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The Polymorphism Association of Visfatin Gene in CAD Patients of North Indian Population

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Abstract

Visfatin is the adipocytokine that plays a major role in inflammatory processes of atherosclerosis and could be associated with developing coronary artery diseases Hence the study aimed to develop a correlation between frequency distribution of rs61330082 gene polymorphism of visfatin with the development of coronary artery disease. The study was performed on 150 North Indian subjects in which 90 patients were chosen as cases based on CAD diagnosis after performing coronary angiography and rest remaining 60 patients were taken as controls. Biochemical investigation such as lipid test, and glycated Haemoglobin (HbA1c) was performed along with visfatin gene polymorphism that was further compared between CAD patients and healthy controls. The study shows that the mean level of various biochemical parameters was significantly higher in CAD patients (**p<0.001**). Also, there was a significant association of CC genotype (**p<0.001**) in CAD patients than in healthy control. Our findings indicate a potential connection between the CC genotype of rs61330082 with the CAD population.

Keywords: visfatin, coronary artery disease, rs61330082

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1. Introduction

Coronary artery diseases (CAD) are the most common cause of morbidity and mortality in most countries [1], and hence, it is important to target some novel markers that can identify the determinants of risk for developing this disease. Coronary artery diseases are the generic designation for the three forms of cardiac diseases, i.e. angina pectoris unstable angina (UA), stable angina (SA), sudden cardiac death, and acute myocardial infarction (AMI), In most cases, abnormal outcomes of inadequate blood flow result in the development of atherosclerosis, which causes a narrowing of the coronary arteries; thus is also often called coronary heart disease (CHD) in general term or ischemic heart disease (IHD) [2-3]. To that, a recently discovered adipokine multifunctional enzyme known as Visfatin, often referred to as nicotinamide phosphoribosyl transferase (NAMPT), is been found to play an important role in cardiac disease. It is mainly found in visceral adipose tissue and mimics insulin in lowering plasma glucose levels [4]. Visfatin is a regulator of the intracellular nicotinamide adenine dinucleotide (NAD) pool [5-7]. Its gene is located on the 7q22.3 complement strand and consists of 11 exons and 10 introns that span a 34.7-kb region [8]. Visfatin influences the activity of NAD-dependent enzymes via its NAD biosynthesis activity [9], thereby acting as a driver or a pacemaker of metabolism by enhancing cellular proliferation and tipping the balance toward cell survival after a genotoxic insult [10]. Accordingly, it has been regarded as a molecular link between metabolism and cancer [11]. It is expressed both intracellularly and extracellularly [12], in which intra-cellular is been found to function in cellular aging and survival [14], and also being responsible for transmitting inter-organ signals [13]. In addition to, extracellular visfatin has cytokine-like

activity and is regarded as an adipokine; also known as a pre-B-cell colony-enhancing factor. [14]. Visfatin has been associated with inflammation which is a key component in the development and progression of CAD. The elevated levels have been reported in individuals with CAD, and it may contribute to the inflammatory processes that play a role in atherosclerosis. [15] Also, it has been found that endothelial dysfunction, which refers to impaired functioning of the inner lining of blood vessels, is a critical step in the development of atherosclerosis. Visfatin has also been suggested to contribute to endothelial dysfunction, thereby promoting the formation of atherosclerotic plaques. [16] Circulating visfatin levels have been linked to different inflammatory diseases as well as metabolic problems, according to research. Notably, several studies have hypothesized connections between indicators of insulin resistance, circulating visfatin levels, and CAD [17-19]. Also, the studies show elevated fasting plasma glucose and insulin levels have been associated to the promoter variant rs61330082, which is represented by the T-allele. Additionally, this variant has various degrees of relationship with other SNPs in the 5' region, particularly those located in the putative promoter section (rs1319501, 948 G > T). In various populations, this SNP displays significant or moderate linkage disequilibrium (LD) with other SNPs. The purpose of the current investigation is to look into the potential correlation between the rs61330082 SNP and CAD in individuals from North India [20].

2. Materials and Methods

The study involved a cohort of 90 patients ranging in age from 30 to 70 years, who sought medical care at Integral Hospital in Lucknow. These patients were categorized based on the presence or absence of coronary artery disease (CAD). Patients admitted for angiography were classified as cases, while individuals without CAD were selected as controls for this investigation. Blood samples were gathered from participants in both fasting and postprandial states, and subsequently subjected to analysis.

2.1. Subject Selection

Inclusion Criteria

Patients with a confirmed diagnosis of CAD determined through angiography or ECG and elevated cardiac marker levels were eligible.

Exclusion Criteria

Patients with conditions such as cardioembolic embolic stroke, cerebral venous sinus thrombosis, CNS vasculitis, and hemorrhage due to trauma, tumor, vascular malformations, and coagulopathy were excluded. Individuals with bacterial or viral infections, inflammatory diseases, thyroid, liver, kidney disorders, or any form of cancer were excluded. Pregnant individuals were excluded. Those unable to provide written informed consent were excluded.

2.2. Control Selection

The patients enrolled for the category of control in the study should not have a history of ischemic heart disease, and endocrine or metabolic diseases and hence to confirm that the trade mill test is performed and after fulfilling the criteria are taken as control [12].

2.3. Sample Collection and Preparation

Whole blood samples were collected and processed at the Department of Medical Biochemistry, IIMS&R, Lucknow. Peripheral blood samples, totaling 4 ml each, were obtained from both patients and controls. Of this, 1 ml was dedicated to DNA extraction, while the remaining 3 ml was used for serum separation. The collected samples were stored at -20°C for future use. The study was approved by Integral Institute of Ethical Committee of Medical Science and Research IIMS&R, Integral University (IEC/IIMS&R/2019/34)

2.4. Serum Separation

Blood samples were collected in a plain vial and centrifuged for 10 minutes at 4000 rpm at 40°C. After the collection of sera, it was stored at -200 °C until further analysis.

2.5. Estimation of Anthropometric and clinical parameters

Estimations of Height, weight, Hip and Waist Circumference, WH Ratio, Blood Pressure, HbA1c, serum lipid profile (Total cholesterol, High-density lipoprotein-cholesterol, and triglycerides), HbA1c were done by using commercially available kits.

2.6. DNA Extraction

High molecular weight DNA extraction was conducted using the high salting out method, following the outlined protocol. In a 1.5 ml Eppendorf tube, 500 µl of EDTA blood is collected. Tubes are then centrifuged at 12,000 rpm for 5 minutes at 4°C. The resulting final pellet is subjected to in 100 µl of 70% ethanol, and finally, the dried pellet is autoclaved with HPLC-grade water. Further quantification is done through optical density (OD) measurements at 260 nm and 280 nm were used to determine the quantity and quality of DNA.

2.7. Genotyping and amplification of DNA

The isolation of genomic DNA from blood samples was achieved by GeNetBio genomic DNA isolation kit (Korea), adhering to the manufacturer's instructions. 13. Primer sequences were designed using Beacon Designer 7.5 from PREMIER Biosoft International (USA) and then synthesized by TIB MOLBIOL (Germany). The oligonucleotide sequences were as follows:

Forward - 5'-CCGGTAAAACACAGGGAAGAT-3' and **Reverse -5'-ATTCTATCTGGGGGCAGTGAT-3'**

Subsequent genotyping was then performed through specific PCR reactions and the amplification of fragments was achieved through the utilization of restriction fragment length polymorphism (RFLP).

2.8. Statistical analysis

ANOVA and chi-squared tests were employed to analyze group differences. A two-tailed test was used to evaluate the Hardy-Weinberg equilibrium for both the patients and the controls. Assuming a dominant mode of inheritance, odds ratios (ORs) for CAD problems linked to each genotype were calculated using logistic regression analysis, along with 95% confidence intervals (CIs). In addition, logistic regression was used to determine the characteristics that, when compared to controls, increased the chance of more significance

3. Results and discussion

3.1. Genotype and allele distribution of visfatin rs61330082 polymorphism

The visfatin rs61330082 SNP genotype distributions observed were consistent with the expected frequencies from Hardy–Weinberg equilibrium across all study groups (Table 1). The findings indicated a notable increase in the occurrence of visfatin rs61330082 CC genotype in CAD cases compared to controls (51.1% vs 25.0 %) in Table 2a and 2b.An gel image also been added to bring out more clearance. In the comparison between the control group (n = 60) and the group with coronary artery disease (CAD) (n = 90), several variables were analyzed, yielding the following results the age of the case and control does not show any significance implying the age does not correlate with generating CAD. On the contrary markers such as total cholesterol (TC), TAG, HbA1c, LDL-HDL-C, VLDL-C, and SBP (mmHg) and DBP (mmHg) show significance implying that generating CAD is closely linked with elevated levels of glycosylated hemoglobin, lipid level and blood pressure.

3.2. PCR-RFLP

PCR products were analyzed on 1.8% agarose gel; the visfatin gene fragments rs61330082 were 344 bp in length. Restriction digestion of the PCR products rs61330082 with MvaI yielded (344, 233, and 111 bp) bands on 2% agarose gel, respectively. In the analysis of the rs61330082 genotype distribution between the group without coronary artery disease (CAD) (N=60) and the CAD group (N=90), notable differences were observed in the genotype distribution. Among individuals without CAD, 41.7% had the GG genotype, 33.3% had the TG genotype, and 25.0% had the CC genotype. Conversely, in the CAD group, the distribution significantly differed, with only 15.6% having the GG genotype, 33.3% having the TG genotype, and a higher proportion, 51.1%, having the CC genotype. Significance testing using the chi-square test revealed a statistically significant association between the rs61330082 genotype and CAD (χ^2=15.48, $p<0.001$). Particularly noteworthy there is a substantially higher frequency of the CC genotype in the CAD group compared to the group without CAD. Further analysis showed that individuals with the CC genotype had a significantly higher odds ratio (OR) of developing CAD compared to those with the GG genotype, with an OR of 5.48 (95% CI: 2.28-13.15). Similarly, individuals with the TG genotype also exhibited higher odds of CAD compared to GG genotype carriers, with an OR of 2.68 (95% CI: 1.13-6.36). These findings underscore the potential role of the rs61330082 genotype as a genetic risk factor for CAD development, with the CC genotype conferring a particularly elevated risk. This highlights the importance of genetic variations in predisposing individuals to CAD and underscores the need for further investigation into the mechanistic pathways underlying this association. In our present study, we aimed to develop a correlation between the frequency distribution of rs61330082 gene polymorphism of visfatin with the development of coronary artery diseases. Importantly, the non-CAD group demonstrated decreased HbA1c levels, and reduced LDL cholesterol, and triglycerides, while simultaneously exhibiting elevated total adiponectin levels.[20] Also, some of the previous studies show elevated visfatin levels which are associated with coronary artery disease (CAD) and acute coronary syndromes even after correction for classic cardiovascular risk factors such as cholesterol, smoking, hypertension, diabetes, and obesity. In addition, within the North Indian CAD patient population, we identified a correlation between the G-allele of the rs61330082 SNP and CAD. While there is a trend towards higher plasma visfatin levels in carriers of the risk G-allele, the allelic association with CAD persisted independently of plasma visfatin concentrations [21]. Notably, this same allele has previously been associated with elevated insulin and glucose levels, as well as an increased visceral/subcutaneous fat visfatin mRNA expression ratio. Visfatin exists in both intracellular and extracellular compartments, with the intracellular pool potentially offering a more accurate reflection of its impact on tissues [22]. However, the relationship between intracellular visfatin and plasma concentrations remains unclear. Also, limited literature exists on genotype-related effects on plasma visfatin levels. [23] associating the -1535C > T polymorphism (rs61330082) with heightened pro-inflammatory status and increased visfatin levels. However, this allelic association was not been replicated in a North American cohort. Disparities in cohort composition may contribute to these inconsistent genetic outcomes, with the Brazilian cohort consisting of subjects from diverse ethnic backgrounds while the North American cohort comprised exclusively of non-Hispanic white individuals. [24]. Variances in subject selection criteria, based on coronary angiography in the Brazilian cohort and clinical data in the North American cohort, further differentiate the two groups. Deviations from Hardy-Weinberg equilibrium in the North American cohort could stem from the presence of heterozygotes and a shortage of GG homozygotes among CAD patients [25]. Another study conducted on a Swedish cohort identified an association between the G allele of rs1319501, a promoter SNP in complete linkage disequilibrium with rs61330082, and myocardial infarction. Conversely, in a distinct population of Spanish rheumatoid arthritis patients, no associations were found between rs61330082 and cardiovascular events or carotid artery intima-media thickness [26-27].

Table1. Inter group Comparison of Age and Biochemical Parameters

Variable	Control group	CAD	Significance
	(n = 60)	**(n = 90)**	
Age (years)	49.56 ± 10.22	51.27 ± 10.23	p=0.317
TC (mg/dL)	162 ±18.35	251 ± 56.84,	**p<0.001***
TAG (mg/dL)	115.1±28.38	228.70 ± 47.82,	**p<0.001***
HbA1c %)	5 (4.56–6.2)	8.9 (7.90–9.96)	**p<0.001***
Dyslipidemia (%)	50 (83.3)	86 (95.6)	p=0.012
LDL-C (mg/dL)	89.22± 24.49	189.40±49.82	**p<0.001***
TC/HDL-C	3.26 ±2.7	4.79 ± 9.8,	p=0.163
HDL-C (mg/dL)	52.47±4.19	30.92±4.95,	**p<0.001***
VLDL-C (mg/dL)	33.55±5.03	48.33±8.99	**p<0.001***
SBP (mmHg)	122.67 ± 8.87	140.32 ± 21.89	**p<0.001***
DBP (mmHg)	72.67 ±10.55	85.00 ± 12.26	**p<0.001***

* p values under < 0.05 were used to evaluate statistical significance
SBP- systolic blood pressure, DBP- Diastolic blood pressure, TC- total cholesterol, TAG- Triacyl glyceride, HDL-C -High-density lipoprotein cholesterol, VLDL-C Very-low-density lipoprotein, LDL-C- low-density lipoprotein cholesterol, hemoglobin A1C

Table 2a. Association of rs61330082 with CAD

rs61330082	Without CAD		CAD group		significance	OR (95% CI)
Genotype	N=60		N=90			
	No.	%	No.	%		
GG	25	41.7	14	15.6	Ref.	-
TG	20	33.3	30	33.3	**p=0.024**	2.68 (1.13-6.36)
CC	15	25.0	46	51.1	**p<0.001***	5.48 (2.28-13.15)
*p values under <0.05 were used to evaluate statistical significance						

Table 2b. Allele frequency CAD vs control

Groups	Sample size	rs61330082 (C/T)	
		C	T
Control (Non-CAD)	**60**	62	72
CAD	**90**	135	72
CAD:- cardiovascular disease			

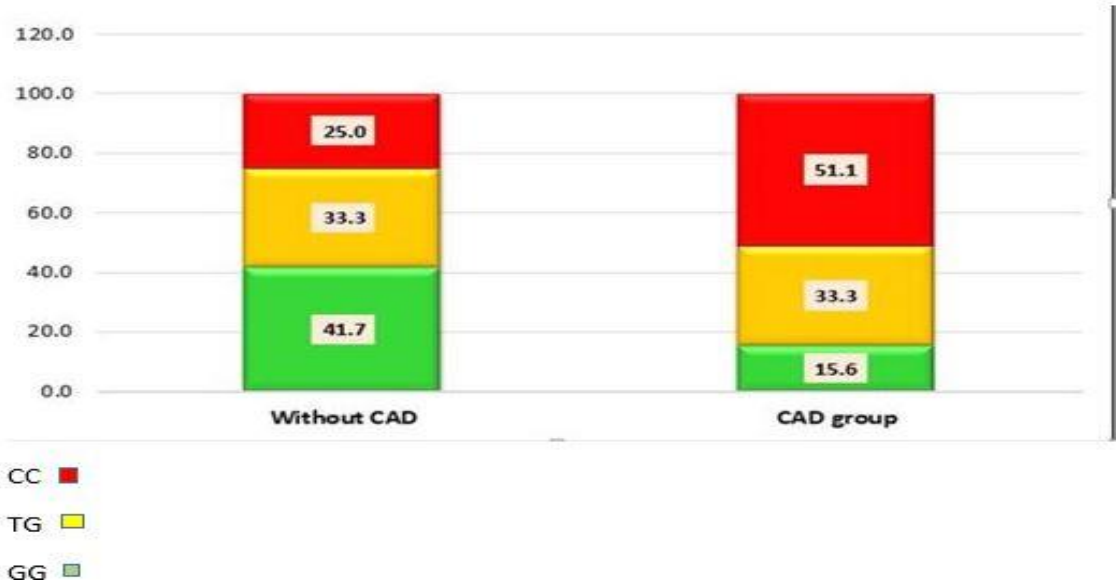

Figure 1. Comparison of rs61330082 without CAD and CAD group

The T allele of rs61330082, along with other visfatin gene SNPs, has been linked to fasting plasma insulin and glucose levels in French-Canadian individuals. The genetic underpinnings of these allelic associations remain unclear [28-29]. The evolving role of adipokines in endothelial dysfunction adds a new dimension to our understanding of the relationship between obesity particularly increased abdominal fat and CAD risk [30]. In recent years, omentin-1 and visfatin subjects have attracted a number of researchers &It has been reviewed that omentin-1 and visfatin play a significant role in vascular health and CAD [31].

4. Conclusions

Our findings indicate a potential link between visfatin rs61330082 genotype CC levels and the presence of CAD in the subjects studied. Furthermore, the observed polymorphism might be connected to CAD in specific populations. However, to establish a more comprehensive understanding, further investigations involving larger cohorts and extended haplotype analyses are warranted to validate the association of visfatin variants with coronary artery disease.

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Conflict of interest

The authors declare no conflict of interest.

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