (68.18%) were males and 28 (31.81%) were females. Proportion of advanced stage of CKD (stage 5) was more among females (75%) than males (50%). Gender of the patients was seen to be associated with CKD stages significantly ($p<0.05$).

Table 2: Distribution of stages of CKD according to gender

CKD stage	Total n(%)	Male n(%)	Female n(%)	$\chi 2$	p-value
3	9 (10.22)	9 (15.0)	0 (0.00)	6.859	**0.032**
4	28 (31.81)	21 (35.00)	7 (25.00)		
5	51 (57.95)	30 (50.00)	21 (75.00)		
Total	**88 (100)**	**60 (100)**	**28 (100)**		

*p-value by Chi-square test

Table 3 and table 4 shows the results of ANOVA and Post-hoc Tukey test. These tests were applied to see the difference in mean value of HbA1c and MDA among different stages of CKD & control group.

ANOVA test found a significant difference in the mean values of HbA1c among CKD stages and control group. CKD stage3, 4, 5 & control group had mean HbA1c value as 5.90±2.40%, 6.94±2.85%, 6.96±2.60% and 4.65±0.49% respectively. Post-hoc Tukey test depicted no significant difference in mean HbA1c level among CKD stage3, 4 & 5. However, mean HbA1c value of control differed significantly from all the stages of CKD.

As far as MDA is concerned, its mean level in stage3, 4 and 5 of CKD and in the control group was 4.05±1.26 nmol/ml, 5.59±2.18 nmol/ml, 8.62±3.00 nmol/ml, and 3.32±0.83 nmol/ml respectively. ANOVA test found a significant difference between these mean values also. On Post-hoc Tukey test difference in mean MDA level between control & stage3 was observed to be non-significant. However, it was significant between CKD stage3 & 4, CKD stage3 & 5, CKD stage4 & 5 and control & CKD stage4 or 5.

Table 3: Comparison of HbA1c and MDA among stage 3, 4 and 5 CKD patients**:**

Groups	n		HbA1c (%)	MDA (nmol/ml)
Stage3	9	Range	4.2-13.0	2.0-8.7
		Mean±SD	5.90±2.40	4.05±1.26
Stage4	28	Range	4.3-13.4	1.7-10.0
		Mean±SD	6.94±2.85	5.59±2.18
Stage5	51	Range	4.3-14.0	3.9-15.8
		Mean±SD	6.96±2.60	8.62±3.00
Control group	**88**	Range	3.7-5.6	2.2-7.1
		Mean±SD	4.65±0.49	3.32±0.83
ANOVA	F		20.297	81.311
	p		**0.000**	**0.000**

*p-value by ANOVA

Table 4: Post-hoc Tukey test for HbA1c and MDA among Stage 3, 4 and 5 CKD patients and control group

Groups Comparison		HbA1c (%)	MDA (nmol/ml)
Difference between	Stage3 vs. Stage4	0.201	**0.015**
	Stage3 vs. Stage5	0.169	**0.000**
	Stage3 vs. Control	**0.048**	0.439
	Stage4 vs. Stage 5	0.987	**0.000**
	Stage4 vs. Control	**0.000**	**0.000**
	Stage5 vs. Control	**0.000**	**0.000**

*p-value by Post-hoc Tukey test

Discussion-

Our study found that more than half (57.95%) of the patients were in stage5 of CKD. CKD stage 4 & 3 comprised 31.81% and 10.22% respectively.

Choudhary N[13] also found stage 5 to be commonest (70.4%)) followed by stage 4 (25.2%) and stage 3 (4.3%). Indian CKD registry also stated that advanced stage i.e. stage 5 constituted the maximum number of patients at presentation followed by other lower stages in descending order of occurrence. It followed same pattern all over the country, excluding the North Zone where stages 1-3 were more common.[14] This is in contrasts with the study by George C et al. where he observed stage3 CKD to be more common (79.2%) than stage4 (15.6%) & stage5 (5.2 %).[15]

Present study found that proportion of stage3, 4 and 5 in the age category 20-39 years was 15.38%, 38.46% and 46.15% respectively. As the age advances, the proportion of severe CKD increased but this association was not found to be significant. Indian CKD registry also documented that as the stage of disease progresses from stage 1-4, the mean age of CKD patients increases however stage 5 of the disease comprised of younger patients than CKD stage III & CKD stage IV.[14]

In present study, females were found to be associated with advanced stage of CKD (stage 5) more (75%) than males (50%). This association of gender with CKD stage was seen to be statistically significant ($p<0.05$). This is in contrast to findings by Zhu X et al. where no such association of stages of CKD and gender was seen. They found that males and females to be almost equally distributed in all stages of CKD (24.4% & 23.8% in stage 3, 20.4% & 21.4% in stage 4 and 55.1% & 54.7 % in stage 5).[16]

The present study depicted that mean HbA1c of CKD (all stages) is significantly higher than the control group. This may be due to stress caused by CKD itself. However, difference in mean value of HbA1c among CKD stage3, 4 and 5 was found to be non-significant.

Present study observed that mean MDA level in stage 4 (5.59±2.18 nmol/ml) and 5 (8.62±3.00 nmol/ml) CKD patients was significantly higher compared to control group (3.32±0.83 nmol/ml). Level of MDA was also found to be significantly increased as the disease progressed. These findings are in accord with the research by Sridhar AV et al.[17] and Kuchta A et al.[18] as they also documented mean MDA to be significant & high in CKD patients than control. It was also observed in their study that the levels of these markers increased progressively with increased progression of renal disease. Similar results are reported in earlier studies.[19,20] Another study by Zhu X et al. also found significant difference in MDA level between control group and all stages of CKD but there was non-significant difference among CKD stage 3 & stage 4 or among CKD stage 4 & 5. However significant difference was reported between stage 3 and stage 5.[16] All these research findings support the notion that oxidative stress increases as stages of CKD progress.[21,22]

Conclusion-

Our study found that gender is significantly associated with CKD stage, females being affected more with advanced stage of CKD than males. CKD is a condition in which HbA1c and oxidative stress level denoted by MDA are more than the control group and MDA increases progressively as the disease advances. It could further lead to the progression of renal dysfunction and the complications. So, routine evaluation of these parameters and keeping a dietary check like intake of antioxidant rich diet & restriction of lipids & glucose can help in prevention of progression of CKD resulting into better outcome of the disease.

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