Pakistan J. Zool., pp 1-9, 2023. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20230124100125

Comparative Analysis of Serum Adiponectin and its Genetic Polymorphisms in Metabolic Syndrome and Healthy Group-an Observational Study

Uzma Zafar[1]*, Zaima Ali[1], Ambreen Tauseef[2] and Saba Khaliq[3]

[1]Department of Physiology, Lahore Medical and Dental College, University of Health Sciences, Lahore, Pakistan
[2]Department of Physiology, CMH Lahore Medical College and Institute of Dentistry, Lahore, Pakistan
[3]Department of Physiology, University of Health Sciences, Lahore, Pakistan

ABSTRACT

The present study was executed to determine the association of adiponectin gene polymorphisms rs1501299 +276 G>T and rs266729 -11377 C>G with metabolic syndrome and to compare serum adiponectin levels in its polymorphic genotypes. It was an observational study conducted at University of Health Sciences, Lahore. Study approval was granted by institutional Review/Ethical Board. Informed consent was taken from all the participants. The venous blood sample was taken after an overnight fast of 8-10 h. Blood was secured for DNA analysis and biochemical tests. Adiponectin rs266729(-11377 C>G) and rs1501299(+276G>T) genotypes were determined by ARMS PCR. Serum adiponectin levels were measured by ELISA. The current study included 200 metabolic syndrome cases and 200 healthy subjects. The minor allele 'G' of -11377 C>G [p=0.013; OR (CI)=1.47(1.08-1.999)] and the major 'GG' genotype of +276 G>T [(p=0.027; OR(CI)=1.56(1.05-2.32)] were associated with the increased risk of metabolic syndrome. The major 'CC' genotype of -11377 C>G was associated with the decreased risk [(p=0.004; OR (CI)=0.55(0.36-0.83)] of metabolic syndrome. Serum adiponectin was higher significantly in the wild genotype (CC) of -11377 C>G in cases and controls. Serum adiponectin correlated inversely with blood pressure, waist circumference and fasting serum glucose; and it exhibited positive correlation with serum high density lipoproteins in the controls while no significant relation was found in the cases. The major "GG" variant of adiponectin +276 G>T was associated with the increased risk whereas the major "CC" variant -11377 C>G was associated with the decreased risk of metabolic syndrome. It is concluded that the serum adiponectin levels were significantly less in the minor "GG" variant of -11377 C>G in metabolic syndrome as well as in the healthy group.

Article Information
Received 24 January 2023
Revised 20 June 2023
Accepted 09 May 2023
Available online 30 June 2023
(early access)

Authors' Contribution
UZ designed the study. UZ, ZA, AT and SK did field work, performed data analysis, wrote and gave final approval of the manuscript.

Key words
Adiponectin gene polymorphism, Metabolic syndrome, Genotype variants

INTRODUCTION

The world-wide metabolic syndrome is regarded a major public health issue (Manaf *et al*., 2021). It is a multicausal disorder and develops in a slow gradual way. atherogenic dyslipidemia, central obesity and Metabolic syndrome is a collection of derangements such as hypertension. Almost all these features are intimately associated with insulin resistance (Fahed *et al*., 2022). The complications of this disorder such as diabetes mellitus and cardiovascular catastrophes exert huge burden on patients and health care set up (Howlader *et al*., 2021). Diagnostic criteria for metabolic syndrome has been defined by various organizations, "International Diabetes Federation" presented a set criteria in which large waist circumference representing intra-abdominal fat accumulation was considered a mandatory component of metabolic syndrome (Han and Lean, 2016). Current literature has revealed that adipose mass is an active endocrine organ rather than an inert fat depot. It integrates various endocrine, inflammatory and metabolic signals that regulate homeostasis. A variety of

* Corresponding author: uzma.zargham@gmail.com, uzma.zargham@lmdc.edu.pk
0030-9923/2023/0001-0001 $ 9.00/0

adipocytokines have been documented to be released from adipose tissue into the circulation. These include resistin, adiponectin, interleukin-6, tumor necrosis factor alpha and many more (Cinkajzlová *et al*., 2022).

Adiponectin enhances insulin sensitization at tissue level by modulating cellular and metabolic functions. Although adipose organ is the major site of origin of adiponectin, it is also synthesized in skeletal muscles, liver and bone marrow cells. Adiponectin produces its physiological effects by acting on skeletal muscles predominantly through adiponectin receptor 1 and liver cells predominantly through adiponectin receptor 2. Once these receptors are activated serial signal transduction is through activation of adenosine monophosphate dependent protein kinase pathway and peroxisome proliferator activated receptor gamma pathway resulting in an increased fatty acid oxidation and decreased hepatic glucose synthesis (Yibby *et al*., 2020). Low serum adiponectin levels are found to be associated with insulin resistance, metabolic syndrome, type 2 diabetes mellitus and perivisceral/central adiposity (Nguyen, 2020).

Genome-based linkage studies on adiponectin gene have revealed direct association of adiponectin genetic polymorphisms with insulin resistance and type 2 diabetes mellitus. The rs1501299 G>T genetic variant of adiponectin at 276th position of intron 2 was intimately linked with type 2 diabetes mellitus and pathogenesis of insulin resistance. The wild GG genotype of 276 G>T instead of TT was found to be a risk for type 2 diabetes mellitus and related with the low adiponectin levels (Alimi *et al*., 2021a). Adiponectin promoter polymorphism rs266729 -11377 C>G was also reported to be significantly associated with the risk of nonalcoholic fatty liver disease and altered blood lipid profile in obese subjects (Gasparotto *et al*., 2017; Zheng *et al*., 2022). However, a previous study from Pakistan did not show significant association between adiponectin promoter polymorphism and type 2 diabetes mellitus (Nadeem *et al*., 2017). Another study from Pakistan reported lack of association between adiponectin rs1501299 G>T polymorphism and polycystic ovaries (Ahmed *et al*., 2019). The conflicting diverse results might be due to variable sample size or genetic, ethnic or clinical heterogeneity of different populations. There is still no definite or recognized genomic DNA marker for clinical application. The present study was executed to determine the association of adiponectin gene polymorphisms rs1501299 +276G>T and rs266729 -11377 C>G with metabolic syndrome and to compare serum adiponectin levels in different polymorphic genotypes.

MATERIALS AND METHODS

Study population

It was an observational cross-sectional study conducted at the Physiology Department of University of Health Sciences. Total 400 participants were recruited; 200 were cases of metabolic syndrome and 200 controls. Study population was estimated by WHO calculator. Significance level was taken as 0.05 and power of study was 80%. Sample size equation utilized was "Hypothesis test for two population proportions"; minor allele proportion of rs266729 -11377C>G was considered (Hashemi *et al*., 2013). Subjects were selected from Sheikh Zayed Hospital, Lahore from June to November 2016.

Diagnostic criteria of metabolic syndrome

Metabolic syndrome identification was based upon "International Diabetes Federation" definition. All recruited male patients were centrally obese with waist circumference of more or equal to 90 cm plus two out of the four other features stated as; (1) triglycerides in serum ≥ 150 mg/dl or on its treatment, (2) high density lipoprotein cholesterol less than 40 mg/dl or on its treatment, (3) Blood pressure greater than 130/85 or on antihypertensives, and (4) Fasting serum glucose > 100mg/dl or on its treatment (Fahed *et al*., 2022).

Age-matched controls were taken from healthy population. Those were selected as controls; having waist circumference ≤ 90 cm and no evidence of hypertension or diabetes mellitus. There was also no history of intake of anti-lipid, anti-diabetic and anti-hypertensive medications. Blood pressure and plasma glucose (fasting and 2 h postprandial) of the controls were checked on two separate days. Subjects selected were having fasting plasma glucose less than 100mg/dl, 2 h after meals less than 140 mg/dl and BP less than 130/85. All those having evidence of chronic infective or inflammatory metabolic states were excluded (Sinnott *et al*., 2015).

Anthropometric and biochemical measurements

After subject selection informed consent was undertaken followed by collection of demographic and clinical measurements. Waist circumference was taken mid-way between the last rib (its lower border) and the top rim of the antero-superior iliac crest. Blood pressure was measured from the left arm in sitting position after the rest of 10-12 min. Two measurements were taken after 5 min interval and an average of two was noted. The venous blood sample was taken from the subjects after an overnight fast of 8-10 h (Sinnott *et al*., 2015). Blood was secured at -200 °C for DNA extraction and analysis; serum was stored at -800 °C for biochemical tests. Serum adiponectin levels were measured by ELISA using ELISA

kits (Elabscience, Germany) (E-EL-H0004).

Analysis of adiponectin gene polymorphisms

DNA extraction done with the Favor-Prep Blood Genomic DNA Extraction Kit (Taiwan, China) as per given guidelines. Adiponectin rs266729(-11377 C>G) genotype was determined by ARMS PCR with following primers:

Outer F5'-GGACTGTGGAGATGATATCTGGGGGGCA-3',
R5'-TGGCCTAGAAGCAGCCTGGAGAACTGGA-',
Inner F5' CTTGCAAGAACCGGCTCAGATCCTCCC-3',
R5' GAGCTGTTCTACTGCTATTAGCTCTGC-3'

PCR reaction made was of total 15µL containing 0.3 µL of each primer, 2 µL (50ng) genomic DNA, 7.5µL 2X master mix and 4.3µL double distilled water. The PCR cycling conditions were initial denaturation for 5 min at 95°C followed by 30 cycles of denaturation for 30 sec at 95°C, annealing for 30s at 62°C, extension for 27 sec at 72°C with a final extension at 72°C for 5 min to allow for complete extension of all PCR products. The amplicons were run for 45 min on 3% agarose gel stained with ethidium bromide. The PCR product was checked under UV gel documentation system (Bio Rad, USA). The product sizes for rs266729 were 299-bp for control band, 155-bp for C allele, and 201-bp for G-allele (Hashemi *et al*., 2013).

Adiponectin rs1501299 (+276G>T) genotype was determined by ARMS PCR with following primers;

Outer F5'-GAGCTGTTCTACTGCTATTAGCTCTGC-3'
R5'-GAATATGAATGTACTGGGAATAGGGATG-3'
Inner F5'-CCTCCTACACTGATATAAACTATATGAGGG-3'
R5'TGTGTCTAGGCCTTAGTTAATAATGAACGA-3'

PCR reaction made was of total 15µL containing 0.3 µL of each primer, 2µL (50ng) genomic DNA, 7.5µL 2X PCR master mix and 4.3µL double distilled water. The PCR cycling conditions were initial period of denaturation for 5 min at 95°C initially, followed by 30 denaturation cycles for 30 sec at 95°C, annealing for 27 sec at 61°C and extension for 25 sec at 72°C followed by a final extension at 72°C for 5 min to allow for complete amplification of all PCR products. The amplicons were run for 45 min on 3% agarose gel stained with ethidium bromide. The PCR product was checked under ultra-violet gel documentation system (Bio Rad, USA). The product sizes of rs1501299 were; for G allele 244-bp, T allele 292-bp and control band 476-bp (Hashemi *et al*., 2013).

Statistical analysis

The data analysis was conducted using SPSS version 22.0 (Statistical Package for Social Sciences). Mann-Whitney U was applied to compare non-normally distributed quantitative variables between the cases and the controls. Allelic and genotypic proportions were checked by Hardy Weinberg equation with online (OEGE) tool of gene epidemiology. Distribution was consistent with Hardy Weinberg law p=0.05 (Bińkowski, 2022). For the comparison of the frequency and determination of the genotypic association with the study groups, three genetic models (Co-dominant, wild/dominant, and mutant/recessive) were designed. With chi-square test ($\chi 2$) gene and allele frequencies/proportions of two groups were compared followed by the calculation of the odds ratio. Logistic regression analysis was utilized to find the association between metabolic syndrome and gene frequencies after control of all genotype models. Serum adiponectin was compared between the groups by Mann-Whitney U statistics. Correlation of serum adiponectin with metabolic syndrome related traits was determined with spearman test. A p of less than 0.05 was taken as of statistical significance.

RESULTS

Clinical characteristics of the study population

Study population included 200 cases of metabolic syndrome and 200 healthy controls. The mean (± standard deviation) age of Met S group was 48.03 (±8.03) years and of healthy was 47.04 (±8.19) years. No significant difference was found among the mean ages. Duration course of metabolic syndrome in 60% subjects was less than one a year and in the rest 40% it was more than a year. In the metabolic syndrome study group; 80% were diabetics, 10% were with impaired fasting sugar, 79% hypertensives or on its treatment, 92% were with impaired lipid profile. Anthropometric and biochemical parameters such as waist circumference, blood pressure, serum triglyceride, insulin and glucose levels were higher significantly in cases as compared to the controls. These characteristics were already described in a previous study (Zafar *et al*., 2019).

Genotype frequency of rs266729 -11377 C>G and rs1501299 +276 G>T

Allelic and genotype frequencies of adiponectin polymorphisms are presented in the Tables I-III. The overall frequency of the major allele C of rs266729 -11377 C>G was 0.71 and the minor allele G was 0.29 in the present study. The minor allele 'G' was significantly associated with the increased risk of metabolic syndrome [p=0.013; OR (CI)= 1.47(1.08-1.999)] and the major 'CC' genotype was associated with the decreased risk [(p=0.004; OR (CI)=0.55(0.36-0.83)] of metabolic syndrome.

The overall frequency of the major allele G of rs1501299 G>T was 0.75 and the minor allele T was 0.25 in the present study. The major 'GG' genotype of +276 G>T was associated with the increased risk [(p=0.027; OR(CI)= 1.56(1.05-2.32)] of metabolic syndrome. These associations remained significant after controlling the confounders such as age and serum lipid status.

Table I. Comparison of genotypes and allelic frequency of adiponectin rs266729 (-11377 C>G) polymorphism among study groups.

Geno-types	Metabolic syndrome (%)	Healthy group (%)	p value	Odds ratio and confidence interval
Co-dominant model				
CC	83(41.5%)	111(55.5%)	0.02*	Odds ratio not computed
CG	100(50%)	76(38%)		
GG	17(8.5%)	13(6.5%)		
Total	200	200		
Allelic model				
G	134(33.5%)	102(25.5%)	0.013*	1.47(1.08 to 1.99)
C	266(66.5%)	298(74.5%)		
Total	400	400		
Recessive model				
GG	18(9%)	14(7%)	0.461	1.31(0.63 to 2.72)
CG+CC	182(91%)	186(93%)		
Total	200	200		
Dominant model				
CC	83(41.5%)	112(56%)	0.004*	0.55(0.36 to 0.83)
CG+GG	117(58.5%)	88(44%)		
Total	200	200		

A Chi square test was utilized to calculate the p value, Confidence interval and Odds ratio. A "p" of < 0.05 is statistically significant*.

Table II. Comparison of genotypes and allelic frequency of adiponectin rs1501299 (276G>T) polymorphism among study groups.

Geno-types	Metabolic syndrome (%)	Healthy group (%)	p value	Odds ratio and confidence interval
Co-dominant model				
GG	120((60%)	96(48%)	0.055	
GT	72(36%)	93(46.5%)		
TT	8(4%)	11(5.5%)		
Total	200	200		
Allelic model				
G	312(78%)	285(71.25%)	0.000*	2.67(1.99 to 3.59)
T	88(22%)	115(28.75%)		
	400	400		
Dominant model				
GG	119(59.5%)	97(48.5%)	0.027*	1.56(1.05 to 2.32)
GT+TT	81(40.5%)	103(51.5%)		
Total	200	200		
Recessive model				
TT	8(4%)	11(5.5%)	0.481	0.72(0.28 to 1.82)
GT+TT	192(96%)	189(94.5%)		
Total	200	200		

A Chi square test was utilized to calculate the p value, Confidence interval and Odds ratio. A "p" of < 0.05 is statistically significant*.

Table III. Association of adiponectin genotypes with metabolic syndrome after controlling for co-variates.

Co-variates	B	Sig	Exp(β)	CI
Rs266729dominant	0.599	0.005*	0.55	0.36 to 0.83
Rs266729recessive	0.070	0.860	0.49	0.49 to 2.32
Rs1501299dominant	-0.466	0.028*	1.56	1.05 to 2.33
Rs151299recessive	0.049	0.921	0.39	0.39 to 2.78
Age	0.301	0.240	0.82	0.82 to 2.23
Fasting lipids	-0.304	0.966	0.67	0.67 to 1.52

Binary logistic regression analysis was applied to calculate p-value, confidence interval and Exp(β) coefficient. A "p" of < 0.05 is statistically significant*. Study groups are taken as dependent variable.

Comparison of serum adiponectin among the study groups

Serum adiponectin level (Table IV) was significantly higher in controls as compared to the cases (p=0.000). On comparison of serum adiponectin levels in different adiponectin genotypes its level was significantly less in metabolic syndrome (p=0.028) and healthy group (p=0.008) in the minor 'GG' genotype of -11377 C>G.

Table IV. Comparative analysis of serum adiponectin between the study groups and within each group in different genotype models.

Serum adiponectin levels (in µg/ml)	Metabolic syndrome	Healthy group	p value*
Among study groups	2.88 (1.04-2.44)	4.01 (2.67-5.8)	0.000
rs266729 (-11377 C > G) minor genotype model within the study groups			
GG	1.8 (0.44-2.3)	2.03 (0.93-3.18)	
CG+CC	3.07(1.19-4.04)	4.46 (2.88-5.97)	
p-value** (within groups)	0.028*	0.008*	
rs1501299 (276G > T) major genotype model within the study groups			
GG	2.67(0.92-4.23)	3.92(2.5-5.9)	
GT+TT	3.04(1.61-4.93)	4.85(2.88-5.83)	
p-value**(within groups)	0.704	0.274	

*Mann Whitney U test was applied to compare serum adiponectin levels between metabolic syndrome and healthy group. **Mann Whitney U test was applied to compare serum adiponectin levels in risk genotypes of adiponectin within each study group. Serum adiponectin- expressed as median (interquartile range) due its non-normal distribution. A "p" of < 0.05 is statistically significant.

Correlation of serum adiponectin with metabolic syndrome traits

There was significant correlation of serum adiponectin with blood pressure, waist circumference, serum high density lipoproteins and triglycerides in the healthy group (Table V) however no significant correlation of serum adiponectin was found with these traits in the metabolic

syndrome group. Linear regression analysis was applied (Table VI) taking serum adiponectin as a dependent variable and controlling all the covariates such as age, waist circumference, blood pressure, serum triglycerides, high density lipoproteins and glucose. Correlation of waist circumference with serum adiponectin (Fig. 1) remained significant (p=0.001).

Table V. Correlation of serum adiponectin with metabolic syndrome traits.

Variables	Metabolic syndrome rho(p-value)	Healthy group rho(p-value)
Systolic blood pressure	0.031(0.736)	-0.322(0.027*)
Diastolic blood pressure	0.058(0.531)	-0.305(0.037*)
Waist circumference	-0.099(0.289)	-0.298(0.042*)
Fasting serum glucose	0.013(0.188)	-0.291(0.047*)
Fasting serum high density lipoproteins	0.036(0.702)	0.308(0.035*)
Fasting serum triglycerides	-0.065(0.487)	0.218(0.141)

Spearman rho test was applied to determine the correlation of serum adiponectin with the traits of metabolic syndrome. A p of less than 0.05* is of statistical significance.

Table VI. Linear regression analysis to determine the significant correlation of adiponectin after controlling the co-variates.

Predictors of adiponectin	Standardized coefficients beta	t	signifi-cance
Systolic blood pressure	-0.014	-0.124	0.902
Diastolic blood pressure	-0.003	-0.028	0.978
Waist circumference	-0.293	-3.34	0.001*
Fasting serum glucose	-0.041	-0.458	0.648
Fasting serum high density lipoproteins	0.001	0.014	0.980
Fasting serum triglycerides	0.210	0.210	0.834

Linear regression analysis is applied to determine significant predictor of serum adiponectin. Serum adiponectin is dependent variable. Other co-variates are independent. A p of less than 0.05* is of statistical significance.

DISCUSSION

In the present study, two adiponectin gene polymorphisms rs1501299 (+276 G>T) and rs266729 (-11377 C>G) were evaluated for their possible association with the metabolic syndrome. The wild type or major 'G' allele and GG genotype of adiponectin +276 G>T genetic

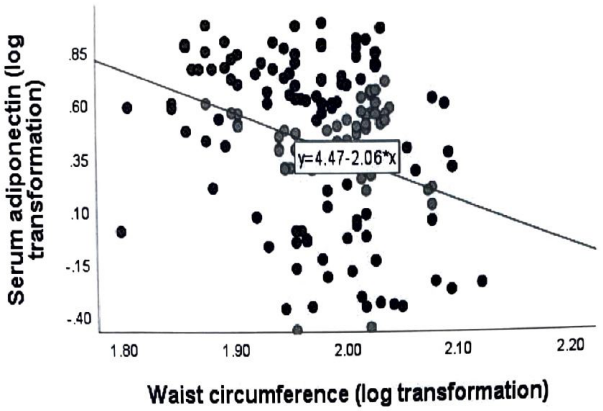

Fig. 1. Correlation of waist circumference with serum adiponectin.

variant was found to be associated significantly with the risk of metabolic syndrome. Our study reported major allelic frequency of 0.75 for G allele and minor allelic frequency of 0.25 for the T allele. However, a study on non-diabetic Korean men revealed minor T allelic frequency of 0.38 and significant association of mutant or TT genotype with low adiponectin levels (Song *et al*., 2018). Another study on post-menopausal North-West Indian women reported minor allelic frequency of 0.35 and significant association of minor TT genotype with the increased cardiovascular risk and oxidative stress (Amrita *et al*., 2021). In our study the proportion of wild GG genotype was considerably higher in metabolic syndrome than the control group. The minor allelic frequency as determined in our study (0.25) was comparable to that of another study on Iranian population (0.28), however no significant association of rs1501299 polymorphism was found with type 2 diabetes mellitus in Iranians (Alimi *et al*., 2021b). In a study on Chinese coal mine workers minor allelic frequency was 0.26 and major GG genotype was reported to be the risk genotype for hypertension (Hu *et al*., 2022). In Saudis major GG genotype was associated with increased risk of type 2 diabetes mellitus. This result is in concordance with our study, however minor allele frequency in Saudi population was higher as compared to ours, i.e., 0.4 versus 0.25 (Al-Nbaheen, 2022). In another meta-analysis, T allele of +276G>T was found to reduce the risk of coronary artery disease in Caucasians and Asians (Yang *et al*., 2012).

In the current study, the wild or major CC adiponectin genotype of rs266729 (-11377 C>G) was found to be associated significantly with the decreased risk whereas minor allele G was associated with the increased risk of metabolic syndrome. This finding is in agreement with that

of a previous systematic review that reported considerable correlation of adiponectin gene rs266729 C>G with non-alcoholic fatty liver disease (Zheng *et al*., 2022). The minor allelic G frequency in this study was 0.29 which is comparable to the minor allele frequency of 0.30 reported in another study from Pakistan on Rajput population. However, no association of minor rs266729 C>G genotype was found with type 2 diabetes mellitus in that study (Nadeem *et al*., 2017). In a study on Iranian population significant association of rs266729 C>G minor genotype was found with type 2 diabetes mellitus and minor allelic G frequency (0.3) in this study was also comparable to the frequency reported in the present study (Alimi *et al*., 2021b). The rs266729 minor genotype was also reported to be associated with the increased risk of type2 diabetes mellitus and metabolic syndrome in Veitnamese Kinh population (Truong *et al*., 2022). Another meta-analysis revealed that rs266729 C>G variant was associated with the increased risk of type2 diabetes mellitus in Asian population (Chu *et al*., 2013).

Serum adiponectin level in the present study was significantly less in centrally obese, insulin resistant, metabolic syndrome group as compared to the healthy group. Several postulated mechanisms may explain decrease adiponectin production of the adipose tissue in metabolic syndrome; such as hypoxia, oxidative insult, increased tumor necrosis factor alpha levels in obese adipocytes. Chronic gradual inflammation results in low adiponectin levels in obese metabolically unhealthy subjects (Marques *et al*., 2021; Singhal *et al*., 2022; Sparrenberger *et al*., 2019; Yosaee *et al*., 2019). Similar findings were reported by the studies from Pakistan conducted upon diabetic and pre-diabetic subjects (Ahan *et al*., 2014; Najam *et al*., 2014). The results of this study were also in concordance with those of a previous study on Brazilian adolescents; that reported downward trend in adiponectin with the increasing traits of metabolic syndrome (Sparrenberger *et al*., 2019).

In the present study, serum adiponectin levels were significantly less in metabolic syndrome as compared to the healthy controls however no significant correlation of serum adiponectin was found with anthropometric or biochemical traits of insulin resistance in metabolic syndrome. These results were not in agreement with those of the previous studies that reported positive association of serum adiponectin with components of metabolic syndrome (Marjani *et al*., 2022; Yang *et al*., 2022; Zaha *et al*., 2020). In our population mean adiponectin levels were very low as compared to the other populations. Dysregulated adiponectin production by obese and inflamed adipose tissue might result in alteration of adiponectin relation with anthropometric and biochemical parameters in the diseased group in our study (Nigro *et al*., 2014). In the healthy group significant inverse correlation of serum adiponectin was found with blood pressure, waist circumference and serum glucose and positive correlation was there with serum high density lipoproteins. These results were supported by the previous studies that reported association of serum adiponectin with anthropometric measurements in healthy non-diabetic population (Bonneau *et al*., 2014; Foula *et al*., 2020; Sparrenberger *et al*., 2019; Zaidi *et al*., 2022).

Serum adiponectin was also low significantly in the recessive GG genotype of rs266729 in both healthy and metabolic syndrome group however no significant difference was observed in different genotypes of rs1501299. The impact of rs266729 polymorphism on serum adiponectin suggests that it is a heritable trait and its expression can be modified by genetic variations. This result is also consistent with a previous study conducted on type2 diabetic Jordanians (Alfaqih *et al*., 2022). Another study conducted on Iranian population reported lack of association of rs266729 -11377 C>G genotype with type 2 diabetes mellitus and serum adiponectin levels in its different genotypes (Karimi *et al*., 2018). The diversity of results suggests that the heterogenous and multifactor heritability of metabolic syndrome is a challenge for understanding the factors associated with this condition and related traits (Rana *et al*., 2022).

CONCLUSION

The major "GG" genotypic variant of adiponectin +276 G>T was significantly associated with the increased risk whereas the major "CC" variant -11377 C>G was associated with the decreased risk of metabolic syndrome. Serum adiponectin levels were significantly less in the minor "GG" variant of -11377 C>G in metabolic syndrome as well as in the healthy group. Adiponectin gene variants by regulating adiponectin expression can be the possible immune modifiers. Further studies are required to ascertain the role of genetic variants and adiponectin in prediction of metabolic syndrome and its traits.

ACKNOWLEDGEMENT

We are thankful to the administration University of Health Sciences, Lahore.

Funding

This study was approved by the institutional review/ ethical board (No: UH/education/126-16/1267/5-5-2016).

IRB approval

The study was approved by the Advanced Studies &

Research Board of University of Health Sciences, Lahore (No: UHS/Educational/126-16/1267).

Ethical statement

Informed written consent was taken from the participants of the study.

Statement of conflict of interest

The authors have declared no conflict of interests.

REFERENCES

Ahan, S., Ahmed, S., Ahmed, S. and Nauman, K., 2014. Status of serum adiponectin related to insulin resistance in prediabetics. *J. Pak. med. Assoc*., **64**: 184-188.

Ahmed, W., Tasleem, R. and Shehzad, S., 2019. Association of adiponectin gene 276G> T polymorphism with polycystic ovary syndrome in Pakistani women. *Biomed. Res*., **30**: 218-223. https://doi.org/10.35841/biomedicalresearch.30-18-1182

Alfaqih, M.A., Al-Hawamdeh, A., Amarin, Z.O., Khader, Y.S., Mhedat, K. and Allouh, M.Z., 2022. Single nucleotide polymorphism in the ADIPOQ gene modifies adiponectin levels and glycemic control in type two diabetes mellitus patients. *Biomed. Res. Int*., **2022**: 6632442. https://doi.org/10.1155/2022/6632442

Alimi, M., Goodarzi, M.T. and Nekoei, M., 2021. Adiponectin gene polymorphisms and risk of type 2 diabetes: An updated evidence for meta-analysis. *Diabetol. Metab. Syndr*., **13**: 1-19. https://doi.org/10.1186/s13098-021-00749-x

Alimi, M., Goodarzi, M.T. and Nekoei, M., 2021. Association of ADIPOQ rs266729 and rs1501299 gene polymorphisms and circulating adiponectin level with the risk of type 2 diabetes in a population of Iran: A case-control study. *J. Diabetes Metab. Disord*., **20**: 87-93. https://doi.org/10.1007/s40200-020-00715-w

Al-Nbaheen, M.S., 2022. Effect of genetic variations in the ADIPOQ gene on susceptibility to type 2 diabetes mellitus. *Diabetes Metab. Syndr. Obes*., **15**: 2753-2761. https://doi.org/10.2147/DMSO.S377057

Amrita, J., Mahajan, M., Bhanwer, A. and Matharoo, K., 2021. Association of AdipoQ gene variation (rs1501299) and oxidative stress with cardiovascular disease in North West Indian population of Punjabi women. *J. med. Biochem*., **40**: 49-59. https://doi.org/10.5937/jomb0-24704

Bińkowski, J.M.S., 2022. *Gene-Calc [Computer software]*. Available from: www.gene-calc.pl. https://gene-calc.pl/hardy-weinberg-page. Gene-Calc [Computer software]. 2018-September [cited 27-12- 2022].

Bonneau, G.A., Pedrozo, W.R. and Berg, G., 2014. Adiponectin and waist circumference as predictors of insulin-resistance in women. *Diabetes Metab. Syndr. Clin. Res. Rev*., **8**: 3-7. https://doi.org/10.1016/j.dsx.2013.10.005

Chu, H., Wang, M., Zhong, D., Shi, D., Ma, L., Tong, N. and Zhang, Z., 2013. ADIPOQ polymorphisms are associated with type 2 diabetes mellitus: A meta-analysis study. *Diabetes Metab. Res. Rev*., **29**: 532-545. https://doi.org/10.1002/dmrr.2424

Cinkajzlová, A., Mráz, M. and Haluzík, M., 2022. Adipose tissue immune cells in obesity, type 2 diabetes mellitus and cardiovascular diseases. *J. Endocrinol*., **252**: R113-127. https://doi.org/10.1530/JOE-21-0159

Fahed, G., Aoun, L., Bou Zerdan, M., Allam, S., Bouferraa, Y. and Assi, H.I., 2022. Metabolic syndrome: Updates on pathophysiology and management in 2021. *Int. J. mol. Sci*., **23**: 786. https://doi.org/10.3390/ijms23020786

Foula, W.H., Emara, R.H., Eldeeb, M.K., Mokhtar, S.A. and El-Sahn, F.A., 2020. Effect of a weight loss program on serum adiponectin and insulin resistance among overweight and obese premenopausal females. *J. Egypt Publ. Hlth. Assoc*., **95**: 1-8. https://doi.org/10.1186/s42506-020-00060-z

Gasparotto, A.S., Borges, D.O., Zandoná, M.R., Ramos, M.J., Meihnardt, N.G. and Mattevi, V.S., 2017. Adiponectin promoter polymorphisms are predictors of lipid profile improvement after bariatric surgery. *Genet. mol. Biol*., **40**: 736-742. https://doi.org/10.1590/1678-4685-gmb-2016-0241

Han, T.S. and Lean, M.E., 2016. A clinical perspective of obesity, metabolic syndrome and cardiovascular disease. *JRSM Cardiovasc. Dis*., **5**: 2048004016633371. https://doi.org/10.1177/2048004016633371

Hashemi, M., Bojd, H.H., Nasab, E.E., Bahari, A., Hashemzehi, N.A., Shafieipour, S., Narouie, B., Taheri, M. and Ghavami, S., 2013. Association of adiponectin rs1501299 and rs266729 gene polymorphisms with nonalcoholic fatty liver disease. *Hepat. Mon*., **13**: e9527. https://doi.org/10.5812/hepatmon.9527

Howlader, M., Sultana, M.I., Akter, F. and Hossain, M.M., 2021. Adiponectin gene polymorphisms associated with diabetes mellitus: A descriptive

review. *Heliyon*, **7**: e07851. https://doi.org/10.1016/j.heliyon.2021.e07851

Hu, X., Xi, Y., Bai, W., Zhang, Z., Qi, J., Dong, L., Liang, H., Sun, Z., Lei, L. and Fan, G., 2022. Polymorphisms of adiponectin gene and gene lipid interaction with hypertension risk in Chinese coal miners: A matched case-control study. *PLoS One*, **17**: e0268984. https://doi.org/10.1371/journal.pone.0268984

Karimi, H., Nezhadali, M. and Hedayati, M., 2018. Association between adiponectin rs17300539 and rs266729 gene polymorphisms with serum adiponectin level in an Iranian diabetic/pre-diabetic population. *Endocr. Regul*., **52**: 176-184. https://doi.org/10.2478/enr-2018-0022

Manaf, M.R.A., Nawi, A.M., Tauhid, N.M., Othman, H., Rahman, M.R.A., Yusoff, H.M., Safian, N., Ng, P.Y., Manaf, Z.A. and Kadir, N.B.Y.A., 2021. Prevalence of metabolic syndrome and its associated risk factors among staffs in a Malaysian Public University. *Sci. Rep*., **11**: 1-11. https://doi.org/10.1038/s41598-021-87248-1

Marjani, M., Dolab, N., Kamkar, M.Z., Amiriani, T., Yuzugulen, J. and Marjani, A., 2022. Gender and body mass index-related serum level of adipokines and metabolic syndrome components in bipolar patients who received lithium and valproic acid. *Metab. Syndr. Relat. Disord*., **20**: 79-87. https://doi.org/10.1089/met.2021.0078

Marques, V., Afonso, M.B., Bierig, N., Duarte-Ramos, F., Santos-Laso, Á., Jimenez-Agüero, R., Eizaguirre, E., Bujanda, L., Pareja, M.J. and Luís, R., 2021. Adiponectin, leptin, and IGF-1 are useful diagnostic and stratification biomarkers of NAFLD. *Front. Med*., **8**: 683250. https://doi.org/10.3389/fmed.2021.683250

Nadeem, A., Mumtaz, S., Naveed, A.K., Mansoor, Q., Aslam, M. and Ismail, M., 2017. Association of ADIPOQ C-11377G (rs266729), TNF-[alpha] G-308A (rs1800629) and TNF-[alpha] G-238A (rs361525) single nucleotide polymorphisms with type 2 diabetes mellitus in Pakistani population. *J. Coll. Phys. Surg. Pak.,* **27**: 625-631.

Najam, S.S., Awan, F.R. and Baig, S.M., 2014. Serum adiponectin levels in diabetes, obesity and gender in Punjabi subjects from Faisalabad, Pakistan. *J. Pak. med. Assoc*., **64**: 1186-1188.

Nguyen, T.M.D., 2020. Adiponectin: Role in physiology and pathophysiology. *Int. J. Prev. Med*., **11**: 136. https://doi.org/10.4103/ijpvm.IJPVM_193_20

Nigro, E., Scudiero, O., Monaco, M.L., Palmieri, A., Mazzarella, G., Costagliola, C., Bianco, A. and Daniele, A., 2014. New insight into adiponectin role in obesity and obesity-related diseases. *BioMed. Res. Int*., **2014**: 658913. https://doi.org/10.1155/2014/658913

Rana, S., Ali, S., Wani, H.A., Mushtaq, Q.D., Sharma, S. and Rehman, M.U., 2022. Metabolic syndrome and underlying genetic determinants. A systematic review. *J. Diabetes Metab. Disord*., **21**: 1095-1104. https://doi.org/10.1007/s40200-022-01009-z

Singhal, A., Punia, V.S., Bharti, A., Mittal, S., Mishra, P.R. and Kumar, P., 2022. A study of correlation of adiponectin levels in metabolic syndrome. *Eur Med J.,* https://doi.org/10.33590/emj/10146572

Sinnott, M., Kinsley, B.T., Jackson, A.D., Walsh, C., O'Grady, T., Nolan, J.J., Gaffney, P., Boran, G., Kelleher, C. and Carr, B., 2015. Fasting plasma glucose as initial screening for diabetes and prediabetes in Irish adults: The diabetes mellitus and vascular health initiative (DMVhi). *PLoS One*, **10**: e0122704. https://doi.org/10.1371/journal.pone.0122704

Song, J., Yoon, S.R., Kim, O.Y., 2018. T allele at ADIPOQ rs1501299 G/T polymorphism is more susceptible to the influence of circulating adiponectin on arterial stiffness in nondiabetic men. *Diabetol Metab. Syndr*., **10**: 1-9. https://doi.org/10.1186/s13098-018-0345-2

Sparrenberger, K., Sbaraini, M., Cureau, F.V., Teló, G.H., Bahia, L. and Schaan, B.D., 2019. Higher adiponectin concentrations are associated with reduced metabolic syndrome risk independently of weight status in Brazilian adolescents. *Diabetol. Metab. Syndr*., **11**: 1-9. https://doi.org/10.1186/s13098-019-0435-9

Truong, S., Tran, N.Q., Ma, P.T., Hoang, C.K., Le, B.H., Dinh, T., Tran, L., Tran, T.V., Le, L.H.G. and Vu, H.A., 2022. Association of ADIPOQ single-nucleotide polymorphisms with the two clinical phenotypes type 2 diabetes mellitus and metabolic syndrome in a Kinh Vietnamese population. *Diabetes Metab. Syndr. Obes*., **15**: 307. https://doi.org/10.2147/DMSO.S347830

Yang, H.S., Lee, G.H., Kim, D., Lee, K.R. and Hur, M., 2022. Association of serum adiponectin biomarker with metabolic syndrome components in koreans with extremely high HDL cholesterol levels in general health checkup. *Metabolites*, **12**: 1086. https://doi.org/10.3390/metabo12111086

Yang, Y., Zhang, F., Ding, R., Wang, Y., Lei, H. and Hu, D., 2012. Association of ADIPOQ gene polymorphisms and coronary artery disease risk: A meta-analysis based on 12 465 subjects.

Thromb. Res., **130**: 58-64. https://doi.org/10.1016/j.thromres.2012.01.018

Yibby, M.L.G.A.A. and Torresb, F., 2020. Adiponectin and leptin adipocytokines in metabolic syndrome: What is its importance? *Horm. Mol. Biol. Clin. Investig*., **2014**: 37-45.

Yosaee, S., Khodadost, M., Esteghamati, A., Speakman, J.R., Djafarian, K., Bitarafan, V. and Shidfar, F., 2019. Adiponectin: An indicator for metabolic syndrome. *Iran. J. Publ. Hlth*., **48**: 1106. https://doi.org/10.18502/ijph.v48i6.2921

Zafar, U., Khaliq, S., Ahmad, H.U. and Lone, K.P., 2019. Serum profile of cytokines and their genetic variants in metabolic syndrome and healthy subjects: A comparative study. *Biosci. Rep*., **39**: BSR20181202. https://doi.org/10.1042/BSR20181202

Zaha, D.C., Vesa, C., Uivarosan, D., Bratu, O., Fratila, O., Tit, D.M., Pantis, C., Diaconu, C.C. and Bungau, S., 2020. Influence of inflammation and adipocyte biochemical markers on the components of metabolic syndrome. *Exp. Ther. Med*., **20**: 121-128. https://doi.org/10.3892/etm.2020.8663

Zaidi, H., Aksnes, T., Åkra, S., Eggesbo, H.B., Byrkjeland, R., Seljeflot, I. and Opstad, T.B., 2022. Abdominal adipose tissue associates with adiponectin and TNFα in middle-aged healthy men. *Front. Endocrinol*., **13**: https://doi.org/10.3389/fendo.2022.874977

Zheng, Y.T., Xiao, T.M., Wu, C.X., Cheng, J.Y. and Li, L.Y., 2022. Correlation of adiponectin gene polymorphisms rs266729 and rs3774261 with risk of nonalcoholic fatty liver disease: A systematic review and meta-analysis. *Front. Endocrinol*., **13**: https://doi.org/10.3389/fendo.2022.798417