

Familial Hypercholesterolemia: Screening, Diagnosis And Management of Patients.

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Abstract: Familial hypercholesterolemia (FH) is a genetic disorder of lipoprotein metabolism resulting in elevated serum low – density lipoprotein cholesterol leading to increased risk for premature cardiovascular disease (CVD). The diagnosis of this condition is based on clinical features, family history, altered lipids profiles and aided more recently by genetic testing. As the cardiovascular disease burden is dependent on the degree and duration of exposure to raised LDL- Cholesterol levels, early diagnosis, and initiation of treatment is essential. This review aims to highlight the screening, diagnosis, goals of therapy and management option in patients with FH. India ranks at high position along with other countries. During the last several years, our knowledge about possible biomarkers of familial hypercholesterolemia has increased, parallel the development of new therapeutic approaches. The search for a peripheral biomarker that could be utilized diagnostically has resulted in an extensive amount of studies that employ several biological approaches including the assessment of tissues, genomics, proteomics and metabolomics.

Keywords: Familial hypercholesterolemia, low- density lipoprotein, LDL receptor, Apo B, PCSK9 gene mutation.

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I. Introduction

Familial hypercholesterolemia (FH) is a genetic disorder of lipoprotein metabolism, characterized by elevated plasma total –cholesterol commencing from childhood, and manifestation of atherosclerosis in adulthood¹. Men who have familial hypercholesterolemia have heart attacks in their 40's to 50's, and 85 percent of men with the disorder have a heart attack by age 60. Women who have familial hypercholesterolemia also have an increased risk for heart attack, but it happens 10 years later than in men (so in their 50's and 60's). An accelerated development of CVD is observed after menopause². Clinical management of FH focuses on early detection and control of hypercholesterolemia to decrease the risk of atherosclerosis and to prevent premature cardiovascular disease. Establishing an accurate diagnosis of FH is often difficult. Despite its significant prevalence and considerable benefit associated with its early detection and treatment, FH is often under diagnosed in many countries. Systematic genetic screening for mutation in those at risk of FH has been found to be cost effective and will help in better prognosis. In India only few studies have been carried out on genetic diagnosis. Thus, the aim of the study is to evaluate the diagnostic utility of total lipid profile and lipid related genetic variants such as Low Density Lipoprotein Receptor (LDLR), Apolipoprotein B (APOB), and the Proprotein Convertase Subtilizing/kexin type 9 (PCSK9) in the clinical management of FH.

Genetics of FH

Familial hypercholesterolemia is inherited in families in an autosomal dominant manner. In autosomal dominant inherited conditions, a parent who carries an altered gene that causes the condition has a 1 in 2 (50 percent) chance to pass on that altered gene to each of his or her children.

LDL – Receptor gene

The LDL receptor gene is located on the short arm of chromosome 19 (19 p 13.1-13.3). It comprises 18 exons and spans 45kb and the protein product contains 839 amino acids in natural form. It contains the information for a protein called LDL receptor expressed on liver cells that is responsible to clear up LDL from the blood stream. One in 500 individuals carries one altered gene causing familial hypercholesterolemia^{3,4}. These individuals are called heterozygote. More rarely, a person inherits the gene mutation from both parents, making them genetically homozygous⁵. Individuals who are homozygous have a much more severe form of hypercholesterolemia, with heart attack and death often occurring before age 30⁶. Plasma LDL is inversely

related to the activity of LDL receptor (LDLR). There are five major classes of Familial Hypercholesterolemia due to LDLR mutation^{3,4}

- Class I – LDLR is not synthesized at all.
- Class II – LDLR is not properly transported from the endoplasmic reticulum to the Golgi apparatus for expression on the cell surface.
- Class III – LDLR does not properly bind with LDL on the cell surface of a defect in either Apo lipoprotein B 100 (R3500Q) or in LDLR.
- Class IV – LDLR bound to LDL does not bound properly cluster in Clathrin – coated pits for receptor mediated endocytosis.
- Class V – LDLR is not recycled back to the cell surface

Apo B gene

Apo B in its Apo B 100 form is the main Apo protein or protein part of lipoprotein particle. Its gene is located on 2nd chromosome (2p24 – p23) and is between 21.08 and 21.12mb long. Familial Hypercholesterolemia is often associated with mutation of R3500Q, which causes replacement of arginine by glutamine at position 3500. The mutation is located on a part of the protein that normally binds with LDL receptor and the binding is reduced as the result of the mutation. Like LDLR, the number of abnormal copies determines the severity of Hypercholesterolemia⁷.

PCSK9 gene

Mutations in the Proprotein Convertase Subtilizing/kexin type 9 (*PCSK9*) gene were linked to autosomal dominant (i.e. requiring only one abnormal copy) FH in a 2003 report⁸. The gene is located on the first chromosome (1p34.1-p32) and encodes a 666-amino acid protein that is expressed in the liver. It has been suggested that PCSK9 causes FH mainly by reducing the number of LDL receptors on liver cells⁹. Over 1,500 mutation of the low-density lipoprotein (LDLR) gene has been detected by Brown and Goldstein^{10,11}. Janine Genschel and Peter Thomas identified two novel mutations in the LDL receptor gene in patient with FH. Apo 3500 and Apo3551 defects were excluded by PCR. The mutation C122Y was found in a single 66 yrs. old female with CVD. The subject had one healthy daughter with normal concentration of total and LDL cholesterol. The E296X mutation was found in a family (father and two children) with hypercholesterolemia. None of these subjects had cardiovascular disease. The missense mutation C122Y is in the exon 4 and results in amino acid exchange of cysteine by tyrosine at codon 122 in the ligand binding domain of the LDL receptor. The nonsense mutation E296X is in the first codon of exon 7¹².

Livia Pisciotto and Claudio Olive¹³ sequenced Apo B and PCSK9 genes in two patients with the clinical diagnosis of homozygous FH who were heterozygous for LDLR gene mutations. One female patient (LDL-C 13.39 mmol/L) was heterozygous for an LDLR mutation (p.E228K) inherited from her father (LDL-C 8.07 mmol/L) and a PCSK9 mutation (p.R496W) from her mother (LDL-C 5.58 mmol/L). She and her sister (LDL-C 11.51 and 10.47 mmol/L, Xanthomatosis and carotid atherosclerosis) were heterozygous for an LDLR mutation (p. Y419X) inherited from their mother (LDL-C 6.54 mmol/L) and a PCSK9 mutation (p. N425S) probably from their deceased father. The LDL-C levels in double heterozygotes of these two families were 56 and 44% higher than those found in simple heterozygotes for the two LDLR mutations, respectively. The two PCSK9 mutations are novel and were not found in 110 controls and 80 patients with co-dominant hypercholesterolemia. These observations indicate that rare missense mutations of PCSK9 may worsen the clinical phenotype of patients carrying LDLR mutations¹³.

Though single gene disorder plays a crucial role in the etiology of FH, linkage studies have exposed that many cases of FH are caused by numerous unexceptional genetic variations. Interplay of these genetic variations together with environmental factors remains the leading cause of hypercholesterolemia in general population^{14,15}.

II. Screening For FH

Screening tests for FH included risk assessment questionnaires, biochemical tests and combination of both. The ideal strategy to screen for FH is currently a controversial issue. The lipid guidelines advocated “targeted screening” which compared a fasting lipid profile test with risk factors for FH such as family history of premature cardiovascular diseases (CVD), Dyslipidemia or obesity¹⁶. An alternative approach to screening is termed ‘cascade screening’ in which we actively screen for disease among the first and second degree relatives of patients diagnosed by the targeted screening. An equally important question is what to screen—lipids OR genes? Genetic screening strategy involves searching for the common genes causing FH among the patients and their close relatives¹⁷. Recent National Institute for Health and Care Excellence (NICE) guidelines recommend a DNA testing on all patients diagnosed with FH and subsequent genetic screening among their close relatives to augment case detection rates. Sometimes a significant number of patients clinically diagnosed with FH are

tested negative by genetic screening¹⁸. In such patients' genetics testing is expected to have a very low yield and is unlikely to be cost effective. Hence genetic screening is likely to benefit only where a definite mutation is identified. In other cases, a strategy of lipid profile, based cascades screening is preferable.

Clinical features of familial hypercholesterolemia:

The major features of familial hypercholesterolemia are^{19,20}

- High levels of total cholesterol and LDL cholesterol.
- A strong family history of high levels of total and LDL cholesterol and/or early heart attack.
- Elevated and therapy-resistant levels of LDL in either or both parents.
- Xanthomas (waxy deposits of cholesterol in the skin or tendons). (Fig.A, B, B)
- Xanthelasma (cholesterol deposits in the eyelids). (Fig.C)
- Corneal arcus (cholesterol deposit around the cornea of the eye). (Fig.D)
- If angina (chest pain) is present, it may be a sign of heart disease.

Individuals who have homozygous familial hypercholesterolemia develop xanthomas beneath the skin over their elbows, knees and buttocks as well as in the tendons at a very early age, sometimes in infancy. Heart attacks and death may occur before the age of 30.



Familial hypercholesterolemia diagnosis:

Diagnosis of familial hypercholesterolemia is based on physical examination and laboratory testing. Physical examination may find xanthomas and Xanthelasma (skin lesions caused by cholesterol rich lipoprotein deposits), and cholesterol deposits in the eye called corneal arcus.²¹

Laboratory testing includes blood testing of lipid profile²², studies of heart function and genetic testing. Blood testing of cholesterol levels may show: increased total cholesterol usually above 300 mg/dl (total cholesterol of more than 250 mg/dl in children) and LDL levels usually above 200 mg/dl. Studies of heart function, such as a stress test, may be abnormal. Genetic testing may show an alteration (mutation) in the LDL receptor gene²².

Management of familial hypercholesterolemia:

The overall goal of treatment is to lower the risk for atherosclerotic heart disease by lowering the LDL cholesterol levels in the blood stream. Atherosclerosis is a condition in which fatty material collects along the walls of arteries. This fatty material thickens, hardens and may eventually block the arteries. Atherosclerosis happens when fat and cholesterol and other substances deposits in the arteries to form a hardened material called plaque. The plaques make the arteries less flexible and more difficult for blood to flow leading to stroke and heart attack.

Lifestyle changes

The first step in the treatment for an individual who has heterozygous familial hypercholesterolemia is changing the diet to reduce the total amount of fat consumed up to 30% of the total daily calories. This can be done by limiting the amount of beef, pork, and lamb in the diet; cutting out butter, whole milk and fatty cheeses as well as some oils like coconut and palm oils; eliminating egg yolks, organ meats and other sources of saturated fat from animals. Dietary counseling is often recommended to help people to make these changes in their eating habits²³. Therapeutic lifestyle adjustments are important part in the management of FH. This specific dietary manipulation, physical activity, limitation of alcohol intake, and total avoidance of tobacco products. Recent guidelines recommend a low-calorie diet with a total fat intake of $\leq 3\%$ of total dietary intake including

≤8% of saturated fat and < 75/1,000 kcal cholesterol for these patients. However, dietary restriction are noted to have a more effective in lowering the lipids²³.

Drug therapy

Drug therapy is usually necessary in combination with diet, weight loss and exercise, as these interventions may not be able to lower cholesterol levels alone.

Bile acids sequester

The guideline issued by National Heart, Lung and Blood institute (NHLBI) advised treatment with bile acid sequestrates, the lowest age recommended for initiation being 10 years²⁴. This was based on the excellent long term safety profile of this drugs group, owing to lack of their systematic absorption. They bind with bile acids in intestine, thereby, preventing their systematic absorption. This results in a greater conversion of cholesterol to bile acids and an enhanced production of LDL receptors by the liver. Cholestyramine and colestipol were the most frequently used drugs. However, they proved less favorable due to their modest efficacy (10-20% LDL reduction) and gastrointestinal intolerance²⁵.

Statin

Statins are currently the first line of drugs in the treatment of FH. They inhibit the rate limiting step in cholesterol synthesis, thus increasing the expression of LDL receptor, resulting in the rapid clearance of LDL from the blood. Among the various statins available, the food and drug administration (FDA) has approved of pravastatin in children over 8 year of age and lovastatin, atorvastatin, and simvastatin above the age of 10 years²⁶. The statin therapy remains controversial, as this can potentially hamper the production of steroid hormones in the body²⁷. A recent Cochrane review²⁸ and two meta- analysis²⁹ of the placebo- controlled trials on statin in children and adult with FH showed no major side effects about growth, sexual development, muscle and liver toxicity, concurrently they show good efficacy in lipid lowering with 26.5% mean relative reduction in LDL-cholesterol levels. There are specific recommendations about monitoring patients with statin therapy. Creatine Phosphokinase (CK) to assess muscle toxicity, Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) to assess liver toxicity are mandatory prior to initiation of statins. Drug therapy should be interrupted when CK levels reach five times, and AST and ALT three times over normal limits²³. Follow-up measurement is recommended 1-3 months after starting the drugs and yearly thereafter. The same drug at a lower dose or a different statin may be introduced after a drug free interval of 3 months. Other drugs may be tried if the patient does not tolerate statin despite these measures.

Ezetimibe:

Ezetimibe is a new class of cholesterol absorption inhibitors that acts on the brush border of the small intestinal epithelium. The specific site of its action is believed to be the epithelial cell.³⁰ As their mechanism of action is not based on the expression of LDL receptors, they are especially beneficial in the management of Homozygous FH. Clinical trials have displayed their efficacy in reducing LDL levels when given alone³¹ or in combination with statins^{32, 33}. The US food and Drug Administration (FAD) has approved of Ezetimibe therapy in children over the age of 10 years. Current guidelines recommended drug initiation before 18 years of age only in patients intolerant to statins and in patients who fail to realize lipid goals with statin monotherapy.

Surgical options:

In addition to the therapies, surgical option including bypass and portocaval shunt has been tried earlier in refracting cases. To the significant involved and the need for treatment before the onset of clinical effects of atherosclerosis, these never become a popular choice of treatment. Recently successful pediatric liver transplant has been done for the treatment of FH. However, in view of the scarcity of donor liver available and complexities of the transplant and post- transplant management such a decision should be taken only after carefully assessing the risk benefit ratio^{34,35}.

III. Summary

Most diseases in adulthood are a result of complex interactions between genetic and environmental factors. Coronary heart disease (CHD) is one of the largest killers in the Western population. In India too, the prevalence of CHD is on the rise. Moreover, the disease is seen in the younger population, which is a cause of great concern. Thus, it becomes imperative to identify the risk factors leading to premature heart diseases. The genetic evidence of CHD is rapidly emerging. Familial Hypercholesterolemia (FH), though not the only cause of CHD is certainly one of its major concern.

The genetic association between LDLR gene and APO B gene PCSK9 gene with Familial Hypercholesterolemia has been highlighted. The biochemical parameter in serum such as total cholesterol, triglyceride, High Density

Lipoprotein, Low Density Lipoprotein, Apo lipoprotein A and B; and CK, AST, ALT can be assessed along with genetic markers in Familial Hypercholesterolemia patients, as they are diagnostically effective and significant. The effects of drug therapy on these biochemical measurements can shed light on the mechanism to improve the therapeutic methods and drugs for familial hypercholesterolemia.

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