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ARAŞTIRMA / RESEARCH

Association between synovial fluid prostanoid levels and ultrasonographic findings in knee osteoarthritis

Diz osteoartriti bulunan hastalarda sinoviyal sıvılarındaki prostanoid düzeyleri ve ultrasonografik bulgular arasındaki ilişki

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Abstract

Purpose. The aim of this study was to investigate the association between prostanoid [thromboxane (TxA2) and prostacyclin (PGI2)] levels in synovial fluid and infrapatellar fat pad (IPFP)/ suprapatellar fat pad (SPFP) thickness as well as other clinical findings in knee steoarthritis (OA) patients.
Materials and Methods: 16 patients with knee OA with effusion were included. The average pain levels were evaluated using a Visual Analogue Scale (VAS). The pain, stiffness, and physical functions were evaluated using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). The levels of 6-keto-PGF1α, stable metabolite of PGI2, and TxB2, stable metabolite of TxA2, were measured in synovial fluid using an enzyme immunoassay.
Results: 6-keto-PGF1α levels were significantly greater than TxB2 levels in synovial fluid of patients with OA. TxB2 levels and TxB2/6-keto- PGF1α ratio were negatively correlated with SPFP and cartilage thickness, respectively. IPFP thickness was positively correlated with SPFP thickness and effusion volume. WOMAC scores were positively correlated with VAS-activity and VAC-evening scores.
Conclusion: In comparison to 6-keto- PGF1α levels in synovial fluid, TxB2 levels seem to be more associated with ultrasonographic findings in patients with OA.
Keywords:. Prostacyclin, thromboxane A2, fat pads, cartilage, knee osteoarthritis

Öz

Amaç: Bu çalışmada, diz osteoartriti olan hastaların sinoviyal sıvılarındaki prostanoidlerin [tromboksan (TxA2) ve prostasiklin (PGI2)] düzeyi ile infrapatellar ve suprapatellar yağ yastığı kalınlığı ve diğer klinik parametreler arasındaki ilişkinin değerlendirilmesi amaçlanmıştır.
Gereç ve Yöntem: Çalışmaya efüzyonlu diz osteoartriti bulunan 16 hasta dahil edilmiştir. Ağrı düzeyleri görsel analog skala (VAS) ile değerlendirilmiştir. Ağrı, sertlik ve fiziksel fonksiyon osteoartrit indeksi (WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index) ile değerlendirilmiştir. PGI2'nin kararlı metaboliti olan 6-keto-PGF1α ve TxA2'nin kararlı metaboliti olan TxB2 düzeyleri enzim immunoassay yöntemi ile ölçülmüştür.
Bulgular: Sinoviyal sıvıda ölçülen 6-keto-PGF1α düzeyleri TxB2 düzeylerine göre anlamlı derecede yüksek bulunmuştur. TxB2 düzeyleri ile suprapatellar yağ yastığı kalınlığı ve TxB2/6-keto- PGF1α oranı ile kıkırdak kalınlığı arasında negatif korelasyon bulunmaktadır. Infrapatellar yağ yastığı kalınlığı ile suprapatellar yağ yastığı kalınlığı ve efüzyon hacmi arasında pozitif korelasyon bulunmaktadır. WOMAC değerleri ile VAS-aktivite ve VAS-gece skorları arasında pozitif korelasyon bulunmaktadır.
Sonuç: 6-keto-PGF1α düzeylerine kıyasla, sinoviyal sıvı TxB2 düzeyleri osteoartritli hastalardaki ultrasonografik bulgular ile daha çok ilişkili bulunmuştur.
Anahtar kelimeler: Prostasiklin, tromboksan A2, yağ yastığı, kıkırdak, diz osteoartriti

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INTRODUCTION

Osteoarthritis (OA) is characterized by cartilage degeneration, destruction, hyperplasia, and synovitis of the articular cartilage[1]. The knee is an important target site for OA. Although several studies were performed in order to investigate the mechanism underlying knee OA, the pathogenesis of this disease remains unclear[2]. Current treatments are used for relieving the pain and functional restriction however there were no effective pharmacological treatments to modify the course of the disease[3].

Obesity is an important risk factor for both the incidence and progression of OA[4]. In earlier, the effect of obesity on OA was thought to be associated with mechanical overload. However, this hypothesis cannot explain the increased risk of OA in non-weight bearing joints (such as shoulder or hand) among obese patients [2,4,5]. Furthermore, loss of body fat but not body weight produced symptomatic relief of knee OA[6]. A metabolic link between obesity and OA has been also suggested and this link can be associated with the mediators released from adipose tissue. Adipose tissue acts as an active endocrine organ[2] and dysregulation of several mediators released from adipose tissue in obesity-related disease could be involved in the OA development[5]. The enhanced prevalence of obesity worldwide increases the importance of elucidating the relationship between obesity and OA[7]. In addition, novel therapeutic approaches will emerge for the treatment and prevention of OA by fully revealing the effect of obesity on the development of OA. For this purpose, recent studies have investigated on the role of local adipose tissues in the knee joint in the pathogenesis of OA[8]. Infrapatellar fat pad (IPFP) and suprapatellar fat pad (SPFP) are the main adipose tissues located in the knee joint consist of adipocyte, immune cells, vessels and, nerve fibers. These adipose tissues can interact with other tissues around them by releasing several mediators. In parallel, they can influence joint homeostasis and be involved in the destructive processes in OA[9]. The amount of this adipose tissue increases with obesity and plays an important role in the initiation and progression of knee OA[10]. Han *et al.* demonstrated that IPFP signal intensity alteration was significantly and positively correlated with knee structural abnormalities and clinical symptoms in older adults [11]. On the other hand, another study indicated that the maximal area of IPFP was negatively correlated with differences in knee pain in older female adults[12]. Further studies were necessary in order to elucidate the associations between IPFP and the pathogenesis of OA.

Several studies including ours indicated that prostanoids were released from adipose tissue [13-18] and there are involved in the pathogenesis of obesity[19-21]. Among prostanoids, prostacyclin (PGI_2) and thromboxane (TxA_2) have opposite actions[22]. Therefore, TxA_2/PGI_2 ratio reflects the pathological condition[23]. The role of PGI_2 and TxA_2 on the development or progression of OA is not fully elucidated. In this study, our aim is to determine the association between prostanoids (PGI_2 and TxA_2) levels in synovial fluid and local fat pads (IPFP, SPFP) thickness located in knee joints as well as other clinical findings.

MATERIALS AND METHODS

Study population

This study performed on 16 patients who were administered at the Outpatient Clinic of the Istanbul Physical Therapy Rehabilitation Research and Training Hospital from June to December 2019. In this period, all the patients who met the inclusion criteria and gave informed consents were included to this study successively. The inclusion criteria were as follows: having knee OA with effusion, chronic symptomatic knee pain. Effusion was determined by physical examination and verified by ultrasonography. In order to determine OA severity of patients, radiologic findings were investigated and Kellgren and Lawrence classification was used. According to Kellgren and Lawrence classification, grades 2-3 patients were included to study. Patient without effusion is not included to study.

The exclusion criteria were as follows: younger than 18 years old, a significant trauma during the last 3 months, other severe meniscus or ligament injuries that could lead to knee pain, a history of knee surgery, the presence of inflammatory joint disease, a hematological disease, an infection. Patients with meniscus or ligament problem determined by physical examination. Furthermore, if the patients have MR results for abnormal high meniscal signal intensity, patients with grade 3-4 were excluded. Written informed consents were obtained from all the participants. Biruni University Ethics Committee (approval number 37-10) approved our study protocol in accordance with the Declaration of

Helsinki. The sociodemographic characteristics were recorded.

Measures

Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)

The pain, stiffness, and physical functions were investigated by WOMAC, which is among the most commonly used knee OA assessment tools. The patients replied 24 questions, grouped into 3 subscales (pain, stiffness, and physical function). In Likert scale, there are five alternative answers to every question (0= none, 1= mild, 2= moderate, 3= severe, 4= extreme). The maximum score in likert scale is 20 points for pain, 8 points for stiffness, and 68 points for physical function. they were summated to provide a single value in which the three component subscales were equally weighted (WOMAC total score). Acceptability, reliability, validity and responsiveness of the Turkish version of WOMAC osteoarthritis index were performed by Tuzun et al.[24,25,26].

Visual Analogue Scale (VAS)

The average pain levels during activity and at rest and evening were investigated by VAS score between 0 and 10, where 0 indicated no pain and 10 indicated the worst pain. Reliability and validity of the Turkish version of VAS were performed by Gur *et al.* The results were expressed as a mean ± standard error of the mean (SEM) [25, 27, 28]. After getting 16 patients VAS and WOMAC scores, the evaluation of results was performed by the investigator who was blinded to ultrasonographic findings and prostanoid levels.

Cartilage and infrapatellar/suprapatellar fat pad thickness measurement

The distal femoral cartilage and IPFP and SPFP thickness were measured using a MyLab series ultrasonography (USG) device (Esaote Biomedica, Italy) and a high resolution 7–12 MHz linear probe. The patient was kept in a prone position, with his/her knee in full flexion and ankle in a neutral position. The probe was placed on the lateral margin of the patella in an axial position. The femoral cartilage was observed as anechogenic between hyperechogenic bone cortex and suprapatellar fat.

The distance between the fine hyperechogenic line on the cartilage surface of the synovial space and the sharp hyperechogenic line on the bone surface was measured as the cartilage thickness. Three (mid-point) measurements were taken from each affected knee as follows: lateral condyle (LFC), intercondylar area (ICA), and medial condyle (MFC) [25,29,30]. During measurement of fad pad thickness, patients were in the supine position with legs flexed at around 90° and the probe was positioned vertically of the patellar tendon. Cartilage and infrapatellar/suprapatellar fat pad thickness measurements were performed by the experienced clinician who was blinded to the clinical data.

Prostanoid measurements

Synovial fluid specimens were collected by inserting a needle with a syringe into the joint space of the knee. The specimens were kept in serum tubes and centrifuged at 3000 rpm for 15 min at 4°C. The supernatants were collected into Eppendorf tubes and kept at -80°C until use [31,32]. The levels of 6-keto-$PGF_{1\alpha}$, stable metabolite of PGI_2, and TxB_2, stable metabolite of TxA_2, were measured in synovial fluid using an enzyme immunoassay (EIA) kit according to the manufacturer's instructions. TxB_2 (Item No. 501020) and 6-keto-$PGF_{1\alpha}$ EIA kits (Item No. 515211) were obtained from Cayman Chemical (Ann Arbor, MI, USA).

The prostanoid concentrations in synovial fluid were expressed as pg/ml. Technical replicates were used to ensure the reliability of single values. Measurement of prostanoids was performed by the investigator who was blinded to ultrasonographic and clinical findings.

Statistical a**nalysis**

All results obtained from different patients (n) were expressed as a mean ± standard error of the mean (SEM). The normality of distribution was determined by the Kolmogorov-Smirnov test. When there is a normal distribution, Pearson's correlations were performed, correlation coefficients (r values) were calculated. When the data is not normally distributed, Spearman correlations were performed. Since BMI and WOMAC values were not normally distributed, Spearman correlations were performed between BMI or WOMAC values and other parameters. All the other parameters were normally distributed and Pearson's correlations were performed.

Paired Student's t-test was used to compare TxB_2 and 6-keto-$PGF_{1\alpha}$ values obtained from same patient. P-value <0.05 indicates data significantly different. Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA).

RESULTS

Table 1 indicates the clinical characteristics of patients with knee OA. The 16 patients included 5 men and 11 women. The mean age was 64.5±2.8 years (range 45-82). The total WOMAC score was 67.14±4.26. In subscales of WOMAC, pain score was 13.36±1.22, stiffness score was 5.36±0.65, and function score was 48.43±2.88. VAS score were given in Table 1.

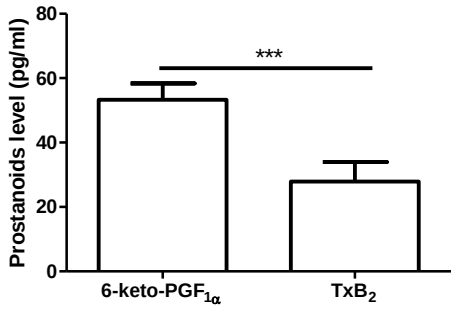

Figure 1. 6-keto-PGF$_{1\alpha}$ levels and TxB$_2$ levels in synovial fluid of patients with osteoarthritis.

Values are means ± SEM, derived from n patients (n=16). *** indicates p<0.001 and data significantly different (Paired Student's t-test) between 6-keto-PGF$_{1\alpha}$ levels and TxB$_2$ levels.

Table 2 indicates the ultrasonographic findings of patients with knee OA. Effusion volume was also presented in Table 2. SPFP thickness was found significantly lower versus IPFP (Table 1). Cartilage thickness-MFC was significantly lower than cartilage thickness-LFC and cartilage thickness -ICA (Table 2).

Correlations between ultrasonographic and clinical findings as well as prostanoid levels measured in synovial fluids

6-keto-PGF1α levels were significantly greater than TxB2 levels in synovial fluid of patients with osteoarthritis (Figure 1). Table 3 and Figure 2 show correlations between 6-keto-PGF1α and TxB2 levels and ultrasonographic/clinical measures. Our results demonstrated that there was a negative correlation between TxB2 levels and SPFP thickness (Table 3, Figure 2A). SPFP thickness was positively correlated with IPFP thickness (Table 3, Figure 2B). Furthermore, the TxB2/6-keto-PGF1α ratio was negatively correlated with cartilage thickness- ICA (Table 3, Figures 2C). IPFP thickness was positively correlated with effusion volume (Table 3, Figures 2D). WOMAC scores were positively correlated with VAS-activity and VAC-evening scores (Table 3, Figures 2E, 2F). Cartilage thickness-MFC values were positively correlated with cartilage thickness-IFC (Table 3, Figure 2G). On the other hand, 6-keto-PGF1α levels were not correlated with any ultrasonographic or clinical findings (Supplementary Table 1)

Table 1. Demographic and clinical characteristics of patients with knee osteoarthritis

Sexe (male; female)	5;11
Age (years)	64.5±2.8
Body mass index (kg/m2)	30.63±1.69
Visual analog scale- activity	8.21±0.46
Visual analog scale- rest	2.86±0.55
Visual analog scale- evening	4.86±0.68
WOMAC Osteoarthritis Index, total	67.14±4.26
WOMAC Osteoarthritis Index, pain	13.36±1.22
WOMAC Osteoarthritis Index, stiffness	5.36±0.65
WOMAC Osteoarthritis Index, function	48.43±2.88

Data are expressed as mean±SEM. WOMAC, Western Ontario McMaster Universities.

Table 2. Ultrasonographic findings of patients with knee osteoarthritis

Suprapatellar fat pad thickness (mm)	6.00±0.35
Infrapatellar fat pad thickness (mm)	7.93±0.32***
Cartilage thickness-MFC (mm)	1.87±0.10
Cartilage thickness-LFC (mm)	2.12±0.12#
Cartilage thickness-ICA (mm)	2.23±0.13#
Effusion volume (ml)	8.76±1.19

Data are expressed as mean±SEM. ICA, intercondylar area; MFC, medial femoral condyle; LFC, lateral femoral condylar area. *** indicates p<0.001 versus suprapatellar fat pad thickness, # indicates p<0.05 versus cartilage thickness-MFC.

Table 3. Spearman or Pearson correlation results

	r	P
Effusion Volume vs Infrapatellar fat pad thickness	0.55	0.05
Suprapatellar fat pad thickness vs Infrapatellar fat pad thickness	0.62	0.03
Suprapatellar fat pad thickness vs TxB_2 levels	-0.56	0.04
Cartilage thickness-LFC vs Cartilage thickness-MFC	0.68	0.01
Cartilage thickness-ICA vs TxB_2/6-keto-$PGF_{1\alpha}$	-0.60	0.03
Activity VAS vs WOMAC	0.52	0.05
Evening VAS vs WOMAC	0.68	0.01
TxB_2 vs TxB_2/6-keto-$PGF_{1\alpha}$	0.81	0.00

Regression coefficient (r) and statistical significance (p) are reported for each test. p-value < 0.05 is significant ICA, intercondylar area; MFC, medial femoral condyle; LFC, lateral femoral condylar area; VAS-visial analog scale; WOMAC, Western Ontario McMaster Universities.

Table 4. Spearman or Pearson correlations between variables

Spearman or Pearson correlation (r,P)													
	6-keto-$PGF1\alpha$ (pg/ml)	Effusion Volume (ml)	Suprapatellar fat pad thickness	Infrapatellar fat pad thickness	Cartilage thickness-LFC (mm)	Cartilage thickness-MFC (mm)	Cartilage thickness-ICA (mm)	Rest VAS	Activity VAS	Evening VAS	WOMAC	TxB_2 (pg/ml)	TxB_2/6-keto-$PGF_{1\alpha}$
6-keto-$PGF_{1\alpha}$ (pg/ml)		0.42,0.15	-0.31,0.31	0.29,0.33	0.24,0.43	0.02,0.94	0.33,0.27	-0.23,0.42	-0.16,0.58	-0.02,0.95	-0.29,0.31	0.42,0.12	-0.10,0.72
Effusion Volume (ml)	0.42,0.15		0.41,0.18	0.55,0.05	0.15,0.65	-0.12,0.71	0.25,0.44	0.16,0.61	-0.01,0.98	-0.09,0.78	-0.13,0.68	-0.29,0.33	-0.53,0.06
Suprapatellar fat pad thickness (mm)	-0.31,0.31	0.41,0.18		0.62,0.03	-0.06,0.85	0.23,0.44	-0.16,0.60	0.41,0.17	-0.31,0.31	-0.05,0.87	-0.17,0.57	-0.56,0.04	-0.32,0.29
Infrapatellar fat pad thickness (mm)	0.29,0.33	0.55,0.05	0.62,0.03		0.09,0.77	0.16,0.60	-0.30,0.33	0.15,0.63	-0.49,0.09	-0.24,0.43	-0.27,0.38	0.03,0.91	0.02,0.94
Cartilage thickness-LFC (mm)	0.24,0.43	0.15,0.65	-0.06,0.85	0.09,0.77		0.68,0.01	0.42,0.15	-0.10,0.75	-0.51,0.07	-0.28,0.36	-0.46,0.11	0.15,0.64	0.06,0.85
Cartilage thickness-MFC (mm)	0.02,0.94	-0.12,0.71	0.23,0.44	0.16,0.60	0.68,0.01		0.39,0.19	0.27,0.38	-0.41,0.17	0.14,0.64	-0.30,0.32	-0.08,0.81	0.02,0.94
Cartilage thickness-ICA (mm)	0.33,0.27	0.25,0.44	-0.16,0.60	-0.30,0.33	0.42,0.15	0.39,0.19		0.03,0.92	0.10,0.74	0.40,0.17	0.01,0.97	-0.29,0.33	-0.60,0.03
Rest VAS	-0.23,0.42	0.16,0.61	0.41,0.17	0.15,0.63	-0.10,0.75	0.27,0.38	0.03,0.92		0.23,0.44	0.30,0.29	0.49,0.08	-0.31,0.28	-0.05,0.86
Activity VAS	-0.16,0.58	-0.01,0.98	-0.31,0.31	-0.49,0.09	-0.51,0.07	-0.41,0.17	0.10,0.74	0.23,0.44		0.48,0.08	0.52,0.05	-0.03,0.92	-0.07,0.82
Evening VAS	-0.02,0.95	-0.09,0.78	-0.05,0.87	-0.24,0.43	-0.28,0.36	0.14,0.64	0.40,0.17	0.30,0.29	0.48,0.08		0.68,0.01	-0.16,0.59	-0.14,0.64
WOMAC	-0.29,0.31	-0.13,0.68	-0.17,0.57	-0.27,0.38	-0.46,0.11	-0.30,0.32	0.01,0.97	0.49,0.08	0.52,0.05	0.68,0.01		-0.07,0.82	-0.02,0.96
TxB_2 (pg/ml)	0.42,0.12	-0.29,0.33	-0.56,0.04	0.03,0.91	0.15,0.64	-0.08,0.81	-0.29,0.33	-0.31,0.28	-0.03,0.92	-0.16,0.59	-0.07,0.82		0.81,0.00
TxB_2/6-keto-$PGF_{1\alpha}$ ratio	-0.10,0.72	-0.53,0.06	-0.32,0.29	0.02,0.94	0.06,0.85	0.02,0.94	-0.60,0.03	-0.05,0.86	-0.07,0.82	-0.14,0.64	-0.02,0.96	0.81,0.00	

Regression coefficient (r) and statistical significance (p) are reported for each test. p-value < 0.05 is significant

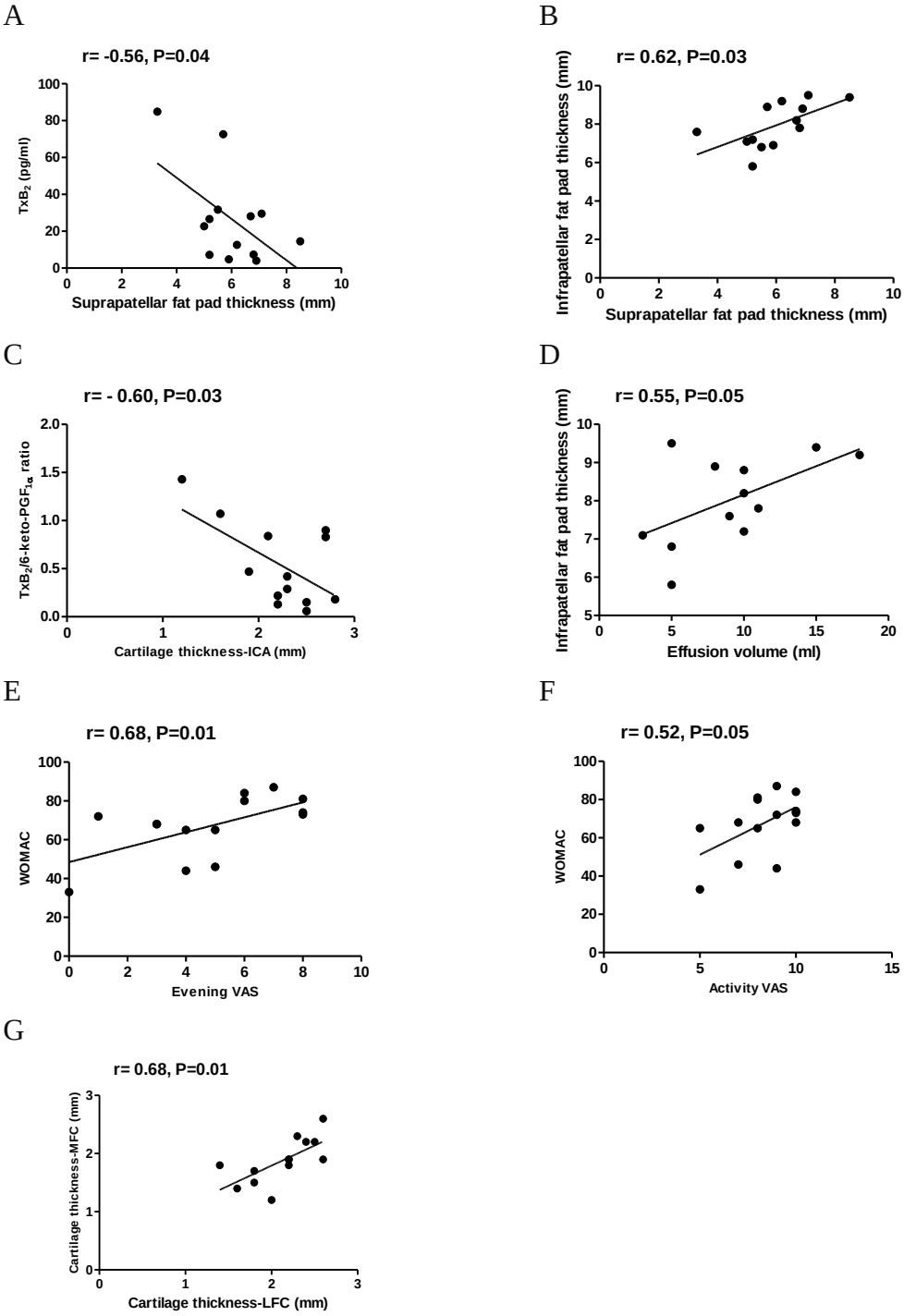

Figure 2. Correlations between ultrasonographic and clinical findings as well as prostanoid levels measured in synovial fluids. p<0.05 indicates data significantly different (Pearson and Spearman correlation). ICA, intercondylar area; MFC, medial femoral condyle; LFC, lateralfemoral condylar area; VAS-visial analog scale; WOMAC, Western Ontario McMaster Universities.

DISCUSSION

Obesity is an important risk factor for the development of OA. The main mechanism for the association between obesity and OA could be related to both biomechanical and metabolic mechanisms[33]. In this aspect, local adipose tissues in the knee joint (IPFP and SPFP) have gained attention in recent years. Several studies suggested that the mediators released from these adipose tissues can be involved in the pathogenesis of OA [33,34]. Prostanoids are released from adipose tissue and they are involved in pain and inflammation[13,22]. Therefore, we focused on the association between prostanoid levels and local fat pad thickness as well as other clinical parameters in knee OA patients. Our results demonstrated that TxB_2 levels were negatively correlated with SPFP thickness and TxB_2/6-keto-$PGF_{1\alpha}$ ratio was negatively correlated with cartilage thickness. On the other hand, we did not find any association between PGI_2 levels and clinical parameters.

Non-steroidal anti-inflammatory drugs (NSAIDs) are effective for pain relief, as well as symptoms of OA. Their mechanism of action is mediated through the inhibition of prostanoids. Therefore, several studies focused on the role of prostanoids in the development of OA. In patients with OA have significantly lower levels of prostanoids in synovial fluid as compared to patients with rheumatoid arthritis, Reiter's disease, acute gouty arthritis. There were no significant correlations between leucocyte cell counts and prostanoids levels measured in synovial fluids[35].

Among prostanoids, several studies focused on the role of PGE_2 in the development or progression of OA. Egg et al. demonstrated that PGE_2 was the most predominant prostanoid measured in synovial fluid of patients with OA[35]. On the other hand, the contributions of other prostanoids including PGI_2 and TxA_2 were not fully elucidated, even though PGI_2 has been defined as an important mediator of inflammation and pain [36-38]. Furthermore, PGI_2 also plays role in angiogenesis which involves in cartilage loss and exudate formation in arthritis models[39].

The role of PGI_2 in the development of OA is investigated in rat models of OA. In these rats, there was up-regulation of 6-keto-$PGF_{1\alpha}$ levels in synovial fluids 3 days after MIA injection and 6-keto-$PGF_{1\alpha}$ levels returned to basal levels by day. Treatment with IP receptor antagonist reduced pain. Furthermore, IP receptor knock out mice exhibited a 91% reduction in arthritis score [38]. In line with this finding, early up-regulation of 6-keto-$PGF_{1\alpha}$ levels was found in the rat arthritis model [40]. Another study also demonstrated PGI_2 and PGI_2 receptor (IP receptor) were produced by synovial fibroblasts from patients with OA[39]. In our results, we demonstrated 6-keto-$PGF_{1\alpha}$, stable metabolite of PGI_2, was detectable in synovial fluids of patients with OA. Even though the role of PGI_2 was shown in the pathogenesis of OA in animal models, we could not detect any correlation between 6-keto-$PGF_{1\alpha}$ levels and clinical parameters of patients with OA.

There is limited study which investigates the contribution of TxA_2 in OA development in literature. In our results, TxB_2, stable metabolite of TxA_2 was detected in synovial fluids of patients with OA. 6-keto-$PGF_{1\alpha}$ levels were significantly greater than TxB_2 levels in synovial fluids of patients with OA. Similarly, another study performed in patients with rheumatoid disease demonstrated that PGI_2 was the major prostanoid in synovial fluids [41]. Our results demonstrated no correlation between 6-keto-$PGF_{1\alpha}$ and TxB_2 levels.

Timur *et al.* demonstrated that TxB_2 levels were not related to clinical parameters in OA patients [42]. In the present study, we also did not detect any correlations between TxB_2 levels measured in synovial fluid and clinical parameters including WOMAC, VAS, cartilage thickness. Interestingly, our results indicated that TxB_2 levels were negatively correlated with SPFP thickness. Although IPFP thickness was also positively correlated with SPFP thickness, we could not detect any correlation between TxB_2 levels and IPFP thickness. Another study demonstrated that the release of TxB_2 from IPFP of patients with OA was not different from those obtained from without OA [42].

In comparison to IPFP, studies performed for the investigation of the role of SPFP in OA were limited. There were conflicting results regarding the prevalence of SPFP edema with mass effect and its correlation with clinical symptoms[33]. In the present study, there was no association between SPFP thickness and pain scores of patients. In accordance with this finding, another study demonstrated that SPFP edema with a mass effect is rarely associated with knee pain [43].

Several studies indicated that the ratio of TxA_2/PGI_2 levels reflects the pathological condition [23] however,

it was not investigated in regards to OA. Our results demonstrated that TxB_2/6-keto-$PGF_{1\alpha}$ ratio was negatively correlated with cartilage thickness-ICA. Several studies demonstrated that the reduction of cartilage thickness was strongly associated with radiographic progression [44].

Effusion has been suggested to be a precursor of OA outcomes[45]. We also demonstrated that effusion volume was positively correlated with IPFP thickness. In line with our results, a recent study demonstrated that accelerated knee OA was characterized by greater effusion volume and/or IPFP signal intensity however in this study correlation between IPFP thickness and effusion volume was not examined [46]. The increased effusion was associated with an increased risk of knee pain [47-49]. However, our results did not demonstrate any correlation between effusion volume and pain scores. WOMAC has been developed for the evaluation of knee and/or hip OA and it is widely used for the assessment of OA [50]. Our results demonstrated that WOMAC scores were positively correlated with evening VAS and activity VAS. Similar results have been found by Papathanasiou *et al.* [51].

This is the first study for the determination of the association of synovial prostanoid levels with local fat thickness and clinical status in knee OA patients. Since there is still lack of information on what degree fat tissue is involved in OA, the issue is an up-to-date subject. However, there are some limitations in our present study. One of them is that a relatively limited number of patients were included. Multicenter studies with higher numbers of patients with OA and control subjects are needed. Furthermore, more studies are necessary in order to investigate the mechanism of action of prostanoids in OA.

In conclusion, our study demonstrated that TxB_2 levels and TxB_2/6-keto-$PGF_{1\alpha}$ ratio were negatively correlated with SPFP and cartilage thickness, respectively. However, we could not detect any correlations between 6-keto-$PGF_{1\alpha}$ levels and ultrasonography findings. In comparison to 6-keto-$PGF_{1\alpha}$ levels, TxB_2 levels seem to be more associated with ultrasonographic findings in patients with OA. Furthermore, neither TxB_2 nor 6-keto-$PGF_{1\alpha}$ levels correlated with demographic and clinical parameters in patients with OA. Further studies are necessary in order to investigate the participation of prostanoids in OA.

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