Original Research Article

The most common mutations in the CFTR gene in the population of Bosnia and Herzegovina

ABSTRACT

Aims: Cystic fibrosis is an autosomal recessive multisystem disease caused by a mutation of the CFTR gene. To date, more than 1900 mutations of this gene are known. Studies have shown that the most common mutation is delF508. In Bosnia and Herzegovina, the prevalence of individual mutations in the general population has not been thoroughly studied, so this study aimed to determine the prevalence of the mutation concerning the countries of the region and the rest of the world.

Study design: Retrospective study.

Place and Duration of Study: Thirty-nine patients with suspected Cystic fibrosis were referred to the Center for Genetics of the Medical Faculty in Sarajevo between 2018-2020.
Methodology: 29 common CFTR gene mutations were analysed with the ELUCIGENE CF29 v2 kit (Elucigene Diagnostics, UK) using four multiplex PCR.

Results:
The most common mutation in our study was the F508 deletion, present in 11 patients (68.75%). R347P and G542X mutations were confirmed in two patients in the heterozygous state in combination with delF508 (M), i.e. 6.25% of each of these mutations. G21+1G>T was found in a homozygous state in one patient, while in another, it was in a heterozygous state in combination with delF508(M) mutation, i.e. 12.5%. Mutation 2184 delA, was found in one patient in the homozygous state with a total frequency of 6.25%.
Conclusion: This research suggests that patients with cystic fibrosis in BiH are most often carriers of the delF508 mutation. Determining the most common mutation of the CFTR gene in a particular population, in addition to gene therapy, is also essential in prenatal diagnostics. Considering the existence of many mutations and that it is impossible to test them all, targeting the most common mutations in a specific population during prenatal testing can confirm with great certainty that the child or parents are or are not carriers of the CFTR mutation.

Keywords: [Cystic fibrosis, mutation F508, heterozygous, homozygous }

1. INTRODUCTION

Cystic fibrosis is a genetic multisystem disease that predominantly affects the lungs and other organs such as the pancreas, liver, intestines, etc. Viscous and dense content in the lumen of the canaliculi prevents the secretion of exocrine

glands, which leads to various clinical manifestations such as chronic destructive bronchitis with polymicrobial lung colonization, chronic hepatitis, exocrine pancreatic insufficiency, intestinal obstruction, infertility, growth retardation, etc. (1). The pathophysiological basis of this disease is a mutation in the CFTR gene (cystic fibrosis transmembrane conductance regulator), which regulates the expression of the chloride channel on epithelial cells, resulting in increased viscosity of secretions on the surface of the epithelium (2). Cystic fibrosis is inherited in an autosomal recessive manner. The gene that causes cystic fibrosis was discovered in 1989 and is located on chromosome 7, at position 7q13. More than 1900 mutations of this gene have been defined to date, of which 1500 lead to the manifest clinical presence of cystic fibrosis (3,4). Mutations are grouped into five classes, according to the CFTR protein defect, namely, class one (defective synthesis), class two (defective processing and maturation), class three (defective channel regulation), class 4 (reduced channel conductance), and class 5 (reduced amount of CFTR protein with normal function) (5). By far, the most common, and also the first identified mutation, is the deletion of codon 508, which leads to the loss of phenylalanine from the amino acid sequence and belongs to class two (6). There are also frequent cases of complex heterozygotes with one mutation on a single chromosome and a completely different mutation on the other, in which case the severity of the clinical presentation is determined by the "milder" mutation (3). With the progress of treatment modalities, the life expectancy of patients with cystic fibrosis is continuously increasing, and today in developed countries, the life expectancy exceeds 40 years. More than 50% of patients are adults (7).

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

The research included 39 people suspected of cystic fibrosis, who were referred for molecular analysis to the Center for Genetics of the Faculty of Medicine from 2017 to 2020. All relevant data on the subjects are in the appropriate protocols of the Center.

Taking the sample was preceded by information about the goals and diagnostic potential of the analysis and the signing of the voluntary consent of the subjects that the results, without revealing the identity of the subjects, can be used for scientific purposes and published. Along with each biological sample, the respondents also filled out questionnaires with appropriate questions relevant to the analysis.

Genomic DNA was extracted from 200 µL of whole blood using QIAamp DNA Mini Kit (*QIAGEN GmbH*, Germany). We employed the protocol for purifying total DNA from whole blood using a microcentrifuge (8). The concentration of the obtained DNA was determined by Qubit 3.0 Fluorometer (*ThermoScientific*, USA). 29 common CFTR gene mutations (D1152H, 1717-1G>A, G542X, W1282X, N1303K, ΔF508, 3849+10kbC>T, 394delTT, 621+1G>T, S1251N, G551D, R117H, R1162X, R334W, A455E, 2183AA>G, 3659delC, 1078delT, ΔI507, R347P, R553X, E60X, 3120+1G>A, 2789+5G>A, 1898+1G>A, 711+1G>T, G85E, 2184delA, and R560T) were analysed with the ELUCIGENE CF29 v2 kit (*Elucigene Diagnostics*, UK) using four multiplex PCR. The thermal condition of PCR were 35 cycles of a Chain reaction of denaturation step at 94^0C for 30 s, annealing at 58^0C for 120 sec and elongation at 72^0C for 60 s, after a single initial denaturation step at 95^0C for 5 min and followed by a single step at 72^0C for 5 min. Electrophoresis of the PCR product was performed in a 3% agarose gel with ethidium bromide (0.1 mg/ml) at 80 V, and 2 A. Visualization was performed in a UV transilluminator (*Uvitec*, Cambridge).

3. RESULTS

Figure 1 shows the results regarding the age of 16 subjects out of 39 tested, with a present mutation of the CFTR gene. Out of the total number of patients in the testing period, 12 were under 18, while four were over 18 years old. Important

information is that this study included a four-member family (husband, wife, and two children), where the model of autosomal recessive inheritance of cystic fibrosis was shown as an example.

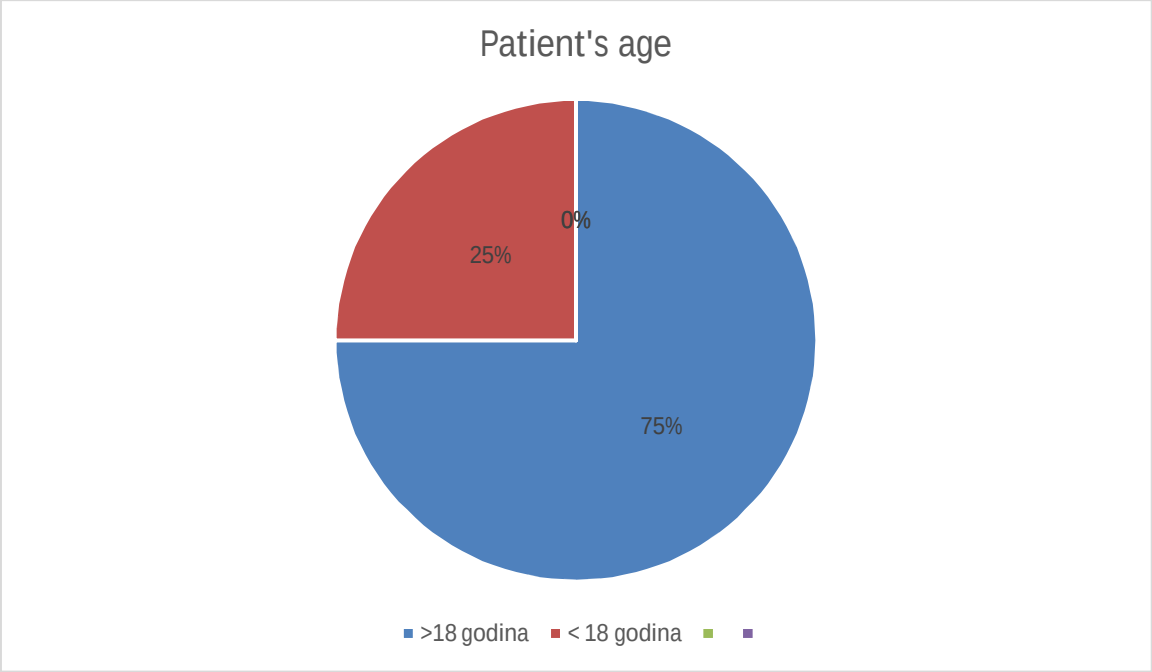


Figure 1. Age of patients with CFTR gene mutation

Analysis of DNA material from 16 subjects confirmed the presence of five mutations in the CFTR gene. Table 1 shows the percentage of detected mutations, with the most prevalent mutation being delF508, which is present in 11 patients, 6 in the homozygous state (delF508 (M)), and in five patients in the heterozygous state (delF508 (M)/ delF508 (N)), which is a percentage of 68.75% (Figure 2). R347P and G542X mutations were confirmed in two patients in the heterozygous state in combination with delF508 (M), i.e. 6.25% of each of these mutations. The fourth type of mutation G21+1G>T was found in one patient in the homozygous state, while in the other, it was in the heterozygous state in combination with the delF508(M) mutation, a percentage of which is 12.5%. The fifth mutation 2184 delA, was found in one patient in the homozygous state with a total frequency of 6.25%.

Table 1. Present mutations on the CFTR gene in homozygotes and heterozygotes

Mutation	Homozygous	Heterozygous	**Percentage representation % (n=32)**
delF508	6	5	***68.75***
R347P	/	1	***6.25***
G542X	/	1	***6.25***
G21+1G>T	1	1	***12.5***
2184 delA	1	***/***	***6.25***
			Total : **100**

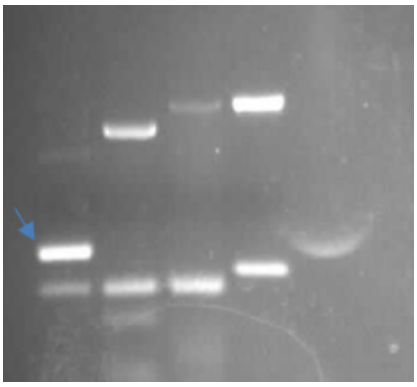
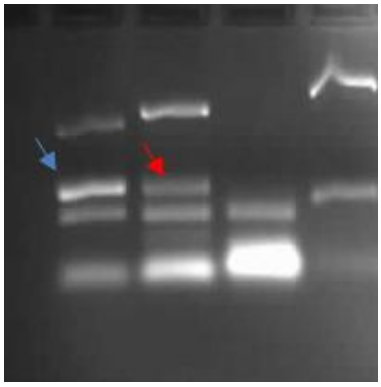
a)  b) 

Figure 2. a) Homozygous mutation F508 (M); b) Heterozygous mutation F508 (M) (blue arrow) and F508 (N) (red arrow)

DISCUSSION

Cystic fibrosis is a complex disease; therefore, the various symptoms and severity can vary from person to person. Many factors (genetics, age at CF diagnosis, environment, diet) can also affect a person's health and disease course.

The exact incidence of cystic fibrosis in Bosnia and Herzegovina is unknown since there is no national registry of patients. Accordingly, numerous epidemiological data regarding this disease in our country are unknown. To expand the data on the frequency and distribution of mutations of the CFTR gene in our country, we analysed 39 patients, 16 of whom had a proven mutation of the CFTR gene. It was impossible to find a similar study in the PubMed database that refers to the population of Bosnia and Herzegovina at the time of this paper's publication. Five mutations were found, with different percentages. The most common mutation in our study was the F508 deletion, presented in 11 patients (68.75 %). Such a result is from studies done in the region and the world. CF in about 70% of cases results from mutation F508 in the CFTR gene. The remaining 30% of cases refer to more than 1,700 mutations in the specified gene.

As for the surrounding countries, the percentage presentation of delF508 is 72.28% in Serbia and Montenegro (9), in Croatia 58.33% (10), and in Slovenia (62.7-68.6%) (11). For the sake of comparison, some countries, such as Denmark, show a higher rate of this mutation presence (87.2 %). In contrast, some countries have a significantly lower percentage of this mutation (e.g. Algeria, with the world's lowest presentation of 26.3%) (12). R347P and G542X mutations were confirmed in two patients in the heterozygous state (together with delF508), which is a percentage of 6.25% for each of the mentioned mutations. R347P is a missense with an overall worldwide frequency of about 0.2%. The patients described initially with this mutation were compound heterozygotes with the delta F508 mutation and had a very mild course of CF, suggesting that R347P, like other missense mutations, causes a mild phenotype. However, severe cases of carriers of this mutation have also been described (13). The study by de Garcia et al. (2005) showed that the relationship between delF508/R347P and delF508/G21 1G T mutations resulted in significantly lower spirometry values during follow-up and a lower survival rate in end-stage lung disease than in patients with at least one class III or IV mutation. (14) G542X, one of the most common mutations in European populations (2.6%), was detected in 6.1% of CF alleles in Mediterranean countries and is found in the countries of the region (9). G21+1G>T was found in a single patient in the homozygous state and another patient in the heterozygous state with the delF508 mutation, a percentage of 12.5%. The 621 1G T mutation has an average frequency of 0.54% in the European population, being the most common in central Greece (5.72%) (15).

2184 delA mutations were found in one patient in the homozygous state, with a percentage of 9.375%. Because the 2184 insA mutation is not included in standard commercial tests, routine DNA diagnostics have not identified this allele. However, some targeted studies have shown a high frequency of this mutation in certain countries, such as Ukraine (16). In the results tabular presentation of our study, one family was included, for which the analysis determined that the mother and father were heterozygous for delF508, and two male children, one of whom was homozygous and possessed only F508(M), and the other was heterozygous and possessed F508 (N). This data shows the autosomal recessive mode of inheritance of cystic fibrosis.

A better understanding of the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) protein structure and the consequences of CFTR gene mutations has enabled the development of new therapies targeting specific defects underlying CF. Some of these therapies are clinical, and some are in preclinical development (17). Moni and Al Basheer, in their study, explained the use of monoclonal antibodies as one of the potential future targeted biological drugs for the treatment of CF (18).

Recently, more and more hope has been placed in the CRISP method as one of the significant methods in treating CF. Many studies have been done to try to replace the mutated gene with a “correct version” of the CFTR gene (19, 20). Although some gene therapies are already being used in CF patients, much research is still needed to treat patients with CF successfully.

4. CONCLUSION

This research suggests that patients with cystic fibrosis in Bosnia and Herzegovina are most often carriers of the delF508 mutation. This aligns with a similar study that showed similar results in the surrounding countries and the rest of the world. Determining the most common mutation of the CFTR gene in a particular population, in addition to gene therapy, is also essential in prenatal diagnostics. Considering the existence of many mutations and that it is impossible to test them all, targeting the most common mutations in a specific population during prenatal testing can confirm with great certainty that the child or parents are or are not carriers of the CFTR mutation.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

“All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.”

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