Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

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

Eiji Sasaki (e_sasaki@hirosaki-u.ac.jp)
Hirosaki University https://orcid.org/0000-0002-4859-8473
Daisuke Chiba
Hirosaki University Graduate School of Medicine
Seiya Ota
Hirosaki University Graduate School of Medicine
Yuka Kimura
Hirosaki University Graduate School of Medicine
Shizuka Sasaki
Hirosaki University Graduate School of Medicine
Yuji Yamamoto
Hirosaki University Graduate School of Medicine
Maika Oishi
Hirosaki University Graduate School of Medicine
Kaori Iino
Hirosaki University Graduate School of Medicine
Masataka Ando
Hirosaki University Graduate School of Medicine
Mika Kumagai
Hirosaki University Graduate School of Medicine
Eiichi Tsuda
Hirosaki University Graduate School of Medicine
Yasuyuki Ishibashi
Hirosaki University Graduate School of Medicine

Research article

Keywords: Early knee osteoarthritis, estrogen, menopause, antimullerian hormone, inflammation, osteoporotic osteoarthritis

Posted Date: October 6th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-84980/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

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[1], and its radiographic prevalence among adults aged > 40 years in Japan was up to 42% in males and 62.4% in females[2]. Disadvantages of progressed KOA include high treatment costs, decreased productivity, and absence from work[3]; therefore, early diagnosis is crucial for early treatment and preventative interventions[4]. 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[5]. The major causal factor of EKOA and definitive KOA (DKOA) was sex[6,7]. The radiographic prevalence of knee OA in females were 1.2–2.8 times higher than that in males[5,8–12], and cartilage loss progressed more rapidly in females[13]. Estrogen deficiency after menopause is known to cause a reduction in bone mineral density (BMD)[14], and suppress cartilage and subchondral bone remodeling[15–16]. 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[17]. Few reports exist to investigate the relationship between menopausal transition and EKOA, which are observed among females ≥ 50 years old.

Both osteoporosis and KOA are attributed to menopause[18–19]. However, the possibility of osteoporosis causing the development of KOA is uncertain. Osteoporotic osteoarthritis is a particular type of OA[20], and both low and high bone mineral densities (BMDs) are associated with KOA[19]. More recent epidemiological studies revealed that knee pain in those with EKOA were related to bone fragility and bone marrow lesions (BMLs)[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[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 P□NP, 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 0and 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 $\geq$ 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/m^2)	22.0 ± 2.9	22.8 ± 3.5	23.8 ± 3.5*
Bone mineral density (g/cm^2)	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-Mullerian 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 odds 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			χ^2 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	0.78	8.28
Estradiol	0.636	0.399	-	0.873	1.27	0.260	20.00	0.15	0.56	7.17
Luteinizing hormone	0.677	0.487	-	0.866	3.33	0.068	10.27	0.28	0.78	9.13
Follicle-stimulating hormone	0.697	0.509	-	0.886	4.22	0.040	9.65	0.30	0.78	8.28
Prolactin	0.513	0.286	-	0.740	0.01	0.909	16.32	0.18	0.33	2.31
Testosterone	0.513	0.325	-	0.702	0.02	0.890	0.21	0.33	0.44	1.62

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%	10.9%
Definitive knee osteoarthritis	12.9%	10.0%	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	0.496	-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 P□NP	-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 P□NP	-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[7,26]. A systematic review estimated that the total odds ratio of females was up to 1.68 (95%CI: 1.37–2.07)[27]. Framingham study showed that the prevalence of OA in females was 36% and 1.2 times higher than that in males[11]. In Japan, the prevalence of OA in females were 1.5 to 2.8 times higher than males[5,8–9]; 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[28]. Further, representative therapy decreased the incidence of knee OA[29]. Although one systematic review concluded that there was no clear association between female hormonal aspects and knee OA[30], 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[31–32]. AHM decreases during menopausal transition, as previous reports found that the AMH reduces three years before menopause[17]. 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[19]. 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 90 s, several studies have reported that higher BMD is related to the incidence of KOA[33]. 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[21]. Furthermore, others also

reported a pathogenic mechanism since excessive bone resorption takes place in the early phase of OA[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[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[37]. Bone abnormalities in the early stage of KL are likely to occur in the middle-aged female population[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[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[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[23,42]; this predicts future joint space narrowing over 5 years[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[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[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 P□NP: 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.

Funding

This study was supported in part by JST COI Grant Number JPMJCE1302, a Grant-in-Aid from the Japanese Society for the Promotion of Science (Nos. 21500676, 18K16606, 18K09091), Health Labor Sciences Research Grant, JOA-Subsidized Science Project Research from the Japanese Orthopedic Association, and Grant of Japan Orthopaedics and Traumatology Research Foundation (No. 421).

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.

Acknowledgements

We would like to thank the all of stuffs helping us for conductiong Iwaki Health Promotion Project, and Editage (www.editage.jp) for English language editing.

References

1. Lawrence RC, Helmick CG, Arnett FC, Deyo RA, Felson DT, Giannini EH, et al. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. Arthritis Rheum. 1998;41:778-99.
2. Yoshimura N, Muraki S, Oka H, Mabuchi A, En-Yo Y, Yoshida M, et al. Prevalence of knee osteoarthritis, lumbar spondylosis, and osteoporosis in Japanese men and women: the research on osteoarthritis/osteoporosis against disability study. J Bone Miner Metab. 2009;27:620-8.
3. Hiligsmann M, Cooper C, Arden N, Boers M, Branco JC, Luisa Brandi M, et al. Health economics in the field of osteoarthritis: an expert's consensus paper from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). Semin Arthritis Rheum. 2013;43:303-13.
4. Luyten FP, Bierma-Zeinstra S, Dell'Accio F, Kraus VB, Nakata K, et al. Toward classification criteria for early osteoarthritis of the knee. Semin Arthritis Rheum. 2018;47:457-63.
5. Sasaki E, Ota S, Chiba D, Kimura Y, Sasaki S, Yamamoto Y, et al. Early knee osteoarthritis prevalence is highest among middle-aged adult females with obesity based on new set of diagnostic criteria from a large sample cohort study in the Japanese general population. Knee Surg Sports Traumatol Arthrosc. 2020;28:984-94.
6. Nevitt MC, Felson DT. Sex hormones and the risk of osteoarthritis in women: epidemiological evidence. Ann Rheum Dis. 1996;55:673-6.
7. Cimmino MA, Parodi M. Risk factors for osteoarthritis. Semin Arthritis Rheum. 2005;34:29-34.
8. Muraki S, Oka H, Akune T, Mabuchi A, En-yo Y, Yoshida M, et al. Prevalence of radiographic knee osteoarthritis and its association with knee pain in the elderly of Japanese population-based cohorts: the ROAD study. Osteoarthritis Cartilage. 2009;17:1137-43.
9. Omori G, Narumi K, Nishino K, Nawata A, Watanabe H, Tanaka M, et al. Association of mechanical factors with medial knee osteoarthritis: A cross-sectional study from Matsudai Knee Osteoarthritis Survey. J Orthop Sci. 2016;21:463-8.
10. KELLGREN JH, LAWRENCE JS. Osteo-arthrosis and disk degeneration in an urban population. Ann Rheum Dis. 1958;17:388-97.

11. Felson DT. The epidemiology of knee osteoarthritis: results from the Framingham Osteoarthritis Study. Semin Arthritis Rheum. 1990;20:42-50.
12. Zhang Y, Xu L, Nevitt MC, Aliabadi P, Yu W, Qin M, et al. Comparison of the prevalence of knee osteoarthritis between the elderly Chinese population in Beijing and whites in the United States: The Beijing Osteoarthritis Study. Arthritis Rheum. 2001;44:2065-71.
13. Hanna FS, Teichtahl AJ, Wluka AE, Wang Y, Urquhart DM, English DR, et al. Women have increased rates of cartilage loss and progression of cartilage defects at the knee than men: a gender study of adults without clinical knee osteoarthritis. Menopause. 2009;16:666-70.
14. Black DM, Rosen CJ. Postmenopausal Osteoporosis. N Engl J Med. 2016;374:2096-7.
15. Xu X, Li X, Liang Y, Ou Y, Huang J, Xiong J, et al. Estrogen modulates cartilage and subchondral bone remodeling in an ovariectomized rat model of postmenopausal osteoarthritis. Med Sci Monit. 2019;25:3146-53.
16. Richette P, Corvol M, Bardin T. Estrogens, cartilage, and osteoarthritis. Joint Bone Spine. 2003;70:257-62.
17. Iino K, Tarakida A, Abe K, Taniguchi R, Higuchi T, Takahashi I, et al. Role of antimüllerian hormone as a biomarker of the menopausal transition. Menopause. 2013;20:218-22.
18. Zheng W, Ding B, Li X, Liu D, Yokota H, Zhang P. Knee loading repairs osteoporotic osteoarthritis by relieving abnormal remodeling of subchondral bone via Wnt/β-catenin signaling. FASEB J. 2020;34:3399-412.
19. Dequeker J, Aerssens J, Luyten FP. Osteoarthritis and osteoporosis: clinical and research evidence of inverse relationship. Aging Clin Exp Res. 2003;15:426-39.
20. Im GI, Kim MK. The relationship between osteoarthritis and osteoporosis. J Bone Miner Metab. 2014;32:101-9.
21. Ota S, Chiba D, Sasaki E, Kumagai G, Yamamoto Y, Nakaji S, et al. Symptomatic bone marrow lesions induced by reduced bone mineral density in middle-aged women: a cross-sectional Japanese population study. Arthritis Res Ther 2019;21:113
22. Chiba D, Sasaki E, Ota S, Maeda S, Sugiyama D, Nakaji S, et al. US detection of medial meniscus extrusion can predict the risk of developing radiographic knee osteoarthritis: a 5-year cohort study. Eur Radiol. 2020;30:3996-4004.
23. Inoue R, Ishibashi Y, Tsuda E, Yamamoto Y, Matsuzaka M, Takahashi I, et al. Knee osteoarthritis, knee joint pain and aging in relation to increasing serum hyaluronan level in the Japanese population. Osteoarthritis Cartilage. 2011;19:51-7.
24. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis. 1957;16:494–502.
25. Roos EM, Roos HP, Lohmander LS, Ekdahl C, Beynnon BD. Knee Injury and Osteoarthritis Outcome Score (KOOS)--development of a self-administered outcome measure. J Orthop Sports Phys Ther. 1998;28:88–96.

26. Lawrence RC, Felson DT, Helmick CG, Arnold LM, Choi H, Deyo RA, et al; National Arthritis Data Workgroup. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. Arthritis Rheum. 2008;58:26-35.
27. Silverwood V, Blagojevic-Bucknall M, Jinks C, Jordan JL, Protheroe J, Jordan KP. Current evidence on risk factors for knee osteoarthritis in older adults: a systematic review and meta-analysis. Osteoarthritis Cartilage. 2015;23:507-15.
28. Szoeke CEI, Cicuttini FM, Guthrie JR, Clark MS, Dennerstein L. Factors affecting the prevalence of osteoarthritis in healthy middle-aged women: data from the longitudinal Melbourne Women's Midlife Health Project. Bone. 2006;39:1149-55.
29. Jung JH, Bang CH, Song GG, Kim C, Kim JH, Choi SJ. Knee osteoarthritis and menopausal hormone therapy in postmenopausal women: a nationwide cross-sectional study. Menopause. 2018;26:598-602.
30. de Klerk BM, Schiphof D, Groeneveld FP, Koes BW, van Osch GJ, van Meurs JB, et al. No clear association between female hormonal aspects and osteoarthritis of the hand, hip and knee: a systematic review. Rheumatology (Oxford). 2009;48:1160-5.
31. Cate RL, Mattaliano RJ, Hession C, Tizard R, Farber NM, Cheung A, et al. Isolation of the bovine and human genes for Müllerian inhibiting substance and expression of the human gene in animal cells. Cell. 1986;45:685-98.
32. de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC. Antimüllerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril. 2002;77:357-62.
33. Burger H, van Daele PL, Odding E, Valkenburg HA, Hofman A, Grobbee DE, et al. Association of radiographically evident osteoarthritis with higher bone mineral density and increased bone loss with age. The Rotterdam Study. Arthritis Rheum. 1996;39:81-6.
34. Bultink IE, Lems WF. Osteoarthritis and osteoporosis: what is the overlap? Curr Rheumatol Rep. 2013;15:328.
35. Bijlsma JW, Berenbaum F, Lafeber FP. Osteoarthritis: an update with relevance for clinical practice. Lancet. 2011;377:2115-26.
36. Guermazi A, Niu J, Hayashi D, Roemer FW, Englund M, Neogi T, et al. Prevalence of abnormalities in knees detected by MRI in adults without knee osteoarthritis: population based observational study (Framingham Osteoarthritis Study). BMJ. 2012;345:e5339.
37. van Oudenaarde K, Jobke B, Oostveen AC, Marijnissen AC, Wolterbeek R, Wesseling J, et al. Predictive value of MRI features for development of radiographic osteoarthritis in a cohort of participants with pre-radiographic knee osteoarthritis-the CHECK study. Rheumatology (Oxford). 2017;56:113-20.
38. Sellam J, Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. Nat Rev Rheumatol. 2010;6:625-35.
39. Herrero-Beaumont G, Pérez-Baos S, Sánchez-Pernaute O, Roman-Blas JA, Lamuedra A, Largo R. Targeting chronic innate inflammatory pathways, the main road to prevention of osteoarthritis progression. Biochem Pharmacol. 2019;165:24-32.

40. Baker K, Grainger A, Niu J, Clancy M, Guermazi A, Crema M, et al. Relation of synovitis to knee pain using contrast-enhanced MRIs. Ann Rheum Dis. 2010;69:1779-83.
41. Xie C, Chen Q. Adipokines: new therapeutic target for osteoarthritis? Curr Rheumatol Rep. 2019;21:71.
42. Ishijima M, Watari T, Naito K, Kaneko H, Futami I, Yoshimura-Ishida K, et al. Relationships between biomarkers of cartilage, bone, synovial metabolism and knee pain provide insights into the origins of pain in early knee osteoarthritis. Arthritis Res Ther. 2011;13:R22.
43. Sasaki E, Tsuda E, Yamamoto Y, Maeda S, Inoue R, Chiba D, et al. Serum hyaluronic acid concentration predicts the progression of joint space narrowing in normal knees and established knee osteoarthritis - a five-year prospective cohort study. Arthritis Res Ther. 201;17:283.
44. Arendt EA, Berruto M, Filardo G, Ronga M, Zaffagnini S, Farr J, et al. Early osteoarthritis of the patellofemoral joint. Knee Surg Sports Traumatol Arthrosc. 2016;24:1836–44
45. Iijima H, Fukutani N, Aoyama T, Fukumoto T, Uritani D, Kaneda E, et al. Clinical impact of coexisting patellofemoral osteoarthritis in Japanese patients with medial knee osteoarthritis. Arthritis Care Res (Hoboken). 2016;68:493–501

Figures

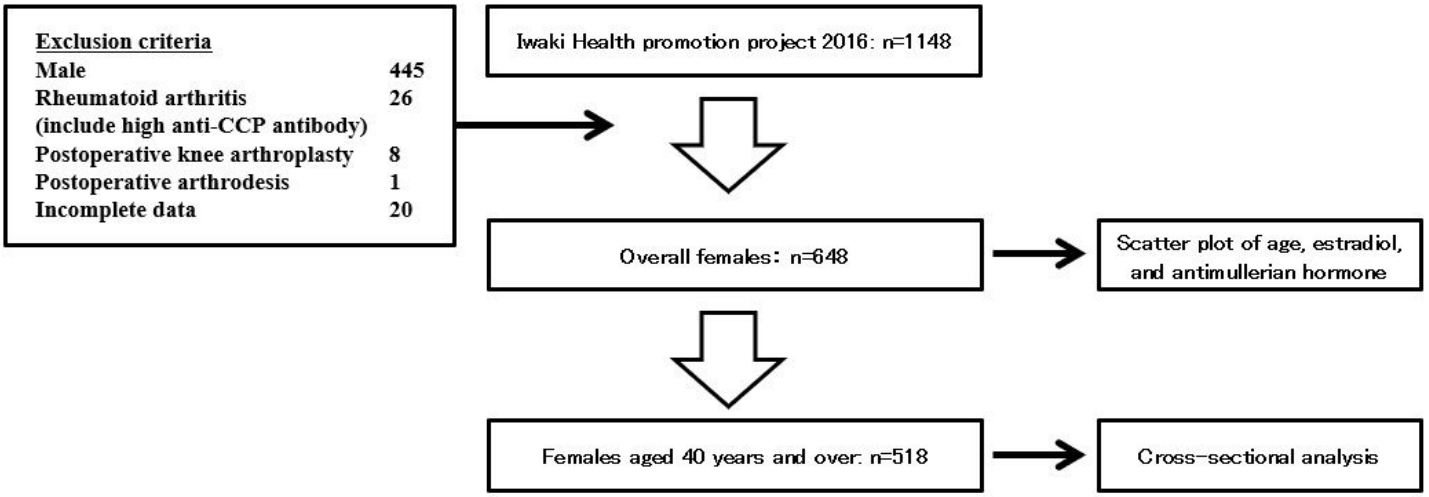

Figure 1

Flow of participants enrollment in Iwaki Health promotion project.

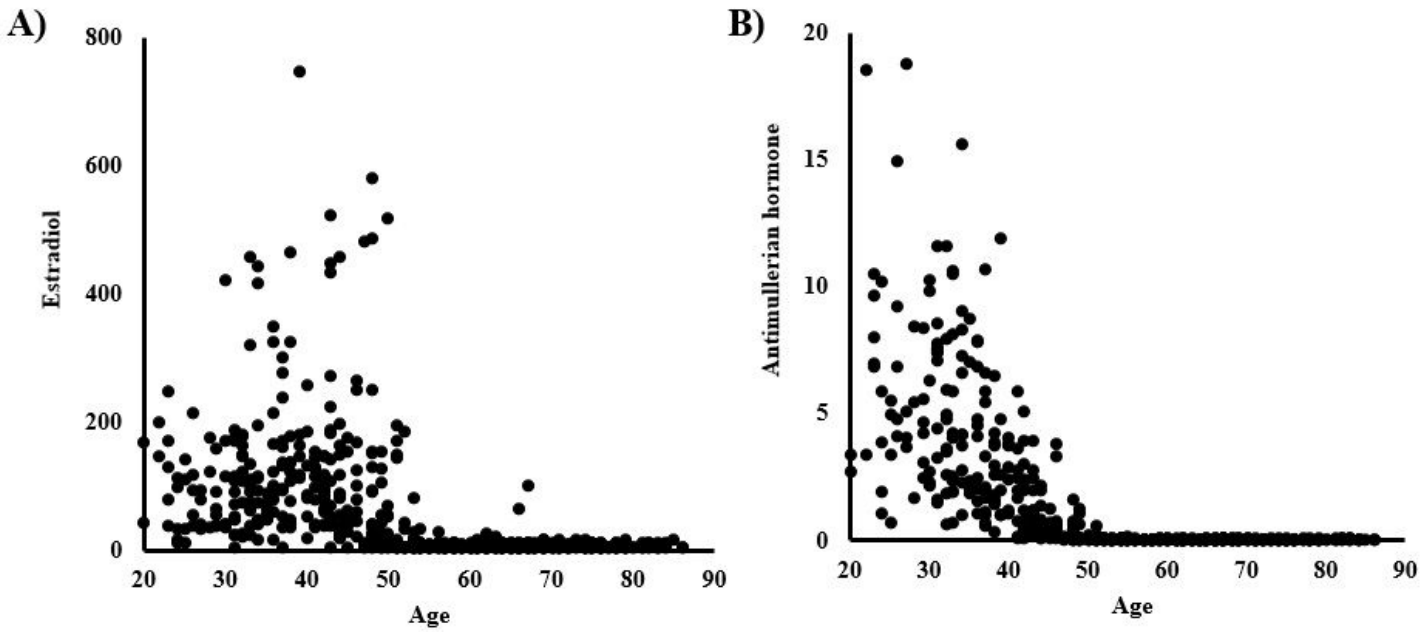

Figure 2

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

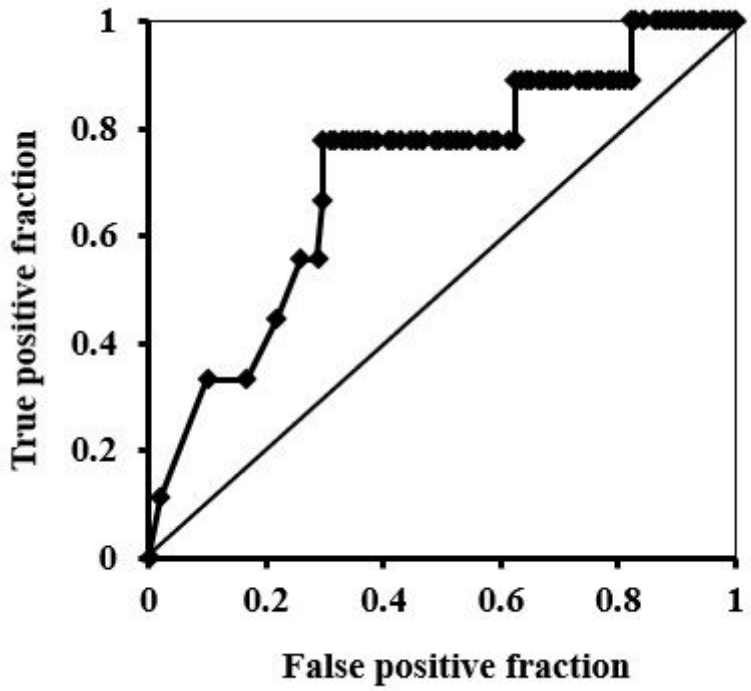

Figure 3

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