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Association of melatonin and glutathione peroxidase enzyme in patients with type 2 diabetes mellitus

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Abstract

Diabetes mellitus (DM) is a group of diseases characterized by high blood glucose levels resulting from a defect in the body's ability to produce and/or use insulin. It is believed that oxidative stress plays important role in the development of vascular complications in type 2 diabetes. The objectives: to determine the serum concentrations of endogenous melatonin and glutathione peroxidase (GPx) in cases of Type 2 DM and compare it with normal controls and to assess the correlation between melatonin and GPx. A case control study was done on 70 patients with diabetes mellitus type 2 according to ADA definition of DM type 2 recruited from Al Imamain Al-Kathemeaain medical city, Baghdad, Iraq who compared with 70 age, BMI and gender matched healthy control group in the levels of serum melatonin, serum glutathione peroxidase (GPx), fasting blood glucose (FBG) level, glycated hemoglobin (HbA1c), lipid profile, serum urea and serum creatinine. The activities of GPX enzyme (5.3021±1.2098) were significantly (p=0.037) lower than those of controls (6.6185±2.06877) which is accompanied with a significant reduction in the melatonin levels in patients (13.7652±2.75756) comparing with controls (17.3398±2.80153) with a significant positive correlation between GPX activity and melatonin levels in both patients and control groups. Inconclusions; melatonin levels showed to be reduced significantly in diabetic patient which may play an essential role in reducing the defense mechanism against ROS via affecting the activity of GPx enzyme.

Keywords: Diabetes mellitus, Glutathione peroxidase, Melatonin, Oxidative stress, Reactive oxygen species (ROS)

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Introduction

Diabetes mellitus (DM), or simply diabetes, is a group of diseases characterized by high blood glucose levels that result from defects in the body's ability to produce and/or use

insulin. It is a condition primarily defined by the level of hyperglycemia giving rise to risk of microvascular damage (retinopathy, nephropathy and neuropathy) [1, 2].

Free radicals are reactive chemical entities that are short lived species containing one or more unpaired electrons. They can also be considered as necessary evil for signaling involved in normal process of differentiation and migration. The free radicals induce damage to cells by passing the unpaired electron resulting in oxidation of cell components and molecules. They are generally very unstable and very much reactive [3].

Oxidative stress causes healthy cells of the body to lose their function and structure by attacking them. It is believed that oxidative stress plays important role in the development of vascular complications in diabetes particularly type 2 diabetes [4]. ROS level elevation in diabetes may be due to decrease in destruction or/and increase in the production by catalase (CAT—enzymatic/non-enzymatic), superoxide dismutase (SOD) and glutathione peroxidase (GSH–Px) antioxidants. The variation in the levels of these enzymes makes the tissues susceptible to oxidative stress leading to the development of diabetic complications. According to epidemiological studies, diabetic mortalities can be explained notably by an increase in vascular diseases other than hyperglycemia [5].

The aim of the current work was to determine the serum concentrations of endogenous melatonin and glutathione peroxidase (GPx) in cases of Type 2 DM and compare it with normal controls. The present work also aimed to assess the correlation between melatonin and GPx and determine the correlation of these markers with different variables like (HBA1C, fasting blood sugar, Lipid profile).

Method

A case control study was done on 70 patients with diabetes mellitus type 2 according to ADA definition of DM type 2(Fasting blood glucose ≥7.0 mmol/L) or (126 mg/dL) & HbA1c 6.5% (48 mmol/mol) with age ranged from 20-65 years old (mean±SD; 41.16 ±15.09) recruited from Al Imamain Al-Kathemeaain medical city, Baghdad, Iraq who compared with 70 age, BMI and gender matched healthy control group with age ranged from 18-65 (mean +SD 44.41±14.73)

Inclusion criteria

Patients with type 2 diabetes will be included according to ADA definition of DM type 2 (Fasting blood glucose ≥7.0 mmol/L) or (126 mg/dL) & HbA1c 6.5% (48 mmol/mol).

Only healthy individuals (volunteers including medical staff, relatives, friends will be included within the (control) group free from diabetes mellitus disease confirmed by fast blood glucose level test (less than 6.1 mmol\L).

Exclusion criteria

- Patients with type 1 diabetes.
- Patients with liver or pancreatic inflammation.
- Patients with any type of cancer or tumor.
- Patients taking insulin, supplements, Sedative medications (CNS depressants), Birth control pills (Contraceptive drugs), Anticoagulant / Antiplatelet drugs.(

Blood sampling

Ten milliliters of blood were put into serum separating tube (SST) and left to clot for 15-30 min at room temperature then were centrifuged at 8000 rpm for 15 min, the separated sera were divided into small aliquots and store at (-20oC) until assayed for the evaluation the following according to manufacturer instructions:

1. Serum level Melatonin by ELISA.
2. Serum level of glutathione peroxidase (GPx) by ELISA.
3. Fasting blood glucose (FBG) level.
4. Glycated hemoglobin (HbA1c).
5. Lipid profile.
6. Serum Urea.
7. Serum Creatinine .

The study was approved by the local Ethical Committee of the College of Medicine, Al-Nahrain University, Baghdad, Iraq. In addition, an informed written consent for participation in the study was signed by the participant or the legal guardians of the investigated subjects according to the Helsinki principles.

Statistical analysis

Results were expressed as mean ± standard deviation (SD) and all statistical comparisons were made by means of independent t-test and Analysis of variance (ANOVA) test with P ≤0.05 was considered statistically significant. The correlation was done between all parameters using Pearson correlation test. All statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) software 20.

Results

Table 1.

Demographic characteristics of the patients with DM in comparison with controls.

	Group	N	Mean	Std. Deviation	P-value
Age	cases	70	46.16	15.09	0.62
	control	70	44.41	14.73	
BMI	cases	70	28.1000	9.16570	0.892
	control	70	28.7857	7.60575	

Some demographic characteristics of the studied groups were summarized in table (1) and figure (1).

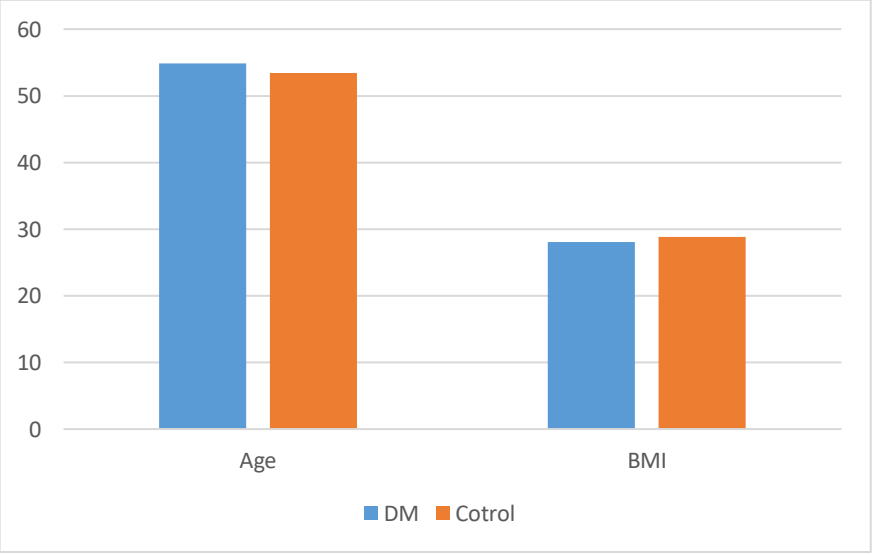

Figure 1.

Demographic characteristics of the patients with DM in comparison with controls.

Results demonstrated in table (2) revealed that there were non-significant differences between the gender distribution and the smoking habit between cases and control as represented by the odd ration and Chi^2 results obtained

Table 2.

Comparison in the gender and smoking habit distribution between cases and controls

		Gender		Smoking	
		Male n (%)	Female n (%)	Smoker n (%)	Non-smoker n (%)
Cases		29 (41.4)	41 (58.6)	22 (31.4)	48 (68.6)
Control		36 (51.4)	34(48.6)	31 (44.3)	39 (55.7)
Odd ratio	95% CI	0.34-1.3		0.28-1.12	
	p-value	0.24		0.1	
Chi^2	Phi	-0.11		0.28	
	p-value	0.21		0.2	

Results illustrated in table (3) revealed that the activities of GPX enzyme were significantly lower than those of controls which is accompanied with a significant reduction in the melatonin levels. Moreover, the levels of HDL in diabetic patients were significantly lower than that of controls whereas TG and TC levels were significantly higher than those of controls. Additionally, urea and creatinine levels were elevated significantly in diabetic patients in comparison with controls, but the majority of patients showed a normal urea and creatinine levels. As an indices of blood glucose levels, fasting blood sugar and HbA1c in diabetic patients were significantly higher than those of healthy controls' levels

Table 3.

The levels of all studied parameter in diabetic patients in comparison with controls

	Group	N	Mean	Std. Deviation	P-value
GPX	cases	70	5.3021	1.20987	0.037
	control	70	6.6185	2.06877	
MT	cases	70	13.7652	2.75756	0.025
	control	70	17.3398	2.80153	
HDL Ch	cases	70	38.2338	5.80673	<0.001
	control	70	43.2615	7.73239	
TG	cases	70	169.8235	52.54250	<0.001
	control	70	129.3846	40.71728	
TC	cases	70	195.7206	65.05104	<0.001
	control	70	157.8615	43.72745	
Creatinine	cases	70	.9344	.21658	<0.001
	control	70	.6250	.19702	
Urea	cases	70	45.9351	11.23329	<0.001
	control	70	37.4769	11.01151	
FBS	cases	70	158.0294	67.13941	<0.001
	control	70	84.7538	15.42120	
HbA1c	cases	70	8.3990	2.00116	<0.001
	control	70	5.2281	.37010	

As shown in tables (4 and 5), there were several significant correlations among the parameters subjected to the current study some of them negative correlations whereas the others are positive.

Table (4) showed the correlations among the studied parameters in controls which revealed that the levels of GPX were significantly and positively correlated with the melatonin levels. Additionally, TG levels showed positive significant correlations with the BMI, total cholesterol and creatinine levels. Furthermore, creatinine levels also showed to be positively and significantly correlated with the levels of urea. On the other hand, creatinine levels showed

to be negatively and significantly correlated with the HbA1c levels. Moreover, HDL levels were negatively and significantly correlated with both of TG and total cholesterol.

Table 4.

The correlation between all studied parameters in controls

		MT	BMI	HDL Ch	TG	TC	Creatinine	Urea	FBS	HbA1c
GPX	r	0.843	-0.137	0.032	0.083	0.033	-0.263	-0.033	-0.005	-0.009
	p	<0.001	0.641	0.799	0.513	0.797	0.263	0.792	0.969	0.942
MT	r		-0.089	-0.079	0.124	0.034	-0.298	0.043	-0.044	0.032
	p		0.762	0.530	0.325	0.791	0.202	0.736	0.728	0.802
BMI	r			0.280	0.552	0.204	-0.878	0.254	-0.304	0.282
	p			0.331	0.041	0.485	0.05	0.38	0.291	0.329
HDL Ch	r				-0.499	-0.417	0.061	-0.149	-0.218	-0.218
	p				<0.001	0.001	0.8	0.236	0.082	0.083
TG	r					0.538	0.465	0.239	0.211	0.105
	p					<0.001	0.039	0.056	0.092	0.410
TC	r						0.359	0.173	0.148	0.084
	p						0.12	0.169	0.24	0.509
Creatinine	r							0.548	0.223	-0.459
	p							0.012	0.345	0.042
Urea	r								0.052	-0.151
	p								0.68	0.233
FBS	r									0.174
	p									0.168

Results postulated in table (5) showed the correlations among all the studied parameters in diabetic patients in which there were higher number of positive and negative correlations than that obtained in controls. In a similar manner to that in controls, GPX and melatonin were positively and significantly correlated. It was also demonstrated that the levels of fasting blood sugar in diabetic patients were positively and significantly correlated with levels of melatonin, triglyceride, total cholesterol, creatinine, and urea. Furthermore, HbA1c levels were positively and significantly correlated with the levels of total cholesterol, creatinine, urea, and fasting blood sugar. Additionally total cholesterol levels were correlated positively and significantly with the levels of TG and creatinine. On the other hand, HDL levels were correlated negatively and significantly with triglyceride and total cholesterol levels.

Table 5.

The correlation between all studied parameters in diabetic patients

		MT	BMI	HDL Ch	TG	TC	Creatinine	Urea	FBS	HbA1c
GPX	r	0.395	0.748	0.071	-0.133	-0.138	0.021	0.152	0.112	0.007
	p	0.001	0.462	0.563	0.280	0.263	0.938	0.215	0.362	0.952
MT	r		0.550	0.086	-0.062	-0.025	0.153	0.173	0.312	0.133
	p		0.629	0.491	0.618	0.840	0.571	0.162	0.010	0.283
BMI	r			-0.563	-0.620	0.853	0.518	0.657	0.952	0.368
	p			0.620	0.574	0.349	0.427	0.543	0.198	0.760
HDL Ch	r				-0.320	-0.415	-0.134	-0.071	-0.110	-0.135
	p				0.008	0.000	0.621	0.565	0.372	0.271
TG	r					0.568	0.417	0.048	0.294	0.186
	p					0.000	0.108	0.699	0.015	0.129
TC	r						0.504	0.201	0.389	0.297
	p						0.047	0.100	0.001	0.014
Creatinine	r							0.564	0.515	0.513
	p							0.023	0.041	0.042
Urea	r								0.271	0.278
	p								0.026	0.022
FBS	r									0.610
	p									0.000

Discussion

In the current work all subjected individuals either patients or controls were non-significantly differ from each other in age, BMI, gender, and smoking habit to exclude any effect of these variables on the oxidative status of all subjects in an attempt to elucidate the effect of the melatonin levels on the oxidative status of diabetic patients that represented as an activity of GPX enzymes. Results obtained in the current study revealed that the levels of GPX enzyme were significantly reduced in diabetic patients in comparison with controls which indicate that one of the most important defense mechanism against ROS were defective which is consistent with previous work which demonstrated that oxidative stress plays important role in the development of vascular complications in diabetes particularly type 2 diabetes6 given that ROS level elevation in diabetes may be due to decrease in destruction or/and increase in the production by catalase (CAT- enzymatic/non-enzymatic), superoxide dismutase (SOD) and glutathione peroxidase (GPX) antioxidants. The variation in the levels of these enzymes makes the tissues susceptible to oxidative stress leading to the development of diabetic complications [7].

Moreover, results illustrated in the current research revealed that the levels of melatonin were significantly lowered in diabetic patients in a comparison with controls which showed to be decreased in parallel with the activities of GPX enzyme that may indicate the

mechanism of melatonin's antioxidant action which is in agreement with several studies demonstrated that melatonin antioxidant activity originate from its ability to improve the activities of antioxidant enzymes such as SOD [8].

Melatonin works via multiple means to limit oxidative stress. While melatonin is capable of directly or indirectly scavenging toxic oxygen species [9], it has other means at its disposal for combatting free radical damage. When a molecule such as melatonin merely renders one of its delocalized electrons to neutralize a free radical, this action is achieved without receptor intervention [10]. It is well documented, however, that melatonin's ability to limit oxidative stress sometimes also relies on its interaction with melatonin membrane receptors that are present in many, perhaps all, cells [11,12]

These antioxidant actions of melatonin rely on an interaction with membrane receptors located on the cell membrane or on intracellular organelles [8]; membrane receptors for melatonin also may exist in all organisms13. These receptor-mediated actions of melatonin are indirect and likely involve stimulation of antioxidant enzymes, e.g., glutathione peroxidase (GPX), superoxide dismutase (SOD1, 2), SIRT3, etc. [14]. When melatonin acts via receptors to carry out its antioxidant actions, it can achieve this effect at much lower concentrations than those required when it functions as a direct free radical scavenger. This relates to the fact that the signal transduction pathways associated with receptors serve to magnify the response [8].

The above-mentioned mechanism of melatonin role as an antioxidant consistent with the correlation results obtained in the present work that showed a significant directly proportional relationship between melatonin and GPx enzymes.

Conclusion

Melatonin levels showed to be reduced significantly in diabetic patient which may play an essential role in reducing the defense mechanism against ROS via affecting the activity of GPx enzyme.

Ethical Approval

The study was approved by the Ethical Committee.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

All authors shared in conception, design of the study, acquisition of data, and manuscript writing, the critical revising and final approval of the version to be published.

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