Altered serum levels of melatonin, antioxidant enzymes and oxidative stress in individuals with diabetes mellitus type 2

Alteración de los niveles séricos de melatonina, de las enzimas antioxidantes y el estrés oxidativo en individuos con diabetes mellitus tipo 2

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Abstract

Background and aim: Dysregulation of melatonin, is thought to play a role in the development of type 2 diabetes mellitus. The objective of this study was to evaluate the relationship between circulating melatonin, lipid peroxide, total antioxidant capacity (TAC), nitric oxide (NO), superoxide dismutase (SOD) concentrations in adults with and without type 2 diabetes mellitus. Included in the study were 46 patients, newly diagnosed with type 2 diabetes mellitus and 46 healthy control subjects. The patients and control subjects were asked to come to the clinic in the morning, after an 8-hour fast. At that time, 5 mL of venous blood was collected, to determine biochemical indicators. Melatonin level was assessed by ELISA technique. Results: In T2DM subjects, mean values of serum melatonin, NO, TAC, and lipid peroxide were found to be significantly decreased compared to the control group (39±4.7versus 100.9±26.5pg/ml)), (26.67±3.18 versus 34.2±3.2μmoles/L), (188.5±27.3 versus 263±32.62μmol/ mg of protein), and (3.32±2.62 versus 6.1± 2.68nmole/ mL) respectively. Whereas serum fasting blood glucose (FBG), and SOD level was increased in the diabetic groups compared to the control group (181±22.9 versus 101±3.8 mg/dL) (5.79±2.03U/mL, versus 3.68±1.53 U/mL), respectively. Conclusions: Our study supports the hypothesis that abnormal production of oxidative stress markers, NO, and melatonin can contribute to the pathogenesis of T2DM.

Keywords: Oxidative stress, T2DM; melatonin; Lipid peroxide

Resumen

Antecedentes y objetivo: se cree que la desregulación de la melatonina desempeña un papel en el desarrollo de la diabetes mellitus tipo 2. El objetivo de este estudio fue evaluar la relación entre las concentraciones circulantes de melatonina, peróxido lipídico, capacidad antioxidante total (TAC), óxido nítrico (NO), superóxido dismutasa (SOD) en adultos con y sin diabetes mellitus tipo 2. Se incluyeron en el estudio 46 pacientes, recién diagnosticados con diabetes mellitus tipo 2 y 46 sujetos de control sanos. Se pidió a los pacientes y sujetos control que acudieran a la clínica por la mañana, después de un ayuno de 8 horas. En ese momento se recolectaron 5 mL de sangre venosa, para determinar indicadores bioquímicos. El nivel de melatonina se evaluó mediante la técnica ELISA. Resultados: Los niveles medios (media ± DE) de melatonina sérica, NO, TAC y peróxido lipídico en el grupo control fueron (100,9±26,5 pg/mL), (34,2±3,2 μmol/L), (263±32,62 μmol/ mg de proteína) y (6,1±2,68 nmol/mL), respectivamente. De manera similar, en pacientes con DM2 se obtuvieron niveles medios de (39±4,7pg/mL), (26,67±3,18 μmol/L), (188,5±27.3 μmol/mg de proteína) y (3.32±2.62n nmol/mL) para los respectivos parámetros En sujetos con DM2, se encontró que los valores medios de melatonina sérica, NO, TAC y peróxido lipídico se redujeron significativamente, mientras que la glucemia sérica en ayunas (FBG) y el nivel de SOD aumentaron en los grupos diabéticos en comparación con el grupo control (181±22,9 frente a 101±3,8 mg/dL) (5,79±2,03U/mL, frente a 3,68±1,53 U/mL), respectivamente. Conclusiones: Nuestro estudio apoya la hipótesis de que la producción anormal de marcadores de estrés oxidativo, NO y melatonina puede contribuir a la patogénesis de la DM2.

Palabras clave: Estrés oxidativo, DM2; melatonina; peróxidos lipídicos.

Introduction

The pancreas is a vital endocrine-exocrine organ that produces several hormones and enzymes. Its enzymes help in the digestion of carbohydrates, fats, and proteins whereas its hormones such as insulin control blood glucose levels. In patients with diabetes, the absence or insufficient production of insulin causes hyperglycemia. Diabetes mellitus (DM), is a chronic and endocrine disorder of multiple etiologies that produces multiple biochemical sequelae, characterized by hyperglycemia and hyperlipidemia, is associated with long-term damage dysfunction and eventually the failure of organs especially the eyes, kidneys, nerves, heart, and blood vessels. It was estimated that it affects about 438 million people by 2030 worldwide[1-5].

Free radicals and oxidative stress play a significant role in pathogenesis and progression of diabetic vascular complications. Oxidative stress is a condition induced in the body when the amount of reactive oxygen species (ROS) in cells surpasses the capacity of antioxidant.T2D is a clinical syndrome described as a metabolic disturbance in which mitochondria play a key role. In fact, mitochondria are the main source of reactive oxygen species (ROS) and participate in redox homeostasis and other functions, such as apoptosis and Ca^{2+} metabolism and ATP (adenosine triphosphate) production or heat generation. ROS can act as signaling molecules, but when their production is exacerbated, they induce mitochondrial dysfunction and a decrease in ATP production[6,7].

Melatonin is an indole neuroendocrine hormone (N-acetyl-5-methoxy-tryptamine), a tryptophan derivative secreted mainly by pinealocytes and released into the blood using enzyme machinery during the dark phase of the day it can regulate and affect the function of multiple organs, effectively control the immune system's function, play an anti-stress role, and regulate the sleep cycle[8-10].

This study aimed to evaluate serum levels of melatonin, lipid peroxide, TAC, NO, and SOD in patients with type 2 diabetes mellitus.

Materials and methods

Subjects

The present study was carried out in the Department of Biochemistry, Tikrit Teaching Hospital/ Tikrit/ Iraq, during the period January 2004 to December 2016-2017.

The total number of subjects included in the study was 80 and divided into two groups. Group I was consisting of 40 normal healthy subjects as controls while group II consisted of 40 patients with Type II diabetes. The study included subjects of age between 39-62 years for both groups. An ethical approval was taken from Scientific Ethical Committee of Kirkuk Teaching Hospital

Biochemical analysis. After an overnight fast, blood was taken from a forearm vein. The blood was clotted and immediately centrifuged to separate serum; melatonin was determined using an Enzyme Immunoassay Kit. Serum glucose, lipid peroxide, TAC, NO, and SOD activity were measured colorimetrically using commercially available kits on a fully auto analyzer of Clinical Biochemistry Laboratory. Descriptive variables were analyzed between groups using a t-test. Analyses were performed using SPSS ver. 16.0 (SPSS Inc., Chicago, IL, USA). All data are presented as mean ± standard deviation. Statistical significance was set at P≤0.05. The data are presented as mean ±SEM

Results

Serum FBG, and melatonin in diabetic patients and the Control Group.

This study reveals that the level of FBG, which was significantly elevated in diabetic patients was 181±22.9 mg/dL as compared with apparently healthy women which were 181±22.9 mg/dL at a P<0.01. While the mean serum of melatonin, in diabetic patients was 39±4.7 pg/mL, compared with the apparently healthy group 100.9±26.5. As shown in Table 1.

Table 1: Serum Level of FBG, and melatonin in diabetic patients and the control group.

Study group	Diabetic group	Control group
FBG (mg/ dL)	101 ± 3.8	181 ± 22.9*
Melatonin (pg/ml)	39±4.7**	100.9±26.5

*P<0.01; **P<0.001

This study reveals that serum level of lipid peroxide, and NO, was significantly reduced in diabetic patients was 3.32±2.62 nmol/mL, and 26.67±3.18 µmol/L as compared with an apparently healthy group which was 6.1±2.68, and 34.2±3.2 µmol/L at a P value of p<0.01.

Table 2: Serum Level of Lipid peroxide, and NO in diabetic patients and the control group.

Study group	Diabetic group	Control group
Lipid peroxide (nmole/mL)	3.32± 2.62	6.1± 2.68*
NO (µmoles/L)	26.67 ± 3.18	34.2 ± 3.2*

The mean serum level of TAC and SOD in this study was found among the diabetic study group (188.5±27.3 µmol/mg of protein) and (3.68 U/mL), respectively. The result was significantly lower in the diabetic group compared to control (263±32.62 µmol/mg of protein), and (5.79 ± 2.03 U/mL) respectively, as shown in the following Table 3.

Table 3: Serum Level of TAC, and SOD in diabetic patients and the control group.

Study group	Diabetic group	Control group
TAC (µmol/mg of protein)	188.5 ± 27.3	263 ± 32.62**
SOD (U/ml)	3.68± 1.53	5.79 ± 2.03***

*** P<0.0005

Discussion

Melatonin may influence diabetes not only by regulating insulin secretion but also by protecting against reactive oxygen species since pancreatic β-cells are very susceptible to oxidative stress because they possess only low-anti-oxidative capacity. It can increase membrane fluidity, as well as the activity of the electron transfer chain (ETC) and ATP production, mitochondria membrane potential while reducing oxidative stress. In our study, melatonin patients were found to have significantly lower serum levels. Because melatonin is a novel marker of inflammation and is increased in the immune-inflammatory state may be involved in the genesis of diabetes as it induces a phase shift in insulin secretion. In contrast, dysregulation of circadian insulin secretion is an essential feature of type 2 diabetes[9-11].

The results show a decrease in nitric oxide level might be due to chronic hyperglycemia which stimulates the production of advanced glycation end products (AGEs), and overflow of the polyol pathway, protein kinase C (PKC), and hexosamine pathways, along with depletion in the nicotinamide adenine dinucleotide phosphate (NADPH). NAD(P)H+ is an important cofactor for the enzymes in the metabolism of the reactive oxygen species (ROS). Then, excessive ROS, such as superoxide anion (O_2^-), react rapidly with NO radicals, forming the peroxynitrite anion, which is a toxic oxidant capable of damaging several biological molecules, leading to tissue injury[12,13].

The observed high levels of plasma MDA in diabetic patients reflect lipid peroxidation which is the consequence of oxidative stress. The increase in the level of MDA correlates with hyperglycemia in these patients because of the self-oxidation of glucose and could generate free radicals[14].

The specific role of antioxidants is to neutralize rampaging free radicals and thus reducing their capacity to damage. They act as radical scavengers, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, and synergist[15]. Total Antioxidant Capacity (TAC), defined as the moles of oxidants neutralized by one liter of solution, is a biomarker measuring the antioxidant potential of body fluids[16].

Patients with type 2 diabetes in the current study had significantly lower serum TAC levels than control subjects. The increased TAC levels could paradoxically reflect a high OS evidenced by increased MDA levels in the patient group that has stimulated the compensatory up-regulation of antioxidants. These results could be related to the fact that reactive oxygen species are more numerous in patients with diabetes[17]. Our results are in line with the results of other studies[18].

Superoxide dismutase (SOD) is widely distributed in oxygen-metabolizing cells and has been supposed to protect such cells against the deleterious action of superoxide radicals, it is important because it is the first line of defense against pro-oxidant molecules[19]. Decreased activity of these enzymes was stated in our study in patients compared with the control group. Due to the depletion of the antioxidant defense system following the over generation of free radicals, as well as glucose autooxidation results in the formation of hydrogen peroxide which inactivates SOD. Accumulation of hydrogen peroxide may be one of the explanations for decreased activity of this enzyme[20,21].

Conclusions

This study concludes that the abnormally high levels of oxidative stress in diabetics coupled with a simultaneous decline in antioxidant defense mechanism results in complications in diabetes mellitus.

Conflict of Interest

All authors declare no conflict of interest

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