RESEARCH ARTICLE

Genetic association of rs373001 polymorphism in mir-130b gene with susceptibility to dyslipidaemia and correlation with lipid profile

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Abstract

Objective: To find the association and correlation of rs373001 polymorphism in micro-ribonucleic acid-130b gene with the risk of dyslipidaemia.

Method: The case-control study was conducted from April 2 to December 13, 2024, at the Department of Biochemistry and Molecular Biology, National University of Medical Sciences, Rawalpindi, Pakistan, in collaboration with the Chemical Pathology Laboratory, Army Medical College, Rawalpindi, and comprised dyslipidaemia cases and healthy controls. Genomic deoxyribonucleic acid extraction was done from blood samples using an organic method, and tetra-primer amplification refractory mutation system-polymerase chain reaction, followed by agarose gel electrophoresis. Data regarding lipid profile and fasting blood glucose was noted for all the participants. Data was analysed using SPSS 26.

Results: Of the 300 subjects, 150(50%) were in each of the two groups. There were 86(57%) males and 64(43%) females among the cases with mean age was 49.1±11.6 years, and 76(52%) females and 72(48%) males with 43.3±15.6 years among the controls. The rs373001 polymorphism of micro-ribonucleic acid-130b gene showed a C>T transition. The C allele (reference allele) frequency was n=123 (41%) in cases and n=144 (48%) in controls, while T allele (risk allele) frequency was n=177 (59%) in cases and n=156 (52%) in controls. The cases had 120(80%) CT, 1(0.7%) CC and 29(19.3%) TT genotypes, while the controls had 142(94.7%) CT, 1(0.7%) CC and 7(4.7%) TT genotypes. The TT genotype was a risk (adjusted odds ratio: 4.8, p<0.001) in recessive genetic model, while CT was protective (adjusted odds ratio: 0.2, p<0.001) in over-dominant model. A correlation was noted between genotypes and triglycerides levels (p=0.039) without confounders age and gender, but when adjusted with these confounders, no correlation was found (p>0.05).

Conclusion: There was a positive association of the TT genotype of rs373001 polymorphism in micro-ribonucleic acid-130b gene with the risk of dyslipidaemia development in recessive genetic model and log-additive pattern of inheritance.

Key Words: Low-density lipoprotein, High-density lipoprotein, microRNAs, Polymorphism, Lipid metabolism.

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Introduction

Dyslipidaemia is a multifactorial disorder, and, according to the National Cholesterol Education Programme Adult Treatment Panel-III guidelines (NCEPATP-III), it is defined as high levels of triglycerides (TG <150mg/dl normal) or high levels of low-density lipoprotein cholesterol (LDL-C <100mg/dl optimal) with low levels of high-density lipoprotein cholesterol (HDL-C 40mg/dl in men and 50mg/dl in women normal).[1] It has two categories; primary and secondary dyslipidaemia. Primary dyslipidaemia is hereditary that involves the genetic predispositions passed down through families.[2] Secondary dyslipidaemia is triggered by extrinsic or modifiable factors, including diabetes mellitus (DM_, chronic kidney disease (CKD), hypothyroidism, lifestyle factors, medications and dietary habits.[3] Dyslipidaemia increases the risk of cardiovascular diseases (CVDs) and atherosclerosis, leading to high morbidity and mortality.[4]

In Pakistan, the overall incidence of dyslipidaemia appears to be quite high. Sub-analysis of the second National Diabetes Survey of Pakistan, carried out across the country among 10,834 adults aged at least 20 years, indicated 90% women and 83.9% men had low HDL levels, 48.9% had hypertriglyceridaemia and 39.3% had high LDL-C. It was determined that the three main risk factors for dyslipidaemia were DM, obesity and hypertension (HTN), and the overall prevalence of dyslipidaemia was 96% in Pakistan.[5]

Dyslipidaemia plays a role in the development of different disorders, particularly CVDs and metabolic syndromes. The raised LDL-C levels in dyslipidaemia destroy the endothelial cells in the blood vessels, which results in

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inflammation and leads to the development of atherosclerosis.[6] The TG levels are raised in dyslipidaemia, resulting in their deposition in the pancreas which causes inflammation and pancreatitis.[7] Micro-ribonucleic acids (miRNAs) are one of the epigenetic mechanisms of regulating many lipid metabolism genes expression. They are 18-22 bases non-coding, single-stranded RNAs that are posttranscriptional modulators in humans[8] and are used as blood-based biomarkers of underlying physiological conditions.[9] The micro-ribonucleic acid-130b (miR-130b) gene is located on chromosome 22, and regulates the expression of many genes, such as Peroxisome proliferator-activated receptor gamma (PPARγ), Adenosine triphosphate (ATP)-binding cassette subfamily A member 1 (ABCA1), Sterol Regulatory Element-Binding Proteins (SREBPs), 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-COA) and low-density lipoprotein receptor (LDLR) of lipoprotein metabolism and cholesterol homeostasis. It also regulates genes like insulin-induced gene 1 (INSIG1) and PPAR gamma coactivator 1 alpha (PGC1-α).[10] The rs373001 single nucleotide polymorphism (SNP) of miR-130b gene, to our knowledge, has not been previously investigated for its association with dyslipidaemia in the Pakistani population. The current study was planned to fill the gap in literature by finding the association and correlation of rs373001 polymorphism in miR-130b gene with the risk of dyslipidaemia.

Materials and Methods

The case-control study was conducted from April 2 to December 13, 2024, at the Department of Biochemistry and Molecular Biology, National University of Medical Sciences (NUMS), Rawalpindi, Pakistan, in collaboration with the Chemical Pathology Laboratory, Army Medical College (AMC), Rawalpindi. Approval was obtained from the ethical review committee of AMC, written informed consent was taken from all the participants, and the study was conducted in line with Helsinki guidelines.[11] The sample size was calculated using the GeneQuanto Software.[12] using a log-additive genetic model with gene effect size 1.5-2.0, two-tailed significance level (α) 0.05, power 80%, and estimated error rate 20%. The sample was raised using non-probability sampling technique from among males and females visiting the Pakistan Emirates Military Hospital (PEMH), Rawalpindi, aged 25-70 years. Those with types I and II DM, gestational DM (GDM) or obesity, and individuals on lipid-lowering therapy were excluded.

Genotypic association analyses were performed using SNPstats software under pre-specified inheritance models, including codominant, dominant, recessive, over-dominant and log-additive models. The biochemical tests of lipid profile and fasting blood glucose (FBG) were performed for each subject, and data was recorded along with demographic details. The genomic deoxyribonucleic acid (DNA) was extracted through the organic phenol-chloroform method[13] from whole blood samples of both the cases and controls, and the visualisation of determined DNA was done through a gel documentation system (Bio-Rad Laboratories, Hercules, California, USA).

The genotyping of rs373001 polymorphism with transition C>T of the miR-130b gene was performed through Tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). The four primers, including outer forward, outer reverse, inner forward and inner reverse primers, were used in a single PCR reaction, and then gel electrophoresis was performed. Reverse outer primer sequence was 5′AACAGGGAAAGAGTGTCGGGCAGGCCTG 3′; the forward inner primer was 5′CCCTTGGGCTTGCAGGCCCTCCCTTA3′; the forward outer primer was 5′CCGCAGGTGCTCTGACGAGGTTGCACTA3′; and the reverse inner primer was 5′TGGAGAAGGGAGCGAATTGGGTAGGGAGG3′. The primers were designed from the coding region of the studied gene miR-130b by using Primer 1 plus output software. Concentration for the inner primers was 1.3 picomoles per microliter (pmol/µl), and for the outer primers it was 1pmol/µl. The final optimised PCR temperature was 72.9ºC.

Demographic, biochemical and genotypic data was analysed using SPSS 26. and SNPstats biological software. The allelic and genotypic frequencies were calculated using chi-square test. Adjusted odds ratios (AORs) with 95% confidence intervals (CIs) were calculated to assist the association of genetic variant with dyslipidaemia. SNP exact test for Hardy-Weinberg equilibrium was used. Analysis of covariance (ANCOVA) was performed to examine the relationship between rs373001 genotypes and lipid profile parameters, with TG, HDL-C, LDL-C and total cholesterol (TC) treated as dependent variables. Age and gender were included as covariates. The distributional assumptions of ANCOVA were evaluated prior to analysis. Lipid parameters showed approximately normal distributions, and the residuals from the models were visually inspected using histograms and Q-Q plots to confirm normality. $P < 0.005$ was considered statistically significant.

Results

Of the 300 subjects, 150(50%) were in each of the two

Table-1: Demographic and biochemical parameters of dyslipidaemia cases and healthy controls (n=300).

Parameter	Group I (n=150)	Group II (n=150)	p-value
Age (years)	49.10 ± 11.64	43.31 ± 15.63	<0.001
BSF (mg/dL)	96.84 ± 1.80	73.44 ± 10.98	<0.001
TG (mg/dL)	285.21 ± 144.10	106.28 ± 30.11	<0.001
HDL (mg/dL)	38.98 ± 15.00	45.24 ± 8.12	<0.001
LDL (mg/dL)	123.74 ± 42.54	84.30 ± 22.82	<0.001
TC (mg/dL)	197.99 ± 50.66	167.44 ± 29.39	<0.001

FBS: Fasting blood sugar, TG: Triglycerides, HDL: High-density lipoprotein, LDL: Low-density lipoprotien, TC: Total cholesterol.

Table-2: Genotypic frequencies of rs373001 polymorphism of micro-ribonucleic acid-130b (miR-130b) gene in dyslipidaemia cases and healthy controls.

Genotype	Healthy Controls (n=150) n (%)	Dyslipidaemia Cases (n=150) n (%)
C/C	1 (0.7%)	1 (0.7%)
T/C	142 (94.7%)	120 (80%)
T/T	7 (4.7%)	29 (19.3%)

Table-3: Association of rs373001 genotypes with dyslipidaemia under different genetic models (adjusted by age and gender).

Pattern of Inheritance	Genotype	Cases	Controls	Crude OR (95% CI)	AOR (95%CI)	p-value
Codominant	C/C	1 (0.7%)	1 (0.7%)	1.0	1.0	< 0.001
	C/T	142 (94.7%)	120 (80%)	0.7 (0.05-12.9)	0.7 (0.05-12.9)	
	T/T	7 (4.7%)	29 (19.3%)	3.8 (0.2-70.8)	3.8 (0.2-70.8)	
Dominant	C/C	1 (0.7%)	1 (0.7%)	1.0	1.0	0.9
	C/T-T/T	149 (99.3%)	149(99.3%)	0.9 (0.05-14.6)	0.9 (0.05-14.6)	
Recessive	C/C-C/T	143 (95.3%)	121 (80.7%)	1.0	1.0	< 0.001
	T/T	7 (4.7%)	29 (19.3%)	4.8 (2.1-11.8)	4.8 (2.1-11.8)	
Over dominant	C/C-T/T	8 (5.3%)	30 (20%)	1.0	1.0	< 0.001
	C/T	142 (94.7%)	120 (80%)	0.2 (0.1-0.5)	0.2 (0.1-0.5)	
Log additive	---	---	---	4.2 (1.8-9.6)	4.2 (1.8-9.6)	< 0.001

AOR: Adjusted odds ratio, CI: Confidence interval.

groups. There were 86(57%) males and 64(43%) females among the cases with mean age was 49.1±11.6 years, and 76(52%) females and 72(48%) males with 43.3±15.6 years among the controls. The biochemical parameters showed significant difference between the groups (Table 1). The rs373001 polymorphism of miR-130b gene showed a C>T transition. The C allele (reference allele) frequency was 123 (41%) in cases and 144 (48%) in controls, while T allele (risk allele) frequency was 177 (59%) in cases and 156 (52%) in controls. The cases had 120(80%) CT, 1(0.7%) CC and 29(19.3%) TT genotypes, while the controls had 142(94.7%) CT, 1(0.7%) CC and 7(4.7%) TT genotypes (Table 2).

The TT genotype was a risk factor (AOR: 4.8, p<0.001) in recessive genetic model, while CT was protective (AOR: 0.2, p<0.001) in over-dominant model (Table 3). A correlation was noted between genotypes and TG levels (p=0.039) without confounders age and gender, but when adjusted with these confounders, no correlation was found (p>0.05).

Discussion

In the current study, the association of rs373001 polymorphism in miR-130b gene with dyslipidaemia in Pakistani sample was analysed. The database has reported this SNP gene variant, but the literature, to our knowledge, was not available regarding it association with dyslipidaemia in the Pakistani population. The current study is the first in this regard, and the findings suggest that rs373001 polymorphism in miR-130b gene is significantly associated with the increased risk of developing dyslipidaemia. The TT genotype in recessive and log-additive genetic models of T allele was found to be a risk factor. This association may be biologically plausible because miR-130b is known to regulate the expression of genes involved in lipid metabolism, such as those controlling lipid synthesis, transport, and clearance. Therefore, genetic variation in miR-130b could disrupt these regulatory pathways, leading to impaired lipid homeostasis, and the manifestation of dyslipidaemia. These evidences suggest that specific genetic variation is playing a crucial role in disturbing lipid metabolism, and causes dyslipidaemia. These findings are in line with previous reports highlighting the involvement of miR-130b gene in the risk of development of coronary artery disease (CAD) due to its involvement in lipid profile dysregulation.[14] The genetic predisposition of TT genotype of rs373001 polymorphism in miR-130b gene might affect its transcriptional processing and stability. This variation might influence the functional interaction with target genes of lipid metabolism.

The majority of samples in both cases and controls were observed in a heterozygous state carrying CT genotype. The results suggested that the C allele was protective, masking the effect of the T allele in dominant and over-dominant genetic models. This implies that the CT genotype might have a protective role compared to the TT genotype. The CT genotype showed a more protective

role in the males with AOR 0.1 than in females with AOR 0.4. According to the SNPstats results, the stronger protective role found in males compared to females indicated that gender-related factors might influence miR-130b expression. This difference could be due to hormonal or genetic variations that affect how miR-130b is regulated, which may then impact lipid metabolism pathways.

The Hardy-Weinberg equilibrium test showed significance ($p<0.001$) for both the cases and controls, and suggested that the population had more heterogeneity, and there were some environmental factors or genetic interactions which were responsible for the variations in the allelic frequencies of the studied polymorphism. A study has reported that deviations from Hardy-Weinberg equilibrium in genetic research on complex diseases, such as dyslipidaemia, may highlight genetic interactions or environmental influences.[15] The small sample size in the current study might have been a cause of genetic drift.

The current study has limitations because of a sample size that was because of time and resource constraints. Moreover, the study was limited to one city, meaning the findings may not be generalizable to other geographical areas. Finally, the study population could be stratified based on ethnicity to find true association of rs373001 in miR-130b gene, but due to limited resources and time, it was not done.

The current findings have highlighted the need to study the rs373001 polymorphism on a large scale to find its true association with miR-130b gene in disease development.

Conclusion

The rs373001 polymorphism of miR-130b gene was found to have a significant association with the risk of dyslipidaemia development in the studied population in recessive and log-additive genetic models with TT genotypes.

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AUTHORS' CONTRIBUTIONS:

NF: Concept, design, literature review, data collection, analysis, drafting, final approval and agreement to be accountable for all aspects of the work.
AM: Concept, design, revision, drafting, data interpretation, final approval and agreement to be accountable for all aspects of the work.
AR: Concept, design, review, critical analysis, drafting, final approval and agreement to be accountable for all aspects of the work.
UN: Concept, data collection, review, data interpretation, final approval and agreement to be accountable for all aspects of the work.