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IL1RN VNTR Polymorphism and kidney damage in sickle cell anemia patients

LVKS Bhaskar[1*], Smaranika Pattnaik[2]

[1]Department of Zoology, Guru Ghasidas Vishwavidyalaya, Bilaspur, India
[2]Department of Biotechnology and Bioinformatics, Sambalpur University, India

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ABSTRACT

Introduction: Sickle cell anemia (SCA) is a chronic illness associated with acute and chronic hemolytic anemia, recurrent vaso-occlusion episodes, intense pain, progressive multiple organ damage, and early mortality. Inflammation plays a significant role in the pathophysiology of SCA. Elevated levels of pro-inflammatory cytokines are involved in worsening the degree of kidney damage in SCA patients.

Objectives: The present study aimed to assess whether *IL1RN* VNTR polymorphism is associated with kidney damage in patients with SCA.

Patients and Methods: We have investigated 190 SCA patients (104 with Normal kidney function and 86 with kidney damage). Creatinine-based estimated glomerular filtration rate (eGFR) was calculated to assess kidney function. Interleukin-1 receptor antagonist gene (*IL1RN*) variable number tandem repeats (VNTR) genotypes were analyzed using PCR-electrophoresis. The association between *IL1RN*-VNTR and kidney damage was evaluated by using χ^2 test. Odds ratios (OR) and 95% CI were calculated. The relationship between kidney damage and fetal hemoglobin (HbF) and their interaction with *IL1RN*-VNTR genotypes, was investigated using a Mantel-Haenszel (M-H) stratified analysis.

Results: There were no significant differences in genotype frequencies between SCA patients with or without kidney damage (P=0.107). Furthermore, no significant interactions between *IL1RN* VNTR and HbF on determining kidney damage were found.

Conclusion: These results conflict with the biological plausibility that interleukin levels modulate SCA pathophysiology and may deserve further exploration.

Implication for health policy/practice/research/medical education:
Inflammation plays a significant role in the pathophysiology of sickle cell anemia. Although there is no significant association between IL1RN-VNTR and kidney damage in this study, in vitro and in vivo data supporting the role of interleukin in pathophysiology of sickle cell anemia.

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Original

Introduction

Sickle cell anemia (SCA) is caused by a single nucleotide substitution mutation in the sixth codon of the beta-hemoglobin gene that alters the DNA sequence from GAG to GTG. The mutation changes the sixth amino acid in the beta globin subunit of adult hemoglobin A from glutamic acid to valine, resulting in hemoglobin S (HbS). The HbS in deoxygenated state participated in polymerization of hemoglobin, forming the "sickle cells" (1). SCA is a chronic illness with symptoms that appear during the first year of life. SCA is inherited in an autosomal recessive pattern. SCA is linked to acute and chronic hemolytic anemia, recurrent vaso-occlusion episodes, intense pain, progressive multiple organ damage, and early mortality (2). Fetal hemoglobin (HbF), the major hemoglobin during pregnancy, is elevated in SCA patients in adulthood. These inherently elevated HbF levels would tend to decrease the severity of SCA by inhibiting the polymerization. Hence medications that increase the levels of HbF are used in the treatment of SCA (3). Hydroxyurea, a potent, HbF inducer is approved for the treatment of SCA. Owing to better survival and migration of population, the universal burden of SCA is growing continuously. Epidemiological data showed that 1% of the global population affected by

***Corresponding author**: LVKS Bhaskar, Email: lvksbhaskar@gmail.com

SCA and SCA patients' expected number increase to more than 400 000 between 2010-2050 (4).

Several lines of evidence indicated that inflammation plays a significant role in the pathophysiology of SCA (5). Persistently elevated levels of pro-inflammatory cytokines have been documented during the development and progression of vaso-occlusion in SCA (6, 7). Infarction and reperfusion injury occurring due to red blood cells (RBCs) vascular occlusion within the renal cortex, medulla and calyces, leading to sickle cell nephropathy (SCN). The presence of elevated levels of IL-1 (IL-1α and IL-1β) in chronic kidney disease (CKD) suggests that pro-inflammatory cytokines are involved in worsening the degree of renal damage (8). IL-1Ra is a naturally occurring antagonist for the IL-1 receptor and functions to block further IL-1 activity and facilitate the termination of inflammatory response (9).

The gene encoding IL-1ra (designated IL1RN) is located in chromosome 2q14 region. The intron 2 of the *IL1RN* gene contains 86-bp sequence as repeat elements (rs2234663) associated with inflammation in many diseases, including SCA (10). Of the five known alleles of this repeat element, homozygotes for allele 2 is reported to be associated with more intense inflammatory responses (11). Although there is no known link between *IL1RN* VNTR and CKD, allele 2 of *IL1RN* VNTR has been linked to diabetic nephropathy (12).

Objectives

The present study aimed to assess whether *IL1RN* VNTR polymorphism is associated with kidney damage in patients with SCA.

Patients and Methods

Study design

A total of 190 SCA patients (55.8% males and 44.2% females) visiting the outpatient department of Sickle Cell Institute Chhattisgarh (SCIC), Raipur, India, were consecutively selected for this study. All sickle cell subjects confirmed by hemoglobin electrophoresis. Values of complete blood count (CBC) and hemoglobin HPLC were collected from the patient's records. Following an overnight fast, three ml of blood sample was collected from each participant. Serum creatinine and blood urea were analyzed by using standard assay methods on ILab 650 automatic analyzer. In the present study, estimated glomerular filtration rate (eGFR) was assessed separately in adults and children (≤17 years) respectively using chronic kidney disease epidemiology collaboration (CKD-EPI) equation (13) and Schwartz equation (14, 15). Patients with eGFR >140 mL/min/1.73 m^2 is denoted as glomerular hyperfiltration (GHF), eGFR<89 to 60 mL/min/1.73 m^2 as renal insufficiency (RI) and eGFR ≤59 mL/min/1.73 m^2 as renal failure (RF) *(16)*. DNA was extracted from whole anticoagulated blood following the standard protocol (17).

IL1RN VNTR genotyping

IL1RN VNTR polymorphism was genotyped by Polymerase chain reaction (PCR) and gel electrophoresis as per Tai et al (18). Briefly, the forward 5'-CTC AGC AAC ACT CCT AT-3' and reverse 5'-TCC TGG TCT GCA GGT AA -3' primers were used. PCR was performed on 40 ng of DNA in a 10 µL reaction containing 5 µL of 2× PCR Master Mix RED (Ampliqon, Denmark), 2.8 µL nuclease free water, and 10 pM of forward and reverse primers using an ABI 2700 PCR machine. PCR conditions were as follows; 5 minutes at 95°C for initial denaturation followed by 35 cycles of 95°C for 40 seconds for denaturation, 59°C for 35 seconds for annealing and 72°C for 40 seconds for extension and a final cycle at 72°C for seven minutes for the final extension. The PCR amplicons were separated by 2% agarose gel electrophoresis and identified with ethidium bromide staining. The amplicon sizes were determined by comparing them against a 100 bp ladder. The PCR amplicon sizes were recorded and converted into alleles as described elsewhere (19).

Statistical analysis

The Pearson's chi-square test was used to compare the distribution of *IL1RN* VNTR genotypes in SCA patients with and without kidney damage. To determine the impact of various genotypes on the relationship between kidney damage and SCA patients, odds ratios (OR) with 95% confidence intervals (CIs) were calculated. The kidney damage and HbF levels in each genotype were assessed using the M-H stratified analysis, which involved stratifying the study subjects based on IL1RN genotypes. SPSS statistical software was used to conduct all statistical analysis. The significance level was thought-out $P<0.05$.

Results

About 190 SCA patients were studied, 106 males (55.8%) and 84 females (44.2%). Their mean age was 16.5 ± 9.3 years. Participants with normal kidney function (NKF) and kidney damage had almost similar hematological profile (Table 1). However, individuals with kidney damage showed higher HBF levels (21.2 ± 7.1%) than those with NKF (18.3 ± 6.3%; $P=0.025$). A significantly higher level of creatinine was found in kidney damage patients than in the NKF group (Table 1). No significant differences were found in blood urea, total bilirubin (TB), direct bilirubin (DB), SGOT and SGPT levels in SCA patients with kidney damage compared to patients with normal kidneys (Table 1). Genotyping of *IL1RN* VNTR showed that the a1a1 genotype is the most frequent genotype, followed by a1a2, a2a2 and a1a3 genotypes. The distribution of *IL1RN* VNTR genotypes in SCA patients with or without kidney

Table 1. Distribution of various hematological variables according to kidney function in SCA patients

Variable	NKF (n=104)	Kidney damage (n=86)	*P* value
Age (y)	17.8±9.4	15.0±9.0	0.044
BMI (kg/m^2)	16.6±3.7	15.4±2.5	0.013
Hb (g/dL)	8.5±1.8	8.4±1.7	0.616
HbF %	18.3±6.3	21.2±7.1	0.003
Hematocrit %	25.1±5.6	25.1±4.9	0.979
TLC (×10^9/L)	11.2±5.3	11.1±6.0	0.938
PLT (×10^9/L)	324.7±164.5	332.0±172.1	0.766
RBC (×10^{12}/L)	3.0±0.7	3.2±2.3	0.411
MCV (fL)	86.3±10.9	86.1±9.5	0.885
MCHC (g/L)	33.8±2.3	33.7±2.0	0.730
MCH (pg)	29.1±4.2	29.0±3.7	0.875
RDW-CV	18.4±3.0	18.0±2.3	0.337
TB (mg/dl)	2.4±1.6	2.4±1.8	0.787
DB (mg/dl)	0.4±0.6	0.4±0.4	0.349
SGPT (U/L)	20.7±13.9	25.5±22.3	0.069
SGOT (U/L)	44.7±27.4	52.5±30.2	0.065
Blood urea (mg/dL)	16.0±8.6	18.4±12.3	0.114
Creatinine (mg/dL)	0.6±0.2	0.7±0.3	0.002
No. of blood transfusions	6.6±10.6	7.5±17.2	0.641

BMI: Body mass index; Hb: hemoglobin; HbF: fetal hemoglobin; TB: total bilirubin; DB: direct bilirubin.

damage is not statistically significant ($P=0.107$; Table 2). When the a1a1 genotype was set as the reference, both a1a2 and a2a2 genotypes were not associated with the risk for kidney damage (OR, 1.37; 95% CI, 0.69-2.74; for a1a2; and OR, 0.27; 95% CI, 0.06-1.28; for a2a2, respectively, Table 2). Among SCA patients with kidney damage, 26 (30.2%) showed GHF with an average age of 16.8 ± 6.4 years, and 60 (69.8%) showed renal insufficiency (RI) with a mean age of 14.3±9.9 years. The distribution of *IL1RN* VNTR genotypes in GHF and RI groups indicates that the *IL1RN* VNTR was not associated with kidney damage development ($P=0.824$). When compared to the a1a1 genotype, the a1a2 and a2a2 genotypes have not contributed to the risk of kidney damage in SCA patients (OR, 0.96; 95% CI, 0.34-2.72; for a1a2; and OR, 0.42; 95% CI, 0.25-7.06; for a2a2, respectively, Table 3). The univariate analysis revealed that the SCA patients with HbF >20% presented a significantly greater risk of kidney damage (Table 2). However, higher HbF is not associated with the type of kidney damage (Table 3). No evidence of heterogeneity of the effect of HbF on kidney damage was observed among different genotypes of the *IL1RN* VNTR. This indicated that genotype had no confounding effect on the association between kidney damage and HbF. For *IL1RN* VNTR genotypes the Mantel-Haenszel (M-H) combined OR for HbF was 2.22 and 95% CI is 1.22-4.04 (Table 4).

Discussion

Sickled erythrocytes contribute to the development of inflammation in SCA by participating in pathological mechanisms including, vaso-occlusion and hemolytic processes in blood vessels (20). The damaged sickle erythrocytes and activated endothelial cells can produce a pro-inflammatory environment leading to inflammatory vasculopathy (21). Endothelial dysfunction in the

Table 2. Association between *IL1RN* VNTR and kidney damage in Sickle cell anemia

SNP	Genotype	NKF	Kidney damage	OR (95% CI)	*P* value*
IL1RN VNTR	a1a1	73	61	Reference	
	a1a2	20	23	1.37(0.69-2.74)	
	a2a2	9	2	0.27 (0.06-1.28)	0.107
	a1a3	2	0	-	
HbF	<20%	61	35	Reference	
	>20%	43	51	2.07(1.16-3.70)	0.014

OR:odds ratio; CI: confidence interval; HbF: fetal hemoglobin.
* χ^2 test.

Table 3. Association of *IL1RN* VNTR with the type of kidney function

Gene	Genotype	Kidney damage		OR (95% CI)	*P* value*
		Hyperfiltration	Renal Insufficiency		
IL1RN VNTR	a1a1	18	43	Reference	
	a1a2	7	16	0.96 (0.34-2.72)	0.824
	a2a2	1	1	0.42 (0.25-7.06)	
HbF	<20	10	25	Reference	
	>20	16	35	0.88 (0.34-2.25)	0.781

OR:odds ratio; CI: confidence interval; HbF: fetal hemoglobin.
* χ^2 test.

Table 4. Association between kidney damage and HbF stratified by *IL1RN* VNTR genotypes

Gene	Genotype	OR (95% CI) for HbF	*P* value*
IL1RN VNTR	a1a1	1.98 (0.99-3.94)	0.555
	a1a2	2.55 (0.72-8.96)	
	a2a2	1.50 (0.85-2.64)	
	a1a3	-	
M-H combined		2.22 (1.22-4.04)	

M-H: Mantel-Haenszel risk.
*Homogeneity test *P* value.

glomerular endothelium would promote an activated, pro-inflammatory environment resulting in kidney damage (22).

Kidneys are sensitive to the effects of RBC sickling and therefore susceptible to damage in SCA patients. The renal manifestations in SCA patients include tubular disturbances, hematuria, proteinuria, and CKD (23). SCA induced pathophysiological changes of the kidney are known to cause anatomical and functional alterations in kidney. Previous studies also demonstrated that the kidney damage is unrelated to hypertension or low hemoglobin levels, implying that SCN is not solely caused by vaso-occlusive events or adaptations to long-standing anemia (24). One of our previous studies demonstrated that most SCA patients exhibit GHF followed by renal insufficiency. Moreover, this study showed that the SCA patients with kidney damage have a higher level of HbF (25). This study revealed that the *IL1RN* VNTR polymorphism was not found to be associated with kidney damage in SCA patients. The univariate analysis revealed that the higher HbF (>20%) is contributed to kidney damage. The *IL1RN* VNTR did not exhibit a confounding effect on the relationship between kidney damage and HbF. The carriers of *IL1RN* allele 2 showed significantly increased production of IL 1β in peripheral blood mononuclear cells (26). A recent study found that non-hematopoietic IL-1R deficiency or treatment with an IL-1R antagonist protects against increased stroke size in SCA patients (27).

Conclusion

There is a great deal of superior in vitro and in vivo data supporting the role of interleukin in the pathophysiology of SCA, and it cannot be cast into doubt by a genetic association study that does not examine all functional polymorphisms or measure IL-1 levels. However, the lack of correlation between kidney damage and *IL1RN* polymorphism is worthy of report but must be carefully interpreted.

Limitations of the study

This study has three main limitations, which have to be pointed out. First, we did not measure levels of IL-1 in the study; thus it has inherent drawbacks and precludes causal inferences. Second, only one VNTR polymorphism of *IL1RN* was examined in this study, and it is highly encouraged to incorporate other polymorphisms in the IL-1 family gene complex. Finally, it was a nested study; hence there was a possibility of selection bias. In summary, there was no suggestive evidence for association of *IL1RN* VNTR in the development of kidney damage in SCA.

Author's contribution

Conceptualization: SP. Methodology: LVKSB and SP. Validation: LVKSB and SP. Formal analysis: LVKSB. Investigation: LVKSB. Resources: LVKSB and SP. Data curation: LVKSB and SP. Writing—original draft preparation: LVKSB. Writing—review and editing: LVKSB and SP. Visualization: SP. Supervision: SP. Project administration: LVKSB. Funding acquisition: LVKSB.

Conflicts of interest

The authors declare that they have no competing interest.

Ethical issues

The research followed the ethical principles of the Declaration of Helsinki. The Institutional ethics committee of Sickle Cell Institute Chhattisgarh, Raipur approved this study. Adult subjects have given written informed consent, and parents of the child or the child's guardian provided written consent on behalf of child. Besides, ethical issues (including plagiarism, data fabrication and double publication) were completely observed by the authors.

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