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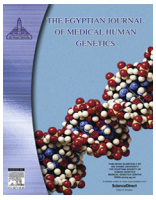

Case Report

Asymptomatic hemochromatosis case with HFE c.1007−47G>A, c.340+4T>C heterozygous mutations and alpha globin −3.7 kb deletion

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ABSTRACT

Background: Hereditary hemochromatosis is a disease associated with iron deposition which is caused by the mutations in "hereditary Fe (iron)" (HFE) gene.
Case: The 16-year-old male patient was diagnosed with hereditary hemochromatosis after c.1007−47G>A heterozygous c.340+4 T>C heterozygous mutations were detected in HFE gene analysis after a suspicion of hemochromatosis due to increase of hemoglobin value from 14.8 g/dL to 16.8 g/dL and the level of ferritin from 68 ng/ml to 300 ng/ml in routine check-up controls in two-years period. In addition, due to low mean corpuscular volume (MCV) (76 fL), and mean corpuscular hemoglobin (MCH) (26 pg) levels, gene mutation analysis was carried out and the patient was also shown to carry α thalassemia −3.7 deletions.
Conclusion: Early diagnosis of hemochromatosis is important in terms of prognosis and morbidity. We aimed to emphasize that we can easily diagnose the disease by performing genetic analysis in cases with suspected hemochromatosis even they have no complaints.

1. Introduction

Hemochromatosis is the abnormal accumulation of iron in parenchymal organs, leading to organ toxicity. It is the most common autosomal recessive genetic disorder and the most common cause of severe iron overload [1]. Hemochromatosis is classified as primary and secondary. In some diseases with deposition of iron, there is no secondary disorder that causes the disease and they are classified as primary hemochromatosis. Secondary hemochromatosis develops secondary to another disease such as thalassemia with ineffective erythropoiesis [2].

Hereditary hemochromatosis (HH) is a disorder of iron metabolism with genetic and environmental factors involved in pathogenesis and causes progressive iron accumulation in various parenchymal organs. In 1996, Feder et al. identified hereditary Fe (iron) gene mutations (HFE) responsible for HH. With the detection of these mutations, a great deal of improvement has been made in the identification of the disease [1]. The most common type of HH seen due to changes in the HFE gene is called HFE-associated HH. Along with the identification of new mutations, other subgroups of the disease have also been identified and they are referred to as non-HFE related HH (non-HFE).

The HFE gene encodes a 343-amino acid HFE protein which is a major histocompatibility complex (MHC) class 1 molecule and found in the HLA-A locus on chromosome 6. HFE protein is a transmembrane protein with intracellular and extracellular components and plays an important role in the regulation of iron metabolism [3]. Two important mutations in the HFE gene have been identified. C282Y mutation takes place with tyrosine substitution instead of the cysteine in the codon 282 whereas H63D mutation is formed by aspartate passage instead of histidine in the codon 63. The biological effect of the first mutation is stronger than the H63D mutation. In their study, Feder et al. found that 83% of the HH cases were homozygous (C282Y) and 8% were heterozygous (C282Y/H63D). Conformational structure of the protein is impaired and the iron absorption increases after HFE gen mutation. HH type 1 Classical hereditary hemochromatosis is characterized by HFE gene mutation [4]. Juvenile hemochromatosis, defined as type 2; type 2A if a hemojuvelin (HJV) mutation is present, or type 2B if a hepsin (HAMP) mutation is present [5]. Type 3 hemochromatosis occurs as a result of transferrin receptor 2 (TfR-2) mutation. Type 4 haemochromatosis occurs due to ferroporphyrin mutation [6].

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The most practical, easy and feasible method for the screening in hereditary hemochromatosis is the measurement of ferritin and transferrin saturation values. The analysis of genetic mutations in susceptible cases is important in diagnosis [7]. Direct genetic screening should be performed in the first degree relatives of individuals who are defined with hereditary hemochromatosis [8].

2. Case report

A 14-year-old male patient was admitted to the Pediatrics Clinic for routine check-up. His physical examination was normal and blood analysis revealed: erythrocyte (RBC) count 6.07 $\times$ 10^6/uL (4.1–5.1 $\times$ 10^6/uL), hemoglobin (Hb) 14.6 g/dL (13–16 g/dL), hematocrit (Hct)%43.1 (%37–49), mean corpuscular volume (MCV) 71 fL (73–95 fL), mean corpuscular hemoglobin (MCH) 24.1 pg (26–30 pg), ferritin 68 ng/ml (13–68 ng/ml). On peripheral blood smear, mild hypochromia and anisocytosis observed. His family history was clear. There was no significant finding in his or family's history.

After 2 years, at the age of 16, he was admitted to the Pediatrics clinic for a routine check up once more. His physical examination was normal. However, it was found that RBC count was 6.07 $\times$ 10^6/uL, Hb was14.6 g/dL, Hct was 43.1%, MCV and MCH were 71 fL and 24.1 pg, respectively, ferritin level was 300 ng/ml. The patient had no changes in his nutrition status or was not on medication in that period. Serum iron, iron binding capacity, and other acute phase reactants were normal. Due to low levels of MCV and MCH, he was suspected as having thalassemia triat, however, no abnormal hemoglobin was detected in his hemoglobin electrophoresis (Hb A2%2.1). He was diagnosed as alpha thalassemia due to detected $-\alpha$3.7, $-\alpha$4.2 single gene deletion in "Multiplex Ligation-Dependent Probe Amplification" (MLPA) method (Fig. 1). Erythropoietin levels analysed for polycythaemia were normal (7 IU/L) and a clinically insignificant wild type (normal) genomic sequence involving JAK2-V617F mutation was detected in DNA point mutation mini sequence analysis. With the pre-diagnosis of hereditary hemochromatosis, c.1007−47G>A heterozygous c.340+4 T>C heterozygous mutations were detected in HFE gene mutation analysis (Fig. 2). Previously, c.1007−47G>A mutation has been reported to be associated with iron overload and c.340+4 T>C mutation is defined as the polymorphism associated with hemochromatosis [9].

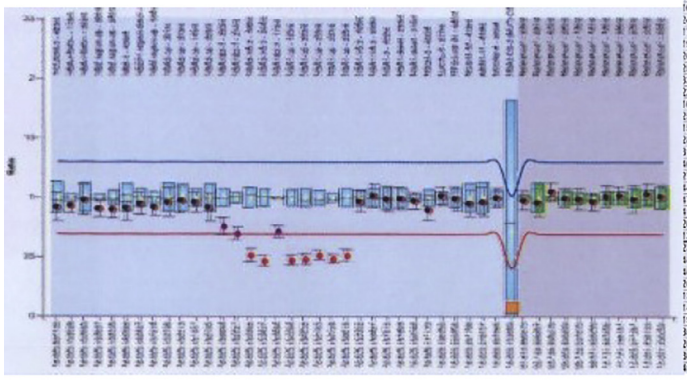
3. Discussion

For suspected hemochromatosis cases, ferritin level and fasting transferrin saturation should be measured at first [10]. HFE gene mutation analysis was performed in our case because of an increase in ferritin levels in two-years period, and the patient was diagnosed as hereditary hemochromatosis due to the detected heterozygous mutations for −47G>A/+4T>C. According to our knowledge, these mutations are reported in Turkish population for the first time.

Ferritin increases in neoplasms and infections as an acute phase reactant. Alcoholic liver disease, Hepatitis C infections, and non-alcoholic steatohepatitis may also elevate ferritin levels [11]. Therefore, ferritin is not a specific marker for hemochromatosis and transferrin saturation should be evaluated concurrently. In case of normal levels of both ferritin and transferrin saturation, hemochromatosis should be ruled out [12]. Transferrin saturation levels above 45 are valuable for the diagnosis and HFE mutation should be analysed. Prior to 1996 the diagnosis of hemochromatosis was made by liver biopsy. However, after the detection of HFE gene mutations, genetic analysis substitute liver biopsy [13,14]. Our case was diagnosed as hereditary hemochromatosis based on unaccompanied additional complaints, negative acute phase reactants, increase in ferritin levels in two-years period, and detection of HFE gene mutation.

Today, C282Y and H63D mutations are detected in whole blood by polymerase chain reaction (PCR). Accumulation of iron in the body and detection of homozygous C282Y mutation is sufficient for the diagnosis of the disease [15]. C282Y homozygosity is the most common mutation for hemochromatosis and proceeds with maximum iron accumulation. While the patients with both C282Y/H63D heterozygosity show moderate iron accumulation, H63D homozygotes and C282Y heterozygotes are usually normal [16]. These two mutations are previously defined in Turkish population [7]. Genetic analysis may be useful for diagnosis of hemochromatosis. However, only for patients with hepatic fibrosis and cirrhosis liver biopsy is supportive [17].

Alpha-thalassemia is a hereditary anemia that results from a defective synthesis of alpha-globin. Revise alpha-thalassemia can be inherited or acquired and is originated by defects or deletions in one or more genes of the four alpha-globin genes. Hypochromic and microcytic cells characterize alpha-thalassemia [18].



) [nt]	Gene-Exon	Chr.band	hg18 loc.	Height	Area	Ratio*
163	POLR3K-3	16p13.3	16-000.037132	602	4971	0.92
178	HBA-HS40--	16p13.3	16-000.103528	1124	8206	0.94
182	HBA-HS40--	16p13.3	16-000.103585	645	4689	0.99
164	HBZ region-up	16p13.3	16-000.133637	630	5337	0.91
146	HBZ region-up	16p13.3	16-000.139336	777	5783	0.91
138	HBZ-1	16p13.3	16-000.142592	609	5440	0.92
192	HBZP1 region-	16p13.3	16-000.149467	1052	8512	0.96
184	HBM region-up	16p13.3	16-000.157274	1100	6988	0.92
191	HBA2-up	16p13.3	16-000.159798	951	7217	0.96
173	HBA2-up	16p13.3	16-000.160313	1047	7870	0.98
145	HBA2-up	16p13.3	16-000.161951	1267	7766	0.96
128	HBA2-up	16p13.3	16-000.162190	1064	7753	0.92
120	HBA1&2-1	16p13.3	16-000.166644	830	5806	0.76
114	HBA1&2-1	16p13.3	16-000.162921	722	5396	0.7
160	HBA2-intr.2	16p13.3	16-000.163362	574	3805	0.52
144	HBA2-intr.2	16p13.3	16-000.163427	611	4564	0.47
172	HBA1&2-3	16p13.3	16-000.163484	841	6372	0.72
190	HBA1-up	16p13.3	16-000.164084	589	3591	0.48
102	HBA1-up	16p13.3	16-000.164602	620	3992	0.49
156	HBA1-up	16p13.3	16-000.165160	670	4789	0.52
137	HBA1-up	16p13.3	16-000.165728	461	4681	0.49
126	HBA1-up	16p13.3	16-000.166018	629	4275	0.52
165	HBA1-intr.2	16p13.3	16-000.163362	1071	8035	0.97
150	HBA1-intr.2	16p13.3	16-000.163427	1406	10444	1.02
152	HBA1-3	16p13.3	16-000.167572	1108	7274	0.99
183	HBA1-down	16p13.3	16-000.167890	1188	8246	1
110	HBA1-down	16p13.3	16-000.169744	1010	7026	0.98
100	HBQ1-3	16p13.3	16-000.171170	814	6841	0.9
177	LUC7L-5	16p13.3	16-000.196305	1174	8937	1.02
145	HBA2-intr.01	16p13.3	16-000.229854	662	5443	1
172	RGS11-10	16p13.3	16-000.261758	781	6980	0.96
118	AXIN1-11	16p13.3	16-000.278107	1045	8464	0.97
162	DECR2-4	16p13.3	16-000.397524	1274	9207	1
136	HBA2 CS-3 (MU	16p13.3	16-000.163569	101	743	8%
138	Reference*	01q41	01-213.880610	1197	8033	0.98
181	Reference*	02q33.1	02-199.896807	989	8588	0.96
109	Reference*	03q29	03-194.895216	1087	8590	1.05
169	Reference*	05p15.31	05-009.490899	1201	8350	1
130	Reference*	05q31.1	05-132.037610	1454	9216	0.99
141	Reference*	08p23.1	08-011.650000	1267	8201	0.97
196	Reference*	10q26.3	10-131.447456	1398	9814	1.01
156	Reference*	11q22.2	11-101.726161	1287	10358	1.02
108	Reference*	12p13.31	12-005.971281	1256	8285	0.99
100	Reference*	13q14.3	13-051.409733	1126	7759	1.03
154	Reference*	15q26.3	15-097.300059	895	7268	1.01

Fig. 1. (A and B) Heterozygous α thalassemia −3.7 deletion determined by MLPA method (SALSA MLPA P140 HBA probemix, MRC Holland).

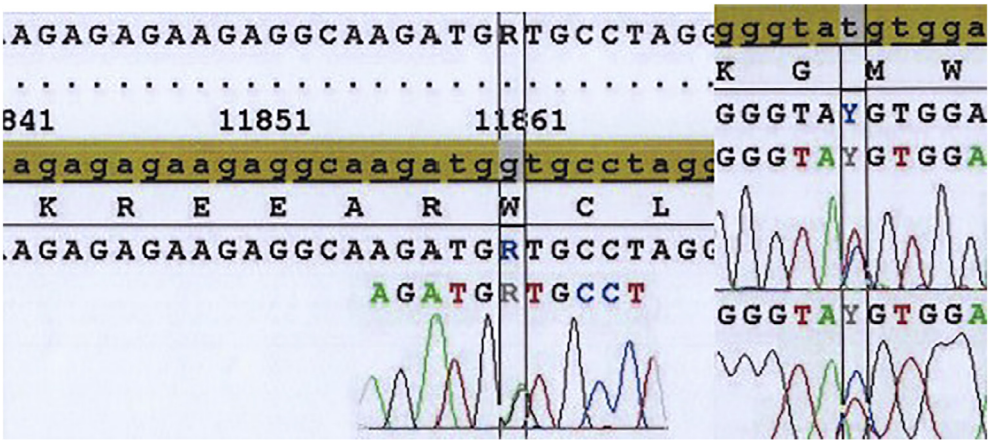


Fig. 2. (A) Heterozigot c.1007−47G>A mutation, (B) Heterozigot c.340+4 T>C mutation.

Agostinho et al. showed that the allelic frequencies of H63D mutation in the alpha-thalassemia for Caucasians was 13.70%. and for the C282Y mutation the frequency observed 2.38% [19]. Besides, Pietrangelo et al. found compound heterozygous patients (H63D/C282Y) in the alpha-thalassemic patients [20].

In a study carried out in Hong Kong, iron overload in alpha-thalassemia was not related to hemochromatosis mutations [21]. However, more data about the role of *HFE* mutations in alpha-thalassemic patients are still necessary.

4. Conclusion

Although there are no complaints or symptoms, hemochromatosis can be detected incidentally by routine pediatric examination and blood analysis, and its diagnosis can be made easily by mutation analysis.

References

[1] Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996;13(4):399–408.
[2] Bacon BR, Britton RS. Hemochromatosis. In: Mark Feldman, editor. Gastrointestinal and Liver disease pathophysiology, diagnosis, management. Canada: Saunders Elsevier; 2006. p. 1589–99.
[3] Whittington CA, Kowdley KV. Haemochromatosis. Aliment Pharmacol Ther 2002;16:1963–75.
[4] Ahmad KA, Ahmann JR, Migas MC, et al. Decreased liver hepcidin expression in the HFE knockout mouse. Blood Cells Mol Dis 2002;29:361–6.
[5] Papanikolaou G, Samuels ME, Ludwig EH, et al. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nat Genet 2004;36:77–82.
[6] Kawabata H, Fleming RE, Gui D, et al. Expression of hepcidin is downregulated in TfR2 mutant mice manifesting a phenotype of hereditary hemochromatosis. Blood 2005;105:376–81.
[7] Simsek H, Sumer H, Yilmaz E, et al. Frequency of HFE mutations among Turkish blood donors according to transferrin saturation. J Clin Gastroenterol 2004;8:671–5.
[8] Tavill AS. Diagnosis and management of haemochromatosis. Hepatology 2001:331321–8.
[9] Khusainova RI, Khusnutdinova NN, Litvinov SS, Khusnutdinova EK. Analysis of H63D mutation in hemochromatosis (HFE) gene in populations of central Eurasia. Genetika 2013;49(2):269–78.
[10] Murtagh LJ, Whiley M, Wilson S, et al. Unsaturated iron binding capacity and transferring saturation are equally reliable in detection of HFE hemochromatosis. Am J Gastroenterol 2002;97(8):2093–9.
[11] Adams PC, Reboussin DM, Barton JC, et al. Hemochromatosis and iron-overload screening in a racially diverse population. N Engl J Med 2005;352(17):1769–78.
[12] McCullen MA, Crawford DH, Dimeski G, et al. Why there is discordance in reported decision thresholds for transferrin saturation when screening for hereditary hemochromatosis. Hepatology 2000;32(6):1410–1.
[13] Janssen MC, Swinkels DW. Hereditary haemochromatosis. Best Pract Res Clin Gastroenterol 2009;23(2):171–83.
[14] Kaczorowska-Hac B, Luszczyk M, Antosiewicz J, Ziolkowski W, Adamkiewicz-Drozynska E, Mysliwiec M, et al. HFE gene mutations and iron status in 100 healthy polish children. J Pediatr Hematol Oncol 2017 Jul;39(5):e240–3. doi: https://doi.org/10.1097/MPH.0000000000000826.
[15] Adams PC, Barton JC. Haemochromatosis. Lancet 2007;370(9602):1855–60.
[16] Pietrangelo A. Hereditary haemochromatosis – a new look at an old disease. N Engl J Med 2004;350(23):2383–97.
[17] Clark P, Britton LJ, Powell LW. The diagnosis and management of hereditary haemochromatosis. Clin Biochem Rev 2010;31(1):3–8.
[18] Higgs DR, Vickers MA, Wilkie AO, Pretorius IM, Jarman AP, Weatherall DJ. A review of the molecular genetics of the human alpha-globin gene cluster. Blood 1989;73:1081–104.
[19] Agostinho MF, Arruda VR, Basseres DS, Bordin S, Soares MC, Menezes RC, et al. Mutation analysis of the HFE gene in Brazilian populations. Blood Cells Mol Dis 1999;25:324–7.
[20] Pietrangelo A. Hereditary hemochromatosis – a new look at an old disease. N Engl J Med 2004;350:2383–97.
[21] Chan V, Wong MS, Ooi C, Chen FE, Chim CS, Liang RH, et al. Can defects in transferrin receptor 2 and hereditary hemochromatosis genes account for iron overload in HbH disease? Blood Cells Mol Dis 2003;30:107–11.