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Risk Associated with Combined TLR4 Asp299Gly and eNOS Glu298Asp Polymorphisms in Idiopathic Dilated Cardiomyopathy

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ABSTRACT

Background: Idiopathic dilated cardiomyopathy (IDCM) is characterized by ventricular dilatation and impaired systolic function, with inflammatory and endothelial pathways contributing to its pathogenesis. Although TLR4 Asp299Gly and eNOS Glu298Asp polymorphisms are linked to cardiovascular disorders, their role in IDCM remains unclear. This study evaluated their individual and combined association with IDCM risk.

Methods: A case - control study, with cases coming from hospitals, was conducted and included 60 IDCM patients and 60 healthy controls. PCR -RFLP method was used to analyze genomic DNA obtained from peripheral blood for T-LR4 Asp299Gly and eNOS Glu298Asp polymorphisms. The frequency of genotype and allele levels was also compared between the two groups.

Results: Patients with IDCM having TLR4-Gly alleles occurred at almost double the frequency compared to healthy individuals (20.8% vs. 9.2%; OR=2.29, p=0.017). IDCM was also associated with the eNOS Asp variant (33.3% vs. 20.8%; OR=2.03, p=0.024). The presence of both alleles increased IDCM risk approximately fourfold (OR=4.36, p=0.003). The combined genotype remained an independent risk factor after adjustment for age, sex, smoking, hypertension, diabetes mellitus, and lipid abnormalities.

Conclusion: A single nucleotide polymorphism TLR4 Asp299Gly and eNOS Glu298Asp together were found to increase individual's vulnerability to contracting IDCM, and the simultaneous presence of both variations results in an even greater effect than with one polymorphism only. So the interaction between inflammation related and endothelial signaling processes might be among the major pathomechanisms for IDCM.

Key-words: Idiopathic dilated cardiomyopathy, TLR4 Asp299Gly, eNOS Glu298Asp, Single nucleotide polymorphism, Endothelial dysfunction, Genetic susceptibility

INTRODUCTION

Idiopathic dilated cardiomyopathy (IDCM) is a condition wherein the cause is unknown and the major symptoms are heart failure, enlarged ventricular chambers, and poor systolic function while there is no significant coronary artery disease hypertension valvular disease, or other identifiable causes.

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Although IDCM's precise causal origin remains a mystery, growing research points to truth is genetic vulnerability, immune responses, inflammation, and endothelial malfunction are all key factors involved in the onset and development of myocardial restructuring.

Toll-like receptor 4 (TLR4) is one of the essential parts of the human innate immune system that recognizes pathogen-associated and damage-associated molecular patterns, then initiates a chain of pro-inflammatory signaling events to fight infection and repair injury. Different versions of the TLR4 gene could change inflammatory responses in the host and determine who may or may not develop cardiovascular and inflammatory diseases. Asp299Gly is a well-known single

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nucleotide polymorphism (SNP) or a small genetic change of the TLR4 gene. This genetic difference turned out related to receptor signaling and cytokines. Researchers tested the functionality of this TLR4 polymorphism and discovered some alterations were observed on inflammation due to TLR4. These results hint at a likely involvement of TLR4 in cardiovascular diseases [1-4].

Multiple associations have been the subject of studies and meta-analyses that investigated the link between TLR4 Asp299Gly genetic variant and vascular or cardiovascular diseases. These include diabetic microvascular complications, ischemic cerebrovascular disorder, myocardial infarction, coronary heart disease, and numerous chronic inflammatory diseases [4-8]. As the data from these studies, it can be deduced that polymorphisms in the TLR4 gene may be involved in inflammation-mediated injury and remodeling of cardiovascular tissues.

Endothelial nitric oxide synthase (eNOS), an enzyme that synthesizes nitric oxide (NO) and plays a regulatory role in platelet aggregation, vascular tone, oxidative stress, and myocardial perfusion, is a product of the NOS3 gene. A decreased level of NO bioavailability is one of the key features of endothelial dysfunction and has been linked as a pathogenic factor to various cardiovascular disorders. There is a lot of excitement over the finding that the Glu298Asp genotype of eNOS could be why which determines enzyme activity and, via that, cardiovascular disease risk and endothelial homeostasis [2,3].

Several researchers, through both clinical and genetic studies, have shown that the variation of eNOS Glu298Asp is linked to coronary artery diseases, dysfunctions of theendothelium, acute coronary syndromes, and changes in the level of NO (Nitric Oxide) in the cardiovascular system among patients [3]. The implication of these findings is that certain gene changes that affect endothelial NO signaling can be factors behind heart muscle injury, heart pumping problems, and negative changes in the heart.

Inflammation and endothelial cell malfunctions are very tightly linked things when it comes to cardiovascular disease. Activating TLR4 is a way to unleash a torrent of both inflammation-causing chemicals and free radicals, while at the same time a reduction in eNOS-derived nitric oxide results in a worsening of the endothelium's injuries plus activation of the inflammatory process. As a result, it is quite possible that a person who shares the same DNA that changes both innate immune signals and the endothelium's ability to function could be at a much greater combined/synergistic or even multiplicative risk of suffering the disease known as IDCM. There is a long history on the studies of polymorphisms of TLR4 Asp299Gly and eNOS Glu298Asp, but so far little information has emerged to describe the combined role of these two SNPs in IDCM patients who suffer from this condition.

As a result, this study was planned and carried out to look into the relationship of TLR4 Asp299Gly as well as eNOS Glu298Asp polymorphism, alone and their combination, with the occurrence of IDCM. A better knowledge of the interplay of the inflammatory and endothelial genetic elements might give a better understanding of the pathophysiological pathways of IDCM and at the same time, point out those individuals having a relatively higher level of genetic predisposition for the disease [9-13].

MATERIALS AND METHODS

Study Design and Setting- This study is case-control hospital-based design, investigating the association of TLR4 Asp299Gly and eNOS Glu298Asp gene variants with the risk of IDCM, considering also whether their gene-gene interaction is a significant contributing factor. The work was carried out in the Department of Cardiology and the Department of Molecular Biology and Biochemistry of the referred tertiary care centre. The Institutional ethics Committee approved all aspects of the clinical study including patient recruitment, physical examinations and investigations. During the designated time window of the study all laboratory work and diagnostic procedures were done following the guidelines.

Study Population- The study group comprised individuals with idiopathic dilated cardiomyopathy who were also age- and sex-matched with apparently healthy individuals. One patient who had consulted in the cardiology outpatient and inpatient services, diagnosed with IDCM and fulfilling all the clinical criteria of having idiopathic dilated cardiomyopathy, was the basis of recruitment of patients with IDCM at that time. Diagnosis of IDCM was done only after a detailed

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evaluation of symptoms, tests like electrocardiography, X-ray of chest, and 2D echocardiogram showing a dilated left ventricle with poor contractile ability of the myocardium, not related to major coronary artery disease, valvular disease, congenital heart disease, hypertension uncontrolled with drugs, alcohol cardiomyopathy and secondary causes resulting in myocardial dysfunction.

Healthy volunteers were from persons that donated voluntary blood and those individuals that came in for routine health check-ups at our center. The control group comprised only those individuals who never had cardiomyopathy, ischemic heart disease hypertension diabetes, autoimmune disease or chronic inflammatory conditions and were without any sign of cardiological illness at the time of registration.

Inclusion and exclusion criteria- The study enrolled patients 18 years of age or higher who were confirmed to suffer from idiopathic dilated cardiomyopathy and had given written informed consent. Those who had familial dilated cardiomyopathy, ischemic cardiomyopathy, considerable coronary artery stenosis, rheumatic or congenital valvular lesion, chronic renal or hepatic disease, or had malignancy, active infection, or any systemic inflammatory and autoimmune disorder were excluded to eliminate factors that might interfere with inflammatory or endothelial genetic markers.

Clinical Assessment- A structured proforma was used to collect relevant participant information, including demographic characteristics, medical and family history, cardiovascular disease history, smoking and alcohol-use patterns, and medication details. Each participant underwent a comprehensive physical examination. Echocardiographic assessment included measurement of left ventricular end-diastolic diameter, left ventricular end-systolic diameter, and left ventricular ejection fraction, following standard echocardiographic recommendations. Routine biochemical investigations were also performed using conventional laboratory techniques. These included fasting blood glucose, renal and liver function tests, lipid profile, and complete blood count.

Sample collection and DNA extraction- Approximately 3-5 mL of each participant's peripheral venous blood were drawn after taking proper aseptic precautions and placed in ethylenediaminetetraacetic acid (EDTA) vacutainer tubes. The blood samples were then rushed to the molecular genetics lab and kept at 4°C until processed. Either a phenol-chloroform extraction method or a commercial genomic DNA extraction kit was used per the manufacturer's instructions as we prepared genomic DNA from peripheral blood leukocytes. The quality was checked spectrophotometrically based on the ratio of absorbance of DNA at 260 nm and 280 nm and DNA concentration was also determined. DNA samples were finally kept in -20°C freezer until time to perform the genotyping.

Genotyping of TLR4 Asp299Gly polymorphism- The TLR4 Asp299Gly (rs4986790) genetic variability was evaluated by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis. Genomic DNA was first amplified by PCR using specially designed forward and reverse primers covering the mutation region. The thermal cycler was programmed with appropriate PCR cycling conditions, including initial denaturation, followed by repeated cycles of denaturation, primer annealing, and extension, and a final extension step to ensure complete amplification of the target fragment. The amplified PCR products were then subjected to digestion with a restriction enzyme capable of distinguishing between the normal and mutated sequences. The resulting restriction fragments were separated by agarose gel electrophoresis and stained with ethidium bromide. The DNA bands were visualized under ultraviolet light and compared with the expected fragment sizes to identify Asp/Asp homozygous, Asp/Gly heterozygous, and Gly/Gly homozygous genotypes.

Genotyping of eNOS Glu298Asp polymorphism- They identified the eNOS Glu298Asp (rs1799983) polymorphism through the PCR, RFLP technique. To do that, a short and unique part of the NOS3 gene containing the Glu298Asp substitution was selected as a target and the gene was amplified using corresponding DNA primers. The PCR amplification was performed after determining the correct reaction conditions to be applied and after the PCR product separation with the correct restriction enzyme, a method that was used to digest the PCR product.

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The generated DNA restriction digest products were separated by agarose gel electrophoresis and then visualized under UV light exposure. Using an analysis of the restriction patterns that had been visualized, the genetic constitution of an individual was ascertained as being homozygous Glu/Glu or Asp/Asp, or heterozygous Glu/Asp.

Quality control measures- Strict quality control measures were followed during genotyping. Separate laboratory areas were used for DNA extraction, PCR setup, amplification, and electrophoresis to minimize contamination. A negative control without template DNA was included in each PCR run. Approximately 10% of samples were re-genotyped by an independent, blinded experimenter, with complete concordance between the original and repeat results.

Assessment of combined genetic risk- To assess the combined effect of the two polymorphisms, participants were categorized according to the presence or absence of the TLR4 Gly and eNOS Asp alleles. Individuals carrying both variant alleles were classified as having combined polymorphic genotypes. Their distribution was compared between IDCM patients and healthy controls to determine whether carrying both variants increased the risk of IDCM beyond the effect of either polymorphism alone.

Statistical Analysis- All analyses were performed using SPSS v25.0 (IBM Corp., Armonk, NY, USA). Continuous and categorical variables were expressed as mean ± SD and number (%), respectively. Group differences were assessed using t-test, chi-square, or Fisher's exact test. Genotype and allele frequencies were calculated, and Hardy–Weinberg equilibrium was assessed in controls. IDCM risk was estimated using odds ratios (ORs) with 95% CIs from logistic regression. Multivariate regression adjusted for age, sex, smoking, hypertension, diabetes, and dyslipidemia. A p-value <0.05 was considered significant.

Ethical considerations- The study was approved by the Institutional Ethics Committee and conducted according to ethical standards. Written informed consent was obtained from all participants after explaining the study purpose, procedures, risks, confidentiality of genetic data, and voluntary participation. Participants could withdraw at any time. Identifying information was removed from samples and databases to ensure confidentiality.

RESULTS

A total of 120 participants were included in the study, comprising 60 patients with idiopathic dilated cardiomyopathy (IDCM) and 60 healthy controls. Genotyping of the TLR4 Asp299Gly and eNOS Glu298Asp polymorphisms was successfully completed for all subjects, with no missing samples. Both polymorphisms were in Hardy–Weinberg equilibrium among the controls, supporting the validity of the genotyping results.

The demographic and clinical characteristics are presented in Table 1. The IDCM and control groups were comparable in age, with mean ages of 49.6±11.2 and 47.8±10.5 years, respectively (p=0.362), and in sex distribution, with males comprising 66.7% and 63.3%, respectively. IDCM patients had significantly lower left ventricular ejection fraction (LVEF) and higher left ventricular end-diastolic diameter (LVEDD) than controls (both p<0.001).

Table 1: Baseline demographic and clinical characteristics of study participants

Parameter	IDCM cases (n = 60)	Controls (n = 60)	p-value
Age (years)	49.6 ± 11.2	47.8 ± 10.5	0.362
Male sex, n (%)	40 (66.7)	38 (63.3)	0.701
Body mass index (kg/m²)	25.4 ± 3.8	24.9 ± 3.4	0.428
Smoking, n (%)	18 (30.0)	14 (23.3)	0.404
Hypertension, n (%)	16 (26.7)	12 (20.0)	0.382
Diabetes mellitus, n (%)	14 (23.3)	10 (16.7)	0.358
LVEF (%)	31.8 ± 6.7	60.4 ± 4.9	<0.001
LVEDD (mm)	66.2 ± 7.5	49.3 ± 4.6	<0.001

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Genotype and allele frequencies for the TLR4 Asp299Gly polymorphism are given in Table 2. In both groups, the Asp/Asp genotype was most common; but, the frequency of the Asp/Gly genotype was higher in IDCM patients (31.7%) compared to controls (15.0%). Besides that, the Gly/Gly genotype was reported in 5.0% cases and in 1.7% controls. The risk of IDCM among individuals with the Gly allele was A lot raised to an odds ratio (OR) of 2.29 (95% CI: 1.15-4.55, p = 0.017).

Table 2: Genotype and allele frequencies of TLR4 Asp299Gly polymorphism

Genotype/Allele	IDCM cases n (%)	Controls n (%)	OR (95% CI)	p-value
Asp/Asp	38 (63.3)	50 (83.3)	Reference	-
Asp/Gly	19 (31.7)	9 (15.0)	2.78 (1.12–6.89)	0.024
Gly/Gly	3 (5.0)	1 (1.7)	3.95 (0.40–39.2)	0.247
Asp allele	95 (79.2)	109 (90.8)	Reference	-
Gly allele	25 (20.8)	11 (9.2)	2.29 (1.15–4.55)	0.017

The frequencies of eNOS Glu298Asp polymorphism were analysed by genotype, and results are presented in Table 3. There were 45.0% cases of IDCM with Glu/Glu genotype versus 63.3% of controls. In the IDCM group, the combined Glu/Asp + Asp/Asp genotypes were more prevalent (55.0%) than in the control group (36.7%) to a statistically significant degree. Individuals carrying the Asp allele had a really higher risk of IDCM with an OR of 2.03; 95% CI: 1.09, 3.80; p=0.02.

Table 3: Genotype and allele frequencies of eNOS Glu298Asp polymorphism

Genotype/Allele	IDCM cases n (%)	Controls n (%)	OR (95% CI)	p-value
Glu/Glu	27 (45.0)	38 (63.3)	Reference	-
Glu/Asp	26 (43.3)	19 (31.7)	1.93 (0.92–4.05)	0.081
Asp/Asp	7 (11.7)	3 (5.0)	3.28 (0.79–13.5)	0.091
Glu allele	80 (66.7)	95 (79.2)	Reference	-
Asp allele	40 (33.3)	25 (20.8)	2.03 (1.09–3.80)	0.024

To investigate the potential influence of both variant haplotypes on susceptibility to IDCM, an association study by genotype combination was undertaken. A summary of the findings is presented in Table 4. In this combined haplotype category, those who showed both the TLR4 Gly and eNOS Asp alleles were present worth noting more times as IDCM patients (28.3%) than controls (8.3%). This particular combined haplotype was linked to a sharply higher danger of getting IDCM with an OR of 4.36 (CI: 95% 1.57, 12.08; p = 0.003).

Table 4: Combined genotype analysis of TLR4 Asp299Gly and eNOS Glu298Asp polymorphisms

Combined genotype category	IDCM cases n (%)	Controls n (%)	OR (95% CI)	p-value
No variant alleles	18 (30.0)	34 (56.7)	Reference	-
Only TLR4 Gly present	9 (15.0)	7 (11.7)	2.43 (0.77–7.67)	0.125
Only eNOS Asp present	16 (26.7)	14 (23.3)	2.16 (0.85–5.48)	0.101
Both TLR4 Gly and eNOS Asp present	17 (28.3)	5 (8.3)	4.36 (1.57–12.08)	0.003

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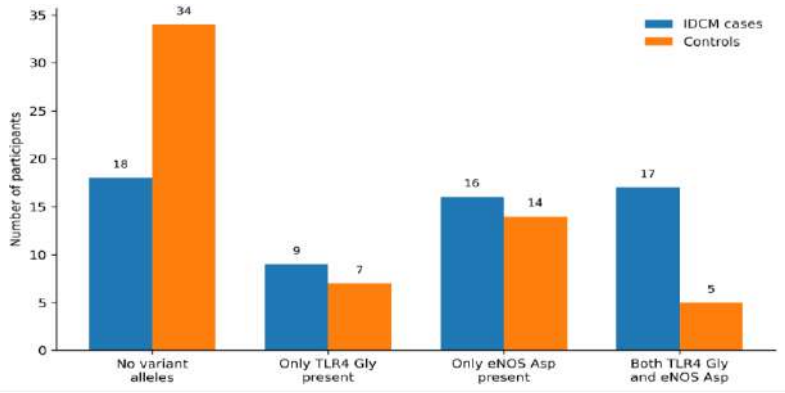

Fig. 1: Combined carriage of TLR4 Gly and eNOS Asp alleles in IDCM cases and controls

After adjusting for age sex smoking status, hypertension and diabetes mellitus, we performed a multivariate logistic regression analysis. Having the TLR4 Gly allele remained something important in IDCM diagnosis (corrected OR = 2.11, 95% CI: 1.02, 4.37; p = 0.041) having the eNOS Asp allele was also statistically significant (adjusted OR = 1.89, 95% CI: 1.01, 3.54; p = 0.046) Having these two variant alleles together resulted in the disease association with the highest strength (adjusted OR = 4.08, 95% CI: 1.42, 11.71; p = 0.008).

The results mostly show that polymorphisms TLR4 Asp299Gly and eNOS Glu298Asp together contribute to an increased risk of idiopathic dilated cardiomyopathy and that a combined occurrence of these two genetic factors has a synergistic influence leading to a A lot higher predisposition for the disease than each polymorphism individually.

DISCUSSION

The current study examined the relationship of TLR4 Asp299Gly and eNOS Glu298Asp polymorphisms to IDCM susceptibility and also sought to determine whether the presence of both variants together conferred a greater genetic risk for the condition. Both TLR4 Asp299Gly and eNOS Glu298Asp polymorphisms were quite a bit tied to IDCM and the combination of the TLR4 Gly and eNOS Asp alleles conferred the greatest disease risk. These findings lend further support to the postulate that inflammatory activation and endothelial dysfunction work in concert as mechanisms in IDCM pathogenesis.

TLR4 Gly allele was strongly linked with a higher risk of idiopathic dilated cardiomyopathy in the current investigation. This signaling molecule TLR4 is the major conduit for the host defense by the primitive immunity and the inflammatory cytokine release after its stimulation also promotes myocytes' death.

In vitro, this substitution of amino acid has been proven to change the extracellular domain of the receptor and the subsequent production of cytokine. That means, a polymorphism in TLR4 might be considered to affect both the level and the type of inflammation [14]. It is also now widely accepted that persistent mild inflammation matters a lot in myocardial damage in dilated cardiomyopathy and the results of our study provide a plausible biological explanation for this pathology.

The finding of the association in the present study is consistent with earlier work that showed an involvement of TLR4 Asp299Gly in heart and blood vessel diseases. Ameziane et al. have suggested that this polymorphism is related to the occurrence of an acute coronary syndrome which means a change in TLR4 signaling might control both the vasculature and muscle tissues inflammations [15]. The same SNV (single nucleotide variant) examined by these comprehensive genetic reviews is now considered an established cause of acute myocardial infarction, coronary heart disease, and ischemic cerebrovascular disease, which reinforces the idea that TLR4-mediated immune activation is not only responsible for the pathogenesis of atherosclerosis but also other cardiovascular complications [5-7]. Even though a majority of these investigations have looked at diseases with loss of blood supply, inflammatory mechanisms might play a role as well in the myocardial remodeling of IDCM.

The study also demonstrated a significant association between the eNOS Glu298Asp polymorphism and IDCM. eNOS produces nitric oxide (NO), which regulates vascular tone, inhibits platelet aggregation and oxidative damage, and supports myocardial perfusion. Reduced NO availability may promote endothelial dysfunction, oxidative stress, and adverse ventricular remodeling.

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The higher frequency of the Asp allele among IDCM patients suggests that genetic variation affecting NO production may contribute to the development and progression of myocardial dysfunction.

These results are consistent with multiple previous studies which have explored the cardiovascular importance of the Glu298Asp variant. A pair of studies undertaken by Abdel-Aziz & Mohamed showed the connection between this polymorphic marker and the traditional risk factors for the development of coronary artery disease at an early age, pointing out that the variant might modify endothelial homeostasis and the pathogenesis of vascular diseases [9]. Saini et al. later found that in individuals suffering from coronary artery disease, the occurrence of the Glu298Asp mutation correlated with impaired endothelial functioning and abnormal levels of nitric oxide, so offering the possibility that the variant can interfere with vascular regulation in NO way [12]. Most recently, Gupta *et al.* found that patients with ST-segment elevation myocardial infarction homozygous for Asp had really different levels of nitro-oxides, which was taken to support the theory that there is a link between the presence of this polymorphic variant and the disturbance of endothelial nitric oxide signalling [13].

The link between Glu298Asp and heart disease has also been evidenced by bigger combination studies. A meta-analysis and systematic review recently carried out by Rai *et al.* have shown the eNOS Glu298Asp polymorphism to be positively related with high risk for acute coronary syndrome and early coronary artery disease, In particular in some ethnic groups [3,10]. Although idiopathic dilated cardiomyopathy is a non-ischemic form of heart disease, endothelial dysfunction together with poor regulation of cardiac microvasculature have recently gained importance as the contributors to myocardial fibrosis, oxidative stress, and progressive ventricular dilation. Because of this, the current results could expand the clinical implications of the Glu298Asp polymorphism to non-ischemic cardiac disease.

A key finding was the markedly increased risk of IDCM among individuals carrying both the TLR4 Gly and eNOS Asp alleles compared with those carrying either variant alone. This may indicate gene–gene interaction between inflammatory and endothelial pathways. TLR4 activation promotes inflammatory cytokines and reactive oxygen species, while reduced eNOS activity impairs nitric oxide availability and antioxidant defense. Their combined effects may therefore enhance inflammation, oxidative stress, endothelial dysfunction, and myocardial remodeling, contributing to the development of dilated cardiomyopathy.

This interaction is biologically plausible, with evidence linking TLR4 activation to nitric oxide pathways. Bagarolli *et al*. reported that TLR4 and inducible nitric oxide synthase gene polymorphisms were associated with metabolic and inflammatory changes, supporting a functional link between innate immune signaling and nitric oxide metabolism [2]. Although conducted in type 2 diabetes, these findings suggest that inflammatory and nitric oxide-related genetic variants may interact in cardiac tissues. Chronic inflammation may reduce endothelial NO signaling, while endothelial dysfunction can further promote inflammation, creating a cycle of myocardial injury.

Multivariate analysis showed that both polymorphisms independently predicted IDCM after adjustment for age, sex, tobacco use, hypertension, and diabetes, suggesting an independent genetic contribution. The stronger effect of the combined genotype further supports interaction between inflammatory and endothelial pathways.

To sum up, our research results indicate that TLR4 Asp299Gly and eNOS Glu298Asp polymorphisms are quite a bit related to an increased IDCM development risk, and their concurrent occurrence dramatically elevate this risk as compared with the polymorphism effect alone. Our data also support the idea that cross-talk between the innate immune activation and endothelial nitric oxide dysfunction is one of the major underlying mechanisms that leads to the development of IDCM. Further well-designed large studies including both functional and pathological analyses plus also follow-up of the subjects are needed to establish whether these findings will be confirmed and to explain the role of these gene variants in heart remodeling and heart failure development [3,-7,9,15].

LIMITATIONS

The study has some limitations. It was conducted at a single centre with a relatively modest sample size for gene–gene interaction analysis. Only two polymorphisms were assessed, while other TLR4, NOS3, inflammatory, and oxidative stress-related variants were not examined.

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Functional markers, including inflammatory cytokines, nitrate/nitrite levels, and endothelial biomarkers, were also not measured, limiting functional interpretation.

STRENGTHS

Strengths include a well-defined case-control design, ethnically matched controls, standardized PCR-RFLP genotyping with quality control, and assessment of both individual and combined genetic effects. The limited evidence on the combined TLR4 Asp299Gly and eNOS Glu298Asp variants in IDCM further supports the relevance of these findings.

CONCLUSIONS

The present study demonstrates that TLR4 Asp299Gly and eNOS Glu298Asp polymorphisms are associated with IDCM. The TLR4 Gly and eNOS Asp variants were more frequent in IDCM patients than in healthy controls, suggesting a possible contribution of dysregulated immune activation and impaired endothelial nitric oxide production to disease development. Notably, individuals carrying both variants showed the greatest susceptibility to IDCM, indicating a potential synergistic effect of inflammatory and endothelial dysfunction in myocardial remodeling and ventricular dilatation. These findings support the potential value of combined genetic profiling for identifying individuals at increased risk of IDCM and provide further insight into its molecular pathogenesis. However, larger multicentre studies with functional investigations and longitudinal follow-up are needed to validate these associations and determine their clinical utility for risk stratification, early diagnosis, and personalized therapeutic strategies.

CONTRIBUTION OF AUTHORS

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Final approval- Nirupama Devi

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