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Homozygous familial hypercholesterolemia: Treatments and needs for LDL apheresis in Tunisia

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ABSTRACT

Familial hypercholesterolemia is an autosomal dominant inherited disorder caused by mutations in the low density lipoprotein receptor gene, the proprotein convertase subtilisin/kexin type 9 gene, the apolipoprotein B gene, and more rarely, the autosomal recessive hypercholesterolemia adaptor protein.

The worldwide prevalence is about 1 case per million for homozygous, and 1 per 500 for heterozygous. In some population, the prevalence of FH is greater, presumably due to founder effects. In Tunisia, the frequency is about 1 per 165 for heterozygous and 1 per 125000 for homozygous.

Treatment typically involves lipid-lowering drugs as well as mechanical removal of plasma LDL by means of apheresis. Statins are the most prescribed drugs for HoFH. Frequently, Statins alone do not lower LDL-cholesterol level to therapeutic level. Combination with other pharmacological drugs such as Ezetimibe or Fenofibrate may enhance the LDL-cholesterol reduction. In the case of Statins intolerance, LDL apheresis is the best treatment option.

The purpose of this review is to provide current perspectives on therapies available for FH patients, particularly LDL apheresis, in an effort to encourage the development of this therapy in Tunisia.

KEYWORDS

LDL apheresis;
homozygous familial hypercholesterolemia;
Treatments.

INTRODUCTION

Homozygous familial hypercholesterolemia (HoFH) in an inherited disorder caused primarily by mutation in the low density lipoprotein receptor (*LDLR*) gene. Three other genes lead to similar phenotype with varying severity: apolipoprotein B-100 (ApoB-100), proprotein convertase subtilisin/kexin (*PCSK9*), and more rarely the autosomal recessive hypercholesterolemia (*ARH*) adaptor protein[1].

The LDL receptor is mainly located in the liver[2,3]. its main function is removing the LDL particles from the plasma by endocytosis[2]. More than 1200 mutations were reported, covering the entire gene.

HoFH patients with mutations in the *LDLR* gene may be true homozygous (having the same mutation in both *LDLR* alleles) or compound heterozygous (having different mutations on each *LDLR* alleles).

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HoFH patients present severe hypercholesterolemia and if untreated, cholesterol levels range between 580-1160 mg/dl (15-30 mmol/L)[4]. At a clinical level, FH is associated with cholesterol deposits in several tissues. Cutaneaous deposits lead to xanthomas and xanthelasmas, corneal deposits lead to corneal arcus and deposits in vascular walls are at the origin of premature coronary heart disease (CHD)[5].

In addition, HoFH patients frequently develop either valvular or supravalvular aortic stenosis[6,7].

FH is one of the most common genetic disorders. The prevalence of HoFH is estimated to be 1 case per 1 million, whereas the heterozygous form (HeFH) is estimated at 1 case per 500 persons[7]. In some populations, the prevalence of FH is greater, presumably due to founder effects[1]. Among French Canadians, the prevalence of HeFH is 1 in 270 and is 1 in 275000 for HoFH[8]. The prevalence of HoFH is 1 in 100000 among the Lebanese. In Tunisia, the prevalence of HoFH was estimated to be 1 in 125000 and 1in 165 for HeFH[9].

Although, a high prevalence of FH was reported among Tunisian population, only pharmacological treatments are available, and there is no LDL apheresis. In this review we focus on treatment available for FH patients, in particular LDL apheresis. then we expose the clinical and genetic characteristics of Tunisian FH patients, and we concluded on the necessity to develop LDL apheresis in Tunisia.

CURRENT LIPID LOWERING THERAPY

Dietary treatment

For FH patients, lifestyle modifications to lower LDL-cholesterol level and reduce other coronary vascular disease (CVD) risk factors should be introduced despite the modest and variable degree of LDL-cholesterol reduction[10]. For FH patients a diet containing less than 7% saturated fat and less than 200 mg of cholesterol is to be advised.

Pharmacological treatments

(a) Statins

Statins are Hydroxy-3-MethlyGlutaryl-Coenzyme A (HMG-CoA) reductase inhibitor. They inhibit the rate-limiting step in cholesterol synthesis by reducing the conversion of HMG-CoA to mevalonate. The consequently decreased intracellular cholesterol levels induce an upregulation of the LDL receptor which leads to an increased clearance of LDL-cholesterol and a decreased plasma LDL-cholesterol level[11].

Statins are the most commonly prescribed drugs for FH patients[12]. and may be started early in the first year of age or at initial diagnosis in patients with HoFH[13]. Observational data from large FH cohorts suggest that long-term statin treatment removes the excess lifetime risk of CVD due to FH and reduces it to a level similar to that of the general population[14].

(b) Bile acid sequestrants

Use of bile acid sequestrants such as cholestyramine showed that they only have minor effects on lowering cholesterol levels[15].

Fenofibrate from the fibrate class is another drug with the license for use on children[3]. It works by activating the peroxisome proliferator-activated receptor type alpha. Fenofibrate was assumed to be able to lower LDL-cholesterol; however, Tonstad *et al.* reported that there was little evidence for its effectiveness on children, although it was well tolerated[16].

(c) Ezetimibe

Ezetimibe represents the first of a new class of agents, the cholesterol absorption inhibitors. It binds to a protein called Niemann-Pick C1-like 1 (NPC1L1) protein transporter, which reduces the delivery of intestinal cholesterol to the liver. As a result, the LDL receptor expression is upregulated and the clearance of LDL-cholesterol from plasma is increased[17].

Ezetimibe is able to reduce the LDL-cholesterol level by 15-25% from baseline in monotherapy[18]. The combination with simvastatin represents the most common combined therapy, due to the fact that ezitimibe can add an extra 20% reduction in LDL-cholesterol to that seen with Statins alone[19].

Lipid apheresis

Hypercholesterolemic patients with high level of LDL-cholesterol cannot be treated sufficiently by diet and drugs. Lipid apheresis can reduce this atherogenic lipoprotein. Moreover, lipoprotein (a) (Lp(a)), a prothrombotic proatherogenic lipoprotein,which is not

amenable to any conservative treatment, is co-eliminated by LDL apheresis[20].

Since 1975, LDL-cholesterol has been removed from patients' blood with a treatment called "plasma exchange" (plasma apheresis)[21].

Since 1981, Stoffel *et al*.[22] combined these two concepts by using a cell separator to perfuse plasma through a column containing anti-LDL antibodies, a procedure called LDL apheresis.

Today LDL apheresis is the major indication for performing extracorporeal blood purification. In many countries, including the Unites States, Japan and many European countries, LDL apheresis is a treatment of choice for patients with HoFH, particularly those refractory to Statins[23].

Five different techniques of LDL apheresis are in current use: immunoadsorption (IA), dextran sulphate-cellulose adsorption (DSA), heparin extracorporeal LDL precipitation system (HELP), double filtration plasmapheresis (DFPP) or lipidfiltration and direct adsorption of lipoprotein using hemoperfusion (DALI).

(a) Immunoadsorption (IA)

The commercially available system consists of a continuous flow cell separator which pumps plasma through twin columns containing polyclonal sheep antibodies to human apoB-100 coupled with sepharose 4B gel. The result is an acute reduction in LDL-cholesterol and Lp(a) of about 55% after each procedure [24].

(b) Dextran sulphate-cellulose adsorption (DSA)

Dextran Sulphate-covalently bound to cellulose beads selectively binds very low density lipoprotein (VLDL) and LDL but not high density lipoprotein (HDL)[25]. Studies in FH patients showed an acute reduction in LDL-cholesterol of 75-80% and in Lp(a) of 65-70%[26].

(c) Heparin extracorporeal LDL precipitation (HELP) system

This system involves on-line precipitation of LDL through the addition of heparin to plasma. Precipitation of LDL occurs without addition of cations if the pH is sufficiently low, the precipitate being removed by filtration. The net results of this procedure are acute reductions in LDL-cholesterol and Lp(a) of around 60% [27]. Side effects were mild and infrequent and hemorrhagic complications were not observed despite a 50%decrease in fibrinogen [28].

(d) Double filtration plasmapheresis (DFPP) or lipid filtration

In this procedure plasma is separated from blood cells by a hollow first fiber filter and then perfused through a second filter which selectively retains useful plasma components, such as HDL, but discards larger molecular weight components including LDL. Thermofiltration involves warming plasma to 38°C prior to DFPP, which increases the amount of LDL removed and reduces the amount of HDL lost [28]. In comparative studies DFPP was almost as efficient as DSA in lowering both LDL cholesterol and the LDL/HDL ratio and as effective as HELP in removing Lp(a) and fibrinogen[29].

(e) Direct adsorption of lipoprotein using hemoperfusion (DALI)

DALI LDL-apheresis is the first LDL-adsorption system which directly adsorbs atherogenic lipoproteins from whole blood[30]. Thus, no plasma separation is necessary, which simplifies the extracorporeal circulation and markedly improves the user-friendliness of the system. The adsorbent consists of modified polyacrylate ligands immobilizedon Eupergit®. Its mechanism of action hinges on the electrostatic interaction of the negatively charged polyacrylate adsorber ligands with the positively charged Apo B moiety of LDL, Lp(a)and VLDL[31]. Treatment of a large volume of blood (1.6 blood volumes) acutely reduced LDL-cholesterol by 66-77% and Lp(a) by 59-73%[32] without reducing HDL-cholesterol or fibrinogen.

Direct adsorption of LDL from whole blood can also be achieved with dextran sulphate, using larger beads than those used in the standard Liposorber columns developed for plasma[32]. This system is now in clinical use.

TABLE 1 compares the efficacy of the different techniques described below.

Acute decrease in LDL-cholesterol range from 49 to 76%, averaging over 60% and differ little between various methods. The data suggest that IA and DFPP decrease HDL-cholesterol more than other methods.

Described methods are usually performed to lower the LDL-cholesterol level. After 2 to 4 weeks patients

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TABLE 1 : Mean percentage reduction of plasma lipoproteins and fibrinogen with different methods of LDL apheresis[28]

	IA (%)	DSA (%)	HELP (%)	DFPP (%)	DALI (%)
LDL-cholesterol	62-69	49-75	55-61	56-62	53-76
HDL-cholesterol	9-27	4-17	5-17	25-42	5-29
Lp (a)	51-71	19-70	55-68	53-59	28-74
Triglycerides	34-49	26-60	20-53	37-49	29-40
Fibrinogen	15-21	17-40	51-58	52-59	13-16

will return to the baseline level and regular sessions of apheresis are so required to lower levels of cholesterol after rebounds.

LDL apheresis has been shown to have a beneficial effect on aortic and coronary atherosclerosis on HoFH patients and also to reduce the risk of CAD on patients with HeFH[4].

Studies have also shown that HoFH patients treated by LDL apheresis can be free from cardiovascular disease[33]. Furthermore, two clinical studies with event-based assessment have demonstrated remarkably beneficial outcomes of long-term LDL apheresis using dextran sulfate cellulose columns plus adjunctive cholesterol-lowering drug therapy in the prevention of cardiovascular events in HeFH with CAD[34].

Side effects of LDL apheresis are infrequent, mild, and have mainly consisted of lightheadedness, nausea, vomiting, and hypotension.

Indications and guidelines for therapeutic use for LDL apheresis

The indications for LDL apheresis have been reviewed recently. And in the USA[35].the Food and Drug Administration (FDA) have approved the use of DSA and HELP apheresis in 3 categories of patients:

a) Functional FH homozygotes, with LDL-cholesterol >13mmol/L
b) Functional FH heterozygotes, with LDL-cholesterol >7.8mmol/L
c) Functional FH heterozygotes, with documented CHD and LDL-cholesterol >5.2 mmol/L.

Liver transplantation

Operative strategies to reduce blood cholesterol have been proposed previously. However, as it is known that most of the LDL receptors are located in the liver, this procedure has become a treatment of choice for affected, non-responsive to routine pharmacologic treatments patients[36].

Liver transplantation is possibly curative for HoFH and may be best performed as early as possible to limit vascular complications[37,38]. Given the reductions in risk and the potential for cure for HoFH, several successful liver transplantations from non-FH donors have been conducted in patients before appreciable vascular lesions appeared[37]. However, liver transplantation is limited by a lack of donor organs and the need for ongoing postoperative immunosuppression.

Other potential treatment options for HoFH, including portacaval shunting, partial ileal bypass surgery, and gene therapy are proposed but have not been shown to be effective.

FUTURE THERAPIES

Several therapies under investigation may be proved beneficial for treatment of HoFH.

Mipomersen (ISIS 301012) is a therapeutic antisense that targets Apo B-100 mRNA. The long-term safety and effects of this medication are being evaluated not only in HoFH but also in subjects with severe HeFH and in subjects intolerant to statin treatment[39,40].

Lomitapide (AEGR-733; BMS-201038), a microsomal triglyceride transfer protein (MTP or MTTP) inhibitor, interrupts VLDL assembly and secretion in the liver. Lomitapide current tests demonstrated that it reduced LDL-cholesterol levels and Apo-B levels by 50.9% and 55.6%, respectively, from base-line after 4 weeks of treatment[41].

Other potential therapies include PCSK9 inhibitors, which target wild-type PCSK9 and may lead to an increased expression of the LDL receptor. Published results of PCSK9 inhibitors (antisense and monoclonal antibodies) in non-human primates demonstrate 50–70% reductions in circulating LDL that was transient with the antibody inhibitor[42-44].

CLINICAL AND BIOLOGICAL ASPECTS OF ADH IN TUNISIA AND CASCADE SCREENING

Studies on ADH in Tunisia started in 1993 with the

work of NM Slimane and coworkers. They estimated a high frequency of this disease for heterozygous (about 1/165). Beside they noted an attenuated phenotypic expression of ADH[9,45].

Indeed, the analysis of 91 ADH patients showed that the prevalence of CHD in Tunisian ADH heterozygous after 30 years old was 23.5% for men and 29.4% for women. All of them went through life without developing any tendon xanthomas (except one female aged 62). The mean total cholesterol level for heterozygous was 7.04 ± 1.40 mmol/L and was higher than the one reported in China (6.1 ± 1.2 mmol/L) [46]. but lower than in Japan (8.8±2.0 mmol/L)[47]. or in Italy (8.49±1.66 mmol/L)[50]. The same observation was made concerning LDL-cholesterol levels.

Concerning homozygous patients, xanthomas were present for all of them, CHD was present for 10% of them before 9 years old, for 71% between 10 and 19 y.o. and for 100% above 20 y.o.. Therefore, CHD in Tunisian ADH homozygous appears to have a later onset than in other homozygous populations. Indeed, CHD occurs for 50% of the Afrikaners ADH homozygous patients before 9 y.o.[51] .and for 25% in Japan before 10 y.o.[47]. Their mean life expectancy was 13 y.o. compared with 17 y.o. in Japan[47] .and 21 y.o. in Italy[48]. The mean total cholesterol level for homozygotes reported was 17.52±3.12 mmol/L[47]. similar to those reported in other populations.

A recent study in Tunisia showed that 24% (9 out 38) of the ADH patients carrying an heterozygous mutation in the *LDLR* gene have a LDL-cholesterol level under the 60th percentile of an age-and gender-matched reference population[50]. This discrepancy between the clinico/biological and molecular phenotype observed reveals the existence of factors that decrease the severity of the disease. In a previous study, we identified one of these factors as the traditional Tunisian diet which is enriched in polyunsaturated fats[9].

GENETIC DEFAULTS CAUSING ADH IN TUNISIA

Primary genetic studies were focused on *LDLR* gene. Recently, we started research on *PCSK9* gene variation. Concerning *APOB* gene, studies were realized to search for the p.Arg3500Gln mutation. Studies were carried on 102 patients from 19 unrelated ADH families.

TABLE 2 : Molecular defects reported in Tunisian ADH patients

Exon	cDNA modification	Mutation at peptide level	Type of mutation
	On *LDLR* gene		
2_5*	g.11205052_11217736 del12684	p.Gly23_Arg 271del	Major rearrangement
3	c.267C>G	p.Cys89Trp	Missense
4	c.443G>C	p.Cys148Ser	Missense
5	c.796G>A	p.Asp266Asn	Missense
5_6*	g.11216885_11219249 del2364	p.Ala231_Cys312del	Major rearrangement
7*	c.1027G>T	p.Gly343Cys	Missense
8-9*	c.1186+1G>A	p.Glu380_Gly396del	Splice site
10*	c.1477-1479del/insAGAGACA	p.Ser493ArgfsX44	Frame shift
12-13	c.1845+1G>A	?	Splice site
15*	c.2299delA	p.Met767CysfsX21	Frame shift
17*	c.2446A>T	p.Lys816X	Non sense
	On *PCSK9* gene		
1	c.63_64 ins CTG	p.leu21 dup/Tri	Insertion
3*	c.520C>T	p.Pro174Ser	Missense
9	c.1420A>G	p.Ile474Val	Missense
12	c.2009G>A	p.Gly670Glu	Missense

*** Mutations identified only in Tunisian patients.**

Mutations identified in Tunisian ADH patients are presented in TABLE 2.

In the *LDLR* gene, we identified 11 mutations in the different exons of the gene, from them 7 were novels. Mutations were nonsense, frame shift, missense and major rearrangement. The mutation p.Ser493ArgfsX44 in exon 10 appears to be the most frequent mutation[9,45,50-52].

Concerning the *PCSK9* gene, our team identified a novel missense mutation named c.520C>T (p.Pro174Ser) localized in exon 3. Study indicates that this new *PCSK9* variant is able to reduce the severity of FH, very probably acting as a loss-of-function variant. This funding should be confirmed by in vitro experiments[50].

CONCLUSION

Familial hypercholesterolemia is a serious genetic disorder affecting patients at an early age.

In Tunisia, it is well demonstrated the high prevalence of HoFH with a 1 case per 125000. Special ef-

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forts are required to identify individuals with ADH in Tunisia as they are at high risk of premature coronary heart disease.

Patients can benefit only from medical drugs, because no LDL-apheresis is available in the country.

Therefore, there is a great need for the development of such technique in Tunisia. Indeed, LDL apheresis is a very important therapeutic tool for managing patients at high risk for premature CAD, and it represents the best option for HoFH patients in particular who are resistant to or intolerant of statin therapy.

The decision to start LDL apheresis should be made carefully, with every attempt given to maximize drug therapy options, and guided by regular and detailed cardiovascular assessments.

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