Epidemiological study on the relationship among menopausal condition, bone fragility, and knee osteoarthritis

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Abstract

Background: Knee osteoarthritis (KOA) occurs more often in middle-aged females. While this age-group experiences comorbid osteoporosis with menopause, its influence on KOA has not been clarified. This epidemiological study aimed to investigate the relationship between menopausal conditions, bone mineral density (BMD), and KOA.

Methods: A total of 518 female volunteers who participated in the Iwaki cohort study were enrolled and divided into groups (pre- and post-menopause). Antimullerian hormone (AMH) was measured as a predictive marker for menopause in the pre-menopausal subjects. Weight-bearing anterior-posterior knee radiographs were classified by Kellgren-Lawrence grade, and grade ≥ 2 was defined as definitive KOA (DKOA). Early KOA (EKOA) was defined by Luyten's criteria, and BMD was measured at a distal radius. The relationship between menopausal condition, BMD, and KOA was analyzed by ROC and regression analysis.

Results: Fifty-two participants (10.0%) were diagnosed with EKOA and 204 (39.4%) with DKOA. A total of 393 (75.9%) females began menopause, and the prevalence of DKOA was up to 48.1% and >12.0% in pre-menopause females (p < 0.001, Odds ratio: 6.79). From the ROC analysis in pre-menopausal females, cut-off value of AMH for detecting EKOA was 0.08 ng/ml (AUC: 0.712, p5%CI: 0.527 to 0.897, p-value: 0.025, Odds ratio: 8.28). Regression analysis showed that lower AMH was related to EKOA (p=0.035, Odds ratio: 5.55) and DKOA (p=0.032, Odds 1.59), and lower BMD and high turnover bone metabolism were correlated with DKOA.

Conclusions: KOA increased after menopause and was correlated with lower BMD. Furthermore, reduction in AMH was a valuable biomarker for the detection of EKOA.

Introduction

Knee osteoarthritis (KOA) is a common cause of chronic pain and disability in elderly people, and its radiographic prevalence among adults aged > 40 years in Japan was up to 42% in males and 62.4% in females. Disadvantages of progressed KOA include high treatment costs, decreased productivity, and absence from work; therefore, early diagnosis is crucial for early treatment and preventative interventions. A recent epidemiological study revealed that the highest prevalence of early KOA (EKOA) was observed in females aged ≥ 50 years, owing to factors such as age, female sex, obesity, and previous knee injury. The major causal factor of EKOA and definitive KOA (DKOA) was sex. The radiographic prevalence of knee OA in females were 1.2–2.8 times higher than that in males, and cartilage loss progressed more rapidly in females. Estrogen deficiency after menopause is known to cause a reduction in bone mineral density (BMD), and suppress cartilage and subchondral bone remodeling. Despite the relevance of estrogen in evaluating chondral and bony metabolism, it is not easily clinically monitored because irregular menstrual cycles induce unstable female hormone patterns during menopausal transitions. Antimullerian hormone (AMH) has been found to be a new biomarker to
predict menopause; it decreases three years prior to menopause\textsuperscript{17}. Few reports exist to investigate the relationship between menopausal transition and EKOA, which are observed among females $\geq 50$ years old.

Both osteoporosis and KOA are attributed to menopause\textsuperscript{18-19}. However, the possibility of osteoporosis causing the development of KOA is uncertain. Osteoporotic osteoarthritis is a particular type of OA\textsuperscript{20}, and both low and high bone mineral densities (BMDs) are associated with KOA\textsuperscript{19}. More recent epidemiological studies revealed that knee pain in those with EKOA were related to bone fragility and bone marrow lesions (BMLs)\textsuperscript{21}. Further studies are needed to establish the relationship between bone fragility and KOA.

The purpose of this study was to investigate the association between menopausal transition and the radiographic severity of KOA by using serum biomarkers, with special focus on AMH. Furthermore, we examined the association between menopause related bone fragility and KOA in middle-aged community-dwelling females. We hypothesized that, based on a relationship among menopause, osteoporosis, and knee OA, bone fragility would affect knee OA. Furthermore, in early stages, AMH would help detect EKOA in middle-aged females.

**Methods**

The participants were volunteers for the Iwaki Health Promotion Project, which is a community-based preventive medicine program that aims at improving average life expectancy by performing general health check-ups\textsuperscript{5,22-23}. The participants were recruited using mass media advertisements and with the help of public health nurses. All participants provided written informed consent, and the study was done in agreement with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards and conducted with the approval of the ethics committee of Hirosaki University Graduate School of Medicine (2016-028).

**Participants**

Out of the 12,000 eligible residents, 1,148 volunteers were enrolled in this study. The exclusion criteria of this study were as follows: male sex (n = 455), patients under treatment of rheumatoid arthritis or with anti-cyclic citrullinated peptide antibody of more than 4.5 U/ml (n = 26), postoperative patients with total knee arthroplasty and arthrodesis (n = 9), and incomplete data (n = 20). After the first exclusion, a total of 648 female participants were included, and their distribution of estradiol and AMH per their ages were shown in a scatter plot (Fig. 1). Data of females aged 40 years and above were considered for further statistical analysis. For an anthropometric evaluation, their heights, weights, and body mass index (BMI) were recorded.

**Blood examination**
Blood samples were taken from all participants before breakfast after a period of ten or more hours without food. Blood analysis were performed by the LSI Medience Corporation (LSI Medience Corp., Tokyo, Japan). This company has an ISO-15189-accredited laboratory, and serum assays were performed under strict conditions. AMH (ng/ml, CLEIA), luteinizing hormone (LH, mIU/ml, CLIA), follicle-stimulating hormone (FSH, mIU/ml, CLIA), estradiol (pg/ml, CLIA), prolactin (ng/ml, CLIA), and testosterone (ng/ml, CLIA) were measured on the female hormones. Further, bone metabolism was evaluated by the following markers: type I procollagen N-terminal propeptide total (total PNP, µg/l, ECLIA), bone alkaline phosphatase (BAP, µg/l, CLEIA), N-terminal telopeptide (NTx, nMBCE/l, EIA), and tartrate-resistant acid phosphatase 5b (TRACP-5b, mU/dl, EIA). Furthermore, pentosidine (pmol/ml, HPLC), and homocysteine (nmol/ml, LC-MS/MS) were measured for bone quality. The inflammation markers, hyaluronan (ng/ml, LA), matrix metalloproteinase-3 (MMP-3, ng/ml, LA), high sensitivity C-reactive protein (hr-CRP, mg/dl, Nephelometry), adiponectin (µg/ml, LA), leptin (ng/ml, RIA), and interleukin-6 (IL-6, pg/ml, CLEIA) were examined.

**Radiographic evaluation**

A radiographic examination of the knee was performed using a digital radiographic device (CXDI-40EG, Canon Inc. Tokyo, Japan). Experienced radiologists and orthopedic surgeons obtained the weight-bearing, full extension, and anterior-posterior radiographs of both knees with foot map positioning on the day of the check-up. The beam was positioned parallel to the floor and aimed at the joint space, and the sequencing was set at 60 kV, 50 mAs, and 80 ms for all participants. The images were converted into JPEG format files. OA severity in each knee was classified as KL grade 0 to 4 using the KL radiographic atlas by two trained orthopedic surgeons (D.C. and S.O.)\(^{10}\). The interclass correlation coefficient from the two surgeons was 0.815. These surgeons were blinded to the sequence in which the radiographs were acquired and the clinical status of the participants. In this study, KL grades 0 and 1 were classified into pre-radiographic OA stage, non-OA, and EKOA. Participants with KL grade 2, 3, or 4 in the most affected knees were diagnosed with DKOA.

**Classification criteria for early OA of the knee**

EKOA was defined based on the classification criteria proposed by Luyten\(^4\). The classification criteria for EKOA were as follows: A) Patient-based questionnaires: the knee injury and osteoarthritis outcome scores (KOOS)\(^{25}\) – two of the following needed to score “positive” (i.e., ≤ 85%): pain (9 items), symptoms (7 items), activities of daily living (ADL) (short version, 7 items), and knee-related quality of life (QOL) (4 items). B) Clinical examination: at least one of the following needed to present: joint line tenderness or crepitus of the knee. C) Radiographs: KL grade zero and one at the standing, extension, and weight-bearing positions. Based on the above criteria, subjects with KL grade 0/1 were classified into non-OA and EKOA.

**Bone mineral density measurements**

The BMD of the forearm was determined by dual-energy X-ray absorptiometry using DCS-600EXV (Hitachi Aloka Medical, Tokyo, Japan) based on a previous report\(^{21}\). Briefly, the region of interest of the BMD was
measured on the non-dominant side, one third of the distal radius, unless there was a history of previous fracture, where the dominant side was measured.

**Statistical analysis**

Demographic data among non-OA, EKOA, and DKOA groups were shown as mean ± standard deviations. Chi-square test for categorical variables and analysis of variance (ANOVA) and Tukey test for continuous variables were performed to compare the three groups' demographic data. To estimate the odds ratio of DKOA by menopause, crude logistic regression analysis was performed, with DKOA as a dependent variable and menopause as an independent variable. To estimate cut-off values of AMH for EKOA among pre-menopausal females, receiver operating characteristic (ROC) analysis was performed. The plot of false positive fraction and 1-true positive fraction build curve and area under the curve (AUC) was calculated. The cut-off point was defined from the nearest point to the true positive. Based on the cut-off value, the prevalence of EKOA and DKOA were estimated. Furthermore, the Spearman's correlation coefficients were calculated using female hormone, bone metabolic markers, and inflammation markers. Finally, to investigate the correlation among menopause, AMH, EKOA and DKOA, logistic regression analyses using a backward stepwise selection method was performed, with the EKOA or DKOA as a dependent variable, and BMI, BMD, bone metabolic markers, and inflammation markers as basal independent variables. In addition to basal independent variable, regression model 1 were set to investigate the risk of lower AMH on EKOA in pre-menopausal females with KL grade zero and one, which included categorized AMH (pre-menopause with AMH ≥ 0.08, pre-menopause with AMH < 0.08, or post-menopause) as independent variables. Subsequently, the same independent variables were used in the regression model 2 to investigate the risk of DKOA in females. Regression model 3 included a categorized post-menopausal period of five years. Data input and analysis were performed using SPSS version 25.0J (SPSS Inc., Chicago, IL, USA). A p-value < 0.05 was considered statistically significant.

**Results**

Out of the 518 participants, 262 (50.6%) were classified as non-OA, 52 (10.0%) participants as EKOA, and 204 (39.4%) participants as DKOA. There were no significant differences of age, BMI, and BMD between non-OA and EKOA groups (Table 1).
Table 1
Demographic data of non-KOA, EKOA, and DKOA groups.

|                                | Non-KOA     | EKOA        | DKOA        |
|--------------------------------|-------------|-------------|-------------|
| Sample number                  | 262         | 52          | 204         |
| Age (y.o)                      | 55.8 ± 10.5 | 58.8 ± 7.8  | 67.2 ± 9.8**† |
| Post-menopause (%)             | 161 (61.5%) | 43 (82.7%)  | 189 (92.6%)* |
| Body mass index (kg/m²)        | 22.0 ± 2.9  | 22.8 ± 3.5  | 23.8 ± 3.5*  |
| Bone mineral density (g/cm²)   | 0.59 ± 0.10 | 0.58 ± 0.09 | 0.52 ± 0.09**† |
| KOOS pain                      | 97.3 ± 6.0  | 75.4 ± 13.8* | 79.5 ± 20.0* |
| KOOS symptom                   | 95.6 ± 5.6  | 78.7 ± 12.8* | 80.2 ± 19.7* |
| KOOD ADL short                 | 97.5 ± 7.0  | 79.2 ± 16.1* | 80.2 ± 21.6* |
| KOOS QOL                       | 89.9 ± 14.3 | 58.2 ± 16.7* | 63.2 ± 27.2* |
| Anti-Müllerian hormone (ng/ml) | 0.39 ± 0.88 | 0.08 ± 0.31* | 0.08 ± 0.46* |
| Estradiol (pg/ml)              | 50.9 ± 95.7 | 19.6 ± 50.8* | 11.0 ± 21.0* |
| Luteinizing hormone (mIU/ml)   | 16.9 ± 11.2 | 19.6 ± 7.2   | 18.5 ± 9.2   |
| Follicle-stimulating hormone (mIU/ml) | 42.5 ± 29.8 | 53.9 ± 23.7* | 50.9 ± 20.6* |
| Prolactin (ng/ml)              | 10.0 ± 9.2  | 10.6 ± 13.1  | 10.5 ± 16.2  |
| Testosterone (ng/ml)           | 0.24 ± 0.09 | 0.24 ± 0.15  | 0.24 ± 0.09  |
| Type I procollagen N-terminal propeptide total (µg/l) | 49.2 ± 24.7 | 54.9 ± 20.6 | 52.6 ± 22.5 |
| Bone alkaline phosphatase (µg/l) | 13.4 ± 6.2  | 14.8 ± 4.6   | 15.6 ± 8.3*  |
| N-terminal telopeptide (nMBCE/l) | 15.0 ± 4.6  | 16.9 ± 4.7   | 16.7 ± 6.5*  |
| Tartrate-Resistant Acid Phosphatase 5b (mU/dl) | 424.7 ± 194.6 | 508.8 ± 200.9* | 509.7 ± 193.9* |
| Pentosidine (pmol/ml)          | 27.7 ± 11.9 | 30.4 ± 11.2  | 33.4 ± 16.3* |
| Homocysteine (nmol/ml)         | 8.2 ± 2.7   | 8.3 ± 1.9    | 9.0 ± 3.3*   |
| Hyaluronic acid (ng/ml)        | 26.3 ± 29.7 | 30.7 ± 24.5  | 90.7 ± 262.6* |

Values are means ± standard deviation of the demographic data. Values in () indicate percentage in each group. Differences among the non-KOA, early knee osteoarthritis (EKOA), and definitive knee osteoarthritis (DKOA) groups were compared by analysis of variance or chi-square test. A p value below 0.05 when compared with non-OA (*) and EKOA (†) were considered statistically significant. EKOA: early knee osteoarthritis, DKOA: definitive knee osteoarthritis, KOOS: the knee injury and osteoarthritis outcome scores, ADL: activities of daily livings, QOL: quality of life.
|                                | Non-KOA     | EKOA       | DKOA       |
|--------------------------------|-------------|------------|------------|
| **Matrix metalloproteinase-3 (ng/ml)** | 35.3 ± 14.3 | 39.7 ± 29.7 | 44.7 ± 34.5* |
| **High sensitivity C-reactive protein (mg/dl)** | 0.06 ± 0.14 | 0.05 ± 0.14 | 0.09 ± 0.18 |
| **Adiponectin (µg/ml)**          | 12.5 ± 6.0  | 12.1 ± 5.0  | 13.2 ± 6.1  |
| **Leptin (ng/ml)**               | 10.0 ± 5.7  | 10.3 ± 5.8  | 12.2 ± 8.5* |
| **Interleukin-6 (pg/ml)**        | 1.3 ± 1.6   | 2.2 ± 6.1   | 1.9 ± 2.2*  |
| **Fitness habit (%)**            | 58 (22.1%)  | 13 (25.0%)  | 48 (23.5%)  |
| **Drinking habit (%)**           | 87 (33.3%)  | 13 (25.0%)  | 52 (25.6%)  |
| **Smoking habit (%)**            | 64 (24.5%)  | 12 (23.1%)  | 30 (14.7%)  |

Values are means ± standard deviation of the demographic data. Values in () indicate percentage in each group. Differences among the non-KOA, early knee osteoarthritis (EKOA), and definitive knee osteoarthritis (DKOA) groups were compared by analysis of variance or chi-square test. A p value below 0.05 when compared with non-OA (*) and EKOA (†) were considered statistically significant.

EKOA: early knee osteoarthritis, DKOA: definitive knee osteoarthritis, KOOS: the knee injury and osteoarthritis outcome scores, ADL: activities of daily livings, QOL: quality of life.

A total of 393 (75.9%) females were 49.4 ± 4.7 years old. The prevalence of OA in post-menopausal women was up to 48.1%, and 12.0% in the pre-menopausal participants (p < 0.001). Regression analysis revealed that the odds ratio of DKOA by menopause was 6.79 (p < 0.001, 95%CI: 3.82–12.07). Correlation between age and estradiol was weak (r=-0.281, p = 0.002) at the pre-menopausal stage, and there was no correlation post-menopause (p = 0.334) (Fig. 1A). On the other hand, the values of AMH in pre-menopausal females were 0.93 ± 1.22 ng/ml, which was highly correlated with age (r = -0.738, p < 0.001); the levels in post-menopausal females were 0.02 ± 0.01 ng/ml, regardless of age (p = 0.126) (Fig. 1B).

Also, the values of estradiol and AMH of EKOA and DKOA were significantly lower than that of the non-OA group (Table 1). ROC analysis in pre-menopausal females with KL grade zero and one showed that lower AMH strongly predicts the presence of EKOA, and its cut-off value was estimated as 0.08 ng/ml. The odd ratio for EKOA was 8.28 (p = 0.024, AUC: 0.712, 95%CI: 0.527–0.897) (Fig. 2) (Table 2). Pre-menopausal women were grouped into two categories based on the cut-off value; the presence of EKOA increased up to 15.0%, compared with 3.5% in those with higher AMH (p < 0.001) (Table 3). Also, the AMHs were negatively correlated with bone absorption and formation markers (Table 4).
Table 2
ROC analysis of female hormones for early knee osteoarthritis.

| Female hormone                      | AUC   | 95%CI  | χ² value | p-value | Cut-off value | FPF  | TPF  | Odds ratio |
|-------------------------------------|-------|--------|----------|---------|---------------|------|------|------------|
| Antimullerian hormone               | 0.712 | 0.527  | -        | 0.897   | 5.05          | 0.025| 0.08 | 0.30       |
| Estradiol                           | 0.636 | 0.399  | -        | 0.873   | 1.27          | 0.260| 20.00| 0.15       |
| Luteinizing hormone                 | 0.677 | 0.487  | -        | 0.866   | 3.33          | 0.068| 10.27| 0.28       |
| Follicle-stimulating hormone        | 0.697 | 0.509  | -        | 0.886   | 4.22          | 0.040| 9.65 | 0.30       |
| Prolactin                           | 0.513 | 0.286  | -        | 0.740   | 0.01          | 0.909| 16.32| 0.18       |
| Testosterone                        | 0.513 | 0.325  | -        | 0.702   | 0.02          | 0.890| 0.21 | 0.33       |

ROC: AUC: area under the curve, 95%CI: 95% confidence interval, FPF: false positive fraction, TPF: true positive fraction.

Table 3
Prevalence of knee osteoarthritis in menopausal transition and post-menopausal stage.

|                                | Pre-menopause | Post-menopause |
|--------------------------------|---------------|----------------|
|                                | AMH ≥ 0.08    | AMH < 0.08     |
| Early knee osteoarthritis      | 3.5%          | 15.0%          |
| Definitive knee osteoarthritis | 12.9%         | 48.1%          |

Prevalence of early and definitive knee osteoarthritis in menopausal transition and post-menopausal stage. In pre-menopausal stage, subjects were divided into two groups according to the cut-off value (0.08 ng/ml).
Table 4
Correlation coefficients among biomarkers of female hormones, bone metabolisms, and inflammations

|                | BMD  | AMH  | E2   | LH   | FSH   | Prolactin | Testosterone |
|----------------|------|------|------|------|-------|-----------|-------------|
| AMH            | 0.491| -0.458| -0.555| 0.595| 0.185 | 0.127     |
| BMD            |      | 0.491| -0.224| -0.334| 0.186 | 0.102     |
| Total P1NP     | -0.243| -0.260| -0.298| 0.198| 0.206 | -0.119    |
| BAP            | -0.365| -0.356| -0.363| 0.243| 0.261 | -0.163    |
| NTx            | -0.284| -0.392| -0.384| 0.328| 0.331 |           |
| TRACP-5b       | -0.465| -0.506| -0.479| 0.351| 0.410 | -0.211    | -0.105      |
| Pentosidine    | -0.298| -0.280| -0.328| 0.136| 0.234 | -0.119    | -0.140      |
| Homocysteine   | -0.214| -0.152| -0.175| 0.141| 0.175 |           |
| sHA            | -0.516| -0.443| -0.388| 0.216| 0.259 | -0.132    |
| MMP-3          |      |       |       |       | 0.095 | 0.132     |
| Hs-CRP         |      |       |       |       | -0.131|           |
| Adipokine      | -0.219| -0.280| -0.208| 0.192| 0.259 |           |
| Leptin         |      | 0.125| 0.162| -0.097| -0.160| 0.122     |
| IL-6           | -0.244| -0.155|       |       |       |           |

Spearman's correlation coefficients were calculated. Only statistically significant correlations (p < 0.05) were shown in this table.

Regarding bone fragility, the BMD of DKOA were lower than EKOA and non-OA (p < 0.001, respectively). Also, the values of BAP (p = 0.002), TRACP-5b (p = 0.002), and NTx (p < 0.001) of the DKOA group were significantly higher than those of the non-OA group. Furthermore, the bone quality markers, pentosidine (p < 0.001), and homocysteine (p = 0.014) were worse in the DKOA group than in the non-OA group. Bone metabolic and quality markers were negatively correlated with BMD, AMH and estradiol concentration, and positively correlated with LH and FSH (Table 4). Further comparison with inflammation markers showed that hyaluronan, MMP-3, leptin, and IL-6 concentrations in the DKOA group were higher than those in the non-OA group. Hyaluronan moderately correlated with BMD (r=-0.516, p < 0.001), AMH (r=-0.443, p < 0.001), and estradiol (r=-0.388, p < 0.001) (Table 4).

Models 1 and 2 logistic regression analysis focusing on AMH reduction at menopausal transition showed that the related AMH below 0.08 ng/ml was significantly related to EKOA (p = 0.035, Odds ratio: 5.55) and DKOA (p = 0.032, Odds ratio: 1.59). In the model 3, analysis focusing on the post-menopausal period
showed that the risk of DKOA increased by 1.24 times every five years post-menopause (p = 0.005) (Table 5).

Table 5  
Related factors for radiographic knee osteoarthritis.

|                           | Standard partial regression coefficient | p-value | Odds ratio | 95% confidence interval |
|---------------------------|----------------------------------------|---------|------------|-------------------------|
| **Model 1: Risk for EKOA in pre-menopausal females (Categorized AMH)** |                                        |         |            |                         |
| AMH reduction             | 1.71                                   | 0.035   | 5.55       | 1.13–27.23              |
| Total PPN                 | -0.05                                  | 0.106   | 0.95       | 0.90–1.01               |
| Leptin                    | 0.12                                   | 0.015   | 1.13       | 1.02–1.25               |
| **Model 2: Risk for DKOA in overall females (Categorized AMH)** |                                        |         |            |                         |
| Body mass index           | 0.16                                   | < 0.001 | 1.18       | 1.10–1.26              |
| Bone mineral density      | -4.40                                  | 0.002   | 0.01       | 0.01–0.19              |
| Hyaluronic acid           | 0.02                                   | < 0.001 | 1.02       | 1.01–1.02              |
| Total PPN                 | -0.01                                  | 0.106   | 0.99       | 0.98–1.00              |
| AMH reduction             | 0.46                                   | 0.032   | 1.59       | 1.04–2.42              |
| **Model 3: Risk for DKOA in overall females (Postmenopausal period by 5 years)** |                                        |         |            |                         |
| Body mass index           | 0.16                                   | < 0.001 | 1.17       | 1.10–1.25              |
| Bone mineral density      | -2.70                                  | 0.086   | 0.07       | 0.01–1.47              |
| Hyaluronic acid           | 0.01                                   | < 0.001 | 1.01       | 1.01–1.02              |
| Post-menopausal period    | 0.22                                   | 0.005   | 1.24       | 1.07–1.44              |

Logistic regression analysis by backward stepwise selection method was performed, with the presence of early knee osteoarthritis or knee osteoarthritis as dependent variable, and body mass index, bone mineral density, bone metabolic markers, and inflammation markers as independent variables. Furthermore, model 1 and 2 included categorized antimullerian hormone (AMH) reduction (pre-menopause with AMH
≥ 0.08, pre-menopause with AMH < 0.08, or post-menopause). Model 3 included post-menopausal period by 5 years after menopause.

**Discussion**

The most important finding of this study was that bone fragility and high turnover bone metabolism with menopause correlated with EKOA and DKOA in middle-aged Japanese females. Furthermore, AMH reduction below 0.08 ng/ml during menopausal transition indicated the presence of KOA from the early stage of this disease. On the other hand, reduction of the AMH and estradiol, which were proof of menopause, indicated lower BMD, high turnover bone metabolisms, and increased inflammation. These results suggested that the decline of female hormone would track KOA from the early stages. Bone fragility and inflammations based on menopause influenced the KOA even in early phase of this disease, which is a target of treatment or preventive interventions for KOA.

Females are at a higher risk of knee OA, and many previous epidemiological studies revealed that the prevalence of OA in females were higher than in males. A systematic review estimated that the total odds ratio of females was up to 1.68 (95% CI: 1.37–2.07). Framingham study showed that the prevalence of OA in females was 36% and 1.2 times higher than that in males. In Japan, the prevalence of OA in females were 1.5 to 2.8 times higher than males; the prevalence was higher than that of Caucasians. Considering the role played by menopause, a longitudinal study from Melbourne Women’s Midlife Health Project showed that those who have never undergone hormone therapy were at a higher risk of developing knee OA. Further, representative therapy decreased the incidence of knee OA. Although one systematic review concluded that there was no clear association between female hormonal aspects and knee OA, the relationship between DKOA and menopause shown in this study is supported by previous studies.

Firstly, this study revealed the application of AMH as a serum biomarker to detect EKOA and DKOA, especially at an early phase of the disease. AMH indicated the presence of EKOA, which proved that the initiation of KOA is in line with the menopausal transition stage. AMH is a glycoprotein dimer, only expressed in growing follicles, and has been identified as a marker of ovarian aging. AMH decreases during menopausal transition, as previous reports found that the AMH reduces three years before menopause. Another advantage of AMH is that it is not affected by menstrual cycles like estradiol, LH, and FSH. AMH helps to detect KOA in its early stages and aids in early intervention.

The influences of bone fragility on OA incidence or progression are controversial. However, this study showed that lower BMD and high turnover bone metabolism of middle-aged females were correlated with the presence of KOA. In the 90s, several studies have reported that higher BMD is related to the incidence of KOA. On the contrary, from the basis of evidences in the most recent decade, Ota et al. revealed that symptomatic BMLs on MRI were related to bone fragility, including lower BMD and high turnover of bone metabolism in middle-aged females without radiographic abnormalities. Furthermore, others also
reported a pathogenic mechanism since excessive bone resorption takes place in the early phase of OA\textsuperscript{34–35}.

Currently, the etiology and pathology of EKOA remain unclear. Our study was important because reduced AMH was associated with the presence of female EKOA, which suggested that initiation of degenerative change was in line with the menopausal transition stage. In the early stages of KOA, the Framingham study, including 710 knees with KL grade 0/1, showed the featured MRI findings in those with knee pain as having BMLs, attrition, and subchondral bone cysts\textsuperscript{36}. Further, the CHECK study, a 5 year longitudinal cohort of general population, showed that the incidence risk factors for knee OA from KL grade 0/1 were BMLs, effusion, and meniscal lesion on MRI\textsuperscript{37}. Bone abnormalities in the early stage of KL are likely to occur in the middle-aged female population\textsuperscript{36–37}. During menopausal transition, bone metabolism changes dramatically, corresponding to the intrinsic hormonal changes in females. Based on this evidence, the current data indicates that such abrupt transitions in menopause predicts not only systemic bone metabolism turbulence but also focal bone abnormalities, which could induce osteoarthritic changes in knee joints.

Another interesting result in this study was that serum AMH concentration in the female population having KOA was associated with serum inflammation markers. Systemic and focal inflammation in synovium play an important role. Inflammation is generally induced by microfragments of cartilages or danger-associated molecular pattern (DAMPS) in synovial fluids and releases several proteases and cytokines, which accelerate the degeneration of articular tissues\textsuperscript{38–40}. The infrapatellar fat pad is a major source of adiponectin in synovial fluid; adiponectins are closely related to the metabolic syndrome and degenerative pathological changes in the cartilage and bone during OA\textsuperscript{41}. In this study, the presence of EKOA and DKOA correlated with an increasing serum hyaluronan concentration, which indicated synovitis, severity of knee OA, and degree of pain\textsuperscript{23,42}; this predicts future joint space narrowing over 5 years\textsuperscript{43}. AMH was a valuable and predictive biomarker for EKOA, which indicated bone fragility and inflammation. However, the utility and reliability of systemic biomarkers in OA diagnosis was not validated; further studies are needed.

This study has several limitations besides the selection bias focusing only on middle- to old-aged females. First, the study sample was the general Japanese population; their background information and confounding factors were not completely evaluated. Second, the imaging examination was limited to the radiographs. Although the MRI and ultrasonographic imaging detected minute structural changes and inflammation of the knee joint\textsuperscript{22,37} especially in EKOA, we could not use these modalities. However, by measuring many inflammation biomarkers, we could evaluate the minute changes of osteoarthritis. Furthermore, regarding radiographic examination, patellofemoral joint OA was not fully examined. Although crepitus was evaluated as one of the symptoms of patellofemoral OA, an imaging analysis was not performed. Imaging of patellofemoral joints could not be performed as a result of time constraints. However, patellofemoral OA should be assessed because of its significant effect on ADL and QOL\textsuperscript{44–45}. Third, this study was a cross sectional study; basal causes of the incidence of knee OA were not
determined. Further, a longitudinal observation was conducted in our cohort to estimate the real incidence of knee OA after AMH reduction and to certify its predictive values. Despite these limitations, this cohort study revealed the association of AMH to predict EKOA during menopausal transition, in addition to the relationship between menopause and osteoporotic knee OA in middle-aged females. In considering the etiology of OA, AMH reduction occurs three years before menopause, which leads to high turnover of bone metabolism and bone fragility. Bone fragility and inflammation at menopause influences knee OA even in the early phase of this disease. Therefore, AMH reduction could detect the EKOA without radiographic abnormalities via bone fragility and synovitis.

**List Of Abbreviations**

ADL: activities of daily living
AMH: antimullerian hormone
ANOVA: analysis of variance
AUC: area under the curve
BAP: bone alkaline phosphatase
BMD: bone mineral density
BMI: bone mass index
BML: bone marrow lesion
DAMPS: danger-associated molecular pattern molecules
DKOA: definitive knee osteoarthritis,
EKOA: early knee osteoarthritis,
FSH: follicle-stimulating hormone
hr-CRP: high sensitivity C-reactive protein
IL-6: interleukin-6
KL grade: Kellgren-Lawrence grade
KOA: knee osteoarthritis,
KOOS: knee injury and osteoarthritis outcome scores
LH: luteinizing hormone
MMP-3: matrix metalloproteinase-3
NTx: N-terminal telopeptide
QOL: quality of life
ROC analysis: receiver operating characteristic analysis
total PNP: type I procollagen N-terminal propeptide total
TRACP-5b: tartrate-resistant acid phosphatase 5b
95%CI: 95% confidence interval

Declarations

Ethics approval and consent to participate
All participants provided written informed consent, and the study was done in agreement with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards and conducted with the approval of the ethics committee of Hirosaki University Graduate School of Medicine (2016-028).

Consent for publication
Consent for publication were obtained from all of participants with consent to participate.

Availability of data and materials
All of data and material are available.

Competing interest statement
The authors declare that they have no conflict of interest.

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Author contributions
ES, DC, and MO contributed to the conception and design of the study. ES, SO, MO, KI, MK and MA contributed to the acquisition of the data. ES, DC, SO, YK, SS, MO, and YY contributed to the analysis and/or interpretation of the data. ES, SO, DC and MO drafted the manuscript. YY, ET, and YI revised the
manuscript critically for important intellectual content. DC, SO, YK, SS, YY, MO, KI, MA, MK, ET, and YI approved the final version of the manuscript to be published. YI is the guarantor. The corresponding author attests that all listed authors meet the authorship criteria and that no other authors meeting the criteria have been omitted.

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**Figures**

**Figure 1**

Flow of participants enrollment in Iwaki Health promotion project.
Figure 2

Scatter plot among age, estradiol (A), and antimullerian hormone (B) levels.

Figure 3

ROC curve of antimullerian hormone for presence of early knee osteoarthritis.