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Research Article

Common Polymorphisms in the Interleukin-22 Gene are Not Associated with Cardiopulmonary Bypass-Associated Acute Kidney Injury

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Abstract

Objectives: Interleukin-22 (IL-22) is an immunomodulatory cytokine which plays an important role in kidney injury and kidney regeneration. In this study, we aimed to investigate the association of IL-22 polymorphisms with the risk of cardiopulmonary bypass-associated acute kidney injury (CPB-AKI) in Iranian population.

Materials and Methods: Patients undergoing coronary artery bypass graft surgery were enrolled in this study (n = 145). 70 patients developed AKI and compared to non-AKI subject (n = 75). Allele and genotype frequencies of IL-22 polymorphisms, rs2227513, rs2227491, rs2227478, were assessed using PCR-SSP method.

Results: The genotype distribution of rs2227513, rs2227491, rs2227478 were in accordance with Hardy-Weinberg equilibrium. Genotype and allele frequencies of the three polymorphisms were not significantly different between AKI and non-AKI subjects.

Conclusion: Taken together, this study has provided the first genetic data on the IL-22 gene polymorphisms in Iran south population and showed that mentioned SNPs cannot be considered as an effective and important factor in susceptibility to CPB-AKI.

Keywords: *Acute Kidney Injury; Cardiac Surgery; Cardiopulmonary Bypass; Polymorphism; Interleukin-22*

Abbreviations

CPB-AKI: Cardiopulmonary Bypass-Acute Kidney Injury; IL: Interleukin; SNP: Single Nucleotide Polymorphism; NCBI: National Center for Biotechnology Information; GAPDH: Glyceraldehyde 3-Phosphate Dehydrogenase; MAF: Minor Allele Frequency; PCR-SSP: Polymerase Chain Reaction-Sequence-Specific Primer; AKIN: Acute Kidney Injury Network

Introduction

Cardiopulmonary bypass-associated acute kidney injury (CPB-AKI) is a common perioperative complication for patients undergoing cardiopulmonary bypass (CPB) which is associated with more than 2-fold increase in early mortality [1]. CPB-AKI is a complex procedure

which is associated with increased production of immune system mediators including both proinflammatory cytokines (tumor necrosis factor-a (TNF-a), interlukin-6 (IL-6) and anti-inflammatory cytokine (IL-10). Apparently, the balance between pro and anti- inflammatory cytokines determines the degree and extent of renal injury [2].

Interleukin (IL)-22 is a member of the IL-10 family and a key component of immune-epithelial cell cross-talk, which is secreted by several types of immune cells including Th1, Th17, Th22 and natural killer T cell (NKT) innate lymphoid cells (ILCs) [3].

Previous studies showed that this cytokine has both protective and inflammatory nature and play an important role in modulating tissue damage in autoimmune disease, infection and malignancy [3]. IL-22`s anti-apoptotic effects and its capability to promote regeneration and proliferation are key features in regulatory roles in tissue repair and wound healing [3]. Feng., *et al*. study in pancreatitis showed that IL-22 inhibits the autophagic pathway and promotes cell survival through up-regulating survival genes such as Bcl-2 and Bcl-X_L, which bind to Beclin-1 and subsequently inhibit autophagosome formation [4]. IL-22 also promotes proliferation of liver stem/progenitor cells in damaged liver [5].

Recent studies have reported IL-22 functional roles in kidney injury and kidney regeneration [6]. Aggarwal., *et al*. showed that the IL-22 receptor (IL-22R) is expressed by renal epithelial cells [7]. Kulkarni., *et al.* using mouse models of AKI, showed that IL-22 enhances tubular epithelial cells (TEC) re-epithelialization. They found that interstitial mononuclear phagocytes are the major source of renal IL-22 and neutralizing endogenous IL-22 impairs epithelial recovery on AKI. The necrotic TECs involve interstitial dendritic cells to support their renewal, through a specific TLR4-IL-22-IL-22R pathway and subsequent STAT3 and ERK1/2 phosphorylation [8]. Xu used a mouse model of renal ischemia/reperfusion injury and found that IL-22R signaling prevents renal cell death via upregulating anti-apoptotic genes of the BCL-2 family and downregulating the proapoptotic Bad gene [9].

However, though there are proven roles of IL-22 in kidney regeneration [6], there has been no report regarding an association between human IL-22 and the AKI. Therefore, for the first time, we conducted this study to evaluate whether single nucleotide polymorphisms (SNPs) of IL-22 (rs2227513, rs2227491, rs2227478) may affect the susceptibility of acute renal injury after cardiac surgery.

Materials and Methods

Study participants

In this study 145 patients who were admitted for cardiopulmonary bypass graft surgery in Jorjani heart center, Bandar Abbas, Iran from January 2018 to August 2019 were enrolled. After surgery, patients classified as acute kidney injury (AKI) defined as 0.3 mg/dL increase in serum creatinine concentration compared to the base line serum creatinine during 48 hour post operation (stage-1 of the Acute Kidney Injury Network, AKIN definition) [10] and non-AKI groups. Urine output was not used as the criteria for AKI diagnoses because of high-dose diuretic therapy. The following exclusion criteria were used: Patients suffering from autoimmune disease, disorders of liver and kidney, repeated hemodialysis, kidney transplants, current inflammatory condition, pre-existing AKI, infectious diseases, those who used addictive substances (except cigarettes), those who underwent coronary angiography 72 hours before blood sampling and those who were treated with immunosuppressive agents or nephrotoxic drugs (NSAID, aminoglycosides). All patients were aware of the sample collection for this study and signed written informed consent. Hypertension was defined as a systolic blood pressure ≥ 140 mm Hg and/or a diastolic blood pressure ≥ 90 mm Hg according to the National Institute of Health definition [11]. Body mass index was calculated by dividing body weight in kilograms by height in meters squared (BMI = kg/m^2). Smokers were defined as subjects who smoked at least 2 cigarettes a day at the time of diagnosis.

Blood sample and laboratory measurements

Before and 48 hours post operation, blood samples were collected. Samples coagulation was performed at room temperature for 30 minutes and after centrifugation (sigma 2-16KL) at 2600g for 10 minutes serum was obtained. Serum creatinine was measured using

Cobas Integra 700 analyzer (Roche Diagnostics, Indianapolis, IN, USA). A structured questionnaire was used to record the demographic data such as age, gender, smoking and family history of disease. Variables including CPB time, cross-clamp time and ejection fraction were extracted from patient's medical records.

Polymorphism analysis

Genomic DNA was extracted from whole blood samples DNA Extraction kit (Thermo Fisher Scientific, USA). Specific primers were designed using Primer3 software and NCBI Primer Blast tool and GAPDH primers were used as the internal positive control (Table 1). SSP-PCR mixture contained 0.4 µl of dNTPs (stock concentration of 15 mM), 0.2 µl of *Taq* DNA polymerase buffer (5 unit/µl), 1 µl of each primer pair (stock concentration of 20 pmol/µl), 0.4 µl of $MgCl_2$ (stock concentration 50 mM), 2 µl PCR buffer 10x, 0.5 µl of prepared DNA and sterile double distilled water to a final volume of 20 µl. The following amplification condition was used: first step, denaturation at 95°C for 5 minutes, second step, denaturation at 94°C for 40s, primer annealing at 60°C and 56°C for rs1053004 and rs744166 respectively for 40s and elongation at 72°C for 1 minute. The second step was repeated for 30 cycles (Bio-Rad Laboratories Inc.). The third step included the final elongation at 72°C for 7 minutes. The amplified fragments of rs1053004 and rs744166 were electrophoresed on 2% agarose (Sigma Chemical Co.) gel, stained with Gel red dye (Sigma Chemical Co.) and photographed.

SNP	Primer sequences	PCR fragment size (bp)
rs2227478	F1 5'- TCCTCATGTGGTTCCGACCCTTT-3' F2 5'-TCCTCATGTGGTTCCGACCCTTC -3' R 5'- AGCAAGGAAGCAAATGCAAG-3'	229
rs2227513	F1 5'- GCGTTTCGGCAAACTTGGTA-3' F2 5'-GCGTTTCGGCAAACTTGGTG -3' R 5'- CCCTCAGGGATAAACAGTGG-3'	432
rs2227491	F1 5'- AGCTACAGTTGTGACGAACA-3' F2 5'- AGCTACAGTTGTGACGAACG-3' R 5'- CCAGACCCCATTCACCACTA-3'	472
GAPDH	F 5'- GCAGCCCTGGAGCCTTCA-3' R 5'- TTACCATATACCCAAGGGAGCC-3'	581

Table 1: *Primers sequences that were used for SSP-PCR amplification.*

Statistical analysis

Categorical variables were analyzed using Chi-square test and presented as percentile. Quantitative variables analyzed using t-test and presented as mean ± SD. Pearson Chi-square test was used for analysis of deviation from Hardy-Weinberg equilibrium (HWE). Power of study was calculated using the Open Source Epidemiologic Statistics for Public Health software, version 3.01. The power of the study was at least 80% to detect significant effects with the odds ratio greater than 2 for the A allele of rs2227478 and rs2227491 and C allele of rs2227513. Relationship between genotypes and alleles were analyzed using logistic regression model and adjusted foe age and gender. $P < 0.05$ was considered as statistical significance. Statistical analysis was performed using the Statistical Package for Social Sciences version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of study participants

In this study, 145 participants were included and 70 patients developed AKI based on AKIN definition. The criteria of AKI definition were not observed in 75 patients and hence those were classified as non-AKI group. Based on AKIN definition, a total of 39 patients

(55.7%) developed stage 1, 20 patients (28.6%) developed stage 2 and 11 patient (15.7%) developed stage 3. Demographic, clinical, and operative variables were shown in table 2. AKI participant age ranged 39-78 years with a mean of 59.1 ± 6.4 and non-AKI subjects age ranged 30 - 79 years with a mean of 58.4 ± 5.1 (P = 0.5). other variables including sex, BMI, blood pressure clinical variables such as creatinine, BUN, diabetes and operative variables were not significantly different between the two groups (P > 0.05).

Variables	AKI (N = 70)	Non-AKI (N = 75)	P value
Age (Year)	59.1 ± 6.4	58.4 ± 5.1	0.5
Sex (%) Male Female	46.6% 41.7%	53.4% 58.3%	0.6
BMI (Kg/m^2)	24.1 ± 4.1	25.3 ± 3.4	0.06
Base line plasma creatinine (mg/dL)	1 ± 0.3	1.03 ± 0.2	0.5
Urine output within 24 hours after surgery (mL/Kg/hour)	1.8 ± 0.4	1.9 ± 0.8	0.3
BUN before surgery (mg/dL)	11.8 ± 9.2	10 ± 1.5	0.1
BUN 24 hours after surgery (mg/ dL)	28.1 ± 7.3	26.5 ± 6.1	0.1
Systolic blood pressure (mmHg)	134.2 ± 27.7	126.4 ± 26.9	0.09
Diastolic blood pressure (mmHg)	75.5 ± 20.3	71.3 ± 12.4	0.1
Cross-clamp Time	84.4 ± 36.5	75.7 ± 30.1	0.1
Bypass Time	131.2 ± 37.8	127.4 ± 38.2	0.5
Ejection fraction (%)	45.4 ± 8.9	48.4 ± 10.4	0.06
Diabetes (%)	54.4%	51.2%	0.7
Hypertension (%)	48.3%	53.8%	0.5

Table 2*: Characteristics and operative variable of the patients with and without acute kidney injury (AKI and non-AKI).*

IL-22 genotype frequencies and risk of AKI

Alleles and genotypes frequencies were analyzed using PCR-SSP method. The selection of polymorphisms evaluated in the present study was based on immunologic and etiopathologic mechanisms associated with previous studies. The minor allele frequencies (MAF) of all selected SNPs were higher than 5%. DNA was extracted from whole blood and genotyping of SNPs was successfully performed for all samples. Ten percent of the samples was randomly selected and PCR-SSP was repeated 5 times and the same results were obtained. Table 3 showed the genotype and allele frequencies of rs2227478, rs2227513 and rs2227491 SNPs. Genotypes frequencies in AKI and non-AKI subjects were in accordance with Hardy-Weinberg equilibrium. Regarding rs2227478, although the frequencies of AG and AA genotypes were higher and the frequencies of GG genotype was lower in non-AKI subjects, but the difference was not statistically significant (P > 0.05). The frequencies of A and G allele of rs2227478 were not significantly different between AKI and non-AKI participants. No significant differences were observed between genotype and allele frequencies of rs2227513 and rs2227491 comparing AKI and non-AKI groups.

Discussion and Conclusion

The compelling evidences about IL-22 important role in the improvement of renal injury [6] suggest that IL-22 gene is a plausible candidate gene for AKI susceptibility. In this study, we investigated whether rs2227513, rs2227491, rs2227478 SNPs are associated with the development of AKI in Iranian patients undergoing coronary artery bypass graft surgery. However, although current studies on IL-22

SNP[1] rs2227478	AKI[2] (n = 70)	Non-AKI (n = 75)	P value
Genotypes, n (%)			
GG	16 (22.9)	14 (18.6)	0.5
AG	42 (60)	44 (58.7)	0.9
AA	12 (17.1)	17 (22.7)	0.4
HWE[3]	0.09	0.1	
Alleles, n (%)			
G	74 (52.9)	72 (48)	0.4
A	66 (47.1)	78 (52)	
SNP rs2227513	**AKI (n = 70)**	**Non-AKI (n = 75)**	**P value**
Genotypes, n (%)			
CC	13 (18.6)	19 (25.3)	0.3
CT	40 (57.1)	44 (58.7)	0.8
TT	17 (24.3)	12 (16)	0.2
HWE	0.2	0.1	
Alleles, n (%)			
C	66 (47.1)	82 (54.7)	0.2
T	74 (52.9)	68 (45.3)	
SNP rs2227491	**AKI (n = 70)**	**Non-AKI (n = 75)**	**P value**
Genotypes, n (%)			
TT	17 (24.3)	15 (20)	0.5
TA	41 (58.6)	45 (60)	0.9
AA	12 (17.1)	15 (20)	0.6
HWE	0.1	0.08	
Alleles, n (%)			
T	75 (53.6)	75 (50)	0.5
A	65 (46.4)	75 (50)	

Table 3: *Allele and genotypes frequencies of IL-22 in AKI and non-AKI subjects.*

1SNP: Single Nucleotide Polymorphism; 2AKI: Acute Kidney Injury; 3HWE: Hardy-Weinberg Equilibrium.

SNPs showed the importance of these variations in different diseases, including malaria and autoimmune disease [12-16], none of the 3 polymorphisms of the IL-22 gene (rs2227513, rs2227491, rs2227478) examined in this study and none of the alleles were associated with the presence or clinical features of CPB-AKI.

However, our results are in agreement with Thompson., *et al*. study which showed no significant difference in frequencies of rs2227513 genotypes between patients suffering from colon cancer and controls [13] and are inconsistent with those studies which suggested that rs2227513 polymorphism might contribute to systemic lupus erythematosus (SLE) [12] and psoriasis [17] susceptibility. Regarding the rs2227491, our findings are consistent with the studies which found no association between rs2227491 and risk of ulcerativa colitis [18], chronic plaque psoriasis [19], SLE [12] and acute myeloid leukemia [20]. Our findings also contradict the Song., *et al*. report showed

the genotype TT in rs2227478 was correlated with the risk of autoimmune thyroid disease and alleles C was linked to the susceptibility of Hashimoto's thyroiditis [21]. In conclusion, although IL-22 has an essential function in kidney injury and regeneration [6] and it was expected to find a probable link between its common polymorphisms and CPB-AKI, the present study, which is the first to report an association of SNPs in IL-22 with risk of AKI, showed that there is no association between rs2227513, rs2227491, rs2227478 SNPs and CPB-AKI and do not support any specific effect of these SNPs on susceptibility to CPB-AKI. However, there are some limitations in our study and caution is still appropriate in generalizing from our genetic association study. This study includes a relatively small sample size. Additional studies with still larger number of patients are required to determine interactions between these SNPs and other potential candidate polymorphisms involved in the etiology of CPB-AK.

Ethical Approval and Consent to Participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. The ethical committee of the Hormozgan University of Medical Sciences confirmed the study (the committee's reference number: IR.HUMS.REC.1397.146).

Consent for Publication

Not applicable.

Availability of Data and Materials

Not applicable.

Conflict of Interests

The authors declare that they have no competing interests.

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Author Contributions

NN and MR developed the concept and prepared the manuscript, ZK performed experimentation, HS performed examination on patients and operation, MR analyzed data, prepared tables and carefully read the manuscript. All authors read and approved the final article.

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