Transplant Immunology xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

Association of chemokine gene variants with end stage renal disease in North Indian population

Vibha Singh, Praveen Kumar Jaiswal, Poonam Tiwari, Rakesh Kapoor, Rama Devi Mittal *

Department of Urology and Renal Transplantation, Sanjay Gandhi Post Graduate Institute of Medical Science, Raebareli Road, Lucknow 226014, Uttar Pradesh, India

ARTICLE INFO

Article history:
Received 11 January 2013
Received in revised form 12 April 2013
Accepted 15 April 2013
Available online xxxx

Keywords:
Chemokine gene polymorphism
ESRD
PCR–RFLP
ARMS-PCR
Haplotype

ABSTRACT

Background & aim: The progression rate of chronic kidney disease (CKD) to its end-stage renal disease (ESRD), and the development and severity of various complications, are indirectly influenced by genetic and epigenetic factors. Chemokines are small inducible pro-inflammatory cytokines, which are implicated in many biological processes like migration of leukocytes, angiogenesis, tumor growth and metastasis. We tested association of four single nucleotide polymorphisms (SNPs) viz. *CCL2I/D*, *CCL2A2518G*, *CXCL12G801A* and *CXCR2(+1208)C/T* among individuals with ESRD (end stage renal disease) and normal healthy controls from North Indian population.

Materials and method: CCL2I/D, *CCL2A2518G*, *CXCL12G801A* and *CXCR2(+1208)C/T* were genotyped in blood samples of hospital-based case–control study comprising of 200 ESRD cases and 200 healthy controls using Restriction Fragment Length Polymorphism (RFLP) and ARMS (Amplification Refractory Mutation Specific) PCR methodology.

Results: A significant association was found in *CXCL12G801A* with ESRD risk. In case of *CXCL12G801A* polymorphism heterozygous (GA) genotype showed significant risk ($p = 0.039$; OR = 1.55) whereas A allele carrier (GA + AA) also exhibited risk with ESRD ($p = 0.045$, OR = 1.59). In *CXCR2(+1208)C/T* polymorphism, the heterozygous genotype (CT) showed significant risk for ESRD ($p = 0.028$; OR = 1.65) and combination of CT + TT demonstrated significant high risk for ESRD ($p = 0.036$; OR = 1.52). In case of *CCL2I/D*, the variant genotype (D/D) showed reduced risk for ESRD patients. Upon analyzing the gene–gene interaction between *CXCR2* and *CXCL12*, the combination (CT–GA) showed 2.65 fold risk for ESRD ($p = 0.018$).

Conclusion: Our results indicated that polymorphism in *CXCL12G801A* and *CXCR2(+1208)C/T* showed high risk for ESRD in North Indian population. However, *CCL2I/D* showed reduced risk and *CCL2A2518G* exhibited no association. Study with large sample size and diverse ethnicity is required to validate these observations.

1. Introduction

Renal failure refers to temporary or permanent damage to the kidneys that results in loss of normal kidney function. The prevalence of chronic kidney disease (CKD) and end-stage renal disease (ESRD) is growing worldwide and therefore, constitutes a serious public health problem, which causes substantial morbidity and mortality [1]. Chronic kidney disease progression has been linked to pro-inflammatory chemokine and markers of inflammation. These markers are also elevated in end-stage renal disease (ESRD). Persistent proteinuria, dyslipidemia, hypertension and smoking are considered established conventional risk factors along with a low estimated glomerular filtration rate (eGFR) and albuminuria which are known risk factors for end-stage renal disease (ESRD) [2,3].

However, it has been assumed that oxidative stress, inflammation and immune processes, may also be important contributors to the pathogenesis of cardiovascular disease as well as progression to ESRD [4]. The inflammatory response involved in renal damage produces a release of pro-inflammatory cytokines and chemokines, which cause an increased inflow of leukocytes, intensification of interstitial nephritis and progressive fibrosis. Alternatively, genetic susceptibility is also considered an important determining factor for the appearance and/or progression of ESRD and its complications [5,6].

Kidney cells produce MCP-1 (monocyte chemoattractant protein-1) in response to a variety of pro-inflammatory stimuli and predictably, its expression has been identified in kidney diseases which involve significant inflammation. A biallelic genetic variation (A/G) in the MCP-1 gene distal regulatory region at position −2518 affects the level of MCP-1 expression in response to an inflammatory stimulus [7]. *CXCL12* has been found to be involved in cell proliferation, cell migration, and cell invasion. The *CXCL12* also known as stromal cell derived factor (SDF-1) has been revealed that a single nucleotide polymorphism (SNP), in this gene may affect the expression of SDF-1 chemokine [8].

* Corresponding author. Tel.: +91 522 2668004 8x4116; fax: +91 522 2668017.
E-mail addresses: ramamittal@gmail.com, rdm_pgi@rediffmail.com (R.D. Mittal).

0966-3274/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.trim.2013.04.004

CXCR-2 is a receptor of interleukin-8, which is involved in acute and chronic inflammatory processes. Polymorphisms in the *CXCR2(+1208)C/T* gene have been associated with chronic inflammatory conditions [9].

We selected these polymorphic markers as they have been reported to be functional and associated with altered immune responses. CCL2, also known as monocyte chemoattractant protein 1 (MCP-1) is the strongest known chemotactic factor for monocytes. Polymorphism in CCL2 gene at distal regulatory region at position −2518 affects the level of CCL2 expression in response to an inflammatory stimulus [7]. Exact mechanism of *CCL2I/D* gene polymorphism affecting the expression of gene is still unclear. CXCL12 gene is associated with cell proliferation, migration and invasion. Single nucleotide polymorphism (SNP) in this gene at *CXCL12G801A* may affect the expression of *CXCL12* chemokine [8]. CXCR-2 gene is a receptor of interleukin 8 and is involved in acute and chronic inflammatory process. Polymorphisms at *CXCR2(+1208)C/T* gene have been associated with chronic inflammatory conditions [9].

Our preliminary data suggest that four polymorphisms showed an association with ESRD protection or development, which could help to predict the risk of developing ESRD. The aim of the present study was to investigate whether single nucleotide polymorphisms (SNPs) of *CCL2I/D* (rs3917887), *CCL22518A/G* (rs1024611), *CXCL12G801A* (rs1801157) and *CXCR2(+1208)C/T* (rs1801032), associated with different immune and inflammatory process could also be associated with development of ESRD in North Indian populations.

2. Materials & method

2.1. Patients and clinical data

The demographic characteristics of the subjects are represented in Table 1. Healthy controls (n = 200), (mean age 33.8 ± 12.8, male = 192 and female = 8) for the study were recruited from Northern India with similar ethnicity having no history of hypertension, diabetes, renal failure, vascular diseases, stroke, and/or cardiomyopathy. They had no recognizable autoimmune disease at the time of assessment. A total number of 200 ESRD patients on hemodialysis (mean age 33.9 ± 11.3; male = 168 and female = 32) were recruited from Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India. Patients were selected having >4.0 mg/dl serum creatinine that had been dialyzing for at least 3 months with no evidence of active inflammation. Stringent diagnostic criteria were used for diagnosing ESRD. Data collected included basic demographic information, pattern, onset of disease, and use of medications. The Institutional Review Board and ethical committee approved the protocol, and informed consent was obtained from the patients and the controls participating in the study.

2.2. DNA extraction and genotyping

Blood samples were collected from patients with ESRD and healthy controls in ethylenediamine tetraacetic acid (EDTA) anticoagulant coded vials. DNA was extracted from peripheral blood lymphocytes using 'salting-out' method [10]. Polymorphisms in chemokines *CCL2I/D*, *CCL2A2518G* and *CXCL12G801A* and *CXCR2(+1208)C/T* genes were analyzed using PCR–RFLP (Polymerase Chain Reaction–Restriction Fragment Length Polymorphism) and ARMS-PCR (Amplification Refractory Mutation System-Polymerase Chain Reaction) method. Primer detail and PCR conditions of *CCL2I/D*, *CCL2A2518G* [11], *CXCL12G801A* [12] and *CXCR2(+1208)C/T* [13] were respectively. The genotyping for all the gene taken in present study was done on 15% Poly Acrylamide Gel Electrophoresis (PAGE) using molecular weight markers and further visualized after staining with ethidium bromide. Positive and negative controls were used in each genotyping assay, and 10% of the samples were randomly selected and run in duplicates with 100% concordance. The results were reproducible with no discrepancy in genotyping.

Table 1
Demographic details of the ESRD patients and healthy controls.

Variables	Controls (n = 200)	ESRD patients (n = 200)	p value
Age (mean ± SD)	33.8 ± 12.8	33.9 ± 11.3	0.103
Gender (male/female)	192/8	168/32	0.348
Causes of ESRD			
Chronic glomerulonephritis (CGN)	–	115 (57.5)	
Chronic kidney disease (CKD)	–	58 (29.0)	
Diabetic nephropathy (DN)	–	23 (11.5)	
Diffuse global sclerosis (DGS)	–	4 (2.0)	

2.3. Statistical analysis

The sample size was calculated using Quanto software, version 1.0 (available from: http://hydra.usc.edu/gxe) with input of following variables: case–control study design, significance level (alpha) >0.05 (2 sided), model of inheritance was log additive, allele frequency was 0.28, and the genetic effect for odds ratio (OR) was 1.50 or greater. The present study achieved 80% of the statistical power. The goodness-of-fit chi square test was used to analyze any deviation from the Hardy–Weinberg Equilibrium in controls. A binary logistic regression model was used to estimate the risk as the OR at the 95% confidence interval. Haplotypes of each individual consisting of single nucleotide polymorphisms (SNPs) in chemokines were constructed, and the maximal likelihood haplotype frequencies were estimated using the expectation–maximization algorithm using the SNP analyzer ver. 1.2 program. Bonferroni's correction was applied in the case of multiple comparisons using the formula pc = p × n (pc represents corrected value where n is the number of comparisons performed). The statistical analysis was done using the Statistical Package for Social Sciences software, version 15.0 (SPSS, Chicago, IL), and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Chemokine gene polymorphisms and ESRD

The present study achieved 80% of the statistical power. The genotype and allele frequencies of chemokine gene polymorphisms in healthy individuals (controls) and ESRD patients are presented in (Table 2). The genotype frequencies of controls were in Hardy–Weinberg Equilibrium (HWE). We found significant association of *CXCL12G801A* and *CXCR2(+1208)C/T* polymorphism with ESRD risk whereas *CCLI/D* polymorphism showed reduced risk with ESRD. *CCL2A2518G* gene polymorphism showed no association with ESRD. In *CXCR2(+1208)C/T* polymorphism, the heterozygous genotype (CT) showed significant risk for ESRD (p = 0.020 OR = 1.65; 95% CI; 1.08–2.51). Combining heterozygous and variant genotype, (CT + TT) exhibited marginal risk for ESRD (p = 0.036, OR = 1.52; 95% CI; 1.02–2.61). In *CXCL12G801A* polymorphism the heterozygous genotype (GA) showed significant risk for ESRD (p = 0.039, OR = 1.55; 95% CI; 1.02–2.36). Combining heterozygous and variant genotype (GA + AA) demonstrated high risk for ESRD (p = 0.045, OR = 1.59; 95% CI; 1.04–3.68) However, at allelic level no significant association of any chemokine genes was observed with ESRD risk.

3.2. Association of CCL2I/D–CCL2A2518G haplotypes with ESRD risk

Recent studies have demonstrated that haplotype analysis may be more affirmative in predicting the disease association compared with an analysis of a single polymorphism, as individual polymorphism is likely to confer modest effects to the risk of ESRD. We, therefore, examined the effects of *CCL2* gene polymorphisms by constructing haplotype sets taking combination IA as a reference. However there was no significant association observed in case of any set of haplotype combinations with ESRD in North Indian population (Fig. 1).

3.3. Gene–gene interaction of chemokine gene polymorphism

To analyze the combined effect of chemokine SNPs taken in the present study, we conducted gene–gene interaction. Five combinations were constructed for chemokines taken for the present study viz. *CXCL12G801A–CCL2I/D*, *CXCL12G801A–CCL2A2518*, *CXCR2(+1208)C/T–CCL2I/D*, *CXCR2(+1208)C/T–CCL2A2518G* & *CXCR2(+1208)C/T–CXCL12G801A*. Combination of *CXCR2(+1208)C/T–CXCL12G801A* only showed statistically significant results viz. CT–GA combination. After applying the Bonferroni correction for multiple variable CT–GA combination remains significant for ESRD risk (pc = 0.018). None of the other combinations

Please cite this article as: Singh V, et al, Association of chemokine gene variants with end stage renal disease in North Indian population, Transpl Immunol (2013), http://dx.doi.org/10.1016/j.trim.2013.04.004