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Original Research Article

Effects of melatonin on bone: a case control study

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ABSTRACT

Background: There is a stereotypical notion of estrogen being the most relevant parameter for bone health in post-menopausal females, but apart from estrogen; advances in research have presented ample evidence that melatonin may also play a critical role in bone health outcomes.
Methods: Our study consisted of 48 post-menopausal females, 24 subjects in the case group and 24 in control groups, to study the differences of certain parameters existing between the two. Serum Melatonin was calculated using ELISA test and Bone Mineral Density (BMD) was evaluated using a portable Ultrasound Bone Densitometer Testing Machine.
Results: A strong Positive Pearson correlation exists between BMD and serum melatonin levels. Pearson correlation coefficient value (r) of 0.96 and 0.95 for the Control and Osteopenic group respectively. Linear regression for control group is (r^2) 0.92. Linear regression for osteopenic group is (r^2) 0.90.
Conclusions: The results of our study exhibited strong interdependence between the serum melatonin levels and their effects on BMD. Osteopenic subjects who had a lower BMD were also found to have relatively lower levels of serum melatonin. Aligning with the results, similarly control group with normal BMD was found to have relatively higher level of serum melatonin.

Keywords: Melatonin, Bone mineral density, Osteopenia, Osteoporosis, Postmenopausal

INTRODUCTION

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone synthesized in the pineal gland within the pinealocytes from amino acid tryptophan. Although melatonin is primarily produced by the pineal gland, it is also produced in the gut, retina, skin, and leukocytes in small quantities. Synthesis of melatonin takes place at night and during the dark phase of the day. The hormone is produced and secreted in a rhythmic pattern influenced by the natural circadian rhythm. The circadian rhythm is an endogenous clock which regulates the normal sleep-wake cycle, the functioning of which is governed by the hypothalamic suprachiasmatic nucleus.[1-4] Melatonin plays a crucial role in sleep-wake cycle, and therefore has a wide array of pharmaceutical applications. It is used for the treatment of sleep disorders, primary insomnia, sleep disturbances in young and elderly suffering from neurodevelopmental abnormalities.[5-7] At physiologic doses, it also induces sleep onset and maintenance, decreases sleep latency, improves sleep efficiency and quality and overall increases the total sleep time.[8,9]

Melatonin and bone

The skeleton being a metabolically active organ, undergoes constant remodelling throughout life. The regulation of bone remodelling exists at both, systemic

and local. The major systemic regulators include parathyroid hormone (PTH), calcitriol, thyroid hormones, growth hormone, glucocorticoids and sex steroid hormones. Factors such prostaglandins, tumor growth factor-beta (TGF-beta), insulin-like growth factors (IGFs), bone morphogenetic proteins (BMP), and cytokines are involved as well. As far as local regulation of bone remodelling is concerned, a large number of cytokines and growth factors that affect bone cell functions have been recently identified. Furthermore, through the receptor activator of NF-kappa B (RANK) ligand/osteoprotegerin (OPG) system, the processes of bone formation and bone resorption are tightly coupled allowing a wave of bone formation to follow each cycle of bone resorption, thus maintaining skeletal integrity.[10] Osteoblast and osteoclast interaction is the key mediator of bone metabolism and remodelling.

Nobuo Suzuki et al studied the effects of melatonin on osteoclastic and osteoblastic cells using a culture system of goldfish scale.[11] Alkaline phosphatase (ALP) and Tartrate-resistant acid phosphatase (TRACP) were used as markers of osteoclastic and osteoblastic cells, respectively. Results of this study suggested that melatonin acts directly on the scale osteoclastic and osteoblastic cells where it suppresses osteoclastic and osteoblastic activities in the scales of goldfish. In another study, melatonin was found to have a dose dependent increase in cell proliferation of normal human bone cells and human osteoblastic cell lines. There was a two-fold increase in cell proliferation with maximal effect at 50 µM concentration. melatonin augmented procollagen type I c-peptide production (a measure of type I collagen synthesis) but not alkaline phosphatase activity or osteocalcin secretion.[12] Several reports suggest that melatonin also possesses anti-aging properties.[13] This may be attributed to melatonin's ability as a potent free radical scavenger and antioxidant at both physiological and pharmacological concentrations. Melatonin has an ability to directly neutralize free radicals, reactive oxygen and nitrogen species. It simultaneously stimulates multiple antioxidative enzymes that increase its efficiency as an antioxidant. Melatonin is highly lipid soluble due to which it can provide protection to intracellular macromolecules and cell membranes from damage caused by activity of singlet oxygen, superoxide anion radical, hydroxyl radical, peroxyl radical, and finally the peroxynitrite anion.[14-16] Thus, the free radical scavenging properties of melatonin plays a part in modulating osteoclast activity in bones.

Age related changes in levels of melatonin

The levels of melatonin change throughout life which plays a pivotal role on various physiological actions relayed by it. During the intrauterine life, the sole fetal source of melatonin being of maternal origin based on the day-night differences experienced by the mother, maternal melatonin reaches fetal umbilical circulation via placenta and relays a temporal circadian signal to the fetus (called "maternal photoperiodic adaptative programming"), preparing the CNS to properly deal with environmental day/night fluctuations after birth. At birth, melatonin levels are almost undetectable. A Melatonin Rhythm is established around 3 months of life.[17] An exponential rise in melatonin levels until a lifetime peak is seen in prepubertal children.[18] Melatonin levels steadily decline reaching mean adult concentrations in late teens.[19,20] The levels stay stable until 35 to 40 years of age, thereafter leading to a decline in amplitude of Melatonin Rhythm and lower levels with ageing, leading to fragmented sleep-wake patterns.[21]

Bone homeostasis

Bone homeostasis is significantly impacted by multiple factors, for post-menopausal women; estrogen and Melatonin are two major contributors in age related bone remodelling. Apart from estrogen and melatonin, heredity, hormonal status, age, and various environmental factors exert modulating effects on bone.[22] Aforementioned discussion described the decline in levels of melatonin with age and Sack et al as well in their study reported a steep decline in melatonin secretion after menopause.[23] This decline in melatonin plasma levels may be an important factor in changes of bone mineral density (BMD) in post-menopausal females. In our study, we investigated the correlation between levels of melatonin and their effect on bones in post-menopausal females.

METHODS

Study design

Current study was based on an analytical observational design with a case-control approach to a population of post-menopausal females.

Location and duration of study

The subjects were screened from participants of women's health awareness camps, organized and coordinated by an NGO, outpatient department of Dr. B.R. Ambedkar medical college and hospital and M. R. Ambedkar dental college & hospital, Bangalore, Karnataka. The sample collection was carried out from February, 2020 to December 2021. Sample collection activities were carried out in a controlled environment and written informed consents were taken from all subjects.

Sampling method

Intravenous blood was drawn for assessment of serum melatonin levels and bone mineral density (BMD) was measured by the use of a portable ultrasound bone densitometer testing machine. All samples were collected in regulated and supervised conditions. A total of 73 postmenopausal females were screened, out of which 52 were found eligible for the study based on inclusion and

exclusion criteria. To eliminate an ascertainment bias, 48 females were included in the study and were sorted into two groups based on BMD: control group; 24 subjects had normal bone mineral density (BMD) and study group (referred as osteopenic group); 24 subjects had osteopenia.

Inclusion and exclusion criteria

Inclusion criteria for current study were: postmenopausal; absence of menstruation for 12 consecutive months or more, bone mineral density; normal BMD - T-score of -1.0 or above=normal bone density. T-score <1 SD below the mean for young healthy adults, osteopenia-T-score between -1.0 and -2.5=low bone density, or osteopenia and moderate level of physical activity.

Exclusion criteria for current study were; history of autoimmune diseases or inflammatory disorders, melatonin based prescription drugs for sleep induction and maintenance therapy, history of systemic diseases, subjects on steroid therapy, hormone replacement therapy (HRT), NSAIDS, diuretics, oral contraceptive pills (OCP), thyroid medications, on prescription drugs that can influence bone metabolism, tobacco smoking/chewing status, supplements that influence bone turnover (vitamin D3 & calcium), significant sleeping disorders of unexplained origin, history of post-irradiation pineal and hypothalamic atrophy or pinealectomy.

Sample size calculation

The formula for sample size determination for the groups when the end result is quantitative data is:

$$n = 2\,\mathrm{SD2}(\mathrm{Z}\alpha/2 + \mathrm{Z}\beta)2/\mathrm{d2}$$

Where the values of SD (standard deviation) and d (effect size) is taken from a previous study done earlier, n=required sample size, $Z_{\alpha/2}$: for statistical power 90% and confidence interval 95%, standard normal variate is constant and taken as 1.96, Z_{β}: For statistical power of 90% and confidence interval of 95%, the value is constant and is taken as 0.95. The estimated sample size for the groups, keeping the power of the study as 90% at confidence interval of 95% and the statistical significance p value≤0.05, according to the formula is 24 respectively.

Procedure

Sample collection was done at 08.00 hours, considering the diurinal variation in melatonin levels. The samples were collected in dim light to minimize the impact of light on the levels of melatonin secretion. 10 ml intravenous blood was drawn from antecubital fossa under aseptic conditions. Collected samples were immediately packed and stored in dark. A sandwich enzyme-linked immunosorbent assay (ELISA) kit for detection of human melatonin is used. The plates are pre-coated with human MT (melatonin) antibody.

Specimen collected was blood serum. Serum sample was allowed to clot for 10-20 minutes at room temperature. Centrifuged at 2000-3000 RPM for 20 minutes. 160 µl of the standard (1680ng/l) with 160µl of standard diluent to generate a 720ng/l standard stock solution. MT present in the serum sample was added to the wells (40 µl), it binds to antibodies coated on the wells. Then biotinylated human MT Antibody (15 µl) was added which binds to MT in the sample. Then streptavidin-HRP (25 µl) is added and binds to the biotinylated MT antibody.

A sealer is applied for cover and the specimen is left for incubation. Incubated for 1 hour at 37°C. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human MT.

The color immediately changes from blue to yellow. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm using a microplate reader to determine its optical density (OD). Results were determined using regression analytics based on the construction of the standard curve plotted on the basis of average value of OD.

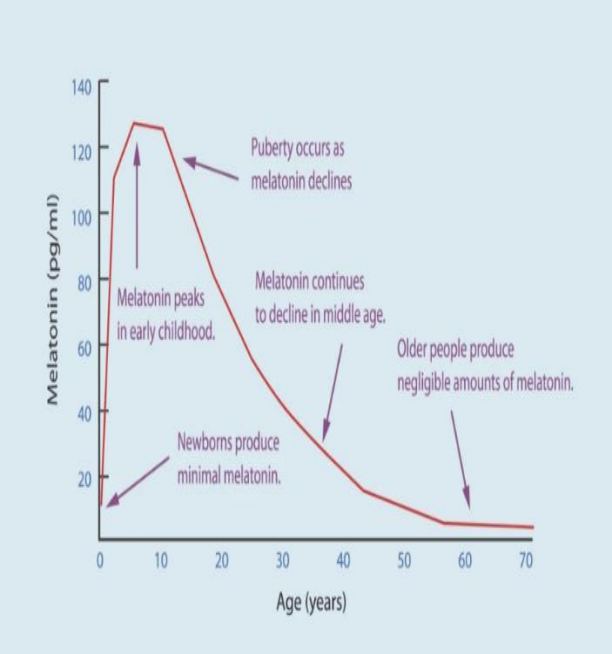

Figure 1: Age related changes in melatonin.[31]

RESULTS

In this case control study, we examined 48 postmenopausal subjects between the age of 46 to 58; mean age of all the participants involved in the study was 50.8.

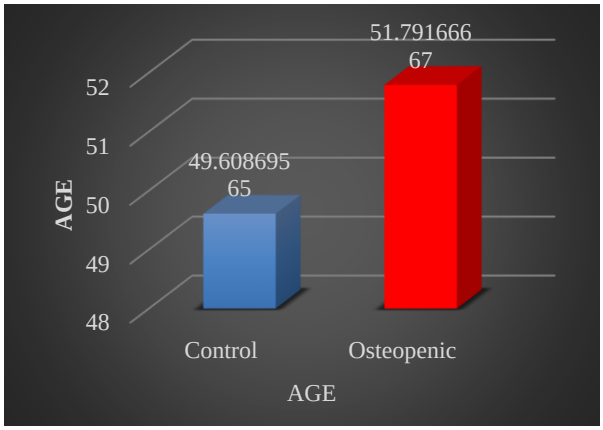

Figure 2: Demographic details of subjects.

These 48 females included in the study and were sorted into two groups based on BMD: control group: 24 subjects had normal bone mineral density, study group (referred as osteopenic group) 24 subjects had osteopenia.

BMD score

Mean BMD score on which the groups were bifurcated is represented in (Figure 3). The mean BMD score of control group was 0.614±0.283. The mean BMD score of osteopenic group was -2.05 (±0.308).

Serum melatonin levels

Mean serum melatonin level was compared for both the groups and a significant difference was noted between them. The control group had a relatively higher serum melatonin 26.440 pg/ml (±2.392) compared to the osteopenic group 19.729 pg/ml (±1.954) and were statistically significant (p<0.01) (Figure 4).

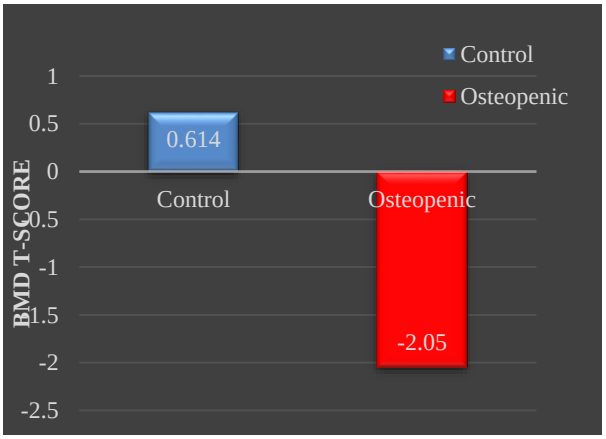

Figure 3: BMD score: control vs. osteopenic.

Relationship between BMD and serum melatonin

The relationship was assessed using a regression model. Control group; the graph we obtained depicts a simple linear line formula y=mx+c, where y is our output variable i.e., level of melatonin; while x is the bone mineral density, m is the slope of our line and c is the constant. The respective values we obtain from our model is m=8.078 and c=21.48. Therefore, our linear regression line is y=8.078x+21.48 which has the Pearson correlation coefficient value (r) of 0.96 and the 'goodness of fit' value (r^2) for our model is 0.92, was found statistically significant (p<0.01). The large positive value of coefficient clearly shows that the level of melatonin and BMD have a very strong positive correlation. Osteopenic group; in the osteopenic group, we found similar results in terms of the strong positive Pearson correlation. The value of r was found 0.95 while the 'goodness of Fit' (r^2) is 0.90, with statistical significance (p<0.01). The linear line for BMD vs. melatonin for the osteopenia affected group was given as y=5.991x+32.0141.

Thus, osteopenic subjects who had a lower BMD were also found to have relatively lower levels of serum melatonin. The results above clearly displayed there is a strong interdependence between the Melatonin levels and their effects on BMD.

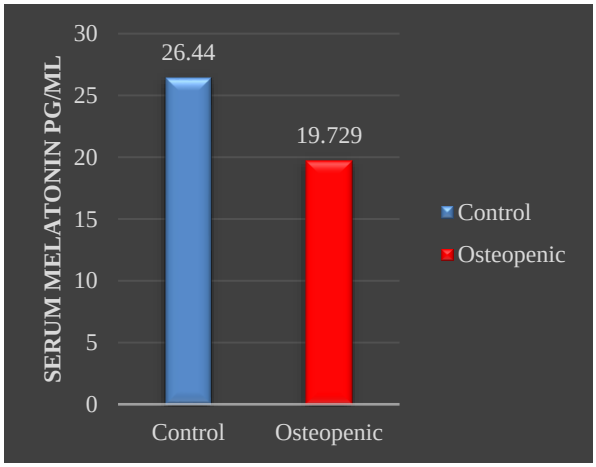

Figure 4: Serum melatonin: control vs. osteopenic.

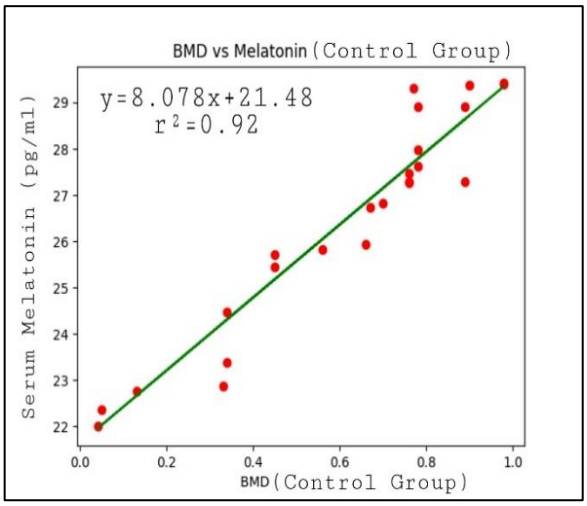

Figure 5: Simple linear regression-BMD vs. melatonin (control group).

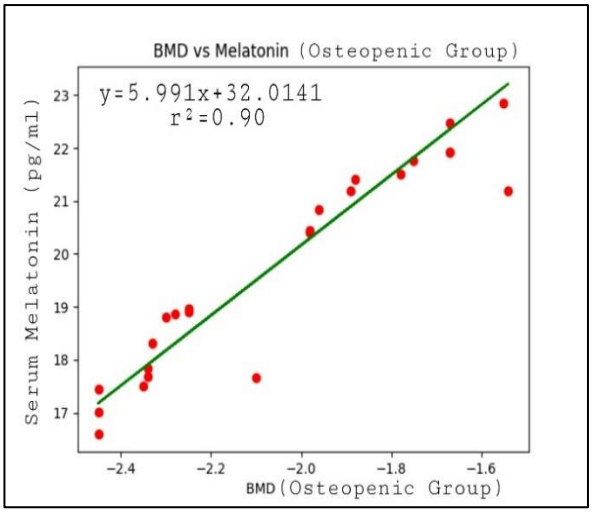

Figure 6: Simple linear regression-BMD vs. melatonin (osteopenic group).

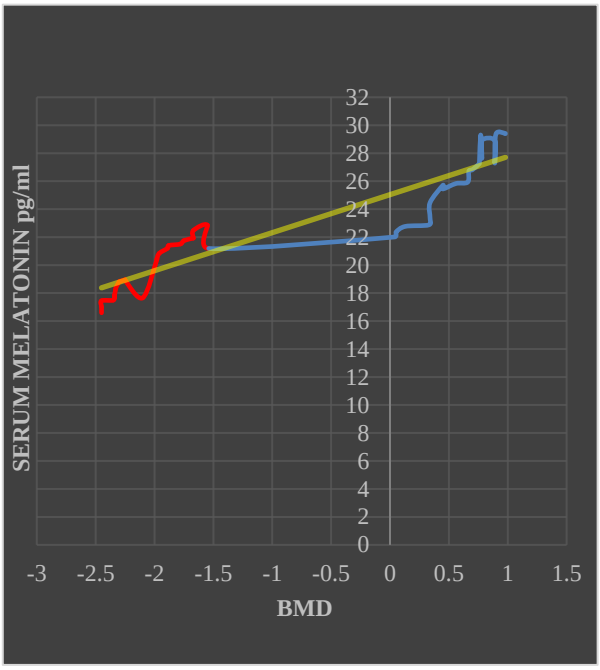

Figure 7: BMD serum melatonin relationship (blue line; control group, red line; osteopenic group, yellow line; trendline for BMD serum melatonin relationship).

DISCUSSION

The bone remodelling cycle is a lifelong process and is crucial for preserving bone integrity and its mineral density. It is a tightly coupled and well-orchestrated process between osteoblasts and osteoclasts, the former responsible for bone formation and the latter for bone resorption. This makes the skeleton a metabolically active organ.[25] Many factors are responsible for influencing bone remodelling including nutritional status, humoral factors, heredity, age and various environmental factors and biomechanical stress; exert modulating effects on bone.[22] Disruption of the bone remodelling cycle and any resulting imbalance between bone resorption and formation leads to metabolic bone disease, most commonly osteopenia and osteoporosis.[25] Estrogen and melatonin both are significant contributors in bone homeostasis as far as female population is taken into consideration. They are skeletally active hormones and influence systemic as well as alveolar bone remodelling. In our study we laid major emphasis on role of melatonin on BMD of post-menopausal females. The results obtained from the study are stated in the aforementioned section. It was clearly depicted that there is a strong interdependence between the melatonin levels and their effects on BMD. Osteopenia and osteoporosis, which are common phenomenon in post-menopausal women and elderly regardless of their sex, the same was shown in a study conducted by Tian et al analysing factors related to osteoporosis. The prevalence of osteoporosis in the entire study population was 9.65% for postmenopausal women and 8.08% for elderly males by WHO criteria, while the rate of osteopenia was 27.09% for postmenopausal women and 26.68% for elderly males.[26]

Keeping the results obtained in our study in mind we can expect a beneficial role that melatonin can play therapeutically in treatment of osteopenia and osteoporosis. In-fact Erdem et al discovered that combined treatment of caffeic acid phenethyl ester (CAPE) and melatonin promotes the maturation of new bone in distraction osteogenesis. These effects are likely a result of a reduction in bone resorption due to inhibition of NF-κB and free oxygen radicals.[27] Melatonin deficiency acts as a central mechanism in the pathogenesis of adolescent idiopathic scoliosis (AIS), a common orthopaedic disorder of unknown etiology and pathogenesis.[28] Melatonin can also be supplemented in patients undergoing radical pinealectomy to maintain the bone homeostasis. Pinealectomy may result in a rapid loss of cancellous bone volume and disrupts the trabecular structure, with increases in formative parameters, such as mineralizing surface, mineralization apposition rate, and adjusted appositional rate in a chicken AIS model.[29]

Micro-CT data revealed that pinealectomized chickens had a greater degree of generalized osteoporosis compared with controls. In chicken, the number of osteoblasts was significantly decreased as a result of pineal loss, while the number of osteoclasts showed no significant difference. These results suggest that melatonin deficiency reduces osteoblast proliferation and leads to the development of osteoporosis.[30]

Limitations

This study has limitations, namely the sampling process. Due to rapid degradation of serum melatonin when exposed to light, it could be challenging to denote the exact values of serum melatonin from the collected samples. Counter measures were taken during collection

and it was made sure that the samples were collected in a dim light and preserved adequately, but there might have been some compromise to this in the likes of unusual caveats to this situation.

CONCLUSION

The results of our study exhibited strong interdependence between the serum melatonin levels and their effects on BMD. Osteopenic subjects who had a lower BMD were also found to have relatively lower levels of serum melatonin. Aligning with the results, similarly control group with normal BMD was found to have relatively higher level of serum melatonin. Hormone replacement therapy estrogen replacement therapy (ERT), has been a mainstay for treating post-menopausal women with osteopathies. ERTs come with their fair share of pros and cons, this opens up a new avenue for melatonin as a potential substitute for ERT, especially in the patients for whom it's an absolute contraindication and can be useful for treatment of bone mineral density disorders. Although there are strong and supportive evidences which justify the role melatonin plays in osteoporosis, the development of successful treatment options that include melatonin have not been implemented. This requires additional investigation, clinical trials and further research advancements.

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