Ailevi homozigot hiperkolesterolemili hastaların uzun dönem izlemi: Bir üniversite hastanesi lipit polikliniğinin 13 yıllık deneyimi

Long-term follow-up of patients with homozygous familial hypercholesterolemia; A 13-year experience of a university hospital lipid clinic

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ÖZET

Amaç: Ailevi hiperkolesterolemi (AH), erken ateroskleroza yol açan aşırı yüksek kolesterol düzeyleri ile karakterize genetik bir hastalıktır. Homozigot AH (HoFH) bulunan olgularda çocukluk döneminden itibaren kardiovasküler olaylar gelişebilmektedir. Bu çalışmada Ege Üniversitesi Tıp Fakültesi, Kardiyoloji Anabilim Dalında izlenen erişkin yaştaki HoFH'li hastalarla olan uzun dönem klinik deneyim sunulmuştur.

Çalışma planı: Çalışmaya 2000-2013 yılları arasında izlenen HoFH'lı 17 hasta (11 kadın, 6 erkek) alındı. Hastalara ait tüm veriler (klinik özellikler, aile öyküsü, lipit düzeyleri, tedavileri, lipit aferezi, izlemde gelişen kardiyovasküler olaylar, kompli-kasyonlar vb) geriye dönük olarak hasta dosya kayıtlarından elde edildi.

Bulgular: Kliniğimize başvuru sırasında yaş ortalaması 31±10 yıl ve ilk tanı yaşı 25±14 idi. Olguların tanı konulduğunda ortalama kolesterol değerleri 625±136 mg/dl idi, tanı sırasında en sık başvuru yakınmaları deri bulguları (%41) ve iskemi ile ilgili yakınmalarıydı. Üç (%18) olgu ise aile öyküsü nedeniyle tarama yapılırken saptandı. Olguların %65'i akraba evliliği çocuğuydu. Ksantom sıklığı %59 olup aort kapak patolojisi %59, karotis plakları %47 ve koroner arter hastalığı %59 olguda saptandı. Tüm olgularda lipit aferezi endikasyonu bulunmasına rağmen tedavinin reddedilmesinden dolayı 10 olguda uygulanabilmişti. Afereze başlama yaşı 27±12 idi (10-42 yaş). Aferez tedavisine uyum %60'tı. Düzenli aferezle deri bulguları kaybolmasına rağmen hastaların hepsinde karotis aterosklerozunda ve aort patolojisinde ilerleme saptandı. Ortalama 43±42 aylık izlemde dört olgu hayatını kaybetti (ortalama yaş 25±5).

Sonuç: HoFH'lı hastalara tanı geç konmaktadır. Aferez tedavisine geç başlandığı için ateroskleroz ve aort darlığı ilerlemektedir. Bu hastalıkla ilgili hekim farkındalığı ve halkın bilinçlendirilmesi gerekmektedir.

ABSTRACT

Objectives: Familial hypercholesterolemia (FH) is a genetic disease characterized by extremely high levels of cholesterol leading to premature atherosclerosis. In homozygous indi-viduals (HoFH) cardiovascular events could develop in child-hood. In this article, long-term clinical experience with adult HoFH patients who were followed in the Department of Cardiology, Ege University Faculty of Medicine was presented.

Study design: Seventeen HoFH patients (11 females, 6 males) who were being followed between the years 2000-2013 were included in the study. All data including clinical characteristics, family history, lipid levels, treatment, lipid-apheresis, cardiovascular events, complications were obtained retrospectively from patient chart records.

Results: Mean age on admission was 31±10 years, age at first diagnosis was 25±14.years. Mean cholesterol level at diagnosis was 625±136 mg/dl. Admission complaints were dermatologic (41%) and ischemic symptoms (41%), whereas 3 patients (18%) were diagnosed during family screening. Sixty-five percent of the cases were children of consanguineous marriage. Xanthomas were present in 59%, aortic valve pathology in 59%, and carotid ar-tery plaques in 47% of the patients. Coronary artery disease was documented in 59% of the cases. Though all patients had indications for apheresis, it could be performed only in 10 patients dur to high refusal rate of the patients. Mean age at the first session of apheresis was 27±12 years (minimum 10-maximum 42) and adherence to apheresis was 60%. After 2 years of regular apheresis skin depositions were vanished, however carotid atherosclerosis and aortic pathology progressed. During a mean follow-up of 43±42 months, 4 patients died (mean age: 25±5 years).

Conclusion: Patients with HoFH are subject to late diagnosis. Due to the delayed initaition of the treatment with lipid apheresis, atherosclerosis and aortic stenosis progress in these patients. The awareness of the physicians and knowledge of the public concerning this disease is warranted.

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Abbreviations:
AS *Aortic stenosis*
FH *Familial hypercholesterolemia*
HeFH *heterozygous hypercholesterolemia*
HoFH *Homozygous hypercholesterolemia*
CAD *Coronary artery disease*
CV *Cardiovascular*
LDL *Low-density lipoprotein*
PCSK-9 *Pro-protein convertase subtilisin/keksin-9*
US ultrasound

Familial hypercholesterolemia (FH) is an inherited metabolic disorder secondary to genetically defective or absent low-density lipoprotein (LDL) receptors. [14] As a consequence of excessively high-serum cholesterol levels, cholesterol accumulate in dermis, tendons, and arterial walls leading to severe cardiovascular (CV) events in young age. Cholesterol typically accumulates in the proximal segments of the vessels. Therefore, stenosis in coronary artery ostium, and aortic valves secondary to accumulation of cholesterol deposits are typical features of the disease. FH demonstrates autosomal dominant inheritance. The untreated cholesterol levels typically range between 250-300 mg/dL in heterozygous individuals, and CV events develop in men by 30-50 years of age, and in women by 40-60 years of age.[5] In homozygous individuals serum cholesterol levels are much higher (500-1000 mg/dl), and severe atherosclerotic events begin from the early childhood.[3,4] Since these patients have defective LDL receptors antilipid drugs are ineffective. If left untreated homozygous patients generally die before the age of 30 years due to accelerated severe atherosclerotic-CV events. [6]

Incidence of homozygous FH (HoFH) has been estimated as one patient per one million people, [17] whereas the incidence of heterozygous form of FH (HeFH) in general population has been accepted as 1/500. However, in recent years, data from closed societies revealed that, FH is more frequent than expected (founder effect). [8-13] The highest FH prevalence was reported from South Africa (prevalence of HeFH, 1in 70). [11] In French Canadians one of every 270 individuals is estimated to have HeFH. [12] In Lebanon, the prevalence of HoFH is much higher (1/10.000). [13] In Turkey the incidence of HoFH is unknown, however when prevalence of consanguineous marriages was taken into consideration, rates higher than predicted should be anticipated. Experience with homozygous patients in our country has been reported as sporadic cases, and data on long-term prognosis is not available. [14-23] Only a few experienced centers have published short-term results of LDL-apheresis from their 5-10 pediatric cases. [24-27] Data concerning long-term outcomes of adult patients are not available.

In this article long-term clinical experience with adult HoFH patients in a university hospital lipid clinic have been presented. At the same time current approach recommended for HoFH has been reviewed.

PATIENTS AND METHOD

This study was conducted by retrospective screening of the medical records of the HoAH patients followed between 2000-2013 in the Lipid clinic of the Department of Cardiology, Ege University Faculty of Medicine. In our lipid clinic which was established in 1996, treatment regimens of the FH patients depend on the lipid profiles, and biochemical parameters evaluated at 3-to-6 month-intervals. Each patient with established diagnosis of FH, undergoes routine screening tests for the detection of atherosclerosis. These screening tests include ECG, echocardiography, carotid, and vertebral artery Doppler ultrasonography (US), ultrasonographic evaluation of renal arteries, and other arteries based on the presence of clinical findings. All of these imaging modalities are performed using standard methods according to the relevant current guidelines. Fundoscopic examination, and exercise stress tests are also components of the routine evaluation of these patients. According to the detected findings, these examinations are repeated at 6-to-12 month intervals. Measurement of thickness of Achilles tendon which is recommended for the follow-up of patients with FH [28] has been routinely performed since 2012. Thickness of Achilles tendon > 9 mm is regarded as xanthoma. [28] In this study, clinical data were obtained from the medical files which included the parameters evaluated during routine follow-up. Age of the patient at the time of diagnosis reason for first admission, detailed family history (including CV disease, hyperlipidemia, xanthoma, consanguineous marriages), presence of CV disease, atherosclerosis in non-coronary arteries, and aortic stenosis (AS) were recorded. Besides, information relevant to all advanced imaging techniques (coronary angiography, tomography, magnetic resonance imaging) performed in case of need was retrieved from patients'medical files. All lipid measurements recorded in patients'files, lipid levels of the patients before and after sessions of apheresis, and complications of apheresis were evaluated

Data are presented as percentages for discrete variables and as mean± SD for continuous variables. Diagnosis of FH was based on extremely increased total cholesterol, and LDL-cholesterol levels, associated with history of familial premature coronary artery disease (CAD), and/or the presence of xanthomas at an early age. Standard Simone-Broome Diagnostic criteria which evaluate presence of typical accumulation of lipid deposits on skin and/or tendons (Table 1). [29] was used for the

identification of the heterozygous family members.

Table 1. Simone-Broome criteria for the diagnosis of familial hypercholesterolemia

For the definite diagnosis of FH

- Presence of indicated levels of cholesterol concentrations, and tendon xanthomas in the patient or in a first- or second-degree relative,

  OR

- DNA based evidence of LDL-R, Apo-B or PCSK-9 mutations

For possible diagnosis of FH

- Presence of indicated levels of cholesterol concentrations, and at least one of the following criteria:
  - Family history of MI in a second-degree relative < 50years of age or in a first-degree relative < 60 years of age

  OR
  - Blood cholesterol level more than > 7.5 mmol/l in a first or second- degree adult relatives (> 6.7 mmol/l in relatives younger than 16 years of age)

Descriptive cholesterol criteria

	Total cholesterol	LDL-cholesterol
Children / Young person	>6.7 mmol/l (260 mg/dl)	>4.0 mmol/l (155 mg/dl)
Adults	>7.5 mmol/l (290 mg/dl)	>4.9 mmol/l (190 mg/dl)

Apo-B: Apolipoprotein B; FH: Familial hypercholesterolemia ; LDL-R: Low density lipoprotein receptor; PCSK-9: Proprotein convertase subtilisin/keksin type 9; MI: Myocardial infarction.

Indication of apheresis therapy was determined as LDL-cholesterol levels >500 mg/dl (>300 mg/dl or non-HDL-cholesterol > 300 mg/dL) in accordance with relevant guidelines.[30-31] Serum lipid levels were measured using standardized methods, and automated analyzers. LDL levels were estimated using both Freidewald formula and in case of suspicion via direct method. Carotid USG findings were evaluated using a linear probe (4-9 MHz), and narrowing of > 69 %, calcification in a plaque, presence of turbulent flow, and peak systolic flow velocity of > 230 cm/sec were reported as indicators of severe carotid stenosis.

RESULTS

Clinical characteristics of 17 patients (11 women, and 6 men) with HoFH are summarized in Table 2. Mean age at admission was 31±10 (17-44) years. Age at the diagnosis was 25±14 years and in only 5 cases diagnosis was made during childhood. Complaints at the time of the diagnosis s were dermatologic (n=7; 41 %), and ischemic (n=7; 41 %). Three patients (18%) were diagnosed during screening for family history of FH. Figure 1 shows the exemplary lipid accumulations of some of our patients. First admissions of all patients were to dermatologists and none of them were questioned for their blood cholesterol levels nor recommended for measurement of their serum lipid levels. In other words diagnosis of FH did not come to any of the attending physician's mind. Moreover, some patients had their xanthelasmas surgically removed, however the surgeons who performed these procedures had not warned their patients about hypercholesterolemia.

In the present study 65 % of patients had consanguineous marriages in their parents. Family history of all cases revealed higher prevalence of early onset CAD. Xanthoma were observed in 10 (59%) patients. Xanthomas were more prominent in patients whose clinical symptoms started in childhood. In all cases, increase in the thickness of Achilles tendon was detected. Thickness of Achilles tendon of 9 patients ranged between 6 mm, and 13.2 mm (mean, 9.4 ± 2.6 mm). Figure 2 shows thickened Achilles tendon. Aortic valve abnormalities including severe AS (n=2), moderate AS (n=4), and mild AS (n=4), were detected in 10 (59%) patients. And one patient had aneurismatic dilation of ascending aorta. In both cases with severe AS, a second narrowing was detected in the supravalvular region (by echocardiography, magnetic resonance imaging, and angiography). Mean carotid intima-media thickness was 1.85±0.83 mm (range, 0.9 mm-3.1 mm).

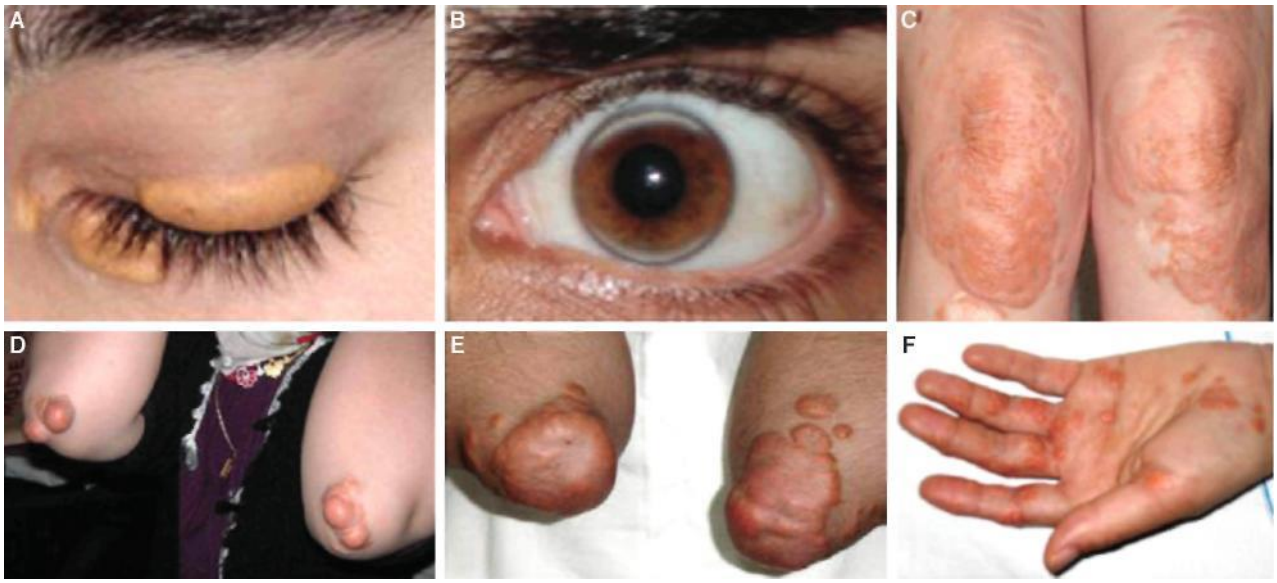

Figure 1. Examples of cholesterol deposits. **(A)** Typical xanthelasma on eyelids (Case 1). **(B) Corneal** arcus lipemia (Case 2). **(C)** Cholesterol deposits on the skin of the extensor surface of the knee joint (Case 3). **(D) Cutaneous xanthomas** of the extensor surface of the elbow**s** (Case16). **(E)** on the skin of the extensor surface of the elbow (Case 1). **(F)** Diffuse cholesterol depositions on the palm (Case 15).

Carotid arterial plaques were apparent in 8 (47%) patients, while severe stenosis was detected in 3 patients. Figure 3 demonstrates the carotid USG of our 2 cases. CAD was detected in 10 (69 %) patients. Detailed history of CAD of the study population is summarized in Table 3.

Lipid values of the patients are shown in Table 4. All of these cases had Type II–a dyslipidemia. At the time of diagnosis mean total cholesterol and LDL-cholesterol levels were 625±136 (447-836) mg/dl and 509±121 (359-747) mg/dl, respectively.

All cases received maxim doses of a statin (atorvastatin 80 mg/day or rosuvastatin 40 mg/day) and ezetimibe (10 mg/day).

Apheresis therapy

All patients had definite indications for apheresis therapy. Three cases had had previously undergone apheresis therapy (Cases 2, 3, and 4). However two of them had discontinued their therapy because of problems of transportation and getting tired of therapy (Cases 2, and 3).

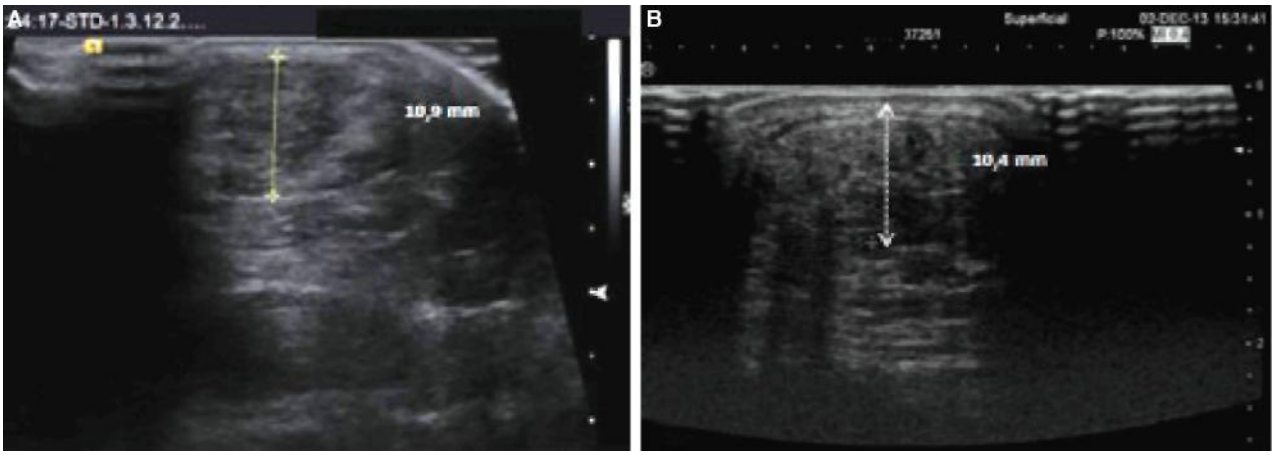

Figure 2. On tranverse ultrasonographic images inhomogenous echogenicity, and thickened Achilles tendon secondary to lipid accumulation. **(A)** Case 7: 10.9 mm-thick Achilles tendon, **(B)** Case 5: 10.4 mm-thick Achilles tendon

Patient No	Gender	Age at diagnos	Reason for admission at diagnosis	Age at first presentation to our clinic	Consangu ineous marriage	TC (mg/dL) at diagnosis	CAD	Valvular disease	Carotid	Xanthoma	Thickness of Achilles tendon (mm)	Apheresis	Final condition
1(SG)	Female	**6**	Angina	18	Yes	824 768	Yes	Moderate AS	With plaque	Diffuse	Not measured Not meaasured	Initiated Initiated (previous 5 sessions, then discontinued) at 3. year he requitted	Died Died
2 (AK	Male	**10**	Xanthoma	21	No		Yes	Severe AS	With plaque	Diffuse			
3(ŞE)	Female	5	Xanthoma	22	Yes	656	Yes	Severe AS	With plaque	Diffuse	Not measured	Initiated (previously attended for 6 mos, then she quitted)	Died
4(EA)	Female	10	Angina	17	Yes	759	Yes	Moderate AS	With plaque	Dizde	10	Regular attendance for 8 years)	Regular apheresis therapy
5 (OK)	Male	4	Xanthoma	22	Yes	700	Yes	Moderate AS	With plaque	Diffuse	12	Initiated	Regular apheresis therapy
6(MK)	Female	39	Angina	41	Yes	764	Yes	None	Severe stenosis	None	13,2	Initiated	Regular apheresis therapy
7 (EŞ)	Female	41	Screening	42	None	600	None	None	İncreased İMT	None	11	Initiated	Regular apheresis therapy
8 (ET)*	Female	26	Xanthoma	29	Yes	466	None	Mild AS	Severe stenosis	Diffuse	11	Initiated -5. Discontinued after the	Discontinued the treatment
9 (MST)*	Male	20	Xanthoma	40	Yes	492	None	Mild AS	Severe stenosis	Diffuse	Not measured	Initiated- Discontinued after the 1. session	Discontinued the treatment
10 (VT)**	Female	42	Screening	42	None	527	None	None	Normal	None	8	Discontinued after the 1. year	She was using another
11 (NY)**	Female	41	Screening	41	None	499	None	None	Normal	None	6	Declined	She was using another
12 (ŞM)**	Male	32	Angina	35	None	447	Yes	None	Normal	None	Not measured	Declined	He was using another
13 (HM)"	Male	41	Angina	44	None	456	Yes	None	Normal	None	6	Declined	He was using another
14 (YA)	Male	38	Angina	42	Yes	515	Yes	Ascending	Normal ,Dilated aorta	None	8	Declined	He was using another
15 (OK)	Female	29	Angina	29	Yes	605	Yes	Mild AS	With plaque	Diffuse	Not measured	She accepted the therapy, but therapy could not be initiated	Died
16 (KB)	Female	22	Xanthoma	18	Yes	836	?	Moderate AS	With plaque	Diffuse	Not measured	She accepted the therapy, but could not be initiated because her expenses were not covered by SS	Lost to follow-up
17 (RK)	Female	24	Xanthoma	22	Yes	702		Mild AS	With plaque	Diffuse	Not measured	Declined	Lost to follow-up

Tc :Total cholesterol; AS: Aortic stenosis; CAD: Coronary artery disease; İMT: intima media thickness; SS: Social security. * Cases 8-9: uncle, and cousine, ** Cases 10, and 11 siblings *** Cases 12, and 13 siblings.

Table 2 Clinical characteristics of the patients with homozygous familial hypercholesterolemia

Table 3. Detailed coronary artery disease history of the patients with homozygous familial hypercholesterolemia

Patient No	Gender	Age at diagnosis	Reason for admission at diagnosis	CAD	Age at diagnosis of CAD	History of CAD	Final condition
1 (SG)	Female	6	Angina	Yes	8	8 years old:; PTCA was applied on right coronary artery because of anginal complaints, During follow-up period, because of her 3-vessel disease, and severe anginal complaints CABG was recommended, however her family declined	Died (During CABG surgery)
2 (AK)	Male	10	Xanthoma	Yes	11	11 years old ; CABG was offered for his 3-vessel disease , but his family declined	Died (heart failure)
3 (ŞE)	Female	5	Xanthoma	Yes	15	She had undergone CABG because of 2-vessel osteal lesion, and severe anginal complaints; she had anginal attacks during follow-up period	Died (heart failure)
4 (EA)	Female	10	Angina	Yes	10	Severe anginal complaints; exercise stress test positivity; the same year she had undergone CABG for severe 3-vessel osteal lesion	Regular apheresis therapy
5 (OK)	Male	4	Xanthoma	Yes	19	19 years old: he had undergone CABG for 2-vessel osteal lesion following an attack of MI	Regular apheresis therapy
6 (MK)	Female	39	Angina	Yes	28	He had suffered from two recurrent MI attacks, and undergone CABG twice for 3-vessel disease His anginal complaits are still continuing	Regular apheresis therapy
7 (EŞ)	Female	41	Screening	None		-	Regular apheresis therapy
8 (ET)*	Female	26	Xanthoma	None		-	She discontinued the therapy
9 (MST)*	Male	20	Xanthoma	None		-	She discontinued the therapy
10 (VT)**	Female	42	Screening	None		-	She was using another antilipidemic agent
11 (NY)**	Female	41	Screening	None		-	She was using another antilipidemic agent
12 (ŞM)**	Male	32	Angina	Yes	29	29 years old; right, and left coronary arteries were stented following MI; restenosis developed for 3 times	She was using another antilipidemic agent
13 (HM)**	Male	41	Angina	Yes	34	34 years old; right, and left coronary arteries were stented following MI; restenosis: none	She was using another antilipidemic agent
14 (YA)	Male	38	Angina	Yes	35	Anginal complaints; on coronary artery angio grams plaques which causes 30-40 % narrowing in all coronary arteries were detected	She was using another antilipidemic agent
15 (OK)	Female	29	Angina	Yes	29	29 years old, died following an attack of anterior wall MI	Died
16 (KB)	Female	22	Xanthoma	Unknown		?	Lost to follow-up
17 (RK)	Female	24	Xanthoma	Unknown		?	Lost to follow-up

CAD: coronary artery disease; PTCA: Percutaneous transluminal coronary angioplasty; CABG: coronary artery bypass grafting; MI: myocardial infarction.

Only one patient was undergoing regular apheresis therapy for 8 years (Case 4). Initiation of LDL apheresis was recommended to 14 patients in our center. However, one died from CV disease while waiting for the reimbursement process (Case 15). Apheresis could not be initiated in one patient as she was not under the cover of social security (Case 16). Later on, this patient was lost to follow-up. One patient initially accepted the treatment, but she withdrew her informed consent before the first session of the apheresis therapy (Case 17).

Four patients refused apheresis and preferred only drug therapy (Cases 11, 12, 13, and 14). Later on these patients gave informed consent for a multi-center randomized clinical trial with mipomersen therapy. The remaining 7 patients underwent regular LDL-apheresis treatment. Besides, apheresis therapy was re-initiated in two patients who had had previous history of short term apheresis (Cases 2 and 3).

Table 4. Lipid levels of the patients

	Lipid levels			
	At first diagnosis	Following Statin+Ezetimibe	The last session of apheresis * Entry	The last session of apheresis exit*
Total cholesterol (mg/dl)	625±136	523±198	436±92	190±56
Triglyceride (mg/dl)	123±41	109±67	118±59	62±20
HDL-cholesterol	55±13	49±16	46±10	37±12
LDL-cholesterol (mg/dl)	509±121	436±151	364±82	142±56
Apolipoprotein-A1 (mg/dl)	111±28	-	-	-
Apolipoprotein-B (mg/dl)	267±92	-	-	-
Lipoprotein A (mg/dl)	56±48	-	-	-

* It contains t data of the patients who attended the sessions of apheresis therapy.

On a total 9 patients, apheresis therapy was initiated. One patient couldn't tolerate the therapy after the first session (Case 9), and he couldn't be convinced despite intensive efforts. After the 5th session, another patient (Case 8) expressed that she wouldn't tolerate the sessions because of her complaints similar to those reported by Case 9, and she also stated that she couldn't attend the sessions regularly because of her small children needed her care at home. One patient (Case 10) had to discontinue her therapy at the end of the first year because she had a new job far away from the medical center, however later on she gave informed consent for participation in a multi-center clinical trial with mipomersen.

The most frequently encountered adverse events due to apheresis were hypotension and syncope during the sessions.

Anemia was observed in all patients under apheresis therapy. Three patients received transfusion, and others replacement therapy. One patient experienced catheter infection twice. All patients except one were psychologically intolerant to apheresis as a chronic, time consuming and invasive treatment. Psychiatric consultation revealed that all these patients had major depression. Doppler US examinations of all cases who regularly attended apheresis therapy for more than one year revealed the progression of carotid plaques (Cases 2, 3, 4, 5, 6, 7, and 10). On the other hand, all cutaneous lipid deposits vanished or markedly regressed in all patients who received apheresis therapy at least 2 years (Figure 4).

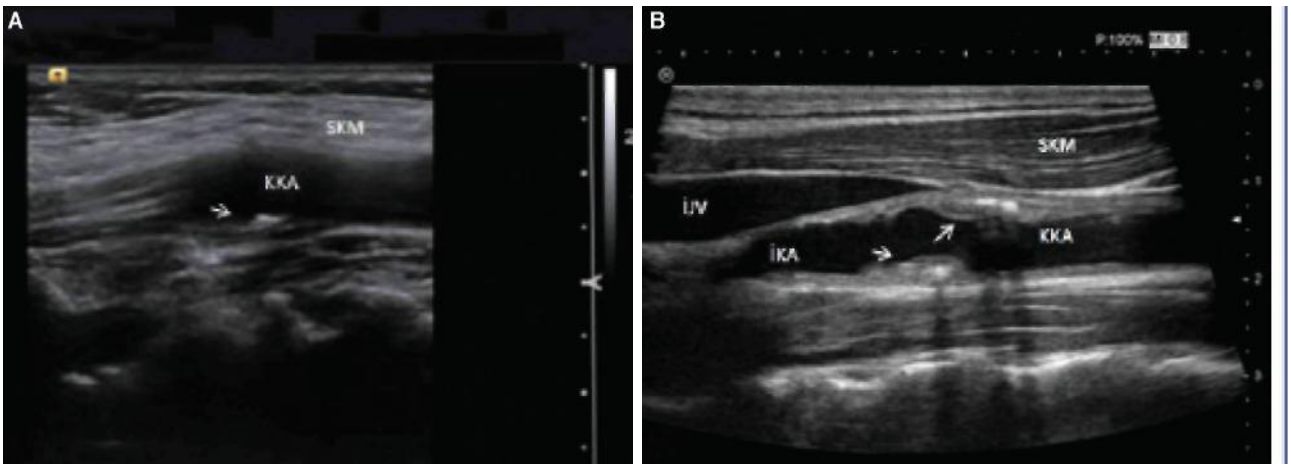

Figure 3. Ultrasonographic evaluation of the carotid artery **(A)** minor calcific plaque on common carotid artery (CCA**)** (Case7). **(B) Diffusely thickened walls of CCA, and internal carotid artery (ICA), calcific plaques which caused luminal narrowing were seen** (Case 5). SKM: Sternocleidomastoid muscle; İJV: Internal jugular vein

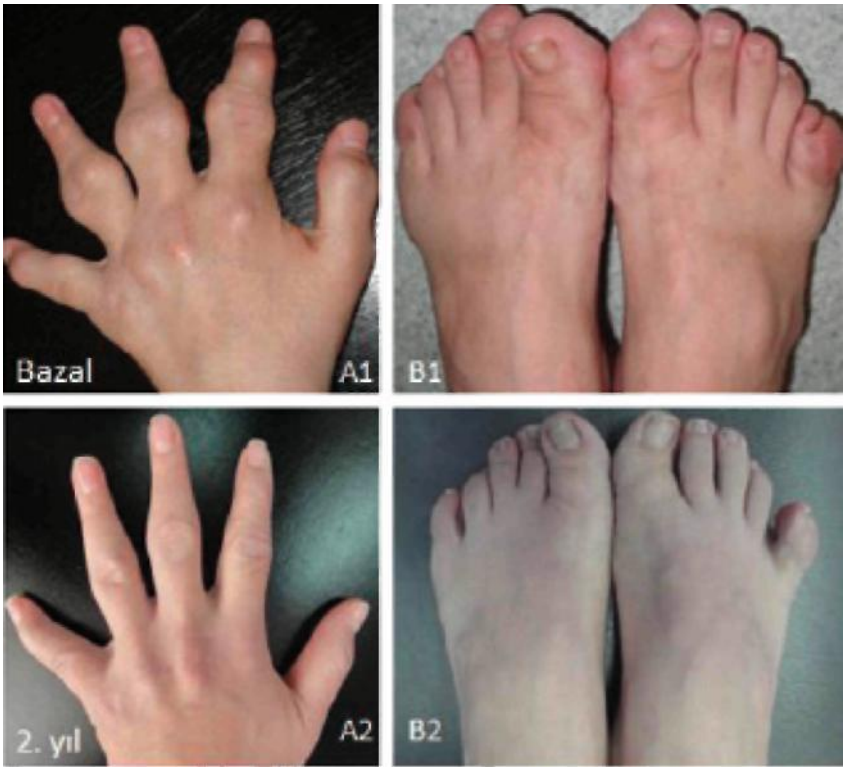

Figure 4. Case 3: Tendon xanthomas on hands (A1), and feet (B1) before apheresis therapy. Marked regression of these xanthomas (A2, B2) at 2. year of the apheresis therapy was seen.

During follow-up evaluation of 10 patients who underwent apheresis, 3 patients (Cases 1,2, and 3) died, 4 patients (Cases 2,8,9, and 10) discontinued their therapy, and currently 4 patients are still receiving regular apheresis (Cases 4, 5, 6, and 7). Compliance rate to apheresis therapy was 60 percent. One of our regular attenders (Case 4) had been bored with long-lasting apheresis therapy since her early childhood and also became depressive as her school were interrupted. Coronary CT angiogram of this patient who had had a coronary artery bypass surgery at the age of 10 revealed the occlusion of one of her saphenous grafts. The second patient (Case 5) who continued to LDL apheresis had undergone coronary artery bypass surgery at the age of 19, and he is still receiving treatment in our center for 2 years. This patient is experiencing difficulties in transportation problem as he is living far very away from our center. One of two female patients who are still undergoing apheresis therapy sessions (Case 6) had had coronary bypass surgery twice in her young age, and she was still struggling with problems of anemia and hypotension. The other patient (Case 7) is experiencing problems of drainage from her fistula during sessions of apheresis.

Cardiovascular events during follow-up

During a mean follow-up period of 48±42 months (2-144 months), two patients were lost to follow-up, and 4 patients were died. One of the deceased (Case 15) had had admitted for the first time to our clinic with acute myocardial infarction, and coronary angiogram revealed severe osteal lesions. She died while waiting for the first session of her apheresis therapy. Other three deceased patients were also in the apheresis therapy group. One of them (Case 1) underwent surgery for persistent coronary ischemia and AS, but she was died during surgery. The second patient (Case 2) underwent regular apheresis therapy for 3.5 years. However, then after he declined apheresis therapy, and died because of advanced heart failure. Case 3, underwent 4.5 years of regular apheresis therapy in our center. However, she died of heart failure a few months after valve surgery. Mean age of the deceased patients was 25±5 (18-29) years. During follow-up period other cases had anginal complaints without any severe CV events.

In order to describe the inherent characteristics of this peculiar disease in a case base more detailed information about the medical history and clinical course of one of our HoFH patients presented:

This was a young female patient (ŞE, Case 3) living in rural area. She was born to a consanguineous marriage. Parents had noted her her xanthomas when she was 5 years old. Although they seek for therapy the attending dermatologist and primary care physicians did not mention of any lipid metabolism disorder. Moreover, her lipid levels were not measured and her family history was not interrogated. Therefore, her family thought that these painless swellings were harmless, and did not seek for further medical information or treatment. Ten years later, when she was 15 years old, with the complaint of severe angina pectoris, she was referred to a university hospital where she underwent coronary artery bypass surgery Since baseline lipid levels were very high, atorvastatin therapy (20 mg/d) was prescribed. As no improvement was observed attending physicians have initiated her to biweekly regular LDL-apheresis therapy. She could maintain her therapy only for 6 months. Afterwards, the family discontinued her therapy because of financial problems and turned back to rural life. During the next 7 years, she was followed up by a primary care physician with atorvastatin 80 mg daily. In 2008, at the age of 22 years she was referred to our lipid clinic with the severe angina complaints. We added ezetimibe (10 mg/d) to her statin therapy, and weekly LDL-apheresis therapy was initiated. Her examination evealed the presence of AS (hypoplasic aorta with a mean gradient of 54/28 mmHg), and moderate pulmonary stenosis. Her coronary bypass grafts were patent on angiographic imaging. However, la severe aneurysmatic dilation on the anastomotic site between saphenous vein, and circumflex artery was detected (Figure 5). She was consulted to department of vascular surgery, and her hypoplasic aorta was not thought to be suitable for reoperation. She continued biweekly regular LDL-apheresis therapy between years 2008 and 2012 in our hospital. During this period, although she was asymptomatic, her AS progressed. As seen in Figure 4 her xanthomas were significantly regressed after two years of regular apheresis therapy. However, she had persistent depressive complaints and psychiatry department initiated treatment with the diagnosis of major depression.

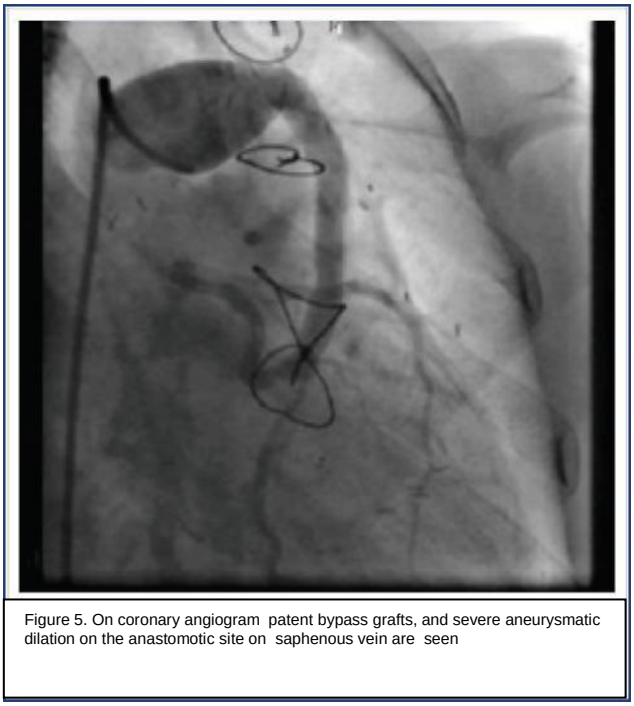

Figure 5. On coronary angiogram patent bypass grafts, and severe aneurysmatic dilation on the anastomotic site on saphenous vein are seen

In 2012, as our center experienced problems in purchasing apheresis materials, she preferred to receive apheresis therapy in another center. In that center, she had undergone surgery for AS, but she died a short-time after surgery at an age of 27 years because of progressive heart failure.

DISCUSSION

Familial hypercholesterolemia is characterized by very high levels of plasma cholesterol and early-onset atherosclerosis.[1-4] LDL-receptor is a glycoprotein which binds to apolipoprotein B (ApoB)-100 on the surface of LDL particles on hepatocytes, and through this pathway it forms, and internalizes LDL-ligand receptor complex.[32] Numerous (>1700) LDL-receptor mutations have been defined which lead to development of FH.[1,2,9] Because of these various types of mutations, plasma cholesterol levels of the patients with FH demonstrate variations depending on the LDL receptor activity.[7,33] Accordingly, patients with < 2% LDL-receptor activity (receptor negative) tend to have very high levels of cholesterol (usually 800-1000 mg/dl), and clinical picture manifests itself with lipid depositions at early childhood. These “receptor negative,” patients do not respond to statins in general and have a poorer clinical outcome. Patients with residual LDL-receptor activity between 2 and 25 %, are defined as “receptor-defective”

In this group, clinical picture becomes manifest at 20 or even 30 years of age dependent on the type of the mutation and residual receptor activity. These patients might respond somewhat better to statins. In our homozygous FH population, the first five patients showed relatively more aggravated onset of manifestations of the disease's clinical picture in early childhood, while the rest had their clinical finding to develop in mid-thirties. This variationof clinical picture is related to the underlying genetic mutations on LDL-receptor. Besides LDL-receptor mutations, Apo-B[34,35] and pro-protein convertase subtilisin/kexin type 9 (PCSK-9) gene [35] mutations can lead to the development of FH. Clinical phenotype originating from all these mutations is variable. For instance, Apo-B mutations exert milder effects among these three mutations. We didn't perform DNA-sequence analysis. Diagnosis of HoFH was made based on clinical manifestations in some and known mutations in other patients. Lack of genetic confirmation in every patient should not be considered as diagnostic limitation. Indeed although a genetic disease, definite diagnosis of FH can be made based on its clinical manifestations.[3,4]

Typical characteristic of HoHF is accumulation of cholesterol in tissues predominantly within dermis. These depositions could be in the form of corneal arcus, xanthalesma on eyelids, and xanthomas on skin and/or tendons (Figure 1). In HoFH, lipid deposits can begin to accumulate on skin in very early childhood which is frequently a prodromal finding of the disease. Therefore, as is seen in our patients usually admit priorly to dermatologists or ophthalmologists. Corneal arcus appearing before the age of 50 years, which is seen in 30 % of the case is an important symptom of FH. [3,4,28] Xanthomas are especially more prominent on extensor tendons, elbows, Achilles tendon, and hands. Our 10 cases had xanthomas which emerged, and became increasingly widespread during childhood, while xanthomas were not observed in other cases. However, even in cases with genetically confirmed FH, xanthomas may not be present in 20-30 % of the cases.[36] In cases without xanthomas, thickening of Achilles tendon (> 9 mm) has been accepted as xanthomas.[28] Thickness of Achilles tendon was increased in all our patients.

The course of atherosclerosis accelerated in HoFH as they are exposed to extremely high levels of cholesterol since birth. Lipids tend to accumulate in especially proximal segments of blood vessels. Depositions in aortic root result in development of valvular and supravalvular AS.

Among our patient population, in cases whose clinical presentation became apparent before the age of 20 years, AS and severe osteal CAD were detected. Type of the genetic mutation depending on the amount of residual receptor activity affects the extent of atherosclerosis, and the age of its onset. The important finding we observed in our patients with AS is that despite regular LDL-apheresis therapy echocardiographic and clinical improvement were not achieved in aortic stenosis, on the contrary AS progressed. This condition might be explained by late initiation of apheresis therapy. In fact initiation of apheresis is recommended at a very early age (< 6-7 years) for the prevention of AS in homozygous patients.[28,30,37] Unfortunately apheresis was initiated in all of our patients after the age of 10 years.

In all patients carotid-intima thickness increased markedly, and calcific plaques were detected. In cases who underwent LDL-apheresis, progresion in carotid lesions were also seen during follow-up. It has been reported that progression or de novo development of CV diseases in 25 % of the patients under regular lipid apheresis.[38] This is observed despite 45-66 % decrease in cholesterol levels relative to baseline values with apheresis. Essentially, apheresis is the most important treatment modality for patients with HoFH. It has been shown that with effective regular LDL apheresis, xanthomas regress (Figure 4), CV events and inflammatory markers decrease, and endothelial functions improve [39,40] Notwithstanding all these favorable effects of apheresis, progression of atherosclerotic process can be explained by the short-lived decreases in lipid levels after apheresis. Indeed, target LDL-cholesterol levels in patients receiving apheresis therapy have not been conclusively defined. Relevant guidelines recommend achievement of at least > 50 % decrease in LDL-cholesterol levels per session of apheresis therapy.[31-41] In our patients generally 50-55 % decrease in LDL-cholesterol levels per session has been achieved. For example, it means that a baseline LDL- cholesterol level of 400 mg/dl will be decreased to 150-200 mg/dl after completion of one apheresis session. However, this level is ensured immediately after the apheresis procedure, and in the following days it rapidly rises back to its baseline values because of unchanged rapidity of Apo-B synthesis.[42]

Another remarkable finding of our study, higher rates of refusal and lower compliance of apheresis therapy. Because of invasive and time-consuming nature of the procedure, the patients get tired of the sessions and become noncompliant to therapy. Initiation of apheresis therapy during childhood and adolescence is an important reason for treatment

noncompliance.
Interruptions in family, school, and professional life adversely contribute to noncompliance to treatment. Because of chronicity of the disease and peculiar characteristics of the apheresis therapy depressive mood develops nearly in all patients which worsens noncompliance to treatment. Besides purchasing necessary materials for apheresis procedures by tender can be delayed, and patients are compelled to live without apheresis therapy. All of these factors can adversely affect the expected benefits from the apheresis therapy.

As is seen, although lipid apheresis is a life-saving procedure in HoFH, in real life compliance to this hard-to-tolerate treatment is challenging, and it is frequently interrupted. Indeed, new treatment modalities which will be more easily applicable and also ensure longer, and effective decrease in blood lipid levels has been investigated. Novel agents targeting Apo-B levels are promising. three new classes of agent have been introduced into the clinical use from the developmental phases. These innovative agents include PCSK-9 inhibitors which prevent degradation of LDL receptors, anti-sense oligonucleotides which inhibits the synthesis of Apo-B (mipomersen), and microsomal triglyceride transfer protein inhibitor lomit apide which inhibits the transfer of Apo-B to very low-density lipoproteins and chylomicrons.[43] Use of mipomersen, and lomitapide in HoFH was approved by FDA in 2012.

In our study, mortality rate was 26.6 percent (4/15, 2 patients who lost to follow-up were excluded from the analysis). The causes of mortality were surgery for aortic stenosis in two, heart failure due to severe aortic stenosis in one, and acute coronary syndrome in the last patient. However common underlying factors of all these deaths are delayed diagnosis, treatment, and misguidance of the physicians. In fact the most important finding of our case series is that the diagnosis delayed in HoFH. Despite emergence of xanthomas during early childhood combined with family history of premature atherosclerosis, diagnosis was always delayed. It is obvious that awareness of primary care physicians about FH is insufficient.
Family screening is important in avoiding delayed diagnosis.[3,4] However only our three cases were diagnosed as FH during family screening. Family screening allows detection of these patients during childhood, and offers them a chance of early diagnosis. Since cases with FH are exposed to higher cholesterol levels from their early childhood, they carry critically higher risks when compared with dyslipidemic cases with similar blood lipid levels, but without any genetic etiology. With early treatment, survival in both homozygous and heterozygous FH patients markedly prolong.
In countries like UK, Holland, and Lebanon, with cascade screening all the first-, and second-degree relatives of a patient diagnosed as FH are screened for lipid levels.[44] In this method genetic tests can be used in case of need. In Holland, with nationwide 'cascade' screening, the number of patients with FH under treatment rocketed from 39% to 93 percent.[45] This is a very important achievement. Indeed in countries having a genetic map for FH, only 15 % of the cases of FH can be diagnosed.[3,4]

FH data related to our country usually come from homozygous case reports presented by one or two centers of apheresis which generally do not contain follow-up information. In only 36 (20 homozygous and 16 heterozygous) cases genetic LDL- receptor sequence analyses have been performed so far, without any follow-up data.[46] Our study is the first to document the long-term clinical course of adult patients with HoFH. With its retrospective design, the results have reflected real-life clinical approach to the disease. On the other hand as reflects the experiences of a lipid clinic of a university hospital, it is more comprehensive, and more consciously conducted than standard approaches.

In conclusion, HoFH is a genetic disease which progresses with accumulation of cholesterol in all over body, and on vessels in the early years of life, and also it is associated with higher mortality secondary to cardiovascular events. Even though a genetic disease it can be clinically diagnosed. And if effective continuous LDL decrease could be achieved survival prolongs significantly. As FH patients are exposed to increased levels of cholesterol since birth early diagnosis is the key point in the success of the management of patients with HoFH. In order to commence early diagnosis and early treatment, awareness of the physicians should be increased. Particularly raising the awareness among primary care physicians regarding high cholesterol levels in children and adolescents would be a substantial step. Public awareness is also an important step. Parents should realize that FH is common disease with high mortality, and that the harms of high cholesterol levels begin in childhood. In order to achieve all these goals, National policy for the diagnosis and treatment of FH should be developed.

Conflict of interest: None declared

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Anahtar sözcükler: Ailevi hiperkolesterolemi; apolipoproteni-B-100/genetik; hastalığa genetik yatkınlık; hiperlipoproteinemi type ll/epidemiyoloji; koroner arter hastalığı; ksantoma; lipit aferezi.

Key vvords: Familial hypercholesterolemia; apolipoprotein B-100/genetics; genetic predisposition to disease; hyperlipoproteinemia type ll/epidemiology; coronary artery disease; xanthoma; lipid apheresis.