Original Article

Role of peroxisome proliferator-activated receptor (PPAR)-α gene in Dyslipidemia

Amir Rashid, Sadaf Jafar, Raja Khalid Yaqub

Departments of Biochemistry, Army Medical College, Rawalpindi and Islamabad Medical Dental College, Islamabad, Pakistan

Objective: To identify the role of peroxisome proliferator-activated receptor (PPAR)-α gene in Dyslipidemia.

Methodology: This experimental study was carried out at Center for research in experimental and applied medicine (CREAM) Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi from December 20, 2016 to June 8, 2017. The study comprised of three groups; Group one included subjects who were healthy, group two was comprised of diabetic without dyslipidemia and group three included diabetics with dyslipidemia. Blood was collected and Primers specific to PPAR-α were designed on the basis of sequence available on website Primer BLAST. RNA was extracted with in first six hours of sample collection using Gene JET RNA purification kit (Thermo Scientific). For first strand cDNA synthesis Revert Aid Premium was utilized. Polymerase chain reaction was used for the isolation, amplification or detection of specific nucleotide sequences in genomic DNA. PCR products were visualized by Agarose gel electrophoresis. After Real time PCR, molecular analysis was quantified by comparative CT Method (ΔΔ Ct method). Positive value means down regulation and negative value means upregulation.

Results: Primers were optimized using conventional PCR. A single band at 224 base pair represented the amplification of PPAR-α gene. The PCR protocol was optimized for PPAR-α gene.

Conclusion: PPAR-α gene has important role in the homeostasis of lipid profile in the body. Upregulation or increased expression of this gene leads to reduction in lipid profile, while down regulation or suppression of this gene leads to deranged lipid levels. (Rawal Med J 202;45:54-57).

Keywords: Dyslipidemia, gene expression, metabolic process.

INTRODUCTION

Most of the deaths in adult life are due to cardiovascular diseases. Lipid profile is an important parameter to measure risk factors. Derangement of cholesterol level especially LDL and HDL are always evaluated in these patients. The dyslipidemia prevalence has increased with the changes in dietary habits, although the exact cause is not understood. The peroxisome proliferator activated receptor (PPAR) ligand activated nuclear transcription factors are responsible for maintaining metabolic lipid homeostasis. PPARα and PPARγ are present in the specified tissues. PPARα deals with tissues related to fatty acid breakdown. Activation of PPAR-α causes up-regulation of numerous genes directly and indirectly which are involved in oxidation of fatty acid. PPARγ is found in adipocytes dealing with lipid and glucose metabolism.[1]

The peroxisome proliferator activated receptor isotypes PPARα, PPARδ and PPARγ are known nuclear transcription factors and these factors express large number of genes which are responsible for regulating glucose, lipid and cholesterol metabolism. These genetic defects can lead to many diseases like cardiovascular and obesity. PPARα agonists can be used for therapeutic purpose for the treatment of dyslipidemia and PPARγ agonists are used for curing Diabetes type ll.[2]

Lipids and lipoprotein metabolism derangements and morbidities result due PPARα gene defects. PPARα gene is the key modulator of lipid metabolism. The C allele was found to be cardioprotective because it was associated with increasing HDL levels, lowering triglycerides and VLDL levels.[3,4]

Peroxisome proliferator activated receptor γ has

been associated with antineoplastic effects in colorectal carcinogenesis. The haplotype analysis showed that women who carried carcinogenic haplotypes of PPARγ were at risk of 67% increase chance of having breast cancer. Estrogen exposure related factors were another cause of carcinogenic stimulation.[5]

The association with hypertriglyceridemia with single nucleotide polymorphism at PPARα,δ,γ was studied. The analysis revealed rs1800206, rs3856806 and rs1805192 were those single nucleotide polymorphs which were responsible for hypertriglyceridemia and gene interaction in between different single nucleotide polymorphs was revealed.[6] A study on Turkish population revealed that PPARγ C161T polymorphism may be linked with increased coronary heart disease risk in diabetics. C161T CT genotype of PPARγ showed negative effects on serum lipid profile in coronary heart disease patients having Diabetes.[7,8] The aim of this study was to identify the role of PPAR-α gene in dyslipidemia.

METHODOLOGY

The present study was approved by institutional ethical committee Army Medical College and Informed consent was taken from all participants. The study comprised of three groups having both male and females. Group one included subjects who were healthy, group two was comprised of diabetic without dyslipidemia and group three included diabetics with dyslipidemia. Each group included 30 subjects. The criteria for lipid and lipoprotein levels were according to the National Cholesterol Education Program.

Participants were diagnosed with dyslipidemia if they had one or more of the following criteria: a plasma concentration of TC of ≥6.24 mmol/L, plasma concentration of TG ≥ 2.26 mmol/L; plasma concentration of HDL-c of <1.04 mmol/L for men or <1.30 mmol/L for women; and a plasma concentration of LDL-c ≥4.14 mmol/L. Blood was collected using aseptic measures and transported to Biochemistry and Molecular Biology laboratory. Primers specific to PPAR-α were designed on the basis of sequence available on website Primer BLAST. RNA was extracted with in first six hours of sample collection using Gene JET RNA purification kit (Thermo Scientific). For first strand cDNA synthesis Revert Aid Premium was utilized. Polymerase chain reaction was used for the isolation, amplification or detection of specific nucleotide sequences in genomic DNA. PCR products were visualized by Agarose gel electrophoresis. After Real time PCR, molecular analysis was quantified by comparative CT Method (ΔΔ Ct method). Positive value means down regulation and negative value means upregulation.

RESULTS

Primers were optimized using conventional PCR. A single band at 224 base pair represented the amplification of PPAR-α gene. The PCR protocol was optimized for PPAR-α gene. Non specific binding was shown by a single band. The annealing temperature was optimized at 59.5C for 35 seconds.

Table 1. Anthropometric and Biochemical Profile of Control.

		Control Group (I)	Without D Group (II)	DD Group (III)	Overall
Age	Mean	35.77± 10.36	56.13	50.70	47.53
	SD	10.36	14.82	11.60	15.01
Weight	Mean	67.57	73.23	71.57	70.79
	SD	9.55	10.24	9.71	10.02
Ht	Mean	1.68	1.68	1.65	1.67
	SD	0.081	0.089	0.078	0.083
BMI	Mean	23.88	25.98	26.39	25.42
	SD	2.57	3.76	3.87	3.58
BSF	Mean	5.02	10.35	13.40	9.59
	SD	0.43	4.08	5.76	5.33
HbA1C	Mean	5.42	8.23	8.01	7.22
	SD	0.59	1.18	1.46	1.71
Total Cholesterol	Mean	4.58	4.90	5.06	4.85
	SD	0.75	1.00	0.92	0.91
TG	Mean	1.33	1.51	2.45	1.76
	SD	0.33	0.23	0.57	0.64
LDL	Mean	2.63	2.64	2.96	2.74
	SD	0.65	0.73	0.82	0.75
HDL	Mean	1.18	1.11	0.85	1.05
	SD	0.23	0.16	0.07	0.22

Body Mass Index was slightly raised in diabetics and diabetics with dyslipidemia. Blood sugar fasting showed marked rise in diabetics and diabetics with dyslipidemia. HbA1C showed minimum difference among diabetics and diabetics with dyslipidemia. Total cholesterol revealed slight difference among the three groups. Triglyceride and LDL levels were raised in diabetics with dyslipidemia, whereas HDL level was decreased (Table 1).

Table 2. Interpretation of gene expression of Dyslipidemia and control groups.

Patient Category	Mean CT genotype of Target PPARα	Mean CT genotype of GAPDH	ΔCT genotype Target-GAPDH	ΔΔCT= ΔCT of patients ΔCT of controls	2-(ΔΔCT)	Interpretation
Patients	21.91	22.18	-0.27	-1.68	2-(-1.68)= 3.20	**Up regulation**
Controls	24.31	22.9	1.41			

After Real time PCR, molecular analysis was quantified by comparative CT Method (ΔΔ Ct method). Positive value means down regulation and negative value means upregulation. The primers for house keeping gene GAPDH was also optimized using Conventional PCR. Single band was obtained which showed lack of non specific binding (Table 2).

DISCUSSION

Lipoprotein (a) act as a risk predisposition to cardiovascular diseases. The association of three single nucleotide polymorphism and the haplotypes of the peroxisome proliferator activated receptor γ gene with the level of lipoprotein (a). It was found out that PPARγ polymorphism rs1086571, rs4684847 and haplotypes might be the genetic predisposition for Lp(a) level. In the present study, PPARα gene was studied and it was found that increased expression of this gene leads to reduction in lipid levels.[9]

Genome wide associated studies revealed that single nucleotide polymorphism linked dyslipidemia are located in non-coding regions. In our study, genome wide scan was not done only likage analysis for PRPPα gene was performed.[10]

The peroxisome proliferator activated receptor gamma did not play any role in transcriptional down regulation of aromatase, which is required for estrogen biosynthesis. There was also no evidence regarding association of pro12A1a PPAR gamma polymorphism with body mass index. In our study, PRPPα gene expression in regard to lipid profile was focused.[11]

The peroxisome proliferator activated receptor showed single nucleotide polymorphism rs1800206 and rs3856806 which were linked to non HDL-C and there was gene interaction among rs1800206, rs3856806, rs135539, rs4253778,rs2016520, rs1805192, rs3856806 and rs709158 which might be influencing the non HDL-C levels. In our study, polymorphism of PPARα gene was not studied but lipid profile was one of the important parameter for gene expression analysis.[12]

Single nucleotide polymorphism of peroxisome proliferator activated receptor rs1800206 of PPARα and rs 1805192, rs53856806 of PPARγ were linked to increased levels of lipid accumulation product. Rs2016520 of PPARγ was linked to low level of lipid accumulation product. There was found to be gene-gene interaction between different SNPs. Polymorphism was not considered in our study but PPARα gene expression in relevance to lipid profile was studied.[13]

Single nucleotide polymorphism rs2016520 was linked with lower down chances of obesity. There was association of rs2016520, rs9794 and rs10865170 with predisposition to obesity.[14] Rs2016520 and rs10865170 were linked to less risk of obesity. rs2016520, rs9794 and rs40865170 were associated with obesity. In our study, PPARα gene increased expression marked lower lipid profile.[15]

Aerobic training effect the glucose metabolism in some individuals, who show PPAR gene polymorphism and their coactivators. PPAR gene polymorphism was not studied in our study.[16]

In an investigation associated with ten SNP. Rs1800206, rs2016520, rs3856806 and rs1805192 were linked to hypertriglyceridemia. Multiple SNP showed gene-gene interaction. Single nucleotide polymorphism was not studied in the present study but only PPARα was considered.[17] We found that there is gene-gene interaction between numerous SNPs. In our study PPARα gene up and down regulation affecting lipid profile was focused.[18]

CONCLUSION

PPAR-α gene has important role in the homeostasis of lipid profile in the body. Upregulation or increased expression of this gene leads to reduction in lipid profile, while down regulation or suppression of this gene leads to deranged lipid levels.

Author contributions:
Conception and design: Raja Khalid Yaqub
Collection and assembly of data: Amir Rashid
Analysis and interpretation of the data: Amir Rashid, Raja Khalid Yaqub
Drafting of the article: Sadaf Jafar
Critical revision of the article for important intellectual content: Prof. Dr. Sajid Raza
Statistical expertise: Mr. Sabir
Final approval and guarantor of the article: Prof. Dr. Sajid Raza
Corresponding author email: Sadaf Jafar: sadafjafar75@gmail.com
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