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RESEARCH ARTICLE

GENETIC STUDIES IN TYPE2 DIABETES IN UNANI CONCEPTS OF TEMPERAMENT; ASSOCIATION BETWEEN PRO12ALA POLYMORPHISM OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-GAMMA 2 (PPAR-A) OF INDIAN POPULATION

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ABSTRACT

Background: Diabetes is a very common metabolic disorder among Indian population. This study validates some of the Unani concepts of humors or temperaments (Phenotypes), with regard to Type 2 Diabetes mellitus. We selected Damvi subjects for our study, with an aim to determine its association with the genetic biomarker- Peroxisome proliferator-activated receptor - Gamma (PPAR-γ) and other biochemical parameters.

Methods: The Unani clinicians randomly selected 100 diabetic damvi patients who were attending the Unani hospital for treatment and 100 healthy volunteers belonging to damvi temperament. Institutional Ethics Committee approved the project. Besides looking at temperaments/ humors as susceptibility factors – we included a genetic factor-PPAR-γ to our investigation. We have genotyped the Proline 12 alanine (Pro12Ala C→G) gene polymorphism by PCR-RFLP.

Results: We found more of CC genotypes in both cases and controls and less number of the hetero genotypes CG in cases compared to controls. Interestingly the homo genotypes GG were not found in this group of patients as well as controls. In our study we observed a significant increase ($p<0.001$) in malondialdehyde (MDA) levels and GST levels using enzyme-linked immunosorbent assay (ELISA). We also found that plasma glucose levels (fasting and postprandial), total cholesterol, triglycerides, liver function tests and renal function tests were very high using Erba Autoanalyzer.

Conclusion: We found that Damvi temperaments (Sanguine- Phenotypes) were more prone to T2DM; hence it is important that damvi phenotypes could be counseled to take preventive measures, also PPAR-γ and antioxidant levels have been identified as biomarkers for T2DM.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes. It is characterized by a cluster of metabolic dysfunctions and cardiovascular risksincluding obesity, insulin resistance, dyslipidemia, atherosclerosis, hypertension, prothrombotic state, and endothelial dysfunction collectively known as the metabolic syndrome (Gross B*et al*., 2007). In spite of worldwide research, the cause and risk factors remain a mystery or more appropriately it has a huge list of risk factors, and the medication is aimed to keep the sugar levels in check. Hence it is believed that environmental and demographic risk factors (e.g., obesity and sedentary lifestyles) also give rise to T2DM. Moreover, the association between lean first-degree family members of diabetic patients with insulin resistance shows the prominent role of genetic factors in this disease (Rhee *et al*., 2006). Recent findings have shown that polymorphism of some genes can influence the risk of T2DM. To illustrate, one of these is Peroxisome proliferator-activated receptor-GAMMA (PPAR-γ) gene polymorphism. PPARs have been identified to be ligand-activated transcription factors that belong to the nuclear receptor super family (Guo *et al*., 2006). PPAR-γ also influences greatly the risk of type 2 diabetes. PPAR γ is a member of the nuclear hormone receptor super family that plays an important role in cellular differentiation and carcinogenesis as well as regulation of metabolism, glucose and lipid homeostasis, and intracellular insulinsignaling events (Michalik *et al*., 2004). PPAR γ exists as two isoforms, γ1 and γ2, generated by alternative promoters and differential splicing of at least three different transcripts

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from the PPAR γ gene on chromosome 3p25. Several polymorphisms in PPAR γ2 have been identified so far and one of the common structural polymorphism in the PPAR γ2 gene was identified as CCA-to-GCA (Pro12Ala) missense mutation in codon 12 of exon B (Yen *et al*., 1997). This substitution possibly results in a conformational change in protein structure and reduced function of the PPAR γ2 gene. In spite of the fact that the association between Pro12Al polymorphism of the PPAR-γ2 gene with insulin sensitivity in diabetic patients has been reported in some populations, (Horiki *et al*., 2004; Tavares *et al*., 2005) a number of conflicting results have heightened the need for further investigation (Stefanski *et al*., 2006; Hamada *et al*., 2006; Stumvoll *et al*., 2002). However such associations have never been discussed by the Unani system of Medicine. Thus, this study was designed to examine whether the association between polymorphism of the PPAR-γ2 gene and T2DM exists in Damvi temperament of an Indian population. Diabetes mellitus is characterized by hyperglycaemia together with biochemical alterations of glucose and lipid peroxidation (Pari *et al*., 2002). Diabetes mellitus is considered to be one of a rank of free radical diseases which propagates complications with increased free radical formation (Baynes, 1991; Varvarovska *et al*., 2002). Oxidative stress is increased in diabetes mellitus owing to the increase in the production of oxygen free radicals and a deficiency in antioxidant defense mechanisms (Gillery *et al*., 1989; Hunt *et al*., 1989). Lipid peroxidation of cellular structures, a consequence of increased oxygen free radicals, is thought to play an important role in atherosclerosis and micro vascular complications of diabetes mellitus (Velazquez *et al*., 1991). Role of GST has also been implicated in the production of ROS. The rise in ROS could also be due to its production in various tissues under diabetic conditions- hyperlipidemia induces ROS through activation of the Glycation reaction and electron transport chain in mitochondria. The formation of AGEs accelerates under the hyperglycemia (Hideaki Kaneto *et al*., 2010). Advanced Glycosylation end products (AGEs), insulin and Angiotensin - 2 also induce ROS through activation of membrane bound NADPH oxidase. Hyperlipidaemia has also been reported as one of the causative factors for increased lipid peroxidation in diabetes mellitus (Hideaki Kaneto *et al*., 2010, Nacitarhan *et al*., 1995; Losada *et al*., 1996).

MATERIALS AND METHODS

Selection of Study Subjects

This study was approved by the Institutional Ethics Committee of CRIUM, following the principles of Helsenki Declaration for patient consent. A specially designed case record form as per the Unani classical text was designed for assessment of humors and the information obtained were recorded from healthy volunteers as well as patients. A total of 100 patients of Type 2 Diabetes were selected on the basis of clinical history by Unani physicians from the OPD clinic of Central Research Institute of Unani Medicine (CRIUM, Hyderabad). To clarify, T2DM was determined by a fasting plasma glucose level after a minimum fast of 12 h or a 2-h post-glucose level were done. The clinical features, their family history and other triggering factors were noted in the CRF proforma as followed by the Unani system of medicine. Generally, Unani system of Medicine recognizes mainly four categories of classification of patients such as Damvi (Sanguine), Balghami (Phlegmatic), Safravi (Bilious) and Saudavi (Melancholic).

A control group of individuals belonging to damvi temperament were selected; they were free of a positive family history of Type 2 Diabetes and other chronic diseases. 100 healthy volunteers served as the controls; they were also systematically examined by Unani physicians at CRIUM and also confirmed by doing fasting plasma glucose levels and 2-h post-glucose levels. As per the Unani concept the disease diabetes is the resultant the patients with a temperament of damvi (sanguine) were identified by the clinical phenotyping criteria as described in Unani classical texts. Healthy volunteers were also identified by the clinical phenotyping criteria and they also belonged to the damvi temperament as in the case of patients. A specially designed case record form as per the Unani classical text was designed for assessment of humours and the information obtained were recorded from healthy volunteers as well as patients. The subjects and patients have also filled in the willingness form to take part in the research process. The CRF"S were then analysed for their dominant clinical phenotype or temperament based on the Unani classics. The dominant clinical phenotype or the temperament obtained in these patients and healthy volunteers had the dominance of the humour -"Dam" which was obtained by a scoring pattern based on clinical phenotyping assessment. After assessment of the dominant clinical phenotype they were further subjected to blood sample collection, genomic DNA isolation, identification and genetic profiling of relevant gene by PCR in relation to the disease Diabetes and also the dominant clinical phenotype.

Blood Sample Collection

Five ml of whole blood was collected by veni puncture from 200 subjects (100 with Type 2 Diabetes and 100 controls). Blood was collected in sterile syringes and was transferred to K2 EDTA vacutainers. The collected samples were transported in an ice box to the molecular biology laboratory. The blood samples were stored at -4°c in refrigerator till further use. Blood were drawn after the approval by the Institute's Ethics Committee (IEC) and after the sanction of the project by the Scientific Advisory committee. Informed consent was taken from the patients prior to collection of blood samples. Biochemical parameters of the study subjects Plasma glucose levels (fasting and postprandial), total cholesterol, TGs, HDL-C, LDL-C, VLDL-C, liver function tests (Bilirubin, SGPT, SGOT and ALP and renal function tests (Urea, creatinine)) were also analyzed by using an Erba Auto Analyser. Results have been presented in Table 2.

Estimation of Serum Lipidperoxidase (MDA) Levels by Enzyme-linked Immunosorbent Assay

Serum levels of lipid peroxidation marker, malondialdehyde (an oxidant) in patients with Diabetes and controls were measured by enzyme-linked immunosorbent assay (ELISA) using the Lipid peroxidation (MDA) assay kit, the kit was supplied by Sigma-Aldrich, USA. Results have been presented in Table 6.

Estimation of Serum Glutathione S Transferase Levels by Enzyme-linked Immunosorbent Assay

Laboratory analysis of GST activity was done by glutathione s Transferase assay kit (supplied by sigma catalog no CS0410)GST activity measured 96 well plate wave length of 340nm under standard condition the amount of enzyme

conjugating one micromole of 1-chloro-2,4-dinitrobenzene (CDNB) with glutathione (GSH) in one minute was defined as one unit activity (µmol/ml/min). Results have been presented in Table 6.

Genetic analysis

For genetic analyses, genomic DNA was isolated from peripheral blood samples by using HiPurA™ blood genomic DNA Purification Kit a column based DNA isolation kit, to get high yield. The DNA was then dissolved in Elution buffer, quality and quantity was checked by agarose gel electrophoresis and nanodrop reading by using Multimode reader. DNA was then stored at -20°C until further use. PCR amplification and genotype determination of Proline 12 alanine (Pro12Ala C→G) was performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism the procedures of PCR-RFLP are given in Table 1 (Liao *et al*., 2006). The appropriate primers were used to amplify the corresponding gene by PCR and the reaction products were digested by using the appropriate enzyme at 60°C. The digested products were analyzed on 2.5% agarose gel stained with ethidium bromide and examined under transillumination. The results after restriction digestion for each gene arepresented in Table 1. Also the genotypes correlated to demographic parameters are presented in Table-3 & 4.

Statistical Analysis

Statistical analyses were performed using the Student's t test. Data are expressed as means + SD. Differences in clinicopathological characteristics between patients and controls were tested by chi-square test for categorical data and Student's t-test for numerical data. Odds ratio (OR) and 95% confidence interval (CI) for the association between genotype and diabetes was computed. A two-sided p-value of less than 0.05 was considered statistically significant.

RESULTS

We present here our results on various biochemical parameters in Damvi subjects suffering from diabetes –(T2DM) and compared it with the non-diabetic healthy controls. Demographic parameters were also recorded for each patient and each control. This is a case control study consisting of 100 cases and 100 controls. All these subjects were selected from those attending the Unani hospital (CRIUM) Hyderabad. Selection of these subjects was based on Unani principles. All the results are presented after statistical analysis in various tables. In this study we found more males than females. Blood samples collected from the study group (100 patients and 100 controls) were divided into differentportions for different types of studies. 1ml sample was used for genotyping studies and the rest for biochemical studies as mentioned above. Similarly the biochemical parameters were also recorded as compared to controls. (Table 2)

Fasting blood sugar

The results of pre and postprandial blood sugar of controls were 99 ± 25 and 99 ± 25, whereas for patients it was 156 ± 57 and 156 ± 57, Both these values were statistically significant ($p<0.0001$) (Table-2)

Lipid profile of the patients

The results of these experiments are presented in Table-2 We found significant differences in the lipid profile of patients when compared to controls.

Other biochemical parameters

The mean (± S.D.) urea level in cases was found to be 28 ± 7.5 whereas in control group it was found to be 24 ± 5.3 ($p= 0.0001$). The mean (± S.D.) creatinine level in cases was found

Table 1. PCR and RFLP Procedures and Products of PPARγ Pro12Ala Gene

Gene	Gene Primers (forward and reverse)	PCR product	Restriction enzyme	Restriction Products
PPAR γ Pro12Ala (Liao et al., 2006)	5'-GCC AAT TCA AGC CCA GTC-3' 5'-GAT ATG TTT GCA GAC AGT GTA TCA GTG AAG GAA TCG CTT TCC-3	270bp	BstUI	CC: 270 bp GG: 43 bp GC: 270 bp, 227 bp,43 bp

Table 2. Clinical and biochemical characteristics of type 2 Diabetic and control subjects

Parameters	Reference Values	Controls	Diabetic Patients	*p* Value
1.Gender				
Male		54	55	
Female		46	45	
2.BMIunits of kg/m^2		27 ± 5.1	28 ± 4.7	<0.0253
3.Glucose level				
FBS	65-110 mg/dL	99 ± 25	156 ± 57	<0.0001
PLBS	90-140 mg/dL	142 ± 50	247 ± 89	<0.0001
4.Lipid Profile				
Triglycerides	25-160 mg/dL	137 ± 63	174 ± 89	0.0009
Cholesterol	50-200 mg/dL	192 ± 35	212 ± 48	0.0007
HDL	35-80 mg/dL	43 ± 12	40 ± 5.7	0.075
LDL	<100 mg/dL Optimal	121 ± 31	141 ± 43	0.0003
VLDL	</= 30 mg/dL	27 ± 9.4	32 ± 10.4	<0.0001
5. Liver function tests				
ALP	15-113 IU/L	84 ± 25	90 ± 27	0.1046
Bilirubin	0.1-1.2 mg/dL	0.7 ± 0.34	0.7 ± 0.35	0.999
ALT	0.5-40 IU/L	26 ± 12	25 ± 11	0.539
AST	0.5-40 IU/L	26 ± 13	25 ± 10	0.542
Urea	13 – 45 mg/dL	24 ± 5.3	28 ± 7.5	<0.0001
Creatinine	0.7-1.4 mg/dL	1.0 ± 0.15	1.0 ± 0.19	>0.999

*p value < 0.05 was considered as Significant.

Values are in mean + SD, *P* value by student's t- test, BMI: body mass index, FBS:Fasting blood sugar, PLBS: Postprandial plasmaglucose, HDL:High-density lipoprotein, LDL: Low-density-lipoprotein, VLDL: Very low density lipoprotein, ALP:Alakaline phosphatase, ALT:Alanine amino transferase, AST:Aspartate amino transferase.

Table 3. GC Genotypes- Clinical and biochemical characteristics of type 2 Diabetic and control subjects

Parameters	Reference Values	Controls	Diabetic Patients	*p* Value
1.Gender +Genotype				
Male-GC		11%	15%	
Female-GC		07%	08%	
2.BMIunits of kg/m^2		25 ± 5.1	29 ± 2.2	<0.001
3.Glucose level				
FBS	65-110mg/dl	104 ± 33	156 ± 43	<0.0001
PLBS	90-140mg/dl	138 ± 31	278 ± 96	<0.0001
4.Lipid Profile				
Triglycerides	25-160 mg/dL	145 ± 42	158 ± 31	0.219
Cholesterol	50-200 mg/dL	204 ± 38	226 ± 40	<0.0001
HDL	35-80 mg/dL	43 ± 4.3	39 ± 6.6	0.015
LDL	<100 mg/dL Optimal	129 ± 31	157 ± 30	0.0021
VLDL	</= 30 mg/dL	31 ± 11.7	30 ± 6.7	0.712
5. Liver function tests				
ALP	15-113 IU/L	87 ± 29	88 ± 33	0.909
Bilirubin	0.1-1.2 mg/dL	0.7 ± 0.2	0.6 ± 0.1	0.0005
Urea	13 – 45 mg/dL	24 ± 6.1	28 ± 6.6	0.015
Creatinine	0.7-1.4 mg/dL	1.0 ± 0.13	1.0 ± 0.2	0.031

Table 4. CC Genotypes- Clinical and biochemical characteristics of type 2 Diabetic and control subjects

Parameters	Reference Values	Controls N=100	Diabetic Patients N=100	*p* Value
1.Gender +Genotype				
Male-CC		43 %	40%	
Female-CC		39%	37%	
2.BMI units of kg/m^2		27 ± 5.0	28 ± 5.0	0.1589
3.Glucose level				
FBS	65-110mg/dl	100 ± 26	155 ± 59	<0.0001
PLBS	90-140mg/dl	143 ± 52	240 ± 87	<0.0001
4.Lipid Profile				
Triglycerides	25-160 mg/dL	135 ± 63	174 ± 94	0.0007
Cholesterol	50-200 mg/dL	191 ± 38	209 ± 49	0.0032
HDL	35-80 mg/dL	43 ± 4.3	39 ± 6.6	0.015
LDL	<100 mg/dL Optimal	120 ± 32	137 ± 45	0.0024
VLDL	</= 30 mg/dL	26 ± 8.6	32 ± 10	<0.0001
5. Liver function tests				
ALP	15-113 IU/L	83 ± 24	90 ± 26	0.0493
Bilirubin	0.1-1.2 mg/dL	0.7 ± 0.3	0.7 ± 0.3	0.999
Urea	13 – 45 mg/dL	24 ± 4.9	28 ± 7.7	<0.0001
Creatinine	0.7-1.4 mg/dL	1.0 ± 0.1	1.0 ± 0.1	0.999

Table 5. Frequencies of PPAR γ Genotype Distributions in Patients with Diabetes and Controls

Gender		Cases(n=100)	Controls(n=100)	P-Value
Male	CC	52.2±11.14	38.43±11.99	<0.0001
	CG	53±10.84	39.8±11.66	<0.001
Female	CC	51.05±7.40	36.30±9.82	<0.001
	CG	52.42±8.12	35.85±2.35	<0.001
Age Distribution				
<50yrsMales	CC	40.84±4.57	34±8.52	<0.0001
	CG	42.16±2.11	34.75±6.35	<0.0001
<50yrs Females	CC	57.38±4.17	34.47±7.76	<0.0001
	CG	Not found	35.85±2.35	
>50yrs Males	CC	59.43±9.35	56.75±4.60	0.0111
	CG	62.28±5.25	60.3±3	0.0002
>50yrs Females	CC	45.05±4.03	58.33±2.35	<0.0001
	CG	52.42±8.12	Notfound	

Table 6. Serum concentration levels of Lipidperoxidase and Glutathione S transferase in Diabetic patients and controls

Parameters	Control group(n=100)	Patients(n=100)	P-value
Lipid Per oxidation (MDA)nmole/µL	1.34±0.33	4.05±1.45	0.001
Glutathione S transferase(µmol/ml/min)	1.099±0.63	1.35±0.67	0.007

Data are expressed as mean ±SD, p value <0.05 was considered statistically significant.

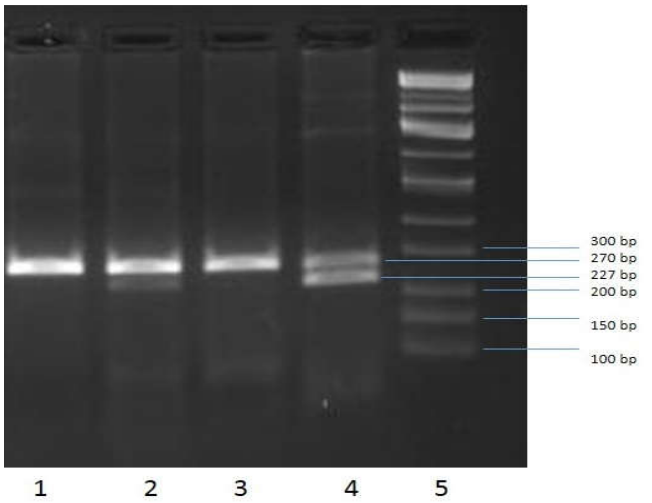
to be 1.0 ± 0.15 whereas in control group it was found to be 1.0 ± 0.19 (p= >0.999). (Table-2)

Frequency of the genotypes

The genotyping results were correlated with the demographic parameters of the subjects and presented in tables (3, 4).

Age wise distribution of the Genotypes is shown in Table (5). We found more of CC genotypes in both cases and controls and less number of the hetero genotypes CG in cases compared to controls. The homo genotypes GG were not found in this group of patients as well as controls. We found that BMI was not significant between cases and controls. (p= 0.02) (Table- 2)

however after genotyping we could find that the GC genotypes showed higher BMI values (Table-3)



This gel picture shows the results of PPAR-gamma 2 gene Pro12Ala polymorphism digested by BstUI ||. Lane 1 for uncut; lanes 2 and 4 for Pro12Ala heterozygotes; lane 3 for Pro12Pro homozygote; lane 5 for 100 bp marker.

Fig.1. Typical representation of PCR amplification and Genotyping of PPAR-Gamma –Pro 12 Ala gene polymorphism

Levels of Serum Lipid peroxidase and GST

We found a very significant rise in the levels of LP and GST in diabetic subjects (p=0.001) as compared to controls. (Table- 6)

DISCUSSION

The PPAR-gamma gene, which is located on chromosome 3, can induce the transcription of target genes after ligand-dependent activation or in a ligand-independent manner. Several genetic variants in the PPAR-gamma gene have been described, the most prevalent being the missense mutation in the PPAR-gamma 2 codon 12 of exon B, involving a C→G substitution at nucleotide 34 that resulted in the exchange of alanine for proline in the position of the PPAR-gamma 2 protein. The non-conservative substitution of proline for alanine may cause a conformational change in the protein and lead to a reduction in the transcriptional activity of PPAR-gamma 2. The frequency of the Ala allele in the control group was consistent with that of the Han population in Beijing and Guangdong in China and that of Japanese, (Hara *et al*., 2000) whereas the frequency of the Ala allele was significantly lower than that in Uygur population (10.4%) of Xinjiang in China and that in Americans (Ridker *et al*., 2003). These data indicate that there is an ethnic difference in Pro12Ala genotype and allele frequency distribution. Tavares *et al*. showed that carriers of Ala12 allele of the PPAR-γ2 gene were more sensitive to insulin resistance when compared to Pro12 carriers in a Brazilian population (Ridker *et al*., 2003). The protection against diabetic nephropathy in Brazilian diabetics caused by presence of Ala allele was reported elsewhere (Tavares *et al*., 2005) It was demonstrated that Ala allele brought about lower development of T2DM in Caucasians (Huguenin *et al*., 2005) Furthermore, our findings were in accord with those of Mori *et al*. in which they found Ala variant of PPAR-γ was associated with a reduced risk for the development of diabetes in Japanese subjects (Mori *et al*., 2001) An association was also reported between increased risk of T2DM and Pro allele of PPAR-γ2 gene in a Russian population (Chistiakov *et al*., 2010) In this study we observed that several biochemical parameters were modulated in diabetic conditions in both male and female patients. However, these Damvi patients did not show raised obesity parameters (<30), except when compared to controls. (as shown in Table-2) Since lipidperoxidation had increased in patients as compared to controls. This can lead to complications as the subjects are unable to deal with the rise in ROS due to the loweredantioxidants leading to hyperlipidemia which causes complications in the patients. ROS is generally produced under diabetic conditions leading to chronic hyperglycemia and subsequent augmentation of ROS deterioration of beta -cell function and increase in insulin resistance (Howard *et al*., 1987; Taskinen, 1992). ROS are also induced by Hyperglycemia and/or Hyperlipidemia under diabetic conditions, which lead to the activation of JNK pathway in pancreatic beta cells. JNK pathway is activated – and hence involved in T2DM, involved in beta cell dysfunction and hencemay lead to T2DM (Young *et al*., 2001).

Similarly, we found that GST levels were also significantly high (p-0.007) in Diabetic patients.Glutathione-s-transferases (GST) are major cytosolic phase II detoxification enzymes which inactivates reactive electrophiles by Glutathione (GSH) dependent mechanism. These enzymes also carry out a range of other functions – they have peroxidase and isomerase activity – they can inhibit the JunN terminal kinase, thus protecting cells from H_2O_2 induced cell death. They are able to bind non-catalytically endogenous and exogenous ligands. GST enzymes are produced to scavenge the ROS and Xenobiotics in normal situations. However, in diabetes the enzyme levels keep on increasing due to absence of its substrate. The increased levels of GST enzymes indicate that their thiol levels have been significantly damaged. Because increase in the level of glucose by insulin resistance by free radicals inhibitsthesignaling pathways, leading to the continuous synthesis of some aminoacids in diabetic cases- as it happens in case of Cysteine synthesis which can increase thus leading to an increased level of GST in T2D compared to healthy controls (Giacco *et al*., 2010). Abnormal lipid metabolism often presents in patients with T2DM. Hypertriglyceridaemia usually accompanies decreased HDL-C, which is also a prominent feature of plasma lipid abnormalities seen in diabetic subjects (Henriksen *et al*., 2010). The low level of HDL-C, which exerts anti-atherogenic and antioxidative effects when present in sufficient amounts, is a key feature of T2DM. The reduced HDL-C levels are often accompanied by elevations in plasma TG levels (Lamarche *et al*., 2010) a process mediated by cholesterol ester transfer protein (CETP). Resistance to insulin likely underlies the changes that occur in lipid parameters of T2DM, and usually it is associated with higher concentrations of total cholesterol and triglycerides, and lower concentrations of HDL-C. The mechanism responsible for hypertriglyceridaemia may be an increased hepatic secretion of VLDL and a delayed clearance of TG-rich lipoproteins, which might mainly be due to increased levels of substrates for TG production, free fatty acids, and glucose. DM is the major cause of renal morbidity and mortality, and diabetic nephropathy is one of chronic kidney failure. Blood urea and creatinine is widely accepted to assess the renal functions. Good control of blood glucose level is absolute requirement to prevent progressive renal impairment. In order to monitor the control of blood glucose level along with blood sugar blood urea can also be important parameter as we found that there is strong correlation of blood sugar and urea level.

Conclusion

It is concluded that the major antioxidant enzymes- GST and LP were significantly affected due to T2DM. Further interesting genotypes were evident in our study as there were more CC genotypes and less number of the hetero genotypes (CG) in cases compared to controls. Surprisingly the homo genotypes GG were absent in this Damvi population of both patients and controls. A significant increase ($p<0.001$) in malondialdehyde (MDA) levels and GST levels appeared to be a key feature in Damvi subjects, hence detection of Antioxidant enzyme levels can be useful biomarkers for early detection of the disease.

Conflict of Interest: None declared

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