Distribution of the chemokine receptor-5 gene in Egyptian breast cancer patients

Amal I.A. Youssef, Amal A. Hassan, Shuzan A. Mohammed, Hebat Allah E.M. Ahmed

Medical Biochemistry and Head of Molecular Biology Unit, Faculty of Medicine, Benha University, Benha, Egypt

Correspondence to Hebat Allah Emam Mohammed Ahmed, MBBCh, El-Shorouq City, Cairo, 11837, Egypt Tel: 01110465803; e-mail: heba.e.attallah@gmail.com

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Background
Chemokine receptor type-5 (*CCR5*)-Δ32, a 32-base pair deletion of the C–C CCR5 gene, is associated with slowed human immunodeficiency virus disease progression in heterozygotes and protection against infection in homozygotes between carriers and noncarriers of each genetic variant.
Aim
The present study aimed to investigate the frequency of the *CCR5*-Δ32 mutation in Egyptian breast cancer (BC) patients.
Materials and methods
We determined the genotypic frequency of wild and mutant variants of *CCR5* in 40 BC patients and 20 healthy individuals using restriction fragment length polymorphism and reverse hybridization.
Results
We found the absence of heterozygous and homozygous mutant gene variants in both BC patients and controls.
Conclusion
No significant difference in the frequency of *CCR5* genotypes and alleles between patients and controls and no association between that gene and BC.

Keywords:
breast cancer, chemokine receptor-5, polymorphism

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Introduction

Breast cancer (BC) is one of the most common malignant diseases in the world. BC has a highest mortality worldwide among women, accounting for 522 000 deaths in 2012 [1]. Among all malignancies, BC is the most common cancer in Egyptian women. It represents about 38% of all reported cancer cases in Egyptian women, with an average age of 49.6 per 100 000 populations [2]. It has been reported that there is a relationship between inflammation and cancer. Leukocyte infiltration in tumor mediated by chemokine and chemokine receptors is regarded as one of the major factors in tumor proliferation, invasion, and progression [3]. In this case, CCL5 and its chemokine receptor type-5 (*CCR5*) have markedly been considered in this process [4].

The C–C *CCR5* is related to the superfamily of the seven-transmembrane G-protein-coupled receptors [5]. It interacts with chemokines that mediate the trafficking and function of memory/effector T-lymphocytes, macrophages, and immature dendritic cells toward the sites of inflammation [6]. After its activation with chemokine ligands, *CCR5* are rapidly phosphorylated at serine and threonine residues within the C-tail and the third intracellular loop [7]. When bound by their ligands, these receptors can be internalized, impairing the subsequent ability to bind their ligands. Once internalized, these receptors tend to recycle to the cell surface in time. Most chemokines activate more than one receptor subtype and like other chemokine receptors, *CCR5* can also bind several chemokines [8,9]. Apart from the role in HIV infection, the *CCR5*-Δ32 mutation seems to have a role in BC.

It was proved that *CCR5* and its ligand CCL5 interaction involved in signaling pathway in BC cell proliferation, cell invasion, metastasis, and angiogenesis [10,11]. One of the most common polymorphism in the *CCR5* gene is a 32 bp deletion polymorphism (denoted as delta *CCR5* or *CCR5*-Δ32). *CCR5*-Δ32 polymorphism leads to a premature stop codon and results in a nonfunctional form of the chemokine receptor that is unable to bind CC chemokine ligands such as CCL5 [12,13] and subsequently leading to major defects in the chemotaxis mediated by these ligands [14]. The aim of this study was

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to determine whether the genetic variant *CCR5*-Δ32 has a relation to BC susceptibility.

Materials and methods

Study participants

Peripheral blood samples were obtained from 20 healthy volunteers free from cancer and 40 women who were clinically and histopathologically diagnosed as BC of different stages. The study was carried out after receiving approval from the ethics committee of Benha Faculty of Medicine and after obtaining informed consent from the included participants, the participants were recruited from the General Surgery Department Faculty of Medicine, Benha University Hospital.

Sample collection and DNA isolation

Peripheral blood samples were collected in a volume of 3 ml and genomic DNA was extracted using 50 ml of lysis solution and 5 ml GENxTRACT resin (ViennaLab Diagnostics, A-1120, Vienna, Austria).

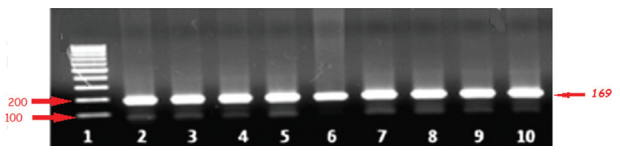
Polymerase chain reaction amplification

Amplification was performed in a single reaction using biotinylated primers (HVD strip kit; HVD Life Science, HVD Vertriebs-Ges.m.b.H., Wurzbachgasse 18, Vienna, Austria). All PCR reagents and DNA templates were kept refrigerated whereas all steps were performed until start of the thermal cycling program, using a G-storm thermocycler.

After getting our target amplified, we went through two assays:

(1) Reverse hybridization: which was performed at 45°C; in a thermoshaker plate.
(2) PCR-restriction fragment length polymorphism: which is performed by PCR with appropriate PCR primers [15] that flank the 32 bp deletion without using restriction endonuclease. The primer set: 5′-AGG TCT TCA TTA CAC CTG CAG C-3′ and 5′-CTT CTC ATT TCG ACA CCG AAG C-3′ were used to amplify a fragment of 169 bp for wild-type and 137 bp for the mutant variant (Fig. 1). The *CCR5*-Δ32 variant was detected by electrophoresis on 3% agarose gel and using ethidium bromide staining. PCR was done for 35 cycles, consisting of a 94°C for 3 min as initial denaturation, denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and further extension at 72°C for 5 min.

Figure 1



PCR results of chemokine receptor type-5 gene on 3% agarose gel. Lane 1: DNA ladder (marker). Lane 2–10: one single 169 bp band in wild-type homozygotes, no mutant type detected.

Statistical analysis

The analysis of data was determined by χ^2-test and Student's *t*-test was used to compare the mean for continuous variables. Microsoft excel 2016 was used. Results were considered significant at *P* value of less than 0.05.

Results

In this case–control study, 40 clinically confirmed breast ductal carcinoma cancer patients and 20 unrelated healthy control individuals were analyzed. The mean ages of patients and controls were 49.9±12.84 and 45.9±11.66 years, respectively. The selected characteristics of the cases and controls are presented in Table 1.

The clinical pathological features of the IDBC group are summarized in Table 2. It observed that more than 90% of patients had positive hormonal receptors

Table 1 Baseline characteristics of breast cancer patients and controls

Variables	BC patients (*n*=40) [*n* (%)]	Controls (*n*=20) [*n* (%)]	*P* value
Age (years) (mean±SD)	49.9±12.84	45.9±11.66	0.386
Menopausal status at diagnosis			0.92
Premenopausal	22 (55)	10 (50)	
Postmenopausal	18 (45)	10 (50)	
Marital status			0.59
Single	8 (20)	6 (15)	
Married	32 (80)	14 (85)	
Systemic diseases			0.78
No systemic disease	16 (40)	10 (50)	
HTN	11 (27.5)	6 (30)	
DM	6 (15)	2 (10)	
HTN+DM	7 (17.5)	2 (10)	
Smoking			0.53
Yes	2 (5)	1 (5)	
No	38 (95)	19 (95)	
Contraception methods			0.79
No contraception	25 (62.5)	10 (50)	
IUCD	10 (25)	7 (35)	
Pills	5 (12.5)	3 (15)	

BC, breast cancer; DM, diabetes mellitus; HTN, hypertension; IUCD, intrauterine contraceptive device.

Table 2 Clinical pathological features of breast cancer patients

	n=40 [*n* (%)]
Estrogen receptor status	
ER positive	37 (92.5)
ER negative	3 (7.5)
Progesterone receptor status	
PR positive	38 (95)
PR negative	2 (5)
HER2 neu status	
HER2 neu positive	37 (92.5)
HER2 neu negative	3 (7.5)
TNM stage at diagnosis	
I	2 (5)
II	10 (25)
III	13 (32.5)
IV	15 (37.5)
Lymph node status	
N0	2 (5)
N1	11 (27.5)
N2	20 (50)
N3	7 (17.5)
Tumor size	
≤5 cm	26 (65)
>5 cm	14 (35)
Tumor grade	
I	7 (17.5)
II	22 (55)
III	11 (27.5)

ER, estrogen receptor; HER2, human epidermal growth factor receptor-2; PR, progesterone receptor; TNM, tumor, node, metastasis.

(estrogen receptor, progesterone receptor, and human epidermal growth factor receptor-2-neu receptors). The majority of patients had node-positive and tumor, node, metastasis stage II disease.

Genotype distribution of *CCR5*-Δ32 polymorphisms are shown in Table 3. The absence of mutant allele was observed in our study.

Gel electrophoresis confirmed the absence of a mutant variant in our study as shown in Fig. 1.

Discussion

Chemokines and chemokine receptors are expressed by many tumor cells, and these molecules can affect both tumor progression and antitumor immune response. Genetic polymorphisms of some chemokine receptors including the *CCR5* receptor gene were found to be closely related to cancer development or involved in metastatic process, including BC [4,16].

Our data did not find any association between *CCR5*-Δ32 polymorphism and BC as the mutant allele was not found in our participants (neither homozygous nor heterozygous).

Table 3 Genotypes of chemokine receptor-5 polymorphisms in this study

Variables	Patients (*n*=40) [*n* (%)]	Controls (*n*=20) [*n* (%)]
$CCR5^{+/+}$ (wild-type homozygous)	40 (100)	20 (100)
$CCR5^{+}$/Δ32 (heterozygous mutant)	0 (0.0)	0 (0.0)
CCR5 Δ32/Δ32 (mutant-type homozygous)	0 (0.0)	0 (0.0)

CCR5, chemokine receptor-5.

These results were in agreement with Salem and Batzer [17], Martinson *et al.* [18], and Voevodin *et al.* [19] who reported that *CCR5*-Δ32 allele was expected to be completely absent among Egyptians from the Sinai, Sudanese, and Yamanis. These findings might not reflect the real frequency of this deletion among the studied populations due to the small size of samples (? 100).

Moreover, this deletion was completely absent to extremely low among individuals from Venezuela, Central and Western Africa, Japanese, Filipino, Korean, Chinese, Brazilian, Indians, and African-Americans [20].

The distribution of the *CCR5*-Δ32 allele varies widely with 21.7% in North American Caucasians, 6.9% in Hispanics, 5.8% in African-Americans, 5.3% in Colombia, 2% in North Africa, and 0.6% in Asian-Americans [21].

In Arabic countries, the frequency of the *CCR5*-Δ32 polymorphism is very low in these populations. Within the Middle East region, the frequency was found to be 2.8% among Bahrainis, followed by 2.5, 2.4, 2.1, and 0.6% in Lebanese, Iranians, Saudis, and Jordanians, respectively [22–25]. Low frequencies have been observed among Kuwaitis, Syrians, and Egyptians from Ismailia (1, 0.6, and 0.5%, respectively) [17,19].

However, other studies reported an association between the *CCR5*-Δ32 allele and BC. Degerli *et al.* [26] studied Δ32 allele of *CCR5* gene in BC and other tumors. They demonstrated that the heterozygote genotype is an independent risk factor for the development of BC.

Guleria *et al.* [14] and Aoki *et al.* [27] have also found no association for Δ32 allele in relation to BC increased susceptibility in Indian and Turkish populations,

respectively.Studies have shown that chemokine receptor systems play an important role in tumor progression [4]. Tumor cells secrete chemotactic factors in a variety of processes, which mediate tumor cell invasion and metastasis through binding with corresponding receptors [28].

Moreover, Azenshtein *et al.* [29] have found that Regulated on Activation, Normal T Expressed and Secreted (RANTES) expression in the *CCR5* ligand occurs in breast tissue and BC cell lines T47D and MCF-7. It is also associated with the degree of malignancy of BC and disease course. The higher the degree of malignancy and the later the course of the disease, the higher is the expression of RANTES. These results indicate that RANTES is involved in BC.

Using immunohistochemical methods Tan [30] has found that high *CCR5* mRNA expression in human BC stem cells accompanied stronger invasion and metastasis capabilities than those of BC cells, which may be a key factor in BC metastasis.

In another study Mañes *et al.* [31] have found that *CCR5* expression is negatively correlated with p53 wild-type gene expression in the progression of breast tumors. *CCR5* may be involved in the progression of BC in a p53 gene mutation-mediated process.

Conclusion

According to the *CCR5* gene, the involvement of *CCR5* gene polymorphism in human immunodeficiency virus pathogenesis and treatment is currently a focus of attention; however, research on the association of *CCR5* gene polymorphism and BC is relatively limited. More studies with a bigger sample size are needed to show the effect of the *CCR5* gene in BC.

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Conflicts of interest

There are no conflicts of interest.

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