INTRODUCTION

Familial hypercholesterolemia (FH) is an autosomal dominant disease caused by mutations in the low-density lipoprotein receptor (LDLR) gene and defects in the apolipoprotein B-100 (apoB), causes Familial ligand defective apoB (FDB).\(^1\)\(^-\)\(^2\) FH is characterized by a selective increase of plasma LDL-C, giving rise to tendon and skin xanthomatosis and to premature coronary heart disease. Plasma LDL-C levels are directly related to atherosclerosis which lead to the development of coronary artery disease. Of the lipoprotein disorders, familial hypercholesterolemia is the most common disorder causing premature coronary artery diseases with autosomal dominant mode of inheritance.\(^3\) Heterozygous familial hypercholesterolemia (HeFH) is single gene disorder that affects ~1 in 500 people but the prevalence is much higher in some population.\(^4\)

Low density lipoprotein (LDL) is the cholesterol carrying lipoprotein in human plasma and major cause of cardiovascular diseases. Lowering the levels of LDL-C is the most important measure to be taken in preventing cardiovascular diseases.\(^5\)

The LDL receptor gene consists of 18 exons on a 45 kb chromosome 19p13. Around 800 mutations have been determined including deletions, insertions and point mutations. These deletions or insertions have Alu sequences. These mutations will affect synthesis, post-translation processing, ligand binding activity and internalization of the LDL receptor. The LDLR gene has 18 exons and Chang \textit{et al.} has given the list of primers for amplification of LDL receptor exons.\(^6\) These receptors mediate endocytosis and receptor-bound
LDL enters hepatocytes and undergoes degradation in lysosomes and the cholesterol remnants. Negative-feedback regulates the number of LDL receptors. A rise in the hepatocyte cholesterol level suppresses the transcription of LDL-receptor genes, and LDL is retained in the plasma. Whereas, decrease in hepatic cholesterol stimulates the transcription of LDLR genes, removing LDL from the plasma.7

The current study was thus undertaken to identify four previously reported LDLR gene point mutations in Indian hypercholesterolemic patients with clinical features of FH.8 The principal aim was to determine the common mutation of low density lipoprotein receptor in hyperlipidemia patients and screening for heterozygous familial hypercholesterolemia in Karachi.

**METHODOLOGY**

The samples were collected from Dr. Ziauddin Hospital and National Institute of Cardiovascular Diseases, Karachi, of patients with or without coronary heart diseases. This study was approved by ethics review committee of Ziauddin University and all participants gave written informed consent. This study was partially funded by Ziauddin University.

240 blood samples were collected from the patients for lipid profiles. The blood samples were collected after informed consent and overnight fasting for lipid profile and in EDTA (ethylenediamine tetra acetic acid) tubes for genotyping. Sample size consisted of 120 cases of hypercholesterolemia and 50 controls (normal lipid profile) collected for genotyping. Sample size was estimated using epi info. computer programme, using prevalence from previous studies.

Inclusion criteria were hypercholesterolemia with or without coronary artery diseases and family history of hypercholesterolemia. Exclusion criteria were Diabetes mellitus, hypertension, renal disease, hypothyroidism and steroid therapy. All subjects aged between 26 to 60 years.

Lipid profile parameters were determined through auto-analyzer (Hitachi kit) for total cholesterol, LDL-C, HDL-C and triglycerides. Cases were divided into classical and probable ones according to Simon Broome Register. Genotyping was done to detect LDL receptor gene mutation and its frequency in the studied group.

The multiplex PCR was performed in a single tube using type specific primers as given. These mutations have been reported previously among Indians of South African population.8 The genomic DNA was extracted from whole blood collected in EDTA tubes with Epicenter DNA Purification Kit (Cat No. MCD85201).9-10

The PCR reactions contained 10 mM Tris/HCl, 50 mM KCl, 1.5 mM MgCl₂, 50 pM of each nucleotide. The total for the PCR reaction mix was 15 µl. In reaction mix composed of 10 µg of DNA template, 20 pmol of each of the eight primers, 2.5 unit Taq DNA polymerase. The thermal cycling regimen consisted of 1 cycle of denaturation 94°C for 5 minutes, following with 35 cycles, 94°C for 45 seconds, annealing at 55°C for 45 seconds and extension at 72°C for 1.5 minutes with the final extension of 72°C for 7 minutes. The amplified product was identified on 2% agarose gel and was stained with ethidium bromide which was visualized under UV light. The different mutations were characterized with 100 bp DNA ladder for common four mutations of LDL receptor gene.

SPSS (version 16.0) package was used to analyse data. Mean value and standard deviation was determined for lipid profile, total cholesterol, LDL-Cholesterol, triglycerides and HDL-Cholesterol for cases and controls. Student’s t-test was applied to compare the mean values; p-value of less than 0.05 was considered significant.

**RESULTS**

One hundred and twenty hypercholesterolemic patients were analyzed for mutations for the LDLR gene. In 42 cases total cholesterol was reported to be > 200 mg/dL and LDL > 160 mg/dL, with family history of coronary artery diseases. Of them 21 cases had developed premature coronary artery diseases and 11 cases reported tendon xanthomas, xanthelasma or arcus cornealis.

Forty two classical cases were found to have high LDL-C, xanthelasmas, tendon xanthomas and arcus cornea with exon 3 and exon 4 mutation of LDLR gene. Other patients had high LDL-C and family history of coronary artery diseases. One of those patients had stroke at 32 years of age and 3 patients had coronary bypass. Seventy eight probable cases with family history of myocardial infarction and raised cholesterol levels in first degree relatives was reported during this study.

![Figure 1: The primer sequencing of different exons for identification of LDLR gene mutations used for the samples with familial hypercholesterolemia by PCR.](image-url)
There were 35% classical and 65% probable cases in these samples. The common mutations identified were located on exon 3 and 4 as shown in Figures 2, 3 and 4, in the various ethnic groups, n=120 hyperlipidemics in Karachi. The forward and reverse primers used for this study were of exons 3, 4, 9, 14 with base pairs, 162, 431, 550 and 496 respectively (Figure 1).

**DISCUSSION**

This study showed 35% classical and 65% probable cases of HeFH in a sample of 120 hyperlipidemic patients from a two tertiary care centres. The cases were divided classical and probable according to Simon Broome Register for diagnosis of heterozygous familial hypercholesterolemia.11 There was a high frequency of low density receptor mutation in patients of hypercholesterolemia in population of Karachi. Goldestein et al. reported that it may result from defect in LDLR that removes LDL from plasma.1 According to Friedrickson Phenotypes it is type IIa.

Clinical characteristics are premature coronary artery disease, tendon xanthomas or arcus cornea. Higher frequency of LDLR gene mutation has been reported in South African Afrikaners and French Canadians.12,13 The frequency of heterozygous FH in most other populations is 1 in 500 except for Lebanon where frequency of heterozygous FH is 1 in 171; in Quebec this frequency is 1 in 270.14

Forty two cases showed LDLR gene mutation, 14 cases with severe hypercholesterolemia had mutation bands in exon 4 and 3 (33%) and 28 had mutation bands in exon 4 (66%). Descamps et al. described LDLR gene mutation in exon 4 which is a common cause of familial hypercholesterolemia in Belgium.15 However, it is of interest that among the probands carrying the FH mutation, some subjects are free of the typical clinical features of FH (tendon xanthoma, arcus cornealis, and coronary heart disease) despite a fairly high level of plasma LDL cholesterol.

In a study conducted in the population of United Kingdom,16 the largest number of LDLR mutations were found in exon 4 (28%) and exons 14 (21%); the other two mutations were found on exon 10 (10%) and exons 3 (10%). It was also reported that 46% of LDLR mutations were found in the ligand binding domain (exons 3-6) and 46% were found in the EGF precursor-like domain (exons 7-14).

Further, Fard-Esfahani et al.17 reported a new missense mutation in exon 4 (445G > T) in Iranian population. In this study also, we have reported that both exon 4 and exon 3 are the common type of mutations. Frequently reported mutation on exon 4 could be as it is the largest exon of LDL receptor gene.

About 700 different mutations in the LDL receptor gene have been reported worldwide. In the Danish FH population, so far 60 different mutations have been localized and the LDL receptor gene has been identified throughout.6 In certain populations a small number of mutations predominate due to founder effects. The spectrum of LDL receptor mutations in Danish FH patients is intermediate between such specific founder populations with 5 predominant mutations (W23X, W66G, W556S, 313 + 1G--> A, 1846-1G--> A) accounting for about 40-50% of FH.18

In a case report by Aslam et al. on a Pakistani family,19 the index case had severe hyperlipidemia and multiple xanthomas all over his body. His father and four other siblings were also diagnosed with familial hypercholesterolemia.19 In this study, tendon xanthomas, xanthelasmas and arcus cornealis were seen in 11 of the classical cases. Xanthomas are associated with increased cardiovascular risk and is the indication for aggressive lipid lowering therapy.

Genetic diagnostic tests will assist in the identification of family members of FH, while improving cardiovascular risk prediction, prevention of disease and treatment efficacy. It is most cost-effective to diagnose FH is to screen close family members. As FH hypercholesterolemia responds well to treatment, early diagnosis can reduce the risk of premature cardiac diseases.20-21
CONCLUSION

In this study, there was a high frequency of HeFH. Further studies should be continued to determine the new cases with HeFH who are at particularly high risk of premature cardiovascular disease. The point mutation on exon 3 and exon 4 of LDLR gene was the most common. PCR is useful for the detection of large rearrangements in the LDL-receptor gene and is a rapid and reliable method for the diagnosis of familial hypercholesterolemia.

REFERENCES