CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1009100

Available online at: http://www.iajps.com Research Article

THE COMMUNICATION OF RS (505151) WITH AMOUNT OF PCSK9 GENE EXPRESSION IN CARDIOVASCULAR DISEASE

Farnoosh Naseri, MSc[1], Elham Moslemi, PhD[2*], Kasra Esfahani, PhD[3]

[1] Faculty of Basic Sciences for Molecular Genetics, East Tehran Branch, Islamic Azad University, Tehran, Iran.

[2] Cellular and Molecular Biology Department, Islamic Azad University, East Tehran Branch, Tehran, Iran.

[3] Associate Professor, National Institute for Genetic Engineering, Tehran, Iran.

Abstract:

Cardiovascular Disease has been proposed as a sickness with considerable complications and consequent disabilities in industrialized countries. One of the main causes of heart diseases is deposition of fat and LDL-Ch in blood vessels and overexpression of PCSK9. This study was designed to investigate PCSK9 gene expression level in patients compared to control groups as well as most prevalent polymorphism of E670G gene. In the current study, 40 patients with CHD (Coronary heart disease) and 20 non - CHD cases were studied. PCSK9 gene expression was evaluated by qPCR and E670G polymorphism (SNP) was determined by RFLP PCR. PCSK9 gene expression in 60 cases including patients and control groups, PCSK9 gene expression was reduced in most of the cases. 75% of samples were patients with A/G genotype; only 10% of patients were G/G genotype. Other samples were homozygous with A/A genotype. Evaluation of PCSK9 gene expression level and its associated SNP (E670G) in patients with cardiovascular disease can be effective in early diagnosis in order to reduce mortality rates of cardiovascular lesions and increase life expectancy.

Key Words: *CVD, Hypercholesterolemia, PCSK9, SNP.*

Corresponding author:

Elham Moslemi,
Cellular and Molecular Biology Department,
Islamic Azad University,
East Tehran Branch,
Tehran, Iran.

QR code

Please cite this article in press as Elham Moslemi et al, ***The Communication of RS (505151) with Amount of PCSK9 Gene Expression in Cardiovascular Disease,*** *Indo Am. J. P. Sci, 2017; 4(10).*

INTRODUCTION:

Cardiovascular disease is an umbrella term referring to any cardiovascular disorder, coronary heart disease, congenital heart defects and heart attack [1]. Cardiovascular diseases are broad disorders leading to a significant percentage of admissions, hospital visits and deaths nowadays. According to statistics, about 45 percent of deaths occur in developed countries and between 25 and 45 percent of the deaths in developing countries are due to cardiovascular disease and also CVD encompasses a broad range of disorders including diseases of the vasculature, the myocardium, the heart's electrical circuit, and congenital heart disease. Hyper tension, high cholesterol, diabetes, obesity and hereditary factors play an important role in cardiovascular disease [2, 3]. One of the main contributing factors in the development of cardiovascular disease is high cholesterol or hyper cholesterolemia. One of the most important forms of hyperactive cholesterolemia, is familial hypercholesterolemia (FH). FH is a dominant autosomal disorder caused by mutations in one of three genes of low-density lipoprotein receptor (LDLR), apolipoprotein B-100 and PCSK9 gene [4]. Familial hypercholesterolemia has different phenotypes which vary from mild to severe. In addition, the clinical phenotypes increase the risk of cardiovascular disease and premature death [5, 6]. The pathological substrate of FH is related to the dysfunctional uptake of LDL particles via its receptor and this can either be caused by mutations in the genes encoding for the LDL receptor (LDLR), apolipoprotein B (apoB), or pro-protein convertase subtilisin/kexin 9 (PCSK9). It is important to diagnose FH at an early age in order to prevent vascular conditions. The diagnosis is based on clinical parameters such as lipid levels, presence of xanthomas, family history, and vascular disease, and a definite diagnosis is based either on the identification of a pathogenic mutation in any of the three well-established FH-causing genes or a probable score derived from clinical characteristics. Due to the presence of benign and non-pathogenic variants associated with genes, there are such phenotypes which can convert FH to mutagenic diseases [7], as mentioned mutation in PCSK9 gene results in destruction of cell surface LDL receptors and prevents cholesterol from internalizing in the liver and its consequent degradation [8]. Eventually, accumulation of cholesterol in blood vessels leads to arteries occlusion[1]. PCSK9 gene is located on chromosome number one. Gain-of-function mutation can prevent liver cells receptors from Destruction. PCSK9 gene codes 74 KD protease which is highly expressed in liver [9, 10]. PCSK9 has an important role in regulating cholesterol homeostasis through increasing circulating LDL-C levels [11]. Studies show that polymorphism E670G (rs505151) plays a significant role in the development of coronary artery disease [12]. The aim of this study is to evaluate PCSK9 gene expression and its related SNP in patients with cardiovascular diseases, which could be helpful in development of prediagnosis of cardiovascular diseases.

MATERIAL AND METHODS:

The study population: In a case-control study, 40 cardiac patients (21 males and 19 females) with an age range of 25 to 77 years (i.e. 53.6 ± 16.98) referring to Shohada hospital and 20 healthy individuals without any history of heart disease were studied. The individuals who have diabetes, high blood pressure, liver disease, and kidney and thyroid disease were avoided. In addition, pregnant women and those taking lipid-lowering medications were excluded from this study. After obtaining written consent from patients, 5 ml of blood were obtained for biochemical tests including total cholesterol, triglycerides, LDL, HDL and FBS and molecular tests.

Extraction of DNA: To determine the specific SNP E670G (rs505151), DNA was extracted by using DNP kit (Cinnagen- Iran). After that, RFLP PCR test using specific forward primers for PCSK9 gene (5' CACGGTTGTGTCCCAAATGG 3') and Reverse primer (3' GAGAGGGACAAGTCGGAACC 5') was done. PCR cycles were as follows: 95c° for 3 min, followed by 35 cycles of 93c° for 30 seconds, 67c° for 30 seconds, 72c° for 30 seconds and final extension on 72c° for 5 minutes. For restriction enzyme digestion 10 µl of the PCR product was mixed with 1µl Eam 1104 I (Fermentas-America) enzyme in a final volume of 30 µl. The mixture was incubated for 16 h at 37c°. Finally, digest product was assessed on 3% agarose gel. Three genotypes A/A (Normal Homozygous) and G/G (Patient homozygous) and genotype A/G (Patient heterozygous) were detected.

RNA Extraction (Evaluation of PCSK9 gene expression): RNA extraction was done using RNX plus (Cinnagen-Iran) and optimized protocols. cDNA synthesis was done using random hexa-nucleotide and oligi dT primers and MMuLv enzyme. Quantitative Real Time PCR was performed as follows: 1X SYBR TM, 0.2 µM F&R primers, 2 µl of cDNA in 20 µl final volume. GAPDH gene expression was used as internal control. 45 cycles; initial denaturation at 95c° for 10 seconds, followed by 95c° for 5 seconds, 59.5c° for 1 minute and 72c° for 30 seconds were The thermal program for the replication of both genes done under the same conditions containing, PCSK9: (90 bp) Forward 5' GAATGCAAAGTCAAGGAGCA3' and Reverse 3'ACTGCAGCCAGTCAGGGT5', GAPDH: (124bp), Forward

5'ATGGAGAAGGCTGGGGCT3', and Reverse 3' ATCTTGAGGCTGTTGTCATACTTCTC5' .

Statistical analysis: All statistical findings were analyzed using SPSS version 20. The relationship between different groups, alleles or genotypes of PCSK9 gene polymorphism were estimated using odds ratio (OR) and 95% confidence intervals. Significant levels were determined through Chi-square test. To analyze the quantitative results of PCSK9 gene expression data were normalized to GAPDH as housekeeping gene. Data analysis was performed using $2^{-\Delta\Delta CT}$, and the significance of differences for gene expression between the control group and the test group was estimated by statistical t-test. P-value less than 0/05 was considered significant [13,15].

RESULTS:

Biochemical analysis of serum in Control and patient groups are listed in Table 1. Accordingly, it was established that the average cholesterol was higher in patients than the healthy group (P<0.05) but no significant differences were observed in the other biochemical markers. Statistical analysis showed the distribution frequency of genotype A/A,A/G,G/G in independent group of patients and control group is statistically significant (P<0.05). Table 2 and Figure 1 show the type of genotype and phenotype resulting from the enzymatic digestion and patient's phenotype respectively. The results of PCSK9 gene expression showed that the average level of PCSK9 gene expression in patient samples was 0.36 compared to 1 in control gene. This implies that the expression of this gene reduces in patients. Also, the average level of PCSK9 gene expression in males and females was not significantly different. Risk factors were compared with the level of PCSK9 gene expression. In this comparison, factors such as TG, Chol, HDL and FBS were not significantly associated with PCSK9 gene expression, but the amount of LDL was significantly correlated with the PCSK9 gene so that overexpression of PCSK9 increases LDL level. Data on comparison of the amount of PCSK9 gene expression with different genotypes are found in chart 1. Based on its results, people with genotype G/G and phenotype are homozygous patients with high expression of the PCSK9 gene. The results of this study show that patient homozygous individuals have a higher expression of the PCSK9 gene. Since the disease, familial hypercholesterolemia is a kind of autosomal dominant, so people with genotype A/G and G/G incidence of the disease. This disease in homozygous people is more widely offered. In addition, chart 2 shows that there is a link between the different genotypes in 40 patients and PCSK9 gene expression. Base on this chart, there is an association of PCSK9 gene expression with a variety of its polymorphisms, that this relationship was not statistically significant.

Table 1: The results of serum biochemical parameters of patients and control groups

Biochemical Factors (mg/dl)	Patients Group (40 Patients)	Healthy Group (20 Healthy)	P-value
Total Cholesterol*	208.2±52.7	170.7±46.4	< 0.05
Triglyceride	175.6± 23.3	153.3±43.6	
Fast Blood Sugar (FBS)	105.15±17	98 ±14.4	
Low Density Lipoprotein (LDL)	123.6±45.5	112.6± 44	
High Density Lipoprotein (HDL)	38.4± 7.5	42.7 ± 12.1	

Table 2: The Percent of Different Genotype in Digestion of Ear I Enzyme

Size	Genotype	Phenotype	Percent of Patient
440 bp	A/A	Normal Homozygous	%17.5
150, 290 bp	A/G	Patient Heterozygous	%72.5
440, 290 bp	G/G	Patient Homozygous	%10

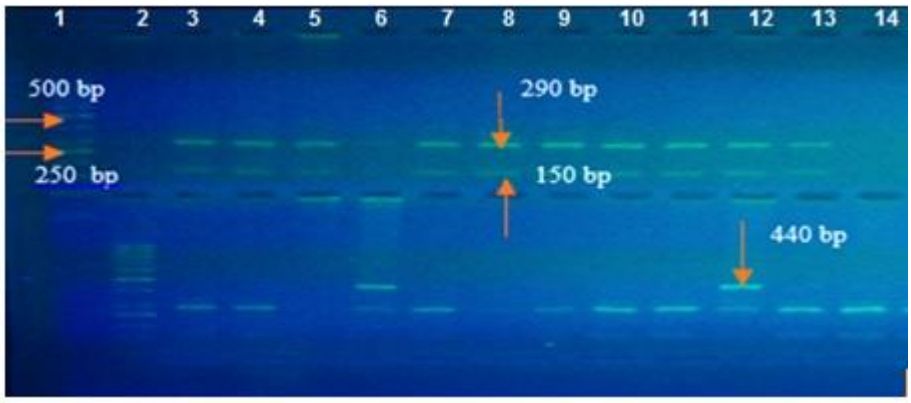

Fig 1: The view of enzymatic digestion on agarose gel 3% for PCSK9 gene. The first well is Fermentase 50 bp size marker and 2-14 wells are some examples of people being studied.

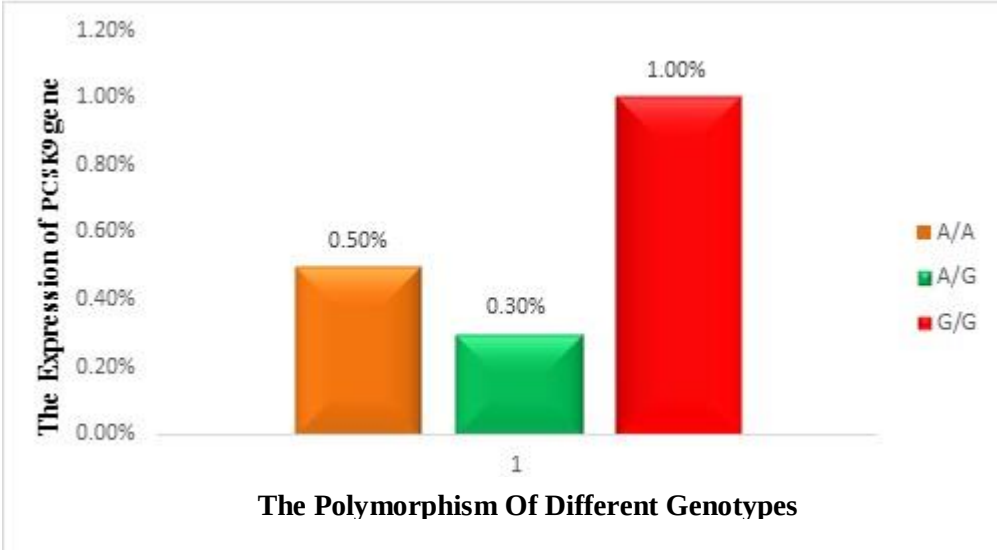

Chart 1: Comparison of PCSK9 gene expression with its different polymorphisms.

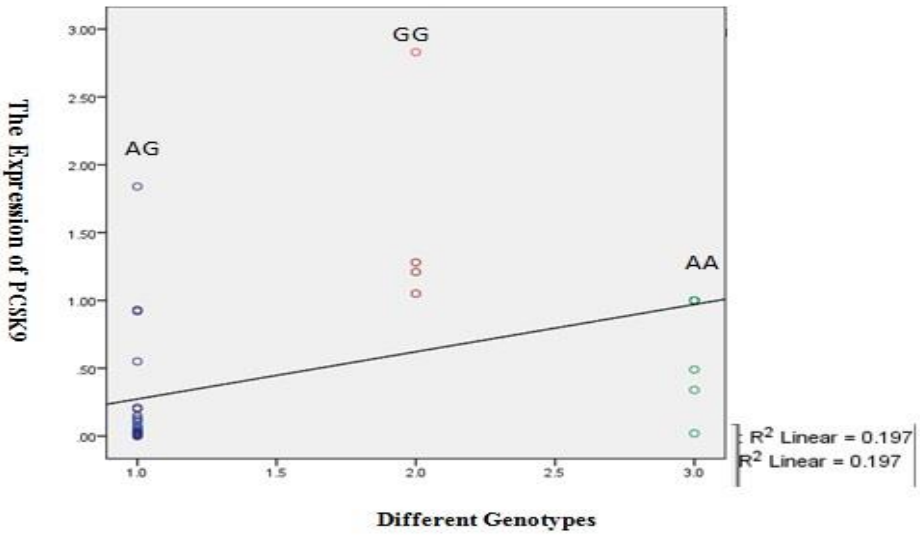

Chart 2: Association of PCSK9 gene expression with a variety of its polymorphisms

DISCUSSION:

Cardiovascular disease is currently one of the three contributing factors in mortality rates and disability of people around the world, and is becoming the main cause of death or disability in most countries. Although the effects of infectious and contagious diseases, due to their nature, are fast and progressive,

consequences and side effects of chronic diseases such as cardiovascular disease are persistent, more costly and debilitating but preventable for patients and the society [16, 17]. Many of the studies reported that overexpression of PCSK9 can be an important factor in the progression of atherosclerosis [14]. Ricardo Jorge and Anna Roubtsora in 2011 studied PCSK9 cycle. According to their study, PCSK9 reduces the LDLR, thus, the role of PCSK9 in the metabolism of fatty tissue is clear. To study the genome and detecting of DNA variants in PCSK9 gene, molecular technical was applied [18, 19]. Ekaterina Cherhagubova in 2012 explored the common genetic variants with low frequency and the effects of PCSK9 level in these positions [20]. PCSK9 is a secreted protein with effects on the plasma LDL and susceptibility to cardiovascular disease [21]. Xin - Lin Zhang in 2015 and others studied the safety and efficacy of conducted PCSK9 antibodies. During these studies, cholesterol was introduced as a major risk factor for cardiovascular disease [22]. Unlike previous study results which showed the increase of PCSK9 gene expression, the results of current study shows that except some cases (which showed PCSK9 high expression), most of 40 studied patients samples demonstrated decreased PCSK9 level [23]. According to the results of the upcoming study, people with genotype G/G and homozygous phenotype are patients with high expression of the PCSK9 gene. In these patients the common polymorphism is E670G. This polymorphism in other examples is heterozygote, but overexpression of PCSK9 gene is associated with heterozygous patient form and low expression of the gene associated with heterozygous patient form. In other words, since the hyper cholesterolemia disease is an autosomal dominant disease, people with genotype A/G and G/G are patients. Homozygous patients show clinical symptoms of hyper cholesterolemia disease more extensively. Based on the results, due to a higher expression of the gene PCSK9 in homozygous patients; gain of function mutation is observed in such patients. Overactivity of PCSK9 gene, leads to further destruction of liver cell surface receptor and broadly reveals hyperlipidaemia. The results of this study showed that nearly 75% of the samples had genotype A/G and were heterozygous patients, and only 10% of patients with genotype G/G phenotype are homozygous patients. Other samples were healthy homozygous A/A. Common mutations in PCSK9 gene can lead to hyper cholesterolemia followed by coronary artery disease. Moreover, patients with homozygous mutations show hypercholesterolemia more extensively, except that the frequency of homozygote patient genotype due to autosomal dominant nature of the disease is less. Due to intensive manifestation of disease in homozygous patients from birth, they even die; therefore, the majority of observed population included heterozygous patients. In addition, the overexpression of PCSK9 gene, leads to high levels of LDL in the blood serum. Thus, it can be concluded that studied polymorphism is more heterozygous and leads to high blood fat. Moreover, overexpression of PCSK9 gene leads to decreased expression of the LDL, but its low expression does not reduce plasma LDL because heterozygous patients with low expression of PCSK9 gene, show hypercholesterolemia with lower incidence. People with genotype G/G increased expression of the PCSK9 gene. people with decreased expression of the PCSK9 gene, have genotype A/G and constant expression of the gene considered genotype A/A. high expression of PCSK9 gene in patients with cardiovascular diseases can lead to high level of LDL-C , G/G genotype and hyperlipidaemia and thus atherosclerosis progression in patients.

CONCLUSION:

In this study, the relationship between E670G polymorphisms in the gene PCSK9 and the risk of cardiovascular disease were studied. The amount of PCSK9 gene expression and its related SNP the E670G (rs505151) in patients with cardiovascular, can be effective for early notification in order to reduce mortality of cardiovascular lesions and increase life expectancy.

REFERENCES:

1.Ahmed W, Whittall R, Riaz M, Ajmal M, Sadeque A, Ayub H, Qamar R, Humphries SE. The genetic spectrum of familial hypercholesterolemia in Pakistan. Clinica Chimica Acta. 2013; 421(3): 219-25.

2.Akamine R, Yatsushiro S, Yamamura S, Kido J-i, Shinohara Y, Baba Y, et al. Direct endonuclease digestion and multi-analysis of restriction fragment length polymorphisms by microchip electrophoresis. Journal of pharmaceutical and biomedical analysis. 2009; 50(5): 947-53.

3.Alves A, Medeiros A, Francisco V, Gaspar I, Rato Q, Bourbon M .Molecular diagnosis of familial hypercholesterolemia: an important tool for cardiovascular risk stratification. Revista portuguesa de cardiologia. 2010; 29(6): 907-21.

4.Rader DJ, Cohen J, Hobbs HH. Monogenic hypercholesterolemia: new insights in pathogenesis and treatment. The Journal of clinical investigation. 2003; 111(12):1795-803.
5.Anda R, Williamson D, Jones D, Macera C, Eaker E, Glassman A, et al. Depressed affect, hopelessness, and the risk of ischemic heart disease in a cohort of US adults. Epidemiology. 1993; 4(4): 285-94.
6.Awan Z, Seidah NG, MacFadyen JG, Benjannet S, Chasman DI, Ridker PM, Genest J. Rosuvastatin, proprotein convertase subtilisin/kexin type 9 concentrations, and LDL cholesterol response: the JUPITER trial. Clinical chemistry. 2012; 58(1): 183-89.
7.Benjannet S, Hamelin J, Chrétien M, Seidah NG. Loss-and gain-of-function PCSK9 variants cleavage specificity, dominant negative effects, and low density lipoprotein receptor (ldlr) degradation. Journal of Biological Chemistry. 2012; 287(40): 337-55.
8.Ariyo AA, Haan M, Tangen CM, Rutledge JC, Cushman M, Dobs A, Furberg CD. Depressive symptoms and risks of coronary heart disease and mortality in elderly Americans. Circulation. 2000; 102(15): 1773-79.
9.Bennette CS, Gallego CJ, Burke W, Jarvik GP, Veenstra DL. The cost-effectiveness of returning incidental findings from next-generation genomic sequencing. Genetics in Medicine. 2014; 17(7): 587-95.
10.Austin MA, Hutter CM, Zimmern RL, Humphries SE. Genetic causes of monogenic heterozygous familial hypercholesterolemia: a HuGE prevalence review. American journal of epidemiology. 2004; 160(5): 407-20.
11.Chackerian B, Remaley A. Vaccine strategies for lowering LDL by immunization against proprotein convertase subtilisin/kexin type 9. Current opinion in lipidology. 2016; 27(4): 345-50.
12.Chen SJ, Sanmiguel J, Lock M, McMenamin D, Draper C, Limberis MP, Kassim SH, Somanathan S, Bell P, Johnston JC, Rader DJ. Biodistribution of AAV8 vectors expressing human low-density lipoprotein receptor in a mouse model of homozygous familial hypercholesterolemia. Human Gene Therapy Clinical Development. 2013; 24(4): 154-60.
13.Havasian MR, Panahi J, Khosravi A. Correlation between the lipid and cytokine profiles in patients with coronary heart disease (CHD)(Review article). Life Science Journal 2012; 9(4): 5772-77.
14.Panahi J , Havasiyan MR, Gheitasi S, Pakzad I, Jaliliyan A, Hoshmandfar R, Havasiyan M. The in Vitro Inhibitory Effects of the Aqueous Extracts of Summer Onion on Candida Albicans. J of Ilam Uni Med Sci 2013; 21(1): 54-9.
15.Havasian MR, Panahi J, Pakzad I, Davoudian A, Jalilian A, Zamanian Azodi M. Study of Inhibitory effect of alcoholic and aqueous extract of Scrophularia striata (tashne dari) on candida albicans in vitro. J of Pejouhesh 2013; 36(5): 19-23.
16.Chernogubova E, Strawbridge R, Mahdessian H, Mälarstig A, Krapivner S, Gigante B, et al. Common and low-frequency genetic variants in the PCSK9 locus influence circulating PCSK9 levels. Arteriosclerosis, Thrombosis, and Vascular Biology. 2012; 32(6): 1526-34.
17.Cohen JC, Boerwinkle E, Mosley Jr TH, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. New England Journal of Medicine. 2006; 354(12): 1264-72.
18.Colbert A, Umble-Romero A, Prokop S, Xu R, Gibbs JP, Pederson S, editors. Characterization of a quantitative method to measure free proprotein convertase subtilisin/kexin type 9 in human serum. MAbs, 2014.
19.Colhoun HM, Robinson JG, Farnier M, Cariou B, Blom D, Kereiakes DJ, Lorenzato C, Pordy R, Chaudhari U. Efficacy and safety of alirocumab, a fully human PCSK9 monoclonal antibody, in high cardiovascular risk patients with poorly controlled hypercholesterolemia on maximally tolerated doses of statins: rationale and design of the ODYSSEY COMBO I and II trials. BMC cardiovascular disorders. 2014; 14(1): 121-27.
20.He X-M, Chen L, Wang T-S, Zhang Y-B, Luo J-B, Feng X-X. E670G polymorphism of PCSK9 gene of patients with coronary heart disease among Han population in Hainan and three provinces in the northeast of China. Asian Pacific journal of tropical medicine. 2016; 9(2): 172-76.
21.McKenney JM, Koren MJ, Kereiakes DJ, Hanotin C, Ferrand A-C, Stein EA. Safety and efficacy of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease, SAR236553/REGN727, in patients with primary hypercholesterolemia receiving ongoing stable atorvastatin therapy. Journal of the American College of Cardiology. 2012; 59(25): 2344-53.
22.Mata P, Alonso R, Pérez-Jiménez F. Screening for familial hypercholesterolemia: a model for preventive medicine. Revista espanola de cardiologia. 2014; 67(09): 685-88.
23Tran NT, Aslibekyan S, Tiwari HK, Zhi D, Sung YJ, Hunt SC, et al. PCSK9 variation and association with blood pressure in African Americans: preliminary findings from the HyperGEN and REGARDS studies. Frontiers in genetics. 2015; 6(4): 1-7.