THE ROLE OF PROINFLAMMATORY CYTOKINES AND SOME HORMONES IN ENDOMETRIOSIS-INDUCED OSTEOPOROSIS

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ABSTRACT

Objective: The aim of this study is to evaluate the effects of proinflammatory cytokines and some hormones, in patients with endometriosis, on the serum levels of bone formation and bone resorption markers.

Subjects and methods: Eighty female patients with endometriosis at their reproductive age subjected to standard criteria were participated in this study. Eighty apparently healthy women were selected to be a normal group for comparison. In addition, assessing the serum levels of tumor necrosis factor alpha [TNF-α], interleukin 1[IL-1], interleukin 6 [IL-6], CarboxyterminalTelopeptides of type I Collagen [CTX1], CarboxyterminalPropeptide of type I Procollagen [PICP], Estradiol [E2], Follicle-Stimulating Hormone [FSH], Thyroid-Stimulating Hormone [TSH], and Parathyroid Hormone [PTH] by EILISA and enzymatic Kits.

Results: The results show that the levels of tumor necrosis factor alpha [TNF-α], interleukin 1[IL-1], interleukin 6[IL-6], C terminal Telopeptides of type I Collagen[CTX1]and C terminal propeptide of type I procollagen[PICP], follicle-stimulating hormone [FSH], thyroid-stimulating hormone [TSH] and parathyroid hormone [PTH] were significantly changed in serum of patients with endometriosisas compared with control healthy group. Whereas, the change of serum estradiol [E2] level was non-significant as compared with control group.

Conclusions: The inflammatory activity of tumor necrosis factor alpha [TNF-α], interleukin 1[IL-1] and interleukin 6 [IL-6] was important in the development of osteoporosis in patients with endometriosisbysy its effect on bone formation and resorption markers. The role of some hormones, follicle-stimulating hormone [FSH], thyroid-stimulating hormone [TSH] and parathyroid hormone [PTH], in the development of osteoporosis in patients with endometriosis was also assessed.

KEYWORDS: Endometriosis, Osteoporosis, Tumor Necrosis Factor Alpha [TNF-α], Interleukin 1[IL-1], Interleukin 6 [IL-6], Estradiol [E2], Follicle-stimulating hormone [FSH], Thyroid-stimulating hormone [TSH], Parathyroid hormone [PTH].

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INTRODUCTION

Endometriosis is a disease in which the endometrial tissue lining is located outside the uterus, that induces a chronic inflammatory response. This ectopic endometrial tissue is mainly seen on the peritoneum, ovaries, recto-vaginal septum, bladder, bowel, and rarely on the diaphragm and the lungs [1,2]. The incidence and prevalence of endometriosis are 5% to 10% of women at their reproductive age, from all ethnic and social groups [3]. Women with reproductive tract anomalies and the resulting obstruction of menstrual outflow are also at higher risk of the disease. The increased parity and prolonged irregular menses reduce the likelihood of the disease, while nulliparity, subfertility, and prolonged intervals since pregnancy are associated with high risk [4].

Endometriosis also has a major effect on the physical, mental and social conditions [5]. In addition, it is a large healthcare problem and exerts an economic burden on the society. The annual cost of endometriosis in the United States in 2002 was about 22 billion dollars [6].

Increased inflammation and altered immune response have been observed in women with this disease. The complex network of locally produced cytokines and immune cells have been found to control the growth and inflammatory behavior of the endometrial implants [7]. These proinflammatory cytokines play the main role in regulation of cell proliferation, activation, adhesion, chemotaxis, motility, and morphogenesis during the pathogenesis of endometriosis. They include interleukins (e.g., IL-1, IL-6, IL-8), tumor necrosis factor-alpha (TNF-α), monocyte chemotactic protein-1 (MCP-1), transforming growth factor-β (TGF-β), and Regulated on Activation Normal T-cell Expressed and Secreted (RANTES) [8]. It has been found that the levels of IL-8, TNF-α, and MCP-1 were higher in early stage of EM and decreased with disease progression, while TGF-β expression decreased in severe disease [9].

Estrogen has many physiologic effects in woman's body and was found to be involved in the pathogenesis of endometriosis. Aromatase is the key enzyme in estradiol biosynthesis, which is important for the growth of endometrial tissue. It has been found that interleukin-6 (IL-6) leads to a higher aromatase activity, thus stimulates endometriotic stromal cells [10].

The bone remodeling process occurs throughout life, and this is regulated by many cytokines and hormones which play a critical role in skeletal homeostasis in health and disease conditions. Osteoporosis is a condition characterized by a low bone mass and altered bone microarchitecture that leads to an increased risk of fractures [11]. It is classified as either primary or secondary. Primary osteoporosis is mainly due to bone loss that occurs with aging, while secondary osteoporosis may result from medications [e.g., glucocorticoids] or diseases [e.g., malabsorption] that adversely affect the skeleton [12]. It can be caused by increased bone resorption and/or decreased bone formation. Clinically, osteoporosis mostly results from a combination of postmenopausal estrogen deficiency and age-related bone loss by bone-resorbing cells (osteoclasts) and immune cells (both originate in the bone marrow from...
hematopoietic cells[13]. Osteoclasts develop from the precursors of mononuclear monocyte-macrophage cell line after stimulation by macrophage colony-stimulating factor [M-CSF] and receptor for activated nuclear factor kappa-B [RANK] ligand [RANKL]. The bone-forming cells [osteoblasts] are mesenchymal in origin and have a common precursor cells with adipocytes [14]. During the normal remodeling process; bone marrow stromal cells and osteoblasts produce RANKL, which binds to the transmembrane receptor RANK on osteoclast precursors and evokes differentiation and activation [15]. This is mediated by the transcription factor, nuclear-factor kappa B [NFkB], which is responsible for activating osteoclastogenesisand also for the inflammatory response [16]. Interleukin-6 [IL-6] regulate osteoclast differentiation and inflammation processes[17]. The main role of cytokines in bone remodeling is by the fact that receptors for the proinflammatory cytokines [IL-1, IL-6, and TNF-α] are present on both osteoclast precursor cells and mature osteoclasts [18].Osteoblasts also produce osteoprotegerin [OPG], a soluble decoy receptor that blocks RANKL and controls the remodeling process. It is essential to the success of RANK/RANKL/OPG system on bone homeostasis [19]. At the molecular level, increased bone resorption and osteoporosis may result partiallyfrom the overproduction of RANKL and other cytokines mediators regulating osteoclast differentiation and function [20,21]. These include cyclooxygenase [Cox]-2, prostaglandin [PGE2], TNF-α, IL-1, IL-6 or IL-11. All of them lead to recruitment and differentiation of pre-osteoclasts [22].Several studies demonstrating the key role of Estradiol (E2) in bone turnover, in both women and men that maintain bone density in women at their reproductive age. This is a major evidence showing the link between hormone levels and altered bone remodeling[23]. Longitudinal studies, over the past ten years, have related the pituitary hormones levels to measurements of bone architecture and bone turnover markers, during the menopausal transition [24]. In fact, the pituitary-bone axis is being widely involved in skeletal regulation, particularly in the context of osteoporosis. Accordingly, recentclues have proved that some pituitary hormones play an important role in bone regulation, such as growth hormone (GH) [25], follicle stimulating hormone (FSH) [26], thyroid stimulating hormone (TSH) [27], prolactin (PRL) [28], and oxytocin [29].Moreover, in the bone, PTH secreted by parathyroid glands acts on an osteoblast cell membrane receptor that activatesadenylate cyclase and increasing intracellular cyclic adenosine monophosphate (cAMP), which increases the cell permeability to calcium (Ca$^{2+}$). This increase in cytosolic calcium activates a pump that drives calcium from the bone to the extracellular fluids (bone resorption). This pump is enhanced by 1,25-(OH)2 VitD3[30].The majority of bone resorption markers are products of degradation of type I collagen that include carboxy-terminalcross-linked telopeptides of type I collagen (CTXI), and pyridinolines [31].The intact serum N-terminal propeptides of type1procollagen [P1NP] are considered as
early bone formation markers, while osteocalcin is a late marker of osteoblastic activity [32]. The serum concentration of P1NP is directly correlated with the amount of new collagen produced by osteoblasts [33]. It is useful for the assessment of bone turnover in postmenopausal women [34]. Accelerated osteoclastic activity increases bone turnover and is associated with low bone mass in both pre- and postmenopausal women [35]. The high levels of resorption markers indicate increased osteoclastic activity and a higher risk for osteoporosis and hip fractures, independent of BMD [36]. The measurement of biomarkers allows the detection of metabolic changes long before alterations in BMD, reducing the sole reliance on BMD testing. A more complete approach would employ biomarkers to assess risk and identify many disease mechanisms, including inflammation, hormonal defects, oxidative stress, nutrient deficiencies, and malabsorption [37].

SUBJECTS & METHODS

This cross-sectional study was carried out in the fertility center in Al Basra city southern of Iraq. This work was conducted from November 2013 until April 2014. A total of 160 women (80 patients and 80 healthy) were enrolled. Eighty endometriotic patients (N=80) were participated in this study, all of them were females at their reproductive age. The mean age of these subjects was (34.2 ± 0.59). Apparently healthy females (N=80), with regular menstrual cycles (28 – 30 days), were selected to participate as a normal control group for comparison with the same age group. The mean age of these subjects was (32.1 ± 0.97). Diagnosis of endometriosis was made by specialist gynecologists. The diagnosis of this endometrial disease depends on the presence of typical symptoms, physical examination and the demonstration of endometrial masses and adhesions on diagnostic imaging, biopsy examination and laparoscopy. Pelvic ultrasonography, endometrial biopsy and laparoscopy were the standard diagnostic tests used for endometriosis disease detection [38,39].

Informed consents were approved and signed by the patients and healthy volunteers who were enrolled in this study. Disposable syringes and needles were used for blood collection. Fasting blood was aseptically collected during mid cycle (14–16 day) by venipuncture, about 10 ml of venous blood samples were collected, from patients and healthy female participants, in plain tubes. After allowing the blood to clot at room temperature (25 ć) for 15 min, blood samples were centrifuged at 3000 rpm for 10-15 min. Fresh serums were used for assessing the levels of the interleukin1(IL-1) [40], interleukin6(IL-6)[41], tumor necrosis factor alpha [TNF-α][42] by using enzyme linked immunosorbent assay[ELISA] KITS. The carboxyterminal telopeptides of type I collagen[CTX1] [43], carboxyterminal propeptide of type I procollagen [PICP][44], and parathyroid hormone (PTH)[45] serum concentrations were also assessed by ELISA Kits. The serum concentrations of follicle-stimulating hormone (FSH)[46], thyroid-stimulating hormone (TSH)[47], and estradiol (E2)[48]
were measured by using enzymatic kits. The study protocol was approved by the Institutional Ethics Committee in in Middle Eastern Arab countries [49]. The statistical analysis of the results was performed by the application of Microsoft Excel 2010[50], the student's t-test was used to determine the significant difference in means of each of the two groups in study, and the Regression analysis and Pearson correlation (r). The results of analysis with (P) values less than 0.05 (P<0.05) were considered-significant.

RESULTS

Demographic Characteristics of Endometriosis patients and Controls: the demographic data of eighty female patients with premenopausal endometriosis and 80 females in the control group were shown in table (1):

**Table 1: Demographic characteristics of endometriosis patients & healthy controls. Data are expressed as mean ± standard error of mean (SEM) or percentage (%).**

<table>
<thead>
<tr>
<th>Category</th>
<th>Controls (N = 80)</th>
<th>Patients (N = 80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE (years)</td>
<td>32.1 ± 0.97</td>
<td>34.2 ± 0.59</td>
</tr>
<tr>
<td>Social Condition:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>27 (33.8%)</td>
<td>18 (22.5%)</td>
</tr>
<tr>
<td>Married</td>
<td>53 (66.3%)</td>
<td>62 (77.5%)</td>
</tr>
<tr>
<td>Occupation:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>40 (50%)</td>
<td>36 (45%)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>40 (50%)</td>
<td>44 (55%)</td>
</tr>
<tr>
<td>Parity</td>
<td>1.7 ± 0.15</td>
<td>3.8 ± 0.29*</td>
</tr>
<tr>
<td>Menstrual cycle regularity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eumenorrhea</td>
<td>79 (98.8%)</td>
<td>21 (26.25%)**</td>
</tr>
<tr>
<td>Amenorrhea</td>
<td>1 (1.3%)</td>
<td>24 (30%)**</td>
</tr>
<tr>
<td>Menorrhagia</td>
<td>0.0 (0.0%)</td>
<td>35 (43.8%)**</td>
</tr>
<tr>
<td>Stage of endometriosis:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>stage 1</td>
<td>---</td>
<td>25 (31.25%)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>---</td>
<td>23 (28.75%)</td>
</tr>
<tr>
<td>Stage 3</td>
<td>---</td>
<td>32 (40.0%)</td>
</tr>
<tr>
<td>Location Of Disease:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uterus</td>
<td>---</td>
<td>38 (47.5%)</td>
</tr>
<tr>
<td>Uterus &amp; ovaries</td>
<td>---</td>
<td>40 (50.0%)</td>
</tr>
<tr>
<td>Other sites</td>
<td>---</td>
<td>2 (2.5%)</td>
</tr>
<tr>
<td>BMI(kg/m²)</td>
<td>23.3 ± 0.18</td>
<td>28.8 ± 0.40*</td>
</tr>
<tr>
<td>WHR</td>
<td>0.8 ± 0.01</td>
<td>0.9 ± 0.00*</td>
</tr>
</tbody>
</table>

* Significantly different at (P<0.05) as compared to the control group values.

** Significantly different at (P< 0.001) as compared to the control group values.
Table 2: The results show that serum levels of interleukins [IL-1, IL-6] and tumor necrosis factor alpha [TNF-α] are significantly higher [P<0.001] in endometriosis patients than that of the control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control(N = 80)</th>
<th>Patients(N = 80)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1 (pg/ml)</td>
<td>24.8 ±4.53</td>
<td>115.2 ± 14.45*</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>1.2 ± 0.07</td>
<td>10.7 ± 1.85*</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>24.6 ± 2.45</td>
<td>80.4 ± 13.29*</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

* Significantly different (p < 0.05) as compared to the control group values. Data are expressed as Mean ± standard error of mean (SEM).

Figure 1: Histogram shows difference in serum concentrations of IL-1 measured in pg/ml, for both control and study groups.

Figure 2: Histogram shows difference in serum concentrations of IL-6 measured in pg/ml, for both control and study groups.

Figure 3: Histogram shows difference in serum concentrations of TNF-α measured in pg/ml, for both control and study groups.
Table 3: The results in show that serum levels of Follicle-Stimulating Hormone (FSH), Thyroid-Stimulating Hormone (TSH) and parathyroid hormone (PTH), measured in different units, are significantly different \( p < 0.05 \) between the control and endometriosis patient groups, except for estradiol they are insignificant; data are expressed as Mean ± SEM.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control(N = 80)</th>
<th>Patients(N = 80)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2 (µg/L)</td>
<td>30.05 ± 1.44</td>
<td>29.73 ±1.98</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>FSH (mU/ml)</td>
<td>6.3 ± 0.60</td>
<td>10.3 ± 0.73*</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>TSH (U/ml)</td>
<td>3.3 ± 0.15</td>
<td>2.2 ± 0.12*</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>34.1 ± 0.65</td>
<td>51.9 ± 1.82*</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

* Significantly different \( p < 0.05 \) as compared to the control group values.

Figure 4: Histogram shows serum concentrations of estradiol measured in µg/L, for both control and study groups.

Figure 5: Histogram shows serum concentrations of FSH measured in mU/ml, for both control and study groups.

Figure 6: Histogram shows difference in serum concentrations of TSH measured in U/ml, for both control and study groups.

Figure 7: Histogram shows difference in serum concentrations of PTH measured in pg/ml, for both control and study groups.
Table 4: The results show that the serum level of C-terminal telopeptide [CTXI] is significantly higher \( p<0.05 \) in endometriosis patients than that of the control group. The level of C-terminal propeptide [PICP] is insignificant. Data are expressed as Mean ± SEM.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (N = 80)</th>
<th>Patients (N = 80)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-terminal telopeptide (CTXI) (ng/ml)</td>
<td>29.3 ± 5.32</td>
<td>103.0 ± 6.92*</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>C-terminal propeptide (PICP) (ng/ml)</td>
<td>18.4 ± 0.84</td>
<td>18.0 ± 0.75</td>
<td>0.7453</td>
</tr>
</tbody>
</table>

* Significantly different \( p < 0.05 \) as compared to the control group.

In this study, many significant positive correlations have been observed between some of the measured biomarkers, as shown in figures 10, 11, 12 and 13.

Figure 8: Histogram shows difference in serum level of C-terminal telopeptide measured in ng/ml, in both control and study groups.

Figure 9: Histogram shows difference in serum level of C-terminal propeptide measured in ng/ml, in both control and study groups.

Figure 10: Correlation between serum level of TNF-α (pg/ml) and serum CTXI (ng/ml).

Figure 11: Correlation between serum level of PTH (pg/ml) and serum CTXI (ng/ml).
Discussion

The current study was undertaken to exclude the effects of factors, other than endometriosis, like age, marriage, occupation and parity, as described in table (1). In this table, the demographic results of endometriosis patients and healthy females were non-significant (P>0.05) with respect to the age. With respect to the social condition of the women enrolled in this work, non-significant deviations were also detected among the single women and the married one in both groups, as shown in table (1). This table also reveals the occupation states that were (50%) employed and (50%) unemployed in the control group, while (45%) employed and (55%) unemployed in the patients group. The above results signifies minimal negligible percentile differences among the socio-economic states of both healthy and endometriotic female groups. These findings may exclude the proposed effects of the socio-economic status imposed on the women recruited in the present study. Identifying these factors during the early and late menopause are important because age and menopause have been associated with high risk of several chronic diseases such as cardiac, breast, endometrial diseases and osteoporosis [51,52]. Several studies have shown that low socio-economic status is associated with early menopause and endometrial disorders in comparison to high socio-economic status [53,54]. The interest in the prevention of osteoporosis (OP) is escalating among general practitioners but the ways are unclear. Osteoporosis causes are multifactorial, the only definite risk factors are advanced age and the menopausal state [55]. The fractures in postmenopausal women, due to osteoporosis, are an important cause of morbidity, mortality, personal and social cost [56], all good reasons for prevention. Table (2) and figures (1),(2), and (3) showed that the serum levels of IL-1, IL-6, and TNF-α respectively, in the patients were significantly higher (p<0.001) as compared to the women in control group. A reasonable interpretation for such elevation in the results is due to increased inflammation and altered immune functions that have been observed in women with endometriosis disease.
In the present study; increased levels of IL-1and-6 and TNF-α have important role in the pathogenesis of osteoporosis, as described by the correlation analysis (figure 10). The obtained results show that certain proinflammatory cytokines, and predominantly IL-1, IL-6 at higher levels are assumed to play a crucial role in inducing osteopenia and idiopathic OP in the developmental age [57]. Interleukine-1exerts its action on bone cells through many pathways; affecting osteoblast receptors, stimulates resorption through enhancing synthesis and release of collagenase by osteoblasts, inhibits osteocalcin synthesis, and stimulates the production of an osteoclast precursor recruitment-inducing factor by osteoblasts and their fusion into polyynuclear osteoclastic cells. It can directly stimulate osteoclasts through receptor binding [58,59]. It also stimulates osteoclast activity by increasing M-CSF production [58], but IL-1 enhances osteoclastogenesis only in the presence of threshold concentrations of RANKL [60]. Salamone et al. [61] found a relationship between IL-1β and the low bone mineralization in patients with idiopathic hypercalciuria, similarly Zheng et al. [62] found the same results in postmenopausal and premenopausal women respectively. Moreover (IL-1, IL-6 and TNF-α) can affect osteoclasts directly via their specific receptors located on osteoclasts or via modulation of the RANK/RANK ligand (RANKL)/osteoprotegerin (OPG) system [63]. Interleukin-6 is a pleiotropic cytokine and has important role in osteoclast differentiation and function. Its expression was upregulated in bone tissue from osteoporotic patients [64], and it may exert its direct inhibitory effect on bone formation through [glycoprotein 130Signal transducer and activator of transcription (STAT-3) or indirect effect on the balance between OPG and receptor activator of nuclear factor β (RANK) and its ligand (RANKL) [65,66]. The effect of IL-6 on RANK-RANKL/OPG was more powerful leading to increased bone turnover which in turn induce bone resorption. This agree with the animal study by Mysliwiec et al [67]. Moreover Chung et al in 2003 and Garnero et al in 2002 support our finding in relation to role of IL-6 and bone mass in pre- and postmenopausal women, respectively [68,69]. There mechanisms by which TNF-α act in osteoclasts are; bone marrow stromal cells and osteoclast precursors express TNF-α receptors. The main process occurs when stromal cells are exposed to TNF-α and produce RANKL, M-CSF, and IL-1, which promote osteoclast formation and activation. Both TNF-α and RANKL are synergistic, and minimal levels of one markedly enhances the osteoclastogenic capacity of the other [70,71]. TNF-α also has antiapoptotic effects on osteoclasts, prolonging their lifespan [72]. The second mechanism occurs when the inflammatory process becomes more severe and TNF-α may promote osteoclast formation by directly stimulating its precursors in the absence of stromal cells responsive to the cytokine through activating transforming growth factor (TGF-β) [73]. The ability of TNF to increase the osteoclastogenic activity of
RANKL is due to synergistic interactions at the level of NFkB and AP-1 signaling [73]. The other target of TNF is the receptor RANK, whose expression in osteoclasts precursors is synergistically upregulated by TNF and RANKL [74]. The present study finds agreement with other study that correlates the elevation of TNF-α and OP [75,76]. In the present study, as shown in table (3) and figure (7), the serum level of parathyroid hormone (PTH) was significantly higher (p<0.001) in endometriosis patients as compared to women in the control group. The possible explanation for this elevation could be the low levels of 25-Hydroxycholecalciferol(25-(OH)VitD3) and increased secretion of PTH. In general, hyperparathyroidism can be primary or secondary to other disease affecting calcium serum levels. In Sweden, primary hyperparathyroidism is prevalent in 3-4% of all postmenopausal women, and is often asymptomatic for many years. In the skeleton, PTH causes cortical bone loss [77], figure (11). It has been found that, PTH indirectly stimulates increased bone turnover and calcium/phosphorus mobilization from bone via binding to cell surface PTH receptors on the osteoblasts [78]. Also in this study, the serum level of follicle-stimulating hormone (FSH) was significantly elevated (P<0.001) in endometriotic patients than healthy controls, as demonstrated in table (3) and figure (5). Furthermore, the serum level of thyroid-stimulating hormone (TSH) was significantly decreased (P<0.001) in patients than healthy women in control group, as demonstrated in table (3) and figure (6). Whereas, table (3) and figure (4) showed that the serum concentration of estradiol (E2) was non-significantly different (P>0.05) in the patients group than the control group. These findings can be explained on the basis of the major evidence showing the link between hormone levels like estrogens demonstrating their key role in bone turnover, in both genders [23]. It has been considered that the primary specific action of FSH is to stimulate ovarian follicle and estrogen synthesis. Despite the well-established reproductive role of FSH, a debate regarding the association of its high circulating levels and bone loss has emerged [79], see figure (12). This came from studies involving women during pre- and perimenopause showing that high serum levels of FSH do correlate with bone mineral density (BMD) or bone resorption markers even before menopause and estradiol's decline [80]. Furthermore, a meta-analysis of ten prospective studies revealed that the rate of spinal BMD loss during perimenopause, when estrogen levels were still elevated, was higher than the rate of loss in the years following menopause, when estrogen levels were much lower [81]. Allan et al. studied the role of FSH in regulating bone loss in a mouse model using a transgenic expression of human FSH in female mice. This study finds that elevated FSH activity in vivo significantly stimulates bone mass through an ovary-dependent pathway, and discovered the positive association of FSH-induced ovarian secretion of testosterone and inhibins ,which inturn suppress pituitary FSH secretion, with elevated bone mass and the absence of direct FSH stimulatory effects on bone [82]. The bone-mediated FSH effects has also been correlated to the immune cell changes
occurring during perimenopause. The T-lymphocytes and inflammatory cytokines, such as TNF-α and IL-7, are strongly involved in hypogonadal bone loss. Cannon et al. found that FSH partially influences BMD by affecting the activity of bone-resorbing cytokines (IL-1β, TNF-α, and IL-6), either by inducing their secretion or by altering their receptor expression. Endogenous FSH concentrations were found to be correlated with the circulating levels of these cytokines [83]. With respect to the bone tissue, the high osteoporotic turnover in hyperthyroidism has been related only to the elevated thyroid hormone (T3 and T4) levels. However, the therapeutic suppression of TSH or subclinical hyperthyroidism, in which TSH is low and thyroid hormones are normal, are both associated with severe osteoporosis suggesting a direct antiresorptive role of TSH [84,85], see figure (13). This means that TSH acts on bone independent of thyroid hormones and that osteoporosis of hyperthyroidism is due to low TSH levels. Many evidences showed that TSH exerts direct effects on skeletal remodeling by interacting with specific receptors expressed on bone cells. The reduced expression of TSH receptor led to the osteoporotic changes, inhibiting bone turnover and, the administration of low doses of TSH in ovariectomized rats improved bone microstructure and prevented osteoporosis [86]. The TNF-α production has been demonstrated to be mediated genetically and is upregulated in TSHR−/−mice, which increases osteoclastic activity and contributes to the osteopenia phenotype. Although, the endometrial tissue is a sensitive target for steroid sex hormones [estradiol (E2), luteinizing hormone (LH), and FSH], and aromatase is the key enzyme in the biosynthesis of estradiol which is important for the development and growth of endometriosis as mentioned previously, the non-significant change of serum estradiol level neglects its effect on the patients enrolled in this study.

As shown in table (4) and figures (8,9), the serum level of carboxyterminal telopeptide (CTXI) was significantly elevated whereas carboxyterminal propeptide (PICP) level was insignificantly different in patients as compared with the control group. Many experimental studies predicting that certain inflammatory cytokines, including IL-1, IL-6, and TNF-α, play an important role in the pathogenesis of osteoporosis[87]. This supports our present study through significant elevations of bone resorption markers [carboxyterminal telopeptide, CTXI] in the serum of endometriosis patients. The high bone remodeling, as demonstrated by a high level of serum bone turnover markers, is associated with increased bone loss and fragility in some patients. Therefore, inflammation has been implicated recently as risk factor for osteoporosis [88]. In conclusion, the high levels of inflammatory biomarkers observed in this study, especially IL-1, IL-6 and TNF-α, along with the abnormal levels of some hormones namely FSH, TSH and PTH predicts bone loss, osteoporosis and increased risk of fractures in patients with endometriosis. These findings suggest that targeted therapy against inflammation and hormonal imbalance may have potential for the prevention of osteoporosis and its sequelae.
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THE ROLE OF PROINFLAMMATORY CYTOKINES AND SOME HORMONES IN ENDOMETRIOSIS-INDUCED OSTEOPOROSIS


دور السايتوكينات الموالية للالتهابات وبعض الهرمونات في حدوث هشاشة العظام لدى مريضات الانتباذ البطاني الرحمي

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هدف الدراسة:

أجريت هذه الدراسة لقياس بعض المؤشرات الخلوية الموالية للالتهابات وبعض الهرمونات لدى مريضات الانتباذ البطاني الرحمي ومدى تأثيرها على تركيز مؤشرات ارتيشات وتكوين العظام وحودة حالة هشاشة العظام لدى النساء المصابات بهذا المرض.

المرض وطرق العمل:

تماثل مريضات اثنين مصابات باذاء الانتباذ البطاني الرحمي في عمر الاتجاه حاضرات للمعايير القياسية واثنان أمرأة صحية في مجموعة الشريحة تم إشراكهن في هذه الدراسة من أجل قياس تركيز كلا من: عامل التخسيس السرطاني نوع ألفا، إنترلوكين 1، إنترلوكين 6، الهرمون المحفز للبيضة، الهرمون المحفز للدراقي، هرمون البالا درقي، مؤشرات تكوين العظام البروتينات وأرشف العظام التيلوبينتيد، ذوات الكركدوكسيل الطرفي للكلاراجين، وقد وجد أنها جميعا قد تغيرت معاونيا لدى المريضات بالمفارقة مع النساء الصحية في مجموعة الشريحة. فقد التغير في مستوى هرمون الأستروجين كان ليس معيونا في كل المجموعتين.

الاستنتاجات:

أن تأثير المؤشرات الموالية للالتهابات مثل عامل التخسيس السرطاني نوع ألفا، إنترلوكين 1 و إنترلوكين 6 كان عاملا مهما في حدوث حالة هشاشة العظام لدى مريضات الانتباذ البطاني الرحمي، فلما بالإضافة إلى التأثيرات المسبقة لبعض الهرمونات المقاومة على عظام المريضات المشتركة في هذه الدراسة.

مفتاح الكلمات:

زهرة الانتباذ الرحمي، هشاشة العظام، عامل النخر السرطاني، الإنترلوكين 1 و 6، الاستروجين، الهرمون المحفز للبيضة، الهرمون المحفز للدراقي، هرمون البالا درقي.

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