

Correlation Between Serum Melatonin and Serum C-Peptide Levels in Uncontrolled Type II Diabetes Mellitus in Comparison with Normal non Diabetic People

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Abstract: Aim of the study: Estimation of Serum Melatonin and Serum C-Peptide levels in Uncontrolled Type 2 Diabetes Mellitus-Correlation with normal people.Objectives: To investigate whether there is a correlation between Serum Melatonin and Serum C-Peptide levels in Uncontrolled Type 2 Diabetes Mellitus.To compare Serum Melatonin and Serum C-Peptide values between the diabetic and non-diabetic population.Materials and Methods: This study was conducted in Sree Balaji Medical College, Chennai, Tamil Nadu during the period of May 2012 to May 2013. Total number of people included in this study were 50. Out of 50, 26 were patients with type 2 diabetes mellitus and 24 were age & sex matched controls. There were 21 male and 29 female patients ranging from 35 years to 85 years.Conclusion:Comparison between patients and controls show that the serum melatonin levels are low in uncontrolled type 2 diabetes mellitus patients and high in the control population. The correlation between Mean fasting blood sugar levels and c-peptide levels with serum melatonin levels is an inverse relationship.

Key words: Melatonin % C-Peptide % Type 2 Diabetes Mellitus

INTRODUCTION

The term "diabetes mellitus" describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. It is classified into 2 types -Insulin dependant Diabetes Mellitus which is type I (IDDM) and Non -Insulin dependant Diabetes Mellitus that is type II(NIDDM). Type I diabetes is an autoimmune disease characterized by a local inflammatory reaction in and around islets that is followed by selective destruction of insulin secreting cells. Type II diabetes is characterized by peripheral insulin resistance and impaired insulin secretion [1].

Melatonin is a circulating hormone that is mainly released from the pineal gland. It is best known as a regulator of seasonal and circadian rhythms, its levels being high during the night and low during the day. Interestingly, insulin levels are also adapted to day/night

changes through melatonin-dependent synchronization. This regulation may be explained by the inhibiting action of melatonin on insulin release, which is transmitted through both the pertussis-toxin-sensitive membrane receptors MT_1 and MT_2 and the second messengers 3',5'-cyclic adenosine monophosphate, 3',5'-cyclic guanosine monophosphate and inositol 1,4,5-trisphosphate [2]. Melatonin may influence diabetes and associated metabolic disturbances not only by regulating insulin secretion, but also by providing protection against reactive oxygen species, since pancreatic β -cells are very susceptible to oxidative stress because they possess only low-antioxidative capacity. On the other hand, in several genetic association studies, single nucleotide polymorphisms of the human MT_2 receptor have been described as being causally linked to an elevated risk of developing type 2 diabetes. This suggests that these individuals may be more sensitive to the actions of melatonin, thereby leading to impaired insulin secretion. Many evidence indicate that melatonin shows an

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outstanding functional versatility, by exhibiting antioxidant, oncostatic, antiaging and immunomodulatory properties [3].

C-peptide is a protein that is produced in the body along with insulin. First preproinsulin is secreted with an A-chain, C-peptide, a B-chain and a signal sequence. The signal sequence is cut off, leaving proinsulin. Then the C-peptide is cut off, leaving the A-chain and B-chain to form insulin [4].

Newly diagnosed diabetes patients often get their C-peptide levels measured as a means of distinguishing type 1 diabetes and type 2 diabetes. C-peptide levels are measured instead of insulin levels because insulin concentration in the portal vein ranges from two to ten times higher than in the peripheral circulation. The liver extracts about half the insulin reaching it in the plasma, but this varies with the nutritional state. The pancreas of patients with type 1 diabetes is unable to produce insulin and, therefore, they will usually have a decreased level of C-peptide, whereas C-peptide levels in type 2 patients are normal or higher than normal. Measuring C-peptide in patients injecting synthetic insulin can help to determine how much of their own natural insulin these patients are still producing, or if they produce any at all.

A C-Peptide Test is Done To:

- C Differentiate between type 1 diabetes and type 2 diabetes.
- C Find the cause of low blood sugar (Hypoglycemia).
- C Check to see whether a tumor of the pancreas (Insulinoma) was completely removed.

AIM: Estimation of Serum Melatonin and Serum C-Peptide levels in Uncontrolled Type 2 Diabetes Mellitus- Correlation with normal people.

Objectives:

- C To estimate the levels of Serum Melatonin and Serum C-Peptide levels in Uncontrolled Type 2 Diabetes Mellitus and normal population(Non-diabetic).
- C To investigate whether there is a correlation between Serum Melatonin and Serum C-Peptide levels in Uncontrolled Type 2 Diabetes Mellitus.
- C To investigate whether there is a correlation between Serum Melatonin and fasting blood sugar levels in Uncontrolled Type 2 Diabetes Mellitus
- C To compare Serum Melatonin and Serum C-Peptide values between the diabetic and non-diabetic population.

MATERIALS AND METHODS

Study Population: This study was conducted in SreeBalaji Medical College, Chennai, Tamil Nadu during the period of May 2012 to May 2013. Total number of people included in this study were 50. Out of 50, 26 were patients with type 2 diabetes mellitus and 24 were age & sex matched controls. There were 21 male and 29 female patients ranging from 35 years to 85 years.

Study Design: This study is a cross-sectional study.

Inclusion Criteria:

- C Male and female patients of age > 40 years were selected.
- C HbA1c of >8
- C Fasting blood sugar of > 200

Exclusion Criteria:

- C Type 1 diabetes mellitus
- C Age >85 years
- C History of insomnia, Psychiatric illness
- C Patients with recent past history of surgery and admission in icu
- C History of stress, renal failure and acute infection.
- C BMI >30, not a diabetic
- C Pregnant women
- C Patient who is not willing to participate in the study
- C Individuals who are cognitively impaired
- C Born blind people.
- C Any other health or mental condition that in the Investigator's opinion may adversely affect the subject's ability to complete the study.

Description of the Study: Patients satisfying the criteria for our study were briefed about the study intention and consent obtained for collecting blood samples.

Patients visiting SreeBalaji Medical College were categorised into 2 groups

- C Uncontrolled Type II Diabetes Mellitus
- C Healthy age and sex matched controls.

Age group of the patients were between 35 and 85 years. Both Male and Female were included in the study. Blood samples were collected from the patients with type 2 uncontrolled diabetes mellitus and healthy age and sex matched controls and the same was sent to the laboratory for estimation of FBS, serum melatonin levels

and serum c-peptide levels. Patients were asked to stop eating and drinking for 8 hours before the blood test for c-peptide levels. Insulin and some oral medicines used to treat type 2 diabetes can change the test results. Hence patients were asked to stop these medicines before the blood test.

Fasting Blood Sugar: After overnight 8 hours of fasting, 5 ml of venous blood sample was collected for estimating fasting blood sugar (mg/dl).

Serum melatonin: Melatonin was assessed by drawing blood in dim light (0 lux) via an indwelling cannula from an antecubital vein for measurement of melatonin levels while the patients were sleeping around 4 a.m in the morning. Particular attention was paid to allowing the participants to remain asleep during the blood collection. Blood was collected without anti-coagulant and serum separated. Estimation of melatonin was done by BG MLT ELISA kit. It is a 1.5 hour solid-phase ELISA designed for the quantitative determination of human melatonin. MLT ELISA kit applies the quantitative sandwich enzyme immunoassay technique.

Serum C-Peptide: Patients already taking oral hypoglycaemic agents were asked to stop the drugs for 3 days. The venous blood samples were taken in the fasting state after 8 hrs of fasting. Blood was collected using standard venipuncture without any anticoagulant and the serum separated. DRG c-peptide ELISA was used in this study to estimate the fasting c-peptide levels. The DRG c-peptide ELISA kit is a solid phase enzyme linked immunosorbent assay, based on the principle of competitive binding. This study was conducted with a sample size of 50 people. Out of 50 people 26 were patients with type 2 diabetes mellitus and 24 were control population. They were selected with the help of the proforma and as per the inclusion and exclusion criteria. The patients and controls were age and sex matched. Their blood samples were taken for FBS, serum c-peptide and melatonin levels at 4 a.m in the morning while they were asleep via an indwelling i.v. cannula. The tests were done, data collected and results were obtained.

RESULTS

Frequency Tables: Table 1 shows the comparison between melatonin & c-peptide, FBS levels in patients & controls. There is an inverse relationship between melatonin & c-peptide levels which is statistically significant as per the p value.

Frequency table 1 shows the frequency distribution of c-peptide levels among patients

C-Peptide Levels among Patients	Frequency	Percent
2.5 - 4.5	16	61.5
4.5 - 6.5	7	26.9
6.5 - 8.5	1	3.8
>8.5	2	7.7
Total	26	100.0

Frequency table 2 shows the frequency distribution of FBS levels among patients

FBS among Patients	Frequency	Percent
<250	16	61.5
250 - 300	9	34.6
300 - 350	1	3.8
Total	26	100.0

Frequency table 3 shows the frequency distribution of melatonin levels among patients

Serum Melatonin among Patients	Frequency	Percent
<15	4	15.4
15 - 25	15	57.7
25 - 35	7	26.9
Total	26	100.0

Frequency table 4 shows frequency distribution of c-peptide levels among controls

C-Peptide Levels among Controls	Frequency	Percent
<0.5	2	8.3
0.5 - 1.0	2	8.3
1.0 - 1.5	6	25.0
1.5 - 2.0	6	25.0
2.0 - 2.5	8	33.3
Total	24	100.0

Frequency table 5 shows the frequency distribution of FBS levels among controls

FBS among Controls	Frequency	Percent
<75	1	4.2
75 - 100	15	62.5
100 - 125	8	33.3
Total	24	100.0

Frequency table 6 shows the frequency distribution of melatonin among controls

Serum Melatonin among Controls	Frequency	Percent
50 - 100	14	58.3
100 - 200	4	16.7
200 - 300	6	25.0
Total	24	100.0

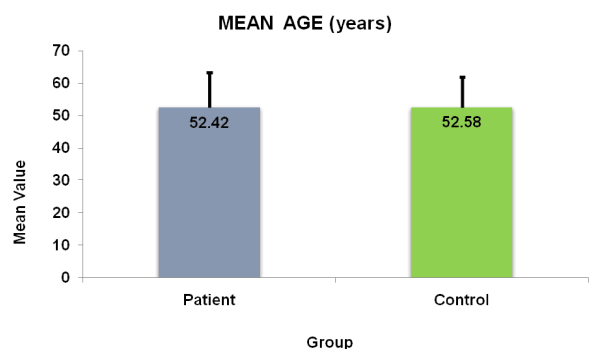


Fig. 1: Mean age of patients and control population was matched

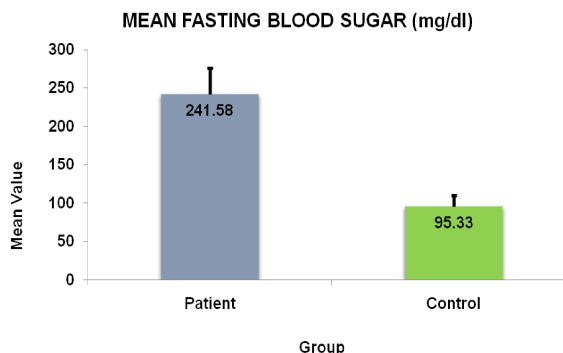


Fig. 4: Similarly mean fasting blood sugar levels were high in the patients compared to control population.

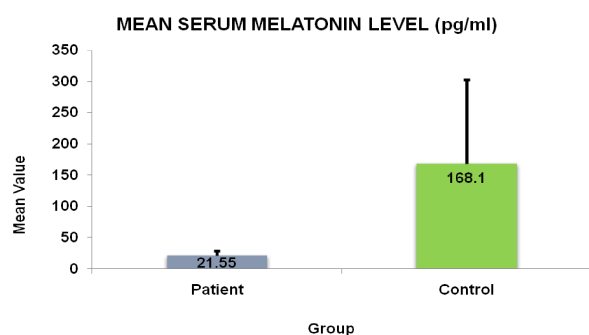


Fig. 2: Bar diagram 2 shows that patients had very low melatonin levels compared to the control population.

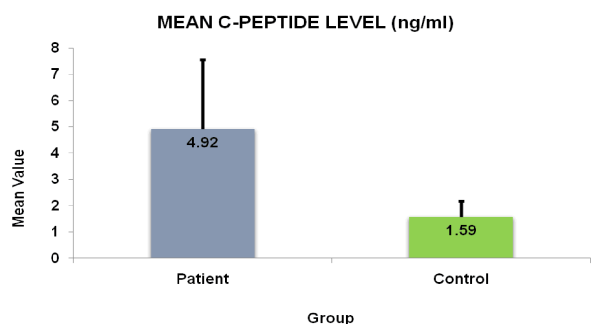


Fig. 3: Whereas here the c-peptide levels are high in the patients compared to the controls.

Table 1: Independent samples T-Test to compare the mean values between patient and controls

Variables	Group	N	Mean	Std. Dev	P-Value
AGE	Patient	26	52.42	10.89	0.956
	Control	24	52.58	9.38	
Serum Melatonin Level (pg/ml)	Patient	26	21.55	6.67	0.001
	Control	24	168.10	134.50	
C-peptide Level (ng/ml)	Patient	26	4.92	2.63	<0.001
	Control	24	1.59	0.58	
Fasting Blood Sugar (mg/dl)	Patient	26	241.58	34.30	<0.001
	Control	24	95.33	14.852	

DISCUSSION

The frequency Table 1 shows the level of fasting c-peptide levels among type 2 diabetes mellitus patients in the present study. Out of 26 patients 16(61.5%) patients had fasting c-peptide levels between 2.5-4.5 ng/ml, 7(26.9%) patients had fasting c-peptide levels between 4.5-6.5, 1(3.8%) patient had c-peptide levels between 6.5-8.5 ng/ml and 2(7.7%) had c-peptide levels more than 8.5 ng/ml. The mean fasting c-peptide level of the study in patients was 4.92 ± 2.63 . The mean fasting c-peptide level of the study in controls was 1.59 ± 0.58 .

In a study done by Bilal *et al.*[5] in north Karnataka, out of 75 patients, 39 (52%) patients had fasting c-peptide levels between 1 - 2 ng/ml, 19(25.4%) patients had fasting c-peptide levels between 0.6-0.9 ng/ml and 13 (17.3%) patients had > 2 ng/ml. Only 4 (5.3%) patients had <0.6 ng/ml. The mean fasting c-peptide level of the study was 1.315 ± 0.811

The frequency table 2 shows that out of 26 patients with type 2 diabetes mellitus 16(61.5%) had FBS levels <250mg/dl, 9 (34.6%) patients had FBS levels between 250-300mg/dl and 1(3.8%) patient had FBS levels between 300-350 mg/dl. The mean FBS level among patients was 241.58 ± 34.30 . The mean fasting glucose level among the control population was 95.33 ± 14.852 .

In the study done by Bilal *et al.* [5] out of 75 patients with type 2 diabetes, 42(56%) patients had fasting plasma glucose level between 200-250 mg/dl, 19(25%) patients had < 200 mg/dl and 14(19%) patients had =250 mg/dl. So the study shows that majority of the patients (42) patients had fasting plasma glucose levels between 200-250 mg/dl. The mean plasma glucose level of the study was 222.92 ± 29.11 .

The frequency Table 3 shows that out of 26 patients 4(15.4%)patients had serum melatonin levels <15 pg/ml,15(57.7%)patients had serum melatonin levels between 15-25 pg/ml,7(26.9%)patients had melatonin levels between 25-35 pg/ml.The majority of the patients with type 2 diabetes mellitus(57.7%) had early morning serum melatonin levels between 25-35 pg/ml.The mean serum melatonin among patients was 21.55 ± 6.67 .The mean serum melatonin among control population was 168.10 ± 134.50 .

Taichii *et al.* [6]conducted the first study to evaluate the relationship between melatonin levels and diabetic retinopathy. Their results show that alteration of nighttime melatonin level was more apparent in patients with PDR.Theyreported that the nighttime and daytime melatonin levels were 35.5 ± 33.4 pg/mL and 2.8 ± 3.0 pg/mL in the nondiabetic group, 23.6 ± 30.1 and 1.0 ± 0.9 pg/mL in the diabetic group, 34.8 ± 37.4 and 1.33 ± 0.7 pg/mL in the NPDR group and 10.9 ± 11.4 and 0.7 ± 0.9 pg/mL in the PDR group. The night time melatonin level was significantly lower in the diabetic group than in the nondiabetic group ($P < 0.03$, Student's t-test) and in the PDR group than in the nondiabetic and NPDR groups ($P < 0.01$ and $P < 0.03$, repeated-measures analysis of variance), but no significant difference was found between the nondiabetic and NPDR groups. The daytime melatonin level did not significantly differ between the nondiabetic and diabetic groups, or between the nondiabetic, NPDR and PDR groups.

Tutuncu *et al.* [7] reported that the circadian rhythm of melatonin secretion was deteriorated in patients with type 2 diabetes and that there was a complex relationship between various components of the autonomic nervous system and nighttime melatonin secretion.

Table 1 shows the correlation between serum melatonin levels and FBS,c-peptide levels in patients and control population.Since the patients and controls were age matched mean age of the patients and controls were almost the same.The mean serum melatonin levels were significantly low in the patient population (21.55pg/ml) compared to the control population (168.10). The mean c-peptide levels(4.92ng/ml) and FBS levels (241.58mg/dl) were high in the patient population compared to the control population (1.59ng/ml&95.33mg/dl) which is statistically significant with a p value <0.001.

Hence there is an inverse relationship between serum melatonin and c-peptide,FBS levels in type 2 diabetes mellitus patients.

Ciarin *et al.* [8] conducted a study to find the association between melatonin secretion and the risk of developing type 2 diabetes. The importance of the study was based on the fact that loss of function mutations in the melatonin receptor are associated with insulin resistance and type 2 diabetes. Additionally, in a cross-sectional analysis of persons without diabetes, lower nocturnal melatonin secretion was associated with increased insulin resistance. They concluded that lower melatonin secretion was independently associated with a higher risk of developing type 2 diabetes.

In my study patients with high c-peptide levels also had high mean fasting blood sugar levels instead of low levels indicating insulin resistance which is the main pathophysiology behind type 2 diabetes mellitus.

Bilal *et al.* [5] in their study found that those with fasting c-peptide levels > 2ng/ml, instead of having lower FBS levels had elevated FBS level above 200mg/dl. 8 patients out of the 13 had >250 mg/dl of FBS. The observations suggest that fasting c-peptide levels and FBS were moderately associated ($p=0.05$). They were moderately positively correlated ($r=0.532$) ($p=0.000$).The positive correlation indicates that as the fasting c-peptide level increases, the FBS level also increases, but not in a linear fashion.It was observed that the increase in fasting c-peptide levels were associated with increased plasma glucose due to insulin resistance and this was proved significant in their study by P2 value of 19.782and p value of 0.05. A positive correlation exists in the study with r value of 0.532.

CONCLUSIONS

- C From this study I draw the following conclusions :-
- C The control population had normal mean fasting blood sugar levels and c-peptide levels. They had high serum melatonin levels in the early morning time as per the normal circadian rhythm.
- C Patients with uncontrolled Type 2 Diabetes mellitus had high mean fasting blood sugar levels and c-peptide levels.They had low serum melatonin levels in the early morning time in contrast to the normal population who have high melatonin levels in the early morning time.
- C The correlation between Mean fasting blood sugar levels and c-peptide levels with serum melatonin levels is an inverse relationship.

- C Lower nocturnal melatonin secretion is associated with increased insulin resistance indicated by the high c-peptide levels in uncontrolled type 2 diabetes mellitus patients.
- C Comparison between patients and controls show that the serum melatonin levels are low in uncontrolled type 2 diabetes mellitus patients and high in the control population.

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