Volume : 4 | Issue : 1 | January 2015 • ISSN No 2277 - 8179

Research Paper

Frequency of 32 bp deletion in Chemokine Co-receptor CCR5 of Hypertension patients

Microbiology

KEYWORDS : Chemokines, Mutation, Hypertension, Diabetes.

Prasad.M.P

Department of Microbiology/Biotechnology, Sangenomics Research Lab, Domlur Layout, Bangalore 560071

ABSTRACT

Chemokines are important regulators in the development, differentiation, and anatomic location of leukocytes and they help in determination of the cells that cross the endothelium and move within the tissue. Chemokines activate leukocytes by binding to selective, seven transmembrane domain, G protein-coupled receptors present on the plasma membrane. The CCR5 protein functions as a chemokine receptor in the CC chemokine group. The natural chemokine ligands that bind to this receptor are RANTES, MIP-1α and MIP-1β. CCR5 is predominantly expressed on T cells, macrophages, dendritic cells and microglia. The CCR5 has multiple variants in its coding region, the deletion of a 32-bp segment results in a nonfunctional receptor, thus preventing HIV R5 entry. In the present study the association between chemokine receptor gene polymorphisms and hypertension was determined. The mutation of CCR5 gene was detected in hypertension patient blood samples collected from various hospitals in and around Bangalore. The study was carried out using DNA isolated from the blood samples with primers related to CCR 5 gene. The PCR product length was found to be 990.53 base pairs. It was found that twelve samples out of twenty showed polymorphism and rest of the samples did not indicate any polymorphism. This suggests that CCR5 mutation is associated with hypertension.

INTRODUCTION:

Chemokines are one of the important regulators in the development, differentiation, and anatomic location of leukocytes. These Chemokines are a group of at least 40 small cytokines involved in cell migration, activation and chemotaxis. These receptors belong to the family of G protein coupled hepta-helical receptors and are primarily expressed on hemopoietic cells. Chemokines are pro inflammatory cytokines with the ability to attract and activate leukocytes. They also modulate the functions of numerous other cell types.

Chemokine receptor 5 is a protein, they encodes the proteins in humans by the *CCR5* gene which is located on chromosome 3 on the short (p) arm at position 21, represented as p21.3-p24. CCR5 has also recently been designated as CD195. The CCR5 protein functions as a chemokine receptor in the CC chemokine group. CCR5-Δ32 is a deletion mutation of a gene that has a specific impact on the function of T cells. CCR5-Δ32 is widely dispersed throughout Northern Europe and in those of Northern European descent. While CCR5 has multiple variants in its coding region, the deletion of a 32-bp segment results in a non functional receptor, thus preventing HIV R5 entry; two copies of this allele provide strong protection against HIV infection.

Homozygotes for a 32-bp deletion allele of *CCR5* (*CCR5*-D*32*), causes a frame shift at amino acid 185, which are relatively resistant to HIV-1 infection (Mary Carrington et al., 1997).

Ahuja *et al*, 1997 studied that the Human CC chemokine receptor 5 (CCR5), mediates the activation of cells by the chemokines macrophage inflammatory protein-1a, macrophage inflammatory protein-1b, and RANTES, and serves as a fusion cofactor for macrophage-tropic strains of human immunodeficiency virus type 1.

Zlotnik and Yoshie, 2000, suggested that many chemokines are clustered in certain chromosomal locations. Two main clusters have been recognized. Many human CXC chemokines that mainly act on neutrophils are clustered at chromosome 4q12–13, while many CC chemokines that mainly act on monocytes are located in another cluster at 17q11.2.

Grimaldi *et al*, 2005, investigate the Deletion of 32bp in the CCR5 gene has been shown to confer resistance to infection by HIV-1 R5 strains. Another mutation, characterized by a thymine to adenine (T to A) nucleotide substitution at position 303 (m303), has shown the same effects as the delta Δ32 mutation, with previous studies having shown that the allele frequency of the CCR5-m303 mutation is 0.014 in African-American and 0.007 in French populations.

MATERIALS AND METHODOLOGY:

Sample collection: The blood samples were collected from hospitals in and around Bangalore in sterile conditions. In the present study, 21 samples were used. Of these 20 were collected from patients having hypertension and one sample is used as control. The blood samples were stored at 4°C.

Isolation of DNA from blood samples by phenol chloroform method: 0.2 ml of blood and 0.8ml of reagent A was mixed at Centrifuged at 1000 rpm for 10 min. The supernatant was discarded and cells were collected. 0.2ml of reagent B and 0.025 ml reagent C was added to the pellet and mixed well. 0.06 ml Tris phenol and 0.06 ml of chloroform and isoamyl alcohol mixture was added and centrifuge at 8000 rpm for 10 min. 0.06 ml chloroform was added to the aqueous layer and Centrifuged at 8000 rpm for 10 min. To the upper aqueous phase double volume of cold absolute alcohol was added and incubated for overnight in 4°C. The samples were then Centrifuged at 12,000 rpm for 10 min and the pellet was dissolved in 80-100 µl of TE buffer and Stored at 4°C for further investigation. The qualitative analysis was carried out with 1.0% agarose gel electrophoresis and quantitative estimation was done using Nanodrop 1000.

Polymerase Chain reaction: The polymerase chain reaction was carried out for the isolated DNA with primers for ccr 5 gene. The PCR conditions for the primer were standardized. The initial denaturation was carried out at 95° C for 5 min. Final denaturation at 94 °C for 30 seconds, Annealing temperature was 62 °C for 30 seconds and Extension was at 72 °C for 30 seconds. This cycle was repeated for 30 cycles. The final elongation was carried out at 72 °C for 10 mins. The PCR products were determined on 1.5% agaore gel.

RESULTS:

The DNA was isolated by phenol chloroform method and the DNA was run on 1% agarose. The quantification of DNA is done by nanodrop analysis.

Each sample was isolated in duplicates. And each tube was measured for its purity and DNA concentration. The results are as above. Purity of DNA was determined using the A260/A280 absorption formula. For DNA, the purity is indicated by a value of 1.5 to 1.8. Anything greater than 1.9, indicates RNA contamination.

Volume : 4 | Issue : 1 | January 2015 • ISSN No 2277 - 8179

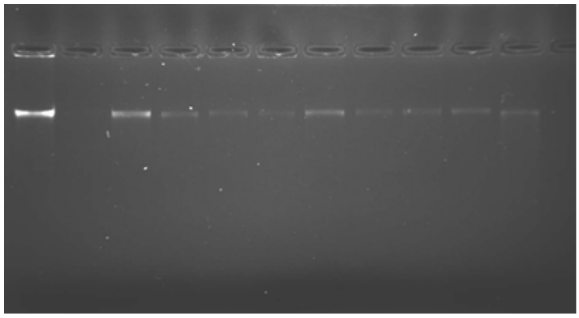

Figure 1: The Qualitative analysis of DNA
Lane 1 shows the control blood (C), while the other lanes were loaded with diabetes with hypertension blood DNA.

PCR: The control (C) showed no bands, indicating no polymorphism of gene. 12 Samples showed clear bands which indicated the polymorphism in the same position in the gene in all the samples. The PCR product was of a 990.53 kb in size (Figure 2-5).

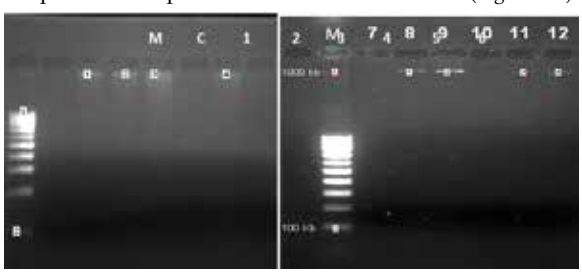

Figure-2: PCR products run on Agarose gel for control and samples 1 to 12.

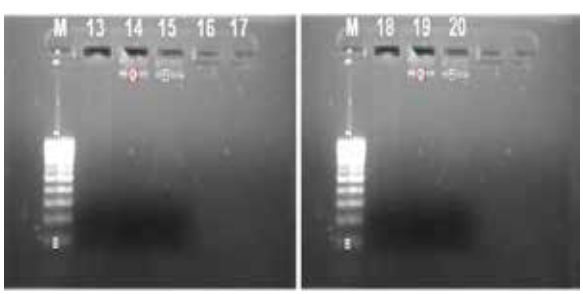

Figure-4: PCR products run on Agarose gel for samples 13 to 20.

DISCUSSION:
The mutation in the CCR5 gene has been having an increased risk of developing hypertension. In this study showed an association between chemokine receptor gene polymorphisms and hypertension. The mutation of CCR5 gene was detected in hypertension patient blood samples.12 samples showed polymorphism out of 20 samples and rest of the samples were not indicating polymorphism. The present investigation results is in concordence with preliminary evidence from a phenotypic expression study of subjects with a CCR5 (Delta) 32/CCR5 (Delta) 32 homozygous genotype, in whom hypertension was one of the most frequent diagnoses (Nguyen et al, 1999).

Giang et al, 1999 studied that the Hypertension and conditions attributable to hemophilia were the diagnoses found to be present in clinical records of CCR5-[DELTA] 32/ [DELTA] 32 study subjects. The blood pressure measurement and treatment history, CCR5 homozygotes has a 2.8-fold higher prevalence of hypertension than age-matched CCR5-+/+ study subjects. The blood pressure variation is usually determined by genetical analysis, the mendelian forms of hypertension result from rare alleles that cause severe hypertension by altering the renin-angiotensin system. It may be noteworthy that CCR5 and AT1, the major angiotensin receptor, are both seven-transmembrane G protein-coupled receptors and that these two receptors share 60% amino acid homology. If the association between CCR5-Δ32/Δ32 homozygosity and hypertension is verified in other populations, a possible interaction with AT1 should be explored (Lifton, 1996).

S Hummel et al., 2005, report amplifications of the CCR5-D32 DNA sequence from up to 2900-year-old skeletal remains from different burial sites in central Germany and southern Italy. Furthermore, the allele frequency of CCR5-D32 in victims of the 14th century plague pandemic in northern Germany was not different from a historic control group.

Bush et al, 2000 investigated and reported a critical role for macrophage recruitment in the pathogenesis of hypertension, experimental support by studying in mice deficient for the CCR2 gene. This and other results implicate the CCR genes in several pathogenic pathways involving the immune system through vascular hypertrophy and macrophage infiltration.

REFERENCE

1. Grimaldi, R, Acosta, AX, Cabral-Oliveira, FC, Brites, C, Galvão-Castro, B. (2005). "Study of the CCR5-m303 mutation in three different ethnic groups from Brazil". Genetics and Molecular Biology, 28, 2, 214-217. | 2. Zlotnik, A and Yoshie, O. (2000). "Chemokines: A New Classification System and Their Role in Immunity". Immunity. 12, 121–127. | 3. Ahuja, S.S, Mummidi, S, Mc Daniel, B.L, Ahuja, S.K. (1997). "The Human CC Chemokine Receptor 5 (CCR5) Gene". J Biol Chem; 272 (49): 30662–30671. | 4. Nguyên, Giang T.; Carrington, Mary; Beeler, Judith A.; Dean, Michael; Aledort, Louis M.; Blatt, Philip M.; Cohen, Alan R.; DiMichele, Donna; Eyster, M. Elaine; Kessler, Craig M.; Konkle, Barbara; Leissinger, Cindy; Luban, Naomi; O'Brien, Stephen J.; Goedert, James J.; O'Brien, Thomas R.(1999). "Phenotypic Expressions of CCR5-[DELTA]32/[DELTA]32 Homozygosity", JAIDS, 22;75-82. | 5. Francis Barin, Gonzague Jourdain, Sylvie Brunet, Nicole Ngo-Giang-Huong, Supawadee Weerawatgoompa, Warit Karnchanamayul, Surabhon Ariyadej, Rawiwan Hansudewechakul, Jullapong Achalapong, Prapap Yuthavisuthi, Chaiwat Ngampiyaskul, Sorakij Bhakeecheep, Chittaphon Hemwutthiphan, Marc Lallemant and the Perinatal HIV Prevention Trial Group(2006). "Revisiting the Role of Neutralizing Antibodies in Mother-to-Child Transmission of HIV-1", Maternal Antibodies and Transmission of HIV-1, JID; 193 (1 June), 1504-11. | 6. Lifton RP.(1996). Molecular genetics of human blood pressure variation. Science 272:676–680. | 7. Mary Carrington, Teri Kissner, Bernard Gerrard, Sergey Ivanov, Stephen J. O'Brien, and Michael Dean, (1997). "Novel Alleles of the Chemokine-Receptor Gene CCR5", Am. J. Hum. Genet. 61:1261–1267, | 8. S Hummel, D Schmidt, B Kremeyer, B Herrmann and M Oppermann, (2005). "Detection of the CCR5-D32 HIV resistance gene in Bronze Age skeletons", Genes and Immunity; 6, 371–374. |