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Serum HDL - Cholesterol Levels In Patients With Molecularly Defined Familial Hypercholesterolemia.

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ABSTRACT:
Background- *familial hypercholesterolemia (FH) is the most common genetic disorder leading to premature atherosclerosis. Typically, it is due to mutation in the LDL receptor gene resulting in elevated total and LDL cholesterol levels. The type of the LDL receptor gene mutation may affect the severity of hypercholesterolemia and consequently the incidence of coronary atherosclerosis. Furthermore, HDL- cholesterol levels have been recently showed to be an independent risk factor for coronary heart disease in the population. In this study we examine the effect of the gene mutation and of common gene polymorphism possibly affecting the lipid metabolism. CETP (cholesterol ester transfer protein), Total cholesterol. HDL cholesterol, LDL cholesterol, Triglycerides levels in patients with the molecularly defines heterozygous familial hypercholesterolemia. We studied 925 women and men from the general population, and scan for the ischemic heart disease, and familial hypercholesterolemia, all from western region of Maharashtra for three mutations in the apolipoprotein B gene: Arg3500Gln, Arg3531Cys, and Arg3500Trp*
Conclusion: *we conclude that HDL cholesterol levels in heterozygous FH patients may be affected by the Apo B gene polymorphism.*

Key words: *HDL Cholesterol, Familial Hypercholesterolemia, Apo B gene polymorphism.*

I. INTRODUCTION:

Elevated plasma low density lipoprotein (LDL) cholesterol levels are related to ischemic heart disease early in life[1,2]. The syndrome results from mutation in the LDL receptor (LDLR) gene. The type of mutation in the LDLR gene may affect the levels of total and LDL- cholesterol, and consequently the incidence of CHD as well as patients responsiveness to statin treatment's. it has been recently reported that high-density lipoprotein(HDL)cholesterol levels is an independent risk factor for CHD in heterozygous FH[3,4]. It is well known that his polymorphism or mutation in APO B gene can affect the HDL levels. APO B in its Apo B 100 form is the main apoprotein or protein part of lipoprotein particles. Its gene is located on 2nd chromosome (2p24-p23) and in between 21.08 and 21.12 mb long[5]. 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 numbers of abnormal copies determine the severity of the hypercholesterolemia[5].

Despite intensive research in this field, the mutation in Apo B has been identified. Arg3500Gln, which is responsible for familial defective apolipoprotein B-100, and Arg3531Cys .however since familial defective Apoliprotein B-100 has with few exceptions been identified only among patients with hyperlipidemia OR familial hypercholesterolemia and not in general population, the estimate of this mutation in the adult general population is only approximate, the effect of the mutation on cholesterol levels are probably overestimated and the risk of ischemic heart disease associated with this mutation is not known. Neither the frequencies of Arg3531Cys and Arg3500Gln, in the general population nor their effect on hyperlipidemia and the risk of ischemic heart disease is known[6].

In this regards, for a practical point of view , in order to be of clinical value for screening, a mutation in Apo B must be both relatively frequent and associated with hypercholesterolemia and thus with an increased risk of ischemic heart disease , we studied the mutation in Apo B to see whether they fulfil these criteria.

II. MATERIALS AND METHODS:

To obtain subjects from the general population we recruited 512 women's and 413 men's from the departments of the medicine, Grant Govt. medical college and sir J.J. group of hospital, Mumbai and Raigad hospital ad

research centre Karjat from2014 to 2019.patients were diagnosed on the basis of relevant diagnosis modalities such as clinical examination along with routine biochemical investigations. Institutional ethical committee clearance was obtained after informed consent, under full aseptic precautions.
Blood samples were obtained after 14 hrs, overnight fast for genetics detection as well as for determinations of lipid parameters .blood samples were centrifuged for 30 mins .(3600)and serum was separated stored at temp.4°C for analysis. The lipid profiles – Total cholesterol, triglycerides, LDL, HLD cholesterol were determined by Enzymatic methods (cholesterol oxidase and peroxidise) Diagnosis kits.

DNA Analysis:

The Arg3500Gln and Arg3531Cys mutation are caused by substitution of adenine for guanine 10699 of complimentary DNA and thymine for cytosine at position 10,791 respectively in exon26 of the APO B gene[7,8]. The presence of either mutation was determined in pooled samples by polymerase chain reaction (PCR). There was a common bond of 334bp and a mutation specific band of 167bp in case of Arg3500Gln and 111 bp in the case of Arg353Cys.

The mutation specific arms, primers, Arg3500Gln and Arg3531cys.

Sr.no	Mutation	Primer sequence ($5^{/}$ -$3^{/}$)	Length of PCR (bp)
		GACCACAAGCTTAGCTTGG	
1	Arg3500Gln	GGGTGGCTTTGCTTGTATG	167bp
		TGCAGCTTCACTGAACACT	
		GACCACAAGCTTAGCTTGG	
2	Arg3531Cys	GGGTGGCTTTGCTTAGCTTGG	111bp
		GAGAAGCCACACTCAAAT	

All the reaction were performed in to a total volume of 50ul,with the concentration of primers as indicated,1U of Taq polymerase,200 umol of each deoxynucleoside triphosphate per litter. 1.5 mmol of magnesium chloride per liter,1× buffer(200Mm TRIS hydrochloride ,p^H – 8.4 and 500 mm potassium chloride) and 0.1 to0.2 ug of FNA. When a mutation was identified in a pooled samples DNA from each subject in the sample was again subjected to PCR, followed by the agarose gel electrophoresis to confirm the diagnosis and to determine zygosity. All patients with familial hypercholesterolemia or ischemic heart disease who have high plasma cholesterol levels and low HDL cholesterol levels were screened for the mutation of Arg3500Cys and Arg3531Cys[9].

Other analysis:

Colorimetric and turbid metric assay were used to measure serum total cholesterol, LDL cholesterol, HDL cholesterol, Triglycerides by enzymatic method[10, 11] (cholesterol oxidase and peroxidise).

Statistical analysis:

We have tested the serum lipids- Total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol between patients of familial hypercholesterolemia and normal controls. And mutation in Apo B gene at Arg3500Gln and Arg3531Cys in FH and normal controls in general population.
To examine the effect the Arg3500Gln and Arg3531Cys mutation on the phenotype in carriers identified in the general population and compared with normal controls using student's t test. Students t test was used to test the statistical significance between the groups at 95% confidence level when the (two sided) p value is less than the conventional 0.05 ; All p value of less than 0.05 was considered to indicated statistical significance.

III. RESULT:

Frequencies of Apo B Mutations: The Arg3500Gln and Arg3531Cys mutations were each identified in 8 of 945 general populations. All 16 subjects were heterozygous for the mutation. 6 patients with Ischemic heart Disease and 2 patients with familial hypercholesterolemia were heterozygous for the Arg3500Gln and one patient with ischemic heart disease was heterozygous for Arg3531Cys mutation. The Arg3500Trp mutation was not identified among the subjects with hypercholesterolemia in general population.
Mutation in the apolipoprotein B gene, Hypercholesterolemia and Risk of Ischemic Heart Disease[12,13].

Table 1.characteristic of the subject who were Heterozygous for Arg3500Gln and Arg3531Cys

Characteristic	Patients with FH (n=2)	IHD (n=6)	Controls (n=8)	IHD (n=1)	Controls (n=8)
Sex M/F	O/2	4-Feb	7-Jan	0/1	6-Feb
Cholesterol mg/dl	394 ±43	367±40	190±30	347±42	180±50
LDL- Cholesterol	278±83	286±50	120±40	255±52	130±45
HDL- cholesterol	48±12	42±16	55±10	42±12	55±10
Triglyceride	130±90	130±80	97±60	159±45	106±30

Phenotypic characteristic of heterozygous carriers of ApoB mutation in the general population:

The characteristics of the 25 heterozygous carriers of Arg3500Gln or Arg3531Cys are shown in table no. 1.

Subject who were heterozygous for Arg3500Gln had significantly higher cholesterol levels, LDL cholesterol levels, and triglyceride levels than the general population as a whole ($p<0.001$) for all comparisons and the carriers of Arg3531Cys mutation also had significantly higher levels of cholesterol, LDL cholesterol, and triglycerides ($p=0.001$, $p=0.001$.$p=0.001$) respectively[14]. Although the percentile for HDL cholesterol ($p=0.001$) tends to be lower among Arg3500Gln heterozygote than the subjects of general population.

Subjects from the general population who were heterozygous for the Arg3500Gln mutation had a significantly increased frequency of Ischemic heart disease, Hypertension as compared with non carriers in general population.

Apo-B mutation and the risk of ischemic heart disease and familial hypercholesterolemia :

Frequencies of an odd ratio for ischemic heart disease and familial hypercholesterolemia among subjects heterozygous for Arg3500Gln.Arg3500Trp, Arg3531Cys.

Groups	No. Of probands /mutations	Frequency of heterozygous 95% confidence interval	Odds Ratio 95% confidence	P- Values
Arg3500Gln				
General population (n=925).	8	0.08(0.03-0.17)	-	-
IHD (n=98)	6	0.53(0.17-1.23)	7.0(2.2-22)	0.003
FH(n=36)	2	5.6(0.7-19)	78(16-388)	0.001
Arg3500Trp				
General population (n=925).	0	0.00(0.00-0.18)	ND	ND
IHD (n=98)	0	0.00(0.00-0.39)	ND	ND
FH(n=36)	0	0.00(0.00-9.7)	ND	ND
Arg3531Cys				
General population (n=925).	8	0.08(0.03-0.17)		-
IHD (n=98)	2	0.53(0.17-1.23)	1.4(0-17)	0.54
FH(n=36)	0	5.6(0.7-19)	ND	ND

The Arg3500Gln mutation was significantly more frequent among patients with ischemic heart disease than the general population.(odds ratio, 7.0;95% confidence interval,2.2 to 22, p= 0.003) table no. 2 and as mentioned above[15], was also significantly more common among patients with IHD in general population[16]. The odd ratio for familial hypercholesterolemia for same mutation was also significant.(odds ratio 78;95% confidence interval,16 to 388 p=0.001)

The Arg3531Cys mutation was not more frequent among patients with IHD than in the general population samples. (Odd ratio 1.4; 95% confidence interval, 0.2 to 11,p= 0.54)table 2 and was not identified among subject with ischemic heart disease in general population or among the patients with familial hypercholesterolemia[17,18].

IV. CONCLUSION:

As among most other population, the frequency of clinically diagnosed heterozygous familial hypercholesterolemia is about 0.2 percent. Approximately 6 percent with clinical familial hypercholesterolemia are carrier of the Arg3500Gln mutation table 2. This is responsible for familial defective Apo lipoprotein B-100. our data indicate that Arg3500Gln occurs at a frequency of 0.8 percent in the general population and is associated with considerably higher than normal plasma cholesterol levels[19] (by 100mg per decilitre) and an increased risk of ischemic heart disease (odds ratio,7.0) .in contrast , the Arg3531Cys mutation, which is just a common, is not in itself associated with hypercholesterolemia or an increased risk of ischemic heart disease[20].
In most population, the estimated frequency of Arg3500Gln ranges from i in 500to 1in 1000 and has been associated with hypercholesterolemia in studies. The generalize ability of our data on Arg3531Cys and Arg3500Trp population is not completely clear. The available data suggest that the frequency of Arg3531Cys is similar to that in general population in our study and that this mutation is not associated with hypercholesterolemia, whereas Arg3500Trp is probably very rare and not associated with hypercholesterolemia[21].

Most studies searching for new mutations in APOB gene potentially associated with hypercholesterolemia have concentrated on screening the presumes receptor binding region patients with hyperlipidemia used reduced binding to the LDL receptors or other in vitro measure of apolipoprotein B Dysfunction as an indicator of probable hypercholesterolemia with the use of these method only three such rare mutations have been identified[22, 23].

It is possible that mutation contributing to detective binding of apolipoprotein B may lie elsewhere in the gene or that there are common genetic variations in APO-B that have only relatively effects on cholesterol levels and are detected by these method[24,25].

In conclusion our result suggest that the Arg3500Gln mutation is at present the only known APO B mutation worth screening for patient with hypercholesterolemia[26] and ischemic heart disease and their relatives.

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