Detection of the splicing defects(c.1845+11c>g), and common polymorphism (1773C>T) in exon 12 of LDL-R gene on chromosome 19 among some Heterozygous FH patients in Tripoli

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Abstract:

Study discusses the genetic basis for familial hypercholesterolemia (FH) in some Libyan patients at Tripoli city, examining the distribution of variants that associated with mutations in exon 12 of low-density lipoprotein receptor (LDL-R) gene on chromosome 19. Genetic analysis was done using polymerases chain reaction-single strand conformation polymorphism (PCR-SSCP) based on DNA technique, to detect splicing defects of the LDL-R gene in exon 12 on chromosome 19, among some Libyans patients probably have heterozygous familial hypercholesterolemia (HeFH). The patient's volunteer's participants in this study were from Tajjora National Cardiac Center, Endocrine and Diabetic Hospital in Tripoli.

The study showed the presence of the splicing defects (1845 +11 C>G)in the exon 12 of the LDL-R gene on chromosome 19 in patients with hypercholesterolemia and ischemic heart disease, as they have family history in hypercholesterolemia and other secondary causes to heart disease such as diabetic, hypertension, chest pain, and obesity. Frequency of HeFH Libyan patients was (0.86%), and the significant of statistical analysis were{p-value>0.05}, which considered high according to clinical diagnostic criteria for HeFH. As well as detection the single nucleotide polymorphisms (SNP) (1773C>T) that alter the exon splicing efficiency, because it is associated with an emerging of the functional genetic variants for mutations in exon-12, where results of molecular diagnosis confirmed that occurrence the mutation of (LDL-R gene) relates to premature coronary artery diseases(P-CAD).

Abbreviation:

FH	Familial Hypercholesterolemia
HeFH	Heterozygous Familial Hypercholesterolemia
LDL-R gene	Low Density Lipoproteins –Receptor gen
PCR-SSCP	Polymerases Chain Reaction-Single Strand Conformation Polymorphism
SNP	Single Nucleotide Polymorphisms
P-CAD	Premature Coronary Artery Diseases
LDL-c	LDL-cholesterol
LDLs	low density lipoproteins
LDLR-DLs	low density lipoprotein receptor - Low density lipoproteins
DLCNS	Dutch Lipid Clinic Network Score

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Introduction:

(FH)is one of the main problems in health systems and global public health threat, which has increased dramatically over the past two decades (Henderson etal., 2016; Nordestgaard and Chapman., 2013). It is considered the first genetic disease of lipid metabolism disorders which manifested clinically and molecularly since birth; it is associated with very high levels of plasma LDL-cholesterol (LDL-c), and (P-CAD)(Henderson et al.,2016).HeFH is monogenic disorder, the most common affecting 1 in 200–250 of the population globally and It is believed that there are 34 million FH case worldwide, and that less than 1% of potential patients with FH have been identified in most countries (Nordestgaardm and Chapman., 2013). Which result from the absent or malfunctioning of LDL-R gene (Citkowtz et al., 2009). It acts as mediator for clearance of low-density lipoproteins (LDLs), which considered the main plasma reservoir of cholesterol (Umans-Eckenhausen et al., 2001). Thus, LDLs bound with LDL-R on the cell surface leads to internalization of low-density lipoprotein receptor -low density lipoproteins (LDLR-LDLs) complex, occurs subsequently disassembled to its components intra the cells. So, the cholesterol derived from disassembling this complex inhibits cholesterol synthesis within the cell (Austin et al., 2004).Thus, Loss of functional activity in LDL-Rs leads to the increased biosynthesis cholesterol, whilst decreased level of metabolism the total cholesterol and LDL-C, resulting in reduction of uptake of LDLs in liver (Dagard et al., 2004 and Leren., 2004).So, mutations that completely prevents synthesis of a molecule functional of (LDL-R) causes more severe elevations in plasma-cholesterol than mutations that permit synthesis of defective LDL-R that reduces functional activity(Leren., 2004).

The prevalence of LDL-R gene mutations among patients with FH is highly variable, and reflects true genetic variation among different populations in world (Williams et al., 1999 and Yuan et al., 2006). More than 700 mutations have been identified with a significant impact on the LDL-R function, ranges from completely absent to approximately just 25% of normal receptor activity (Citkowtz et al., 2009). There are a wide variety of mutations including insertions, deletions, nonsense, has been identified in patients with FH (Hobbs et al., 1992 and Chiu et al., 2005).

The polymerases chain reaction - single strand conformation polymorphism (PCR-SSCP) based on DNA technique analysis represents a powerful technique that will facilitate study of genetic variation in LDL-R gene and thereby increases the understanding of the role of this gene in the development of FH (Heath and Humphries, 2000; Mozas et al., 2004 and Zhu et al., 2007). It acts to improve detection rate of mutations on LDL-R gene, which shows splicing defects that occurred on exon 12. It is also advantageous for detecting if this defect was by mutations at splicing sites on exon 12 or whether is the polymorphism associated with change in lipid levels, because presences the greater number of them on exon 12 (Graham et al., 2005).

Material and Methods:

Samples collection: Blood samples were collected from 412 Individuals, the study group for biochemical investigations:
350 patients of them 233 patients with hypercholesterolemia and coronary artery disease,117 patients suffering from high cholesterol and had no symptoms of cardiac disease.
62 control group in studied sample.
Study group according to clinical diagnostic criteria of HeFH :

For mutational investigations identify 14 patient samples and 2 control sample, aged (28-76) years according to diagnosis criteria for Dutch Lipid Clinic Network Score (DLCNS) for HeFH(Austin et al, 2004; Kane and Havel, 2001;Leonard et al., 1977 and Williams, 1999). Blood samples were stored at ice box and transported to Laboratory of Molecular Genetics, Department of Biology, Faculty of Science and Technology, at Cairo University, Egypt.

Genetic screening: In this study were used single strands correlation polymorphism (PCR-SSCP) technique. the PCR assay was designed using Due I restrictions enzyme for detecting novel mutation (ivs 12+11c>g). For amplification of LDL- R gene on chromosome 19, primers were used:
{5'-CTCTGGGACTGGCATCAGC-3` (position c.1706-52 to c.1706-34) and 5'- GTTCATCTTGGCTTGAGTG-3`(position c.1845+77 to c.1845 +59)}.The primers were designed to amplify between the flanking regions with the exon 12 12 of LDL- R gene, approximately A 269 bp fragment will be amplifies including 52 bp from intron 12 and 77 bp in splicing cryptic of the exon 13 containing c>g mutation. The size of each amplified fragment corresponded to the right size in base pairs (Graham et al., 2005).
A garose gel electrophoresis technique:

Under appropriate conditions, this technique allows to identification of differences between DNA sequences on single strand of DNA from patient samples compared with control samples. These differences are detected by

shifts or variants in the bands that elicited from amplified fragments the gene when separated by a garose gel. This method was carried out according the methods of (Friedman et al., 1996).

To photographed the amplified of DNA fragments from 16 samples were loaded on a garose gel, and used an ultraviolet transilluminator (UV) (340 nm), with gel documentation system (Sambrook et al., 1989). then was analyzed using software named (lab. Image V2.7).

Single strands correlation polymorphism (PCR-SSCP) based on DNA technique:

To confirm the presence of splicing defects in exon 12 on a low-density lipoprotein receptor gene, used the single strands correlation polymorphism (PCR-SSCP) based DNA technique. This method depends on the existence of form aide that works to eliminations the distorted strands of DNA, and separates them by using polyacrylamide gel electrophoresis under fixed conditions (Graham et al., 2005).Also this technique used the single-strand binding protein principle that was carried out according the methods of (Friedman et al., 1996). Was screened 16 samples (14 patient sample and 2 control sample had no related to patients (they did not have high blood cholesterol level or coronary artery disease**N21** and **N25**). Also was occurred hemolysis for some samples, and then excluded in the analysis.

Statistical analysis: Statistical Package for the Social Sciences Software (SPSS version18), was used for data analysis.

Results:

Clinical detection and biochemical investigations for study group according to clinical diagnostic criteria of HeFH (tab.1).

Table (1): Data of study group according to clinical diagnostic criteria of HeFH

Samples	Group	Age (y)	Sex	CVD	Complications	BMI kg/m	T-C mg/DL	LDL-C mg/DL	TG mg/DL	HDL-C mg/DL	DNA variation
1	P	28	M	+	X	37.11	128	740	509	22	_**
2	P	38	M	+	HY	42.52	540	267	700	20	_
3	P	69	F	+	DM_HY	37.08	424	263	311	35	_
4	P	71	F	+	HY_X	45.7	394	261	213	33	_
5	P	53	M	+	DM_HY	29.00	383	260	345	29	+*
6	P	76	F	+	DM_HY	43.34	352	259	424	37	+*
8	P	51	M	+	-	29 .18	348	237	710	21	-
9	R-P	47	M	-	Ob	109	327	220	1262	37	-
10	R-P	39	F	-	Ch-Pa	29	210	218	145	51	-
11	R-P	63	F	-	Ch-Pa	34.30	313	207	159	39	_
12	R-P	49	M	-	DM	28.41	240	200	213	21	_
13	R-P	55	F	-	Ob	89.70	307	197	701	41	_
14	R-P	43	M	-	HY	36.92	301	191	166	28	_
17	R-P	29	M	-	DM	36.00	299	183	303	11	+*
19	R-P	45	M	-	Ch-Pa	33.39	201	159	127	30	_
N -21	C	55	F	-	-	21	69	51	79	64	-
N- 25	C	47	M	-	-	22.31	58	87	83	47	-

Abbreviation of (tab.5); P: Patients as clinical diagnosis, R-p: related with patient, C: Control, Y: Years, M: Male, F: Female, CVD: Coronary artery disease, DM: Diabetes mellitus, X: Xanthomas, HY: Hypertension, Ob: Obesity, C-P: Chest pain, BMI: Body mass index, T-C: Total cholesterol, TG: Triacylglycerol, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, (+): Heterozygous FH, (-): Non Heterozygous FH, (*) Mutant on exon 12 of LDL-R.

(**) Sitosterolemia Disorder: the level of LDL-cholesterol was 740 higher the normal level about 6 fold according to followed a diagnosis criteria of FH, which caused by mutations on ABCG5 gene and / or ABCG8 gene which results in Suppression of receptor gene transcription as described by(Austin et al, 2004;Citkowtz et al., 2009; Kane and Havel., 2001; Leonard et al., 1977 and Williams., 1999). No mutations on exon 12 of LDL-R gene.

Amplified fragments of LDL-R in exon 12.

The resulting amplified fragments of samples that loaded on a garose gel with a DNA marker was used to ensure that the amplified fragments were of the expected (Fig.1).

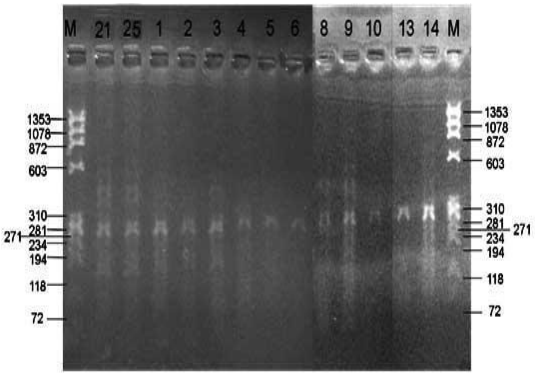

Figure (1) :PCR amplification of exon 12 of LDL-R. Amplified fragments were separated on 2% a garose gel. Lane M: molecular size marker. Lane N-21 to 14: PCR products (269 bp) of 3'end of intron 11, exon 12 and5`end of intron 12 of LDL-R gene.

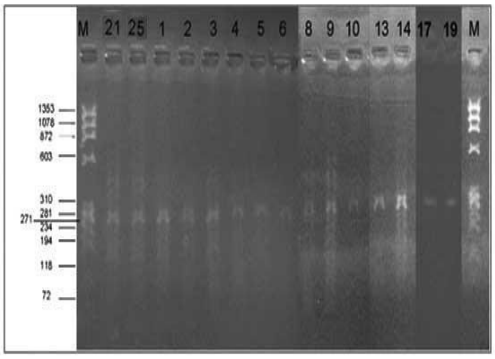

Figure (2):PCR amplification the exon 12 of LDL-R. Amplified fragments were separated on a garose gel (2%) . Lane M: the molecular size marker for determine the correct and normal size (269 bp) by compared with amplified fragments of samples. Lane N-21 to 19: PCR amplified fragments ofeach sample (269 bp) of 3'end of intron

Detection of point mutation by using (SSCP)-based on DNA test:

Results of this study identified 3 HeFH patients, disclosed two variants; splicing defects (1845+11C>G) and single nucleotide polymorphisms (SNPs) (1773C>T) (Fig.3 and 4). Summary the detected of SNPs and splicing defects in patients' samples (Tab. 2 and 3).

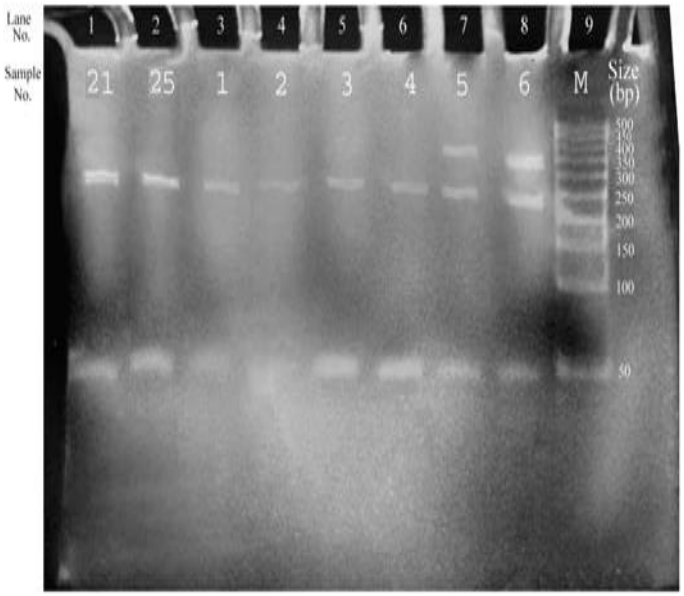

Figure (3) Products of (PCR-SSCP)-based DNA, test for (c.1845+11 C.>G) mutant.

(A) Amplified fragments were migrated on polyacrylamide gel and separated lasted for about 5 hours at 160 volts 25 MA (B) bases highlights refer to specific primers used and DNA sequence of 3' end of intron 11, exon 12 and 5'end of intron 12 of LDL-R gene. (C) Lanes 1and 2-PCR products from volunteer (negative controls). (D) Lanes 3 to 8: PCR products from patients that probably defected by HeFH. (E) Lanes 9: M; molecular size marker. (F) DNA of patient (5) which carried (c.1845+11C>G) variant that produced the additional 21 bp in intron 12, was not shown at control samples. (G) DNA of patient (6) who carried (c.1773C>T) variant, that produced the addition 11 bp in intron 12.

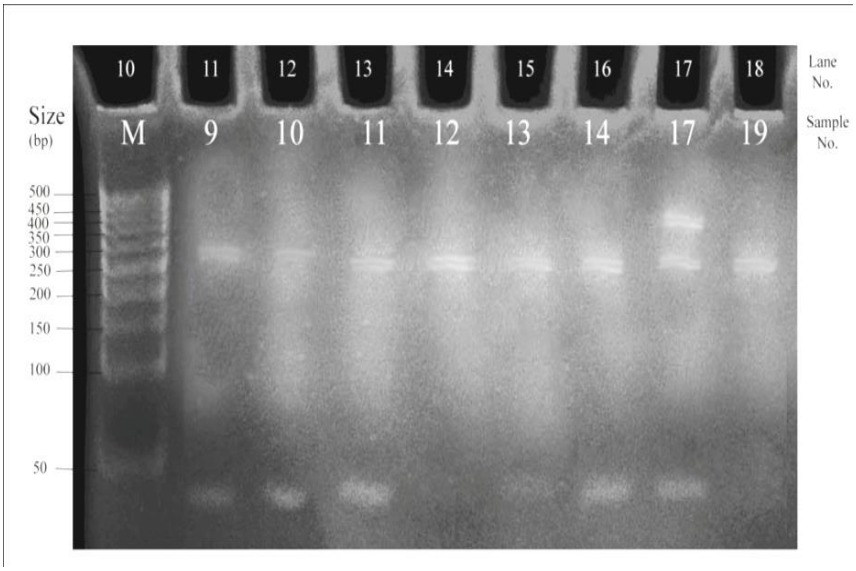

Figure (4) Products of (PCR-SSCP)- based DNA test for (c.1845+11 C.>G) mutant.

(A) Amplified fragments were migrated on polyacrylamide gel and Separated lasted for about 5 hours at 160 volts 25 MA(B) bases highlights refers to specific primers used and DNA sequence of 3' end of intron 11, exon 12 and 5'end of intron 12 of LDL-R gene. (C) Lanes 10: M; molecular size marker. (D) Lanes 11 to 18: PCR products from volunteer (that relative with patients) were possibly affected by HeFH. (E) Lanes 17: patient (17) carried (c.1845+11C>G) variant that produced the additional 21 bp in intron 12, the additional bands were not shown at control samples.

Table (3): Summary the detected of SNPs and splicing defects in patients' samples.

Lanes:	1	2	3	4	5	6	7	8	9
Sample No.	N-21	N-25	1	2	3	4	5	6	M
	-	-	-	-	-	-	-	-	500
	-	-	-	-	-	-	-	-	450
	-	-	-	-	-	-	-	-	400
	-	-	-	-	-	-	-	-	350
	-	-	-	-	-	-	290	280	300
	269	269	269	269	269	269	269	269	250
	-	-	-	-	-	-	-	-	200
	-	-	-	-	-	-	-	-	150
	-	-	-	-	-	-	-	-	100
	-	-	-	-	-	-	-	-	50

Lane 7: PCR-SSCP product from patient (5) who carried (c. 1845 +11 C>G) variants, that produced the addition 21 bp in intron 12. Lane 8: PCR-SSCP products from patient (6) who carried (1773 C>T) variants, that produced the addition 11 bp in intron 12. lanes 1, 2, 3, 4, 5,6: PCR-SSCP products from samples (N21, N25, 1, 2, 3, and 4) appears bands migrates in expected sizes. Lane 9: (M) molecular size marker. (-): Not appears any migrate for bands.

Table (4): Summary the detected of SNPs and splicing defects in patients' samples.

Lanes:	10	11	12	13	14	15	16	17	18
Sample No	M	9	10	11	12	13	14	17	19
	500	-	-	-	-	-	-	-	-
	450	-	-	-	-	-	-	-	-
	400	-	-	-	-	-	-	-	-
	350	-	-	-	-	-	-	-	-
	300	-	-	-	-	-	-	290	-
	250	269	269	269	269	269	269	269	269
	200	-	-	-	-	-	-	-	-
	150	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-	-

Lane 17: PCR-SSCP product from patient (17) who carried (c.1845+ 11C>G) variants, that produced the addition 21 bp in intron 12. Lanes 11, 12, 13, 14, 15, 16, 18: PCR-SSCP products from samples (9, 10, 11, 12, 13, 14, and 19), appears bands migrates in expected sizes. Lane 10: (M) molecular size marker. (-): Not appears any migrate for bands.

Assessment of prevalence rate of HeFH:

Statistical Package for the Social Sciences Software (SPSS version 18) was used for data analysis. Estimated of prevalence rate of familial hypercholesterolemia was on the basis of the frequency of people with mutations in the low-density lipoprotein receptor gene. The prevalence of HeFH was 0.86% of those whose include them the examination and was statistically significant (P>0.05) (Fig.5).

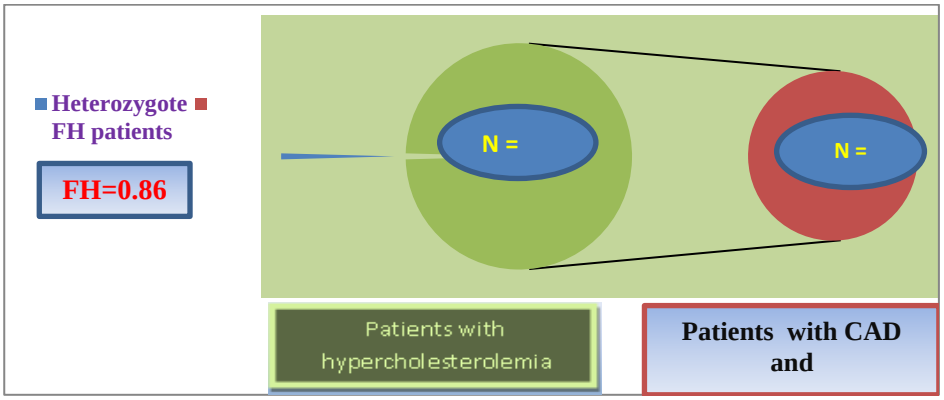

Figure (5): The percentage (%) for HeFH frequency among the studied patients

Discussions:

The study is concerned to detect the splicing defects (c.184511c>g) and polymorphism (1773C>T) on the exon 12 of LDL-R gene at chromosome 19. Also concerned with identifying the correct screening methods for exon-12 of LDL-R gene. The single-strand conformation polymorphism (PCR-SSCP) based DNA technique used performed to improved detection rate for variants rates on the LDL-R gene, and described intron splicing defects which occurred in exon 12.

Based on previous studies (Austin et al., 2004; Heath and Humphries, 2000), that concluded to outcomes comprise 5 percent of FH mutations in genetically heterogeneous patients, where the majority are located within introns 1–8 and intron 12 through the 3'-untranslated region. This pattern corresponds to the distribution of repeat sequences in LDL-R gene, which has a higher frequency of (Alu sequences) than do other genes, and these repeat sequences are also concentrated in introns 1–8 and intron 12 through the 3'-untranslated region (Austinet al.,2004).

(SSCP) analysis is a simple and sensitive technique for mutation detection and genotyping. The principle of SSCP analysis is based on the fact that single-stranded DNA has a defined conformation. Altered conformation due to a single base change in the sequence can makes the single-stranded DNA migrate differently by electrophoresis under fixed conditions. Therefore, DNA samples that have variants shows different bands patterns

(Dong and Zhu, 2005). In this study, was used the single-strand binding protein also known as SSBs or SSBP, are a class of proteins that have been identified in both viruses and organisms from bacteria to humans (Mapelli et al., 2005). That binds with the single strand (coding strand), to keeping them separate to prevent re-annealing in a quick time into double-stranded DNA, and allows for the DNA replication machinery to perform its function, this method was carried out according the methods of (Friedman et al., 1996).

The results of this study detected splicing defects (c.184511c>g) in exon 12, this confirmed by previously described results in studies by (Graham et al., 2005; Mozas et al., 2004 and Zhu et al., 2007). Also confirm presence of polymorphism (1773 C>T) in exon 12, that alter the exon splicing efficiency, because it is associated with an emerging of the functional genetic variants for mutations in exon-12. This consistent with outcome of previous studies by both (Frikke and Schmidt, 2004; Jensen et al., 1996).

This study indicated the existence of (c.1845+11C>G) mutant at two patients (patients5 and 17), were screened exon 12 by polyacryl-amide gel electrophoresis with SSCP-PCR technique, that showed significant difference in the outcome of the amplification between patient samples and control samples. The amplified fragments from two samples which carried (c.1845+11C>G) mutant produced the additional 21 bp in intron 12, which generated in an extra site that produced one band on 162bp whilst was not shown at control samples this consistent with outcomes of (Graham et al., 2005), which confirmed that the c<g variants creates an additional 21bp in restriction site for the enzyme restriction resulting in the mutant allele. This confirms if DNA of patient which carried (c.1845+11c>g) variants resulted in the additional 21bp of intron 12, because this bands were not shown at control samples.

The splicing defects (c.184511c>g) consists of substitution of guanine by cytosine (GC) in nucleotide position 1845 of complementary DNA in the exon-12 of LDL-R gene (tab.1 and 2), These results confirmed by previously results described by both (Frikke and Schmidt, 2004; Graham et al., 2005), that the single nucleotide substitution site in cryptic region (G/C) of the mutant allele on exon-12 of LDL-R gene. This suggests the presence of destruction on the normal splicing in the mature protein sequence in exon 12 of LDL-R

on chromosome 19 in the patients' DNA. Also showed the c.1845+11c<g mutation found in two patients and was not found in any of controls, indicating to the real a mutation, this consistent to the conclusions of previous studies (Graham et al., 2005; Mozas et al., 2004; Zhu et al., 2007).

As well as, the amplified fragments on polyacrylamide gel of (PCR-SSCP) from one patient (patient 6) carried (1773 C>T), that contain an extra site. This new splice site created two putative of SR p40 binding sites, that corresponds with 11 bp additional in intron 12 this consistent with previous studies (Graham et al., 2005: Tveten et al., 2006 and Zhu et al., 2007). Also, these results confirmed by the outcome previous studies of both of Cartegni et al (2003) and Conde et al (2004), that refer to presence the SNP within LDL-R exon 12, may acts on modulate or altere of the LDL-R exon splicing enhancers (LDLR-ESEs).

The SNP (1773C>T) is a common polymorphism Has already been well studied previously, has been evaluated by study of Zhu et al (2007), who referred to as C1773T polymorphism (HincII LDL-receptor restriction fragment length polymorphism), on the minor allele : C/T or T/T,Knew as (rs688) gene. Also, the outcome of previous studies (Cartegni et al., 2003; Conde et al., 2004 and Zhu et al., 2007) suggested that rs688 may modulate the splicing efficiency of exon 12 or its flanking exons.

In recent studies by Tveten et al (2006) and Zhu et al (2007), the SNPs were defined on only one allele, which can generate an ESE (LDLR-ESEs) capable to binding its regulatory protein. and conclude that identified rs688 on an exon 12 acts to modulating two putative SRp40 binding sites. One ESE site altered from the majorallele TGTCAAC to the minor allele TGTCAAT ; this alteration decreases the SRp40 affinity score of binding threshold from 3.04 to 1.50, which below the SRp40 binding threshold in normal allele for (LDLR-ESEs) which up to 2.67.So, the result of this study consistent with previously study by Graham et al (2005), which confirmed that PCR fragment which contained c>t base change, results in an extra site as opposed to the normal allele, creates (two putative SR p40 binding sites) which corresponds with 11 bp additional on polyacrylamide gel.

Based on the above, (SNPs) that alter exon splicing efficiency, an emerging class of functional genetic variants, this confirmed that occurrence the mutations on LDL-R are primary cause of FH , and LDL-R SNPs may alter splicing efficiency and homeostasis of cholesterol in body circulation (Heath and Humphries, 2000).

Based on the frequency of Heterozygous-FH cases in survivors of myocardial infarction and patients with hypercholesterolemia, assuming them more candidates to carryingLDL-R mutations in their genes (Austin et al., 2004; Williams, 1999). The results of statistical analysis indicated that the (3 cases⁄ 350 patients), which indicate to HeFH frequency was 1 case per 117 (0.86%) in Libyans patients with hypercholesterolemia and myocardial infarction. This consistent to the conclusions of studies (Austin et al., 2004; Jansen et al., 2002 and Kathiresan et al., 2008), that showed that prevalence of HeFH cases were a very high in heart patients.

According to clinical diagnostic criteria for HeFH phenotypes: the frequency was 1/500 (0.2%) (Austin et al., 2004; Kane and Havel., 2001; Williams., 1999). Statistical analysis indicated that the p-value >0.05, which considerably is highly frequency, and hence this study consistent with study of Jansen et al (2002) on Lebanese patients, where found FH frequency is (0.60%). This may be due to the usual marriage between relatives in the two community for long periods of time.In contrast, previous study by both Austin (2004) and Yuan (2006),in Japan the HeFH frequency was (0.11%) and Norway (0.22%), it is smaller frequency than identified by clinical diagnostic criteria for HeFH phenotypes in populations, this may be due to development of societies and lackmarriage between relatives in the community.

This study indicated that mutations of LDL-R exon 12 may play important roles in increasing the susceptibility of FH. This is probably due to the relative mutations located on the coding region of LDL-R gene, which affects the structure and function of the protein, causing the decrease of affinity of the receptors for LDL-molecules and may cause the impairer of the receptor's biosynthesis. In conclusion, the data in this study provides preliminary information about the type-I of HeFH cases in Libyan.

في(1773C>T) وتعدد النيوكليوتيدات المفردة (c.1845+11c>g) كشف الطفرة الأرتباطية الأكسون 12على جين مستقبل البروتين الدهني منخفض الكثافة على كروموسوم 19 بين بعض مرضي كولسترول الدم الوراثي متباين اللاقحة (HeFH) في طرابلس

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المستخلص:

الدراسة تناقش الأساس الجيني لمرضى ارتفاع كولسترول الدم الوراثي في بعض المرضى الليبيين بمدينة طرابلس ودراسة توزيع المتغيرات التي تترافق مع طفرات الإكسون12في جين-LDL Rعلى الكرموسوم 19. وذلك باستخدام تقنية سلسلة البوليميريز متعددة أشكال النيوكليوتيدات باستخدام شريط مفرد من المادة الوراثية (PCR-SSCP)، للكشف عن عيوب الربط في مستقبلات البروتين الدهني منخفض الكثافة في أكسون 12على الكرموسوم 19بين المرضى الليبيين المحتمل إصابتهم بارتفاع كولسترول الدممتباين اللاقحة (HeFH). وكان المتطوعون المرضى المشاركين في هذه الدراسة من مركز تاجورا للقلب ومستشفى السكري بطرابلس.

أظهرت هذه الدراسة وجود عيوب الربط (C>G 11 +1845) في اكسون12 في جين-LDL R على الكرموسوم 19 لدي المرضى الذين يعانون من ارتفاع الكولسترول وأمراض نقص ترويه عضلة القلب والذين لديهم تاريخ عائلي من ارتفاع الكولسترول وأسباب ثانوية أخرى لأمراض القلب مثل السكري، ارتفاع ضغط الدم، آلام الصدرو السمنة. كان التكرار النسبي لمرضىHeFH الليبيين (0,86٪)، وقيمة التحليل الإحصائي كانت {0,05<p-value}، حيث تعتبر عالية وفقا للمعايير السريرية التشخيصية لـHeFH. كذلك الكشف عن تعدد النوكليوتيدات المفردة (SNP) (1773C>T) التي تغير كفاءة الربط في الإكسون لأنهيرتبط مع المتغيرات الجينية الوظيفية الناشئة عن الطفرات في اكسون12،حيث أكدت نتائج التشخيص الجزيئي أن حدوث التحور في جين (LDL-R) مرتبط بأمراض الشريان التاجي(P-CAD).

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