International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.12, pp 668-680, 2016

Telomerase enzyme and glutathione peroxidase 1gene as a risk factor in diabetes mellitus type 1 patients in Babylon province

Duaa M. Hadi[1], Maha F. Smaism[2]*, Ali H. Albayati[3]

[1]Student in Clinical biochemistry Dept.,College of Medicine, University of Babylon,Iraq
[2*]Clinical biochemistry Dept.,College of Medicine, University of Babylon, Iraq
[3]College of Medicine, Babylon University,Iraq.(consultant MD/FACE).

Abstract : Type 1 diabetes mellitus is a chronic autoimmune condition. Telomerase is an enzyme that maintainstelomere length in nuclear DNA. The aim of the study is to investigate the relationship between telomerase and glutathione peroxidase 1 GTPX1 concentrationand GPX1 pro 198 Lus gene in diabetes mellitus type 1.This study include (108) persons, their ages between (28-42 years) and body mass index in normal and overweight (68) of them were uncontrolled diabetes type 1 (HbA1c ≥ 6.5), (34) of them are male (M)patients, the other (34) are female(F) patients, and the other (40) apparently healthy as control (C)group included the (20) male(MC) with (20) female(FC).
The present study,observed, a significantly increase in glucose, HbA1c and glutathionein patients when compared with control groups,while significant decrease in telomerase enzyme, TAO-C and GPX1.The results showed significant negative correlation between telomerase enzyme concentration and BMI in patients and control. Also, there is a significant differences in GPX1 levels of TT genotypes in patients compared to CT and CC genotypes, the frequency of TT genotypes in GPX1 gene in male and female were 47% and 61.7% compare to control male(0%) and female(10%) and the odd ratios were(33, CI95% 1.65-656.26) (12.6, CI 95% 1.93-82.08), respectively. there is significant different in glutathione peroxidase and in telomerase concentration depending on different genotyping of GPX1 gene. Conclusion:The TT genotype of the GPX1 gene variation pro 198 Luswas a risk factor to T1DM patients. TIDM is associated with glutathione peroxidase and telomerase concentration in subjects with TT genotypes of GPX1 gene compared to those with CT and CC genotypes.
Key words : telomerase, glutathione peroxidase, type 1 DM, genotypes.

Introduction

Diabetes is a group of heterogenic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both[1]. In along-term happened the damage, dysfunction, and impairmentin different organs, especially the kidneys, nerves,eyes, heart, and blood vessels[2]. Type1Diabetes Mellitus (T1DM) is a chronic autoimmune condition[3]. Its rats for about 10% of all diabetic[4].Telomere are a special heterochromatic structure caps the ends of linear chromosomes, which protects them from degradation and repair activities, and which is therefore essential for ensuring chromosome stability[5].Telomerase is traditionally known as an enzyme that keeps up the telomere length in nuclear DNA, its action is conversely associated with

endothelial senescence, increased telomerase activity can also protect againstreactive oxygen species ROS-induced endothelial dysfunction[6].In normal conditions and in some diseases such asmitochondria and DM, are the sources of reactive species RS[7]. The antioxidant defense system which include vitamins, glutathione,micronutrients and small molecules [9]. In DM this defense system becomes failure and resulting in oxidative stress[10]. Glutathione peroxidase is an important part of the antioxidant defense system[11].GPx needs secondary enzymes (glucose-6-phosphatedehydrogenase andglutathione reductase) and cofactors (NADPH, reduced glutathione and glucose 6-phosphate) to function at high capacity. Glutathione peroxidase1 GPx1 gene .Also known as GPXD and GSHPX1 The protein encoded by this gene belongs to the glutathione peroxidase familyGPX1, GPX2, GPX3, GPX4 and GPX6 utilize a UGA codon that specifies insertion of a selenocysteine residue containing the selenocysteine (Sec,rare amino acid) at its active site which is critical to protein function, members of which catalyze the diminishment of organic hydroperoxides and hydrogen peroxide (H_2O_2) by glutathione, and accordingly protect cells against oxidative damage[12]. GPX1 is found at high levels in tissues presented to high oxygen tensions such as in the lungs, at the cellular elements of blood, liver, kidney and pancreas, furthermore at moderate levels in heart, muscle and brain[13].Glutathione peroxidase1 (GPx1) is an intracellular soluble selenoprotein which transform peroxides such as H_2O_2 ROOH into water and alcohol, The rs1050450 (Pro198Leu) polymorphism is a site located within the GPx1 C-terminal region[14].The proline (CCCCodon) to leucine (CTCCodon) substitution is related to the risk of breast cancer and coronary artery disease[15].

Subjects and methods

Patients

68 patient includes 34 male (M) and 34 female (F) with type1DM, and 40 as controls(C) includes 20 female (FC group) and 20 male (MC group) there age were between (28-42)years at normal and overweight BMI, recruited at Babylon Center for Diabetes and Endocrinology in Margan Teaching Hospital in Babylon / Hilla city. from October 2015 to April 2016. All samples of these groups were diagnosed by physicians.

Ethical Issues:

Approvalby scientific committee in University of Babylon,College of Medicine /Iraq).

Collection of samples

Venous blood tests were drawn from controland patient groups. Seven ml from blood , then put 3 ml in the tubes gel containing, then the serum were obtained until analysis of telomerase and GPX1.The remaining blood (2ml) put in EDTA tubes and was used in the genetic analysis.

Determination the biochemical parameters:

Serum glucose concentration(Fasting) ,glycatedhemoglobin (HbA1c) in whole blood , serum total antioxidant capacity (TAO-C), serum glutathione (GSH) concentration, glutathione peroxidase 1 GPx1 and telomerase concentration are measured by ELISA-kit.

Genotyping analysis

The extraction and purification of the DNA depend on the several studies [16-19].Purity of DNA was surveyed by nanodrop device and electrophoresis on 1% agarose gel as shown in figure 1.

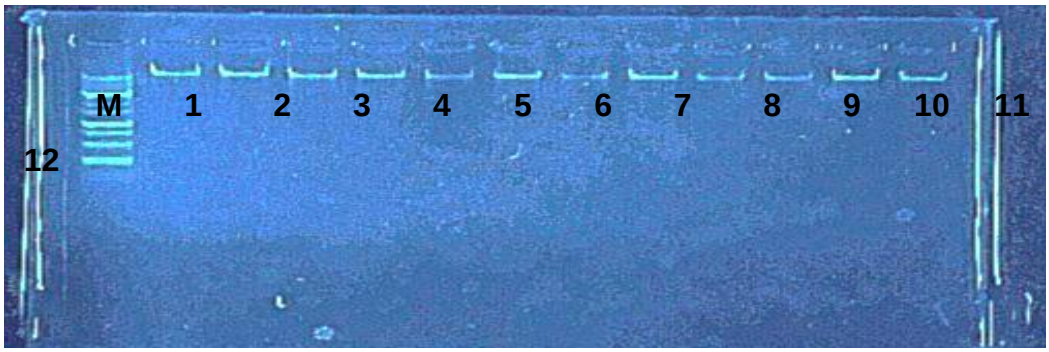

Fig.(1):The DNA extraction in electrophoresis on 1.4% agarose gel.

Amplification C/T codon 338 of GPX1 gene by using the primers[20], table (1).

Table (1): Primer amplification of GPX1 genes.

Primer F+R (5'-3')	Gene	annealing temp.	Amplicon length
F: TGTGCCCCTACGGTACA R:CCAAATGACAATGACACAGG	GPX1	47 C°	338 bp

Amplification conditions forPCR-RFLP analysis of GPX1gene (table 2)

Table (2): Amplification conditions of GPX1 gene

Stage	Temp.(C°)	Time	Function	Cycles
1	95	4 min.	Initial denaturation	
2	95	30 sec.	DNA denaturation	40
	47	1 min.	Primer annealing	
	72	1 min.	Template elongation	
3	72	10 min	Final elongation	
4	8	-	Incubation	Hold

The PCR products for GPX1 gene was electrophoresis on 1.4% agarose gel and staining with syper green to photo documentation.

Statistical analysis

All data were analyzed by student's t-test (SPSS) version 20. Theresults were expressed as mean ± SD. The groups analysis by ANOVA and Fisher-Exact test.The result considered significant at P-Value < 0.05.

Results

Clinic-pathological characteristics of control and patients whose included in this study were showes in table (3).

Also the result shows that fasting serum glucose significantly increased in M and F groups as compared with MC and FC groups respectively at P-value <0.0001, HbA1c was significantly increased in patient groups as compared with control groups as shown in table (4).

Table (3): Age, Body mass index, and waist circumference in diabetic groups compared with control groups; and shows the duration of disease

Parameter	Patient		Control		p-value
	Group	Mean ±SD	Group	Mean ±SD	
No.	M	34	MC	20	-
	F	34	FC	20	-
Age (year)	M	34.058±3.28	MC	35.45±3.6	0.155
	F	34.94±3.37	FC	35.0±3.68	0.952
BMI (kg/m^2)	M	25.728±0.76	MC	28.02±0.6	< 0.001
	F	24.526±0.90	FC	27.62±0.78	< 0.001
Waist circumfer-ence (cm)	M	84.04±2.78	MC	88.84±1.82	< 0.05
	F	84.19±2.98	FC	88.70±1.96	< 0.05
Duration of disease (year)	M	6.67±2.29	MC	-	-
	F	7.0±3.03	FC	-	-

Table (4):Fasting glucose concentration and glycated hemoglobin% for patient and control groups.

parameter	Patient		Control		P-value
	groups	Mean±SD	groups	Mean±SD	
Glucose (mg/dl)	M	251.76±44.2	MC	97.8±10.44	<0.0001
	F	247.41±38.2	FC	104 ±24.0	<0.0001
HbA1c %	M	9.33±0.599	MC	4.94±0.57	< 0.05
	F	9.29± 0.71	FC	5.03±0.54	< 0.05

Also significantly decrease in telomerase enzyme and glutathione peroxidase concentration in patient ($p<0.001$), table 5.

Table (5):Telomerase and glutathione peroxidaseconcentrationin patient and control groups.

groups	No.	(Mean ± SD)	P value
Telomerase (ng/ml)			
Patients	68	3.43 ± 1.47	< 0.001
Control	40	8.63± 2.35	
glutathione peroxidaseng/ml			
Patients	68	23.34 ± 2.33	< 0.001
Control	**40**	**33.97 ± 3.33**	

Also, there is no significant differences ($P >0.05$) between male and female in patient and control groups, table 6.

Table (6):Telomerase concentrationin males and females in both patient and control groups.

parameter	Patient			Control		
	Group	Mean±SD	P-value	Group	Mean±SD	P-value
Telomerase (ng /ml)	M	3.8 ± 1.37	0.106	MC	8.93 ±2.39	0.327
	F	3.07 ±1.50		FC	8.35 ±2.34	

Adult telomerase concentration significant decrease with age ($p<0.001$) at a relatively constant rate fig.(2)and (3).

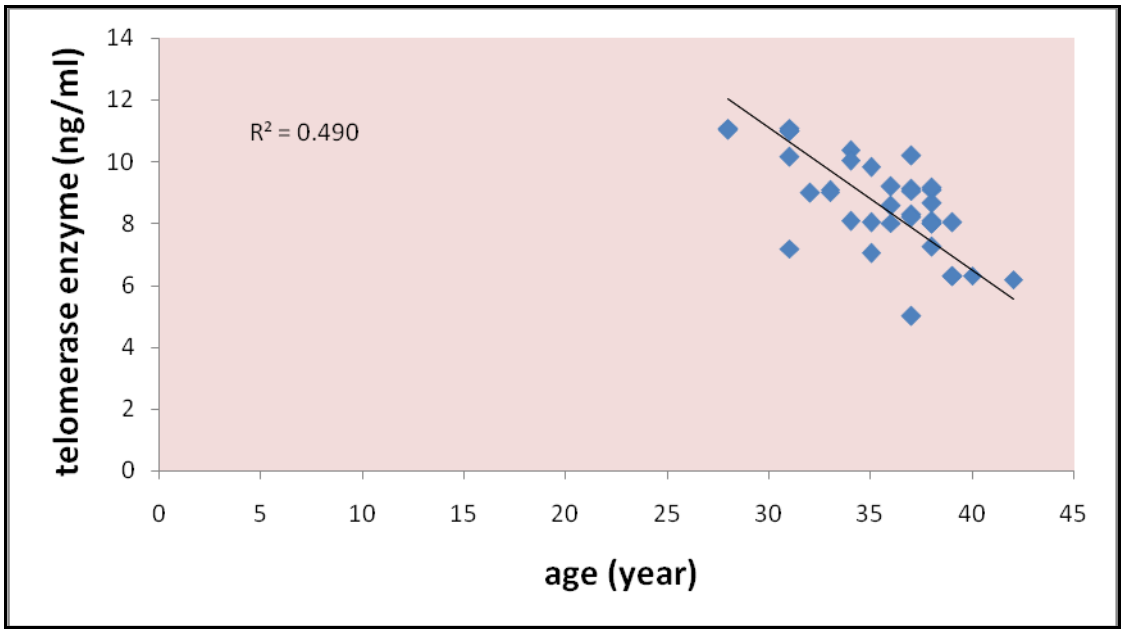

Fig.(2):Correlation between serum telomerase enzyme and age in control.

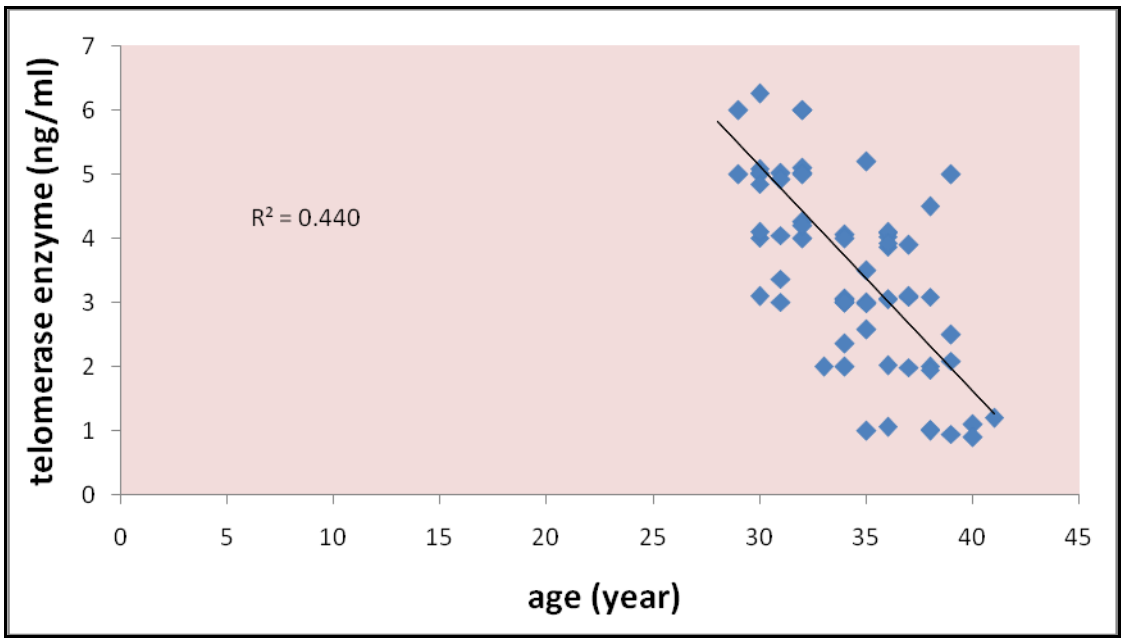

Fig. (3): Correlation between serum telomerase enzyme and age in patients.

The correlation of BMI with telomerase enzyme :

The correlation between telomerase enzyme concentration and BMI in patient and control groupssignificantly negative as shown in figure (4) and (5), respectively**.**

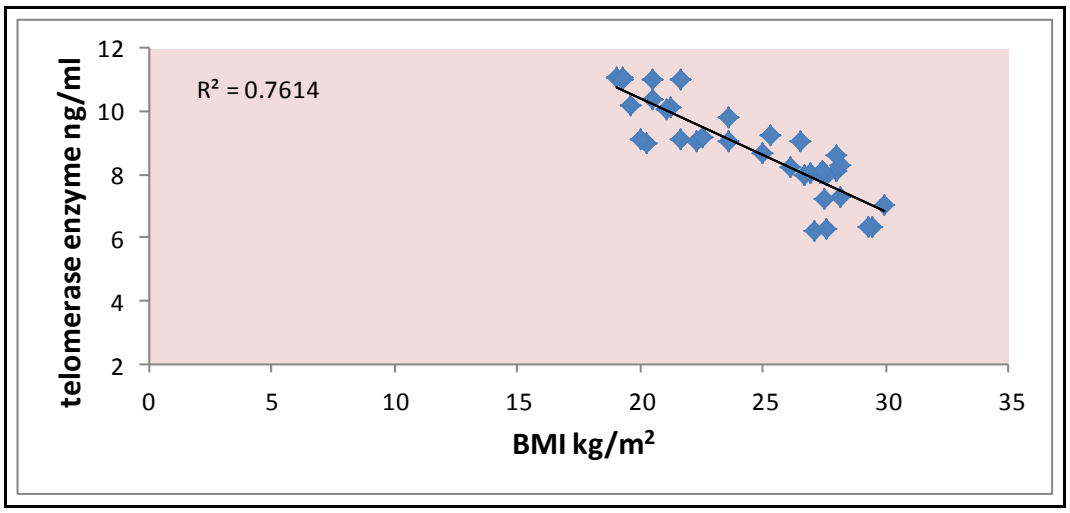

Fig.(4): The correlation between telomerase and BMI in control.

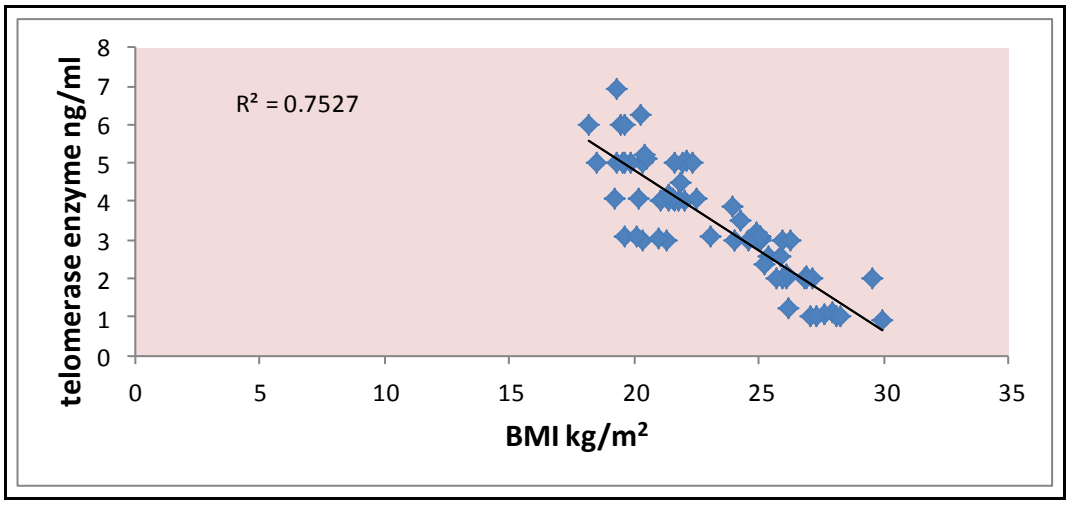

Fig.(5) :The correlation between telomerase and BMI in patients.

Total antioxidant capacity TAO-C, glutathione peroxidase (GPx) and glutathione (GSH) enzymes :

The results were show significant decrease in serum TAO-C, and GPxin patient as compare with control groups ($P < 0.001$), While there is significant increase in glutathione concentration between patient and control ($p < 0.05$), table(7)

Table (7):Total antioxidant capacity TAO-C, glutathione peroxidase (GPx) and glutathione (GSH) enzymes in patient and control groups

Parameters	Patient		Control		*p-value
	gender	mean±SD	Gender	mean±SD	
TAO-C Unit/ml	M	8.44 ±1.79	MC	16.25±1.48	<0.001
	F	8.058±1.61	FC	16.85±1.26	<0.001
GPx ng/ml	M	22.89±2.57	MC	33.15±2.23	<0.001
	F	23.80±2.00	FC	34.80±4.039	<0.001
GSH ng/ml	M	1.33±0.21	MC	1.05±0.17	<0.05
	F	1.34±0.19	FC	1.03±0.14	<0.05

When the oxidative stress increase as in diabetes patients the telomerase enzyme will decrease with decreasing in anti-oxidant defense system, (figure 6).

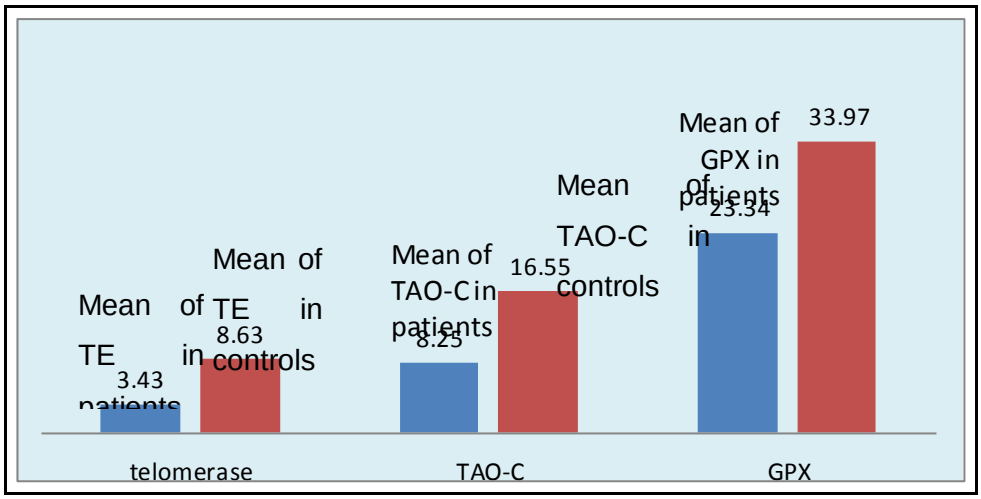

Fig. (6): The correlation between telomerase enzyme, total anti-oxidant and glutathione peroxidase concentration in patients and control.

Glutathione peroxidase 1 (GPx1) Genotyping :

The 198C/T polymorphism of Gpx1. The result showed band in 338 (bp) this due to the C/T polymorphic site in the Gpx1gene,figure7.

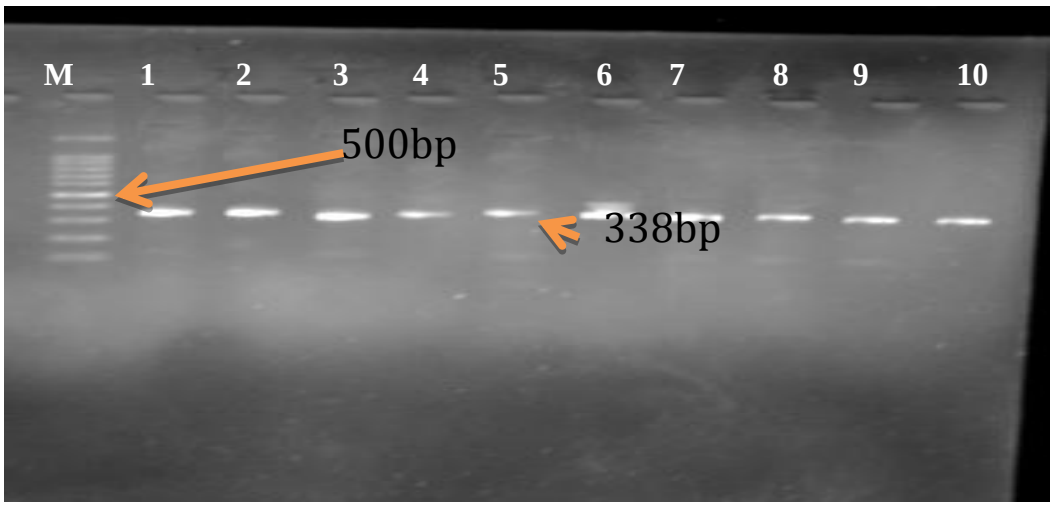

Fig.(7): Electrophoretic pattern of the Gpx1 genotyping, Lane M: DNA ladder, Lanes (1-10) are about 338 bp.

The amplification and digestion were showed two alleles(T and C) and three genotypes: CC(Pro/Pro) was digested by ApaI (RE) into two bands 257and 81, TT(Leu/Leu) .338bp and CT(Pro/Leu) has three bands 338, 257, and81 bp(fig. 8).

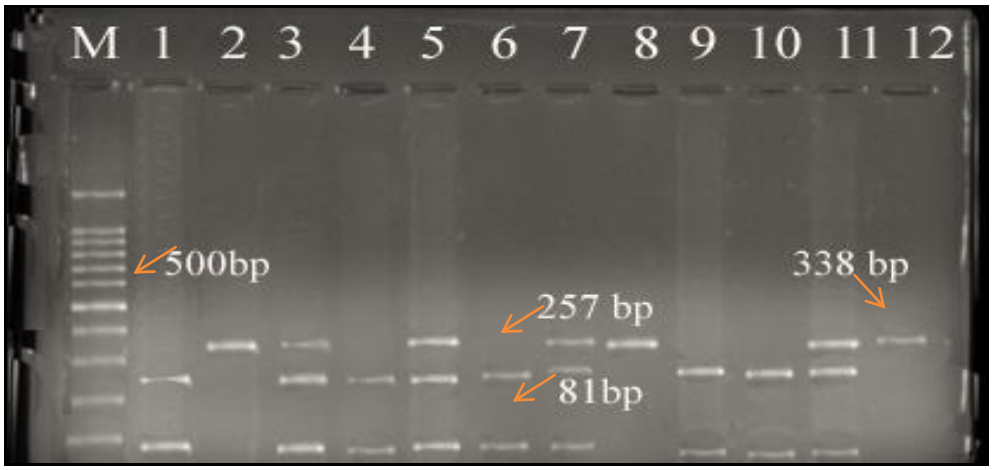

Fig. (8): Electrophoretic resultsfor codon 198 of Gpx1 genotyping , lane M : 100 bp DNA ladder , lane (2,8,12) :single band at 338 bp (TT), lane (3,5,7,11) : band at 338,257,and 81 bp (CT),and lane (1,4,6,9,10) : two bands at 257 and 81 bp(CC).

Genotypes and allele frequency association with disease:

Glutathione peroxidase 1 gene exon 10 codon 198 being Leu/Leu(TT genotype), Pro/Leu (CT genotype), and Pro/Pro(C/C genotype) homozygous, the comparison between C and T allele frequency and evaluated a recessive model of T allele(TT vs. CC+CT) and a dominant model of C allele(CC vs. TT+CT), as shows in table (8 and 9)

Table (8): Genotype of Glutathione peroxidase 1 gene variant in the study subjects.

Group	No.	Genotype			Odd ratio	*P-value	**CI 95%
		TT (-/-)	CT (-/+)	CC (+/+)			
M	34	16(47%)	11(32.3%)	7(20.5%)	33	0.0219	1.65-656.26
MC	20	0(0%)	13 (65%)	7 (35%)			
F	34	21(61.7%)	8(23.5%)	5(14.7%)	12.60	0.0081	1.93-82.08
FC	20	2(10%)	12 (60%)	6 (30%)			

Table (9):Allele frequency, odd ratio and p-value between patient and control in all samples, female and male.

All samples	Control		Patients		OR (95% CI)	P-value*
Allele	Count	Proportion	Count	Proportion		
T	37	0.46	93	0.68	2.51 (1.423-4.440)	0.0013
C	43	0.54	43	0.32	0.39 (0.225-0.703)	
Female	Control		Patients		OR (95% CI)	P-value*
Allele	Count	Proportion	Count	Proportion		
T	24	0.6	50	0.74	1.852 (0.807-4.251)	0.14
C	16	0.4	18	0.26	0.540 (0.235-1.240)	
Male	Control		Patient		OR (95% CI)	P-value*
Allele	Count	Proportion	Count	Proportion		
T	13	0.32	43	0.63	3.572 (1.565-8.152)	0.0020
C	27	0.68	25	0.37	0.28 (0.123-0.639)	

OR: odd ratio , CI: confidence interval , P*: p value of Pearson's chi-square test.

GPX1 genotype association with telomerase and glutathione peroxidase concentration :

There is significant affecting of GPX1 C/T genotype on telomerase and GPx(P-value 0.001) (P-value<0.0005),respectively, as shown in table(10)and (11)

Table (10) Genotypes association with telomerase enzymes

Telomerase (ng/ml)				
Genotype	**N**	**Mean**	**Std. Deviation**	**P-value**
CC	**20**	**4.7370**	**2.65608**	**0.001**
CT	**44**	**6.6184**	**3.45554**	
TT	**43**	**4.2972**	**2.45326**	
Total	**107**	**5.3339**	**3.11146**	

Table (11) Genotypes association with glutathione peroxidase enzymes

Glutathione peroxidase (ng/ml)				
GENOTYPE	**N**	**Mean**	**Std. Deviation**	**P-value**
CC	**21**	**29.5610**	**5.45009**	**<0.0005**
CT	**44**	**29.4732**	**5.16681**	
TT	**43**	**22.8758**	**5.12546**	
Total	**108**	**26.8635**	**6.10008**	

GPx1gene polymorphism and theBMI :

According to their body BMI (18.5- 24.9) kg/m^2 and BMI (25-29.9) kg/m^2, respectively. The normal weight patients versus normal weight controls, and in overweight patients versus overweight controls , as shown in table (12) and (13)

Table (12) : Genotype and allele frequencies of the gene variant in normal and over body mass index in patient and control.

Groups	No.	Genotype			OR (95%CI)	P value
		TT(-/-)	**CT(+/-)**	**CC(+/+)**		
N.W. patient	**39**	**18(46%)**	**15(38.46%)**	**6(15.38%)**	**25.61 (1.206-543.814)**	**0.0375**
N.W. Control	**17**	**0(0%)**	**13(76.47%)**	**4(23.5%)**		
O.W. Patient	**29**	**20(68.96%)**	**6(31.57%)**	**5(17.24%)**	**18.0 (2.93-110.3)**	**0.0018**
O.W. control	**23**	**2(8.69%)**	**11(47.82%)**	**10(43.47%)**		

N.W. = normal weight O.W. = overweight body mass index

Table (13):Allele frequencies of the gene variant in normal and overweight body mass index in patient and control.

Groups	No.	Allele frequency	
		C(+)	T(-)
N.W. patient	**39**	**34.62%**	**65.38%**
N.W. Control	**17**	**61.76%**	**38.23%**
O.W. Patient	**29**	**27.59%**	**79.31%**
O.W. control	**23**	**67.39%**	**32.6%**

Discussion

The mean ± SD of BMI for patient groups were (25.12±1.02), and for control groups were (27.82±0.717), and waist circumference for patient groups were (84.12±2.86), and for control groups were (88.56±1.87) the difference of BMI and waist circumference show significant decrease in patients as compared with controls. This result may be because there diabetic were not controlled and they have long term hyperglycemia, this agree with ADA study that shows patients rarely obese when they present with type 1 of diabetes, the presence of obesity is not incompatible with the diagnosis[21],and this disagreement with (N.Holman*et al*.,2015) study that believes there is a worrying trend towards being a higher BMI in children and young people with type 1 diabetes[22].The elevated level of fasting blood glucose concentration in patient due to their blood glucose is not utilized by all tissues leading to hyperglycemia which agree with (S.Rodriguez*et al*.,2014)[23], and this elevated may be due to the test was made at the fasting state and all patients were without external insulin treatment.

Through HbA1c can be monitor to the long-term glycemic control in diabetic patients, elevated HbA1c levels were associated with impaired insulin secretion and β-cell dysfunction even when insulin resistance was not increased [24,25].

The enzyme telomerase as a biomarker forbiological aging.[26]This study indicate that reduced in telomerase concentration in patient groups may be that a cause of impaired beta-cell. The result are supported by the reduced telomerase activity(shortened telomeres), and associated with type 2 and, since progressive beta-cell failure in type 1 DM [27,28], Also, the study shows no significant difference in telomerase concentration between male and female that's may be because of the telomerase is not gender related enzyme this result agreement with (podlevsky*et al*,. 2008)[29]. Telomere length in proliferative cells is inversely related to the total number of cell divisions, and therefore to biological age[30],and this agreement with the present study.

The result in figures (4 and 5) indicate that BMI including metabolic dysregulation are associated with reduced telomerase activity and shortened telomere length and this agreement with (J.Daubenmier *et al*., 2012) [31].Also, this may indicate a significant association between BMI and mortality rates in the patients with diabetes, and this is agreement with (Katzmarzyk., *et al*., 2013) which indicate that maintaining a healthy waist circumferences and BMI are both important for individuals living with diabetes [32], and in contrast to some previous studies that have documented inverse [33]. There is a complex interaction betweenobesity, insulin and leptin resistance, and the endocrine abnormalities in PCOS[34]. Hyperglycemia is among the causes for oxidative stress conditions, and increase in ROS generation [35]. The study show the level of TAO-C and glutathione peroxidase was significant decrease in patient as compared with control groups p(<0.001) this may be due to hyperglycemia and OS in DM (K.J.Davies*et al*., 2000) [36]Also, this decrease may be due to its association with reducing in their telomerase concentration that's agreement with (G. Saretzki, *et al*., 2009) which showed direct correlation between telomerase that shuttles to mitochondria and a decreased oxidative stress[37].

The result show significant increase of GSH concentration due to reducing the antioxidant reaction that converted it to GSSG, that supported by the GSH [38].Other studies found that GSH levels in type 1 DM patients were significantly less than that in their same age-matched control subjects. These results are disagreement with the present work[30,40].So that, when oxidative stress increase as in diabetes patients the telomerase will decrease with decreasing in anti-oxidant defense system, (figure 6).

The genetic result show significantly association in GPX1–198C/T polymorphisms between the

controls and patients. These results support the hypothesis that genetic variations may modify the risk of type1 diabetes [41]. The study showed significant different in GPX level of TT genotype in patients compared to CT and CC genotype and this may be as a result to increase oxidative stress because hyperglycemia and over production of ROS and so the results in agreement with other study[42], and the frequency of TT genotypes in GPX1 gene in female and male were 47% and 61.7% compare to control male(0%) and female(10%) and the odd ratios were(33, CI95% 1.65-656.26) (12.6, CI 95% 1.93-82.08), respectively. This results indicate that TT genotype was risk factors to diabetic patients. Other studies suggest that many anti-oxidant genes is not related to pathogenesis of diabetes but is associated with microangiopathy expressed as microalbuminuria[43]. Furthermore, this result show there are significant deference in C and T allele between patient and control P-value (0.0013) this differences is specially in male (P-value 0.14) while in females there are not have significant differences (P-value 0.0020).

The result of this study shows significant association of population between telomerase and GPX1genotype (p-value 0.001), and there was also significant difference in distributions of the genotype of the GPx1 Pro198Leu and concentration of glutathione peroxidase in the population (p-value 0.0005).So, that may be the reducing in telomerase concentration due to genetic factors predispose to the decrease in telomere length. Other study suggests that telomeres demonstrated high sensitivity to damage by a high content of guanines (increase the oxidative stress)[44].

Many publications suggested that relationwith increased risk of cancer and DM with the TT (Leu) genotype. In erythrocytes demonstrated significantly lower functional activity and this agreement with this study [45].Several different types of diseases related to defects in antioxidant pathways, such asDM, and cancer [46], previous studies examining the related between ROS and various diseases have revealed that excessive oxidative stress or decreased antioxidant activity can cause several pathologic states[47].

The observation of this study was eighteen mutant genotypes (TT) in patient's normal weight (46%) with zero in control normal weight (0%); in contrast there are twenty of this genotype (TT) in overweight patient (68.96%) with two in control overweight (8.69%),thatmean a single nucleotide polymorphism at codon 198 causing amino acid substitution with type 1 DM , and related with increased risk for disease ($p<0.05$).

References

1. Groop L, Pociot F. (2010), Genetics of diabetes - Are we missing the genes or the disease? Molecular and Cellular Endocrinology. 382(1): 726-739.
2. American Diabetes Association., 2015, Classification and diagnosis of diabetes. Diabetes Care. 38: S8-S16.
3. T. Ransom, R. Goldenberg, A. Mikalachki, A. P. H. Prebtani, and Z. Punthakee, (2013) "Reducing the Risk of Developing Diabetes," Can. J. Diabetes, 37(1): S16–S19.
4. J. Ozougwu, K. Obimba, C. Belonwu, and C. Unakalamba, (2013) "The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus," J. Physiol. Pathophysiol., 4(4):46–57.
5. de L. T. Palm W, (2008) "How shelterin protects mammalian telomeres.," Annu Rev Genet., 42: 301–334.
6. A. M. Beyer, J. K. Freed, M. J. Durand, M. Riedel, K. Ait-Aissa, P. Green, J. C. Hockenberry, R. Garret Morgan, A. J. Donato, R. Peleg, M. Gasparri, C. K. Rokkas, J. H. Santos, E. Priel, and D. D. Gutterman, (2016) "Critical role for telomerase in the mechanism of flow-mediated dilation in the human microcirculation," Circ. Res.,118(5):856–866
7. E. Birben, U. M. Sahiner, C. Sackesen, S. Erzurum, and O. Kalayci, (2012), "Oxidative stress and antioxidant defense.," World Allergy Organ. J., 5(1): 9–19.
8. A. Rahal, A. Kumar, V. Singh, B. Yadav, R. Tiwari, S. Chakraborty, and K. Dhama, (2014) "Oxidative stress, prooxidants, and antioxidants: The interplay," Biomed Res. Int., vol. 2014.
9. E. Lubos, J. Loscalzo, and D. E. Handy, (2011) "Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities.," Antioxid. Redox Signal., 15(7):1957–1997.
10. K. Rahman, (2007) "Studies on free radicals, antioxidants, and co-factors.," Clin. Interv. Aging, 2(2):219–236.
11. B. Halliwell and J. M. C. Gutteridge, (2015) Free radicals in biology and medicine. Oxford University Press, USA.

12. S. P. Shetty, R. Shah, and P. R. Copeland, (2014) "Regulation of selenocysteine incorporation into the selenium transport protein, selenoprotein P," J. Biol. Chem., 289(36):25317–25326.
13. M. Kulak and R. Weigel, (2014) "GPX1 (Glutathione Peroxidase 1)," Atlas Genet. Cytogenet. Oncol. Haematol., 18(7):465–469.
14. M. Naja and H. Ghasemi, (2014) "Lack of association between glutathione peroxidase1 (GPx1) activity , Pro198Leu polymorphism and stenosis of coronary arteries : A population-based prediction," meta gene, 2:722–729.
15. G. Rena, E. R. Pearson, and K. Sakamoto, (2013) "Molecular mechanism of action of metformin: old or new insights?," Diabetologia, vol. 56(9):1898–1906.
16. Al-Gazally, M. E., Al-Awad, A. S., and Kzar, H. H. (2016). "Evaluating the Superoxide Dismutase-1 status in Wild Type and Mutant at codons 12 and 13 of KRAS gene Spectrum for the Patients with Sporadic Colorectal Cancer." International Journal of PharmTech Research 9: 272-279.
17. Al-Gazally, M. E., Obed, A. F., and Al-Saadi, A. H. (2016). "Effect of ACE gene polymorphism of Iraqi patients on ischemic stroke." International Journal of ChemTech Research 9(3): 424-429.
18. Al-Gazally, M.E., , Al-Saadi, A. H., and Radeef, A. H., (2015), Effect of homocysteine on ischemic stroke and myocardial infarction in Iraqi population. International Journal of PharmTech Research, 8(10): 139-145.
19. Al-Gazally, M. E., Al-Awad, A. S., and Kzar, H. H. (2016). "Assessment of antioxidant status in different genotypes/phenotypes at codon 72 of TP53 gene for patients with sporadic colorectal cancer in Babylon province." International Journal of PharmTech Research 9: 280-286
20. E. Eskafi Sabet, Z. Salehi, S. Khodayari, S. Sabouhi Zarafshan, and Z. Zahiri, (2014). "Polymorphisms of glutathione peroxidase 1 (GPX1 Pro198Leu) and catalase (CAT C-262T) in women with spontaneous abortion," Syst. Biol. Reprod. Med., vol. 60(5): 304–307.
21. American Diabetes Association., (2014), "Diagnosis and classification of diabetes mellitus," Diabetes Care, vol. 37(1): 81–90,
22. N. Holman, F. Campbell, N. Sattar, and J. Warner, (2015) "Body mass index in children and young people with Type 1 diabetes in England and Wales," no. March, p. 2015.
23. S. Rodríguez, J. Almeida, and J. C. Pérez, (2014), "Multivessel coronary artery disease , angioplasty and endothelial dysfunction in diabetes mellitus . Case Report," Cuba. Soc. Cardiol., vol. 6(1):110–118.
24. Z. E. Önal, V. Atasayan, T. Gürbüz, E. Hepkaya, and Ç. Nuhoglu, (2014),"Association of glycosylated hemoglobin (HbA1c) levels with Iinsulin resistance in obese children.," Afr. Health Sci., 14(3):533–538,
25. W. H. Herman and R. M. Cohen, (2012). "Racial and Ethnic Differences in the Relationship between HbA1c and Blood Glucose: Implications for the Diagnosis of Diabetes.," J. Clin. Endocrinol. Metab., 97: 1–6,
26. G. Hewitt, D. Jurk, F. D. M. Marques, C. Correia-Melo, T. Hardy, A. Gackowska, R. Anderson, M. Taschuk, J. Mann, and J. F. Passos, (2012). "Telomeres are favoured targets of a persistent DNA damage response in ageing and stress-induced senescence.," Nat. Commun., vol. 3: 708-716,
27. R. Y. L. Zee, A. J. Castonguay, N. S. Barton, S. Germer, and M. Martin, (2010). "Mean leukocyte telomere length shortening and type 2 diabetes mellitus: a case-control study," Transl. Res., 155(4):166–169,
28. J. J. Meier, A. E. Butler, Y. Saisho, T. Monchamp, R. Galasso, A. Bhushan, R. A. Rizza, and P. C. Butler, 2008. "β-cell replication is the primary mechanism subserving the postnatal expansion of β-cell mass in humans," Diabetes, vol. 57, no. 6: 1584–1594,
29. J. D. Podlevsky, C. J. Bley, R. V. Omana, X. Qi, and J. L. Chen, (2008). "The Telomerase Database," Nucleic Acids Res., vol. 36, no. SUPPL. 1,
30. A. S. Astrup, L. Tarnow, A. Jorsal, M. Lajer, R. Nzietchueng, A. Benetos, P. Rossing, and H. H. Parving, (2010). "Telomere length predicts all-cause mortality in patients with type 1 diabetes," Diabetologia, 53(1): 45–48,
31. J. Daubenmier, J. Lin, E. Blackburn, F. M. Hecht, J. Kristeller, N. Maninger, M. Kuwata, P. Bacchetti, P. J. Havel, and E. Epel, (2012). "Changes in stress, eating, and metabolic factors are related to changes in telomerase activity in a randomized mindfulness intervention pilot study," Psychoneuro endocrinology, 37(7): 917–928
32. P. T. Katzmarzyk, G. Hu, W. T. Cefalu, E. Mire, and C. Bouchard, , (2013)"The Importance of Waist Circumference and BMI for Mortality Risk in Diabetic Adults.," Diabetes Care, 36(10): 3128–30.

33. M. R. Carnethon, P. J. D. De Chavez, M. L. Biggs, C. E. Lewis, J. S. Pankow, A. G. Bertoni, S. H. Golden, K. Liu, K. J. Mukamal, B. Campbell-Jenkins, and A. R. Dyer, , (2012)"Association of weight status with mortality in adults with incident diabetes.," Jama, 308(6):581–90.
34. Maha F. Smaism (2016) .Assessment of leptin levels in the different genotypes and leptin receptor genes in the women with polycystic ovary syndrome and diabetes mellitus type 2 in Iraq population. International Journal of PharmTech Research 9(5):269-276.
35. J. L. Rains and S. K. Jain, , (2011). "Oxidative stress, insulin signaling, and diabetes," Free Radical Biology and Medicine, vol. 50, no. 5.567–575
36. K. J. Davies, , (2009)"Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems.," IUBMB Life, vol. 50, no. 4–5, 279–89.
37. G. Saretzki, "Telomerase, mitochondria and oxidative stress," Experimental Gerontology, 44(8): 485–492..
38. D. Giustarini, I. Dalle-Donne, A. Milzani, P. Fanti, and R. Rossi, (2013) "Analysis of GSH and GSSG after derivatization with N-ethylmaleimide," Nat. Protoc., 8(9); 1660–1669.
39. I. Seghrouchni, J. Drai, E. Bannier, J. Riviere, P. Calmard, I. Garcia, J. Orgiazzi, and A. Revol, (2002) "Oxidative stress parameters in type I, type II and insulin-treated type 2 diabetes mellitus; insulin treatment efficiency.," Clin. Chim. Acta., vol. 321(1–2): 89–96.
40. D. Giugliano, A. Ceriello, and G. Paolisso, (1995) "Diabetes mellitus, hypertension, and cardiovascular disease: Which role for oxidative stress?," Metabolism, 44(3): 363–368.
41. J. Zhang, X. Zhang, I. B. Dhakal, M. D. Gross, F. F. Kadlubar, and K. E. Anderson, (2011) "Sequence variants in antioxidant defense and DNA repair genes, dietary antioxidants, and pancreatic cancer risk," Int. J. Mol. Epidemiol. Genet., 2(3): 236–244..
42. M. Nemoto, R. Nishimura, T. Sasaki, Y. Hiki, Y. Miyashita, M. Nishioka, K. Fujimoto, T. Sakuma, T. Ohashi, K. Fukuda, Y. Eto, and N. Tajima, (2007) "Genetic association of glutathione peroxidase-1 with coronary artery calcification in type 2 diabetes: a case control study with multi-slice computed tomography.," Cardiovasc. Diabetol., 6: 23-29.
43. M. Flekac, J. Skrha, J. Hilgertova, Z. Lacinova, and M. Jarolimkova, (2008) "Gene polymorphisms of superoxide dismutases and catalase in diabetes mellitus.," BMC Med. Genet., 9:30-36.
44. G. Saretzki, (2009) "Telomerase, mitochondria and oxidative stress," Experimental Gerontology, 44(8). 485–492.
45. W. Lu, Z. Chen, H. Zhang, Y. Wang, Y. Luo, and P. Huang, (2012) "ZNF143 transcription factor mediates cell survival through upregulation of the GPX1 activity in the mitochondrial respiratory dysfunction.," Cell Death Dis., 3:422-430
46. H. Tang, X. Dong, R. S. Day, M. M. Hassan, and D. Li, (,2010) "Antioxidant genes, diabetes and dietary antioxidants in association with risk of pancreatic cancer," Carcinogenesis, 31(4): 607–613.
47. Y. Zhang, L. Zhang, D. Sun, Z. Li, L. Wang, and P. Liu, (2011) "Genetic polymorphisms of superoxide dismutases, catalase, and glutathione peroxidase in age-related cataract," *Mol Vis 2011 30;17:2325-32. Epub 2011 Aug 30.*

*****