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Risk Factors Affecting the Phenotypic Expression of Heterozygous Familial Hypercholesterolemia in Pakistani Population

Fauzia Imtiaz[1], Wajahatullah Khan[2*], and Ahsan A. Vahidy[3]

[1] Department of Biochemistry, Dow International Medical College, Dow University of Health Sciences, Baba-E-Urdu Road, PO BOX 942,943, Karachi 74200, Pakistan. [2] Biochemistry Department, King Saud University, PO Box 2455, Riyadh 11451, Saudi Arabia. [3] Department of Genetics, University of Karachi, Karachi Pakistan.

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Abstract

Familial hypercholesterolemia (FH) is a genetic disorder of lipoprotein metabolism recognized by high plasma concentrations of low-density lipoprotein cholesterol (LDL-C), tendon xanthomas, and high risk of early coronary heart disease. The phenotypic expression of FH is variable and several genetic and other risk factors contribute to such variability in clinical expression of the disease. FH is an autosomal disorder characterized by increased levels of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and tendinous xanthmata (TX). Clinical phenotype of FH has previously been shown to be associated with increased coronary heart disease (CHD) and premature death. The present study was carried out in a Pakistani population to examine the contribution of environmental factors on the expression of this disease. A detailed examination of the physical and biochemical features of the FH was performed in a cohort of 335 individuals (202 males, 133 females) from Pakistan. Our results indicate that Pakistani females with FH had high levels of TC and LDL-C as compared to Pakistani males. Overall the concentrations of high density lipoprotein cholesterol (HDL-C) were significantly lower for both sexes as compared to the controls (normal). We have also found that high cholesterol levels were associated with increased incidence of CHD in FH Pakistani population where both male and female individuals showed 69% and 31% CHD, respectively. The mean onset age for coronary symptoms was about 38 years in males as compared to 45 years in females. A greater risk of developing CHD has been linked with levels of TC along with a history of smoking in males and presence of hypertension (HTN) in females. Our results indicate that these risk factors possibly affect and contribute in the phenotypic expression of FH in Pakistani population.

Keywords: Phenotypic expression, Familial hypercholesterolemia, Risk factors, Coronary heart disease, Smoking, Hypertension, Total Cholesterol, LDL-C

Introduction

Familial hypercholesterolemia (FH) is a genetic disorder associated with lipoprotein metabolism, where elevated levels of low-density lipoprotein cholesterol (LDL-C) occur due to a mutation in the LDL receptor gene (Austin et al., 2004; Kwiterovich, 2008) to which LDL-C bind and remove it from the bloodstream. However, people with FH have less LDL receptors which result in increased than normal levels of LDL-C in the blood. Transmitted in an autosomal dominant fashion, FH is also characterized by the presence of tendinous xanthomas (TX) and premature atherosclerosis. (Alonso et al., 2009). FH is also one of the most common inherited diseases in the world, with a worldwide frequency of 1 in 500 for heterozygotes and 1 per million for homozygotes or compound heterozygotes (Goldstein et al., 2001). However, a higher incidence rate can be found in certain populations, for example the Afrikaners, Christian Lebanese, Finns, and French-Canadians, because of the founder effects (Goldstein et al., 2001). Patients with heterozygous FH express high levels of plasma LDL-C at a young age, causing atherosclerosis and an increased risk of cardiovascular diseases (Austin et al., 2004). Heterozygous FH patients show about two- to three-fold increase in LDL-C concentrations with a decrease in high-density lipoprotein (HDL-C) levels, along with tendinous xanthomatosis and premature heart disease (CHD) between the age of 35 and 55 years (Gagné et al., 1979; Civeira, 2004). About half of the offspring of an affected parent will have an increased level of cholesterol in the plasma and both genders are equally

* Corresponding author: wkhan@ksu.edu.sa

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affected. Although, a large number of FH cases are due to the mutations in the LDL receptor gene (LDLR), additional contributory genes have also recently been reported for FH. Nevertheless, the mutations in these genes appear to be rare in the populations that have so far been investigated (Abifadel et al., 2003; Rader et al., 2003; Damgaard et al., 2004; Graham et al., 2005).

Advancing age and male gender are strongly associated with CHD risk, with men typically developing disease symptoms 10–15 years earlier than women who generally do not show such symptoms until after menopause (AHA, 2001). However, the course of CHD symptom in FH is different, with some patients expressing events earlier than others inspite of similar increased LDL-C levels. Hence other factors may play a part in CHD risk in these individuals. Earlier studies in European and North-American populations have established the influence of risk factors like age, smoking and hypertension (HTN) in FH individuals (Civeira, 2004). Among the environmental risk factors, smoking is the major contributor and has been associated with a nearly two fold higher risk (Hill et al., 1991). Lack of exercise and the related adiposity, including high intake of saturated fats and a reduced intake of certain vitamins have also been linked with increased risk (AHA, 2001). The mechanism of action of these factors is by determining the differences in the plasma levels of lipids and lipoproteins that are atherogenic and high levels of LDL-C and low levels of HDL-C have been associated with CHD (Castelli et al., 1986).

Evidence for strong genetic component for CHD risk is supported by the association between a family history of early CHD and a personal increased risk (Kardia et al., 2003) in the order of 1.7-fold higher (Hawe et al., 2003). The clinical phenotype for homozygotes is more severe than heterozygotes; however the expression of heterozygous FH is highly variable probably due to other risk factors in addition to the genes affecting the lipoprotein metabolism (Bertolini et al., 2004).

We have previously reported that the cause of FH in Pakistani population is partly due to familial resemblance contributing due to the genes but other risk factors may also play a role in the progression of the FH disease in Pakistani population (Imtiaz, 2009). In the present study, we studied and assessed the contribution of risk factors on the phenotypic expression of FH disease in a Pakistani population.

Materials and Methods

The study, upon written informed consent approval, included a group of 1523 unrelated patients with the clinical diagnosis of heterozygous FH. A detailed examination of the physical and biochemical features of FH was obtained in this cohort.

Clinical Examination

Physical FH Characteristics

The physical characteristics that were examined in FH patients included presence of tendinous xanthoma (TX), xanthelesma, Arcus cornea (AC) and polyarthritis. A xanthelasma is a clearly distinguishable yellowish collection of cholesterol underneath the skin, typically on or in the region of the eyelids. The xanthelasma is a distinctive condition and is called a xanthoma when it becomes bigger and nodular. The TX is clinically characterized by papules and nodules found in the tendons of the hands. The AC is due to the white arc appearance on the cornea as a result of abnormal deposits of phospholipids and cholesterol. The polyarthritis is a condition when any type of arthritis involving five or more joints of the body was present in patients.

Three hundred thirty-five (335) subjects fitting to "definite FH" criteria had increased levels of LDL-C, presence of early heart attacks in the family and tendon xanthomatosis. Five probands were examined and their family pedigree trees were constructed to observe the dominant inheritance pattern of the disease.

Biochemical Analysis

The biochemical tests were performed following overnight (12–14 hrs) fasting. About 5 mL blood samples were drawn to analyze the total cholesterol (TC) levels, triglyceride (TG), HDL and LDL. The concentration of serum cholesterol, HDL-C, and TG were determined enzymatically by the CHOD-PAP and GPO-PAP methods (Boehringer Mannheim, Germany) (Tietz, 1986). The HDL-C was isolated from serum by heparin manganese precipitation of the other lipoproteins. All the lipid profile analysis of TC, HDL-C, LDL-C and TG concentrations were observed in the serum of all 1523 Pakistani individuals diagnosed with FH, cholesterol content of the serum LDL-C was estimated using Friedewald formula (Friedewald et al., 1972).

Statistical Analysis

The obtained data were analyzed for correlation among different risk factors using the Statistical Package for Social Sciences version 12 (SPSS, Inc). Statistically significant differences between groups or among groups for continuous variables were evaluated using Student's t test for unpaired data and ANOVA respectively. The pedigree was made by using the computer based software Cyrillic version 2.10 (Oxford, UK).

Results

Our previous study on the heritability of Pakistani FH population showed that the genetic factors are the major determinant of FH, however because heritability is the proportion of variation due to additive familial effects of genetic and non-genetic sources, therefore we concluded that other non-genetic risk factors may also contribute in the progression of the disease (Imtiaz, 2009). The present study was conducted to examine the involvement of risk factors such as age, sex, HTN, smoking and TC concentrations in the phenotypic expression of heterozygous familial hypercholesterolemia in a cohort of Pakistani population living in the city Karachi. We have studied a total of 1523 unrelated patients with the clinical diagnosis of heterozygous FH. Out of these 1523, only 335 subjects with "definite FH" diagnosis belonged to five probands with cholesterol level >300 mg dl^{-1} (normal range 140-240 mg dl^{-1}). Among them

202 were males and 133 were females. Upon clinical physical examination, out of these 335 individuals (202 males and 133 females) 224 (70%) showed TX. A total of 146 males (72%) and 78 females (58%) aged between 30-40 showed TX symptoms (Table 1). Xanthelesma was detected in 258 individuals (80%), i.e. 148 males (73%; aged 40-50) and 110 females (82 %; aged 30-40). The AC was observed in a total of 224 patients (70%), where 63% males (128 individuals) aged 40-50 and 72% females (96 individuals) aged 30-40 showed the AC symptoms. The polyartheritis symptoms were less common among the observed individuals and only 7 individuals (2%) have this condition. Among the 202 males (aged 40-50), only 2 males (0.9%) and 5 females (3.7%) showed the clinical symptoms of polyartheritis (Table 1). Hence, the occurrence of xanthoma (i.e. tendinous xanthomata and xanthelesma) in the present study in general was about 70% in the patients diagnosed with FH.

A detailed examination of the physical and biochemical features of FH of cohort of 208 females and 156 males showed that females with FH had higher levels of total LDL-C (196 to 358 mg dl^{-1}) compared to the male subjects (201 to 301 mg dl-1) (Figure1), while having lower HDL-C levels (25-55 mg dl^{-1}) in both genders compared to the normal (Figure 1).

Our results also showed that the mean age of FH showing the symptoms in males was earlier than normal, i.e. 38 years compared to 45 years in females. The FH disease prevalence rate was 69% in males compared to 31% in females. The risk factors such as total cholesterol (TC), smoking and HTN were found to be highly correlated with FH appearance. We observed that smoking and HTN were significantly ($p < 0.05$) correlated. Similarly, a high correlation ($p < 0.05$) was found between smoking and TC in the individuals with FH. However, TC and HTN were even more strongly correlated with FH at $p < 0.01$ (Table 2). The risk factors such as smoking and HTN exhibited significant effects in the appearance of the disease. Our study showed that smoking and HTN were significantly correlated with the level of total cholesterol at $p<0.05$ (0.047) and $p<0.01$ (0.000), respectively (Table 2). The smoking in males had profound effect in observed individuals, where we noticed that 70% male smokers were positive for CHD.

Fig. 1. Pedigree showing the presence of FH in every generation of the proband confirming the dominant inheritance pattern. The amount of LDL-C is mentioned as mg/dL.

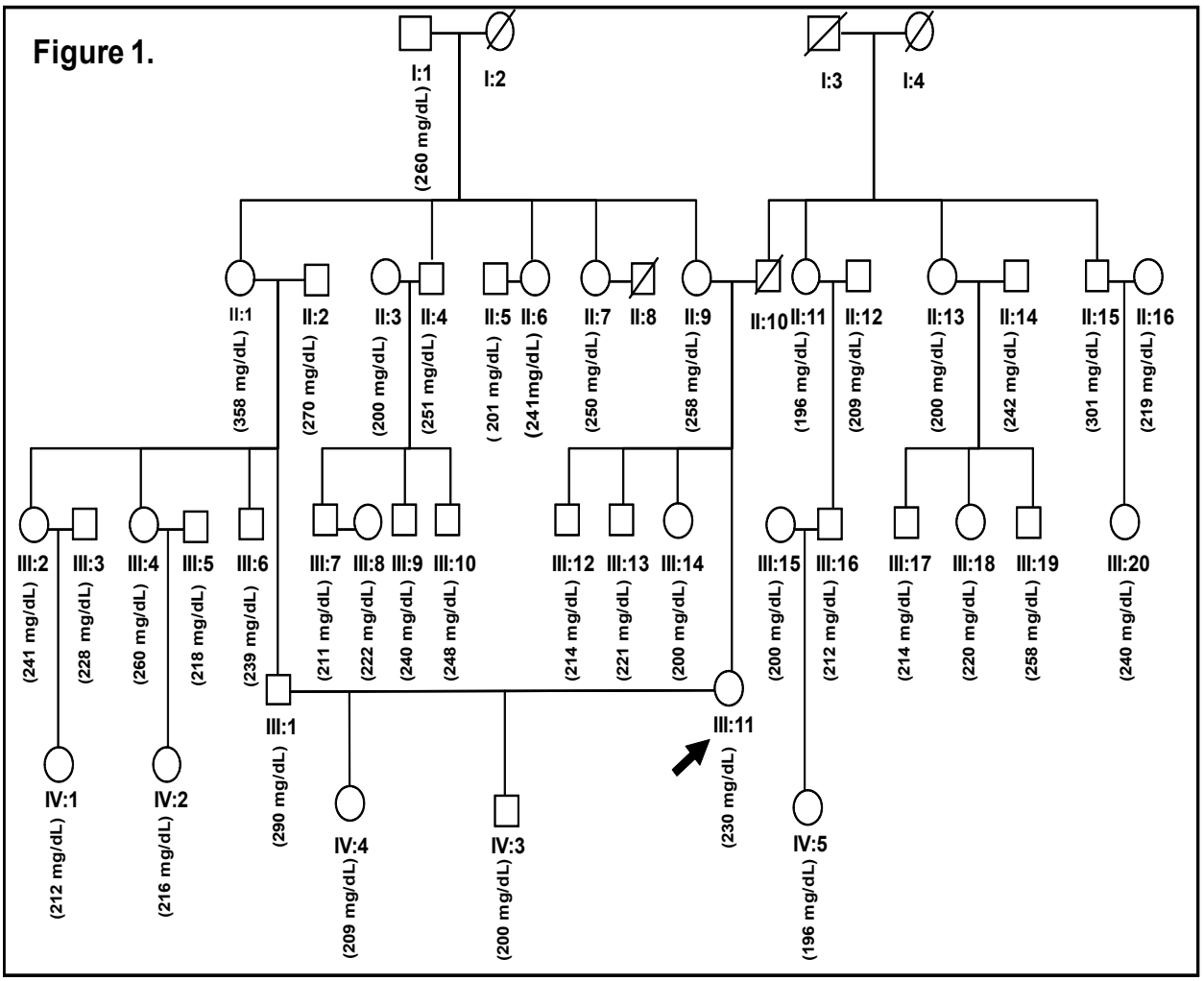

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Table 1. Clinical features of 335 participants with cholesterol level >210 mg/dL in Pakistani population.

Observed Features	Gender	Age (Years)	Total	Observed Cases	Percentage (%)
Tandinous Xanthomata	Male	30-40	202	146	72
		40-50	0	0	0
		50-60	0	0	0
	Female	30-40	133	78	58
		40-50	0	0	0
		50-60	0	0	0
Xanthelesma	Male	30-40	0	0	0
		40-50	202	148	73
		50-60	0	0	0
	Female	30-40	133	110	82
		40-50	0	0	0
		50-60	0	0	0
Arcus cornea	Male	30-40	0	0	0
		40-50	202	128	63
		50-60	0	0	0
	Female	30-40	133	96	72
		40-50	0	0	0
		50-60	0	0	0
Polyarthritis	Male	30-40	0	0	0
		40-50	202	2	0.9
		50-60	0	0	0
	Female	30-40	0	0	0
		40-50	0	0	0
		50-60	133	5	3.7

Table 2. Correlation studies showing relations among smoking, hypertension (HTN) and Total cholesterol levels in Pakistani population.

	Smoking	HTN	Total Cholesterol
Smoking	1.000	0.32*	0.47*
HTN		1.000	0.000**
Total Chlesterol			1.000

Significant at *p< 0.05, ** p< 0.01.

Discussion

FH is a public health problem throughout the world and many heterozygous FH remain undetected until adulthood. The present study was conducted to study the contribution of risk factors (age, sex, smoking and HDL-C concentrations) in the phenotypic expression of heterozygous FH in a Pakistani population. FH may also cause premature coronary artery disease and atherosclerotic plaque formation and is considered as one of the first inherited disorders as being a cause of myocardial infarction (heart attack). In the present study, out of a total of 1523 unrelated patients with the clinical diagnosis of heterozygous FH, only 335 individuals diagnosed with FH belonged to five probands (202 males and 133 females). Similar criteria have previously been used to identify patients with FH based on high plasma levels of total and LDL cholesterol, family history of hypercholesterolemia, tendon xanthomas or corneal arcus, and personal and family history of premature CHD (Goldstein et al., 2001). The FH patients have LDL-C levels almost twice than the normal population (190 to 400 mg dl^{-1}).

The clinical examination of the 335 individuals with FH showed that 72% males and 58% females had TX at ages between 30-40 years. Xanthelesma was found in 80% males (aged 40-50) and in 82 % females (aged 30-40). The AC was observed in 63% males (aged 40-50) and 72% females (aged 30-40). A very few individuals (2% i.e. 7 individuals) showed polyartheritis symptoms among them were only 2 males (0.9%) aged 40-50; while 5 females (3.7%) showed the disease symptoms. The TX is pathognomonic of FH but its identification is not always straightforward and to some extent considered insensitive diagnostic markers. However, other criteria should be taken into consideration for the diagnosis of FH including personal and familial LDL-C levels, history of CHD, and presence of CA before the age of 45 years and xanthomas (Fernando, 2004). Overall, the incidence of xanthoma (i.e. tendinous xanthomata and xanthelesma) in the present study was quite high i.e. 7 out of 10 (70%) cases with FH had it. Recently, Junyent et al. (2005) has reported that the occurrence of TX is very much linked to the FH incidences in individuals with family history of hypercholesterolemia, primary hypercholesterolemia and premature coronary disease. Although TX is pathognomonic of FH but a high variability of xanthoma may occur in FH patients (Descamps et al., 2001). Xanthelasmas occur commonly in heterozygotes, and are rare in homozygotes. The presence of xanthelasmas is not specific for FH and may also occur in individuals with normal lipid levels (Goldstein et al., 2001). The frequency of FH among Caucasians is 1:500, however in some populations there is high heterozygous FH frequency such as French Canadians, Lebanese Christians, South African Afrikaners, Lithuanian Ashkenzai Jews, Druze and Finns (Lehrman et al., 1987; Kotze et al., 1991; Meiner et al., 1991; Leitersdorf et al.,1990; Landsberger et al., 1992; Koivisto et al.,1992). The heterozygote FH individuals have about two fold increase in their plasma cholesterol. The FH subjects show 4-5 time higher age-sex standardized mortality ratios in the general populations (Castro-Orós et al., 2010).

The mean age of males that showed the symptoms of FH in the present study was 38 years, compared to 45 years in females. The incidence rate in the observed males was 69% compared to 31% in females. Similar results have previously been reported where the observed age was strongly associated with high cholesterol levels in men, whereas women had significantly lower non-HDL-C level (Gardner et al., 2000). For women, the age range was 25 to 64 years, whereas for men it ranged from 25 to 54 years. Non-HDL concentration was higher in individuals with increasing age. However, this observation seemed to be not influenced by age as the total cholesterol levels were found to be higher in all age groups. The mean age of males, showing symptoms of FH was 38 $\pm$ 5 years, which is earlier as compared to females (45$\pm$5 years). The FH should immediately be treated upon diagnosis and if left untreated approximately 85% of males and 50% of females will develop a coronary problem before the age of 65 (Civeira, 2004). It is striking that up to 9% of the total premature CHD in eastern Germany and

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Finland has been linked with FH (Koivisto et al., 1993; Baron et al.,1996; Schuster, 2002). Hence, the major cause of death in FH patients has been linked with the occurrence of CHD (Mabuchi et al., 1989; Miettinen and Gylling, 1988). In the present study, we found that the high levels of LDL-C (> 190 mg dl^{-1}) were detected in the proband and in the related family members (Figure 1). The clinical diagnosis of heterozygote FH is associated not only with the high plasma level of LDL-C but also with the incidence of other coronary risk factors (Hill et al, 1991). We have found that the females with FH had higher levels of total LDL-C (196-358 mg dl^{-1}) as compared to the male subjects (201-301 mg dl^{-1}) and with lower levels of HDL-C (25–55 md dl^{-1}). The average age of onset of coronary symptoms was delayed in females, with a mean age of 55 years compared with 48 years for males ($p < 0.05$). Previous studies have shown an association between clinical heterozygous FH and coronary heart disease (Kalina et al., 2001; Umans-Eckenhausen et al., 2002). However, the heterogeneity in FH patients with regards to plasma LDL-C and CHD has also been suggested (Castro-Orós et al., 2010). Multivariate analyses have revealed that in FH heterozygotes and controls, the HDL-C levels may contribute to a greater proportion of the variation in TC to HDL-C ratio than TC (Torres et al., 1996).

A number of factors, including age, sex, smoking and HDL-C concentrations have previously been identified as risk factors for CHD in FH in cross-section studies worldwide (Jansen et al., 2004). There are 100 million people with FH worldwide, mainly heterozygotes, and approximately 85% of males and 50% of females with FH will suffer a coronary event before they are 65 years old, if appropriate preventive measures are not implemented (Fernando, 2004). The risk factors such as smoking and HTN showed a significant correlation in the appearance of the disease. Our study showed that a strong correlation between the risk factors, smoking and HTN, was present in the individuals with FH. The TC and smoking were significantly correlated ($P < 0.05$) in the FH Pakistani population. Similarly, smoking and HTN were also significantly ($P < 0.05$) correlated. But a very strong correlation ($p < 0.001$) between HTN and TC was observed in the studied individuals. Smoking in males had profound effect, where 70% males with positive CHD were smokers. However, the HTN in females was associated with higher incidence of CHD. The HTN has been considered as an independent risk factor for females with FH (Hill et al., 1991). It has been reported that the level of total cholesterol was positively related to the prevalence of systolic HTN and obesity, in addition to smoking (Rywik et al., 1999). A meta-analysis was conducted by Rywik et al. (1999), where they concluded that for every 10% reduction in TC, there was 2.5% decrease in the incidence of CHD. A multivariate analysis performed on a Canadian population indicated that the low HDL-C and smoking were the best predictors of risk and the average age of onset of coronary symptom were delayed in females (55 years) compared to males (48 years) (Weber et al., 1997). The risk of developing heart related problems has also been connected with the lower levels of HDL-C and a history of smoking; however, in women, the heart related problems were mainly associated with elevated TG levels and the presence of HTN (Hill et al., 1991; Neil et al., 2004). Our results are in agreement with earlier published reports (Vuario et al., 1997; Jansen, 2004) on the risk factors like age, sex, smoking and HTN. Our results are consistent with accumulating evidence from a number of cross-sectional population and case control studies that have assessed the role of established and emerging risk factors in individuals with FH. This study on a Pakistani population has shown that FH is linked to risk factors such as age, smoking and HTN in addition to total cholesterol levels and to a larger extent was associated with the development of premature heart diseases as well as in the phenotypic expression in heterozygous FH.

Upon diagnosis the FH patients should immediately be treated and if left untreated, they have a high chance (about 8 times) of early coronary heart disease (CHD). Cholesterol can be accumulated in the coronary arteries of the heart causing angina or heart attacks. Other factors that increase the risk of CHD are smoking and high blood pressure. However, FH is a very treatable condition which can be controlled with the use of cholesterol-lowering drugs combined with a healthy lifestyle. It is now known that people with FH who are treated can expect to have a life expectancy which is the same as the general population. Therefore it is vitally important to diagnose this condition as early as possible so that the right treatment can be started and heart attacks can be prevented.

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