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Peroxisome Proliferator Activated Receptor Gamma (PPARγ) Pro12Ala Gene Polymorphism and Oxidative Stress in Menopausal Women with Cardiovascular Disease from North Indian Population of Punjab

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ABSTRACT The present paper investigated the association of *PPARγ* Pro12Ala polymorphism with cardiovascular disease (CVD) and oxidative stress (OS) in menopausal women from North Indian population of Punjab. 265 diagnosed CVD women as cases and 258 women with no evidence of heart disease as controls were screened for lipid profile, serum malondialdehyde (MDA), serum LDL carbonyl protein and serum superoxide dismutase (SOD). Genotyping was performed by ARMS-PCR method. Significant differences ($p<0.05$) in the levels of hypertension (HTN), lipid profile, MDA, LDL carbonyl protein and SOD were observed between women with and without CVD. However, no significant difference ($p>0.05$) in the distribution of genotype and allele frequency was observed. Further in logistic regression analysis, hypertension (HTN), high density lipoprotein-cholesterol (HDL-C) and OS variables were significantly correlated with CVD but, Pro12Ala was not observed to be an independent predictor of CVD. The paper depicts *PPARγ* (Pro12Ala) polymorphism is not associated with the risk of CVD as such but, significant rise in LDL carbonyl protein in CC homozygotes with CVD implies OS. Both OS and *PPARγ* also act as early indicator of cardiovascular events. Further, studies on association between Pro12Ala polymorphism and CVD should be carried out on a larger population of Punjab.

INTRODUCTION

Cardiovascular disease (CVD) is a disease of heart or blood vessels which commonly includes myocardial ischemia, disturbance of the contraction and/or relaxation of the myocardium, obstruction to the blood flow or an abnormal cardiac rhythm or rate (Loscalzo 2012). It is a multifactorial disease hence, both genetic and environmental factors (Vinukonda et al. 2009; Arnett 2013) contribute to its etiology. Associations of various candidate genes with predisposition to CVD have been extensively reported and polymorphism at multiple genes has been coupled with differential effects of lipid metabolism with this disease. Regarding the susceptibility of genetic markers considerable heterogeneity exists among the studies which may be because of experimental limitations, the intrinsic complexity of the phenotypes and interactions with the environmental factors (Ordovas 2009). Among the reported genes, the most important candidate is peroxisome proliferator-activated

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receptor (*PPAR*) gene. *PPARs* is a family of ligand activated transcription factors belonging to nuclear receptor superfamily (Kliewer and Wilson 1998), which plays a central role in regulating lipid and glucose metabolism (Liu et al. 2016). *PPARγ* signaling pathways affect both cellular and systemic lipid metabolism and has been reported to be associated with various metabolic diseases like diabetes (Liu et al. 2010), obesity (Lwow et al. 2013), dyslipidemia (Li et al. 2015), insulin resistance, metabolic syndrome (Rhee et al. 2006), coronary artery disease (Wu et al. 2012) and cardiovascular disease (Azhar 2010; Wang et al. 2012; Li et al. 2015). Three isoforms α, β and γ of *PPAR* have been identified. *PPARα* is mainly expressed in brown fat, heart, liver and skeletal muscles. *PPARβ/δ* is ubiquitously expressed and *PPARγ* is expressed in adipose tissue, liver, macrophage, cardiac muscles and skeletal muscles Chich et al. (2007). Because of different transcript, translation and tissue distribution, each mRNA transcript of *PPARγ* has different biological functions in a variety of organs and cells (Azhar 2010). *PPARγ* has an additional 28 N terminal amino acid, encoded by exon B located exactly at the differentiative 5' end of the mRNA molecule Tontonoz et al. (1994). Within exon B, the polymorphic site C→G (rs1801282) is found which was first identified by Yen et al. (1997). This nucleotide substitution results in proline to alanine amino acid substitution at codon 12 of *PPARγ* (Pro12Ala), the most commonly studied polymorphism of *PPAR*. Though pathophysiological role of *PPARγ* in cardiovascular disease is perceptible, there are conflicting results regarding association of CVD with *PPARγ* Pro12Ala polymorphism (Rhee et al. 2007; Zafarmand et al. 2008; Galgani et al. 2010; Wu et al. 2012; Wang et al. 2015). Oxidative stress (OS) is a condition where pro-oxidants like superoxide anion ($O_2^{\cdot-}$), hydroxyl ion (OH•), hydrogen peroxide (H_2O_2) also known as reactive oxygen species overwhelm antioxidant capacity of the body resulting in serious cell damage. Enhanced OS strongly modifies circulating lipids and lipoproteins leading to alteration in their biological properties (Florens 2016). Concentration and chemical structure of estrogen hormone has a remarkable effect on OS in the body. At high concentration estrogen metabolites tend to produce antioxidant effect by inhibiting the 8-hydoxylation of guanine DNA bases which show a prooxidant effect (Markides et al. 1998). However, at low concentration especially when the structure contains a catechol it has a prooxidant like effects. These catechol metabolites cause breaks in DNA strands, formation of DNA adducts and oxidation of bases (Wang et al. 2010). Malondialdehyde (MDA), a natural product of lipid peroxidation, binds with proteins and nucleic acids forming DNA adducts such as pyrimidopurinone deoxyguanosine (M1dG) in human beings (Bono et al. 2010). In humans, these adducts may contribute significantly to cancer linked to lifestyle and dietary factors (Marnett 1999). Menopause, a form of reproductive aging is marked by many hormonal variations such as decline in the estrogen levels which cause imbalance in the oxidative processes and also increase the risk of metabolic diseases therefore, menopausal women were the choice for the subjects in the present study. Cardiovascular health awareness among menopausal women is extremely important for primary prevention of the disease. Investigating oxidative stress parameters and genetic markers other than the routine biochemistry investigations may play a pivotal role in diagnostic process of CVD at the earlier stage.

Objectives

Taking into account the paucity of the data on the association of Pro12Ala polymorphism of *PPARγ* gene with CVD in menopausal women from North Indian Punjabi population, the present study has been undertaken. Since, Pro12Ala (C→G) substitution point AF1 is a ligand independent activation function domain, the presence of g allele, as revealed by many studies (Deeb et al. 1998; Schneider et al. 2002; Yamamoto et al. 2002), is related to diminished activity of the g allele of *PPARγ* for PPRE (peroxisome proliferator response element) in target genes. This results in decrease in their expression level. These mutations may result in either gain-of-function or loss-of-function. On the other hand, oxLDL and long chain fatty acids are active ligands for *PPARγ* activation, and ligand binding results in a conformational change in the receptor. This leads to activation of the gene expression. Taking into account the role of *PPARγ* in regulating lipid metabolism which may influence the risk of cardiovascular events, the researchers' interest led to explore the association of *PPARγ* in context to OS for CVD. Wheth-

er this polymorphism influences the risk of CVD independently is yet to be explored. To the best of the researchers' knowledge this is the first study in India to evaluate the association of Pro12Ala polymorphism with oxidative stress in menopausal women of Punjab with CVD.

MATERIAL AND METHODS

Study Design and Selection of Patients

In the present study a cohort of 523 menopausal women from Northern Indian Punjabi population was recruited between August 2011 and February 2014. The cases comprised of 265 menopausal women (mean age, 44±4 years) suffering from CVD from in-patient and out-patient department of Medicine of Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar. The control group from the general population consisted of 258 menopausal women (mean age, 45±4 years) having no evidence of heart disease or any past history of the disease. At enrolment, written, informed consent, detailed clinical history including general, physical and systemic examination was recorded for all the subjects in the form of questionnaire. The protocol was approved by the Institutional ethics committee. The incidence of heart disease increases after both natural and surgical menopause therefore the researchers included both naturally and surgically induced menopausal women. The diagnosis of the heart disease was made by the physician on the basis of clinical symptoms, supportive by documented ECG findings, history of the patient and angiography if required for the disease (O' Rourke and Braunwald 2008). Both the groups were matched for the age at menopause. Women suffering from any chronic disease, acute infections, renal disease, were excluded. Women on hormonal therapy, any antioxidant supplements and lipid lowering drugs at the time of sampling were also excluded from the study for both the groups. For candidate gene association studies, case-control approach is promising for identifying the association of allele/genotype with the disease phenotype. Hence this approach was used for the study design.

Biochemical Analysis

Venous blood sample of all the subjects was collected after 12-hr overnight fasting under aseptic conditions. Serum Total Cholesterol, Triglycerides and HDL-C were performed using ERBA kits (Transasia Bio-medicals Ltd., Solan, India), VLDL-C was calculated by the formula Triglycerides/5 and LDL-C by the formula of Friedewald et al. (1972). Serum Malondialdehyde (MDA) by the method of Buege and Aust (1978). LDL carbonyl protein by Yan et al. (1995) and superoxide dismutase (SOD) by the method of Nandi and Chatterjee (1998).

Genotyping

Genomic DNA was isolated from intravenous blood by inorganic method of DNA isolation (Miller et al. 1988). Genotyping of Pro12Ala in the *PPARγ* gene was done by amplification refractory mutation system polymerase chain reaction (ARMS PCR) method as previously described (Atug et al. 2008). The amplification was carried out in an Eppendorf mastercycler gradient thermal cycler. Conditions for PCR included denaturation at 95°C for 3 min, denaturation at 94°C for 45 sec, annealing at 62°C for 45 sec, primer extension at 72°C for 45 sec followed by final extension at 72°C for 3 min. PCR amplicons were then subjected to electrophoresis on 2 percent agarose gel stained with ethidium bromide.

Statistical Analysis

The statistical analysis was performed using Statistical Package for Social Science program (version 16.0; SPSS Inc., Chicago, IL). For the calculation of sample size and power of the study initially, data from 50 cases and 50 controls were analyzed for obtaining OR. It was later used for the calculation of sample size in the CaTS power calculator (Skol et al. 2006) with the population risk of 12.5 percent for CVD as per the reference Krishnan et al. (2016). 80 percent power was detected with the present sample size. For the analysis of MDA, LDL carbonyl content and SOD levels no established cut-offs were available in the literature therefore, the cut -offs were calculated by ROC (Receiver Operating Characteristic) curves taking sensitivity ranging from 85-90 percent and specificity ranging from 60-85 percent (Fig. 1). The continuous data are expressed as mean ± standard deviation (SD). Student's t-test was used to calculate the mean difference between the continuous variables of the two groups. The significant cut-off has been taken as $p<0.004$ after Bonferroni cor-

rection for multiple comparisons. For data not corrected for Bonferroni, $p<0.05$ is considered to be significant. Categorical variables, frequencies of genotype (scored by gene counting method) and allele in cases and controls were compared using Chi-square (χ^2) test. The genotype distribution analyzed by χ^2 test for both the groups was in the Hardy-Weinberg Equilibrium (HWE). Association of *PPARγ* SNP genotypes with HTN and dyslipidemia was analyzed by chi-square test. Logistic regression analysis was performed to determine the risk factors of CVD after adjusting for confounding factors. Odds Ratio (OR) at 95 percent confidence interval (CI) was calculated indicating the relative odds of the presence of CVD due to the presence of associated risk factors. Since the number of homozygous women for minor allele was small in both cases and controls, the researchers combined CG + GG as a single g allele carrier group for Pro12Ala polymorphism to improve the power of the study.

RESULTS

The comparison of baseline demographic and clinical profile among menopausal women with and without CVD is summarized in (Table 1). Significant increase ($p<0.05$) in the levels of Total Cholesterol, Triglycerides, VLDL-C, LDL-C and a significant decrease ($p<0.05$) in the levels of HDL-C were observed in menopausal women with CVD as compared to menopausal women without CVD. Similarly, a marked rise ($p<0.05$) in the pro-oxidants level such as MDA and LDL carbonyl content and a sharp decline ($p<0.05$) in the antioxidant levels of SOD was observed in CVD group as compared to group without CVD. Percentage occurrence of dyslipidemia and hypertension was significantly more ($p<0.05$) in women with CVD as compared to women without CVD. On the contrary, obesity was found to be more prevalent in subjects without CVD as compared to subjects with CVD. But, the difference was not found to be statistically significant ($p>0.05$) between the two groups. Table 2 illustrates the distribution of genotype and allele frequencies of Pro12Ala polymorphism in *PPARγ* gene. No significant difference ($p>0.05$) in the frequency distribution of genotypes ($p=0.979$) and alleles ($p=0.724$) was observed among the two groups. In Table 3 comparison of lipid profile, pro-oxidants and antioxidant based on *PPARγ* SNP genotype among menopausal women with and without CVD revealed, insignificant difference ($p>0.05$) in the mean levels of TC, TG, VLDL-C, LDL-C, HDL-C, and SOD between homozygous c allele carriers and combined g allele carriers in both cases and control group. Though, increased mean levels of pro-oxidant MDA was found in CC homozygotes as compared to combined g allele carriers but the difference was not found to be statistically significant ($p>0.05$). Interestingly, comparison between CC homozygotes and combined g allele carriers within the CVD group revealed significant difference ($p<0.05$) in the levels of pro-oxidant - LDL carbonyl content. Whereas, in-

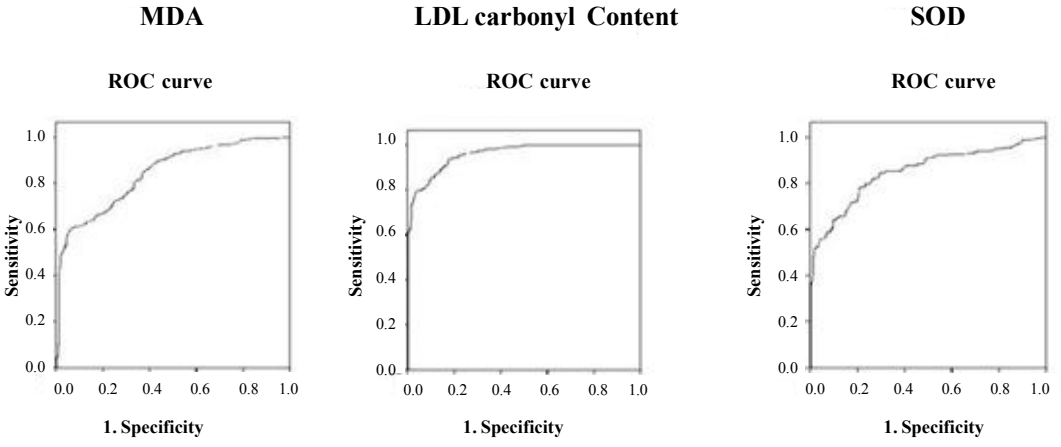

Fig.1. ROC curve of MDA, LDL, carbonyl Protcin and SOD

Table 1: Baseline demographic and clinical profile of menopausal women with and without CVD

Variables	*Subjects*		*p value*
	Menopausal women with CVD (n=265)	*Menopausal women without CVD (n=258)*	
Menopausal age (years)	44.95 ± 4.39	45.17 ± 4.65	0.579
Obesity (%)	53.6	62.8	0.480
Dyslipidemia (%)	62.6	45.0	0.012*
Hypertension (%)	69.0	46.1	0.012*
Total Cholesterol (mg/dl)	224.14 ± 44.29	204.30 ± 32.43	0.012*
Triglycerides (mg/dl)	188.21 ± 52.24	163.14 ± 35.83	0.012*
VLDL-C(mg/dl)	37.39 ± 10.49	32.32 ± 7.20	0.012*
LDL-C (mg/dl)	151.18 ± 40.12	125.44 ± 26.79	0.012*
HDL-C (mg/dl)	35.56 ± 7.13	46.54 ± 6.48	0.012*
MDA(nmoles/ml)	2.18 ± 0.60	1.46 ± 0.40	0.012*
LDL carbonylcontent (nmoles/ml)	24.87 ± 7.00	11.30 ± 3.53	0.012*
SOD (units/ml)	3.44 ± 2.45	7.00 ± 2.79	0.012*

Values expressed as Mean ± SD
*Significant p value after bonferroni correction (the adjusted p value at 0.05 significance level is 0.004)
Comparison of risk factors between menopausal women with CVD and without CVD was done using χ^2 (categorical variables). Comparison of lipid profile, pro-oxidants and antioxidant were done using independent t test (continuous variables). VLDL- C: very low density lipoprotein-cholesterol, LDL-C: low density lipoprotein-cholesterol, HDL-C: high density lipoprotein- cholesterol, MDA: malondialdehyde, SOD: superoxide dismutase.

Table 2: Genotype and allele frequency distribution of PPARγ (Pro12Ala) polymorphism in menopausal women with and without CVD

Genotype/Allele		*Subjects*		*p value*
		Menopausal women with CVD (n=265)	*Menopausal women without CVD (n=258)*	
Genotype	CC	201(75.8)	192 (74.4)	0.979
	CG	58 (21.9)	59 (22.9)	
	GG	06 (2.3)	07 (2.7)	
Allele	c	460 86.8)	443 (86.0)	0.724
	g	70 (13.2)	73 (14.0)	

p<0.05 is considered statistically significant

Table 3: Lipid profile, pro-oxidants and antioxidant levels among menopausal women based on PPARγ SNP genotypes

Variables	*Menopausal women with CVD [mean ± SD]*			*Menopausal women without CVD [mean ± SD]*		
	CC (n=201)	*CG +GG (n=64)*	*p value*	*CC (n=192)*	*CG+GG (n=66)*	*p value*
TC (mg/dl)	223.96 ± 45.19	224.73 ± 41.68	0.903	204.15 ± 32.78	204.73 ± 31.65	0.901
TG (mg/dl)	187.60 ± 53.07	190.12 ± 49.92	0.737	163.26 ± 36.98	162.79 ± 32.52	0.927
VLDL-C (mg/dl)	37.26 ± 10.67	37.81 ± 9.96	0.714	32.34 ± 7.44	32.27 ± 6.50	0.949
LDL-C (mg/dl)	151.02 ± 40.87	151.66 ± 37.95	0.913	125.18 ± 27.35	126.20 ± 25.26	0.790
HDL-C (mg/dl)	35.65 ± 7.20	35.27 ± 6.94	0.707	46.64 ± 6.75	46.26 ± 5.64	0.684
MDA(nmoles/ml)	2.21 ± 0.62	2.08 ± 0.52	0.156	1.48 ± 0.42	1.42 ± 0.33	0.285
LDL carbony content (nmoles/ml)	25.95 ± 6.66	21.47 ± 6.99	0.001*	11.42 ± 3.70	10.95 ± 2.94	0.352
SOD (units/ml)	3.45 ± 2.40	3.41 ± 2.62	0.907	7.02 ± 2.64	6.96 ± 3.20	0.882

*p<0.05 was considered statistically significant.
Independent student's t test was done to compare the variables between the genotypes in CVD group.
As the number of GG genotype was few, they were combined with CG genotype for t test analysis.

significant difference (p>0.05) for LDL carbonyl content was observed among the two genotypes in menopausal women without CVD. Table 4 documents the association of *PPARγ* SNP genotypes with hypertension and dyslipidemia as risk factors. In the CVD group, number of hypertensive homozygous women for wild c allele was found to be more (70.6%) as compared to women with combined g allele (64%) whereas, number of normotensive women with combined g allele (36%) were observed to be more than normotensive c allele carriers (29.4%). But, the difference was not statistically significant (p>0.05). In the control group that is without CVD the percentage occurrence of homozygous c allele and combined g allele hypertensive as well as normotensive women was found to be almost same. No significant association (p>0.05) was observed for the presence of hypertension among CC homozygotes and combined g allele (CG+GG) carriers. For dyslipidemia as risk factor, same scenario was again found in the CVD group as well as in the control group. No significant association (p>0.05) was observed between the *PPARγ* SNP genotypes for the presence of dyslipidemia. Logistic regression analysis was done to understand the association of various risk factors with CVD as dependant variable among menopausal women. Independent variables with p<0.05 in univariate analysis were entered into the model. As shown in (Table 5) after applying logistic regression it was observed that HTN, HDL-C, MDA, LDL carbonyl content and SOD were significantly correlated with CVD after adjusting for confounding factors such as menopausal age, obesity and dyslipidemia. Pro12Ala polymorphism of *PPARγ* was not found to be associated with CVD.

Table 5: Logistic regression analysis of risk factors of CVD in menopausal women

Variables	*p value*	*Odds Ratio (OR)*	*95% CI*
Menopausal age	0.782	0.91	0.46–1.77
Obesity	0.107	0.48	0.19–1.17
Dyslipidemia	0.100	1.79	0.89–3.61
HTN	0.036*	1.97	1.04–3.73
Pro12Ala	0.332	1.43	0.69–2.97
HDL-C	0.001*	6.27	3.22–12.23
MDA	0.001*	4.78	2.47–9.28
LDL carbonyl content	0.001*	25.10	13.05–48.26
SOD	0.001*	9.90	5.01 – 19.56

*p<0.05 for HTN, HDL-C, MDA, LDL carbonyl content and SOD after adjusting for confounding factors such as menopausal age, obesity and dyslipidemia. CI: confidence interval

Table 6: Distribution of minor allele frequency (MAF) for PPARγ (Pro12Ala) polymorphism across different population

Population	*Reference*	*MAF*
German	Blüher et al. 2002	2.0
Korean	Rhee et al. 2007	4.0
Prospect-epic study of sub cohort women	Zafarmand et al. 2008	13.4
South Indians	Haseeb et al. 2009	10.7
Asians	Wu et al. 2012	3.6
Caucasians	Wu et al. 2012	12.1
North Indian (Punjab)	Vats et al. 2013	11.1
Polish postmenopausal women	Grygiel-Gómiak et al. 2015	16.7
Caucasians	Pleskovič et al. 2016	18.5
North Indian menopausal women	Present Study	14.0

DISCUSSION

Interaction between genetic, conventional and environmental factors determines susceptibility of an individual to develop coronary artery disease (CAD). Classical lipid profile deter-

Table 4: Association of PPARγ SNP genotypes with HTN and Dyslipidemia among menopausal women

Variables	*Menopausal women with CVD*			*Menopausal women without CVD*		
	CC (n=201)	*CG +CG (n=64)*	*p value*	*CC (n=192)*	*CG+GG (n=66)*	*p value*
With HTN	142 (70.6)	41 (64)	0.402	87 (45.3)	32 (48.5)	0.762
Without HTN	59 (29.4)	23 (36)		105 (54.7)	34 (51.5)	
With Dyslipidemia	121 (60.2)	45 (70.3)	0.190	84 (43.7)	32 (48.5)	0.600
Without Dyslipidemia	80 (39.8)	19 (29.7)		108 (56.3)	34 (51.5)	

*p<0.05 was considered statistically significant. Chi-square test was used to compare the frequencies of HTN and Dyslipidemia according to different genotypes among menopausal women with CVD and without CVD

mination in cardiovascular disease prediction has already been established. Dyslipidemia is one of the major risk factor for the development of CVD, which usually involves elevated levels of total cholesterol, triglycerides, LDL-C, VLDL-C and a low level of HDL-C. Many studies have proved that high LDL-C, TG and low HDL-C levels are associated with increased risk for the development of CAD (Voight et al. 2012; Bansal et al. 2015). Similarly, in the present study a statistically significant increase in the mean levels of TC, TG, VLDL-C, LDL-C and a pragmatic decrease in the levels of HDL-C were observed in women with CVD when compared with women without CVD. This features the influence of low HDL-C towards CVD risk. As originally thought that low HDL-C might not always cause cardiovascular disease has now engendered renewed interest in unearthing the additional risk factors for the cause. So, the researchers took interest to accentuate other causative CVD risk factors in the study population especially, oxidative stress (OS). The need of exploring the markers of oxidative stress becomes imperative especially in menopausal women due to decline in the estrogen levels which are known to be the natural antioxidants. Menopause thus creates a pro-oxidant state in the body due to which the balance of pro-oxidant and antioxidant gets disturbed. As aforesaid, enhanced oxidative stress strongly modifies circulating lipids and lipoproteins. Oxidized LDLs thus formed, have diverse and potent effects throughout the inflammatory response and play a key role in the process of atherosclerosis. Lipid peroxidation and LDL oxidation are the early event in atherosclerotic lesion formation (Vogiatzi et al. 2009). Therefore, the levels of pro-oxidant MDA, one of the products of lipid peroxidation increases. This state is clearly depicted by the researchers' findings which reveal high levels of prooxidants - MDA and LDL carbonyl content (product of protein oxidation) and low levels of SOD, an antioxidant enzyme when women with CVD were compared to women without CVD. Moreover, decrease levels of HDL-C also favor the pro-oxidant environment because of its antioxidant and anti-inflammatory properties. It is also believed that the production of free oxidative radicals induce endothelial dysfunction which is the initial step of atherogenesis (Vogiatzi et al. 2009). Consistent with this theory the researchers' results indicate the involvement of oxidative stress as an early event in the atherosclerotic process, and several studies have also demonstrated the role of OS in CVD (Upston et al. 2002; Kumar et al. 2008; Augusti et al.2012; Amrita et al. 2016). As described earlier, MDA also forms DNA adducts such as M1dG. Screening large population for its detection may be beneficial. But, since the analysis of MDA adducts formation was beyond the scope of the present study, the study was more focused on genotype association with CVD and OS *PPARγ* is involved in a wide range of metabolic pathways thus it is plausible that its activation or inhibition may show intricate results. Nearly all of the major cells in vasculature express *PPARγ*. Thus, it becomes likely that PPARγ is a key mediator of signaling events which can influence the pathogenesis of CVD (Villacorta et al. 2009). In the present study, frequency of minor g allele for control (14%) was comparable to other world populations (Table 6). Recently, studies showed high MAF than the present study that is in Caucasians (18.5%) (Pleskovič et al. 2016) and in Polish postmenopausal women (16.7%) (Grygiel-Gümiak et al. 2015). Other studies reported were, in North Indian population of Punjab (11%) (Vats et al. 2013), in prospect-epic study of sub-cohort women (13.4%) (Zafarmand et al. 2008) which was nearly close to that of the present study, in Caucasians (12.1%) (Wu et al. 2012), in South Indians (10.7%) (Haseeb et al. 2009). Lower MAF was observed in Korean individuals (4%) (Rhee et al. 2007), in Asians (3.6%) (Wu et al. 2012) and in German subjects (2%) (Blüher et al. 2002). Deviation observed in the frequency of minor allele in North Indian Population of Punjab might be due to the smaller sample size or due to the heterogeneity of the samples. Study from another population included both males and females (Vats et al. 2013) as compared to the present population which comprised of only menopausal women. Still, this finding needs to be confirmed with a larger sample size. However, several variations observed in the association studies across different population confer ethnic heterogeneity which acts as a foremost barrier in defining the genetic predisposition to complex disease like CAD. There have been conflicting results regarding the association of Pro12Ala with CVD risk. In the present study the researchers did not find any consistent direct association of Pro12Ala with CVD risk. This is also in concordance with other studies (Rhee et al. 2007; Dal-

longeville et al. 2009; Vats et al. 2013; Wang et al. 2015). Previous epidemiological clinical studies have assessed protective role of minor g allele for CVD (Doney et al. 2004; Galgani et al. 2010). Studies have also revealed increased risk of g allele with CAD (Vogel et al. 2009; Wu et al. 2012; Li et al. 2015).

Modified LDL (ox-LDL) acts as endogenous activators or ligands for *PPARγ*. They regulate *PPARγ* dependent transcription through mechanism involving scavenger receptor-mediator cellular uptake. Oxidation of LDL and its uptake by macrophages via scavenger receptors such as CD36, and thereby becoming foam cells, and accumulation of these foam cells in the artery are the essential factors underlying atherogenesis (Libby et al. 2011). Exposure to high ox-LDL levels results in increased *PPARγ* dependent receptor expression. *PPARγ* complexes by binding to, and enhancing CD36 promoter activity, increase CD36 expression, which by uptaking oxLDL transforms macrophage into foam cells (Nagy et al. 1998; Kotla and Rao 2015).

The new aspect gained from the present paper is that menopausal women with CVD who were homozygous to c allele variant, when compared to g allele carriers, showed increased levels of LDL carbonyl protein. Upston et al. (2002) reported presence of many other oxidized protein molecules such as 4-hydroxynonenal-modified lysine, dopa, o-tyrosine, m-tyrosine, OH-leucine and OH-valine in early atherosclerotic lesions suggesting oxidation as an early event in the atherosclerotic cascade. In view of the fact that *PPARγ* is highly expressed in vascular smooth muscle cells, monocytes/macrophages, and endothelial cells, presence of pro-oxidant ligands (oxLDL, long chain fatty acids, and eicosanoids) leads to activation of *PPARγ* gene expression for its target cell (macrophages). This results in increased uptake of oxLDL by the macrophages transforming it into foam cells. On the other hand, g allele substitution at AF1 which is ligand independent domain can actively silence the expression of target genes reducing its transcriptional activity which may result in decrease of their expression level as already explained. Thus, this may be one of the reasons for high levels of LDL carbonyl protein observed in CC homozygotes as compared to g allele carriers. The detailed association between the Pro12Ala polymorphism and the above negative modulation of the *PPARγ* activity remains unclear. However, it still needs to be confirmed in more detailed analyses.

Other risk predictors such as hypertension and HDL-C were strongly associated with CVD irrespective of the Pro12Ala polymorphism. Endogenous estrogens are powerful antioxidants which inhibit the generation of reactive oxygen species and increase nitric oxide (NO) bioavailability a powerful vasodilator (Barton et al. 2009). Oxidative stress has been shown to increase blood pressure by reducing the bioavailability of NO. Endothelial dysfunction, the initial step of atherogenesis is typically characterized by the reduction of NO (Lima et al. 2012). Therefore, the loss of estrogens in menopause facilitates the development of HTN in postmenopausal women and increase the cardiovascular risk associated with it. HDL-C appears to be the major carrier of lipid hydroperoxides in plasma. Thus, HDL-C might also lower the risk of atherosclerosis by altering the structures or metabolic fates of oxidized lipids that would otherwise exert atherogenic effects (Bergt et al. 2003).

Dalle-Donne et al. (2006) suggested that *PPARγ* may contribute to the pathogenesis of CVD physiology at a very early stage because they have been shown to control the expression of proinflammatory genes in vascular cell models as reported in the earlier study (Dallongeville et al. 2009). *PPARγ* may add to the pathogenesis of CVD physiology at a very early stage because the present study revealed significant rise in the levels of LDL carbonyl protein in women who were homozygous to c allele suggesting an early event of oxidative stress in the cascade of cardiovascular processes. Li et al. (2015) in their study on meta- analysis also suggested that *PPARγ* Pro12Ala polymorphism could be an early important indicator of an early cardiovascular disease.

CONCLUSION

The results reveal that Pro12Ala polymorphism of *PPARγ* showed no direct significant association with the presence of CVD or cardiovascular risk factors in Punjabi population of menopausal women. However, the role of polymorphism needs to be warranted on a larger sample size. This study has additional strength in that, the findings of favorable LDL carbonyl protein observed in c allele carriers. Moreover, significant association of MDA, LDL carbonyl pro-

tein, SOD, HTN and HDL-C as risk predictors for CVD implicates that interaction of susceptibility genes, environmental and conventional risk factors contributes towards CVD etiology.

RECOMMENDATIONS

The findings of the present study suggest that although, no association of Pro12Ala polymorphism with CVD was observed yet, association of conventional and environmental risk factors with CVD were observed. Further, additional experiments like SNP array would be helpful in validating the role of genetic factors in disease etiology.

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