# Comparison of classical and probable heterozygous familial hypercholesterolemia cases with controls.

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## Abstract

**Backgroud:** Heterozygous familial hypercholesterolemia are either classical cases with high LDL-C levels, tendon xanthomas and LDL receptor gene mutation or probable cases with hypercholesterolemia without known LDL receptor gene mutations.

**Aims & Objectives**: This study was done to compare the severity of hypercholesterolemia and risk of CVD in classical and probable cases of heterozygous familial hypercholesterolemia in tertiary care hospitals of Karachi.
This was a case-series done from June 2008 to July 2010 at Dr Ziauddin Hospital and National Institute of Cardiovascular Diseases, Karachi, Pakistan.

**Methods:** Out of more than 240 patients with dyslipidemia or premature coronary artery diseases 120 patients who had primary hypercholesterolemia and were found to have total cholesterol >230mg/dL and LDL-C >160mg/dL were included in the study. All these cases had premature coronary artery diseases or had a family history of coronary artery diseases. They were grouped as classical or probable cases depending on raised LDL-C, xanthelasmas, xanthoma, premature coronary artery diseases and LDL-R gene mutations. Their blood samples were collected after twelve hours fasting. PCR was done for mutation specific primers for exons 3, 4, 9 and 14.

**Results:** Out of these 120 patients with heterozygous familial hypercholesterolemia, classical cases with LDL-receptor gene mutation were 42(35%) and probable cases without LDL-receptor gene mutations were 78(65%). Eleven (27%) of these classical cases had severe hypercholesterolemia having total cholesterol (>290mg/dL) and LDL-C (>200mg/dL) and were found to have xanthelasmas, xanthomas and arcus cornealis.

**Discussion/Conclusions:** Amongst the HeFH patients from the tertiary care hospitals of Karachi, classical cases with tendon xanthomas and LDL receptor gene mutations had severe hypercholesterolemia with greater risk of CVD, they required more aggressive treatment. Probable cases were also at risk of CVD and needed statin therapy.

**Key words: HeFH** Heterozygous familial hypercholesterolemia, **LDL-R** low density lipoprotein receptor, **PCR** polymerase chain reaction, **TX** tendon xanthomas, xanthelasmas **CVD** Cardiovascular diseases.

## Introduction

Familial hypercholesterolemia is most common autosomal dominant disorder which is characterized by increased LDL-C, tendon xanthomas, arcus cornea, LDL-R gene mutation, family history of hypercholesterolemia and premature coronary artery diseases. Tendon xanthomas develop by the third or fourth decade of life. Thus a family history of coronary heart disease indicates strong genetic cause [1].

Clinical characteristics of high lipid levels are premature coronary artery disease, tendon xanthomas or corneal arcus. The frequency is one in five hundred for heterozygous familial hypercholesterolemia (HeFH) and one in a million that are homozygous famililal hypercholesterolemia. The frequency of HeFH is much more in Afrikaners of South Africa [2] and French Canadians [3]. In autosomal dominant hypercholesterolemia there is one affected parent and positive history of premature coronary heart diseases. First degree relatives have 50% chance of this autosomal dominant disease.

Tendon xanthomas are highly specific for FH in subjects with genetic high LDL cholesterol, and are an important diagnostic criterion. Tendon xanthomas are composed of monocyte-derived foam cells resulting from intracellular accumulation of lipids and connective tissue [4].

In patients with HeFH, the capacity of the liver for catabolism of LDL-cholesterol in a regulated manner is impaired. LDL-cholesterol residence time in plasma is therefore prolonged and the propensity of the cholesterol particles to undergo oxidation increased. Cholesterol deposits within the skin of the eyelids are xanthelasmas , those in connective tissues within and surrounding extensor tendons, especially the Achilles and extensor tendons of the hands, are xanthomas and deposits along the corneal margin are arcus cornealis or corneal arcus. The most dangerous deposits occur within arteries, where they have potential to cause premature coronary artery diseases, stroke and peripheral vascular diseases [5, 6].

The clinical significance of tendon xanthomas (TX) presentation has not been fully established [7]. Because of the lipid and cellular similarities between tendon xanthomas and atherosclerotic plaques, it is probable that heterozygous FH subjects in whom xanthomas develops could also have a higher risk of atherosclerosis [8, 9].

Raised LDL-cholesterol levels in familial hypercholesterolemia are due to deficit in the function of LDL-R gene. The LDL-R gene on chromosome 19p13 and has 18 exons [10] The 'Classical' cases have elevated total cholesterol, LDL-cholesterol, tendon xanthomas or mutation of LDL-R gene. The 'Probable' cases of familial hypercholesterolemia have elevated levels of total cholesterol and LDL-cholesterol with a family history of hypercholesterolemia and coronary artery diseases (Simon Broome Register Group, 1991) [11]. The classical cases of heterozygous familial hypercholesterolemia were reported with mutation at exon 3 and 4. The mutations were further confirmed by DNA sequencing [12].
As no study comparing classical and probable cases of HeFH has been reported on the Asian population of Karachi, Pakistan, it is this research gap that has been addressed as it has been clinically observed that incidence of cardiovascular diseases is very high and one of the main risk factor is HeFH.

## Methods

After interviews of over six hundred patients from Dr. Ziauddin Hospitals and National Institute of Cardiovascular Diseases, Karachi all those hyperlipidemic patients with diabetes, hypertension, thyroid diseases and using drugs causing hyperlipidemia were excluded from the study. A total of 240 patients were screened for hypercholesterolemia after performing lipid profile, having premature cardiac diseases, family history of hyperlipidemia or premature cardiac diseases. Age range of these participants was between 22-60 years. This study was approved by Ethical Review Committee, Ziauddin University. All the participants gave written informed consent.
These patients were screened for diabetes mellitus, hypertension, cigarette smoking and use of drugs as these factors alter the cholesterol levels. The lipid profile was determined by auto analyser, using Hitachi kits. 120 patients with hypercholesterolemia with very high LDL- Cholesterol levels, have premature coronary artery diseases in second and third decades of life, were compared with twenty controls .
The EDTA sample was used for LDL genotyping for the detection of mutation in exons 3, 4, 9 and 14 and PCR was performed. Genotyping was performed with a clear objective of establishing rapid, reproducible and direct molecular diagnostic assays utilizing direct polymerase chain reaction (PCR). PCR was done for LDL-R gene mutation with genomic DNA extracted by using DNA extraction kits (Epicenter, USA) [13].

### *Statistical analysis*

SPSS (16.0) package was used to analyze data. Mean and Standard deviation determined for lipid profile, total cholesterol, LDL- Cholesterol, triglycerides and HDL-

Cholesterol for cases and controls. Students t test was applied to compare means of total cholesterol, LDL-cholesterol, triglycerides and HDL-cholesterol; p value less than 0.05 was considered significant and 0.001 as highly significant.

## Results

Total cholesterol was found significantly increased in 'Classical hypercholesterolemia cases (272 ± 70 mg/dL) as compared to controls (184 ± 27.9 mg/dL). LDL- cholesterol in classical cases was (201± 62 mg/dL), as compared to controls (105.15 ± 22.32 mg/dL) *** p-value was statistically significant. Baseline characteristics of the heterozygous FH patients are given (**Table1**).

The diagnosis of these familial hypercholesterolemia patients was based on clinical features (xanthomas, xanthelasmas, arcus cornealis), lipid profile (high LDL-cholesterol) premature coronary artery disease, family history of premature coronary artery diseases. Eleven of these patients were found to have xanthelasmas, xanthomas and arcus cornealis. (**Figure 1**). They all had family history of premature coronary artery diseases. Pedigrees of two of these patients are shown in (**Figure 2**).

Classical subjects with heterozygous familial hypercholesterolemia with raised LDL-C and mutation in exons 3 and 4 of LDL-R gene as shown in (**Figure 3**).

Probable cases were seventy eight with raised total cholesterol, LDL- C with positive family history of hypercholesterolemia and premature coronary artery diseases.

## Discussion

Xanthelasmas and xanthomas were seen in cases with severe hypercholesterolemia in study by Civeira et al. (2005) [14], their study also shows that tendon xanthomas in familial hypercholesterolemia are associated with cardiovascular risk independent of low-density lipoprotein receptor gene mutation. In our study out of one hundred and twenty subjects who participated eleven had xanthomas, xanthelasmas and arcus cornea with severe hypercholesterolemia and premature ischemic diseases. Tendon xanthomas seen in classical cases in this study are associated high LDL-cholesterol level with increased risk of cardiovascular diseases.

Approximately 26.9% of heterozygous FH patients with genetic diagnosis have tendon xanthomas [8] In this study 27% heterozygous familial hypercholesterolemia patients were found to have tendon xanthomas, xanthelesmas.

According to Simon Broom Register [15] the total cholesterol in HeFH subjects is greater than 7.5mmol/L (>290mg/dL) and LDL – cholesterol is greater than 4.9 mmol/L (> 191mg/dL) [15]. In this study mean total cholesterol in HeFH subjects was greater than 260 mg/ dL and LDL –cholesterol was greater than 192 mg/dL. Their triglycerides were not raised significantly and HDL-cholesterol were low (mean HDL- C< 37 mg/dL).

Descamps et al [16] reported that LDL-R gene mutation is common cause of familial hypercholesterolemia in Belgian population and mutation of exon 4 was most common.

When present in a heterozygous form, most of the mutations identified thus far have been reported to cause a typical clinical picture of FH, with grossly elevated serum LDL cholesterol levels, tendon xanthomas and premature coronary heart disease [17]. In a study done on United Kingdom population, the greatest number of LDLR gene mutations were found in exons 3 (10%), exon 4 (28%), exon 10 (10%) and exons 14 (21%) [18]. Mutations have been reported in exons 3, 4, 9, and 14 of LDLR gene amongst Indians settled in South Africa, which suggests an increased frequency of FH in India [19]. The common mutations were identified at exons 3 and 4 in the various ethnic groups of one hundred and twenty hypercholesterolemia patients in Karachi, Pakistan [20] .

Genetic diagnostic tests assist in the identification of family members with hypercholesterolemia thus improving cardiovascular risk prediction, prevention of disease and treatment efficacy. The most cost effective diagnosis for FH is to screen the family members [21]. The large DNA rearrangement is associated with Alu element. When present in a heterozygous form, most of the mutations identified thus far have been reported to cause a typical clinical picture of FH with grossly elevated serum LDL cholesterol levels, tendon xanthomas and premature coronary heart diseases [22].

In fact, the presence of xanthomas has been associated with very premature cardiovascular diseases, although a recent study from the Simon Broome Register Group in the United Kingdom showed a similar atherosclerosis risk among patients with or without xanthomas. Similarly elevated cardiovascular diseases risk suggests that in adulthood groups of HeFH patients should be treated equally aggressively with HMG Co A reductase inhibitors [23].

## Conclusions

Amongst patients with HeFH from tertiary care hospitals of Karachi, classical cases with severe hypercholesterolemia were found to have LDL receptor gene mutation. The probable cases also had increased risk of premature CVD thus both groups require intensive therapy to prevent CVD morbidity and mortality

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**Table 1: Base Line Characteristics of Heterozygous Familial Hypercholesterolemia**

| **Characteristics** | **Heterozygous Familial Hypercholesterolemia (n=120)** | | | |
|---|---|---|---|---|
| | Classical (n=42) | Probable (n= 78) | Controls(20) | p |
| Age (yrs) | 30 ± 8.6 | 39 ± 8.6 | 30 ± 10 | |
| Male : Female | 22: 20 | 40: 38 | 12:9 | |
| **Lipoproteins** | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | |
| Total Cholesterol (mg/dL) | 272 ± 70 | 267 ± 42 | 184 ± 24 | 0.001 |
| LDL-Cholesterol (mg/dL ) | 201.71± 62.11 | 187± 42 | 105 ± 23 | 0.001 |
| HDL-Cholesterol (mg/dL) | 33 ± 9 | 37 ± 42 | 39 ± 4 | 0.33 |
| Triglycerides (mg/dL) | 215 ± 70 | 194 ± 42 | 190 ± 15 | 0.77 |
| **Clinical features** | **( n= 120)** | | | |
| Xanthomas/ Xanthelasmas | 11 | | | |
| Myocardial Infarction | 70 | | | |
| Family history of CVD/ FH | 120 | | | |

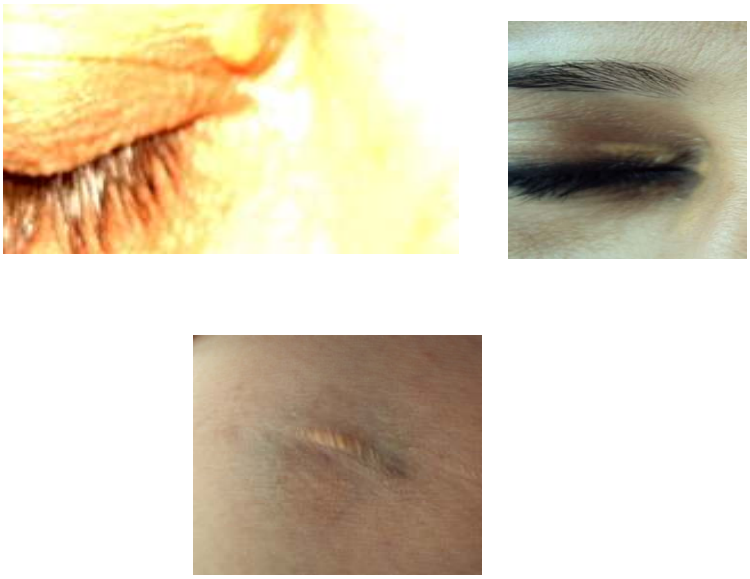

**Figure 1: Xanthelasmas and Xanthomas (Photos of The Study Cases) ***

* The figures show xanthelasmas due to cholesterol deposited on skin of eyelids. Xanthomas on connective tissue in extensor tendon of elbow

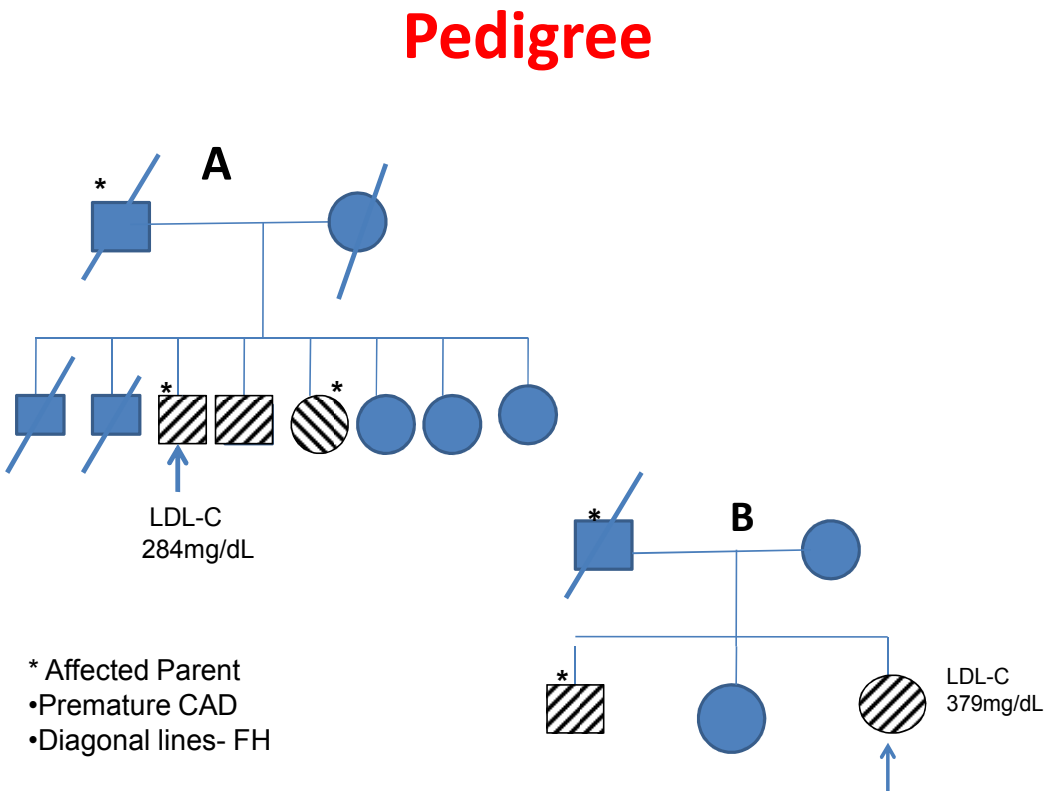


**Figure 2: Pedigree of Two Families with Familial Hypercholesterolemia**

All the cases included in the study had positive family history of hypercholesterolemia and premature coronary artery diseases. Two families pedigree are shown above **Pedigree A,** show Proband (age 35 years) with LDL-C 284mg/dL and had CABAG, he has eight sibling, two brothers and one sister had premature myocardial infarction and father died before forty due to cardiac failure one sister also had CABAG at 30 years of age. **Pedigree B**, Proband (age 32 years) having LDL-C 379 mg/dL, her father died due to cardiac failure in his third decade and two siblings are diagnosed with familial hypercholesterolemia, her younger brother had a CABAG.

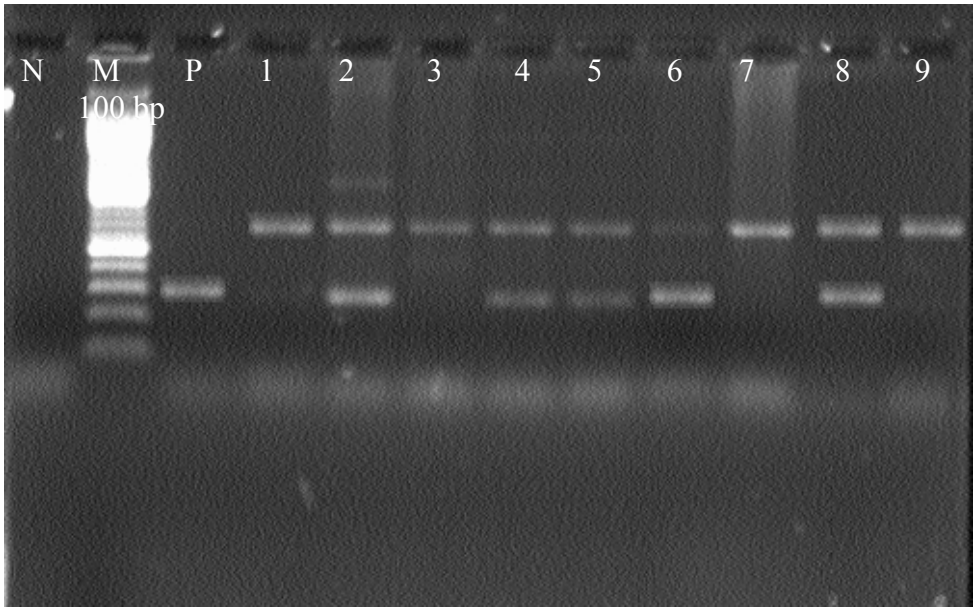


**Figure 3: LDL-R Gene Mutation on Exon 4 and Exon 4 & 3**

The gel analyzed the mutation on exon 3 (162bp) and exon 4 (431bp) for LDL receptor gene. Lane N is the negative control, lane M is the DNA ladder used is 100 bp and lane P is the positive control used showing mutation of exon 3. Lane 1, 3, 7 and 9 shows mutation of exon 4. Lanes -2, 4,5, 6 and 8 show two mutation on exon 3 and 4.