DOI: 10.5281/zenodo.10570258

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THE RELATIONSHIP BETWEEN eNOS & CCR2 GENE POLYMORPHISM IN CHRONIC KIDNEY DISEASE PATIENTS

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ABSTRACT:

Background/Aims: A significant public health concern, chronic kidney disease is frequently brought on by hypertension, diabetes, nephrotoxic drugs and glomerulonephritis. It is possible that some patients' CKD develops earlier than expected even with appropriate treatment, suggesting the possibility of genetic factors contributing to the disease's development.

Materials and Methods: The present study was conducted in the OPD and male and female medicine wards of Dhiraj Hospital, Pipariya, Vadodara, India. The Study duration was 6 months. Based on careful clinical assessment and judgement of treating physician patients having chronic kidney disease with age >18years were selected on the basis of KDIGO Classification of CKD.

Results: During the study period, out of 146 patients selected, 62 met the criteria for the CKD in which CCR-2 and eNOS gene polymorphism were tested and compared.

Conclusions: The investigation of the relationship between CCR-2 and eNOS and the aetiology of chronic kidney disease (CKD), as well as the age at which CKD onset and duration were compared, is a novel discovery in this study. The results of this study show a substantial relationship between the eNOS TT genotype and the CCR-2 GA genotype and stages of renal disease, as well as CKD and declining renal function. However, in the Indian population, CCR-2 and eNOS expression are unaffected by the age at which a disease first manifests, the length of the illness, or the etiology of CKD.

Keywords: CKD, CCR-2, eNOS

1. INTRODUCTION:

Irrespective of the cause, kidney damage or an estimated glomerular filtration rate (eGFR) less than 60 ml/min/1.73 m2 that lasts for three months or more is referred to as chronic kidney disease (CKD)[1]. As chronic kidney disease (CKD) worsens, kidney function is lost, necessitating renal replacement therapy (transplantation or dialysis). Early diagnosis and disease screening are crucial for controlling the CKD epidemic due to the poor prognosis of the condition.

Genetic risk factors are also recommended to contribute to the risk of chronic kidney disease (CKD) due to the variations in the prevalence and risk factors linked with CKD. Sickle cell

trait and two APOL1 risk alleles, for instance, which are prevalent in individuals with African heritage but not European ancestry, may double the risk of chronic kidney disease (CKD)[2].
Recent research indicates a correlation between Diabetic Nephropathy & Nitric Oxide (NO) hence raising the possibility that NO may contribute to the advancement of kidney disorders. NOS is a gaseous lipophilic molecule with a short half-life that is produced in nearly all tissues. It is encoded by three different genes, namely neuronal (nNOS), inducible (iNOS), and endothelial (eNOS).
Furthermore, because NO interferes at several physiologically crucial stages of nephron function, it performs a variety of physiological roles in the kidney, including the regulation of renal and glomerular hemodynamics. NO causes the afferent and efferent arterioles to dilate. It also affects the renal salt handling along different tubule segments, from the thick ascending limb to the distal tubule and the collecting duct, and may raise the glomerular filtration rate (GFR).
The expression of the chemokine receptor-2 (CCR-2) is one of the additional significant markers. MCP1 belongs to the CC family of chemokines, and its chemokine receptor, CCR2, is mostly expressed on monocytes[3]. With a polymorphism known as CCR2-V64I, which converts valine 64 of CCR2 to isoleucine, it has 374 amino acids. Renal inflammation is initiated and amplified by leukocyte chemoattraction mediated by the CCR2 chemokine receptor[4]. Recent research has revealed that chemokines play a role in the pathophysiology of the disease and are therapeutically important in chronic renal failure. Induced by inflammation, CCL2 and its receptor CCR2 are both mechanistically linked to the pathogenesis of numerous chronic diseases, such as rheumatoid arthritis, neurodegenerative diseases, inflammatory bowel disease, obesity, diabetes, cardiovascular disease, and atherosclerosis. Additionally, it has been demonstrated that pharmacological inhibition of CCL2 lowers chronic kidney damage in lupus nephritis[5], enhance renal function in diabetic patients with albuminuria and to enhance podocyte function in diabetic nephropathy. Given that in a number of chronic injury models, suppression of the renin-angiotensin system lowers CCL2-mediated inflammation.
The current study examined the relationship between CCR-2 and eNOS gene polymorphism in CKD patients and examined the variations in genotype and allele frequencies in CKD.

2. METHODOLOGY:

TYPE OF STUDY: Case Control Study.
PLACE OF STUDY: Department of General Medicine, Dhiraj Hospital, Vadodara
DURATION OF STUDY: 6 months.
STUDY SUBJECT: All OPD/IPD patients in SBKS MI&RC diagnosed with CKD (CASES) and patients without CKD (Control) group.
SAMPLE SIZE: 146 (73 in each group)

Following the collection of samples, the samples were refrigerated at -20 degrees before the DNA material was extracted and put through the Polymerase Chain Reaction (PCR).

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Following amplification, restriction digestion was applied to the PCR products using a restriction enzyme.

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A genotype assessment was conducted.

3. OBJECTIVES:

a) Primary Objectives:

- To study the relationship between eNOS genetic polymorphism and CCR2 in patients with and without CKD.
- To examine the variations in CKD allele frequencies

b) Secondary Objectives:

• To look into the relationship between gene polymorphism and CKD duration.

• To evaluate the frequency of various genes and alleles in CKD patients with various aetiology.

4. INCLUSION CRITERIA:

a) Study Group: All OPD/IPD patients of CKD in Dhiraj Hospital, Vadodara.

b) Control Group: All OPD/IPD patients or healthy individuals in Dhiraj Hospital, Vadodara with normal Renal Function were taken as controls.

5. EXCLUSION CRITERIA:

1. Acute Kidney Injury (AKI)
2. Cardiovascular Disease (CVD)
3. Sepsis
4. Critically ill Patients

6. RESULTS:

Table 1: Group wise Distribution of study population

SN		Group	No.	%
1	Cases (CKD Patients)	Group 1	62	50.0
2	Controls (Non CKD Patients)	Group 2	62	50.0
	Total		**124**	**100.0**

Table 2: Comparison of Demographic parameters between Cases and Controls

SN	Parameter	Total	Cases		Controls	
			No.	**%**	**No.**	**%**
1	**Age**					
	≤20 years	4	2	3.2	2	3.2
	21-30 years	17	10	16.1	7	11.3
	31-40 years	22	11	17.7	11	17.7
	41-50 years	17	9	14.5	8	12.9
	51-60 years	29	14	22.6	15	24.2
	61-70 years	24	10	16.1	14	22.6
	≥71 years	11	6	9.7	5	8.1
χ^2=1.380; p=0.967						
			Mean	SD	Mean	SD
			48.95	16.55	49.90	15.15
't'=-0.334; p=0.739						
2	**Gender**					
	Female	50	23	37.1	27	43.5
	Male	74	39	62.9	35	56.5
χ^2=0.536; p=0.464						

On comparing statistically, both the groups were comparable for age and gender.

Table 3: Comparison of Comorbidities between Cases and Controls

SN	Comorbidities	Total	Cases		Controls		Ch-sq. Test
			No.	**%**	**No.**	**%**	

1	Diabetes	67	45	72.6	22	35.5	χ^2=17.176; p<0.001
2	Hypertension	81	57	91.9	24	38.7	χ^2=38.770; p<0.001

In CKD, hypertension (91.9%) and diabetes (72.6%) accounted for the majority of cases, but in non-CKD, very few people had comorbidities. A statistical comparison revealed a noteworthy distinction between the groups with regard to hypertension and diabetes.

Table 4: Comparison of Renal Function Parameters between Cases and Controls

SN	Parameters	Cases		Controls		Student's-t test	
		Mean	**SD**	**Mean**	**SD**	**'t'**	**'p'**
1	Urea (mg/dL)	126.31	38.71	27.09	5.18	20.004	<0.001
2	Creatinine (mg/dL)	8.44	3.24	0.94	0.22	18.196	<0.001
3	eGFR (ml/min/1.73m^2)	16.613	7.30	92.53	23.27	-24.507	<0.001
4	Spot Urine (mg/g)	231.77	156.25	15.95	6.59	10.867	<0.001

In comparison to Controls (27.09±5.18 mg/dl, 0.94±0.22 mg/dl, 92.53±23.27 ml/mn, 15.95±6.59 mg/g), Renal function parameters (Urea, Creatinine, eGFR, Spot Urine) were substantially higher in Cases (126.31±38.71 mg/dl, 8.44±3.24 mg/dl, 16.61±7.30 ml/mn, 231.77±156.25 mg/g).

Table 5: Comparison of Laboratory Parameters between Cases and Controls

SN	Parameters	Cases		Controls		Student's-t test	
		Mean	**SD**	**Mean**	**SD**	**'t'**	**'p'**
1	Calcium (mg/dL)	8.54	0.73	9.08	0.74	-4.052	<0.001
2	Albumin (mg/dL)	3.39	2.94	3.77	0.43	-0.997	0.321
3	Hemoglobin (g/dL)	9.45	1.76	13.23	1.93	-11.398	<0.001

In comparison to Controls (9.08±0.74 mg/dl, 13.23±1.93 mg/g), Cases' serum calcium and haemoglobin levels were considerably lower (8.54±0.73 mg/dl and 9.45±1.76 mg/g, respectively). Serum Albumin levels were equal between the groups, but lower in Cases (3.39±2.94 mg/dL) than in Controls (3.77±0.43 mg/dL).

Table 6.1: Comparison of CCR2 Genotype expression between Cases and Controls

SN	Genotype Expression	Total	Cases		Controls	
			No.	**%**	**No.**	**%**
1	GA	44	30	48.4	14	22.6
2	GG	80	32	51.6	48	77.4
3	AA	0	0	0.0	0	0.0
χ^2=9.018; p= 0.003						

The majority of patients in both groups expressed GG, however the percentage of patients in the Controls group expressed GG at a lower rate than that of the Cases group (51.6% vs. 77.4%). A statistically significant variation in CCR-2 expression was noted between the groups.

Table 6.2: Comparison of Allele expression for CCR2 between Cases and Controls

SN		Groups

	Allele Expression	Total		Cases		Controls		OR (CI)
		No.	%	No.	%	No.	%	
1	G	204	82.26	94	72.58	110	88.71	0.40 (0.20-0.80)
2	A	44	17.74	30	27.42	14	11.29	
χ^2=7.073; p=0.007								

In general, the allele G was expressed more frequently (82.26%). On the other hand, a greater percentage of cases (27.42% vs. 11.29%) possessed the "A" allele than controls. A statistical comparison revealed a substantial correlation between the A allele and CKS. G's phrase had an OR of 0.40 (CI:0.20-0.080).

Table 7.1: Comparison of eNOS Genotype expression between Cases and Controls

SN	Genotype Expression	Total	Cases		Controls	
			No.	%	No.	%
1	GG	42	3	4.8	39	62.9
2	GT	37	23	37.1	14	22.6
3	TT	45	36	58.1	9	14.5
χ^2=49.246; p<0.001						

In the Cases group, the majority of patients (58.1%) expressed TT, whereas the majority of the Control group (62.9%) expressed GG. Based on statistical analysis, a noteworthy distinction was noted in the expression of eNOS between the groups.

Table 7.2: Comparison of Allele expression for eNOS between Cases and Controls

SN	Allele Expression	Groups							
		Total		Cases		Controls		OR (CI)	
		No.	%	No.	%	No.	%		
1	G	121	48.79	29	23.39	92	74.19	0.11 (0.06-0.19)	
2	T	127	51.21	95	76.61	32	25.81		
χ^2=64.053; p<0.001									

Overall, 51.21% of patients had the "T" allele expressed. However, compared to controls, a greater percentage of cases (76.61% vs. 25.81%) displayed the "T" allele. A noteworthy correlation was discovered between the T allele and chronic kidney disease. The G allele's expression OR was 0.11 (0.06-0.19).

Table 8: Association of CCR2 Genotype expression with Renal Function Parameters

SN	Parameters	GA		GG		Student's t-test	
		Mean	SD	Mean	SD	't'	'p'
1	Urea (mg/dL)	104.82	69.04	64.28	45.83	3.859	<0.001
2	Creatinine (mg/dL)	6.96	5.43	3.69	3.46	4.043	<0.001
3	eGFR (ml/min/1.73m^2)	45.00	45.66	58.80	39.53	-1.708	0.090
4	Spot Urine (mg/g)	212.18	191.86	84.84	116.13	4.559	<0.001

GA expression of CCR2 was substantially correlated with elevated renal function measures (eGFR excluded) (Urea, Creatinine, Spot Urine).

Table 9: Association of CCR2 Genotype expression with Laboratory parameters

SN	Parameters	GA		GG		Student's t-test	
		Mean	**SD**	**Mean**	**SD**	**'t'**	**'p'**
1	Serum Calcium (mg/dL)	8.66	0.69	8.87	0.82	-1.418	0.159
2	Serum Albumin (mg/dL)	3.29	0.58	3.71	2.48	-1.034	0.303
3	Haemoglobin (gm/dL)	10.69	2.47	11.63	2.68	-1.861	0.065

Comparable levels of CCR2 expression were seen in albumin, hemoglobin and calcium.

Table 10: Association of eNOS Genotype expression with Renal Function parameters

SN	Parameters	GG		TG		TT		ANOVA	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**'F'**	**'p'**
1	Urea (mg/dL)	31.62	18.86	75.08	45.37	120.11	56.25	45.201	<0.001
2	Creatinine (mg/dL)	1.41	1.66	4.64	3.84	7.79	4.42	35.646	<0.001
3	eGFR (ml/mn)	84.28	28.86	50.24	39.66	30.40	36.97	27.883	<0.001
4	Spot Urine (mg/g)	27.00	50.71	103.49	125.85	231.02	174.12	27.883	<0.001

The TT expression of eNOS was substantially correlated with elevated urea, creatinine, and spot urine.

Table 11: Association of eNOS Genotype expression with laboratory parameters

SN	Parameters	GG		TG		TT		ANOVA	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**'F'**	**'p'**
1	Serum Calcium (mg/dL)	9.13	0.85	8.62	0.67	8.65	0.71	5.969	0.003
2	Serum Albumin (mg/dL)	3.70	0.48	3.90	3.77	3.20	0.50	1.231	0.296
3	Haemoglobin (g/dL)	12.88	2.35	11.24	2.50	10.00	2.27	16.222	<0.001

When eNOS expression was compared statistically, albumin was similar, but hemoglobin and calcium were considerably higher in GG expression (9.13±0.85 mg/dL & 12.88g/dL), followed by TT (8.65±0.71 mg/dL & 10.00±2.27 g/dL) & TG (8.62±0.67 mg/dL & 11.24±2.50 g/dL).

Table 12: Comparison of Albuminuria level between Cases and Controls

SN	Albuminuria Level	Cases		Controls		Chi-sq Test
		No	**%**	**N**	**%**	
1	A1	0	0.0	62	100.0	**χ^2=124.00; p<0.001**
2	A2	38	61.3	0	0.0	

3	A3	24	38.7	0	0.0	

Albumin level A2 (61.3%) was the majority in Group 1, followed by A3 (38.7%). A1 (100.0%) was present in every patient in Group. In terms of albuminuria, there was a statistically significant difference between the groups.

Table 13: Association of CCR2 Genotype expression with Causes of CKD

SN	Causes	GA		GG		Chi-sq Test
		No	**%**	**N**	**%**	
1	Diabetic Nephropathy	21	70.0	19	59.4	**χ^2=1.400; p=0.706**
2	ADPKD(Autosomal Dominant Polycystic Kidney disease)	1	3.3	1	3.1	
3	Glomerulonephritis	2	6.7	5	15.6	
4	Hypertensive Nephrosclerosis	6	20.0	7	21.9	

The causes of CKD were statistically similar across CCR-2 expression levels.

Table 14: Association of CCR2 expression with Age of onset of CKD in Cases

SN	Age of Onset of CKD	GA		GG		Chi-square test
		No.	**%**	**No.**	**%**	
1	≤40 years	11	36.7	15	46.9	χ^2=0.663; p=0.416
2	>40 years	19	63.3	17	53.1	

The onset age of CKD was statistically similar among those who expressed CCR-2

Table 15: Association of CCR2 expression with Duration of CKD in Cases

SN	Duration of Disease	GA		GG		Chi-square test
		No	**%**	**No**	**%**	
1	<5 year	10	33.3	12	37.5	χ^2=0.117; p=0.732
2	≥5 years	20	66.7	20	62.5	

The length of CKD was statistically similar across CCR-2 expression.

Table 16: Association of CCR2 expression with Staging of CKD in Cases

SN	Stage	GA		GG		Chi-square test
		No	**%**	**No**	**%**	
1	Non ESRD (Grade 1-Grade 4)	9	30.0	25	78.1	χ^2=14.480; p<0.001
2	(ESRD) End Stage Renal Disease (Grade 5)	21	70.0	7	21.9	

A statistically significant correlation between end-stage renal disease and GA expression of CCR-2 was found.

Table 17: Association of eNOS expression with Causes of CKD in Cases

SN	Causes	GG		TG		TT	
		No	**%**	**No**	**%**	**No**	**%**
1	Diabetic Nephropathy	1	33.3	16	69.6	23	63.9
2	Glomerulonephritis	1	33.3	4	17.4	2	5.6
3	ADPKD	0	0.0	1	4.3	1	2.8
4	Hypertensive Nephrosclerosis	1	33.3	2	8.7	10	27.8
χ^2=6.519; p=0.368							

The causes of CKD were statistically similar among eNOS expression.

Table 18: Association of eNOS expression with Age of onset of CKD in Cases

SN	Age of onset of CKD	GG		GT		TT		Chi-sq Test
		No	**%**	**No**	**%**	**No**	**%**	
1	≤40 years	2	66.7	10	43.5	14	38.9	χ^2=0.913; p=0.633
2	>40 years	1	33.3	13	56.5	22	61.1	

Age of CKD Onset was statistically similar across eNOS expression.

Table 19: Association of eNOS expression with Duration of CKD in Cases

SN	Duration	GG		GT		TT		Chi-sq Test
		No	**%**	**No**	**%**	**No**	**%**	
1	<5 years	1	33.3	9	39.1	12	33.3	χ^2=0.212; p=0.899
2	≥5 years	2	66.7	14	60.9	24	66.7	

The length of CKD was statistically similar throughout eNOS expression.

Table 20: Association of eNOS expression with Staging of CKD in Cases

SN	Stage	GG		GT		TT		Chi-sq Test
		No	**%**	**No**	**%**	**No**	**%**	
1	Non ESRD (G 1-G 4)	3	100.0	18	78.3	13	36.1	χ^2=12.664; p=0.002
2	ESRD (G5)	0	0.0	5	21.7	23	63.9	

Based on statistical analysis, a noteworthy correlation was found between TT expression of eNOS and end-stage renal disease (ESRD).

7. DISCUSSION:

A significant public health concern, chronic kidney disease is frequently brought on by diabetes, hypertension, nephrotoxic drugs, and glomerulonephritis. It is possible that some patients' CKD develops earlier than expected even with appropriate treatment, suggesting the possibility of genetic factors contributing to the disease's development. CCR-2 and eNOS gene polymorphism in individuals with chronic kidney disease (CKD; Cases) and those

without (Controls) was the focus of the current investigation. In order to do this, the study included 62 CKD patients (Cases) and 62 age- and gender-matched non-CKD patients (Controls).

The current eNOS polymorphism study found that there was a substantial TT genotype association in patients (58.1%) and a GG genotype association in controls (62.9%). T allele is more prevalent and linked to cases (76.61%) in the current study compared to controls (25.81%). The G allele has an odds ratio of 0.11 (0.06-0.19) and is detected in 23.39% of cases compared to 74.19% of controls. Ilhan *et al.* (2016)[10] in their study compared eNOS polymorphism in patients with CKD and healthy controls and reported no statistically significant difference between the groups for genotype frequencies of TT & GT, though smaller proportion of cases had TT genotype, but in our study TT Genotype was significantly associated in CKD Patients. El-Din Bessa *et al.* (2011)[9] compared eNOS polymorphism among ESRD and controls and reported that frequency of TT genotype was significantly increased in ESRD as compared to controls, which is similar to the findings in the present study. Shin *et al.* (2014)[8] compared patients in three groups of normal-albuminuria, microalbuminuria and overt nephropathy and reported higher frequency of eNOS GT Genotype in overt nephropathy group which was statistically non-significant, similarly in present study GT Genotype was statistically non-significant.

In the present study, TT genotype of eNOS is significantly associated with deteriorating renal function with Increased (Spot Urine, Urea, Creatinine) Decreased (Calcium, Hemoglobin & eGFR). Though Serum Albumin was associated with TT genotype, but it is statistically non-significant. Similar findings were reported by Illhan *et al.* (2016)[10] and they observed significantly elevated Urea & Creatinine with TT genotype. Shin *et al.* (2004)[8] reported association of eNOS GT Genotype with rapid deterioration of renal function which was statistically non-significant. Similarly, in the present study GT genotype was non-significant but TT genotype was found to be associated significantly.

In the present study of CCR-2 polymorphism, GG genotype was in higher proportion in controls compared to cases (controls: 77.4% vs. cases: 51.6%), whereas GA Genotype was higher in Cases (48.4%) as compared to Controls (22.6%). Moreover, G allele is more common and associated with controls (88.71%) vs. cases (72.58%). CKD patients/cases expressed A Allele (27.42% *vs.* 11.29%) more commonly. CCR-2 GA genotype expression and A Allele was associated with CKD and deteriorating renal functions with Elevated (Urea, Creatinine, Spot Urine) and Decreased (Calcium and eGFR). Similarly, Sezgin *et al.* (2011)[6] found that the frequency of CCR2 GA genotype was higher in cases and GG genotype was higher in controls, while A allele more in frequency in Cases and G allele more in controls which was statistically significant, which is similar to the present study. Nakajima *et al.* (2002) reported that though the CCR2 was not significantly associated with kidney disease, the frequency of 'A' allele was higher in patients with impaired kidneys, Similarly in present study similar findings of allele was observed. Another study by Elghoroury *et al.* (2018)[7] reported significantly higher frequencies of GA+AA genotype among transplantation, hemodialysis, ESRD-patients as well as those with acute rejection while GG genotype more common in Controls and frequency of the A allele was significantly higher among children with ESRD & those with acute graft rejection, similar findings were seen in the present study. The assessment of the relationship between CCR-2 and eNOS and the aetiology of chronic kidney disease (CKD), as well as the age at which CKD onset and duration occur, is a novel finding of the current study. The current study additionally evaluated the renal disease stage with eNOS and CCR-2. These data are interesting since there are few research that have found similar results recently. Additionally, the majority of current research has assessed either eNOS or CCR-2, but not both genetic polymorphisms collectively.

The results of this study show a substantial relationship between the eNOS TT genotype and the CCR-2 GA genotype and stages of renal disease, as well as CKD and declining renal function. However, in the Indian population, CCR-2 and eNOS expression are unaffected by the age at which a disease first manifests, the length of the illness, or the aetiology of CKD.

7. CONCLUSION:

The findings of the present study:

i. Compared to Controls (27.09±5.18 mg/dl, 0.94±0.22 mg/dl, 15.95±6.59 mg/g), Cases (126.31±38.71 mg/dl, 16.61±7.30 ml/mn, 231.77±156.25 mg/g) had substantially higher renal function indices (urea, creatinine, and spot urine).
ii. Compared to Controls (9.08±0.74 mg/dl, 13.23±1.93, 92.53±23.27 ml/mn respectively), Cases (8.54±0.73 mg/dl, 9.45±1.76 mg/g, and 8.44±3.24 mg/dl, respectively) had substantially lower serum calcium, hemoglobin, and eGFR levels. Serum Albumin levels were equal between the groups, but lower in Cases (3.39±2.94 mg/dL) than in Controls (3.77±0.43 mg/dL).
iii. GA expression of CCR2 &CKD was found to be significantly correlated. While the A allele was substantially linked to CKD, the G allele was often the more commonly detected genotype.
iv. A noteworthy correlation between CKD and TT expression of eNOS was found. The T allele and CKD were substantially correlated.
v. GA expression of CCR2 was substantially correlated with higher renal function indices (Urea, Creatinine, Spot Urine) that were deteriorating. Despite eGFR being lower in certain cases, there was no discernible correlation between eGFR and CCR-2 expression.
vi. CCR2 expression was similar for calcium, albumin, and hemoglobin.
vii. GA expression of eNOS was substantially correlated with declining renal function measures (elevated urea, creatinine, acidic urine, and decreasing eGFR).
viii. CCR2 or eNOS expression was not linked to the causes of CKD, the age at which CKD began, or the length of CKD.
ix. End-stage renal disease was linked to GA expression in CCR2.
x. End-stage renal disease was linked to TT expression in eNOS.
xi. The results above show that, although CKD aetiology and duration are unrelated, GA expression of CCR-2 and TT expression of eNOS are associated with CKD, end stage renal disease, severe albuminuria, and renal dysfunction.

8. CONSENT: As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

9. ETHICAL APPROVAL: As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

10. COMPETING INTERESTS DISCLAIMER: None

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