Serum concentrations of selected endogenous estrogen and estrogen metabolites in pre- and post-menopausal Chinese women with osteoarthritis

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ABSTRACT. The purpose of this study was to investigate whether serum levels of selected endogenous estrogens and their metabolites are involved in the pathogenesis of osteoarthritis in pre- and post-menopausal women with osteoarthritis. Sixty-four patients with osteoarthritis (OA) of the knee, 48 patients with rheumatoid arthritis (RA) of the knee, and 48 healthy women were included in this study. Serum concentrations of estradiol and estrogen metabolites, such as 2-hydroxyestrone, 2-hydroxyestradiol, and 16α-hydroxyestrone, were measured by high performance liquid chromatography-mass spectrometry. Our results show that the serum concentrations of free estradiol and total 2-hydroxyestrone were significantly lower in pre-menopausal women with OA compared to the levels detected in the control groups (RA and healthy women). While serum concentrations of free and total estradiol in post-menopausal women with OA was significantly decreased compared to those of the control groups, the level of total 2-hydroxyestradiol significantly increased in post-menopausal women. Furthermore, the total 2-hydroxyestrone concentration positively correlated with the total estradiol level in pre-menopausal women with OA. In addition, the total 2-hydroxyestradiol level positively correlated with free and total estradiol levels in post-menopausal women with OA. In conclusion, estradiol and estrogen metabolites, including 2-hydroxyestrone and 2-hydroxyestradiol, were found in the sera of pre- and post-menopausal women with OA. Except for free and total estradiol deficiency, a decreased serum level of total 2-hydroxyestrone in pre-menopausal women and an increased total 2-hydroxyestradiol level in post-menopausal women with OA may also correlate with the pathogenesis of female OA.

INTRODUCTION

Osteoarthritis (OA) is characterized by progressive degeneration of articular cartilage and structural changes in the underlying bone, including development of marginal growths, osteophytes, and increased thickness of the periosteum (1). About a century ago, Cecil and Archer described “arthritis of the menopause” as rapid development of hand and knee OA along with menopause. While 2-OHE1 acts as a weak estrogen agonist, 16α-OHE1 is considered to be an estrogen agonist (9). The target tissue-specific biological activities of 2-OHE1 and 16α-OHE1 are distinct and are also different from those of E2 (9). 16α-OHE1 binds to the estrogen receptor (ER) by covalent and non-covalent bonds, with lower affinity than E2 (10). Because it has a low affinity for serum SHBG, 16α-OHE1 is more available for estrogen-sensitive tissues (10). Compared with 16α-OHE1, 2-hydroxyestrone has a reduced binding affinity for ER (11), al-

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Key words: 2-hydroxylated, estradiol, estrogen metabolites, menopause, osteoarthritis.

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though it plays an important role in oxidation/reduction reactions (12). It has been suggested that the metabolism of estrogen may be related to osteoporosis and fracture risk (13, 14). A recent study revealed that lower baseline serum E2 and urinary 2-OHE1 levels are closely related to the development of knee OA in middle-aged women (15). However, it is not yet clear how the levels of estrogen metabolites change with menopause, or whether these changes mediate the pathogenesis of female OA. In the present study, we investigated the serum concentrations of \(E_2\) and estrogen metabolites, such as 2-OHE1, 2-hydroxyestradiol (2-OHE2) and 16\(\alpha\)-OHE1, using high performance liquid chromatography and mass spectrometry (HLPC-MS/MS) in pre- and post-menopausal women with OA or rheumatoid arthritis (RA) and healthy women.

**MATERIALS AND METHODS**

**Subjects**

Thirty-two pre-menopausal outpatients (aged 38-47 yr) and 32 post-menopausal inpatients (aged 55-68 yr) with OA of the knee were designated as the experimental group. The control groups included 48 patients with RA, including 24 pre-menopausal patients (aged 38-48 yr) and 24 post-menopausal patients (aged 54-67 yr), and 48 healthy women, including 24 pre-menopausal women (aged 37-46 yr) and 24 post-menopausal women (aged 56-69 yr). The period of time since menopause was >5 yr for all of the post-menopausal women (both experimental and control groups). All of the subjects were recruited from the Third Affiliated Hospital of Sun Yat-sen University (Guangzhou China). OA patients were recruited from the Department of Orthopedics and RA patients were recruited from the Department of Rheumatism from March 1, 2008 to July 15, 2009. Healthy controls were recruited from the Medical Examination Center from June 20 to July 15, 2009. Baseline characteristics, such as age, disease duration, body height, body weight, and body mass index (BMI), are shown in Table 1. All of the subjects gave written informed consent. All of the studies were permitted by the Institute Ethics Committee.

**Diagnostic criteria for OA and RA**

The patients, who displayed symptoms of knee pain, stiffness (time <30 min) or crepitus, were diagnosed as symptomatic OA if a definite osteophyte was observed in the symptomatic knee on plain radiographs, in accordance with the American College of Rheumatology criteria for OA (16). For all of the patients were included. Smokers, alcohol users, and pregnant women were not allowed for at least 6 weeks.

Patients were considered to have RA if they had at least 4 of the following 7 criteria: morning stiffness (lasting at least 1 h); arthritis of 3 or more joint areas; arthritis of hand joints; symmetric arthritis; rheumatoid nodules; serum rheumatoid factor (<1:40); and radiographic classic changes, such as erosions or unequivocal bony decalcification, localized or most marked adjacent to the involved joints (18). Criteria 1 through 4 must have been present for at least 6 weeks.

None of the subjects (OA, RA, and healthy controls) had received corticosteroid therapy during the last 6 months prior to the investigation and none of them reported using systemic contraceptives, weight-reducing aids, 1.25-hydroxy vitamin D or any HRT. The subjects who had recent histories of medication, malignancy, hypertension, diabetes, viral hepatitis, parenchymal liver disease or fatty liver were excluded. Healthy pre- or post-menopausal women with a history of OA or RA were also excluded. Smokers, alcohol users, and pregnant women were not included in this study.

**Blood collection**

Venous blood was collected from the pre- and post-menopausal women at 08:00 h. Menstrual and menopausal status was based on self-report. In pre-menopausal women, blood was drawn at the follicular phase of the menstrual cycle (days 5-7). After being allowed to clot for 1 h at room temperature, the blood was centrifuged at 2000 g for 10 min to obtain serum that was then frozen at –80 C until assay.

**E2 and estrogen metabolites measurements**

Serum free (unconjugated) and total (conjugated + unconjugated) concentrations of \(E_2\), 2-OHE1, 2-OHE2 and 16\(\alpha\)-OHE1 were measured by HPLC-MS/MS (Agilent 1100-APi 3000, USA) (19). Interassay coefficients of variation (CV) of \(E_2\), 2-OHE1, 2-OHE2 and 16\(\alpha\)-OHE1 were 14.2% (5.9 pg/ml), 12.5% (7.8 pg/ml), 13.8% (6.1 pg/ml) and 14.7% (5.7 pg/ml), respectively. The intraassay CV was 6.7% over the working assay range (4.4-15.7 pg/ml).

<table>
<thead>
<tr>
<th>Table 1 - Baseline characteristics of pre-menopausal and post-menopausal women.</th>
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<tbody>
<tr>
<td><strong>Pre-menopausal women</strong></td>
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<tr>
<td>Subject no.</td>
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<tr>
<td>Age (yr)</td>
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<tr>
<td>Diseases duration (yr)</td>
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<tr>
<td>Body height (cm)</td>
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<tr>
<td>Body weight (kg)</td>
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<td>BMI (kg/m²)</td>
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</table>

Data are shown as Mean±SD. *OA: RA, p=0.033; **OA: Normal, p=0.017; OA: osteoarthritis; RA: rheumatoid arthritis; Normal: healthy women; BMI: body mass index.