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Failure of serum transforming growth factor-beta (TGF-β1) as a biomarker of radiographic osteoarthritis at the knee and hip: A cross-sectional analysis in the Johnston County Osteoarthritis Project

Amanda E. Nelson, M.D.[1], **Fang Fang, M.S.**[2], **Xiaoyan Amy Shi, M.S.**[3], **Virginia B. Kraus, M.D., PhD.**[4], **Thomas Stabler, M.S.**[4], **Jordan B. Renner, M.D.**[1,5], **Todd A. Schwartz, DrPH.**[1,3], **Charles G. Helmick, M.D.**[6], and **Joanne M. Jordan, M.D., M.P.H.**[1]

[1] Thurston Arthritis Research Center, University of North Carolina School of Medicine, Chapel Hill, NC

[2] StatWorks, Inc., RTP, NC

[3] University of North Carolina School of Public Health, Department of Biostatistics, Chapel Hill, NC

[4] Duke University Medical Center, Durham, NC

[5] University of North Carolina, Department of Radiology, Chapel Hill, NC

[6] Centers for Disease Control and Prevention, Atlanta, GA

Abstract

PURPOSE—To assess associations between serum TGF-β1 and radiographic knee and hip osteoarthritis (rOA) in African American (AA) and White men and women.

METHODS—Baseline data from 330 participants in the Johnston County Osteoarthritis Project were used in the analysis. Radiographs were scored with the Kellgren-Lawrence scale and rOA defined as grade ≥ 2. Individual radiographic features (IRFs) were rated 0–3. TGF-β1 was measured using a sandwich ELISA. General linear models were used to estimate associations between lnTGF-β1 and rOA presence, laterality or severity, and IRF presence and severity, adjusting for age, gender, race, and body mass index. Interactions by race and gender were considered significant at $p < 0.1$.

RESULTS—Mean lnTGF-β1 levels were higher among AAs compared to Whites, and among women compared to men ($p<0.009$). Mean lnTGF-β1 levels were higher in those with knee OST, but this association was not significant after adjustment. There were no other significant differences in mean lnTGF-β1 levels by presence, laterality, or severity of knee or hip rOA or IRFs. No race or gender interactions were identified, although a borderline significant association between lnTGF-β1 and knee OST was seen among AAs ($p < 0.06$).

Corresponding Author: Amanda E. Nelson, M.D., Thurston Arthritis Research Center, University of North Carolina School of Medicine, 3300 Thurston Building, Campus Box #7280, Chapel Hill, NC 27599-7280, Phone (919)966-4191, FAX (919)843-7231, Email: AENelson@unch.unc.edu.

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CONCLUSIONS—Although serum TGF-β1 varied by race and gender and several rOA variables, there were no independent significant associations with presence, laterality, or severity of knee or hip rOA by K-L grade or IRFs, suggesting that serum TGF-β1 is unlikely to be useful as a stand-alone biomarker in OA studies. A possible association between TGF-β1 and OST in AAs cannot be excluded.

Keywords

Biomarkers; Transforming growth factor-beta (TGF-β1); Radiography

Introduction

Osteoarthritis (OA) is the most common type of arthritis, affecting an estimated 27 million adults aged 25 years and older (1). Plain radiographs are the standard method for assessing OA presence, severity, and progression. These can be scored either for individual radiographic features (IRFs) such as osteophytes (OST), joint space narrowing (JSN), subchondral sclerosis, and subchondral cysts, or by using a global scale that accounts for these features, such as the Kellgren-Lawrence (K-L) grade (2). We have recently identified differences by race in IRFs of OA at the knee and hip, including variations in JSN and increased OST at these joint sites in AA individuals compared to Whites (3;4). However, plain radiographs have several drawbacks in OA assessment, including insensitivity to change for short term follow up and lack of tight association with patient outcomes such as pain and disability. Biomarkers represent a potentially more sensitive way to evaluate and follow OA patients for the purposes of clinical research and therapeutic trials. Serum and urine biomarkers provide a rapid and relatively non-invasive method of repeated assessment over time, and may be more responsive to therapeutic interventions than the longer-term changes seen on radiographs (5;6).

TGF-β holds promise as a potential serum biomarker of OA. The TGF-β family consists of three closely related isoforms, TGF-β1, -β2, and -β3, with TGF-β1 being the most abundant and most often studied in relation to bone and OA (7). TGF-β1 is a multifunctional growth factor with widespread effects on multiple tissues, including an important role in cartilage matrix metabolism. Elevated TGF-β1 levels have been identified in the synovial fluid of patients with OA (8), and levels after meniscectomy are reflective of future bone and cartilage changes (9). Local administration of TGF-β1 into murine knee joints induces changes consistent with OA, including OST formation (10), while inhibition of endogenous TGF-β (all isoforms) decreases OST size and enhances proteoglycan loss (11). In one of the few studies of TGF-β1 to include AAs, serum levels among patients with end stage kidney disease were higher in AAs with compared to Whites, with no difference by race among healthy controls (12). Serum TGF-β1 has been used as a potential biomarker of OA in a few studies, with varying results (13–15).

We hypothesized that the increased osteophytosis previously observed in AAs compared to Whites may be associated with elevated serum TGF-β1 levels in AAs, and with increased prevalence and/or severity of radiographic osteoarthritis (rOA) in these individuals. Therefore, the current study was designed to test associations of serum TGF-β1 with rOA (both by K-L grade and IRFs) at the hip and knee among participants in the Johnston County Osteoarthritis Project, a community-based cohort of individuals both with and without OA.

Methods

Baseline data from 330 participants in the Johnston County Osteoarthritis Project were used in the analysis. Participants for biomarker assessment were selected to represent approximately equal proportions of AAs, Whites, women, and men, with and without OA, representing a

range of ages, with complete radiographic data at baseline and a 5-year follow up visit. Subject recruitment and overall project design procedures have been described for the parent study (16). In brief, all included participants underwent bilateral weight-bearing anteroposterior radiography of the knees in extension and supine anteroposterior pelvic radiography. Women under 50 years of age were not subjected to pelvic radiography and were excluded from analyses of hip rOA. Radiographs were read without knowledge of participant clinical status by a single radiologist (JBR) using the K-L radiographic atlas (2); inter- and intra-rater reliability for this reader are high (κ=0.86 and 0.89, respectively) (17). Knee rOA was defined as a K-L grade $\geq$ 2 in at least one knee, and laterality as none, unilateral, or bilateral involvement of the knees; hip rOA was similarly defined. Radiographic severity was defined for knees and for hips as none for K-L grades of 0 or 1, mild for K-L grade equal to 2, and moderate/severe for K-L grades of 3 or 4, with the overall grade for an individual defined by the higher grade for each joint site. Results were comparable using a 4-level variable with K-L 1 as a separate category, so the combination of K-L 0 and 1 was defined as "none" in analyses. For IRFs at the knee, both JSN and OST were evaluated as absent or present in either knee; severity was defined at the most affected knee according to the Burnett atlas (0=none, 1=mild, 2,3=moderate/severe); IRFs at the hip were similarly defined (18). After collection at the baseline clinic visit, blood and sera were separated and stored on ice. Sera were frozen within 8 hours of collection at −20°C, then transferred for long-term storage to a −86°C environment. TGF-β1 was measured using a sandwich ELISA kit from Biosource International (Camarillo, CA). This method includes an extraction step to release TGF-β1 from latent complexes, thus measuring total serum concentrations. Manufacturer reported precision was 5.5–6.2% intra-assay, and 6.6–7.9% inter-assay.

The natural logarithm transformation was used to produce near-normal distributions for TGF-β1 (lnTGF-β1) in analyses. Descriptive statistics were calculated for demographic and clinical variables (race, gender, age, and body mass index (BMI)), knee and hip rOA, and lnTGF-β1; differences were assessed by two-sample t-tests. General linear models were used separately for knee and hip rOA to obtain adjusted (least squares means) estimates for lnTGF-β1 and rOA presence, laterality, and severity as well as the presence and severity of IRFs. Models were adjusted for age, gender, self-reported race, and BMI. Interactions by race and gender were assessed and considered significant at a p-value < 0.1. Stratified analysis was performed using general linear models to assess differences in IRFs by race. All analyses were performed using SAS version 9.1 (Cary, NC).

Results

The mean age (±SD) of the sample (n=330) was 60.5 ± 9.4 years, with a mean BMI of 30.3 ± 6.9 kg/m^2; 42% were AA and 39% were men. Fifty-four percent of participants had knee rOA and 22% had hip rOA. The overall median serum TGF-β1 was 16.75 ng/mL (range 6.1–40.9ng/mL); mean serum lnTGF-β1 was 2.82 ± 0.34 ng/mL. Mean serum lnTGF-β1 was higher among AA participants compared to Whites (p=0.009), and among women compared to men (p=0.001, Table 1). There were no significant associations between lnTGF-β1 levels and age or BMI (data not shown). No significant differences were identified for mean serum lnTGF-β1 levels by the presence of rOA status at the knee or the hip in unadjusted analyses (Table 1).

Compared to those without hip rOA, lnTGF-β1 levels were higher in those with bilateral hip involvement, but these differences were not statistically significant before or after adjustment (p >0.5; data not shown). Small numbers in more severe categories precluded meaningful analysis of hip rOA by severity. There were no associations between lnTGF-β1 levels and unilateral or bilateral knee rOA (all p values >0.3; data not shown). Compared to those without knee rOA, mean lnTGF-β1 was higher in moderate/severe knee rOA (p=0.080); this association

was attenuated after adjustment (p=0.188, Figure 1). There was no evidence for a linear trend between increasing lnTGF-β1 and increasing knee rOA severity.

Mean serum lnTGF-β1 levels by IRFs of OA at the knee and hip are shown in Table 3. There were no significant associations between serum lnTGF-β1 levels and presence of JSN or OST at the hip. There were no associations between mean lnTGF-β1 and the presence or severity of JSN at the knee (Figure 2). Significant associations between increasing lnTGF-β1 levels and knee OST presence and severity in unadjusted analyses (p=0.034 and 0.030, respectively) were attenuated after adjustment (p=0.136 for both, Figure 2).

There were no significant interactions between lnTGF-β1 and race or gender for rOA outcomes, either by K-L grade or IRFs. Nonetheless, stratified analysis by race was carried out for IRFs at the knee, given our a priori hypothesis regarding the possibility of racial differences. This analysis again showed no association between JSN presence or severity at the knee in AA or White individuals (data not shown). However, there was a borderline significant association between elevated lnTGF-β1 and OST presence among AA individuals (mean lnTGF-β1 for no OST: 2.77 (95% CI 2.65–2.88), for OST present: 2.90 (2.83–2.97), p=0.056 after adjustment for age, gender, and BMI) that was not seen among Whites (no OST mean lnTGF-β1: 2.76 (2.69–2.83), OST present: 2.79 (2.72–2.86), p=0.613). Similarly for OST severity at the knee, a borderline significant association was observed among AAs for increasing lnTGF-β1 levels with mild and moderate/severe OST, compared to none (figure 3); there was no association between OST severity and lnTGF-β1 levels among Whites.

Discussion

In our population of AA and White individuals with and without hip and knee rOA, serum TGF-β1 levels did not differentiate OA from non-OA states, and did not vary significantly by severity or unilateral vs. bilateral involvement. After adjustment for age, BMI, gender, and race, there were no associations between serum TGF-β1 levels and JSN or OST at the knee or the hip. Despite higher serum TGF-β1 levels among AAs and women, no race or gender interactions were seen for OA outcomes, indicating no differences in the association of TGF-β1 levels and OA in these subgroups. However, there was a suggestion of an association between OST and lnTGF-β1 among African Americans only, in a stratified analysis.

Our results are in agreement with a prior study by Otterness et al, who found that serum TGF-β1 did not discriminate between subjects with and without hip or knee OA defined using American College of Rheumatology radiographic criteria (13). A study of the same biomarkers using clinical outcomes identified only a borderline significant correlation between serum TGF-β1 and change in clinical status over a year, a category including such variables as physician and patient assessments of change in disease status, and changes in stiffness and pain. The number of subjects in the study was small (n=39), as was the r^2 for the identified correlation (r^2=0.17, p=0.05) (14). A larger study (n=193) in a cohort undergoing a diet and exercise intervention (15) found a negative correlation between baseline serum TGF-β1 and baseline K-L grade at the knee (r=−0.19, p=0.01), but no association with follow-up outcomes including clinical, functional, or radiographic measures, or with change in the biomarker level and change in outcome measures over 18 months (15).

There are several limitations to the use of systemic biomarkers in OA, including often incomplete assessment of potential confounders (race, gender, medical comorbidities) and the effect of joint sites other than those under study. In the current study, we had radiographic information only for the hip and the knee, and we had a small sample size for more severe outcome levels. Finally, we focused on a single marker at a single time point and so cannot

specifically comment on the potential of TGF-β1 as a marker of progression. Baseline TGF-β as a predictor of incident or progressive knee rOA will be the subject of future study.

This study has significant strengths. It is the largest study of TGF-β1 in OA to date and includes African American and White men and women and radiographic data from two large joint sites. We identified higher levels of TGF-β1 in AAs and women. Despite the relatively large size of our sample, given the lack of apparent independent significant associations between serum TGF-β1 and rOA presence, severity, or laterality, or JSN or OST, it seems unlikely that this biomarker will be useful as a stand-alone biomarker in future OA studies. However, the potential importance of TGF-β1 among AA individuals warrants further investigation.

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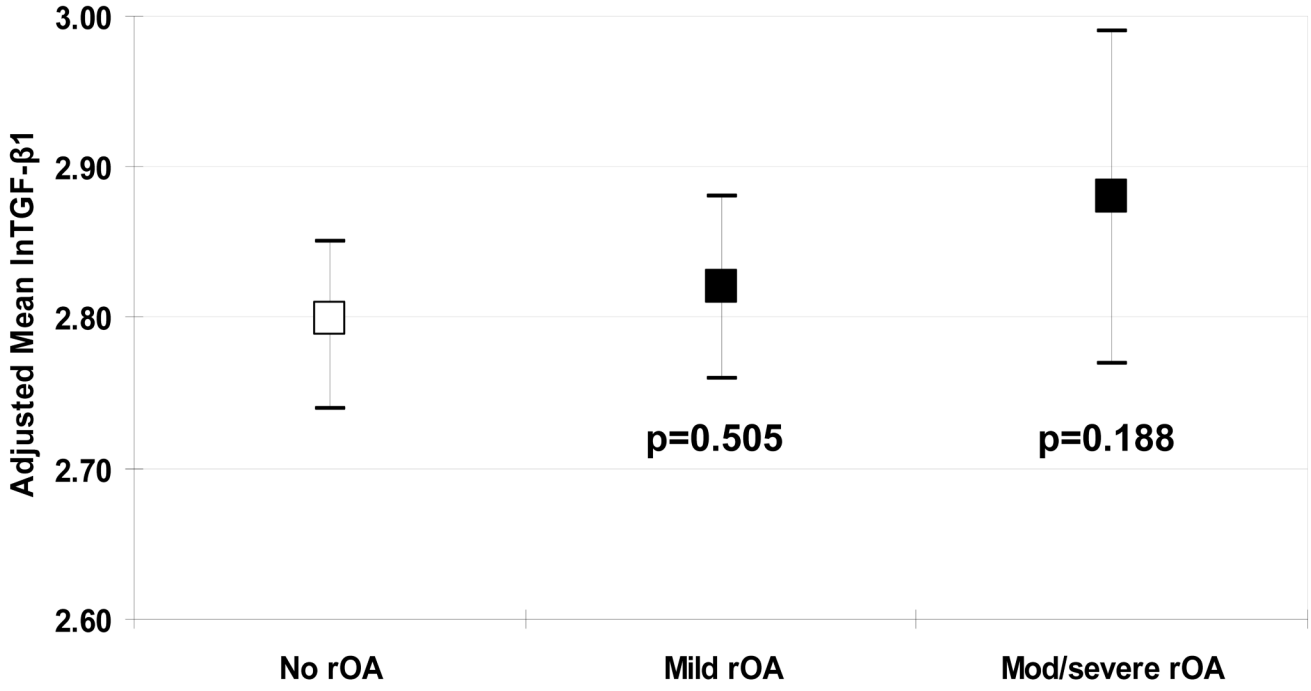

Figure 1.
Adjusted Mean lnTGF-β1 by Knee rOA Severity
The open box is no rOA, all p values given are compared to no rOA.

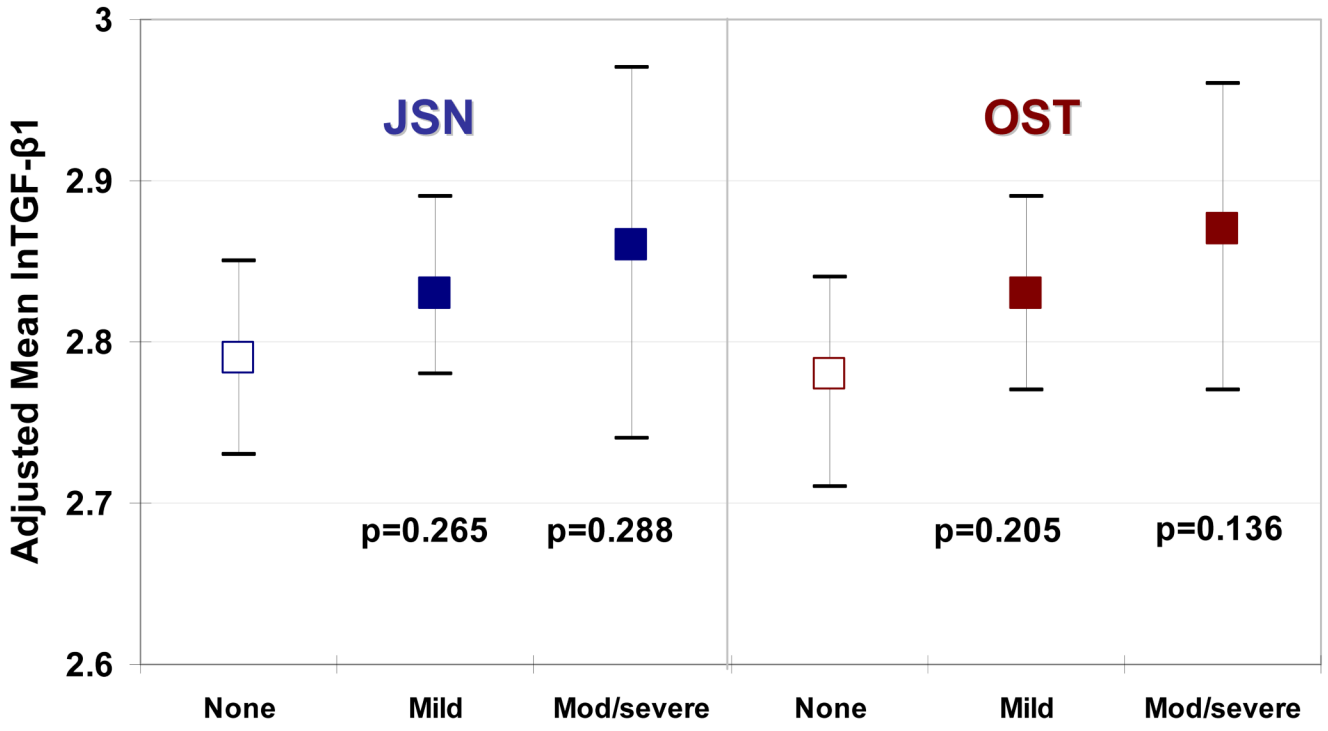

Figure 2.
Adjusted Mean lnTGF-β1 by Knee JSN and OST.
The left side of the figure shows mean lnTGF-β1 by Knee JSN, with no JSN as the referent; p values are compared to no JSN. The right side of the figure shows mean lnTGF-β1 by Knee OST, with no OST as the referent; p values are compared to no OST.

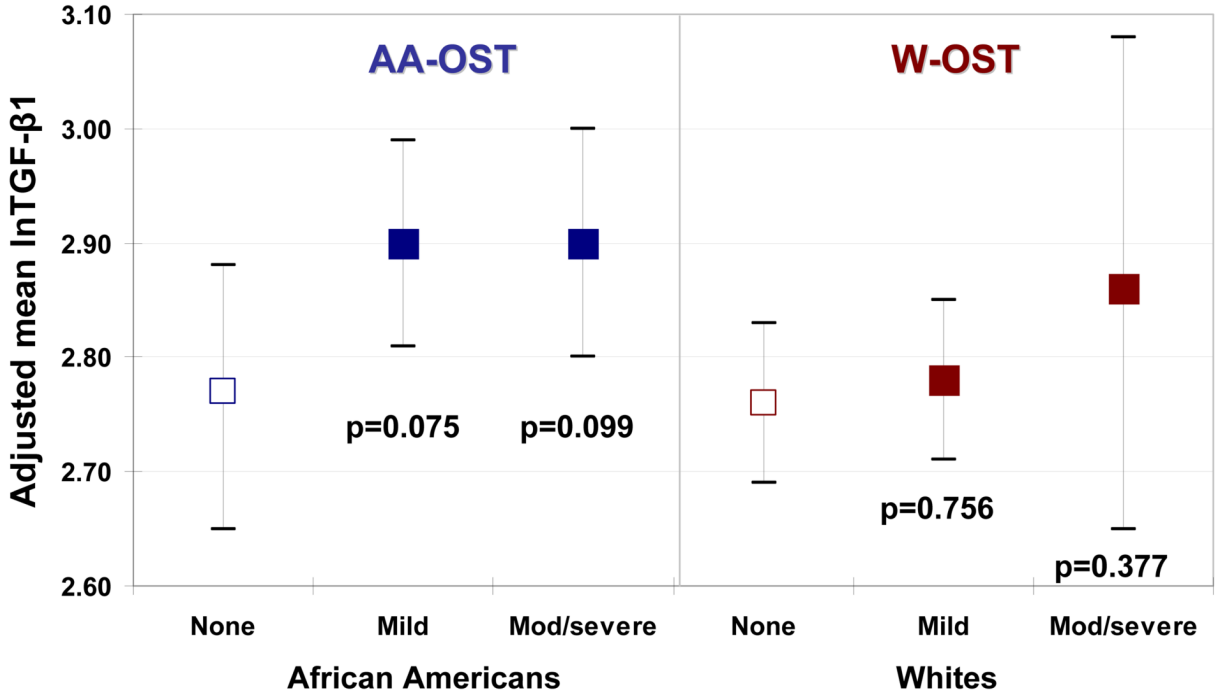

Figure 3.

Adjusted Mean lnTGF-β1 by OST severity, stratified by race.

The left side of the figure shows mean lnTGF-β1 by Knee OST among African Americans, while the right side of the figure shows lnTGF-β1 by Knee OST among Whites. No OST is the referent, all p values are compared to no OST.

Table 1

Serum TGF-β by race, gender, and rOA* status

	n	Serum TGF-β, median (range), ng/mL	Unadjusted Mean lnTGF-β (95% CI)	p value†
Race				
White	190	15.80 (6.10–40.80)	2.78 (2.73–2.83)	**0.009**‡
AA	140	18.35 (7.00–40.90)	2.88 (2.82–2.93)	
Gender				
Men	130	15.20 (6.10–37.00)	2.74 (2.69–2.80)	**0.001**‡
Women	200	18.30 (6.20–40.90)	2.87 (2.82–2.92)	
Knee rOA				
Absent	152	16.35 (6.10–40.80)	2.79 (2.74–2.85)	0.185
Present	178	17.30 (6.20–40.90)	2.84 (2.79–2.89)	
Hip rOA §				
Absent	241	16.50 (6.10–40.90)	2.80 (2.76–2.85)	0.713
Present	68	16.35 (8.30–34.20)	2.82 (2.74–2.90)	

*rOA defined as K-L grade ≥ 2 at the knee or hip

†for differences in unadjusted mean lnTGF-β

‡significant in adjusted models containing age, BMI, race, gender; p=0.016 for race and p= 0.001 for gender

§For hip analyses, n=309 due to exclusion of women under 50 years of age

Table 2

Unadjusted and adjusted* mean lnTGF-β1 by individual radiographic features of OA

	n†	Unadjusted	95% CI	p value‡	Adjusted*	95% CI	p value‡
Hip JSN							
Absent	226	2.81	2.76–2.85	0.937	2.80	2.78–2.85	0.857
Present	80	2.81	2.74–2.88		2.81	2.74–2.89	
Hip OST							
Absent	155	2.79	2.74–2.84	0.296	2.80	2.74–2.85	0.608
Present	149	2.83	2.78–2.88		2.82	2.76–2.87	
Knee JSN							
Absent	140	2.78	2.73–2.84	0.093	2.79	2.73–2.85	0.217
Present	189	2.85	2.80–2.90		2.84	2.79–2.89	
Knee OST							
Absent	126	2.77	2.71–2.83	**0.034**	2.78	2.72–2.84	0.136
Present	200	2.85	2.80–2.90		2.84	2.79–2.89	

*adjusted for age, BMI, race, and gender

†n=306 for hip JSN, 304 for hip OST, 329 for knee JSN, and 326 for knee OST

‡p value compared to absent or none