RESEARCH ARTICLE

No Association between the *CCR5Δ32* Polymorphism and Sporadic Esophageal Cancer in Punjab, North-West India

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Abstract

Background: Chemokines and their receptors influence carcinogenesis and cysteine-cysteine chemokine receptor 5 (*CCR5*) directs spread of cancer to other tissues. A 32 base pair deletion in the coding region of *CCR5* that might alter the expression or function of the protein has been implicated in a variety of immune-mediated diseases. The action of antiviral drugs being proposed as adjuvant therapy in cancer is dependent on *CCR5* wild type status. In the present study, distribution of *CCR5Δ32* polymorphism was assessed in North Indian esophageal cancer patients to explore the potential of using chemokine receptors antagonists as adjuvant therapy. Materials and Methods: DNA samples of 175 sporadic esophageal cancer patients (69 males and 106 females) and 175 unrelated healthy control individuals (69 males and 106 females) were screened for the *CCR5Δ32* polymorphism by direct polymerase chain reaction (PCR). Results: The frequencies of wild type homozygous (*CCR5/CCR5*), heterozygous (*CCR5/Δ32*) and homozygous mutant (*Δ32/Δ32*) genotypes were 96.0 *vs* 97.72%, 4.0 *vs* 1.71% and 0 *vs* 0.57% in patients and controls respectively. There was no difference in the genotype and allele frequencies of *CCR5Δ32* polymorphism in esophageal cancer patients and control group. Conclusions: The *CCR5Δ32* polymorphism is not associated with esophageal cancer in North Indians. As the majority of patients express the wild type allele, there is potential of using antiviral drug therapy as adjuvant therapy.

Keywords: Chemokine - esophageal cancer - *CCR5Δ32*

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Introduction

Tumor growth and metastatic dissemination develop through a complex molecular dysregulation involving cell migration, invasion, resistance to apoptosis, and immune escape mechanisms. Human and murine epithelial cancers contain a leukocyte infiltrate (Balkwill and Mantovani, 2001; Mantovani et al., 2002) and express a complex network of cytokines and chemokines (Wilson and Balkwill, 2002). Chemokines bind to cell surface receptors that belong to the G-protein-coupled receptor (GPCR) family, controlling diverse biological and pathological processes from immunosurveillance, inflammation, and cancer. Evaluation of different tumor types demonstrated that chemokines and their receptors might play roles in cancer cell growth and metastasis (Muller et al., 2001; Arya et al., 2003). Cysteine-cysteine chemokine receptor 5 (CCR5) is a chemokine belonging to the super family of the seven-transmembrane-G-protein coupled receptors. *CCR5* is located on 3p21.31, comprising of three exons and encodes a 352 amino acid protein which is a member of ß-chemokine receptors family of integral membrane proteins. The *CCR5Δ32* arises from a 32bp deletion causing a frameshift at amino acid 185 which results in protein truncation. In animal studies, it has been suggested that *CCR5* and its ligands play a role in the pathogenesis and progression of vascular disease (Veillard et al., 2004; Potteaux et al., 2006; Zernecke et al., 2006). The potential role of *Δ32* allele in pathogenesis of cancer has been evaluated with conflicting results in various gastrointestinal tract (GIT) cancers including pancreatic cancer (Duell et al., 2006), gall bladder cancer (Srivastava et al., 2008), oral cancer (Weng et al., 2010; Tanyel et al., 2013) and gastric cancer (Gawron et al., 2011). Apart from GIT cancers, the association of *CCR5Δ32* has also been evaluated in other cancers like skin cancer and bladder cancer (Zafiropoulos et al., 2004), cervical cancer (Zheng et al., 2006), osteosarcoma (von Luettichau et al., 2008), breast cancer (Manes et al., 2003; Zafiropoulos et al., 2004; Degerli et al., 2005; Aoki et al., 2009; Guleria et al., 2012; Eskandari-Nasab et al., 2014) and prostate cancer (Balistreri et al., 2009).

Intermediate and strong expression of *CCR5* has been reported in colorectal cancer (Erreni et al., 2009), melanoma (Seidl et al., 2007), hepatoma (Sutton et al., 2007), Hodgkin lymphoma (Aldinucci et al., 2008), oral (Chuang et al., 2009) and prostate cancer (Zhang et al., 2010). Positive serum *CCR5* expression has also

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been associated with a poor prognosis in gastric cancer (Sugasawa et al., 2008). But there is no published data on *CCR5Δ32* in esophageal cancer.

CCR5 genotyping has been described as a new diagnostic and prognostic strategy for therapy optimization (McDermott et al., 2010). Antiviral drugs are being proposed as adjuvant therapy for cancer (Velasco-Velazquez et al., 2012). As these drugs are dependent for their action on wild type *CCR5* forming functional protein, the present study was an attempt to determine the distribution of *CCR5Δ32* deletion in esophageal cancer patients. Identification of *CCR5* and/or *CCR5* ligand genes variants and their therapeutic response could allow physicians to tailor preventive therapies for patients. To the best of our knowledge, the present study is the first on distribution of the *CCR5Δ32* in esophageal cancer patients from Punjab, a state in North West India.

Materials and Methods

Study design and collection of samples

In total, 175 clinically confirmed sporadic esophageal cancer patients were selected from Sri Guru Ram Das Institute of Medical Sciences and Research, Vallah, Sri Amritsar, Punjab. Patients who had received chemotherapy, radiotherapy or blood transfusion before surgery or had prior history of any cancer were excluded from the study. For each subject, a detailed personal history including age, habits, habitat, family history of cancer or any other disease and clinical details was collected in a pre-tested structured questionnaire. After informed consent, 5 ml peripheral venous blood sample from 175 esophageal cancer patients and 175 age and gender matched unrelated healthy control individuals was collected. Control subjects were randomly selected from Amritsar city and villages adjoin Amritsar city. Individual who had family history of any type of cancer or any other chronic disease or on regular medications were not included in the control group. This study was carried out in accordance with the Declaration of Helsinki after approval by the ethics committee of Guru Nanak Dev University, Amritsar, Punjab, India.

DNA extraction and CCR5Δ32 genotyping

The genomic DNA was extracted from EDTA anticoagulated whole blood using standard phenol chloroform method (Adeli and Ogbonna, 1990). *CCR5Δ32* (rs333) genotyping was performed by polymerase chain reaction (PCR) amplification of genomic DNA using previously published primer sequences (Apostolakis et al., 2005). A segment of 320bp of the *CCR5* covering the site of deletion was amplified by PCR from the genomic DNA of the subjects. The PCR reaction was set in 10μl reaction volume containing 50ng DNA, 1X Taq buffer with 1.5 mM MgCl2, 0.3μl dNTPs mixture (Bangalore GeNei), 6 picomole of each primer (Sigma, St. Louis, MO, USA), 0.5 U Taq DNA Polymerase (Bangalore GeNei). The PCR conditions were initial denaturation at 95°C for 5 minutes followed by 35 cycles with denaturation at 95°C for 45s, annealing at 59°C for 30s and extension at 72°C for 45s, and final extension at 72°C for 10 min in a Mastercycler gradient, (Eppendorf, Germany). A negative control without template DNA was included in each reaction. PCR products were analyzed on ultraviolet transilluminated 2.3% ethidium bromide stained agarose gel. A band of 320bp indicated wild type homozygous (CCR5/CCR5) and two bands of 320bp and 288bp indicated heterozygous (CCR5/*Δ32*) genotype (Figure 1). Genotyping was performed without knowledge of case/control status. The validity of PCR based assay was proven by sequencing of different genotypes (Figure 2).

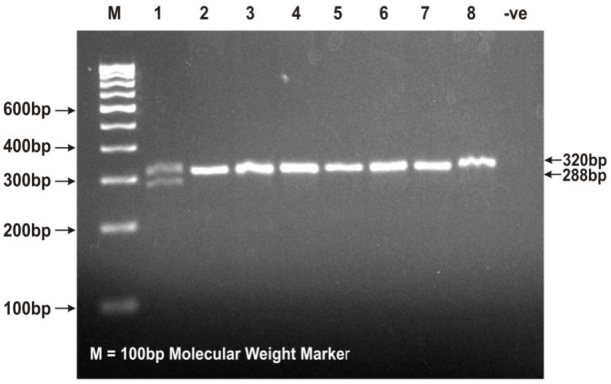

Figure 1. Gel Photograph Showing CCR5 Genotypes. Lane 1: heterozygous genotype (CCR5/Δ32), Lane 2-8: wild-type homozygous genotype (CCR5/CCR5), -ve: negative control

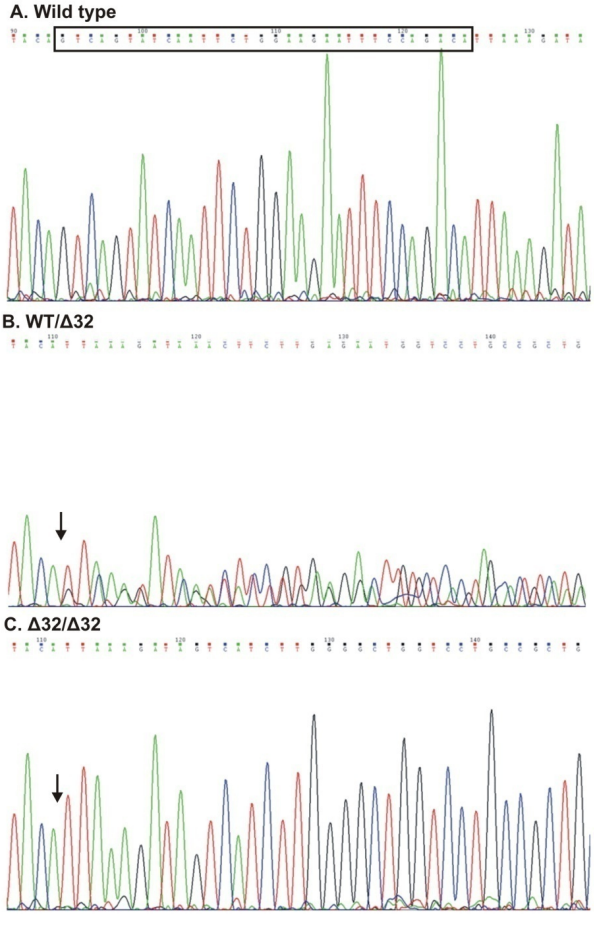

Figure 2. A Part of Electropherogram Showing WT/WT (A), WT/Δ32 (B) and Δ32 /Δ32 Genotype (C) of CCR5Δ32 Polymorphism

Data analysis

The data was analyzed using SPSS (Version 16, SPSS Inc, Chicago, IL). Differences in genotypic and allele frequencies of *CCR5Δ32* variant between patients and controls were evaluated by gene count and χ^2 test. Odds ratio (OR) with confidence interval (CI) was also calculated. A cut off values for p≤0.05 were considered statistically significant.

Results

In this case control study, 175 clinically confirmed sporadic esophageal cancer patients (69 males and 106 females) and 175 unrelated healthy control individuals (69 males and 106 females) were analyzed. The mean ages of patients and controls were 56.22±13.26 and 56.0±13.0 years respectively. Higher incidence of EC was observed among females (60.57%) as compared to males (39.43%). The selected characteristics of the cases and controls are presented in Table 1. The frequencies of wild type homozygous (CCR5/CCR5), heterozygous (CCR5/*Δ32*) and homozygous mutant (*Δ32*/*Δ32*) genotypes were 96.0 *vs* 97.72%, 4.0 *vs* 1.71% and 0 *vs* 0.57% in patients and controls respectively (Table 2). CCR5/*Δ32* genotype frequencies were not significantly different between esophageal cancer patients and control group (p=0.204).

Table 1. Baseline Characteristics of Esophageal Cancer Patients and Controls

Characteristics		Patients n(%)	Controls n(%)
No. of subjects		175	175
Gender	Males	69 (39.43)	69 (39.43)
	Females	106 (60.57)	106 (60.57)
Age in Years	Mean ± SD	56.22±13.26	56±13.0
	Range	24-84	24-84
Habitat	Rural	138 (78.86)	138 (78.86)
	Suburban	7 (4.0)	7 (4.0)
	Urban	30 (17.14)	30 (17.14)
Habits	Vegetarian	104 (59.43)	94 (53.71)
	Non vegetarian	71 (40.57)	81 (46.29)
	Smoker	14 (8.0)	32 (18.29)
	Alcoholic	42 (24.0)	6 (3.43)
Histology	Adenocarcinoma	11 (6.29)	-
	Squamous Cell Carcinoma	164 (93.71)	-
Clinical Stage	I	12 (6.86)	
	II	116 (66.29)	
	III	37 (21.14)	
	IV	10 (5.71)	

Table 2. Distribution of CCR5Δ32 Genotypes and Allele in Esophageal Cancer Patients and Controls

	Patients n (%)	Controls n (%)	OR (95%CI)	p value
Genotypes				
WT/WT	168(96.0)	171 (97.72)	Reference	
WT/Δ32	7 (4.0)	3 (1.71)	2.37 (0.60-9.34)	0.204
Δ32/Δ32	-	1 (0.57)	-	
Alleles				
WT	343 (98.0)	345 (98.57)	Reference	
Δ32	7 (2.0)	5 (1.43)	1.41 (0.44-4.48)	0.56

*n=Number of subjects, Figures in parentheses represent frequency; OR=Odds Ratio; CI=Confidence interval

Discussion

Chemokines belong to the family of chemotactic cytokines that are considered to be the main regulators of leukocyte trafficking under homeostatic and inflammatory conditions. They may be involved in the regulation of immune cell migration and activation, tumor angiogenesis, tumor cell proliferation and metastasis (Dias et al, 2001; Coussens and Werb, 2002; Payne and Cornelius, 2002). Genetic variations in chemokine receptor genes can affect several chemokine functions including the control of leukocyte infiltration into tumors and the initiation of primary tumor growth and survival (Ghilardi et al., 2008). It has been documented that 32 base pair deletion in the coding region of *CCR5* might alter the expression or function of the protein (Sidoti et al., 2005). It has been implicated in a variety of immune-mediated diseases (Yang et al., 2004; Kaimen-Maciel et al., 2007). The molecular basis of the protective effect of *CCR5Δ32* is poorly understood. It is still unclear, whether the *CCR5Δ32* deletion may have an effect on the expression of genes which communicate immunological responses or the protective effect of the *CCR5Δ32* deletion is completely caused by the lack of functional CCR5.

In the present case control study, genotypes and allele frequencies of CCR5/*Δ32* polymorphism did not differ significantly between esophageal cancer patients and control individuals (p>0.05). Thus *Δ32* was not a risk factor for esophageal cancer. Similar to our report, no correlation has been reported between *CCR5Δ32* deletion and inflammatory bowel disease (Rector et al., 2001) and gastric cancer (Gawron et al., 2011). This was contrary to previous reports where CCR5/*Δ32* was reported to confer significant risk for gall bladder cancer (Srivastava et al., 2008), cervical cancer (Singh et al., 2008) and was an independent risk factor for the development of breast cancer (Degerli et al., 2005).

High frequency of *CCR5Δ32* allele has been reported in several Caucasian populations (Lucotte, 1997; Martinson et al., 1997; Libert et al., 1998; Stephens et al., 1998). In the present study, the frequencies of CCR5/*Δ32* heterozygotes and *Δ32* /*Δ32* homozygotes were 1.71% and 0.57% in the control group even though the population has a Caucasian and Indoscythian admixture (Bhasin et al., 1992). A complete absence of *Δ32*/*Δ32* homozygotes and low frequency of CCR5/*Δ32* heterozygotes between 0-6% have previously been reported in some populations of North West India (Majumder and Dey, 2001).

It has been reported that heterozygous subjects express a lower amount of functional receptors as compared to wild type homozygotes (Benkirane et al., 1997). *CCR5* disruption has been demonstrated to inhibit tumor growth in pancreatic cancer (Tan et al., 2009). Mice expressing *CCR5* have showed enhanced local tumor growth and an impaired response to vaccine therapy as compared to knockout mice (van Deventer et al., 2005). Blocking the homing of cancer cells to metastatic sites is a desirable characteristic in a true antimetastatic drug (Perret

and Crepin, 2008). *CCR5* inhibition with Maraviroc or Vicriviroc reduces breast cancer cellular invasion without affecting cellular viability indicating that *CCR5* antagonists might be useful as adjuvant therapy for basal breast tumors with *CCR5* overexpression (Velasco-Velazquez et al., 2012). *CCR5* inhibitors have been reported to prevent cancer cell growth in prostate cancer (Zhang et al., 2010), hepatoma cells (Sutton et al., 2007), and lung cancer (Borczuk et al., 2008). In the earlier study, we reported significant association of RP genotype of p.R72P polymorphism of TP53 with increased risk of esophageal cancer in North Indians (Kaur et al., 2014). In the present study, we did not observe any difference in the allele frequency of *Δ32* allele in esophageal cancer patients and control group, *CCR5Δ32* deletion may not be associated with risk to esophageal cancer in the studied population. Also majority of the subjects being homozygous for *CCR5* wild type allele, it may be worthwhile to use antiviral drug therapy as adjuvant therapy in these patients.

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