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Different circulating levels of monocyte chemoattractant protein-1 and interleukin-8 during the menopausal transition

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ABSTRACT

Objective: The aim of the present study was to clarify the changes in circulating cytokines and chemokines in women during the menopausal transition by using a detailed classification.
Materials and methods: A total of 554 women were recruited for this study from the outpatient clinic of the Department of Obstetrics and Gynecology, Tokushima University Hospital. We divided the women into seven stages by menstrual regularity and FSH level: mid-reproductive stage, late reproductive stage, early menopausal transition, late menopausal transition, very early postmenopause, early postmenopause and late postmenopause. We measured serum concentrations of nine cytokines (IL-1β, IL-5, IL-6, IL-7, IL-8, IL-10, TNF-α, MIP-1β and MCP-1).
Results: Serum IL-8 concentrations in postmenopausal women were significantly ($p = 0.001$) higher than those in women in the mid- or late reproductive stage and women in early or late menopausal transition. Serum MCP-1 levels in women in late menopausal transition and postmenopause were significantly ($p < 0.001$) higher than those in women in the mid- or late reproductive stage and women in early menopausal transition. MCP-1 level showed a significant positive correlation ($r = 0.215$, $p < 0.01$) with FSH level in women in menopausal transition.
Conclusion: By using a detailed classification of menopausal transition, patterns of changes in IL-8 and MCP-1 levels during the menopausal transition were found to be different. IL-8 level showed a high level after menopause, while MCP-1 level showed a high level in menopausal transition. MCP-1 may be sensitive to hormonal change and may be involved in the development of estrogen deficiency diseases.

1. Introduction

Many cytokines and chemokines are involved in the pathogenesis, development and progression of atherosclerosis and osteoporosis. It has been reported that interleukin (IL)-1, IL-4, IL-6 and tumor necrosis factor (TNF)-α are mediators of atherogenesis [1,2] and that IL-1, IL-6 and TNF-α act as bone-resorbing cytokines [3,4]. It has also been reported that the expression of IL-10 was detected in atherosclerosis plaque [5] and that elevated IL-10 level was associated with an increased risk for future cardiovascular events in menopausal women with established coronary atherosclerosis [6]. In addition, cytokines are involved in not only pathogenesis, development and progression of the diseases but also symptoms of the diseases such as major depression and hot flashes [7,8]. It has been reported that circulating IL-6 level was increased in women with major depressive disorders [9] and that levels of IL-8 and macrophage inflammatory protein (MIP)-1β were high in women with severe hot flashes [8].

Since progression of atherosclerosis and decrease in bone mineral density occur after menopause, it is thought that changes in these cytokines and chemokines also occur after menopause due to estrogen deficiency. In previous studies, these cytokines and chemokines were compared in premenopausal women and postmenopausal women according to the presence of estrogen. It has been reported that circulating levels of IL-6 and IL-18 in postmenopausal women were higher than those in premenopausal women, while TNF-α level was lower in postmenopausal women [10]. Macrophage colony-stimulating factor level in late postmenopausal women has been demonstrated to be significantly higher than that in premenopausal women [11]. We showed that levels of IL-2, granulocyte/macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) were significantly increased in postmenopausal women for whom less than 5 years had passed since menopause, while IL-4 level was significantly increased in postmenopausal women for whom more than 5 years

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had passed since menopause [12]. However, changes in all cytokines and chemokines might not be associated with menopause. Levels of some cytokines might change before complete estrogen deficiency.

Endocrinological hormones are regulated by a feedback mechanism via the hypothalamus–pituitary–ovarian axis. Therefore, endocrinological features during the menopausal transition are complicated. Since changes in cytokines and chemokines are closely related to these endocrinological changes, features in these cytokines and chemokines may also be complex during the menopausal transition. The menopausal transition is characterized by variations in cycle length and changes in endocrinological hormones. The American Society of Reproductive Medicine proposed the Stages of Reproductive Aging Workshop (STRAW) staging system based on these characteristics [13], and various changes during the menopausal transition have been distinguished by using this system. Changes in cytokines and chemokines during the menopausal transition should be investigated by a more detailed classification than simply a comparison between premenopause and postmenopause.

The aim of the present study was to clarify the changes in circulating cytokines and chemokines in women during the menopausal transition by using a detailed classification.

2. Subjects and methods

2.1. Subjects

The subjects of this study (age range: 40–65 years) were recruited from the outpatient clinic of the Department of Obstetrics and Gynecology, Tokushima University Hospital. A total of 554 women who were being screened for gynecological cancer were enrolled in this study. Informed consent for participation in this study was obtained from each women. The Ethics Committee of Tokushima University Hospital approved the study. Reviews of medical histories and the results of physical examinations and blood chemistry tests showed that all of the women were in good health. None of the subjects had taken any medication known to influence the immune system for at least 1 year. Subjects suspected of having infectious diseases, inflammatory disorders, malignancy or autoimmune diseases, of being undernourished, or of abusing alcohol or drugs were excluded according to the SENIEUR protocol [14]. We divided the women into seven stages by menstrual regularity and follicle-stimulating hormone (FSH) level: (1) women with a regular menstrual cycle and normal FSH level (mid-reproductive stage: MRS, $n = 96$), (2) women with a regular menstrual cycle and elevated FSH level ($\geqslant$10 m IU/ml) (late reproductive stage: LRS, $n = 43$), (3) women with an irregular menstrual cycle and elevated FSH level (early menopausal transition: EMT, $n = 24$), (4) women who had an irregular menstrual cycle in which the interval of amenorrhea was more than 2 months and who had elevated FSH level (late menopausal transition: LMT, $n = 87$), (5) women for whom less than 1 year had passed since menopause (very early postmenopause: VEPM, $n = 66$), (6) women for whom more than 1 year and less than 5 years had passed since menopause (early postmenopause: EPM, $n = 121$) and (7) women for whom more than 5 years had passed since menopause (late postmenopause: LPM, $n = 117$). Venous blood samples were drawn into tubes between 8 a.m. and 10 a.m. Samples obtained were frozen at −70 °C until use for analysis. The assay was run at the same time.

2.2. Measurement of serum cytokine concentrations

Since IL-1β, IL-5, IL-6, IL-7, IL-8, IL-10, TNF-α, MIP-1β and monocyte chemoattractant protein-1 (MCP-1) are involved in the pathogenesis, development and progression of atherosclerosis [15], we measured levels of these nine cytokines and chemokines by using a Bio-Plex human cytokine assay kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA) as previously described [12]. The intra- and inter-assay coefficients of variation were 2.0–10.0% and 3.5–16.1%, respectively. The sensitivity levels were 1.1 pg/ml for IL-6 and MIP-1β, 0.5 pg/ml for IL-7 and IL-8, 0.8 pg/ml for IL-1β and IL-5, 0.9 pg/ml for IL-10, 3.0 pg/ml for TNF-α, and 6.7 pg/ml for MCP-1.

2.3. Measurements of serum concentrations of hormones

Serum luteinizing hormone (LH) concentration was measured by using a kit (TFB Co., Tokyo, Japan) and serum FSH concentration was measured by an IRM using a commercially available kit (TFB Co., Tokyo, Japan). The intra- and inter-assay coefficients of variation (CVs) both ranged from 3% to 4%. Serum estradiol concentration was measured by using a kit (Siemens Healthcare, Los Angeles, CA). Intra- and inter-assay CVs were 4.5–8.0% and 3.2–12.1%, respectively, and the sensitivity of the assay was 2.5 pg/ml.

2.4. Statistical analysis

Data are presented as medians with 25–75th percentile ranges. The Kruskal–Wallis rank test was used to compare differences between multi-groups and Steel–Dwass adjustment was used for a multiple comparison test. Correlations between variables were determined using Spearman's rank order analysis. To determine differences between groups, the Mann–Whitney U test was used if data were not normally distributed. A probability of $p < 0.05$ was considered significant. Box plots show median, 25th and 75th percentiles as boxes and 10th and 90th percentiles as error bars. All statistical analyses were carried out using Excel (2010; Microsoft Corporation, Redmond, WA) with the add-in software Statcel 3 (OMS, Tokyo, Japan) and SPSS statistics version 20.0 (IBM, Armonk, New York).

3. Results

3.1. Characteristics of the subjects

Background characteristics such as age, body mass index (BMI), and levels of LH, FSH and estradiol in the seven groups are shown in Table 1. There was no significant difference in BMI in the seven groups.

3.2. Serum levels of cytokines and chemokines

As can be seen in Table 2, IL-8, MCP-1 and IL-7 showed significant changes during the menopausal transition ($p = 0.001$, $p < 0.001$ and $p = 0.001$, respectively). Serum IL-8 concentrations in women in LPM were significantly higher than those in women in LRS and LMT. In addition, serum MCP-1 levels in women in LMT, VEPM, EPM and LPM were significantly higher than those in women in MRS. Serum IL-7 level showed a significantly lower level in women in LPM. Other cytokines and chemokines including IL-1β, IL-5, IL-6, IL-10, TNF-α and MIP-1β did not show significant differences among the groups.

3.3. Correlations of MCP-1 and IL-8 with hormones

Changes in levels of MCP-1 and IL-8 during the menopausal transition were different since an increase in MCP-1 level was found in late menopausal transition and increase in IL-8 was observed after menopause. Therefore, we examined the correlations