Evaluation the Levels of Melatonin, Glutathione Peroxidase and Superoxide Dismutase Enzymes in Prediabetic Individuals

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Abstract

Background   Prediabetes is a serious health condition where blood sugar levels are higher than normal, but not high enough yet to be diagnosed as type 2 diabetes. Diabetes mellitus (DM) is a disease characterized by elevated blood glucose levels due to the inability of the body to yield or use insulin or both. It is believed that oxidative stress may play an important role in the development of vascular complications in patients with type 2 diabetes mellitus.

Objective   To evaluate the serum levels of endogenous melatonin and its association with superoxide dismutase (SOD) and glutathione peroxidase (GPx) in prediabetes individuals in comparison with normal individuals as controls.

Methods   Endogenous melatonin and SOD concentrations were measured in sera of 50 prediabetes individuals in comparison with 50 volunteers enlisted as normal controls aged from 20 to 65 using enzyme-linked immunosorbent assay (ELISA); volunteers enlisted as normal. Serum levels of lipid profile, urea, creatinine, GPx, fasting blood glucose and plasma level of glycated hemoglobin (HbA1c) were measured. All individuals were matched for body mass index and sex.

Results   Serum levels of melatonin, SOD and GPx enzymes in prediabetic patients were significantly lower than those of controls (p=0.036, p=0.024, and p=0.044; respectively) in prediabetic individuals when compared with controls with a significant positive correlation of theses biomarker levels in prediabetic individuals as compared with the control group.

Conclusion   Decreased levels of melatonin in prediabetic subjects may play an essential role by influencing decreased levels of SOD and GPx enzymes, which are considered major defense mechanisms against ROS that may lead to the development of diabetes (type 2).

Keywords   Melatonin, superoxide dismutase, glutathione peroxidase, oxidative stress, prediabetes

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List of abbreviations: ADA = American Diabetic Association, BMI = Body mass index, CNS = Central nervous system, Chi2 = Chi-square test, FBS = Fasting blood sugar, WHO = World health organization

Introduction

Diabetes is a family of disorders that is characterized by hyperglycemia. It is caused by an absolute or relative insulin deficiency [1-3]. In diabetes mellitus type 2, there is a spectrum of disorders ranging from insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance. The chronic complication of diabetes mellitus type 2 may lead to diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy [4].

Prediabetes is a metabolic state lying between diabetes and normoglycemia. It can be classified according to World health organization (WHO) to impaired glucose

tolerance and impaired fasting glucose, it remains a state of high risk for developing diabetes with a yearly conversion rate of 5-10%. Observational evidence suggests as association between prediabetes and microvascular complications of diabetes and the risk of macrovascular disease [5].

Free radicals are highly reactive, short-lived, and unstable electrons that may contain one or more unpaired electrons. They can generate and be involved in the normal process of differentiation and migration. Accumulation of free radicals causes damage to cells by disrupting membranes and perhaps causing cancer and atherosclerosis [6-9].

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 [10-12]. Increasing the levels of ROS in diabetes mellitus may be due to an increase in the destruction or a decrease in the production of catalase superoxide dismutase (SOD) and glutathione peroxidase (GPx) antioxidants. Fluctuations in the levels of these enzymes make tissues vulnerable to oxidative stress, leading to the development of diabetes complications. According to epidemiological studies, significantly more deaths from diabetes can be explained by an increase in vascular pathologies other than hyperglycemia [13-15].

The aim of the current work was to determine the serum concentrations of endogenous melatonin, GPx and SOD in cases of prediabetes and compare it with normal controls. The present work also aimed to assess the correlation between melatonin, GPx and SOD and determine the correlation of these markers with different variables like glycated hemoglobin (HbA1c), fasting blood and sugar (FBS).

Methods
Study design case-control study
The present study was done on 50 prediabetic patients according to American Diabetic Association (ADA) definition of prediabetes (Fasting blood glucose 6.1-6.9 mmol/l) or (110-125 mg/dl) and HbA1c (5.7% to 6.4%) with age ranged from 20-65 years old (mean±SD; 34.23±9.75) recruited from Al-Imamain Al-Kadhimain Medical City, Baghdad, Iraq who compared with 50 age, body mass index (BMI) and sex were matched with a healthy control group with age ranged from 18-57 year (mean±SD 36.2±12.71).

Inclusion criteria
Patients with prediabetes were included according to ADA definition of prediabetes [16]. 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 or 2 diabetes.
- Patients with liver or pancreatic inflammation.
- Patients with any type of cancer or tumor.
- Patients taking insulin, supplements, sedative medications (central nervous system depressants), birth control pills (contraceptive drugs), anticoagulant /antiplatelet drugs).

Blood sampling
Blood sample was collected from all 100 subjects from Al-Imamain Al-Kadhimain Medical City, Baghdad, Iraq with the approval of the Institutional Board Review (IRB) of College of Medicine, Al-Nahrain University.
In addition, an informed written consent for participation in the study was signed by the participant according to the Helsinki principles. Participants' consent was taken after explaining to them the nature and goals of our study that may help them and the community for better health care, promising the

participants to protect their private information, for which participants fully understood and agreed.

Serum levels of melatonin, GPx and SOD were measured by enzyme-linked immunosorbent assay (ELISA) technique. The ELISA kits used in the study for melatonin was (Sunlong, melatonin kit Catalog No. SL1169Hu), SOD kit No. (SL3490Hu), GPx kit No. (SL2786Hu). The glucose company kit was (Biosystem S.A.) and kit no. (15011). The HbA1c company kit was (Bio-Rad system) and kit no. (Variant II TURBO 270-2601A).

Statistical analysis
Statistical analysis was carried out by using SPSS version 23 and Microsoft excel 2013. The numerical data were expressed as mean±SD. Comparison between mean serum levels of melatonin, GPx, and SOD of cases and controls were performed by t-test. Pearson correlation test was done between parameters within each group (prediabetes and controls).
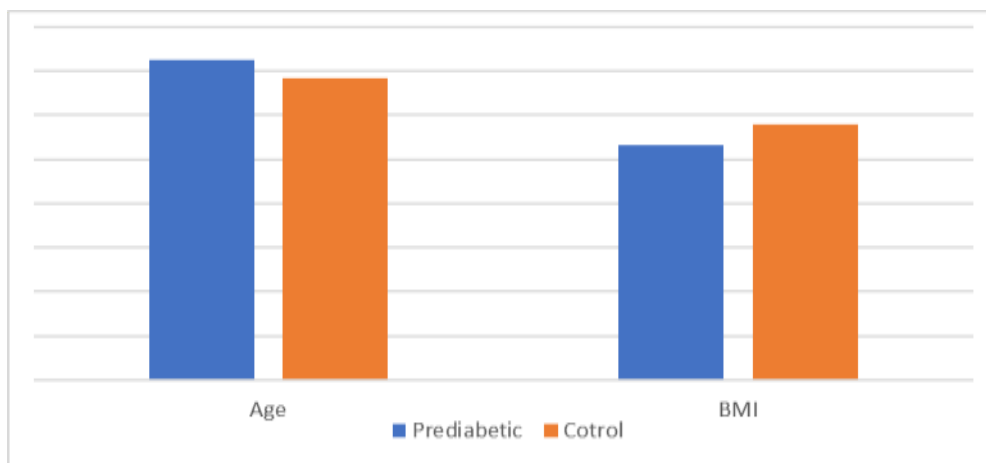
Results
Age and BMI of the studied groups were summarized in table (1) and figure (1). Table (1) showed non-significant differences in age and BMI between diabetic patients and controls

Table 1. Age and body mass index of the prediabetic patients in comparison with controls

Parameter	Group	N	Mean	Std. Deviation	P-value
Age (yr)	Cases	50	36.2	12.71	0.54
	Control	50	34.23	9.75	
BMI (kg/m^2)	Cases	50	26.6	7.16	0.8
	Control	50	28.95	7.89	

BMI: Body mass index

Figure 1. Age and body mass index of the prediabetic patients 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 Chi-square test (Chi2) results obtained.

Results illustrated in table (3) revealed that the levels of FBS in prediabetic patients were non-

significantly differ from those of controls (p value 0.16). However, the levels of HbA1c in prediabetic patients were significantly higher than those of healthy controls' levels (5.84 vs 5.14 %) respectively; (p value <0.001).

Table (4) shows that the levels of melatonin, SOD and GPx enzymes were significantly lower (p=0.036, p=0.024 and p=0.044) respectively than those of controls.

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	26 (52)	24 (48)	20 (50)	30 (60)
	Control	27 (54)	23 (46)	22 (44)	28 (56)
Chi2	Phi	0.012		0.28	
	p-value	0.92		0.2	

Table 3. Comparison of fasting blood sugar and glycated hemoglobin between prediabetes with controls groups

Parameter	Group	N	Mean	Std. Deviation	P value
FBS (mg/dl)	Cases	50	89.2	18.66	0.16
	Control	50	83.8	12.91	
HbA1c (%)	Cases	50	5.84	0.36	<0.001
	Control	50	5.14	0.31	

Table 4. Comparison of melatonin, superoxide dismutase and glutathione peroxidase between prediabetes with controls groups

Parameter	Group	N	Mean	Std. Deviation	P value
Melatonin (pg/ml)	Cases	50	13.75	1.36	0.036
	Control	50	15.68	4.05	
SOD (ng/ml)	Cases	50	1.01	0.18	0.024
	Control	50	1.21	0.37	
GPx (pmol/ml)	Cases	50	5.27	1.37	0.044
	Control	50	6.92	3.56	

From the given data in the table (5), melatonin has no significant correlation with age, BMI, FBS and HbA1c in both groups. However, it has highly significant positive correlation with SOD and GPx in control group (p value <0.001), yet, in prediabetes group, significant positive

correlation with SOD, and insignificant correlation with GPx (p value 0.034, 0.387) respectively.

Regarding correlation of SOD with other parameters that shown in table (6), like melatonin, it also has significant correlation

with melatonin and GPx in control group (p value <0.001) and insignificant correlation with GPx in prediabetes (p value 0.748). Additionally, it has significant negative correlation with HbA1c in prediabetes cases that is insignificant in control group (p value 0.025, 0.316) respectively.

Table 7 shows correlation of GPx with other parameters, and as mentioned above, it has highly significant correlation with melatonin and SOD just in control group but insignificant in control group. Also, like SOD, GPx has negative correlation with HbA1c only in prediabetes cases but not in control group (p value 0.028, 0.383) respectively.

Table 5. The correlation between melatonin and other parameters in prediabetes and controls groups

Parameter		Melatonin (pg/ml)	
		Cases	Controls
Age (yr)	r	0.398	0.125
	p	0.141	0.424
BMI (kg/m^2)	r	-0.284	-0.208
	p	0.585	0.496
SOD (ng/ml)	r	0.464	0.507
	p	**0.034**	**<0.001**
GPx (pmol/ml)	r	0.03	0.485
	p	0.387	**<0.001**
FBS (mg/dl)	r	0.032	-0.020
	p	0.892	0.888
HbA1c (%)	r	-0.267	0.192
	p	0.254	0.169

Table 6. The correlation between superoxide dismutase and other parameters in prediabetes and controls groups

Parameter		SOD (ng/ml)	
		Cases	Controls
Age (yr)	r	0.421	0.148
	p	0.118	0.343
BMI (kg/m^2)	r	-0.144	<0.001
	p	0.786	0.999
Melatonin (pg/ml)	r	0.464	0.507
	p	**0.034**	**<0.001**
GPx (pmol/ml)	r	0.045	0.636
	p	0.748	**<0.001**
FBS (mg/dl)	r	0.301	0.099
	p	0.198	0.478
HbA1c (%)	r	-0.498	0.140
	p	**0.025**	0.316

Table 7. The correlation between glutathione peroxidase and other parameters in prediabetes and controls groups

Parameter		GPx (pmol/ml)	
		Cases	Controls
Age (yr)	r	-0.126	0.071
	p	0.655	0.652
BMI (kg/m^2)	r	0.493	-0.220
	p	0.321	0.471
Melatonin (pg/ml)	r	0.03	0.485
	p	0.387	**<0.001**
SOD (ng/ml)	r	0.045	0.636
	p	0.748	**<0.001**
FBS (mg/dl)	r	0.034	0.181
	p	0.888	0.191
HbA1c (%)	r	-0.491	0.122
	p	**0.028**	0.383

Discussion

In the current work, all subjected individuals either patients or controls were non-significantly differed from each other in age, BMI, gender, and smoking habit to exclude any effect of these variables on the oxidative status of them in an attempt to elucidate the effect of the melatonin levels on the oxidative status of prediabetic patients that represented as a level of SOD and GPx enzymes.

The levels FBS were not significantly different between the two groups, indicating that FBS levels may not be a good predictor of prediabetes. However, the levels of HbA1c were significantly higher in prediabetic patients than in healthy controls, and within the range of prediabetes according to ADA definition of prediabetes suggesting that HbA1c may be a more reliable marker for prediabetes [16,17].

Melatonin is a hormone secreted by the pineal gland and is known to play an important role in regulating the sleep-wake cycle. Additionally, melatonin has been shown to have antioxidant and anti-inflammatory effects, which may be beneficial in the management of various health conditions such as diabetes and cardiovascular diseases. In this study, melatonin was significantly lower in prediabetic cases than controls. According to these results melatonin may be used as a biomarker of inflammation and it may have a role in the genesis of diabetes because it triggers a phase shift in insulin secretion. In contrast, impaired in the regulation of daily insulin secretion is an essential feature of prediabetes that may be ended with type 2 diabetes [18]. Melatonin may protect the individuals from being prediabetic and thus regulating insulin secretion and protecting against ROS since the β-cells of pancreas are objective for oxidative stress because they have low antioxidative capacity [19].

Melatonin can elevate electrical gradients between the two sides of the inner mitochondrial membrane leads to increase the production of adenosine triphosphate (ATP) by increasing the activity of the electron transport chain (ETC) and increased membrane fluidity, while reducing oxidative stress [20].

Results obtained in the current study revealed that the levels of SOD and GPx enzymes were significantly reduced in prediabetic patients in comparison with controls, which indicate that one of the most important defense mechanisms against ROS were defective. One of the explanations for decreased activity of SOD in these patients may be due to the accumulation of hydrogen peroxide (H_2O_2). The

Cu/Zn-SOD considered as primary catalytic cellular defense that protects cells and tissues against potentially destructive reactions of ROS. It has been observed that SOD can be rapidly induced in some conditions when cells or organisms are exposed to oxidative stress. The inhibition of Cu/Zn SOD by nonenzymatic glycation, which is the other cause for H_2O_2 production [17,21]. Fluctuations in the levels of these enzymes make tissues vulnerable to oxidative stress, leading to the development of diabetes complications prediabetes.

Strong positive correlation was found in this study between melatonin with SOD and GPx in control groups which was less significant or insignificant in prediabetes group, which indicate non-parallel reduction in their levels in prediabetes, which may contribute to the pathophysiology of prediabetes.

These results agree with another studies which demonstrated that melatonin antioxidant activity originates from its ability to improve the activities of antioxidant enzymes such as SOD [22,23]. Melatonin acts in multiple ways to reduce oxidative stress; while melatonin can remove toxic oxygen species directly or indirectly, it also has other means at its disposal to combat free radical damage. When a molecule like melatonin only transfers one of its unpaired electrons to neutralize free radicals, this action is accomplished without the receptor's involvement [24]. However, it is well documented that melatonin's ability to reduce oxidative stress sometimes also depends on its interaction with melatonin membrane receptors located on many, possibly all, cells [25,26]. These antioxidant actions of melatonin depend on interaction with transmembrane receptors located on the cell membrane or on intracellular organelles. Membrane receptors for melatonin may also be present in all living organisms. The receptor-mediated actions of melatonin are indirect and likely involve stimulation of antioxidant enzymes, for example, SOD, GPx, etc [27]. When melatonin acts via receptors to carry out its antioxidant actions, it can achieve this effect at concentrations much lower than those required when it acts as a direct scavenger of free radicals. This relates to the fact that the signal transduction pathways associated with the receptors amplify the response [22,27]. 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 SOD and GPx enzymes [28].

In conclusion, melatonin levels decreased significantly in prediabetic individuals, which may play an important role in reducing the defense mechanism represented by the activity of SOD and GPx enzymes against producing of ROS, which may lead to a progression of prediabetes to type 2 diabetes mellitus.

It is important to note that this study has some limitations, such as small sample size and lack of control for confounding variables like diet and physical activity. Further research is necessary to confirm the findings and investigate other potential factors that may contribute to the development of prediabetes. Overall, the results of this study contribute to a better understanding of the biochemical markers associated with prediabetes and may help in the development of more effective prevention and treatment strategies.

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