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Serum melatonin levels alter in type 2 diabetes mellitus individuals along with IL-2, IL-15 and TNF-α

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Abstract:
Background & Aims: According to our knowledge, there is a lack of sample study in this specific region of our country, regarding the relationship of melatonin with anti- and pro-inflammatory cytokines. Therefore, the novelty of this present study, we have taken the opportunity to determine the relationship of melatonin with anti- (IL-2) and pro-inflammatory (IL-1β, IL-15, and TNF-α) cytokines in 2DM subjects. Methods: After obtaining the approval from the institutional ethical committee, the present study was commenced. The study was conducted in Department of Biochemistry, Malwanchal University, Indore, India. Total two hundred and thirty individuals are recruited in to this present study after the approval from Institutional ethical committee. Age & sex matched one hundred and fifteen human non-2DM individuals were taken into healthy control group. One hundred and fifteen subjects, on treatment for 2DM were included in second group. Results: Statistical significant differences were observed in serum melatonin levels and post-prandial blood glucose when compared between 2DM subjects and healthy controls. We also observed statistical significances in the serum values of IL-2, IL-15, IL-1β, and TNF-α when compared between 2DM subjects and healthy controls. The study observed steady upward positive correlation between melatonin and IL-2 levels in healthy controls. On the contrary the study observed a negative association between serum melatonin and IL-15 levels and also between melatonin and TNF-α in 2DM subjects. Conclusion: The authors conclude from the study that alterations in the study parameters in 2DM group are due to altered balance between anti-inflammatory and pro-inflammatory cytokines production. This altered balance is due to low production of melatonin in 2DM subjects than healthy controls.

Keywords: Hormones, type 2 diabetes mellitus, melatonin, cytokines, dys-balance
Study Design: Observational Study.

1. INTRODUCTION:

The life's processes are interconnected mutually to maintain the integrity and fidelity of the organism's survival [1,2]. One such process which unifies or connected with every system is metabolism. The metabolism should be regulated in a harmonious way to sustain a normal and healthy vital process and this is done with the help of hormones, which are regulatory and communicative substances between organs and tissues [3,4]. The hormones affect distant cells by binding to specific receptors on/in the target cell and results in a change in cell function by virtue of signal transduction pathway via activation/deactivation. These signal molecules cause rapid physiological anabolic effects or slower genomic responses where the hormones acting through their receptors results in increased expression of cytokines and eventually physiological functions due to target proteins [5]. Henceforth, any disorder in physiological balance inflicts metabolic disorders and can be considered as the derangement in harmonious well being of the individual.

Non-insulin dependent diabetes mellitus (2DM) is the most frequent type of diabetes, nevertheless, its main cause is yet to be clarified. Several environmental and genetic parameters are believed to be involved. Either genetic or contributing factors (lifestyle) or both in conjunction play a vital role in the development of 2DM [6-8]. There is a plethora of observational studies, which shows that the obese people are at a greater risk of developing pre-diabetic state (IR) and 2DM [9,10].

Interleukins (ILs) are a group of cytokines (signaling molecules) that are produced by the leucocytes including monocytes, macropahges, T lymphocytes, and endothelial cells. Interferons (IFNs) are the signaling factors released by the lymphocytes. ILs also act as the inflammatory markers as their involvement with inflammation is potent and modulator. As 2DM is a complex low grade inflammatory metabolic disorder [11,12], hence there should be an involvement in the initiation and development of the disorder related to the balance of cytokine production. In conjunction, several studies have showed that IL-1β, IL-2, IL-15, IFN-gamma and Tumor necrosis factor-alpha (TNF-α) are associated with the regulation of expression of 2DM and its genes [13-16]. Petrica et al [13]., reported an association between specific serum and urinary ILs in the inflammatory response in 2DM patients with diabetic kidney disease. Tangvarasitticha et al [16]., showed elevated levels of TNF-α and observed IR in T2DM individuals when compared with healthy human controls. Phosat et al [14]., observed elevation of inflammatory cytokines, oxidative stress and insulin resistance are associated with 2DM women subjects. These inflammatory cytokines are positively associated with 2DM and may have a causal relation with an increased oxidative stress in 2DM subjects. More importantly, these cytokines expression differ from person to person and in different societies.

Melatonin is synthesized in the pineal gland and other tissues [17,18], which influences several endocrine and biological functions. It is documented in the literature that the size of the pineal gland decreases in size as the age advances [17]. Melatonin releases maximally early in the morning, peaks between 2 am to 5 am and maintains sleep pattern in circadian rhythm and seasonal functions. Melatonin synthesis occurs largely at night and in low quantity in the day time [19]. Melatonin is useful in insulin action based on the finding that pinealectomy induces insulin resistance [20]. Genome-wide studies across the globe showed the association of melatonin with 2DM and also with the risk of developing of 2DM in susceptible individuals [21-24]. Berbets et al [25]., in their report mentioned that serum melatonin levels are negatively correlated with IL-1β and TNF-α in individuals experiencing placental insufficiency. On the

other side, some studies after supplementation of Melatonin observed decrease in pro-inflammatory cytokines production including IL-1β and TNF-α, at baseline these variables were altered [26]. Further, melatonin is profoundly known as antioxidant hormone for its action at reducing oxidative stress [17-20]. Nevertheless, these findings imply that melatonin deficiency leads to dys-balance between anti- and pro-inflammatory cytokines, which leads to oxidative stress. Considering the connection from the studies [19-26] it is not coincidental to say that melatonin and anti- and pro-inflammatory cytokines are inter-related to each other.

However, according to our knowledge, there is a lack of sample study in this specific region of our country, regarding the relationship of melatonin with anti- and pro-inflammatory cytokines. Therefore, the novelty of this present study, we have taken the opportunity to determine the relationship of melatonin with anti- (IL-2) and pro-inflammatory (IL-1β, IL-15, and TNF-α) cytokines in 2DM subjects.

The null hypothesis of the present study is that serum melatonin has no effect on anti- and pro-inflammatory cytokines in 2DM subjects. Alternate hypothesis is that serum melatonin has an effect on anti- and pro-inflammatory cytokines in 2DM subjects.

2. MATERIALS & METHODS:

After obtaining the approval from the institutional ethical committee, the present study was commenced. The study was conducted in Department of Biochemistry, Malwanchal University, Indore, India. Total two hundred and thirty individuals are recruited in to this present study after the approval from Institutional ethical committee. Age & sex matched one hundred and fifteen human non-2DM individuals were taken into healthy control group. One hundred and fifteen subjects, on treatment for 2DM were included in second group. The diagnosis of 2DM was made according to the norms laid by American Diabetes Association. The diagnosis of 2DM group subjects was done by the consultants of Medicine department of Malwanchal University. A physical examination was performed on all subjects by a qualified doctor per established standard methods. Exclusion criteria were 2DM individuals, more than five years of known duration of 2DM, and with known complications. As far as healthy controls inclusion criteria, are 2DM and those who are not on supplementations intake, and having no other complications. Five mL of fasting venous blood were drawn into fluoride and plane vials, after informed written consent from all the study group subjects with a disposable syringe & needle, under all aseptic conditions. Plasma and serum was separated by centrifuging the blood at 3000 rpm for 20 minutes. Separated samples into aliquots of plasma and serum were stored at -20^{o} C until assayed. Serum melatonin was estimated by using the Elisa Assay method purchased from Eagle Biosciences laboratories. Reagents mixing were done if required as to the instructions laid in respective kit manual. Serum cytokines were estimated with multi-analyte Elisa array kit bought from Qiagen laboratories. Plasma glucose was estimated by Glucose-oxidase and peroxidase method according to the instructions presented in the kit manual.

Statistical analysis:

IBM SPSS version 20 software was used to perform the statistical analysis. Column charts were used to show the mean values of variables of the present study. Unpaired student t test was used to estimate the statistical difference between the two groups of different variables used in the present study. Any association between the two variables was estimated by using the scatter diagrams.

3. RESULTS:

Post-prandial blood glucose, anti-inflammatory and pro-inflammatory cytokines were estimated in the present study along with melatonin levels in both the groups. Column chart (Figure 1) showing the mean values of melatonin, IL-2, IL-15, IL-1β, IFN-γ, and TNF-α in 2DM and healthy controls. Lower levels of IL-1β and TNF-α have been observed in the healthy control subjects when compared with 2DM subjects. In addition, we observed statistical difference between these two groups in case of IL-1β and TNF-α serum levels. On the other hand, higher serum levels have been observed in case of melatonin, IL-2, and IL-15 in healthy control individuals than 2DM individuals. Interestingly, we observed statistical difference in these parameters when compared between 2DM subjects and healthy control subjects. The mean level of post-prandial blood glucose is 196±62.3 in 2DM group subjects and the mean plasma glucose level of healthy controls is 123.7±11.5.

In figure 2, we tried to find the association between the serum levels of melatonin and IL-2 in healthy controls. We observed steady upward positive correlation ($y = 0.170x + 46.32$) between melatonin and IL-2 levels in healthy controls. On the contrary in figure 3, we observed a negative association ($y = -0.016x + 3.828$) between serum melatonin and IL-15 levels in 2DM subjects. Scatter diagram (Fig. 4) showing negative relationship ($y = -0.051x + 8.204$) between melatonin and TNF-α in 2DM subjects.

Figure 1: Column chart showing the mean values of melatonin, IL-2, IL-15, IL-1β, IFN-γ, and TNF-α in 2DM and healthy controls

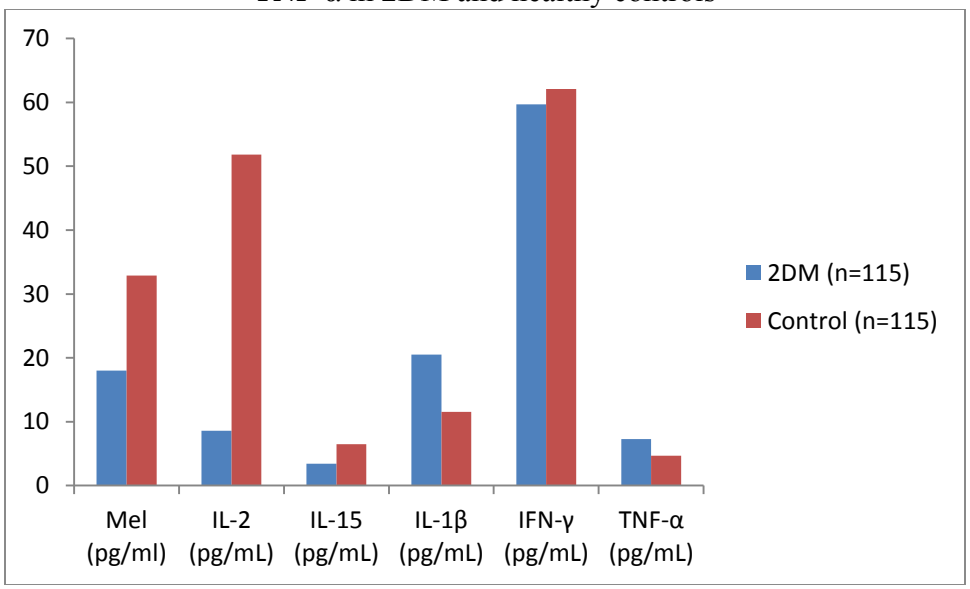


Figure 2: Scatter diagram showing relationship between melatonin and IL-2 in control subjects

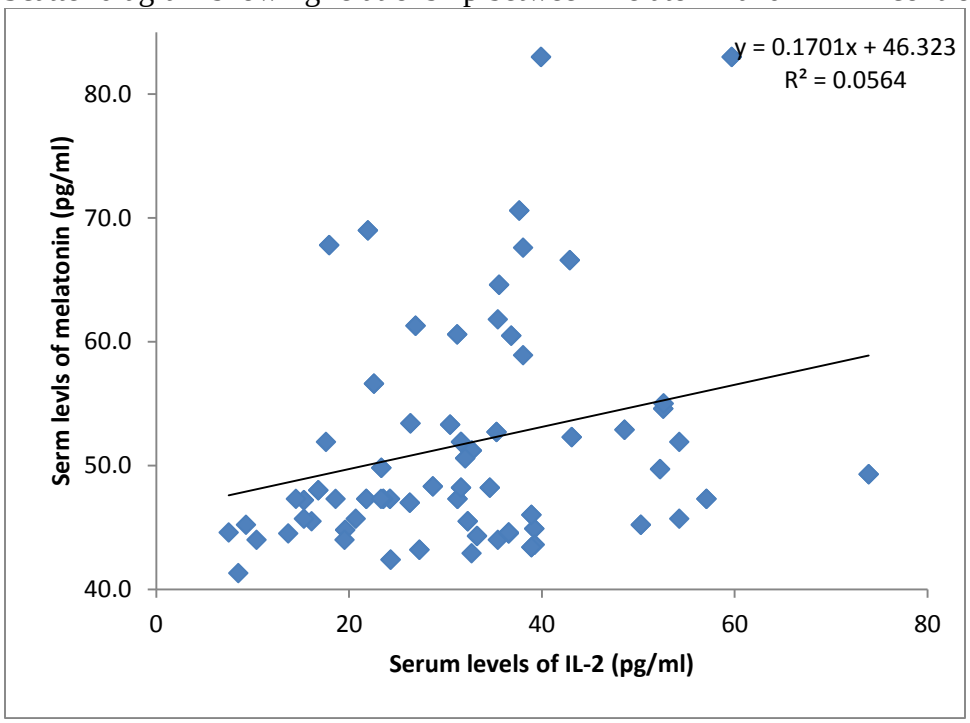


Figure 3: Scatter diagram showing relationship between melatonin and IL-15 in 2DM subjects

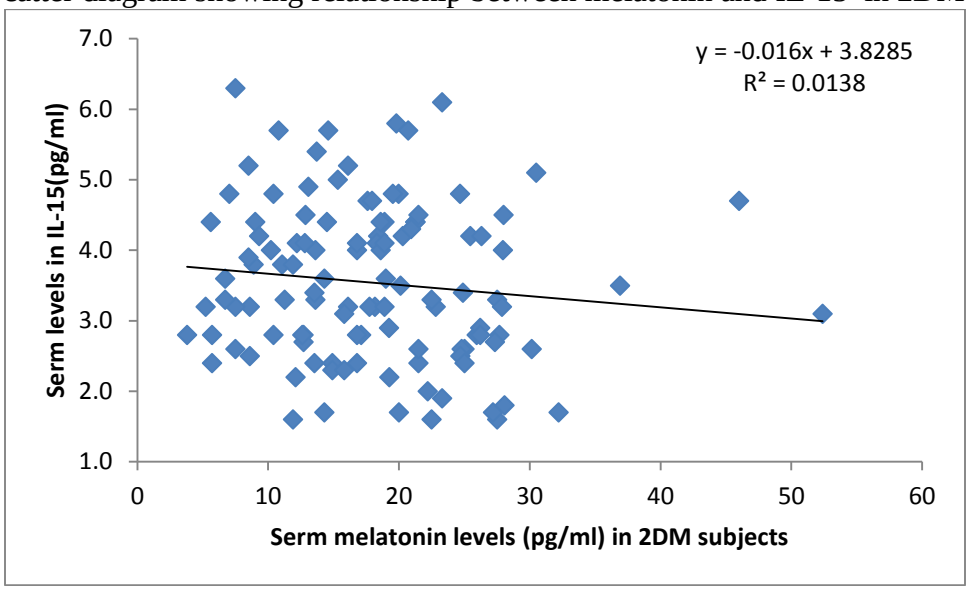
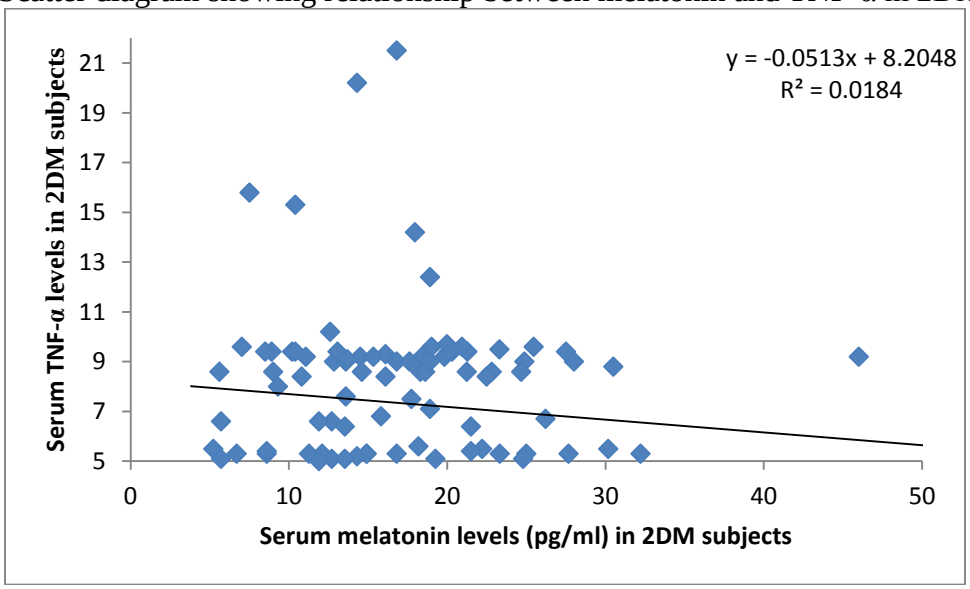


Figure 4: Scatter diagram showing relationship between melatonin and TNF-α in 2DM subjects



4. DISCUSSION:

Statistical significant differences were observed in serum melatonin levels and post-prandial blood glucose when compared between 2DM subjects and healthy controls. Melatonin is a hormone synthesized in the pineal gland and other tissues, which influences several endocrine and biological functions [17]. In addition, cross-talk between melatonin and insulin is necessary to maintain the harmonious balance between normoglycemia and hyperglycemia. Studies all over

the world have reported that hyperglycemia exists in individuals where there is disturbance between this cross-talk [17,18,20,21-24]. Genome-wide studies showed the association of melatonin with hyperglycemia and also with the risk of developing of 2DM in susceptible individuals [21-24]. Previous studies have shown that melatonin is useful in insulin action based on the finding that pinealectomy induces insulin resistance and hyperglycemia [17,18,20]. Thus, hyperglycemia has been reported in subjects experiencing lower levels of melatonin. In conjunction with other study findings, the present study also observed hyperglycemia in 2DM subjects. We infer the present study findings that lower levels of melatonin are responsible for post-prandial hyperglycemia in the 2DMsubjects. On the contrary, one study has been demonstrated that melatonin cannot significantly reduce blood sugar levels in male Wistar rats.

We also determined serum IL-2, IL-15, IL-1β, IFN-γ, and TNF-α in both the groups of the study. We observed statistical significances in the serum values of IL-2, IL-15, IL-1β, and TNF-α when compared between 2DM subjects and healthy controls. We also observed steady upward positive correlation between melatonin and IL-2 levels in healthy controls. Physiologically, IL-2 is anti-inflammatory cytokine helpful for the regulation of white blood cells especially T-lymphocytes which are responsible for immunity of the individual [13-16]. In a recent study conducted on role of interleukin-2 observed significant lower levels of IL-2 in newly diagnosed 2DM individuals when compared with non-diabetic volunteers [27]. In a study conducted by Bosek et al [28]., they observed increased levels of IL-2 in 2DM subjects when compared with counter group individuals. They demonstrated this increase in IL-2 towards selection criteria of colon cancer individuals having 2DM [28]. As of present study, we have included 2DM subjects who were having less than 5 years of duration of the disease. The results of our study show that one of the main reasons for lower levels of IL-2 is due to lower levels of melatonin values in the 2DM subject group. However, higher levels of melatonin values and IL-2 values in the control group and statistical significant differences between control and 2DM subjects in our study could be explained not only due to higher values of melatonin but also due to normoglycemia in healthy control subjects. The studies also observed that individuals with T2DM are affected with vascular diseases in relation to lower anti-inflammatory cytokines levels [16-18].

The other result that we observed is that, a negative association between serum melatonin and IL-15 level and also between melatonin and TNF-α in 2DM subjects. The increase in IL-15 and TNF-α is sufficient enough to initiate complications because 2DM individuals have lower melatonin levels than their healthy controls. There are many possible interpretations can be inferred of this correlation in subjects 2DM due to complex pathogenesis [29]. Manohar et al [30]., recently suggested that increased IL-15 is identified as widely distributed cytokine in various tissue types, including heart, liver, and kidney, which is a critical mediator of inflammation. Turillazzi et al [31] has been well demonstrated that IL-15 actively involved in the initiation and development of inflammation in cardiovascular diseases. In some studies, it has been reported, up-regulated IL-15 levels were found in both human and animal atherosclerotic lesions [32,33]. In addition, various risk factors entailing elevated hyperglycemia and diabetes are involved in the initiation and development of atherosclerotic lesions [34].

Several studies have indicated the disruption of TNF-α level in 2DM, obesity, and nephropathy subjects [34-36]. Significantly higher serum levels of TNF-α was found in the peripheral neuropathy patients and its correlation with nerve conduction velocity in type 2 diabetes mellitus [37]. Our results were in accordance with other studies. Interesting fact from our study is that, the absence of significant correlation in the control group between melatonin and TNF-α in healthy control group, as opposed to the 2DM group, can be inferred as remarkable determinant.

Pro-inflammatory cytokines IL-15 and TNF-α play a vital role in the development of secondary complications like cardiovascular and atherosclerotic lesions. Since subjects with 2DM in our study had increased IL-15 and TNF-α, our results suggest strongly this view. Therefore, 2DM is a common metabolic disorder associated with slow and progressive inflammation altering in the cytokine production including IL-15 and TNF-α [25,26,29].

Since hyperglycemia and lower melatonin level exits in 2DM, a plausible mechanism exists by which it may initiate the cascade of events that result in the development of diabetic complications. However, there is no consensus on either the cause of alteration in the balance between anti-inflammatory and pro-inflammatory cytokines in diabetes or the role played by lower melatonin levels in diabetic complications. Whereas some investigators have suggested that pro-inflammatory cytokines increase has a causative role in the development of diabetic complications, others have proposed that pro-inflammatory cytokines may merely be the common side-effects of tissues abnormalities in the occurrence of secondary complications of 2DM. The most important limitation in our study is that the circadian rhythm of melatonin was not considered in the study. Hence, the authors of the present study tried to compensate this drawback by taking blood samples from all subjects at the same time.

Therefore in the present study, the authors reject the null hypothesis that is serum melatonin has no effect on anti- and pro-inflammatory cytokines and accept the alternate hypothesis that is serum melatonin has an effect on anti- (IL-2) and pro-inflammatory (IL-15 and TNF-α) cytokines in 2DM subjects.

5. CONCLUSION:

The authors conclude from the study that alterations in the study parameters in 2DM group are due to dys-balance between anti-inflammatory and pro-inflammatory cytokines production. This dys-balance is due to low production of melatonin in 2DM subjects than healthy controls. Therefore, in patients with 2DM, any compensation mechanism may become insufficient, but it has not been extensively studied in a multicountric, multicentric which should include all ethnic population.

Conflict of interest:

The authors of the present study do not possess any conflict of interest among themselves.

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