A family screening of CD19 gene mutation by PCR-RFLP

ABSTRACT

Introduction and aim. Mutation(s) in the gene encoding the CD19 molecule affect CD19 protein expression and primary immunodeficiency (PID) occurs. The PCR-RFLP method, which is faster and cheaper than other mutation detection methods, is rarely used in the diagnosis of PID. The study aimed to genetically identify CD19 deficiency, which is a PID, using the PCR-RFLP method.

Material and methods. A total of 8 patients and two healthy controls were included in the study and the relevant region genotypes in the CD19 gene were determined by performing PCR-RFLP analysis.

Results. The index case, newborn baby and mother were also included in the study. It was determined that the index case (P6) was homozygous mutant, the newborn baby (P7) and mother (P8) had heterozygous genotype. Based on this situation, one child (P1) was found to be homozygous mutant, mother (P2), father (P3) and other children (P4 and P5) had heterozygous genotype in the family, which was determined to be related to the first case.

Conclusion. In our study, it has been shown that PCR-RFLP is a method that can be used in the diagnosis of PID by determining genotypes using PCR-RFLP, and especially in terms of rapid genetic testing of family screenings.

Keywords. CD19, PID, RFLP

Introduction

Primary immunodeficiencies (PID) are a group of diseases that result from the development and/or dysfunction of various components of the immune system. In recent years, many genes that cause PID have been identified. Primary antibody deficiencies, mainly predisposed to bacterial infections, can be caused by mutations in genes involved in B cell differentiation. Mutations in these genes inhibit immature B cell differentiation in bone marrow and cause agammaglobulinemia and low B cell in peripheral blood. Although many genes (CD20, CD21, CD81, CD225) play a role in B cell development, one of the most important is the CD19 gene. The CD19 is a gene located in the p11.2 region of chromosome 16, and consists of 15 exons and 7.41 kb length (NC_000016.10). This gene encodes the CD19 protein, a transmembrane glycoprotein of the immunoglobulin (Ig) superfamily. CD19 is also expressed in follicular dendritic cells as well as in normal and neoplastic B cells, which expression begins for the B cells in the bone marrow and continues until the plasma cell. In addition, CD19 forms a complex with CD21, CD81 and CD225 on the surfaces of mature B cells (Fig. 1).

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This complex co-operates with the B cell receptor (BCR) and stimulates both BCR-dependent and BCR-independent intrinsic B cell signaling pathways. Despite the mutation in the CD19 gene, normal B cell differentiation occurs in the bone marrow, and the absolute number of B cells in the peripheral blood is normal. However, stimulation on BCR is impaired and the number of memory B cells in peripheral blood is decreased. This results in hypogammaglobulinemia and impaired antigen-specific humoral response.\textsuperscript{7,8}

Fig. 1. B cell and CD19 complex

The first case of PID due to mutation in the CD19 gene was described by us in a 10-year-old girl.\textsuperscript{9} Following the identification of this case, a village screening of 208 cases was carried out and carrier was detected in 20 cases. Screening was performed by determining flow cytometric CD19 expressions.\textsuperscript{10} She was admitted to us from the same village that was related to the previously described patient. According to the clinical and laboratory results of the patient, it was thought that CD19 gene mutation might be present and genetic identification was aimed. In order to determine the mutation, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method which was used in DCLRE1C mutation detection.\textsuperscript{11}

Aim

Therefore, in this study, it was aimed to determine CD19 gene mutation by PCR-RFLP which is a cheap, reliable and fast method.

Material and methods

Patients

The index family has 4 children (Fig. 2). The patient was admitted to our clinic at 7 years old with complaints of frequent illness.

The history of the patient revealed that she had a fever 3-4 times a month and had a frequent fever since she started school and diagnosed at Idiopathic Thrombocytopenic Purpura (ITP) at 5 years old. Parents had a consanguineous marriage. It was learned that there was a baby who had intrauterine exitus at the age of 6 months in the family. We were learned that patient was related to the first case described by van Zelm et. al. in 2006.\textsuperscript{11} As a result of this information and laboratory examination, it was thought that CD19 gene mutation might be present. The region to be studied for mutation was selected as the previously defined region (c.973_973insA; p.Arg325AlafsX4; GenBank: AH005421.2). The patient (P1), mother (P2), father (P3), sister (P4), brother (P5), the patient identified in 2006 (P6), newborn baby of P6 (P7) and mother of P6 (P8) were included in the study. The studies were performed with 2 healthy controls (C1 and C2). Patient recruitment and the studies reported herein were approved by Institutional Review Board at the Necmettin Erbakan University Meram Medical Faculty. Written informed consent was obtained from participating patients’ guardians and healthy controls.

Flow cytometry

Peripheral lymphocyte subgroup study of the patient and family members were performed. After the study, cell counts were performed with Becton Dickinson Canto II (BD Biosciences, Heidelberg, Germany) flow cytometry and their analysis were performed. In addition, CD19 expression was performed with monoclonal antibody from three different clones (SJ25C1, 4G7, HIB19).

PCR and PCR-RFLP

Polymerase chain reaction (PCR) primers covering mutation regions in CD19 gene were designed (forward primer: 5’-CCTGAGGAGGAAAAGAAT-3’ and reverse primer: 5’-GGAAACAGTAAGTGCAAGGATA-3’). The mutation regions were amplified by PCR using these primers. A PCR protocol was used and cycling conditions were initial denaturation of 95°C for 10 minutes followed by 45 cycles of 95°C for 40 seconds, annealing beginning at 49.9°C and 72°C 15 seconds. A final extension of 72°C for 10 minutes was applied. Resulting PCR products were visualized by 1% agarose gel. DNA ampl-
DNA fragments of 118 bp were obtained after PCR amplification. The PCR products were incubated for 1 hour at 65°C with BsmI (New England Biolabs Inc.). DNA fragments obtained after PCR-RFLP procedure were visualized on 3 % agarose gel and genotypes were determined as wild type, heterozygous and homozygous mutant.

In the absence of mutation, enzyme digestion was performed in both alleles and 94 and 24 bp DNA fragments were obtained. In the case of heterozygote, three DNA fragments will be obtained: 118 bp, 94 bp and 24 bp.

Results
In the peripheral lymphocyte subgroup analysis, CD3+ total T, CD4+ helper T, CD8+ cytotoxic T and CD16+CD56+ NK cell numbers were found to be normal but there was no CD19 expression on CD20+B cells. The patient had low IgG (347 mg/dl) and IgA (25 mg/dl) levels. In addition, the hepatitis B vaccine response was negative (0.74 mIU/ml; normal range 0-10 mIU/ml). Clinical and laboratory characteristics of the P1 are shown in Table 1.

| Parameters                  | P1                                      |
|-----------------------------|-----------------------------------------|
| Clinical features on admission | Frequent illness during school Fewer ITP |
| Age of diagnosis (years)    | 7                                       |
| Lymphocytes (counts/mm³)    | 3700                                    |
| CD3+ (counts/mm³)           | 2479                                    |
| CD4+ (counts/mm³)           | 1369                                    |
| CD8+ (counts/mm³)           | 814                                     |
| CD19+ (counts/mm³)          | 0*                                      |
| CD20+ (counts/mm³)          | 740                                     |
| CD16+CD56+ (counts/mm³)    | 222                                     |
| IgG (mg/dl) (842-1943)      | 347*                                    |
| IgA (mg/dl) (62-390)        | 25*                                     |
| IgM (mg/dl) (54-392)        | 88                                      |

* below normal value

CD19 gene mutation analysis with PCR-RFLP was performed as a result of clinical and laboratory analysis. BsmI enzyme was used for PCR-RFLP. 118 bp DNA fragment was obtained after PCR-RFLP due to lack of enzyme cleavage in P1 and P6 patients previously known to be mutations were determined to be homozygous mutant genotype. DNA fragments of 118 bp, 94 bp and 24 bp were obtained in P2, P3, P4, P5, P7 and P8 and evaluated as heterozygous. Because the enzyme cuts both alleles in healthy controls, 94 bp and 24 bp bands were obtained and genotypes were evaluated as homozygous normal. All results of PCR-RFLP are shown in Figure 3.
though PCR-SSCP allows identification of a potentially causal unknown SNP that cannot be identified by PCR-RFLP, it has more complex steps than PCR-RFLP. However, it is used in many applications because it does not require complex steps to perform PCR-RFLP. On the other hand, PCR RFLP is easier to process in terms of time and interpretation experience. Other of the reasons we prefer this method is that we have used it safely in the identification of DCLRE1C gene mutations. Therefore, the PCR-RFLP method was preferred in the study. That study also identified DCLRE1C gene mutation and carrier beers and the results were confirmed by DNA sequence analysis. In our experience, the disadvantage of this method is that it can only be used to detect known mutations.

CD19 is a basic molecule that promotes the proliferation and survival of mature B cells. It forms a whole together with CD81 and CD225. The CD19 protein forms a whole with CD21, CD81 and CD225 on the surface of mature B lymphocytes. This complex works with the B cell receptor and plays an important role in the regulation of events after antigen stimulation. To date, recurrent sinusopulmonary infections, pyelonephritis, and gastritis have been reported in patients with CD19 gene mutations identified. In our case, these findings were absent and there was a history of recurrent fever with bronchiolitis that had only been repeated once. Inadequate antibody responses, decreased memory B cell count and hypogammaglobunemia were seen in CD19 deficiency and in our case, it was laboratory compatible with the literature.

Conclusion
As with other diseases, the early detection of genes that cause primary immunodeficiency is important both for the course of the disease and for additional treatment options. In addition, identifying carriers and informing families about this issue is of critical importance. Although the PCR-RFLP method is a known method, it has been applied by us for the first time in the diagnosis of PID. Therefore, we believe that PCR-RFLP method can be used both in detection of known mutations and also in family screening.

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Declarations
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Author contributions
Conceptualization, I.R., S.K. and Ş.N.G.; Methodology, E.K., M.A.K. and S.K.; Software, S.K. and H.K.; Validation, M.A.K., H.K. and S.K.; Investigation, M.A.K., H.K., S.N.G., E.K., S.K. and I.R.; Resources, M.A.K. and H.K.; Data Curation, M.A.K.; Writing – Original Draft Preparation, M.A.K., H.K. and I.R.; Writing – Review & Editing, I.R., S.K., E.K. and Ş.N.G; Visualization, M.A.K., E.K. and S.K., Supervision, I.R. and S.K.; Project Administration, M.A.K., S.K., E.K., Ş.N.G. and I.R.

Conflicts of interest
The authors declare no conflict of interest.

Data availability
The data used in the study and the data about the method can be accessed by contacting the corresponding author.

Ethics approval
The study was approved by Necmettin Erbakan University; protocol number (2017/818).

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