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*Original Article*

# Distribution of monocyte chemoattractant protein-1 (MCP-1 A-2518G) and chemokine receptor (CCR2-V64I) gene variants in hyperbilirubinemic newborns

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**Abstract:** Hyperbilirubinemia is one of the most crucial syndromes, which is characterized by high levels of bilirubin, especially when it occurs in newborns. Bilirubin has cytoprotective properties with an antioxidant function and plays several major roles in the inflammation process with its members such as chemokines. The monocyte chemoattractant protein-1 (MCP-1) is a member of the C-C chemokine family and it has been associated with the inflammatory process. There are no data on the chemokine and its receptor genotypes in hyperbilirubinemic newborns to show their distribution. The aim of this study is to investigate the genotypic relationship of MCP-1 and its receptor CCR2-V64I with hyperbilirubinemia in Turkish newborns. A total of 85 newborns were included in the study: 20 infants with hyperbilirubinemia (hyperbilirubinemic group) and 65 infants without hyperbilirubinemia (non-hyperbilirubinemic group). Genotyping of MCP-1 A-2518G and CCR2-V64I gene polymorphisms were detected by PCR-RFLP, respectively. MCP-1 GG genotype in patients was higher than the controls and this genotype had 2.69 times higher risk for hyperbilirubinemic neonates (P: 0.20). The frequency of MCP-1 A-2518G G+ genotype in patients was higher than the controls (55.0% and 38.5%, respectively). The results of our preliminary study suggest that MCP-1 G+ genotype has the ability to increase the hyperbilirubinemia risk of newborns. These results should be focused on to research on a larger scale to confirm the findings.

**Keywords:** CCR2, hyperbilirubinemia, MCP-1 and newborn

## Introduction

Hyperbilirubinemia is one of the most important and critical syndromes in newborns. Even though the hyperbilirubinemia causes critical health problems, it becomes more important when it occurs during the neonatal stage. Neonatal hyperbilirubinemia results from the larger hemoglobin mass, a lower plasma albumin level, an immature biliary secretory system, and the absence of bacterial flora. It might be occur due to the risks of blood incompatibilities, deficiency of glucose-6-phosphate dehydrogenase (G6PD), and pyruvate kinase, hereditary spherocytosis, or defective hemoglobin synthesis [1]. Hyperbilirubinemia may also have protective role in preventing hepatocellular damage in cholestasis, by counteracting bile-acid induced apoptosis in hepatocytes and suppressing the generation of reactive oxygen species (ROS) by the cells [2]. Hyperbilirubinemia is characterized by a high level of bilirubin [3]. Bilirubin is known for its serious role in the inflammation process. The antiinflammatory, antiapoptotic, antiproliferative, and antioxidative effects of biliverdin (BV) and bilirubin, and their effects on the immune response can be very important in many diseases.

The inflammatory response and immune-cell infiltration are induced by several cytokines, chemokines and their receptors [4]. Chemokines are important determinants of an early inflammatory response. They regulate cell trafficking in the organism as migration and infiltration of monocytes/macrophages. Monocyte chemoattractant protein-1 (MCP-1) is the best known of the C-C chemokine family. MCP-1 is located at 17q11.2 and its receptor is named CCR2. MCP-1 and its receptor CCR2 have been researched in various diseases. The research

**Table 1.** PCR and RFLP procedures and products of MCP1 and CCR2

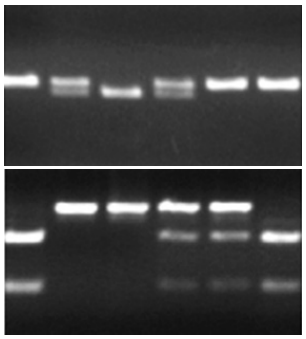
| | Primers (forward and reverse) | Restriction enzyme | Restriction products | |
|---|---|---|---|---|
| MCP1 | 5'-TCTCTCACGCCAGCA CTGACC-3' | PvuII | 149 bp, 173 and 24 bp bands |  |
| | 5'-GAGTGTTCACATAGG CTTCTG-3' | | | |
| CCR2 | 5'-TTGGTTTTGTGGGCAACATGATGG-3' | BseJI | 234 bp, 159 and 75 bp bands | |
| | 5'-CATTGCATTCCCAAAGACCCACTC-3' | | | |

**Table 2.** Demographic characteristic of newborns enrolled in the study

| | Hyperbilirubinemia | Non-hyperbilirubinemia | *P* Value |
|---|---|---|---|
| Number of neonates | 20 | 65 | |
| Peak TSB (mg/dl) | 16.70 ± 3.97 | 8.45 ± 2.58 | 0.000 |
| Gestational week | 38.6 ± 1.08 | 37.9 ± 1.02 | 0.012 |
| Birth weight (g) | 2970.5 ± 425.5 | 3382.4 ± 444.4 | 0.001 |
| Male/female (n) | 9/11 | 37/28 | NS |
| Fed | | | |
| Breast fed, n (%) | 13 | 49 | |
| Breast + Bottle fed, (%) | 7 | 15 | NS |
| Bottle fed, n (%) | 0 | 1 | |

has shown the relationship between MCP-1 and the effects of chemokines on the disease development, hematopoiesis, cell trafficking and homing, angiogenesis and malignancy such as the diseases like autoimmune disorders, pulmonary disease, transplant rejection, and cancer and vascular disease [5]. CCR2 has dual roles in both the pro-inflammatory and anti-inflammatory actions. The pro-inflammatory role of CCR2 is dependent on antigen presenting cells and T-cells, whereas the anti-inflammatory role of CCR2 is dependent on CCR2 expression in regulatory T cells. Several polymorphisms of chemokine and chemokine receptors have been found to the dysregulate chemokine system, suggesting that they may interfere with inflammatory processes and other diseases [6-9].

In this context, we aimed to show that the genotypic distribution of the monocyte chemoattractant protein-1 (MCP-1 A-2518G) and chemokine receptor (CCR2-V64I) and their relationship could be determinative for inflammatory and/or anti-inflammatory effects in hyperbilirubinemic newborns.

## Material and method

### *Subjects*

*A total of 85 newborns were included in the study:* 20 infants with hyperbilirubinemia (hyperbilirubinemic group) and 65 infants without hyperbilirubinemia (non-hyperbilirubinemic group). The study was approved by the Ethics Committee of Istanbul University, Istanbul Faculty of Medicine, and conducted in accordance with the Declaration of Helsinki. In this study, neonates with hyperbilirubinemia were defined as newborn infants with a peak total serum bilirubin (TSB) level of more than 15 mg/dl in the first 10 days of life. The control group consisted of 65 newborns with a peak TSB level less than 15 mg/dl in the first 10 days of life. All infants were born at 37-42 weeks' gestation and they had no risk additional factors (maternal diabetes, polycythemia, infection, birth asphyxia, hypothermia, hypoglycemia, cephalohematoma, hypothyroidism, glucose-6-phosphate dehydrogenase (G6PD) deficiency or hemolysis for any reason).

### *Polymorphism analysis*

Genomic DNA was extracted from isolated lymphocytes using a standard nonorganic procedure [10]. The extracted DNA was used for characterization of the following polymorphic DNA repair genes. Polymerase chain reaction (PCR) was used to analyse the polymorphic

**Table 3.** The distribution of MCP-1 A-2518G and CCR2-V64I genotype frequencies are in patients and control groups

| Genotype/Allele | Controls N: 65 | % | Patients N: 20 | % | *P*-value |
|---|---|---|---|---|---|
| MCP-1 A-2518G | | | | | |
| AA | 40 | 61.5 | 9 | 45.0 | |
| GG | 4 | 6.2 | 3 | 15.0 | |
| AG | 21 | 32.3 | 8 | 40.0 | 0.29 |
| A + (AA + AG) | 61 | 93.8 | 17 | 85.0 | |
| A-(GG) | 4 | 6.2 | 3 | 15.0 | 0.20 |
| G + (GG + AG) | 25 | 38.5 | 11 | 55.5 | |
| G-(AA) | 40 | 61.5 | 9 | 45.0 | 0.19 |
| CCR2-V64I | | | | | |
| wt/wt | 47 | 72.3 | 16 | 80.0 | |
| 64I/64I | 5 | 7.7 | 0 | 0 | |
| wt/64I | 13 | 20.0 | 4 | 20.0 | 0.43 |
| Wt + ( wt/wt + wt/64I) | 60 | 92.3 | 20 | 100 | |
| Wt-(64I/64I) | 5 | 7.7 | 0 | 0 | 0.25 |
| 64I + (64I/64I + wt/64I) | 18 | 27.7 | 4 | 20.0 | |
| 64I-(wt/wt) | 47 | 72.3 | 16 | 80.0 | 0.35 |

regions of the PCR products by using suitable primers for MCP1 and CCR2 regions [11, 12].

Restriction fragment length polymorphism (RFLP) was performed for genotyping by digesting the PCR products with PvuII for MCP1, BseJI for CCR2, respectively. The PCR products were visualized using electrophoresis through a 2% agarose gel. The size of the PCR products was defined through three distinct patterns of bands seen on the gel for both polymorphisms. For CCR2, 149 bp, 173 and 24 bp; for MCP-1, 234 bp, 159 and 75 bp bands were evaluated, respectively (**Table 1**).

*Statistical analysis*

SPSS 11.0 software was used for statistical analysis. The limit of statistical significance was $P < 0.05$. Chi square test and Fisher test were used to assess the differences of genotype and allele frequency between the two groups. Comparison of intergroup demographic data was determined using Student's t-test. Allele frequencies were performed using the gene counting method.

## Results

We classified our patients with regard to hyperbilirubinemic newborns and the demographic data for the study groups are given in **Table 2**.

**Table 3** shows the distributions of genotypes of MCP-1, A-2518G, and CCR2-V64I genotype frequencies among the patients and controls. Distribution of all allele and genotype frequencies among patients and control subjects were in accordance with Hardy-Weinberg equilibrium except CCR2 control group. Nevertheless, there were no findings observed that were statistically important in MCP-1, A-2518G, and CCR2-V64I genotypes between the controls and patients ($P > 0.05$). The MCP-1 GG genotype in patients were higher than the controls and this genotype had a 2.69 times higher risk for the hyperbilirubinemic neonates (P: 0.20, OR: 2.69, 95% CI: 0.54-13.20). Moreover, frequencies of MCP-1 and A-2518G G+ genotypes were higher in patients than the controls (55.0% and 38.5%, respectively). According to the results, MCP-1 G+ genotype has the ability to increase the risk for neonates (P: 0.19, OR: 1.96, 95% CI: 0.71-5.38). Multivariate logistic regression analysis also has been analyzed and there were no significant results have been found (**Table 4**).

## Discussion

Hyperbilirunemia is clinically important situation when it identified in newborns and makes the disease important and open access for severe studies to understand which molecular marker could be used for the hyperbilirubinemic newborns.

In our study there were increasing frequencies in MCP-1, GG, and G+ genotypes in patients although no statistically meaningful results were found. It is thought that MCP-1, GG, and G+ genotypes may be related to the development of hyperbilirubinemia.

Chemokines play a major role in the recruitment of monocytes, neutrophils, and lymphocytes with chemotaxis through the activation of G-protein-coupled receptors (GPCRs) in inflammation and tissue injury [13]. MCP-1 is one of the members of the chemokine family that is well-known and is the most investigated [14]. As reported earlier, A2518G polymorphism in the regulatory region of the MCP-1 gene impacts on MCP-1 expression in response to inflammatory stimulation [15]. MCP-1 is mediated by the CC chemokine receptor 2 (CCR2) as a G-coupled

**Table 4.** Results of multivariate logistic regression analysis

| Variables in the equation | | B | S.E. | Wald | Sig. | Exp (B) | 95.0% C.I. for EXP (B) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Step 1 | Sex | 0.444 | 0.528 | 0.706 | 0.401 | 0.642 | 0.228 | 1.806 |
| | MCP GG | 0.558 | 0.885 | 0.397 | 0.529 | 0.573 | 0.101 | 3.245 |
| | MCP G(+) | 0.580 | 0.562 | 1.065 | 0.302 | 0.560 | 0.186 | 1.685 |
| | CCR2 wt/wt | 0.362 | 0.640 | 0.320 | 0.572 | 0.696 | 0.199 | 2.439 |

Variable(s) entered on step 1: Sex, MCP GG, MCP G(+), CCR2 wt/wt.

receptor and it influences the cell. [16]. CCR2 is also the most researched receptor in the chemokine family [17]. However, monocyte chemoattractant protein-1 (MCP-1 A-2518G) and chemokine receptor (CCR2-V64I) mutations have been studied extensively in different inflammatory diseases. There are no studies in the literature about newborns.

In conclusion, our study suggests that the MCP-1 polymorphism may increase GG alleles by leading the low-level connection between MCP-1 and its receptor and elevated biological activity of the MCP-1/CCR2 system. Our preliminary study has brought us the genotypic distribution of the monocyte chemoattractant protein-1 (MCP-1 A-2518G) and its relationship may be effective to use as a molecular marker in hyperbilirubinemic newborns due to its role in anti-inflammatory processes.

Even though we found no statistically significant results, we found high frequencies of poltmorphic GG alleles which bring us it suggests that the antiinflammatory effects could be a result of MCP-1 being unable to bind tightly/effectively to its receptor. This unsuccessful union of MCP-1 and its receptor may lead to the high levels of bilirubin.

Until this topic has been researched on a larger scale, it is not possible to explain through which mechanisms genotypes of chemokines result in neonatal hyperbilirunemia.

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## Disclosure of conflict of interest

None.

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