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# CCR5 Δ32 and CCR5-59029 Allele Frequency among Hepatitis C Virus Infected and in Non-HCV Infected Saudi Population

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**Abstract:** Background: Saudi Arabia has been known as a high hepatitis B virus (HBV) endemic area and hepatitis C virus (HCV) becomes a major health problem in Saudi Arabia. Chemokines and their receptors control immune cell migration during infections and play an important role in the pathogenesis of chronic hepatitis C. We studied the frequency of genetics polymorphism of chemokine receptor such as CCR5-Δ32 and CCR5 59029 in a cohort of Saudi normal and hepatitis C virus (HCV) infected individuals. Aim: The aim of the study was to determine the ccr5-Δ32, ccr5-59029 allele frequency and genotype distribution in hepatitis c virus infected patients and in non-HCV infected individuals of the Saudi population. Methods: In this study genotyping and allele frequencies of CCR-59029 mutation were determined in Saudi normal and HCV infected populations using PCR and PCR restriction fragment length polymorphism (PCR-RFLP) assays. Results: We found that the CCR5-59029A/A was (42%) in Saudi HCV patients and the G/G was (40%) in normal Saudi people. In this study individuals with A/a genotype were more likely to acquire HCV infection than individuals with G/G genotype (OR 2.08, p=<0.001). Conclusion: These data implicate that CCR-59029 A allele as susceptible allele for HCV infection while CCR5-59029 G allele as a protective allele for HCV infection among Saudi patients.

**Key words:** Chemokines · HCV · Polymorphisms · CCR5 · CCR5-59029

## INTRODUCTION

Virus (HBV) and hepatitis C virus (HCV) are the three most commonly identified worldwide. This disease represents a major public health problem in Saudi Arabia. According to the Saudi Ministry of Health (MOH) data, viral hepatitis ranked the most common reportable viral disease after chickenpox in 2007, with almost 9000 new cases diagnosed in that year ( 52% HBV, 32% HCV and 16% HAV)[1].

HCV-infection leads to chronic liver inflammation in the majority of patients. A substantial proportion of patients develop fibrosis or cirrhosis, causing HCV-related morbidity and mortality. Multiple factors influence the progression of fibrosis, including gender, age at infection and alcohol consumption [2]. In addition, genetic factors influence progression of fibrosis [3].

Chemokines constitute the largest family of cytokines, with more than 50 distinct members. According to NH2-termnal cysteine motifs, the chemokines are divided into the C, CC, CXC and CX3C subfamilies. These proteins act on at least 16 different receptors belonging to the same class of the seven transmembrane domain receptors which are associated with the heterotrimeric G1 proteins [4].Initially, chemokines were characterized as the inflammatory mediators. It is now becoming clear that the chemokine system is involved in much physiological and pathological process, such as inflammation, tumorigenesis, hematopoiesis, development and embryogenesis CCR5 has several polymorphisms of the promoter and exon regions and these are associated with HIV-1 diseases progression. A 32-base pair deletion in the CCR5 gene (CCR5-32) result in loss of a functional CCr5 protein and this confers some protections against

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infection with HIV-1. A polymorphism at position 59029 in the CR5 promoter is related to the rate of HIV-1 disease progression. Hepatitis C infection is major cause of chronic liver disease worldwide, affecting at least 170 000 000 people [5]. Different studies have showed that CCR5 32 mutation has a role or association with HCV infection. Thus, researches have focused on the distribution of this mutant allele (CCR5-32) in different human population. The study of Theolen *et al*. showed that frequency of he CCR5-32 mutant allele was not increased n Belgian HCV infected patients [6]. Konishi *et al* study showed that ccr5- 59029g/g was significantly associated with a higher probability of a sustained interferon response in chronic hepatitis c patients in Japan. Chronic liver disease due to HBV and HCV infection in Saudi Arabia represent a national health problem with high economic burden for the care of the decompensate Patient [7]. In Saudi Arabia viral hepatitis ranked the second most common reportable viral diseases n 2007. The prevalence rate for HCV in Saudis though less studied than hepatitis b it's again variable in different regions at different periods [8].

The aim of the study was to determine the ccr5-Δ32, ccr5-59029 allele frequency and genotype distribution in hepatitis c virus infected patients and in non-HCV infected individuals of the Saudi population.

## MATERIALS AND METHODS

**Subjects:** We enrolled 100 patients with chronic hepatitis C anti-HCV negative healthy individuals (healthy control) at King Abdul Aziz Hospital-Jeddah. All participants were Saudi and unrelated. Informed consent was obtained from all participants. This study was approved by the Scientific Research Committee of Faculty of Applied Medical Sciences, King Abdulaziz University. All participants were free to withdraw from the study at any time. If any adverse effects had occurred, the experiment would have been stopped, with this being announced to the Human Subjects Review Board. However, no adverse effects occurred and so the data of all the participants were available for analysis.

**Molecular Typing of Ccr5-δ32 Genotype:** Genomic DNA was extracted from 5ml of peripheral blood cells using DNA extraction kit (qiagen, hilden, Germany) according to the manufacturers instruction CCR5-Δ32 was detected by sizing PCR amplicon as described by cook *et al* (1998). Genomic DNA (50ng) primers amplify 180-bp (wild-type) and 148-bp (-Δ32deletion) fragments of the CCR5 gene, respectively. The PCR products were resolved on 5% acrylamide gel.

Table 1: Primers used for CCR5-Δ32, CCR5- 59029 DNA Typing

| | PCR Primers ( sense /antisense) |
|---|---|
| CCR5-Δ32 | 5' -CTT CAT TAG ACC TGC AGC TCT-3' |
| | 5'- CAG AGC CCT GTG CCT CTT CTT -3' |
| CCR5- 59029 (G/A) | 5'- CCC GTG AGC CCA TAG TTA AAA CTC-3' |
| | 5'- TCA CAG GGC TTT TCA ACA GTA AGG-3' |

**Molecular Typing of ccr5-59029 Genotype:** CCR5-59029g/a alleles were determined using restriction fragment length polymorphism (RFLP) analysis. Genomic DNA was amplified using the sequence- specific primer shown in Table 1. The PCR products were digested with Bsp 1286 I (New England biolabs, Beverly, Mass, USA). This restriction site was present in CCR5- 59029G, but not in the59029A allele. The products were resolved on 5 % acrylamide gel.

**Statistical Analysis:** The $\chi 2$ test or two-tailed Fisher's exact test compared the frequencies of the CCR-32, CCR5 -59029. Based on gene frequencies predicted phenotype frequencies were calculated according to the Hardy-Weinberg equation and compared with the observed frequency using the $\chi 2$ test assessed variables using the logistic regression model. The model was simplified in a stepwise fashion by removing variables with $P > 0.05$. P values $< 0.05$ were considered statistically significant. Statistical analyses were carried out using SPSS for Windows Version 10.0 (SPSS, Inc. Headquarters, 233 South Wacker Drive, Chicago, USA).

## RESULTS

The distribution of CCR5 Δ32 genotypes reveals an overall no significant differences were observed for any of the allele and genotype frequencies between control and patient group (Table 2). This table reveals that all examined patients and controls have nearly equal percentage of alleles and genes, this indicates non significant differences between examined groups.

The distribution of CCR5 Δ32 genotypes is shown in Table 3. The frequencies of the CCR5 - 59029 alleles 62% in healthy controls and 44% among patients with chronic hepatitis C. significantly differed between the controls and the HCV patients. The frequencies of CCR5- 59029 G/G genotypes significantly differed between the healthy controls and the HCV patients (Table 3). This table shows that the mutant GG is highly significant among control (40%) is compared with HCV patients ($P > 0.001$) which is highly significant this reveals that its protective genes.

Table 2: CCR5 Δ32 deletion frequencies (%) in different groups of Saudi HCV patients.

| | Control (100) | | HCV POS(100) | | |
|---|---|---|---|---|---|
| Alleles | No. | % | No. | % | P value |
| CCR5 | 100 | 99 | 100 | 99 | 1.0* |
| Δ32 | 1 | 1 | 1 | 1 | |
| Genotype | | | | | |
| CCR5/ CCR5 | 99 | 99 | 99 | 99 | 0.36* |
| CCR5/ Δ32 | 0 | 0 | 1 | 1 | |
| Δ32 / Δ32 | 1 | 1 | 0 | 0 | |

No significant difference. = *

Table 3: CCR5 59029 frequencies (%) in group of Saudi HCV patients. (100 patients)

| | Control=100 | | Saudi HCV patient | | |
|---|---|---|---|---|---|
| Allele | No. | % | N0. | % | Odds Ratio 95% Confidence interval OR (95 C1) |
| A | 7 | 38 | 112 | 56 | 2.08 (1.37-3.160)**P =<0.001 |
| G | 124 | 62 | 88 | 44 | |
| Genotype | | | | | |
| AA | 16 | 16 | 42 | 42 | * P<0.001 |
| AG | 44 | 44 | 28 | 28 | |
| GG | 40 | 40 | 30 | 30 | |

* = Significant difference.

** = Highly significant difference.

Furthermore, AA allele is highly significant among patients group (42%), which indicates that this allele is considered as susceptibility allele.

## DISCUSSION

The epidemiology of viral hepatitis in Saudi Arabia has undergone major changes, concurrent with major socioeconomic developments over the last two to three decades since the 1980s [1]. Infection with hepatitis C virus (HCV) is a major global health problem that affects approximately 3% of all individuals worldwide. Most of these patients develop a chronic infection that results in various levels of hepatic inflammation and fibrosis. Due to a substantial risk of disease progression, chronic hepatitis C is a leading cause of liver cirrhosis, hepatocellular carcinoma and liver transplantation [9].

This study aimed to determine the ccr5-Δ32, ccr5-59029 allele frequency and genotype distribution in hepatitis c virus infected patients and in non-HCV infected individuals of the Saudi population. However, results revealed that CCR5-59029A/A was (42%) in Saudi HCV patients and the G/G was (40%) in normal Saudi people. Also, individuals with A/a genotype were more likely to acquire HCV infection than individuals with G/G genotype. Results of this study were supported and agreed my previous studies.

A direct correlation between HIV infection and mutation in the chemokine receptor (CCR5) gene has been established [10]. CCR5 also serves as an entery co-receptor for primary human immunodeficiency virus strains that infect monocytes and macropages [11]. The frequency for the CCR5 -delta was 2.5% among HIV-1 seronegative Lebanese, this frequency in the Lebanese population is consistent with that in the origin of the mutation in the northern Europe. This could be attributed to a gene flow into the Middle East from northern Europe [12].

A previous study of CCR5 Δ32 allele frequency by polymerase chain reaction in a Belgian cohort of 163HCV-infected patients and 310 healty control subjects showed a no significant difference between HCV patients and normals that means that the CCR5 Δ32 mutant allele is not a risk factor for hepatitis C virus infection [6].

A cohort study of 139 patients with hepatitis C and 100 healthy bood donors were analysed for both polymorphisms using real-time polymerase chain reaction (PCR) and Light Cycler technology. CCR5 Δ32 allele was detected in 15 of 278 HCV chromosomes (5.4%) and 15 0f 200 control chromosomes (7.5%). The CCR2-V641 allele was present in24 of 278 HCV chromosomes (8.5 %) and 19 0f 200 control chromosomes (9.5%). So, CCR5 Δ32 and CCR2-V641 polymorphisms are not related to the response to Spanish HCV infected patients [13]. However, Low frequency of CCR5 Δ32 allele may be related to higher genetic susceptibility to HIV-1 infection in Iranians [14].

Also, Genomic DNA samples from 333 German patients with chronic HCV infection and 125 normal were screened by PCR for the presence of CCR5 Δ32 polymorphism. Allele frequencies of CCR5 Δ32 polymorphism did not differ significantly between the two groups (7.6% and 9.5% respectively) and control subjects (10.4%). These results confirm that CCR5 Δ32 and CCR2-V641 polymorphisms are not related to the response to German HCV infected patients [15].

A study on 377 Korean patients with HBV infection who were classified into groups according to their infection into the spontaneous clearance group (Sc) and carrier group (CC) found that the genotype frequencies of CCR5 A59029G significantly differ between the SC group (n=138) and CC group (n=239) ($p<0.05$). The CCR5 59029A allelic genotype was associated with an increased risks of chronic infection rather than spontaneous clearance ($p=0.002$) and the presence of the CCR5 59029G allele was significantly associated with the spontaneous clearance of HBV ($p=0.001$) [16].

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