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Original Research Article

Association of serum estrogen and melatonin levels with bone mineral density of post-menopausal women- a case-control study

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ABSTRACT

Background: A skeleton is a metabolically active organ that constantly undergoes remodelling throughout life. Various factors influence the process of bone formation and resorption. Menopause is a crucial stage in a woman's life and a female's body undergoes many significant changes during this stage. Hormone-related changes occur not only due to menopause but also due to aging; the two crucial hormones we emphasized in our study are- estrogen and melatonin; and their manifestation in the skeletal system of a postmenopausal population.
Methods: Our study consisted of 48 post-menopausal females, 24 subjects in the case group, and 24 in the control groups, to study the differences in certain parameters existing between the two. Serum estrogen and melatonin were calculated using the ELISA test; bone mineral density (BMD) was evaluated using a portable ultrasound bone densitometer testing machine.
Results: The factors (estrogen and melatonin) have a direct strong linear relation with BMD. Pearson correlation coefficient was found to be positive (r) 0.92, indicating a strong correlation. The 'goodness of fit' (r^2) of multiple linear regression was found to be 0.8485 (roundabout 85%), indicating strong positive results.
Conclusions: The results of our study exhibited strong interdependence of BMD on serum estrogen and melatonin. Osteopenic subjects who had a lower BMD were also found to have relatively lesser levels of serum estrogen and melatonin. Aligning with the results, similarly, the control group with normal BMD was found to have relatively higher serum levels of both hormones.

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

INTRODUCTION

Estrogen is a category of sex hormone responsible for the development and regulation of the female reproductive system and secondary sex characteristics.[1,2] The three major endogenous estrogens have estrogenic hormonal activity: estrone (E1), estradiol (E2), and estriol (E3).[1] Estradiol, is the most potent and prevalent.[1] Another estrogen called estetrol (E4) is produced only during pregnancy. Estrogens are a class of steroid hormones; in the ovary, estrogen synthesis begins in theca cells with androgen synthesis and ends with the conversion of androgens to estrogens in granulosa cells by the enzyme aromatase. In the male gonad, estrogens are synthesized in the Leydig cells, Sertoli cells, and mature spermatocytes.[3] Like other steroid hormones, estrogens enter passively into the cells and bind to the estrogen receptors, which then regulate the transcription of downstream estrogen-responsive genes. Estradiol is also produced in several extragonadal organs, including the adrenal glands, brain, adipose tissue, skin, pancreas, and other sites yet to be identified.[4,5]

The effects of estrogen on various systems of the body are described as follows: breast: estrogen is responsible for the development of mammary gland tissue,

parenchymal, and stromal changes in breast tissue at puberty in females. Development of mammary ducts during puberty and pregnancy, and functions to secrete breast milk in postpartum lactation. Contraception: ethinyl estradiol, a component of oral contraceptive pills, functions to suppress the release of gonadotropin-releasing hormone (GnRH) and pituitary release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) thereby preventing ovulation. Uterus: estrogen helps proliferate endometrial cells in the follicular phase of the menstrual cycle, this thickens and prepares the endometrial lining for pregnancy. Vagina: estrogen supports the proliferation of epithelial mucosa cells of the vagina and the vulva. In the absence of estrogen, the vaginal and vulvar mucosal epithelium becomes dry and presents with symptoms of dryness known as vulvovaginal atrophy.[6] Bone: during puberty, estrogen aids in the development of long bones and the fusion of the epiphyseal growth plates. Bones are protected by estrogen due to suppression of osteoclast activity, preventing osteoporosis in both estrogenic-deficient and postmenopausal women.[7] Cardiovascular: estrogen influences plasma lipid profile by increasing the high-density lipoproteins (HDL) and triglyceride levels while lowering low-density lipoproteins (LDL) and total plasma cholesterol and thereby reducing the risk of coronary artery disease (CAD) in early use in postmenopausal women.[8]

Age-related changes in levels of estrogen

Women are born with their full complement of oocytes and during their reproductive years, these oocytes are gradually depleted through ovulation and atresia. The decreased numbers of oocytes secrete less inhibin B, decreasing the ovarian negative feedback on follicle-stimulating hormone (FSH). The resultant increase in FSH level leads to more follicular recruitment and accelerated follicular loss, with preservation of estradiol levels in the early menopausal transition. Eventually, the depletion of follicles results in variability in the ovarian response to FSH, widely fluctuating estrogen levels, and loss of the normal reproductive cycle. When all the ovarian follicles are depleted, the ovary is unable to respond to even high levels of, FSH, and estrogen levels decline. The postmenopausal period is characterized hormonally by an elevated FSH (>30 mIU/ml) and low estradiol levels manifesting into various signs and symptoms (Table 1).[9]

Melatonin

Melatonin (N-acetyl-5-methoxy tryptamine) is a hormone synthesized in the pineal gland within the pinealocytes from the amino acid tryptophan. 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 that regulates the normal sleep-wake cycle, the functioning of which is governed by the hypothalamic suprachiasmatic nucleus.[10,11] Melatonin plays a crucial role in the sleep-wake cycle and therefore has a wide array of pharmaceutical applications. It is used for the treatment of sleep disorders, primary insomnia, and sleep disturbances in young and elderly suffering from neurodevelopmental abnormalities.[12,13] 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.[14,15]

Table 1: Signs and symptoms of menopause.

Signs and symptoms of menopause
Menopausal transition
Menstrual irregularity
Hot flashes
Night sweats
Sleep disruption
Post-menopause
Vaginal dryness
Vulvovaginal atrophy
Lower urinary tract symptoms
Dyspareunia

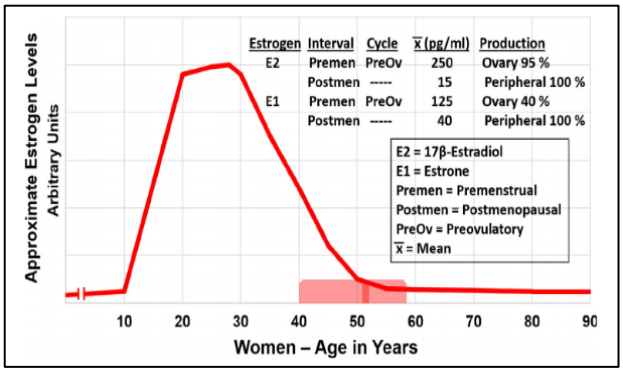

Figure 1: Approximate production of estrogens (profile) in women with age.[50-51]

Age-related changes in levels of melatonin

The levels of melatonin change throughout life which plays a pivotal role in various physiological actions relayed by it. During intrauterine life, the sole source of fetal melatonin is of maternal origin based on the day-night differences experienced by the mother, maternal melatonin reaches fetal umbilical circulation via the placenta and relays a temporal circadian signal to the fetus (called "maternal photoperiodic adaptative programming"), preparing the central nervous system (CNS) to adapt to environmental day/night fluctuations after birth. Noticeably, at birth, melatonin levels are almost undetectable. A melatonin rhythm is established around 3 months of life.[16] An exponential rise in melatonin levels until a lifetime peak is seen in prepubertal children.[17] Melatonin levels steadily start to decline after reaching mean adult concentrations in the

late teens.[18,19] The levels stay stable until the age of 35 to 40 years, thereafter leading to a decline in the amplitude of melatonin rhythm and lower levels with aging, leading to fragmented sleep-wake patterns.[20]

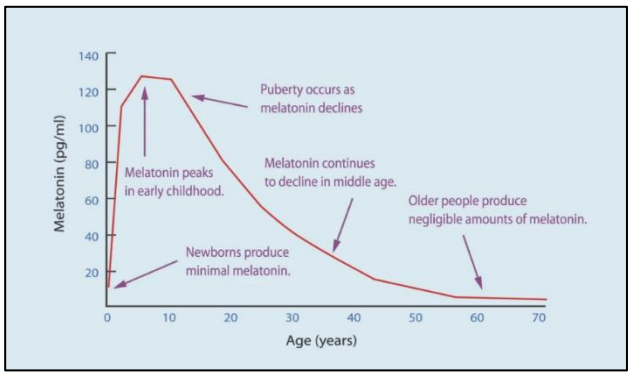

Figure 2: Age related changes in melatonin.[52]

Role of estrogen and melatonin in bone homeostasis

The skeleton is a metabolically active organ. It undergoes constant remodelling throughout life. The regulation of bone remodelling is both systemic and local. The major systemic regulators of bone remodelling include parathyroid hormone (PTH), serum calcium, Vitamin D, calcitriol, and hormones such as growth hormone, glucocorticoids, thyroid hormones, and sex hormones. Factors such as insulin-like growth factors (IGFs), tumour growth factor-beta (TGF-beta), prostaglandins, bone morphogenetic proteins (BMP), and cytokines are involved as well. In the local regulation of bone remodelling, several cytokines and growth factors that affect bone cell functions have been identified. Furthermore, through the RANK/receptor activator of NF-kappa B ligand (RANKL)/osteoprotegerin (OPG) system the processes of bone resorption and formation are tightly coupled allowing a wave of bone formation to follow each cycle of bone resorption, thus maintaining skeletal integrity.[21] Osteoblast and osteoclast interaction is the key mediator of bone metabolism and remodelling.

Estrogen is the major hormonal regulator of bone metabolism. At menopause, there is a significant reduction in estrogen levels which leads to osteopenia and osteoporosis. Several studies have shown that estrogen influences bone metabolism by influencing the basic multicellular units (BMUs) which include osteoclasts, osteoblasts, and osteocytes within the bone remodeling cavity.[22] Estrogen maintains bone homeostasis by inhibiting osteoblast and osteocyte apoptosis.[23,24] Estrogen suppresses osteoclast formation and activity as well as induces osteoclast apoptosis.[22,25-27] Estrogen decreases osteoclast formation by inhibiting the synthesis of the osteoclastogenic cytokine RANKL by osteoblasts and osteocytes. Moreover, estrogen stimulates these bone cells to produce osteoprotegerin (OPG), a decoy receptor of RANK in osteoclast, thus inhibiting osteoclastogenesis.[28-30]

Suzuki et al studied the effects of melatonin on osteoclastic and osteoblastic cells using a culture system of goldfish scale. Alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRACP) were used as markers of osteoclastic and osteoblastic cells, respectively. Results suggested that melatonin acts directly on the osteoclastic and osteoblastic cells. It suppresses the osteoblastic and osteoclastic activities in the scales of goldfish.[31] 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.[32] Several reports suggest that melatonin also possesses anti-aging properties.[33] This may be attributed to melatonin's ability as a potent free radical scavenger and antioxidant at both physiological and pharmacological concentrations. Melatonin can 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 protect intracellular macromolecules and cell membranes from damage caused by the activity of singlet oxygen, superoxide anion radical, hydroxyl radical, peroxyl radical, and finally the peroxynitrite anion.[34,35]

Thus, melatonin's free radical scavenging properties play a part in modulating bone osteoclast activity. Bone homeostasis is significantly impacted by multiple factors, for post-menopausal women- estrogen and melatonin are two major contributors to age-related bone remodelling. Apart from estrogen and melatonin, heredity, hormonal status, age, and various environmental factors exert modulating effects on bone.[36] This decline in melatonin and estrogen plasma levels may be an important factor in changes in bone mineral density (BMD) in post-menopausal females. In our study, we investigated the various associations and correlations between levels of melatonin and estrogen and their effect on bones in post-menopausal females.

METHODS

Study design

Our study used an analytical observational design with a case-control approach to a population of post-menopausal females.

Location and duration of research

The subjects were screened from participants of women's health awareness camps, organized and coordinated by an NGO, an outpatient department of Dr. BR Ambedkar medical college and Hospital, and MR Ambedkar dental college and 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 consent was taken from all subjects.

Sampling method

Intravenous blood was drawn for assessment of serum estrogen and 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). Study group (referred to as osteopenic group)- 24 subjects had osteopenia.

Inclusion and exclusion criteria

Inclusion criteria for the current study were: postmenopausal- absence of menstruation for 12 consecutive months or more. Bone mineral density (BMD) 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. Moderate level of physical activity.

Exclusion criteria for the 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), and thyroid medications. On prescription drugs that can influence bone metabolism. Tobacco smoking/chewing status. Supplements that influence bone turnover (vitamin D_3 and calcium). Significant sleeping disorders of unexplained origin. History of post-irradiation pineal and hypothalamic atrophy or pinealectomy. Subjects consuming a supplement with a high dose of biotin (also termed as vitamin B_7 or B_8, vitamin H, or coenzyme R) can interfere with estrogen laboratory evaluation.

Sample size calculation

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

$$n = \frac{2SD^2(Z_{\frac{\alpha}{2}} + Z_\beta)^2}{d^2}$$

The values of SD (standard deviation) and d (effect size) are taken from a previous study done earlier.[37] n: required sample size. $Z_{\alpha/2}$: for statistical power 90% and confidence interval 95%, the 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 was taken as 0.95. The estimated sample size for the groups, keeping the power of the study as 90% at a confidence interval of 95% and the statistical significance p value ≤0.05, according to the formula was 24 respectively.

Procedure for sample collection of estrogen and melatonin

10 ml intravenous blood was drawn from the antecubital fossa under aseptic conditions. A common blood withdrawal was done for estrogen and melatonin tests.

Assay procedure for serum and plasma for estradiol

Secure the desired number of coated wells in the holder. Dispense 25 µl of standards, specimens, and controls into appropriate wells. Dispense 100 µl of estradiol-HRP conjugate reagent into each well. Dispense 50 µl of rabbit anti-Estradiol (E2) reagent to each well. Thoroughly mix for 30 seconds. It is very important to mix them completely. Incubate at room temperature (18-25°C) for 90 minutes. Rinse and flick the microwells 5 times with distilled or deionized water (should not use tap water). Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds. Incubate at room temperature (18-25°C) for 20 minutes. Stop the reaction by adding 100 µl of stop solution to each well. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

Assay procedure for serum melatonin

Sample collection was done at 08:00 hours, considering the diurnal 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 the antecubital fossa under aseptic conditions. Collected samples were immediately packed and stored in dark.

A sandwich enzyme-linked immunosorbent assay (ELISA) kit for the detection of human melatonin was used. The plates were pre-coated with human MT (melatonin) antibodies. Specimen collected as blood serum. The 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 (1680 ng/l) with 160 µl of standard diluent to generate a 720 ng/l standard stock solution. MT present in the serum sample was added to the wells (40 µl), and 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) was added and binds to the biotinylated MT antibody. A sealer was

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. A substrate solution is then added and color develops in proportion to the amount of human MT. The color immediately changed from blue to yellow. The reaction was terminated by the addition of an acidic stop solution and absorbance was measured at 450 nm using a microplate reader to determine its optical density (OD).

Calculation of results

Calculate the mean absorbance value (A450) for each set of reference standards, controls, and samples. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in pg/ml on a linear-linear graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of estradiol and melatonin in pg/ml from the standard curve. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations. Results were determined using regression analytics based on the construction of the standard curve plotted based on the average value of OD.

RESULTS

In this case-control study, we examined 48 postmenopausal subjects between the age of 46 to 58; the mean age of all the participants involved in the study was 50.9.

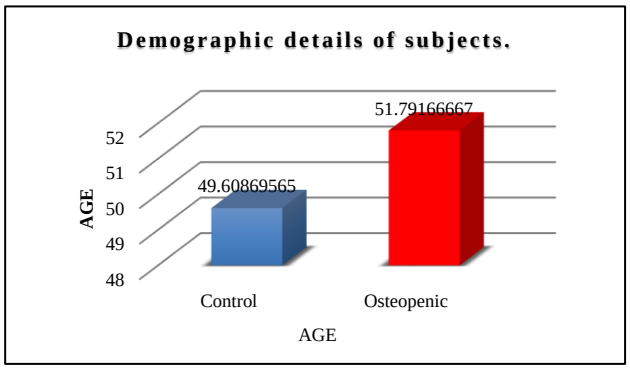

Figure 3: Demographic details of subjects.

These 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). Study group (referred to as osteopenic group)- 24 subjects had osteopenia.

BMD score

The mean BMD score on which the groups were bifurcated is represented in (Figure 4).

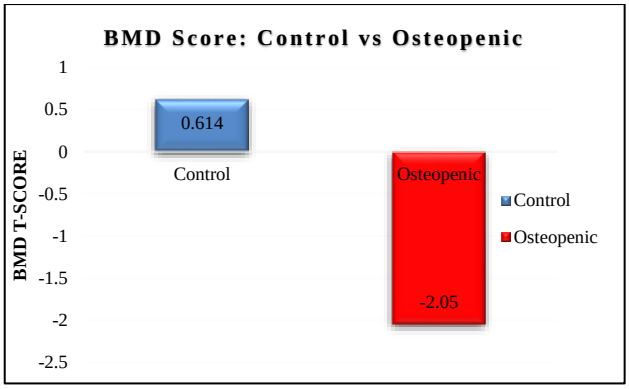

Figure 4: BMD score: control versus osteopenic.

The mean BMD score of the control group was 0.614±0.283. The mean BMD score of the osteopenic group was -2.05±0.308.

Serum estrogen and melatonin levels

Mean serum estrogen and melatonin levels were compared for both groups and a significant discrepancy was noted. The control group had a relatively higher serum estrogen 30.19±2.23 pg/ml compared to the Osteopenic group 23.67±2.08 pg/ml. The control group had a relatively higher serum melatonin 26.440±2.392 pg/ml compared to the osteopenic group 19.729±1.954 pg/ml represented in Figure 5.

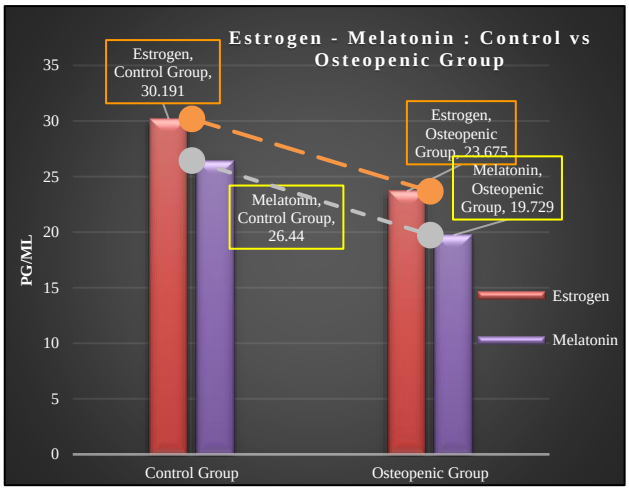

Figure 5: Estrogen-melatonin: control versus osteopenic group.

Relationship between BMD and serum estrogen and melatonin

The relationship was assessed using a regression model. Simple linear regression between BMD versus serum estrogen (control and osteopenic group); BMD versus serum melatonin (control and osteopenic) and multiple regression between target variable- BMD versus serum estrogen and melatonin (control and osteopenic) are shown.

Simple linear regression between BMD versus serum estrogen (control and osteopenic group)

The linear regression line obtained was y=26.0875x+6.678, for the formula y=mx+c. The Pearson correlation coefficient (r) factor for the control group was found to have a positive 0.85 value. A graph has also been represented for the same and the goodness of fit value (r^2) was found to be 0.82. In the osteopenia affected group, we found similar results in terms of the strong positive Pearson correlation.

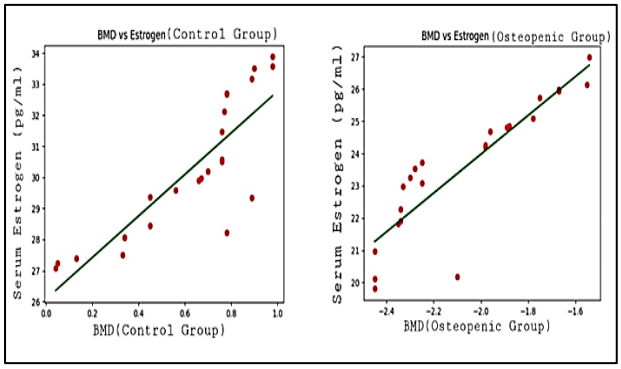

Figure 6: Simple linear regression between BMD versus serum estrogen (control and osteopenic group).

The value of the coefficient touched 0.8929 while the 'goodness of fit' (r^2) was 0.80. The linear line for BMD versus estrogen for the osteopenic group was given as y=36.015x+6.018. The statistical results display that BMD and serum estrogen was linearly interdependent (strong positive linear correlation).

Simple linear regression between BMD versus serum melatonin (control and osteopenic)

The linear regression line was y=8.078x+21.48, for the formula y=mx+c. The control group had a Pearson correlation coefficient value(r) of 0.96 and the 'goodness of fit' value (r^2) of 0.92. In the osteopenic group, we found similar results in terms of the strong positive Pearson correlation.

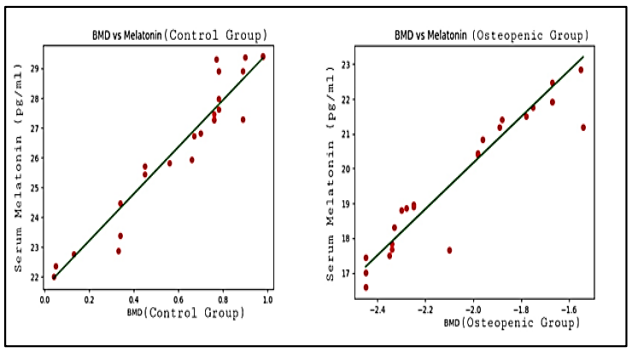

Figure 7: Simple linear regression between BMD versus serum melatonin (control and osteopenic).

The value of r was found 0.95 while the 'goodness of fit' (r^2) was 0.90. The linear line for BMD versus melatonin for the osteopenia affected group was given as y=5.991x+32.0141. Thus, osteopenic subjects with a lower BMD were also found to have relatively lesser serum melatonin levels. The results above clearly displayed there is a strong interdependence between melatonin levels and their effects on BMD.

Multiple linear regression: estrogen, melatonin versus BMD

We built a multiple linear regression polynomial model to understand the correlation and effects of the level of serum estrogen and melatonin on bone mineral density. On calculations, the correlation factor was found to be positive (r) 0.92, which indicates that both the factors (estrogen and melatonin) have a direct strong linear relation with BMD. While its graph was plotted, the goodness of fit (r^2) of the graph was found to be 0.8485 (roundabout 85%), indicating strong positive results.

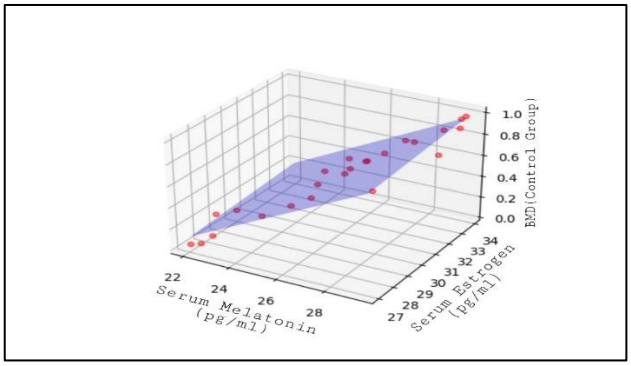

Figure 8: Multiple linear regression- control group.

For osteopenic subjects, the correlation factor (r) was on the higher positive end, 0.8959, and the goodness of fit (r^2) of the graph was 0.802.

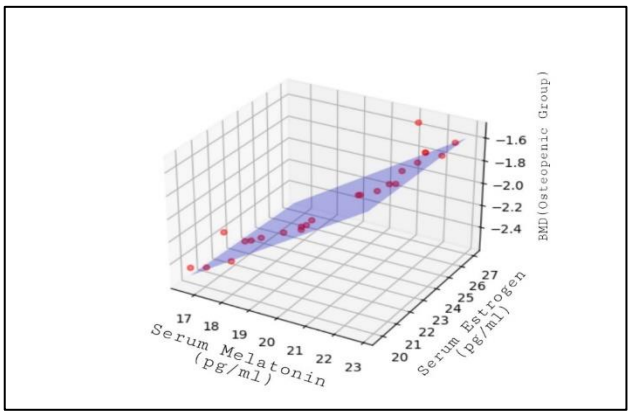

Figure 9: Multiple linear regression- osteopenic group.

Thus, the above results clearly show whether a patient falling under BMD controlled group or osteopenia

affected group possesses strong positive multiple linear relations between them.

DISCUSSION

Menopause is considered a crucial step in a woman's life and has many pronounced effects on overall health. There are mixed opinions about menopause amongst females; for many, this change is liberating, relieving them from the anxieties of childbearing, and pain or discomfort related to their reproductive organs. Some women may view menopause negatively, associating it with aging, which in many cultures has significant negative connotations. The transition to menopause is accompanied by a multitude of symptoms (Table 1). Along with these symptoms, various physiological changes also occur about a shift in hormonal status.

Hormonal changes are a critical part of normal aging. These changes have various physiological manifestations. Menopause is a state defined by the cessation of estrogen production by the ovaries. This withdrawal of estrogen can cause significant changes in various systems of the body, as described in the aforementioned introduction section. One of the most significant changes is in the skeletal system. Estrogen has a direct and indirect influence on osteoclasts, osteoblasts, and osteocytes.[22,24,26,27,38] Loss of estrogens or androgens decreases defence against oxidative stress in bone, and this accounts for the increased bone resorption associated with the acute loss of these hormones.[39] Apart from estrogen, there is also a steep decline in serum melatonin post-menopause as described by Sack et al in their study.[40] Melatonin through its anti-oxidant properties does play a role in bone remodelling. In our study, we showed a correlation between these essential influential factors and their effects on bone homeostasis.

Lindsay et al in their study on oophorectomized women showed that estrogen replacement can prevent this bone loss. Even after a substantial loss has occurred, estrogen will slow further loss or even increase bone mass.[41] Many similar studies have shown similar effects of estrogen replacement and its protective effects against bone loss. Therefore, estrogen replacement therapy (ERT) is indicated both for prevention and treatment. Postmenopausal women with low bone mass or osteoporotic fractures should be offered ERT. Bone mass measurement may persuade those reluctant to take ERT to reconsider.[42] Patients opting to undergo estrogen replacement therapy are predisposing themselves to many benefits and side effects. ERT is known to have cardio-protective benefits, on the other hand, it significantly increases the risk of endometrial cancer due to unopposed estrogen; patients with an intact uterus are given supplemental progestin for endometrial cancer risk reduction but simultaneously it also attenuates the cardio-protective benefits.[8,43] Another major concern is the possible increase in the incidence of breast cancer with ERT.[44]

Previously, estrogen replacement therapy (ERT) has been used mainly in younger postmenopausal women, but it is also effective in older women who are more than 20 years past menopause.[45] However, side effects such as breast tenderness and recurrence of menstrual bleeding may be poorly tolerated in older women.

Melatonin deficiency acts as a central mechanism in the pathogenesis of adolescent idiopathic scoliosis (AIS), a common orthopedic disorder of unknown etiology and pathogenesis.[46] Keeping the results obtained in our study in mind, we can expect a beneficial role that melatonin can play therapeutically in the treatment of osteopenia and osteoporosis. Erdem et al discovered that the combined treatment of caffeic acid phenethyl ester (CAPE) and melatonin promotes the maturation of new bone in distraction osteogenesis.[47] Melatonin can also be supplemented in patients undergoing radical pinealectomy to maintain bone homeostasis. Pinealectomy may result in a rapid and marked loss of cancellous bone volume and profoundly disrupts trabecular structure, with increases in dynamic formative parameters, such as mineralizing surface, mineralization apposition rate, and adjusted appositional rate in a chicken AIS model.[48] These results suggest that melatonin deficiency leads to a reduction in the proliferation of osteoblasts, leading to the development of osteopenia and osteoporosis.

Although ERT provides a great therapeutic edge, the consequent side effects, contraindications, and poor tolerance should be taken into consideration. For this reason, several alternatives to ERT are being explored. Raloxifene, a compound that was originally developed as an anti-estrogen, can prevent bone loss in animals and decrease bone turnover in humans and does not cause uterine hyperplasia.[49] The pros and cons of ERT open a new avenue for the treatment of bone mineral density disorders with melatonin supplementation. Although there are strong and supportive pieces of evidence that justify the role melatonin plays in osteoporosis, the development of successful treatment options that include melatonin has not been implemented. This requires additional investigation, clinical trials, and further research advancements.

This study still has limitations, namely the sampling process. Due to the 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 dim lighted setting 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 serum estrogen and melatonin levels and their

effects on BMD. Osteopenic subjects who had a lower BMD were also found to have relatively lower levels of serum estrogen and melatonin. Aligning with the results, similarly, the control group with normal BMD was found to have a relatively higher level of serum estrogen and melatonin. Hormone replacement therapy- estrogen replacement therapy (ERT), has been a mainstay for treating post-menopausal women with osteopathy. 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 the treatment of bone mineral density disorders. Although strong and supportive evidence justifies the role melatonin plays in osteoporosis, the development of successful treatment options that include melatonin has not been implemented. This requires additional investigation, clinical trials, and further research advancements.

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