

Association analysis between *ARG1* gene polymorphisms and idiopathic dilated cardiomyopathy

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Abstract

The current study aimed at investigate the potential association of *ARG1* polymorphisms in subjects affected by idiopathic dilated cardiomyopathy (IDCM).

We have investigated 352 subjects affected by IDCM and 352 population-matched healthy controls by exploiting case-control study. The serum lipids were quantified using spectrophotometric assay, serum arginase activity was done by enzyme colorimetric assay and 2 polymorphisms (rs2781666 and rs2781667) in *ARG1* were typed by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) to find out disease associate allele/haplotype segregating in subjects affected by IDCM.

Significantly high arginase activity was found to be associated with IDCM subjects when compared with population-matched healthy controls ($P < .0001$). The higher arginase level in IDCM subjects is negatively correlated with nitrite and nitrate ($r = -0.4687$, and $r = -0.6435$, respectively) in our study. There was a significant difference in the distribution of rs2781666 and rs2781667 genotypes of *ARG1* polymorphism in patients and controls ($P < .0001$). Similarly, variant allele T at both loci showed a significant association with the disease phenotypes ($P < .0001$). Haplotype TT at rs2781666G/T and rs2781667C/T also showed a significantly association ($P < .0001$).

To our knowledge, this is the first report to show a significant involvement of *ARG1* polymorphisms to produce IDCM symptoms in subjects originating in Pakistan.

Abbreviations: ARG1 = arginase1, BMI = body mass index, CAD = coronary artery disease, cGMP = cyclic guanosine monophosphate, CI = confidence interval, DNA = deoxyribonucleic acid, EC = enzyme commission number, ECG = echocardiography, ELISA = enzyme linked immunosorbent assay, HDL = high density lipoprotein, HUGO = human genome organization, IDCM = idiopathic dilated cardiomyopathy, LDL = low density lipoprotein, NADH = nicotinamide adenine dinucleotide, NO = nitric oxide, OR = odds ratio, PCR = polymerase chain reaction, RFLP = restriction fragment length polymorphism, SNP = single nucleotide polymorphism, TC = total cholesterol, TG = triglycerides.

Keywords: arginase, gene, polymorphism, association, idiopathic dilated cardiomyopathy

1. Introduction

Hereditary forms of dilated cardiomyopathy (DCM) account for nearly 40% of the patients, while the etiology remains unidentified in wide variety of patients registered with the

symptoms of DCM and it is considered to be idiopathic. Idiopathic form of dilated cardiomyopathy is a myocardial disease of unidentified cause with left ventricular dilation and abnormal myocardial contractility. This disease is considered as

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All the data adhere to the findings of the present study have been represented in tables and figures here in this paper.

The present study was approved by ethical review board of COMSATS University Islamabad (CUI/Bio/ERB/15/22) and an informed consent was obtained from the study participants.

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multi-factorial, with genetic factors perhaps accounting for a major factor in the disease etiology.^[11] The etiological studies had been focused to identify the genetic factors that may contribute and propagate the development of IDCM. In IDCM, the primary disease causing genes have been identified, functioning in the sarcomere,^[2] while literature shows an additional evidence regarding trait variation due to genetic and environmental predisposition, which provide an opportunity to seek modifier genes mutations enough to produce clinical symptoms.^[3] Some susceptible genes, including interleukin-23R (IL23R; NM_144701)^[4], interleukin-10 (IL10; NM_000572.3),^[5] interleukin-6 (IL6; NM_000600) and TNF-alpha (NM_007115),^[6] and nitric oxide synthase 3 (NOS3; NM_000603)^[7] are widely known to be associated with IDCM in different ethnic groups. Further studies of the single nucleotide polymorphisms (SNP) in modifier genes and their expression in relation to IDCM could be one of the factors in understanding the disease pathophysiology.

Arginase (EC:3.5.3.1) is an important enzyme in urea cycle charge for the cleavage of ammonia from protein catabolism. However, arginase is also expressed in tissues originating outside the liver and are involved in regulation of arginine and nitric oxide metabolism.^[8] Recently, the enzyme was detected in various blood cell types, including macrophages, endothelial cells, and smooth muscle cells.^[9] Experimental studies showed an increased expression of arginase1 (ARG1; NM_001244438) in cardiomyocytes.^[10] Bekpinar and colleagues^[11] have reported that ARG1 (P05089) could be a candidate marker that might increase the individual's susceptibility toward the cardiac abnormalities. High ARG1 levels is adversely correlated with left ventricular ejection fraction in patients.^[11] Consequently, arginase has gained the attention as a novel therapeutic target in heart failure patients because it seems to play a key role in the progression of the disease severity.^[12] However, little is known about genetic variations in ARG1 (HGNC: 663) and their contribution to cardiovascular disease progression/susceptibility. In particular, no data have been available on single nucleotide polymorphism in ARG1 in patients affected by IDCM. Therefore, we aimed to investigate the ARG1 polymorphisms and their association to subjects affected by IDCM.

2. Materials and methods

2.1. Arginase nomenclature

The names and symbols of gene and protein used in this manuscript follow the guidelines of "HUGO Gene Nomenclature Committee". The human gene symbol ARG1 is written in capital letters and italicized, while protein ARG1 is written in normal (standard) font type capital letters. Arginase enzyme follows the enzyme committee nomenclature represented by EC number.

2.2. Study subjects

The study presented here includes the clinical, biochemical, and genetics of the subjects registered to have IDCM. This, case-control, study was conducted from January, 2016 to February, 2018. Study protocol was designed by following 2002 Helsinki Declaration and was approved by ethical review board of COMSATS University Islamabad (CUI/Bio/ERB/15/22). A signed consent was obtained from all participants (IDCM subjects and health control) for the analyses of genetic, biochemical and

demographics. We recruited 352 subjects diagnosed with IDCM (mean age: 45.9±9.3 years). Patients with dyspnea, and palpitation were assessed by 12-lead electrocardiography (ECG), M-Mode following 2-dimensional and Doppler echocardiogram. Diagnosis of the patients was established by a senior cardiologists following the standard criteria for the diagnosis of IDCM described elsewhere.^[13] Evidence from echocardiography, including dilation of the left ventricular with >70 mm of end diastolic diameter followed by <40% left ventricular ejection or <25% fractional shortening were taken as diagnostic measurements in IDCM subjects. The subjects with known cardiovascular diseases other than IDCM like coronary heart disease (CAD) and hypertension were excluded from the study. Moreover, all patients were excluded for all other cardiovascular risk factors except age and gender in the study. Three hundred and fifty-two (352) unrelated population-matched healthy volunteers (mean age 45.5±8.1) were randomly chosen to include in the study. Control samples were taken after the evaluation by normal ECG, and echocardiogram along with no family history of cardiomyopathy and other cardiovascular diseases. Standards were followed for the reduction of bias and confounding effect by matching of the age and gender between the patients and controls.

2.3. Collection of blood samples

Blood samples were collected by standard techniques. The blood samples were left for 30 minutes at room temperature for the purpose to clot and to separate serum that was stored at -80°C for future biochemical and immunological analyses. For genotyping, blood was collected in vacutainers containing ethylenediamine tetra acetic acid (EDTA). DNA was extracted from the whole blood, using non enzymatic salting out method.^[14]

2.4. Biochemical analysis

Lipid biomarkers including total-cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were determined by commercially available kits (Gesana Production srl - Italy) with the help of SPECORD 50 PLUS chemistry analyzer (Analytik Jena, Germany). Nitric oxide quickly oxidized to nitrite and nitrate. Determination of nitrite by common method (Griess reagent) is sensitive and does not allow to measure nitrate from the sample. In the current study, serum nitrite and nitrate were measured using NADH dependent enzyme nitrate reductase using Griess reagent I and II, respectively (Thermo Fisher Scientific, Austria). The 96-well plate, including standard and samples was read at 540 nm using an AMP Diagnostics ELISA reader (Austria). Arginase activity in serum samples was assessed using a colorimetric arginase activity assay according to the manufacturer's instructions (Sigma-Aldrich, USA). Serum samples (100 µL) were filtered through 10 kDa molecular weight cutoff filter before measurement to deplete urea that could interfere with the assay. The loaded samples were diluted up to 500 µL with distilled water and were centrifuged at 14,000 × g for 30 minutes. The process was repeated with 500 µL distilled water and 14,000 × g centrifugation. The supernatant volume was adjusted up to 40 µL for further analysis. 40 µL of each sample supernatant and standard were run on 96 well plates against the sample blank and water by using an AMP Diagnostics ELISA reader (Austria). Absorbance was measured at 430 nm.

2.5. Genotyping of ARG1 single nucleotide polymorphism

Polymorphism at ARG1 rs2781666G/T was typed by the polymerase chain reaction (PCR) using forward (5'-CGGAAGGATCTTTAAGGTGCC-3') and reverse (5'-CCATGTGTCCGATGCAGTTCTG-3') primers. For rs2781667C/T polymorphism of ARG1, forward and reverse primers were 5'-TATGTGCCTCACTTGGAAGG-3' and GTGGCTGTAAGGAAATCTCG -3', respectively. The PCR reaction mixture was comprised of 3 µl of DNA (concentration), 3 µl (concentration) of the forward and reverse primer, 5 µl of 10X PCR buffer (0.2 M of (NH₄)₂SO₄, 0.8 M Tris-HCl (pH 8.8) and 0.2% w/v of Tween-20, 5 µl (25 mM) MgCl₂ (Solis BioDyne, Tartu, Estonia), 1 µl (20 mM) of dNTPs (Solis BioDyne, Tartu, Estonia), and 0.5 µl (5 U/ µl) of Taq DNA polymerase (Solis BioDyne, Tartu, Estonia) in 30.0 µl of PCR grade water. ProFlex PCR System (Applied Biosystems, USA) was used for the amplification of the DNA segments including both SNP sites. The PCR cycling conditions were initial denaturation at 95°C for 10 minutes, 40 cycles at 95°C denaturation for 30 seconds, followed annealing at 60°C for 1 minute, and extension at 72°C for 2 minutes. Final extension was carried out by extension step at 72°C for 10 minutes. The amplified fragments were resolved on 2% agarose gel by electrophoresis and analyzed on gel documentation system (Alphamager System, Proteinsimple, California USA).

TaqI restriction enzyme (Thermo Scientific, USA) was used for RFLP and the genotypes of ARG1 rs2781666G/T polymorphism were assigned by visual inspection of restriction-digested PCR product resolved on 3% agarose gel. Restriction-digestion was carried out in 0.2 mL tubes (Axygen, CA). 20 µl restriction reaction was contained of 12 µl of PCR products, 2 µl of G (10X) buffer (10 mM Tris-HCl, 50 mM NaCl, 10 mM MgCl₂, and 0.1 mg/mL BSA), 0.5 µl of TaqI enzyme followed by 5.5 µl of PCR grade water. Similarly, genotypes were determined for rs2781667C/T polymorphism using Hpy188I.

2.6. Statistical analysis

Statistical analysis was accomplished by the help of MedCalc (MEDCALC Software, Acaciaaan 22 8400 Ostend, Belgium). Analysis of the clinical and basic parameters were done by independent samples *t* test, chi-square test, and One-Way ANOVA followed by post hoc tukey test. Hardy-Weinberg was calculated with the help of Arlequin V3.0. Genotype analysis was done by using chi-square test and Fisher exact test. For statistical power calculation, OSSE online tool (<http://osse.bii.a-star.edu.sg/calculation2.php>) was used both in IDCM subjects and healthy controls. *P* values <.05 reflecting a statistically significant results.

3. Results

3.1. Basic and clinical parameters of the patients and controls

The basic variables and vital signs in the studied subjects and healthy controls are mentioned in Table 1. The disease group comprised of 79.5% male and 20.5% female, whereas, there were 80.7% men and 19.3% women in the healthy control group. There were 23.9% smokers with IDCM, while 25.5% healthy control were noted as a smoker. No significant difference was found in age, BMI, gender, and smoking between the cases and controls (*P* > .05; for each variable; Table 1). Among clinical

Table 1

Baseline and clinical characteristics of patients with IDCM and control subjects.

Characteristics	Patients (n=352)	Controls (n=352)	<i>P</i> value
Age (years)	45.9±9.3	45.5±8.1	.6520 ^{*,‡}
BMI kg/m ²	26.1±2.5	26.2±2.9	.8100 ^{*,‡}
Gender (M/F) (n)	280/72	284/68	.7060 [†]
Smoking (yes/no) (n)	84/268	90/262	.600 [†]
LVEDD (mm)	72.3±0.8	46.6±1.1	<.0001 ^{*,‡}
LVESD (mm)	56.4±1.0	29.8±0.9	<.0001 ^{*,‡}
LVFS (%)	22.0±0.8	36.0±2.1	<.0001 ^{*,‡}
LVEF (%)	36.0±2.7	63.5±3.4	<.0001 ^{*,‡}
TC (mg/dL)	178.1±17.3	178.1±18.2	.7925 ^{*,‡}
TG (mg/dL)	102.3±33.7	102.7±34.5	.9144 ^{*,‡}
LDL (mg/dL)	86.1±15.9	87.3±16.8	.4884 ^{*,‡}
HDL (mg/dL)	35.8±8.1	36.7±8.6	.2622 ^{*,‡}
Arginase (Units/L)	5.01±5.5	0.91±1.2	<.0001 ^{*,‡}
Serum nitrite (µM)	0.404±0.1	0.702±0.2	<.0001 ^{*,‡}
Serum nitrate (µM)	16.97±8.9	29.91±14.7	<.0001 ^{*,‡}

Values are given as mean±SD (standard deviation).

BMI=body mass index, HDL=high-density lipoprotein, LDL=low-density lipoprotein, LVEDD=Left ventricular end-diastolic dimension, LVEF=Left ventricular ejection fraction, LVES=Left ventricular end-systolic dimension, LVFS=Left ventricular fractional shortening, n=number of subjects, TC=total cholesterol, TG=triglycerides.

^{*} *P* values were calculated by using the independent samples *t* test.

[†] *P* values were calculated by Chi-square test.

[‡] *P* values were calculated on log-transformed scale.

variables, TC, TG, LDL, and HDL were not significantly linked with the IDCM (*P* > 0.05; for each; Table 1). However, serum arginase showed an increased activity at 5.01±5.5 units/L in IDCM when compared with (0.91±1.2 units/L) in healthy controls. Significant association was observed between serum arginase and IDCM (*P* <.0001; Table 1). Moreover, serum nitrite levels were significantly lower in IDCM as compared to controls (0.404±0.1 µM vs 0.702±0.2 µM, respectively) (*P* <.0001; Table 1). A similar trend of low concentrations of serum nitrate in IDCM (16.97±8.9 µM) when compared with (29.91±14.7 µM) controls (*P* <.0001; Table 1).

3.2. Correlation of arginase with serum nitrite and nitrate

The correlation between serum arginase activity and serum nitrite and nitrate is shown in Figure 1. Serum arginase activity was negatively correlated with nitrite (*r* = -0.4687; *P* <.0001; Fig. 1A), and nitrate (*r* = -0.6435; *P* <.0001; Fig. 1B) in IDCM.

3.3. Single nucleotide polymorphisms (SNP) in ARG1

The genotype and allelic frequencies of rs2781666G/T and rs2781667C/T for all subjects are mentioned in Table 2. The distribution of genotype for rs2781666G/T and rs2781667C/T polymorphism among the healthy subjects was in Hardy-Weinberg equilibrium ($\chi^2=2.41$; *P* =.1206 and $\chi^2=1.15$; *P* =.2835, respectively). Significant differences were present for rs2781666 and rs2781667 genotypes in IDCM versus healthy controls ($\chi^2=27.9$; *P* <.0001 and $\chi^2=22.3$; *P* <.0001, respectively, Table 2). The rs2781666-T variant allele was significantly linked with the increased risk of IDCM (OR=2.5; 95%CI=1.89-3.30; *P* <.0001; T vs G, Table 2). Moreover, ARG1 rs2781667T showed a tight linkage with IDCM (OR=2.02; 95%CI=1.51-2.71; *P* <.0001; T vs C, Table 2).

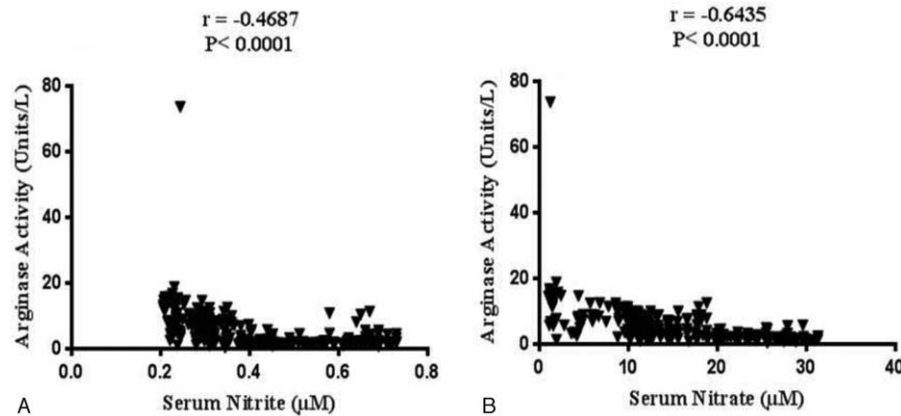


Figure 1. Scatter plots showing the relationship between arginase activity and serum nitrite and nitrate among the patients. Notes: Scatter plots show that arginase activity was negatively correlated with (A) nitrite ($P < .0001$), and (B) nitrate concentrations ($P < .0001$) in IDCM patients. The data represent mean and \pm SE and the analysis was carried out by linear correlation. (r = correlation coefficient).

3.4. Quantitative analysis of serum arginase in different genotypes of ARG1

IDCM subjects showed a varying activity of arginase in rs2781666G/T and rs2781667C/T polymorphism (Fig. 2 A and B). Subjects with the ARG1 variant genotype at rs2781666 showed higher activity of arginase than the cases with GG genotype. Carriers of the TT genotype showed maximal (mean 14.1 ± 1.5 units/L) arginase followed by carriers of GT (mean 7.8 ± 6.5 units/L) and GG (2.02 ± 1.2 units/L) genotype, respectively. There was significant difference in arginase activity among the carriers of different genotype of rs2781666G/T ($P < .0001$ for GG vs GT; GG vs TT, and GT vs TT, Fig. 2A). Similarly, subjects with the variant genotype of rs2781667 showed higher activity of arginase than the cases with the CC genotype. Carriers of the TT genotype showed maximal (mean 13.4 ± 3.1 units/L) arginase activity followed by carriers of CT (mean 7.6 ± 3.1 units/L) and CC (2.2 ± 1.3 units/L) genotype, respectively (Fig. 2B). A significant association was observed for arginase activity in

subjects with IDCM carrying mutated genotypes of rs2781667 ($P < .0001$ for CC vs CT; CC vs TT, and CT vs TT, Fig. 2B).

3.5. Quantitative analysis of serum nitrite and nitrate in different genotypes of ARG1

Patients with IDCM showed a varying concentrations of serum nitrite and nitrate in rs2781666 and rs2781667 polymorphism followed by post hoc tukey analysis (Fig. 3). Subjects with variant TT of rs2781666 showed a minimal level of nitrite ($0.224 \pm 0.017 \mu\text{M}$) followed by patients with GT ($0.28 \pm 0.06 \mu\text{M}$) and GG genotype ($0.494 \pm 0.13 \mu\text{M}$) ($P < .0001$ for GG vs GT; GG vs TT, and GT vs TT, Fig. 3A). Nitrate concentrations were significantly lower in cases having rs2781666-TT ($1.65 \pm 1.2 \mu\text{M}$) followed by carriers of GT ($10.88 \pm 4.7 \mu\text{M}$) and GG ($22.7 \pm 6.2 \mu\text{M}$) genotype ($P < .0001$ for GG vs GT; GG vs TT, and GT vs TT, Fig. 3B). Similarly, Subjects with the CT and TT genotype of rs2781667 showed lower concentrations of nitrite than the cases with CC genotype. Carriers of the TT genotype showed minimal (mean $0.25 \pm 0.1 \mu\text{M}$) nitrite followed by cases having heterozygous CT (mean $0.32 \pm 0.1 \mu\text{M}$) and CC (mean $0.46 \pm 0.1 \mu\text{M}$) genotype, respectively ($P < .0001$ for CC vs CT; CC vs TT, and CT vs TT, Fig. 3C). Nitrate concentrations were significantly lower in IDCM segregating rs2781667 TT ($2.3 \pm 3.7 \mu\text{M}$) and CT ($11.2 \pm 6.1 \mu\text{M}$) genotype compared with carriers of CC ($21.6 \pm 6.9 \mu\text{M}$) genotype ($P < .0001$ for CC vs CT; CC vs TT, and CT vs TT, Fig. 3D).

3.6. ARG1 rs2781666/rs2781667 haplotype

The haplotype of ARG1 polymorphism in rs2781666/rs2781667 segregated in IDCM subjects and controls are given in Table 3. The G-T and T-C haplotype were not significantly different between the patients and controls ($P > .05$, respectively). Haplotype T-T of rs2781666/rs2781667 showed a significantly higher frequency (19.6%) in IDCM when compared with healthy controls having 8.4% (OR = 2.6; $P < .0001$; Table 3).

3.7. Power of the study

Statistical power calculation for genotype frequencies indicated 97.6% power for ARG1 rs2781666G/T polymorphism on the

Table 2

Genotype and allele distribution of ARG1 rs2781666G/T and rs2781667C/T polymorphisms in patients with IDCM and healthy control subjects.

	IDCM Patients (n = 352)	Controls (n = 352)	Chi square (P value)
ARG1 rs2781666 G/T polymorphism			
GG genotype	202 (57.4%)	266 (75.6%)	$\chi^2 = 27.9$ $P < .0001^*$
GT genotype	122 (34.6%)	76 (21.6%)	
TT genotype	28 (8.0%)	10 (2.8%)	
G allele	526 (74.7%)	608 (86.4%)	OR = 2.5 95%CI = 1.89–3.30 $P < .0001^\dagger$ (T vs G)
T allele	208 (25.3%)	96 (13.6%)	
ARG1 rs2781667 C/T polymorphism			
CC genotype	215 (61.6%)	270 (76.0%)	$\chi^2 = 22.3$ $P < .0001^*$
CT genotype	113 (33.8%)	74 (22.4%)	
TT genotype	24 (4.6%)	8 (1.6%)	
C allele	543 (77.1%)	614 (87.2%)	OR = 2.02 95%CI = 1.51–2.71 $P < .0001^\dagger$ (T vs C)
T allele	161 (22.9%)	90 (12.8%)	

Values are given as the number of alleles and genotypes with percentages in parenthesis.

* P values were calculated by chi-square test.

† P values were calculated by Fisher exact test.

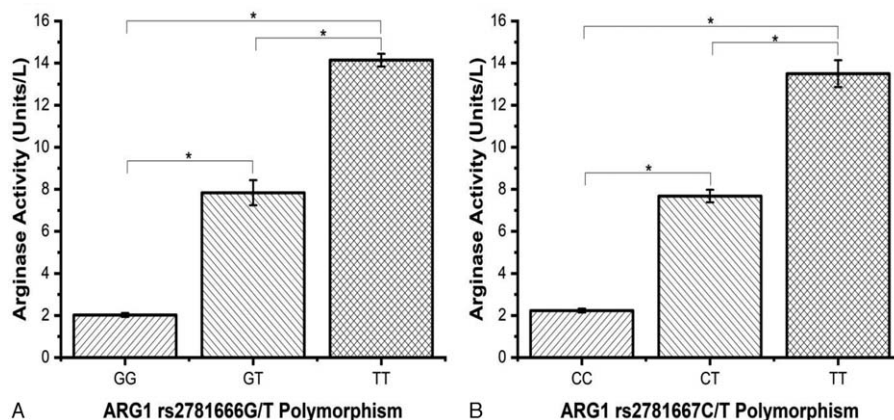


Figure 2. Association between *ARG1* polymorphisms and serum arginase activity in patients with IDCM. The data represent mean ± SE. Serum arginase activity among the carriers of different genotype of (A) rs2781666G/T, **P* < .0001 for GG vs GT, GG vs TT, and GT vs TT, respectively, and (B) 2781667C/T, **P* < .0001 for CC vs CT; CC vs TT; and CT vs TT, respectively. Analysis was done by 1-way-analysis of variance followed by post hoc tukey test.

basis of observed minor allele frequencies in patients and controls. Similarly, the detected frequencies of the minor allele T at *ARG1* rs2781667C/T in patients and controls showed 94.0% power in the study.

4. Discussion

Nitric oxide is a widespread cell signaling molecule generated by endothelial nitric oxide synthase (eNOS) and playing an important role in vascular tone. Impairment of NO synthesis has been recently detected in the development of cardiovascular

disease, including coronary heart disease and essential hypertension. The disease pathophysiology involves a decrease in the concentration of NO by competitive use of L-arginine as a substrate by arginase.^[15,16] In vascular biology, it has been established that NO modulates cardiac contractility by inducing increase in cGMP via soluble guanylyl cyclase. Afterward, cGMP in turn triggers the cardiac contractility via the activation of protein kinase G and direct activation of calcium cycling proteins.^[15] It is suggested that up-regulation of arginase can deplete the cGMP and NO synthesis and resulting with effect on basal contractility of cardiomyocytes.^[16] Therefore, reduced

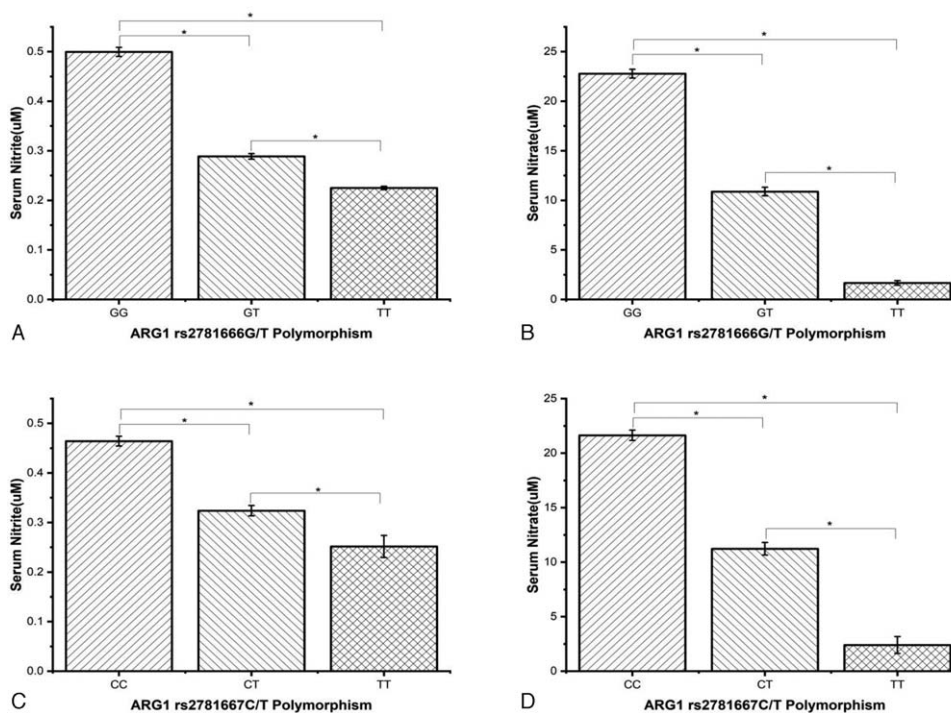


Figure 3. Association among *ARG1* polymorphisms and serum nitrite and nitrate concentrations in patients with IDCM. The data represent mean ± SE. Serum nitrite among the carriers of different genotype of (A) rs2781666G/T, and (C) 2781667C/T. Serum nitrate among the carriers of different genotype of (B) rs2781666G/T and (D) 2781667C/T. Analysis was done by 1-way-analysis of variance followed by post hoc tukey test. **P* < .0001 for each comparison.

Table 3
Distribution of the *ARG1* haplotypes at rs2781666 and rs2781667 in IDCM and healthy control subjects, respectively.

Haplotypes rs278666/rs2781667	Patients	Healthy controls	χ^2	OR (95%CI)	* P Value
G-C	499 (70.8%)	578 (82.1%)		Reference	
G-T	28 (4.0%)	29 (4.1%)	0.16	1.1 (0.63–1.96)	.6810
T-C	40 (5.6%)	38 (5.4%)	0.71	1.2 (0.75–1.97)	.3970
T-T	137 (19.6%)	59 (8.4%)	36.8	2.6 (1.91–3.78)	<.0001

Percentages shown in parenthesis: n=number of subjects, χ^2 =Chi-square, OR = odds ratio, 95% CI=95% confidence interval.

* P value was calculated by Pearson Chi-Square test.

expression of arginase can improve the generation of NO and cGMP in rat cardiomyocytes.^[17] The concept of high arginase activity and their implication in cardiac function is supported by the association of the *ARG1* with high risk in heart failure patients. Arginase was strongly associated with disease phenotype in severe heart failure patients, compared to patients having mild heart failure.^[12] In the current study, we have assessed an association of serum arginase and *ARG1* functional polymorphism at rs2781666 and rs2781667 with IDCM in patients. We found higher arginase activities in IDCM patients and were negatively correlated with serum nitrite and nitrate. Recently, increased levels of serum *ARG1* were found in patients affected by myocardial infarction than in controls. Concordantly, serum arginase has been reported to be linked with reduced ejection fraction in MI patients.^[11] In the current study, it is rational to consider that higher activity of arginase may be involved in pathogenesis of IDCM in patients from Pakistan.

Gene encoding arginase-1 is located on 6q23 consisting of 8 exons with a genomic length of 11.5 kb.^[18] Recently, linked data from the National Center for Biotechnology Information/SNP and HapMap project, a total of 10 SNPs spread out the *ARG1* gene were selected by their locus and were studied in association with cardiovascular disease in different ethnic groups.^[19] Ethnicity is well known to affect the frequencies of SNP and their effects on the disease; therefore, significant progress has been achieved in understanding an association of these tagging SNPs with cardiac disease phenotype through genome-wide association studies.^[20] In previous and current studies, genetic variations in *ARG1* and their association with IDCM has not been studied yet. Therefore, it was of great chance to study that whether single nucleotide polymorphism at *ARG1* rs2781666 and rs2781667, in *ARG1* confer the risk of IDCM. Interestingly, we revealed a significant association between the said SNPs and IDCM. The minor allele at rs2781666 and rs2781667 showed an increase risk IDCM in patients. Contradictions have been reported in the literature about rs278166G/T polymorphism and their association with the cardiovascular disease phenotypes. Sediri and colleagues showed a significant link of variant genotype and allele at rs2781666 with myocardial infarction in patients from a Tunisian male population.^[21] Similarly, reports showed a significant association of *ARG1* rs2781666G/T polymorphism in cardiovascular disease patients,^[19,22] while some have reported a non-significant link with the cardiovascular disease from the Algerian population.^[23] The control subjects in our study showed rs2781666G allele frequency at 75.6%, which is comparable to a case control study of the population of France at (81.7%) and Tunisia at 78.3%.^[19,23] The observed allele G frequency from the current study was in close range reported

from other population. However, this contrariety between the reports have been always associated with difference in ethnic origin, disease phenotype and complex genetic-environment interaction.

Little is known about the *ARG1* rs2781667C/T polymorphism and their association with cardiovascular disease. Therefore, we chose this tagging SNP for the association analysis because it is supposed that T allele creates a binding site for the transcriptional factor NF κ B.^[23] Negative association has been reported between rs2781667C/T polymorphism and myocardial infarction among the patients from a French population.^[19] Another study showed a significant association of rs2781667 variant genotype with systolic blood pressure, but not with diastolic blood pressure.^[22,23] Interestingly, we showed a significant level of association between variant allele T at rs2781667 and IDCM. The novel finding of increased risk of variant allele T at rs2781667 with IDCM must be replicated to validate the pathogenic role of rs2781667C/T polymorphism in the development of IDCM in patients.

Current report showed that *ARG1* rs2781666TT and rs2781667TT were significantly associated with higher activity of arginase, lower nitrite and nitrate in IDCM cases. Little is known about the functional activities of different genotypes in *ARG1* and their relation with the disease. Lacchini and colleagues have reported that the single nucleotide variations in the *ARG1* were associated with reduced levels of arginase activities in patients with clinical erectile dysfunction.^[24] The inconsistency between the current and prior report might be of different phenotypes as the selective up-regulation of arginase-1 has been shown in patients with cardiovascular disease.^[11] In the current study, we showed that variant genotypes at *ARG1* are significantly linked with lower nitrite and nitrate in cases with IDCM. The observations have been supported by similar association of *ARG1* polymorphisms with low levels of serum nitrate and nitrite in patients with essential hypertension.^[22] Further, we observed that T-T haplotype of the *ARG1* at rs2781666 and rs2781667 was significantly associated with the disease in the study population. Similarly, Dumont and colleagues showed 1.8 fold higher risk of variant haplotype at *ARG1* in patients with myocardial infarction.^[19] Therefore, from the previous literature as well as the current study, it can be assumed that the SNP in *ARG1* may confer the risk in patients with IDCM, which may result in enhanced activity of arginase, low nitrite and nitrate in circulation that could have implications in the disease pathophysiology. However, these observations from the current study may cautiously lead to; more comprehensive and larger studies, that would be called to replicate the novel findings from other ethnic groups.

Beside the strength of our study by large sample size with >80% power, there are some limitations. The study is restricted to 2 SNPs in *ARG1* locus. Another limitation of the study is that we have raised activity of arginase in IDCM subject having TT genotypes that do not have clear functional consequences, and that is why there are some inconsistency between us and the other investigators.^[24] The arginase is negatively correlated with nitric oxide metabolites in patients. Moreover, we did not correlate the arginase with clinical findings in patients with IDCM. A comprehensive study may be of great interest to validate the preliminary findings of a novel association of *ARG1* variants and enhanced arginase in patients with IDCM. Moreover, we cannot exclude the interference of ACE Inhibitors used by patients, which may have some effect on the improvement of nitrate and nitrite

concentration. The patients were on oral treatment of captopril, suggested for improvement in heart function. Studies have shown that ACE Inhibitors have a significant role in normalization of nitrate and nitrite.^[22] However, the ACE Inhibitors therapy did not show any effect on the concentration of nitrate and nitrite in patients. Therefore, we recommend that a controlled study must be conducted for the better understanding of nitrate therapy in patients with dilated cardiomyopathy. Data of the present study is promising and one can hope to trial in the clinics after replicating the same work on large number of samples by considering age, gender and other anthropomorphic parameters.

5. Conclusion

This is the first investigation to demonstrate a significant association between *ARG1* rs2781666G/T and rs2781667C/T polymorphism and IDCM in a case-control study. The novel observation of the variant genotype in patients modulates arginase and nitric oxide and needs to be confirmed from other populations.

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