INTRODUCTION

Cataract is defined as the opacification of the lens of the eye resulting in a decrease in vision. About 30% of congenital cataracts are due to monogenic reasons and they show autosomal dominant inheritance. So far, about 25 genes have been defined[1]. Hyperferritinemia Cataract Syndrome (HFCK) (OMIM 600886) is an autosomal dominantly inherited disease characterized by increased serum ferritin levels and bilateral cataract formation in the early period of life. It was first defined by Bonneau et al. and Girelli et al. independently from each
other in 1995[2,3]. Heterozygote mutations in the 5’ untranslated region (UTR) of L-ferritin gene (FTL) have been reported to cause this disease [4]. So far, about 37 mutations have been defined in FTL gene [5]. There is no literature data about its prevalence in Turkish population. However, it is estimated to be about 1:200,000 in Australia [6]. Although one case has been reported from Turkey in literature [7], there are no research articles about which mutations are frequent and about the clinical effects of these mutations. In this study, our purpose was to research the FTL gene mutations which cause HFCS in Central Anatolia and the clinical effects of these mutations.

**MATERIAL METHOD**

**Study Group**

17 patients from 6 families who were referred to Kayseri Training and Research Hospital Medical Genetics Department from Hematology and Ophthalmology Department with a prediagnosis of HFCS between June 2014 and December 2017 were included in the study. The study was carried out in Kayseri Training and Research Hospital Medical Genetics Department. The study was approved by the Local Ethics Research Committee of Erciyes University with protocol number 2018/186 and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All subjects provided written informed consent prior to participation in the study.

Families with high ferritin level in performed serum measurement, those who were found to have cataract in eye examination and families with vertical inheritance since the disease is autosomal dominant were included in the study. Other diseases which caused high ferritin level such as hemochromatosis were excluded from the study.

**Sanger Sequence Analysis**

Genomic DNA was extracted from peripheral blood samples using the DNA isolation kit according to the manufacturer’s instructions (Zinexts Life Science Corp., Taiwan). Exons, exon-intron boundaries, and 5’ and 3’ untranslated region of the FTL (NM_000146) were sequenced using appropriate primers by using the Sanger sequence method. PCR conditions were as follows: Initial denaturation at 94 °C for 5 min; 35 cycles at 94 °C for 30 s and 58 °C for 45 s; 72 °C for 1 min; and a final extension at 72 °C for 5 min. The PCR products were observed with 2% agarose gel electrophoresis. PCR products with enzyme transition were purified using the Exo-SAP kit (Exo SAP PCR purification kit, UAB Corporation, Cleveland, Ohio, USA). Cycle sequence was amplified using Big Dye Terminator, and extension products were purified using the Sephadex. The product was sequenced in both strands initiating from the forward and the reverse primers used in the initial PCR and analyzed on an ABI 3500 Genetic Analyzer (Applied Biosystems, Hitachi, Japan). Bioinformatic analysis was conducted using the SeqScape v2.6 program.
RESULTS

17 patients from 6 families were evaluated. Female/male ratio of the patients was 7/10. The youngest patient was 1 year old, while the oldest patient was 72 years old and the average age was 33.3±19.5. Patients F1P3, F3P7, F3P8, F3P9, F5P12, and F5P16 had not undergone cataract operation yet. The patients’ cataract operation age also differed. Patient F2P5 had been operated at the age of 9, patient F6P17 had been operated at the age of 58. While the levels of ferritin increased, the levels also differed between patients. Table 1 shows the patients’ cataract state, whether they were operated and at which age they were operated, information about ferritin levels and additional findings. Ferritin levels of siblings who were under risk were checked. In addition, the patients’ total blood count and biochemical parameters are listed in Table 2.

In the sanger sequence analysis conducted, all of the patients were found to have c.-160 A>G heterozygous mutation in FTL gene (Figure). This mutation is a genetic change reported in literature previously [8].

DISCUSSION

Ferritin is an iron binding protein storing iron for vital cellular activities and it is the primary intracellular iron storing protein in the body. The protein’s iron free form is called apoferritin; while the iron containing form is called Holoferritin. Each apoferritin consists of 24 subunits containing H-subunit and L-subunit.

Hyperferritinemia Cataract Syndrome is an autosomal dominant inherited rare genetic disease. It is characterized by early onset cataract formation due to L-ferritin accumulation in the lens. Mutations in the 5’untranslated region of FTL gene located in 19q13.1 cause this disease[9,10]. By binding iron responsive elements(IRED) with iron regulated cytoplasmic protein(IRP) forms a complex enabling the inhibition of L-ferritin levels in the 5’untranslated region of FTL gene. Mutations in the FTL gene disrupt the IRE binding with IRPs, shifting the iron mediated down regulations of FTL translations. So far, about 37 mutations have been defined in FTL gene [5]. Frequent mutations are c.-168G>C/T/A, c.-161C>G/T/A and c.-160A>G [9]. Small deletions have also been defined [11]. In our study, we found c.-160A>G mutation in 17 patients from 6 families assessed from the provinces of Kayseri and Nevsehir in Turkey. All our patients had the same mutation. The families were not relatives. This mutation has been reported previously[2,8]. To the best of our knowledge, Tuysuz et al. from Turkey reported +32G>T change in 3 individuals from a family [7]. In addition to having early onset cataract, these three patients had ferritin levels between 659-2000 ng/ml.

In HFCS, ferritin accumulates in all the cells in the body. However, it causes cataract by becoming toxic through forming L-ferritin crystal deposits formation only in lens. Cataract is generally early onset, bilateral and progressive. HFCS penetration may not be complete and patients may have only hyperferritinemia. It has been claimed that the intensity of penetrance and cataract is associated with the location of mutation [10,12]. In our study, cataract was not developed in patients F3P7, F3P9, and F5P12 who were relatively young. However, all of the patients had hyperferritinemia. In addition, although the same mutation was found in all our patients, the differences in cataract development age and changes in ferritin levels bring to mind that factors besides the defined mutation can contribute to the disease.

One of the striking findings in the study was that the youngest patient was 1 year old and oldest one was 72 years old. Patient F5P14 who was 72 years old underwent several tests including a liver biopsy. However, the patient was diagnosed with FTL gene analysis in our
department. Early diagnosis save time for planning the patient’s cataract surgery and it can prevent unnecessary tests.

HFCS prevalence has been shown to be around 1/200 000 in Australia [6]. All of our patients were from the provinces of Kayseri and Nevsehir in Turkey. The total population of these two cities is about 1 700 000. We defined 17 patients from 6 families. This shows that the minimum prevalence is about 1/100 000. However, since ferritin levels were not measured routinely in cataract cases and since the study depended on hospital data, the prevalence of this disease can probably be higher.

The study has some limitations. The number of patients in the study is limited. Further studies with more patient numbers are needed to explain why they have different ferritin levels and the age onset of cataract.

As a conclusion, in the presence of hyperferritinemia, patients should be assessed by a hematologist and be referred to an ophthalmologist in terms of cataract. It should be remembered that this disease is autosomal dominantly inherited and family screening should be performed. This study is the first comprehensive research article for the analysis of hyperferritinemia cataract syndrome and FTL gene molecular genetic analysis from Turkey. In Turkish population, the prevalence of HFCS is about 1/100 000 and the commonly observed mutation is c.-160 A>G mutation.

**Conflict of interest**

The authors declare no potential conflicts of interests.

**Funding**

None.
REFERENCES


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<tr>
<th>Patient No</th>
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Abbrevetions: M,male; F,female; F1P1, family 1 patient 1.

Table 1: It shows the patients' cataract state, whether they were operated and at which age they were operated, information about ferritin levels and additional findings.
Table 2: The patients’ total blood count and biochemical parameters are listed.

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<th>Parameter</th>
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<td>Hb, g/dl</td>
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<td>Iron binding capacity, ug/dl</td>
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Table 2: The patients’ total blood count and biochemical parameters are listed.

Figure Legends

Figure: All patients have heterozygous c.-160 A>G Mutation in FTL gene.

Table 1: It shows the patients’ cataract state, whether they were operated and at which age they were operated, information about ferritin levels and additional findings.

Table 2: The patients’ total blood count and biochemical parameters are listed.