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A study on association of inflammatory cytokine gene polymorphisms in chronic kidney disease patients of southern region of Andhra Pradesh

Lanka Subhashini [1], Dr. K. Vijaya Rachel [2]

1. Research Scholar, Department of Biochemistry and Bioinformatics, Gitam Institute of Sciences, Gitam University, Vishakapatnam.
2. Professor & Head, Department of Biochemistry and Bioinformatics, Gitam Institute of Sciences, Gitam University, Vishakapatnam

Corresponding author
Dr. K. Vijaya Rachel
Department of Biochemistry and Bioinformatics
Gitam Institute of Sciences
Gitam University
Vishakapatnam
Email: rachel.kv@yahoo.com
Contact: 9866007005

Abstract

Background: Chronic Kidney Disease (CKD) is a highly prevalent disease condition in India. There is a lack in knowledge regarding the genes associated with CKD in Indian Population.

Aim: Hence, the present study is aimed to knock the association between IL-6 gene and progression of CKD in people of Andhra Pradesh.

Materials & methods: This was a prospective observational study conducted for a period of 3 years. A total of 579 individuals were involved in the study and were divided into 3 groups i.e, group with early-stage CKD, late-stage CKD and the control group. The general biochemical parameters were assessed and genotyping was done. All the obtained results were analysed statistically.

Results: From the study results, it was found that CKD is more prevalent in men than in women in the study population. Blood urea and serum creatinine levels were high in late-stage CKD than in early and control groups. IL-6 gene was found to be significantly associated with CKD by giving $p<0.005$.

Conclusion: To our knowledge, this was the first study to identify the gene association with CKD in Andhra population. The functional promotor IL6 C-572G(rs1800796) the only SNPs that affect IL-6 gene of CKD population in Andhra Pradesh.

Key words: polymorphism, gene, cytokine

Introduction

The presence of kidney damage or a reduced rate of glomerular filtration (GFR) of less than 60 ml/min/1.73m^2 for a minimum of 3 months is considered as chronic kidney disease (CKD). [1] The major risk factors associated with CKD are overcrowding, poor sanitation, poverty, known and unknown nephrotoxins, pollutants. Added to these, hypertension and diabetes mellitus are also the major contributing factors.[2] According to Global Burden of Disease collaboration, CKD is identifying as a major contributor for morbidity and mortality worldwide.[3] It is estimated that, between 1999 and 2017, the global prevalence and mortality from CKD increased by 29.3 and 41.5% respectively. Literature also proves that, in India, 38% increase in mortality rate is attributed to kidney

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failure between 2001-2003 and 2010-2013. CKD stands as a vital risk player in cardiovascular disease which is the major cause of premature death and disability-adjusted life years. [4]

This sobering data highlights the requirement for screening programs for those who are at risk for CKD. The CKD prevalence rate in various regions ranges from <1% to 13% and the recent literature from International Society of Nephrology's Kidney Disease Data Centre Study reported a prevalence of 17%. Throughout India, the ethology of CKD varies and a few states in Andhra Pradesh, Odisha and Goa have high incidence levels of CKD of unknown etiology. [5]

Reported literature proves that a combined effect of environment and genotype determines the major risk of CKD along with cytokine polymorphisms.[6] The balance between pro and anti-inflammatory cytokines determines the inflammatory response and facilitate the progression of CKD. IL-1, IL-6 and TNF-α are the pro-inflammatory cytokines and IL-2 and IL-8 are the anti-inflammatory cytokines that plays a crucial role in CKD progression. [7] Studies proved that functional SNPs in cytokine gene promotor area might influence the gene promotor activities and gene product levels.[8] But there is paucity in literature whether, these cytokine polymorphisms are contributors of CKD. Various studies failed to show the association between them due to less sample size.

Therefore, the present study is designed to find the association between cytokine gene (IL-6) polymorphism and CKD in larger population.

Materials and Methods

This was a prospective observational study performed during 2020 and 2023. After obtaining ethical clearance from the Institutional Ethical Committee, the study was proceeded further. A total of 579 participants were considered for the study. CKD patients who were diagnosed with CKD according to kidney disease: Improving Global Outcomes (KDIGO) criteria and of age 18-70 were included for the present study. Patients having the history of thyroid disorder, chronic hypertension, gestational diabetes, epilepsy, hypertensive encephalopathy, cardio vascular disease and malignancies were excluded from the study. Detailed clinical history of the patient was collected and laboratory parameters like blood urea, serum creatinine and other parameters were measured in all the participants. e GFR was measured by modification of diet in renal disease.

The study subjects were divided into three groups i. e; patients with eGFR above 45ml/min/1.73 m2 were classified as early-stage CKD and with lower eGFR levels were classified as late-stage CKD. Patients with normal eGFR levels were considered as control subjects.

Estimation of eGFR

SCr was calculated in all participants using an enzymatic method. The eGFR of each participant was calculated from the SCr, age, and sex using the following eGFR equation recently determined

eGFR (ml⁄min⁄1.73m2) = 194 × SCr (mg⁄dl)-1.094 × age-0.287 (× 0.739 if female).

The prevalence of CKD was determined for CKD stages 3-5 (defined as eGFR < 60 ml/min/1.73 m2)

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DNA extraction

Venous blood was collected from the patients during medical visit, stored at -80°C, and processed on the same day. DNA extraction from the blood cells involved an initial treatment with 0.5% SDS lysis buffer followed by treatment with protease K (1 mg/mL, for the digestion of nuclear protein) for 4 h at 60°C. Total DNA was harvested using the Gentra extraction kit and was precipitated using 70% alcohol.

Genotyping

In this study, we selected IL-6 pro-inflammatory cytokine and single nucleotide polymorphisms (SNPs) in the promotor region which had minor allele frequencies in our population based on SNP database and HapMap database. The SNPs were genotyped using a Multiplex PCR-based Invader Assay and reverse primers IL6 (rs1800796).

Statistical Analysis

All the collected information is entered in excel and word documents and genotype distributions were tested ANOVA.

Results

Among total of 579, 220 were considered as early-stage CKD, 210 were considered as late-stage CKD and 149 are without CKD.

Table 1: Clinical parameters of the study subjects

	Early-stage CKD	**Late-stage CKD**	**Control**
Age	53.4±8.2	51.9 ± 8.7	52.1 ± 6.4
Male	112	110	79
Female	108	100	70
Blood urea Male Female	 52mg/dL 50 mg/dL	 76 mg/dL 68 mg/dL	 15 mg/dL 12mg/dL
Serum Creatinine Male Female	 32 mg/dL 28 mg/dL	 40 mg/dL 34 mg/dL	 1.0 mg/dL 0.9 mg/dL

Table 1 demonstrates the clinical parameters of the study population. The mean age of individuals was found to be 53.4 in early onset CKD; 51.9 in late-stage CKD and 52.1 in control group. In total population, total number of males included were 301 and total number of females were 278. Blood urea levels were predominantly higher in late-stage CKD male and female individuals than in comparison with early-stage CKD and control group. Whereas serum creatinine levels were also found to be higher in both male and females of late-stage CKD than in early-stage CKD which depicts the non-functioning of the kidneys.

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Table 2: Genotype frequencies of the study subjects

Genotype	CKD		Control
IL6 C-572G(rs1800796)	Early	Late	
CC	Male -35 Female-36	36 32	24 23
GC	Male -32 Female -36	36 31	25 23
GG	Male-45 Female 36	38 37	30 23

The genotype frequencies noted in the study participants is tabulated in table 2. This also shows CKD is more predominant in males than in females.

Table 3: Mean eGFRs and CKD prevalence with respect to cytokine polymorphism genotype

Genotype	eGFR (ml/min/1.73 m^2) mean ± SD		CKD (eGFR < 60 ml/min/1.73 m^2)	
			Early-stage	Late stage
IL6 C-572G(rs1800796)	Male	95 (78±12.1)	35	36
	Female	91 (77± 18.2)	36	32
CC				
	Male	93 (76±13)	32	36
GC	Female	91 (75± 14)	36	32
	Male	113 (72± 1.2)	45	38
GG	Female	96 (70± 5.3)	36	37
		P value =0.57	P value=0.00*	P value =0.021*

Table 3 demonstrates the mean eGFRs and CKD prevalence with respect to cytokine polymorphism genotype and it was found to be statically significant in early and late-stage CKD in both male and females with $p<0.005$.

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Table 4: Mean eGFRs and CKD prevalence for IL6 C-572G genotypes

Genotype	**eGFR (ml/min/1.73 m^2)**				**CKD (eGFR < 60 ml/min/1.73 m^2)**		
IL6 C-572G	**n**	**mean ± SD**	**b† (95% CI)**		**n**	**OR† (95% CI)**	**P value**
CC Male	95	95 (78±12.1)	0	Reference	71	1	-
Female	91	91 (77± 18.2)	0		68	1	
CG Male	93	93 (76±13)	-1.2	-2.3 -0.2	68	1.14	0.005
Female	91	91 (75± 14)	-1.3	-2.2-0.1	67	1.14	
GG Male	113	113 (72± 1.2)	2.5	-0.1-5.3	83	**0.59**	0.004*
Female	96	96 (70± 5.3)	2.6	-0.1-5.4	39	**0.59**	

Table 4 demonstrates the mean eGFRs and CKD prevalence for IL6 C-572G genotypes. It was found statistically significant with P<0.005.

Discussion

The challenge of patient care now a days seems daunting. Primary health care teachings must be implemented in educating people regarding diet and smoking avoidance, improved antenatal care, screening for non-communicable diseases like diabetes and hypertension. [9] Awareness regarding CKD must increase and timely interventions are vital for better health outcome. Success behind all these relies on all the government and non-government organization and other community members.

The world health organization package of essential noncommunicable disease interventions promises hope for CKD prevention. Early diagnosis of CKD must become a priority in those who are diagnosed with hypertension, autoimmune diseases or the persons who are having family history of CKD. [10] The attempt made in this study throws a lime light to physicians as it is one of the novel studies performed in the community of Andhra Pradesh, India.

As CKD is irreversible disease, cytokines involved in inflammation might be one of the possible explanations for those individuals susceptible to CKD.[11] The evidence of genetic polymorphisms in cytokine genes affecting the risk of CKD is limited. Studies did not focus on the cytokine genes which are identified as susceptible loci for CKD. [12]

A study by Yoshida et al., proved that some genetic variants are associated with CKD in Japanese population where TNFA and IL10 were considered in the study as cytokine genes were not associated. [13] In contrast to that, we selected IL6 gene that is presumed to have association with CKD and found significant results. Several other studies were attempted to find the association between cytokine genes and CKD but failed to identify.[14] In contrast in our study, we successfully found an association between genetic polymorphism and CKD.

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IL-6 accelerates the progression of CKD not only by aggravating kidney injury as described above in the results but also by promoting its complications in case of chronic vascular disease (CVD). Taken together, elevated IL-6 level acts as a trigger for the progression of CKD and its related complications.

Conclusion

To conclude cytokine genes, play a vital role in patients with CKD as it is the major aggravator. The results of the study proved that there was a significant association between gene polymorphisms and the CKD. Hence measures should be taken to diagnose CKD little early and give better life to human beings who are having other chronic diseases.

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