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TO DETERMINE THE RELATIONSHIP BETWEEN CHEMERIN AND MELATONIN LEVELS IN TYPE 2 DIABETES MELLITUS OBESE SUBJECTS

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Abstract:

Purpose: Chemerin and melatonin are known to induce obesity and type 2 diabetes mellitus (T2DM). **Aim:** We aimed to investigate the correlation between chemerin, melatonin, insulin resistance, and glycosylated hemoglobin levels, in addition to the association between chemerin, melatonin, obesity, and T2DM. **Materials & Methods:** The present study comprised of hundred and fifteen obese subjects each in T2DM group and non-diabetics control group. All the subjects of both groups were scrutinized from the out-patient departments of Index Medical college &

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Research Centre, Indore. After taking permission from the Institutional Ethics committee, the authors of the study have initiated the work. **Results:** The BMI was significantly higher in obese T2DM subjects compared to obese non-diabetic subjects (32.6 ± 4.2 and 30.9 ± 0.8). Obese subjects with T2DM had a higher abdominal circumference than obese non-diabetic subjects ($P < 0.05$). Higher levels of serum chemerin concentration was observed in obese T2DM subjects than obese non-diabetic subjects (106.8 ± 9.8 and 78.2 ± 4.1; $P < 0.05$). However, we did not observe a significant difference in the serum melatonin levels between the two groups. In obese subjects with T2DM, the chemerin level correlated positively with glycosylated hemoglobin and HOMA-IR (0.534, $P < 0.009$ and 0.399, $P < 0.014$, respectively). **Conclusion:** Chemerin is associated with obesity, insulin resistance, and T2DM independent of melatonin levels. This study provides the basis for any association between chemerin and melatonin in future studies worldwide.

Keywords: Chemerin; Inflammation; Melatonin; Obesity; Type 2 Diabetes Mellitus

Introduction:

Nearly 2 billion adults aged 18 years and older worldwide are overweight, with 650 million obese, or 39% of the world's population. [1,2]. Obesity kills 2.8 million people annually [1,3]. Atherosclerosis, cardiovascular diseases, cerebrovascular accidents, chronic kidney diseases, and peripheral artery diseases contribute to mortality in obesity and type 2 diabetes mellitus (T2DM) individuals [4-6].

Silent or subclinical inflammation (affected individuals are not aware of it) through cytokine production causes the onset and progression of secondary complications in obese and diabetes mellitus individuals. Cytokines can be anti-inflammatory or pro-inflammatory; one reduces inflammation, and the other causes it. Unbalanced anti- and pro-inflammatory cytokines cause tissue pathology and the increase in inflammatory cytokines induces the severity of inflammation [7,8]. Chemerin, an adipose-produced cytokine, is an inflammatory cytokine [7-9]. In addition, global research links chemerin to energy homeostasis, energy metabolism, endothelial dysfunction, and vascular insufficiency in obesity and T2DM [9-13].

Melatonin is a pineal gland product which regulates energy flow to and from energy stores [14]. It also regulates energy storage (excess lipid stores) and energy expenditure by activating brown and browning white adipose tissue [15-19]. Melatonin synchronizes synthesis of hormone, its secretion, and regulates normal metabolism and it performs these functions via circadian rhythm [14-16]. Further the levels of melatonin are strongly related to insulin resistance, obesity and T2DM [17-19].

Knowing the associations of chemerin and melatonin with obesity comorbidities in research models, chemerin/melatonin-targeted research is thought to improve metabolic disorders such as insulin resistance, obesity, and T2DM [4-19]. Nevertheless, there is little knowledge in the scientific literature about the association of chemerin with melatonin. Therefore, We aimed to

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investigate the correlation between chemerin, melatonin, insulin resistance, and glycosylated hemoglobin levels, in addition to the association between chemerin, melatonin, obesity, and T2DM. As a result, our study is the first to look at the relationship between BMI and melatonin and chemerin in T2DM in a cross-sectional setting.

Materials & Methods:

Place of study:

The present study comprised of hundred and fifteen obese subjects each in T2DM group and non-diabetics (control) group. All the subjects of both groups were scrutinized from the out-patient departments of Index Medical college & Research Centre, Indore. After taking permission from the Institutional Ethics committee, the authors of the study have initiated the work. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Before commencing the present study, from each participant informed consent has been obtained. Exclusion criteria for obese T2DM subjects were individuals more than 50 years in age, and with pathological conditions. The inclusion criteria for obese non-diabetic subjects were not having diabetes, not using supplementations, having no other known pathological conditions, and above 50 years in age. By using standard protocols and considering the exclusion and inclusion criteria of the present study, all the subjects of both groups have undergone a general physical examination by a qualified physician of the medicine department of the hospital where the study had taken place. Height in meters (m) and weight in kilograms (kg) was recorded and using both, we calculated the BMI (by dividing body weight (kg) by the square of height (m2) of each participating subject. After recording the BMI, subjects who were fulfilling the criteria of obesity (≥30 kg/m2) were placed into respective groups.

Collection of samples:

From all the subjects of both groups, under sterile conditions 5ml of fasting venous blood was collected with a disposable syringe & needle into respective vials. Serum samples were aliquoted and stored at 20 ° C until assay after separating the blood through centrifugation of the blood at 3000 rpm for 20 minutes.

Parameters analyzed & instruments used:

Plasma glucose, glycosylated hemoglobin, serum insulin, chemerin, and melatonin were analyzed. Plasma glucose was estimated by using the method Glucose Oxidase and Peroxidase (DPEC – GOD/POD) purchased from Avantor laboratories. Glycosylated hemoglobin was estimated by using the ClinRep complete kit on the BioRad HbA1c analyzers Diamat and Variant. Homeostasis metabolic assessment of insulin resistance (HOMA-IR) was calculated by using the Muniyappa formula.

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Serum chemerin was assessed using an ELISA kit purchased from Invitrogen laboratories. Serum insulin and melatonin was assessed using ELISA assay method purchased from Eagle Biosciences laboratories. These immunoassays were estimated by sandwich ELISA method ROBONIK micro plate reader was used to measure Elisa assay readings. Mixing of reagents was according to the instructions given in the kit manual of respective parameters.

Statistical analysis:

All values obtained were expressed as mean ± standard deviation. Statistical analysis of the results was performed by an unpaired "t" test between the two groups. We used Pearson coefficient correlation to test the correlation between the two parameters. Scattered regression coefficients were used to know the association between the dependent and independent variables. Statistical significant was considered at $P < 0.05$.

Result:

Demographic details were compared between obese subjects with T2DM and obese without diabetes (non-diabetic) in Table 1. Age ranges for obese T2DM subjects between 35-49 years (mean: 48.3 ± 4.3) and 50-64 years for obese non-diabetic subjects (54.6 ± 14.5). The BMI was significantly higher ($P < 0.05$) in obese T2DM subjects compared to obese non-diabetic subjects (32.6 ± 4.2 and 30.9 ± 0.8). Obese subjects with T2DM have a higher value of abdominal circumference when compared to obese non-diabetic subjects ($P < 0.05$).

We observed parameters of fasting blood sugar ($t = 51.42$; $df = 228$; $P < 0.001$, not shown), glycosylated hemoglobin ($t = 5.96$; $df = 228$; $P < 0.05$), and insulin mean levels ($t = 24.38$; $df = 228$; $P < 0.05$) showed a significant difference. Similarly, serum chemerin concentration was significantly higher in obese T2DM subjects than obese non-diabetic subjects (106.8 ± 9.8 and 78.2 ± 4.1; $P < 0.05$), while the difference was not significant for serum melatonin levels (39.1 ± 9.1 and 42.3 ± 4.6; $P > 0.05$) (Table 1).

In obese subjects with T2DM, the level of chemerin correlated positively with glycosylated hemoglobin and HOMA-IR (0.534, $P < 0.009$ and 0.399, $P < 0.014$), indicating a significant relationship with glycemic control and glucose intolerance (Table 2).

Discussion:

The results of the current investigation showed that, while melatonin levels were not substantially different between obese non-diabetic participants and obese subjects with T2DM, serum chemerin was significantly higher in obese subjects with T2DM than obese non-diabetic subjects. Chemerin levels were favorably correlated with insulin resistance and had a substantial positive connection with glycosylated hemoglobin. These results imply that chemerin is involved in the etiology of T2DM through peripheral insulin action-related processes that are independent

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of melatonin. In comparison to control obese non-diabetic participants, our obese people with T2DM exhibit reduced beta-cell function and increased insulin resistance.

Most obese subjects with T2DM in the current study are aged below 50 years and their elevated chemerin levels showed a significant positive correlation with insulin resistance. On the other hand, serum melatonin level was not higher in our obese T2DM subjects than in obese non-diabetic subjects and its correlation with insulin resistance is weak. Literature across the world report that there is decrease in melatonin levels above 50 years of age [20] which indicates some metabolic changes before reaching insulin resistance and T2DM that affect the prone individuals towards the initiation of obesity. These findings might explain why in our study we could not find difference in melatonin level between obese subjects with T2DM diabetic patients and obese non-diabetic subjects since our control mean age was 53.5 years.

The catabolic effect of chemerin on adipose tissue, increased insulin resistance and glucose intolerance in obese rats indicate its important role in development of insulin resistance and diabetes because of obesity [21]. Previous studies reported that chemerin contributes to insulin resistance and T2DM that is associated with obesity [24,25]. In addition, the calorific restriction and exercise effect on waist-hip-ratio and insulin resistance after decreasing chemerin in female T2DM subjects and in obese women indicate its important role in development of insulin resistance and diabetes [22,23].

We found that in obese subjects with T2DM, the higher the level of chemerin, the higher their glycosylated hemoglobin value, which means that chemerin level can be used to predict glycemic control in obese diabetic subjects. A study [27] showed that diabetes was strongly associated with elevated levels of chemerin, which was most significantly elevated in the group of patients with glycosylated hemoglobin mean values of 8.7 %. In our study, the average value for glycosylated hemoglobin in patients with T2DM was 8.2 % and it was positively associated with chemerin level. Since glycosylated hemoglobin is not a direct measure of glycemia, there is a chance that its level might change due to factors unrelated to blood glucose levels such as rate of glycation and turnover of erythrocytes [28,29]. Recently, studies indicated that elevated levels of inflammatory cytokines, including chemerin, were strongly associated with increased risk to occurrence of T2DM [30,31]. Moreover, the finding that chemerin level decreases with good glycemic control would confirm this hypothesis. Interestingly, a previous study showed a significant reduction in chemerin serum level in obese T2DM subjects following treatment with diet and exercise [32,33]. A study reported a correlation between the drop in serum chemerin level and glycosylated hemoglobin value after regular exercise in general population [34]. The correlation between chemerin and glycated hemoglobin suggests that this knowledge could be applied clinically to track the degree of glycemic control in T2DM patients after undergoing medical treatment or making lifestyle changes, as well as in circumstances where glycated hemoglobin cannot be relied upon because of a glycation defect, blood loss, or low hemoglobin levels. The most important limitation of our study is that the study did not consider the circadian

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rhythm of melatonin. Therefore, the authors of this study attempted to compensate for this shortcoming by taking blood samples from all subjects at the same time.

Conclusion:

The current cross-sectional study concludes that chemerin is associated with obesity, insulin resistance, and T2DM independent of melatonin levels. This study provides the basis for any association between chemerin and melatonin in future studies worldwide.

Compliance with Ethical Standards:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflict of interest:

The authors of the study have no competing interests to declare that are relevant to the content of this article.

Funding information:

All authors certify that they have no financial or non-financial interests in the subject matter or materials discussed in this manuscript.

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Table 1: Findings in obese T2DM and obese non-diabetic groups

Variable	Obese T2DM subjects (n=115)	Obese non-diabetic subjects (n=115)	P- value
BMI (kg/mt^2)	32.6 ± 4.2	30.9 ± 0.8	S
Serum insulin (pg/mL)	13.6± 4.1	29.2 ± 5.5	S
HOMA-IR	29.3 ± 7.9	8.3 ± 2.9	S
Glycosylated hemoglobin (g%)	8.2 ± 1.8	6.3 ± 0.9	S
Chemerin (pg/mL)	106.8 ± 9.8	78.2 ± 4.1	S
Melatonin (pg/mL)	39.1 ± 9.1	41.1 ± 10.7	NS

Note: T2DM: Type 2 Diabetes Mellitus; BMI- body mass index, Homeostasis metabolic assessment of insulin resistance (HOMA-IR), S-Significant (<0.05), NS-Not Significant (>0.05)

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Table 2: Showing the pearson correlation and scattered regression coefficients of different variables of obese T2DM and obese non-diabetic groups

	Serum chemerin (pg/mL)		Serum melatonin (pg/mL)	
	R	P	R^2	P
Obese non-diabetic group (n=115)				
Body mass index (kg/mt^2)	0.162	0.39	0.075	0.118
Abdominal circumference (cm)	0.078	0.489	0.068	0.298
Waist-to-hip-ratio (cm)	-0.184	0.193	0.593	0.101
Serum insulin (pg/mL)	0.035	0.65	0.013	0.42
HOMA-IR	-0.137	0.151	0.092	0.733
Glycosylated hemoglobin (g%)	0.088	0.142	0.099	0.089
Obese subjects with T2DM group (n=115)				
Body mass index (kg/mt^2)	**0.251**	**0.024**	0.447	0.048
Abdominal circumference (cm)	**0.233**	**0.024**	0.411	-0.098
Waist-to-hip-ratio (cm)	**0.528**	**0.013**	-0.184	0.049
Serum insulin (pg/mL)	0.023	0.72	0.075	0.118
HOMA-IR	**0.399**	**0.014**	**0.281**	**0.053**
Glycosylated hemoglobin (g%)	**0.534**	**0.009**	0.593	0.101