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Comparison of clinical outcome, cartilage turnover, and inflammatory activity following either intra-articular or a combination of intra-articular with intra-osseous platelet-rich plasma injections in osteoarthritis knee: A randomized, clinical trial

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ABSTRACT

Background: The objective of the study was to determine the changes in clinical outcome (pain and knee activity) and assess bone/ cartilage biomarkers and inflammatory activity in persons with osteoarthritis (OA) knee following a single injection of intra-articular platelet-rich plasma (IA-PRP) and combination of intra-articular, intraosseous PRP (IA+IO-PRP).

Methods: This prospective, randomized, single-blind clinical trial was conducted at a tertiary care teaching hospital in India. Ninety-six persons with OA knee with a Kellgren-Lawrence score of 3 were randomized into three groups- Group-I (IA-PRP), Group-II (IA+IO-PRP)], Group-III, [intra-articular normal saline (IA-NS)]. The primary outcome was a visual analog scale (VAS) for pain. The secondary outcomes were the Knee Injury and Osteoarthritis Outcome Score (KOOS), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), bone/ cartilage turnover biomarkers [C-telopeptide (CTX-II), N-telopeptide (NTX-I), cartilage oligomeric matrix protein (COMP), N-terminal propeptide of collagen type-IIA (PIIANP), and hyaluronic acid (HA)], ultrasonography (USG) findings of the knee joint. The outcome measures were assessed at baseline, 6, and 12 weeks of follow-up.

Results: Compared to IA-NS injection, IA-PRP and IA+IO-PRP injections significantly improved VAS-pain and KOOS scores at 6 and 12 weeks. Furthermore, both PRP groups showed a significant reduction in ESR, CRP, and CTX-II at 12 weeks following PRP injections. In addition, at 12 weeks, the IA+IO-PRP group showed a significant reduction (p=0.009) in NTX-I level. Persons in the IA+IO-PRP group reported significant reductions in the synovial-effusion and infra-patellar bursitis.

Conclusions: Significant clinical improvements were noticed following IA-PRP and IA-IO-PRP injections compared to IA-NS injections. Both PRP groups reported a significant reduction in ESR, CRP, and CTX-II levels at 12 weeks. Persons in the IA+IO-PRP group reported significant changes in u-NTX-I level and knee-USG findings.

Abbreviations: ADL, Activities of daily living; COMP, Cartilage oligomeric matrix protein; CTX-II, C-telopeptides of type-II collagen; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; HA, Hyaluronic acid; IA-PRP, Intra-articular platelet-rich plasma; IA+IO, PRP –Intra-articular and intra-osseous platelet-rich plasma; KOOS, Knee Injury and Osteoarthritis Outcome Score; NTX-I, N-telopeptides of type-I collagen; PIIANP, N-terminal propeptide of collagen type-IIA; OA, Osteoarthritis; PRP, Platelet-rich plasma; QoL, Quality of life; RCTs, Randomized controlled trials; VAS, Visual analog scale.

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Introduction

Osteoarthritis (OA) is a progressive degenerative joint disease that causes the loss of articular cartilage, inflammation of the synovium, and breakdown of the subchondral bone [1]. In its early stage, the subchondral bone undergoes various pathophysiological and structural changes, which include micro-crack, edema-like lesions, and cysts [2–5]. Later, with the progression and severity of the disease, the subchondral marrow also changes; the normal marrow tissue is replaced by fibro-neurovascular mesenchymal tissue [6].

In recent years, intra-articular (IA) platelet-rich plasma (PRP) injection has gained popularity in managing the OA Knee. Platelet-rich plasma, prepared from a patient's whole blood, has been found to have anti-inflammatory and regenerative properties [7]. In the last decade, many studies [8–13] were conducted to measure the efficacy of intra-articular (IA) PRP injections in the OA Knee. Though most of these studies reported positive outcomes in pain relief and knee function following IA-PRP injection, none of these demonstrated complete pain relief and long-term improvements. Therefore, a new approach was proposed to manage the OA knee; the additional application of intra-osseous (IO) injections with intra-articular (IA) injections would provide a better outcome and halt the disease progression more effectively.

Cartilage destruction is not the only feature that determines OA severity. Inflammation is another feature that can persist along with cartilage destruction in persons with OA. The stages of inflammatory activity can determine the degree of synovial hypertrophy, severity, and progression of the disease [14–17]. Erythrocyte sedimentation rate (ESR) and serum C-reactive protein (CRP) levels are well-established inflammatory markers that remain elevated in persons with moderate to severe OA knee [15,16]. Similarly, serum-HA is another inflammatory biomarker that increases with synovium inflammation and cartilage breakdown [18–22]. Along with these inflammatory biomarkers, recent research has also identified several bony-/ cartilage- turnover biomarkers in serum (s-) and urine (u-), which also have demonstrated great potential to measure the bony/ cartilage degradations, determine the prognosis, and responsiveness to OA treatments [18–20]. Among them, urinary- C-telopeptide (u-CTX-II) and N-telopeptide (u-NTX-I), and serum-cartilage oligomeric matrix protein (s-COMP) are established bone/cartilage degradation biomarkers [19,20]. On the contrary, the serum N-terminal propeptide of collagen IIA (s-PIIANP) is cartilage (collagen type-II) synthesis biomarker [20].

Biomarkers are generally used to quantify clinical efficacy, determine dose, and identify between responders and non-responders to treatment[19]. To date, a few authors have tried to measure the bone/ cartilage turnover rate & inflammatory biomarkers in persons with OA knee following PRP injections. This study hypothesized that IA-PRP and intra-articular, intraosseous injections of PRP (IA+IO-PRP) would provide a better positive clinical outcome than intra-articular normal saline (IA-NS) injection in persons with OA knee. Following treatments with PRP injections, the changes in the concentration of inflammatory- and cartilage turnover biomarkers would be parallel with clinical outcomes. Therefore, the objectives of this study were to determine the clinical outcomes, assess inflammatory and cartilage turnover biomarkers following IA-PRP and IA+IO-PRP, and compare their results with those who received IA-NS injections.

Methodology

Study Design

This 12-weeks, prospective, single-blind, randomized, placebo-controlled trial (RCT) was conducted at the All India Institute of Medical Sciences, Bhubaneswar, from October 2019 to March 2022. The institutional ethics committee reviewed the protocol and approved the study. The study was registered at the Clinical Trials Registry (CTRI/2019/10/021708). The signed-informed consent was obtained from each person.

Participants

The persons were recruited from the outpatient clinic of a physical medicine and rehabilitation department. All persons underwent standard clinical and radiological evaluations. The persons were included if they were fulfilling the diagnostic American College of Rheumatology (ACR)) criteria for OA Knee (unilateral/ bilateral). The other inclusion criteria were as follows: (1) age between 40-65 years; (2) severity of knee pain with visual analog scale (VAS) $\geq$ 3 cm (out of 10 cm); (4) duration of pain $>$ 6 months; (5) severity of OA with Kellgren-Lawrence's (K-L) grade III. The persons who (1) underwent surgical or pain interventions (injections) in the index knee; (2) received disease-modifying osteoarthritis drugs (DMOADs) or oral steroids in the last six months were excluded from the study. Persons were also excluded if they had (1) hemoglobin level $<$12.5gm/dl, (2) body mass index (BMI) of $>$40 kg/m2; (3) immunological, hematological, or any systemic diseases; (4) hip joint dysfunction, low-back pain, fibromyalgia, and psychological disorder.

Persons were asked to discontinue the oral/topical pain medications (nonsteroidal anti-inflammatory drugs (NSAIDs) or analgesics; if they were receiving any) at least for two weeks before initiation of treatment until completion of the trial.

Randomization, Blinding

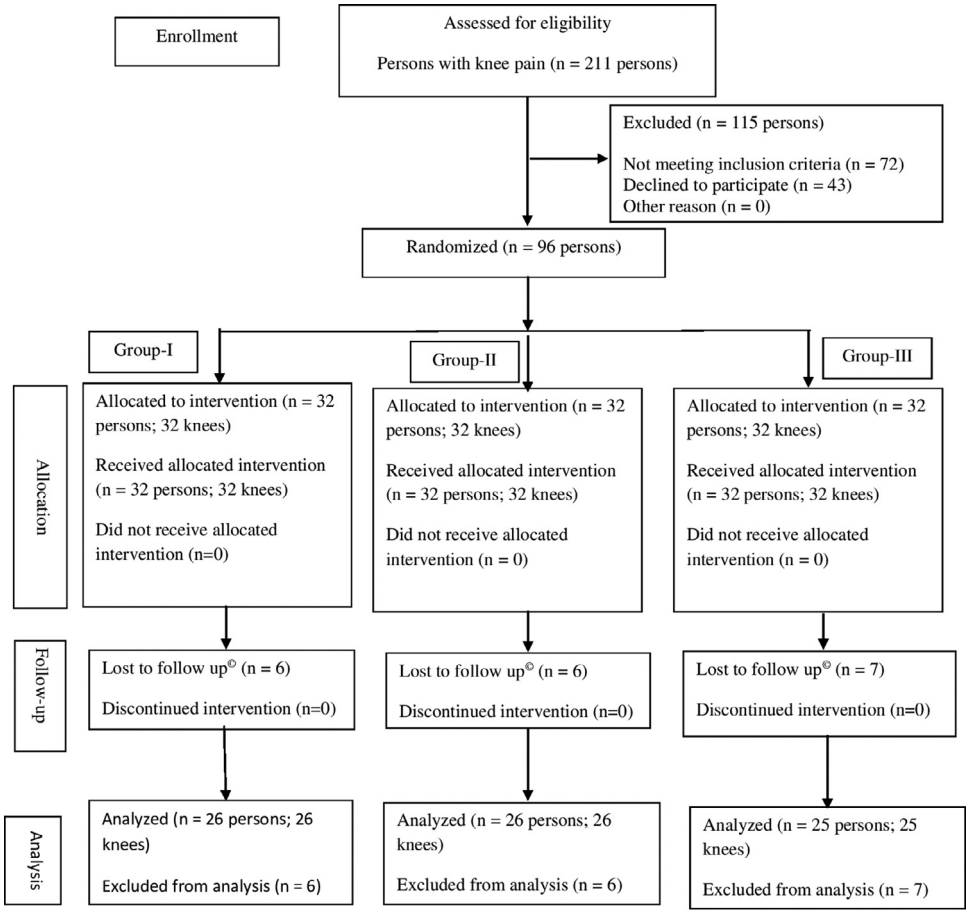
The included persons were randomized into three groups- (1) Group I [intra-articular PRP' ('IA-PRP') group], (2) Group II ['intra-articular, intra-osseous PRP' ('IA+IO-PRP') group], (3) Group III, [intra-articular NS ('IA-NS') group] with computer-generated randomization. One paramedical staff allocated the persons through opaque, sealed envelopes. A schematic diagram of the study persons' recruitment is shown in Fig 1. One independent investigator, who had no role in randomization, intervention, or evaluation, recruited all persons. One physician, who had experience in rehabilitation surgery and pain intervention procedures, performed all interventions.

Clinical assessments

An independent evaluator (Physician) who was not part of the study performed all clinical assessments at baseline and follow-up visits.

Blood and urine sampling, assessment of biomarkers

One nursing officer collected blood (fasting) and urine samples (between 9 AM to 10 AM) from each person at baseline and during follow-up visits. The samples (blood and urine) were then sent to the biochemistry laboratory. The blood samples were initially centrifuged at 4000 rpm for five minutes to obtain the clear supernatant serum. The serum and urine samples were then preserved in a deep freezer (-80degree Celsius). On a later convenient date, the serum and urinary samples (in batches) were analyzed within three months of their collection. The quantitative assessment of each biomarker was done with the enzyme-linked immunosorbent assay (ELISA). The details of the biomarker assessment technique have been reported in **Appendix 1**. One independent physician (biochemistry) assessed all biomarkers.



© Lost to follow up – 6 persons in group-I, 6 in group-II and 7 persons in group-III did not come for follow-up visits. Due to COVID-19 breakout/ continuous lockdown in local area they could not turn-up for follow-up visits. We could not contact them during the study period. However, later all of them came for check-up.

Fig 1. CONSORT (consolidated standard of reporting trials) flow diagram.

Assessment of knee by Ultrasonography (USG)

One radiologist, experienced in musculoskeletal ultrasound, did all USG evaluations. The radiologist was unaware of parsons' selection and intervention procedure. The USG knee joint was performed according to the procedure demonstrated by Bevers K et al.[23,24]. Details of the USG procedure have been reported in **Appendix 1**.

PRP preparation

The PRP was prepared using the Harvest Terumo Smart Prep® 2 system' (Terumo BCT, Colorado, USA). Fifty-five ml of venous blood was collected from the persons allotted in Group-I, whereas 110 ml from persons allotted in Group-II. The details of the PRP preparation technique have been reported in **Appendix 1.**

The cell (leucocyte and platelet) counts were done before (whole blood) and after the preparation of PRP using an automated cell counter (Sysmex X-100) machine. Quantitative analysis of growth factors (platelet-derived growth factor-AB (PDGF-AB) and transforming growth factors beta (TGF-β) from PRP were done using the ELISA method.

Interventions

Irrespective of groups, all interventions were performed under fluoroscopy guidance inside the operation theatre (OT) with proper aseptic preparation. The intra-articular injections (IA-PRP / IA-NS) were given without local or general anesthesia (GA), whereas IA+IO-PRP injections were administered under GA. Before administering the injection, synovial fluid aspiration was done from all persons. Details of intervention procedures (IA and IA+IO) have been described in **Appendix 1**. Fig 2**A** and 2**B** show the tip of the trocar at the femoral condyle and tibial plateau, respectively.

In Group-I, an 8ml PRP solution was injected intra-articularly. In Group-II, 18ml PRP (8ml inside the joint, 5ml in the medial femoral condyle (in subchondral bone), and 5ml in the medial tibial plateau (subchondral bone)) was injected. The volume of injection (at each IO-site) and the intra-osseous sites (subchondral region of medial-femoral condyle and medial-tibial plateau) were decided based on

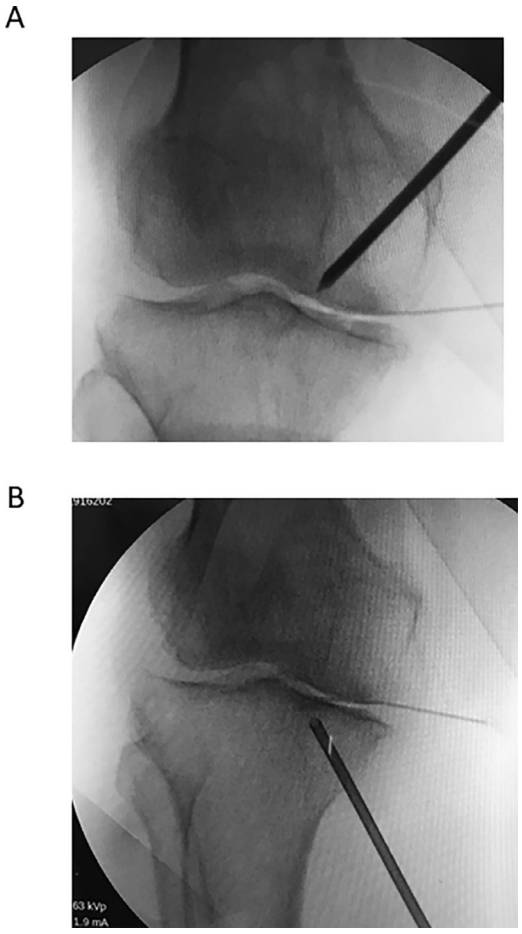


Fig 2. Images shows the tip of trocar at femoral condyle (Fig 2A) and at tibial plateau (Fig 2B).

the studies published on subchondral injections in the treatment of OA knee [6,25,26]. In Group-III, 8ml of 0.9%NS was injected intra-articularly.

Across the groups, all persons were admitted to the hospital indoor-ward for at least 2-days. Persons allotted in group-II were undergone pre-anesthetic checkups (PAC). A single-dose antibiotic (Ceftriaxone 1gm- single dose) was given to all persons allotted to Group-II, as it was mandatory to give a single-dose antibiotic (per institutional protocol) for persons undergoing GA. The antibiotic injection was given inside the OT room just before GA. In contrast, no antibiotics (oral/ injection) were given (pre-or post-intervention) to the persons allotted in Groups -I and -III.

Following injection, passive knee flexion/extension was performed 5-times and rested for 20-minutes. Persons were instructed to apply icepack 3 hours after injection over the target knee joint and then three times a day for 20 minutes each for the next five days. On the day of injection, persons were allowed to stand, weight-bear (partial to complete) on the target knee joint, and walk a few steps (as tolerable, based on the person's condition). From the next day onwards, persons were encouraged to walk (low impact) and gradually increase the walking distance over the next 7-days. From post-injection day 2, the persons were instructed to start the knee exercise program (home-based) for 30 minutes/per day. The exercise program was comprised of passive joint/ soft tissue mobilization (20 repetitions), active ROM exercises (20-repetition), and strengthening (isometric) exercises (20-repetition)]. During strengthening exercises, the persons were advised to increase the intensity of isometrics gradually. At six weeks, all persons were advised to add isotonic knee strengthening exercises (static loading exercises- wall squat hold (20-repetition)) along with existing isometric exercises (20-repetition). The persons were instructed to complete the entire exercise program within 30 minutes.

One physical therapist, experienced in the musculoskeletal-rehabilitation, demonstrated all knee exercises at the baseline (V0) and follow-up visit (V1). The physical therapist was blinded to the person's group.

The persons were not allowed to start new medications (NSAIDs, DMOADs) or receive new interventions (nerve blocks, physical modalities, and exercise programs) during the trial period. However, persons were allowed to consume tablets of acetaminophen (up to 3gm/ day) (1^{st} three days following intervention) in case of severe pain.

Outcomes

The 'visual analog scale (VAS) (10cm) for pain' was used as a primary outcome. Secondary outcomes were (a) Knee Injury and Osteoarthritis Outcome Score (KOOS); (b) assessment of inflammatory activity- ESR and CRP; (c) assessment of bone/ cartilage turnover- CTX-II, NTX-I, COMP, PIIANP, and HA; (c) Ultrasonography (USG) findings of the knee joint.

The KOOS questionnaire was used to assess pain, symptoms, activities of daily living (ADL) function, sport/ recreation, and quality of life (QoL), whose possible scores of each parameter range from 0 to 100 (a score of 100 represents no knee problems)

The ESR was measured from the blood sample. The COMP, PIIANP, HA, and CRP were assessed from serum (s) samples. Whereas the levels of CTX-II and NTX-I were assessed from urinary (u) samples.

The USG knee joint was performed to measure the femoral cartilage thickness and to look for the presence or absence of effusion ($\geq$4mm in supra-patellar recess), synovial hypertrophy ($\geq$2mm in supra-patellar recess), medial meniscal-protrusion (>3mm in medial joint space), infra-patellar bursitis (>2mm infra-patellar bursa), and baker's cyst.

Follow-up

Outcome assessments were done at the baseline visit (V0, pre-intervention), at six weeks (V1), and 12 weeks (V2) post-intervention. The VAS-pain and KOOS scores were recorded at all visits (V0, V1, V2). Similarly, the ESR and quantitative assessment of biomarkers (CRP, CTX-II, NTX-I, COMP, PIIANP, and HA) were performed at V0, V1, and V2. In addition, the USG knee was done at V0 and V2.

Sample Size

Using one-way analysis of variance (ANOVA), a sample size of 27 in each group would achieve 90% power to demonstrate a difference of 1.5 points in a VAS-Pain score with a standard deviation (SD) of 1.5 at the 5% significance level in a 2-sided hypothesis test. Anticipating 10-15% dropouts, the study was designed to enroll 32 persons in each group. The sample size calculation was based on RCT that evaluated a single IA-PRP injection, two IA-PRP injections, and an IA-NS injection for the OA knee [10].

Statistical Analysis

A per-protocol analysis was performed. The results were expressed as mean (SD). The Chi-square test ($\chi 2$) was used to compare binomial variables between 3-groups. A one-way analysis of variance was used to compare the mean values between groups for each domain of a continuous variable, and post hoc tests (the Dunnett procedure) were used to determine the significant difference

Table 1
Baseline Characteristics of the three patient groups (n=96).

	Group-I [IA-PRP] (n= 32)	Group-II [IA+IO-PRP] (n=32)	Group-III [IA-NS] (n=32)	P-value (Between Groups)
Sociodemographic Characteristics				
Age, mean(SD), year	58.91(4.42)	59.28(3.75)	58.81(3.69)	
Sex, male/female, n	16:16	17:15	18:14	0.88
BMI, mean(SD)	26.95(3.09)	26.63(3.46)	26.35(3.03)	0.75
Symptom duration, mean(SD), months	55.69(12.62)	54.69(12.15)	56.25(12.06)	0.88
Right Knee, n (%)	17 (53.1%)	18 (56.2%)	17 (53.1%)	0.96
Persons with DM, n (%)	12 (37.5%)	11(34.4%)	13 (40.6%)	0.87
Clinical Parameters				
Pain VAS (0-10), mean (SD)†	6.22(0.96)	6.39(1.15)	6.05(1.06)	0.43
KOOS-pain (0-100), mean (SD)‡	49.22(10.43)	49.09(9.55)	49.71(8.88)	0.96
KOOS-symptoms (0-100), mean (SD)‡	51.87(11.80)	50.59(10.41)	51.22(11.26)	0.90
KOOS-ADL (0-100), mean (SD)‡	47.28(9.52)	47.66(9.66)	47.34(8.92)	0.98
KOOS-sport/Rec (0-100), mean (SD)‡	29.19(11.73)	30.06(9.92)	30.94(9.97)	0.80
KOOS-QoL (0-100), mean (SD)‡	46.28(11.53)	44.78(10.44)	45.69(10.84)	0.86
Inflammatory Biomarker				
CRP (mg/L), mean (SD)	6.36(3.53)	6.58(2.95)	6.57(3.39)	0.96
Cartilage Turnover Biomarker				
u-CTX-II (ng/mmol Creatinine), mean (SD)	331.60(110.38)	330.00(98.33)	333.11(99.19)	0.99
u-NTX-I (ng/mmol Creatinine), mean(SD)	82.96(31.62)	83.71(29.16)	84.17(30.63)	0.99
s-COMP (ng/ml), mean(SD)	762.63(262.07)	789.41(268.67)	774.20(239.41)	0.92
s-PIIANP (ng/ml), mean(SD)	5.20(3.73)	5.18(3.38)	5.48(3.25)	0.93
s-HA(ng/ml) mean(SD)	5.96(2.90)	6.21(2.73)	6.59(2.56)	0.64
Other blood tests				
ESR (mm/hour), mean(SD)	13.25(5.72)	14.16(5.12)	13.81(6.39)	0.82
Uric acid (mg/dl), mean(SD)	5.40(1.45)	5.35(1.22)	5.46(1.35)	0.95
Ultrasound Findings ₡				
Cartilage thickness (femoral condyle) (mm), mean(SD)	1.54(0.43)	1.56(0.32)	1.53(0.33)	0.96

IA-PRP, Intra-articular platelet-rich plasma; IO+IA-PRP, Intra-articular intraosseous platelet-rich plasma; IA-NS, Intra-articular normal saline; VAS, visual analog scale; KOOS, Knee Injury, and Osteoarthritis Outcome Score; ADL, Activities of daily living; QoL, Quality of life; SD, standard deviation; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; s-HA, serum hyaluronic acid; u-CTX-II, urinary type-II collagen C telopeptide; u-NTX-I, urine Type-I collagen N- telopeptide; s-COMP, serum cartilage oligomeric matrix protein; s-PIIANP, serum N-terminal propeptide of type-IIA collagen
† Score range from 0-10, a lower value indicating less pain.
‡ Score range from 1-100, a higher value indicating less knee problem
₡ A single rater (blinded to group and time point) measured the cartilage thickness on femoral condyle from ultrasonographic (USG) images of target knee joint. Cartilage thickness was measured perpendicular to the surface at three points – at intercondylar notch, medial and lateral condyle (5 mm just medial or lateral from the top of the condyle), with the knee in maximum flexion position. The average cartilage thickness (mean value of the cartilage thickness) was calculated from thicknesses measured at 3-points.

between the two groups. A one-way repeated-measures analysis of variance (ANOVA) with post hoc tests investigated the intra-group differences at various time points (baseline, V1, and V2). The magnitude of changes between two different times in the same group has been reported mean difference with a 95% confidence interval. The SPSS software version 15 (SPSS Inc, Chicago, Illinois) was used. A difference of $p < 0.05$ was considered statistically significant.

Results

Ninety-six persons (32 persons in each group) were recruited. Among them, 77 persons completed all follow-up visits. Fig 1 summarizes participant flow. Out of the 96 persons, 45(47%) were female. The mean age of the participants was 59 years, mean body mass index (BMI) was 26.6 kg/m^2. The mean uric acid level was 5.40 mg/dl. Baseline characteristics were similar between the three groups (Table 1).

Twenty-six persons in Group I, 26 in Group II, and 25 in Group III completed all follow-up visits and were included in the per-protocol analysis.

The biological characteristics and classifications (Mishra[27] and the PAW classification[28] system) of injected PRP in both (Group-I and II) have been summarized in Table 2**.** The concentration of platelets increased 3.93(0.76) times compared to platelet concentrations in blood.

The mean VAS pain scores at the baseline visit and each follow-up visit for all groups have been presented in Table 3. The trend in mean VAS pain scores over time in 3 groups has been reported in Fig 3. Compared to the baseline visit, the MD (95% CI) in VAS pain scores in Group-I was 1.25(0.87 to 1.63) at six weeks and 2.5(1.82 to 3.18) at 12 weeks. The MDs in Group II were 2.04 (1.62 to 2.45) and 3.77(3.27 to 4.27) at six weeks and 12 weeks, respectively. In Group III, the MDs were 0.98(0.67 to 1.29) and 0.68; (-0.11 to 1.47) at six weeks and 12 weeks (**Supplementary table 1**). The MD (95%CI) between two groups (Group-I versus Group-III) and (Group-II versus Group-III) in VAS-pain scores at 6- and 12- weeks have been presented in Table 3**.**

The mean scores of KOOS parameters (pain, symptom, ADL, sports/ recreation, QoL) at baseline and follow-up visits of all three groups have been presented in Table 3. The trend in individual KOOS parameters' scores over time in 3 groups has been reported in Fig 4. Repeated measure ANOVA showed significant changes in all KOOS parameters in Group-I and II (**Supplementary table 1**). The MD (95%CI) between two groups (Group-I versus Group-III) and (Group-II versus Group-III) in KOOS parameters at 6- and 12-weeks have been presented in Table 3.

The mean scores of ESR and s-CRP at baseline and follow-up visits of all three groups have been presented in Table 3. In addition, the change in ESR and individual biomarker levels over time in 3-groups are reported in **supplementary table 2**.

The mean scores of the cartilage turnover markers (u-CTX-II, u-NTX-I, s-COMP, s-PIIANP, s-HA) at baseline and follow-up visits of all three groups have been presented in Table 3. The mean changes in cartilage turnover markers over a period of time can be found in **Supplementary table 2.**

Table 4 demonstrates the overall prevalence of USG abnormalities at V0 and V2. The measurement of cartilage thickness has been demonstrated in **Supplementary figures S1-S3**.

Table 2
Biological Characteristics of PRP

	Group-I [IA-PRP] [Mean (SD)] (n=32)	Group-II [IA+IO-PRP] [Mean(SD)] (n=32)
Blood		
The volume of whole blood collected, mL	54	108
Platelet concentration, 10^9/ L	194.50(29.56)	194.84(29.40)
Leucocyte concentration, 10^9/ L	6.75(1.14)	6.66(1.04)
Platelet-Rich Plasma		
Total volume injected (mL)	8	18
Platelet concentration, 10^9/ L	694.44(101.98)	687.25(109.73)
Leucocyte concentration, 10^9/ L	17.22(2.78)	16.56(2.74)
Mishra classification †	Type 1B	Type 1B
PAW classification ‡	P2-Aα	P2-Aα
Growth factors		
PDGF-AB concentration, ng/mL	30.16(8.34)	31.57(7.71)
TGF-β concentration, ng/mL	94.54(15.07)	96.10(15.18)

IA-PRP, intra-articular platelet-rich plasma; IA+IO-PRP, intra-articular intraosseous platelet-rich plasma; PDGF-AB, platelet-derived growth factor-AB; TGF-β, transforming growth factors beta
† Mishra classification: According to Mishra classification system of Mishra A et al [25].
‡ PAW classification: According to the PAW classification system of Jm D et al [26].

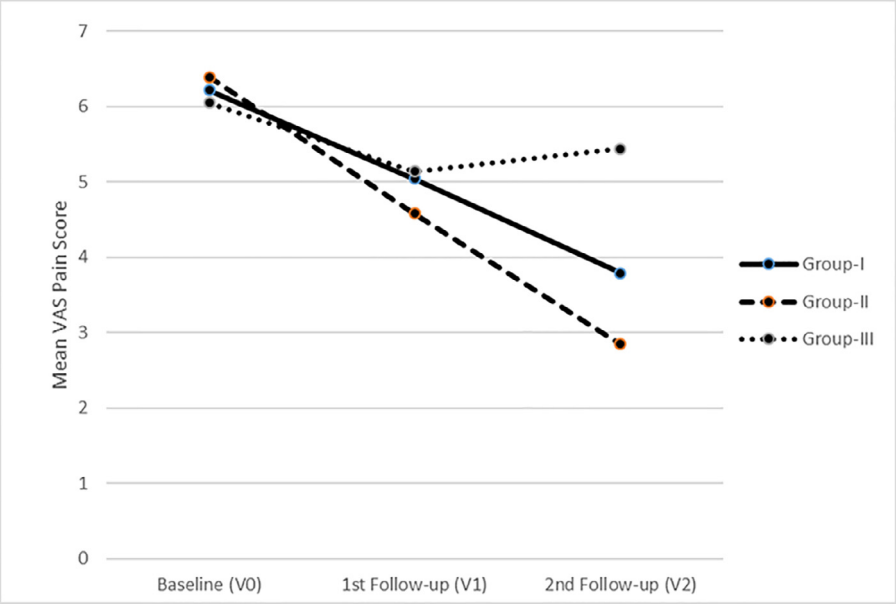


Fig 3. Trends in mean pain scores (VAS) of all groups at baseline and subsequent follow-ups.

Discussion

Compared to 'IA-NS' injections, both IA-PRP and IA+IO-PRP injections showed significant improvement in knee pain and functional activities at 12 weeks. Persons who received IA-NS injections showed no significant changes in clinical parameters at 12 weeks. The study findings were consistent with the articles published on IA-PRP injections in OA knee [10-12,29].

Persons who received IA+IO-PRP injections showed relatively better pain relief and KOOS parameters than IA-PRP injections at V1 and V2. Recently, a few studies [6,25,30-32] were conducted on IA+IO-PRP injection in persons with OA knee. These studies [6,25,30,31] reported additional extra benefits with IA-IO-PRP injections. Similar to their results, we also observed a similar trend in this study. Simultaneous application of 'intra-osseous' and 'intra-articular' PRP injections provided extra growth factors in the subchondral bone/ marrow. Therefore, the direct administration of PRP solution in subchondral bone probably created a better anti-inflammatory response, effectively reduced knee pain, and improved KOOS sub-scores.

The CRP is a valuable inflammatory biomarker linked to synovial inflammation [14,16,17]. This study observed significant changes in ESR and CRP levels in Groups -I and -II following PRP injections. These findings were not surprising as PRP has an anti-inflammatory effect [33]. Cytokines and growth factors present in the PRP solution inhibit the NF-kB signaling pathway (central regulator of the inflammatory path) and reduce the synthesis of IL-1β and TNF-α, which ultimately helps interrupt the inflammatory process [34]. In this study, the PRP solution had an adequate number of platelets and growth factors. The PRP solution was rich in leucocytes.

C-telopeptide-type-II (collagen type-II fragments) and NTX-I (collagen type-I fragments) are the collagenous proteins produced during damage/breakdown of the subchondral bone and articular cartilage [19,20]. In contrast, COMP is a non-collagenous protein resulting from cartilage breakdown [19,20]. In this study, we observed significant reductions in u-CTX-II levels following IA-PRP and u-CTX-II and u-NTX-I levels after IA+IO-PRP injections at 12 weeks; which suggests that compared to NS injections, both PRP injection groups, especially IA+IO-PRP injection group can

Table 3
Primary and Secondary Efficacy Outcomes in Per-Protocol Population

	Group-I [IA-PRP]	Group-II [IA+IO-PRP]	Group-III [IA-NS]	P-Value Between Groups	[Group-I] Vs [Group-III]	[Group-II] Vs [Group-III]
	Mean(SD)	Mean(SD)	Mean(SD)		P value; (95% CI)	P value; (95% CI)
Pain VAS †						
Baseline	6.22(0.96), (*n*=32)	6.39(1.15), (*n*=32)	6.05(1.06), (*n*=32)	0.43	0.74; [-0.42 to 0.77]	0.33; [-0.25 to 0.94)
6-Weeks	5.04(0.87), (*n*=26)	4.58(1.57), (*n*=26)	5.14(1.24), (*n*=25)	0.24	0.94; [-0.90 to 0.69]	0.20;[-1.36 to 0.23]
12-Weeks	3.79(1.21), (*n*=26)	2.85(1.29), (*n*=26)	5.44(1.94), (*n*=25)	0.00	0.00; [-2.60 to -0.70]	0.00; [-3.55 to -1.64]
KOOS Pain ‡						
Baseline	49.22(10.43), (*n*=32)	49.09(9.55), (*n*=32)	49.71(8.88), (*n*=32)	0.96	0.97; [-5.91 to 4.92]	0.95; [-6.03 to 4.80]
6-Weeks	64.31(10.12), (*n*=26)	65.96(11.67), (*n*=26)	55.92(14.96), (*n*=25)	0.01	0.03; [0.57 to 16.20]	0.01; [2.23 to 17.85]
12-Weeks	73.38(9.13), (*n*=26)	76.19(8.76), (*n*=26)	55.16(13.13), (*n*=25)	0.00	0.00; [11.60 to 24.84]	0.00; [14.41 to 27.65]
KOOS Symptom ‡						
Baseline	51.87(11.80), (*n*=32)	50.59(10.41), (*n*=32)	51.22(11.26), (*n*=32)	.90	0.96; [-5.62 to 6.93]	.96; [-5.62 to 6.93]
6-Weeks	68.96(10.27), (*n*=26)	69.58(8.54), (*n*=26)	66.80(9.18), (*n*=25)	.54	0.62; [-3.75 to 8.07]	.47; [-3.13 to 8.69]
12-Weeks	77.11(8.84), (*n*=26)	78.08(8.03), (*n*=26)	58.88(19.08), (*n*=25)	.00	0.00; [10.10 to 26.37]	.00; [11.06 to 27.34]
KOOS ADL ‡						
Baseline	47.28(9.52), (*n*=32)	47.66(9.66), (*n*=32)	47.34(8.92), (*n*=32)	0.98	1.00; [-5.32 to 5.20]	0.99; [-4.95 to 5.57]
6-Weeks	57.61(9.96), (*n*=26)	57.23(10.93), (*n*=26)	53.68(11.91), (*n*=25)	.37	0.34; [-2.98 to 10.85]	0.41; [-3.36 to 10.46]
12-Weeks	65.27(9.36), (*n*=26)	67.31(8.61), (*n*=26)	50.00(14.20), (*n*=25)	.00	0.00; [8.35 to 22.19]	0.00; [10.39 to 24.22]
KOOS Sports/Rec ‡						
Baseline	29.19(11.73), (*n*=32)	30.06(9.92), (*n*=32)	30.94(9.97), (*n*=32)	0.80	0.73; [-7.69 to 4.19]	0.92; [-6.81 to 5.06]
6-Weeks	48.04(9.40), (*n*=26)	47.08(9.41), (*n*=26)	45.20(9.52), (*n*=25)	0.55	0.46; [-3.12 to 8.80]	0.70; [-4.08 to 7.84]
12-Weeks	55.69(6.64), (*n*=26)	55.69(8.34), (*n*=26)	39.56(14.76), (*n*=25)	0.00	0.00; [9.54 to 22.73]	0.00; [9.54 to 22.73]
KOOS QoL Score ‡						
Baseline	46.28(11.53), (*n*=32)	44.78(10.44), (*n*=32)	45.69(10.84), (*n*=32)	0.86	0.97; [-5.55 to 6.74]	0.92; [-7.05 to 5.24
6-Weeks	59.85(9.15), (*n*=26)	59.38(11.57), (*n*=26)	53.28(12.15), (*n*=25)	0.07	0.07; [-0.39 to 13.52]	0.09; [-0.85 to 13.06]
12-Weeks	70.11(7.49), (*n*=26)	74.11(6.68), (*n*=26)	48.92(16.94), (*n*=25)	0.00	0.00; [14.08 to 28.31]	0.00; [18.08 to 32.31]
ESR ¥						
Baseline	13.25(5.72), (*n*=32)	14.16(5.12), (*n*=32)	13.81(6.39), (*n*=32)	0.82	0.89; [-3.80 to 2.67]	0.96; [-2.89 – 3.58]
6-Weeks	10.65(4.36), (*n*=26)	11.92(4.12), (*n*=26)	10.96(5.09), (*n*=25)	0.58	0.96; [-03.17 to 2.58]	0.67; [-1.90 to 3.83]
12-Weeks	8.15(3.93), (*n*=26)	7.65(2.67), (*n*=26)	11.24(6.22), (*n*=25)	0.01	0.03; [-5.92 to -0.25]	0.01; [-6.42 to -0.75]
C-Reactive Protein (CRP) ¥						
Baseline	6.36(3.53), (*n*=32)	6.58(2.95), (*n*=32)	6.57(3.39), (*n*=32)	0.96	0.95; [-2.06 to 1.64]	1.00; [-1.84 to 1.87]
6-Weeks	5.11(3.12), (*n*=26)	4.58(2.68), (*n*=26)	6.14(2.16), (*n*=25)	0.12	0.30; [-2.72 to 0.67]	0.08; [-3.25 to 0.14]
12-Weeks	3.5(2.40), (*n*=26)	3.13(2.14), (*n*=26)	7.10(3.07), (*n*=25)	0.00	0.00; [-5.19 to -1.96]	0.00; [-5.59 to -2.36]
s-HA €						
Baseline	5.96(2.90), (*n*=32)	6.21(2.73), (*n*=32)	6.59(2.56), (*n*=32)	.645	0.55; [-2.17 to 0.90]	0.80; [-1.92 to 1.15]
6-Weeks	5.58(2.17), (*n*=26)	5.38(2.26), (*n*=26)	6.07(2.61), (*n*=25)	.560	0.68; [-1.97 to 1.00]	0.47; [-2.18 to .79]
12-Weeks	5.29(2.23), (*n*=26)	5.14(2.11), (*n*=26)	7.47(4.72), (*n*=25)	.020	0.03; [-4.21 to -0.14]	0.02; [-4.36 to -0.29]
u-CTX €						
Baseline	331.60(110.38), (*n*=32)	330.00(98.33), (*n*=32)	333.11(99.19), (*n*=32)	0.99	0.99; [-59.23 to 56.20]	0.99; [-60.83 to 54.60]
6-Weeks	322.86(119.25), (*n*=26)	316.31(101.86), (*n*=26)	334.99(104.56), (*n*=25)	0.82	0.89; [-80.87 to 56.61]	0.76; [-87.42 to 50.06]
12-Weeks	298.24(112.48), (*n*=26)	296.39(106.52)	348.01(107.16), (*n*=25)	0.17	0.18; [-118.44 to 18.90]	0.16; [-120.29 to 17.05]
u-NTX €						
Baseline	82.96(31.62), (*n*=32)	83.71(29.16), (*n*=32)	84.17(30.63), (*n*=32)	0.99	0.98; [-18.32 to 15.91]	1.00; [-17.57 to 16.66]
6-Weeks	81.66(34.92), (*n*=26)	81.25(32.75), (*n*=26)	86.47(35.76), (*n*=25)	0.84	0.83; [-26.59 to 16.96]	0.81; [-26.99 to 16.55]
12-Weeks	80.04(33.49), (*n*=26)	78.39(33.61), (*n*=26)	89.30(34.99), (*n*=25)	0.47	0.52; [-30.74 to 12.22]	0.41; [-32.40 to 10.57]
s-COMP €						
Baseline	762.63(262.07), (*n*=32)	789.41(268.67), (*n*=32)	774.20(239.41), (*n*=32)	0.92	0.98; [-155.90 to 132.76]	0.96; [-129.12 to 159.53]
6-Weeks	785.37(261.68), (*n*=26)	791.51(264.56), (*n*=26)	763.45(258.73), (*n*=25)	0.92	0.94; [-143.30 to 187.14]	0.90; [-137.16 to 193.27]
12-Weeks	777.84(258.81), (*n*=26)	785.96(252.87), (*n*=26)	767.71(265.92), (*n*=25)	0.97	0.99; [-153.49 to 173.75]	0.95; [-145.37 to 181.86]
s-PIIANP £						
Baseline	5.20(3.73), (*n*=32)	5.18(3.38), (*n*=32)	5.48(3.25), (*n*=32)	0.93	.93; [-2.22 to 1.67]	0.92; [-2.24 to 1.64]
6-Weeks	5.63(3.47), (*n*=26)	5.63(3.25), (*n*=26)	5.14(3.27), (*n*=25)	0.83	.82; [-1.61 to 2.60]	0.82; [-1.61 to 2.60]
12-Weeks	5.81(4.11), (*n*=26)	5.77(3.40), (*n*=26)	4.64(3.11), (*n*=25)	0.42	.40; [-1.08 to 3.43]	0.42; [-1.13 to 3.38]

IA-PRP, Intra-articular platelet-rich plasma; IO+IA-PRP, Intra-articular intraosseous platelet-rich plasma; IA-NS, Intra-articular normal saline; VAS, visual analog scale; KOOS, Knee Injury, and Osteoarthritis Outcome Score; QoL, Quality of life; SD, standard deviation; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; s-HA, serum hyaluronic acid; u-CTX-II, urinary type-II collagen C telopeptide; u-NTX-I, urine Type-I collagen N- telopeptide; s-COMP, serum cartilage oligomeric matrix protein; s-PIIANP, serum N-terminal propeptide of type-IIA collagen

† Score range from 0-10, a lower value indicating less pain

‡ Score range from 1-100, a higher value indicating less knee problem

¥ A higher value indicating more inflammatory activities

€ A higher value indicating more cartilage/ bone degradation

£ collagen type-II synthesis biomarker, a higher value indicating cartilage formation

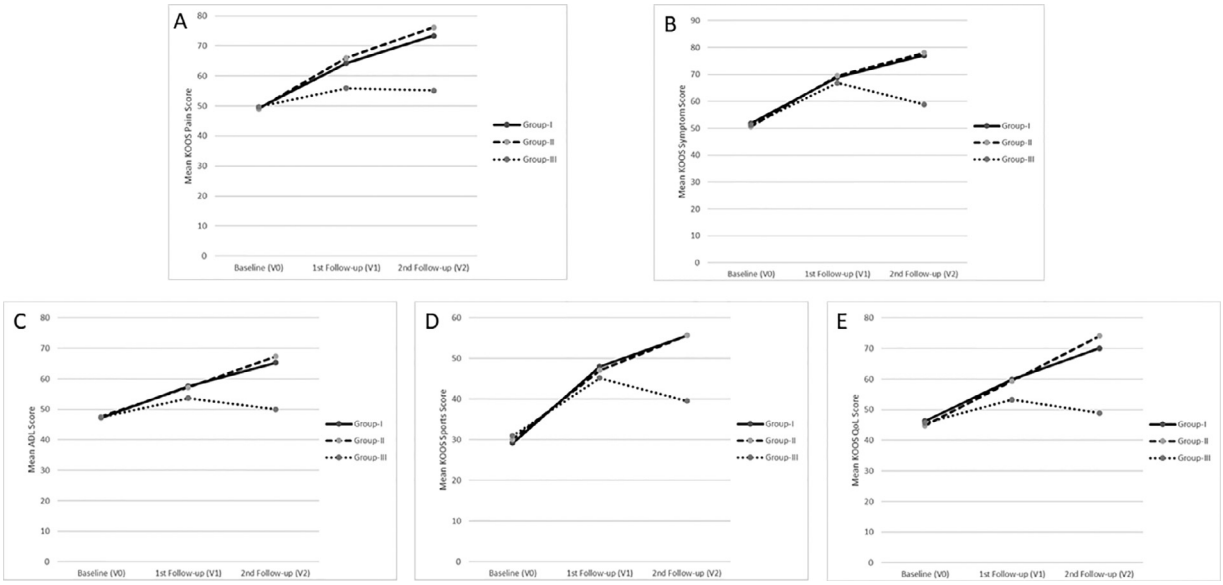


Fig 4. Trends in mean KOOS parameters' scores of all groups at baseline and subsequent follow-ups. (A) mean KOOS pain score; (B) mean KOOS symptoms; (C) mean KOOS ADL scores; (D) mean sports score; (D) mean QoL score.

Table 4
The overall prevalence of USG abnormalities in Per-Protocol Population at two-time points

	Group I [IA-PRP] [No./total (%)][a]	Group II [IA+IO-PRP] [No./total (%)][a]	Group III [IA-NS] [No./total (%)][a]	P-Value[c] (Between three groups)	[Risk Ratio (95% CI)][d] / [Mean difference(95%CI)][e] (Group-I Vs. Group-III)	[Risk Ratio (95% CI)][d] / Mean difference(95%CI) (Group-II Vs. Group-III)
Presence of effusion (≥4mm)[₡]						
Baseline	[18/32(56.2%)][a]	[19/32(59.4%)][a]	[18/32(56.2%)][a]	0.96[c]		
12-Weeks	[7/26(26.9%)][a]	[5/26(19.2%)][a]	[13/25(52%)][a]	**0.03**[c]	[0.52(0.25–1.08)][d]; p=0.08[f]	**[0.37(0.15- 0.89)][d]; p=0.03**[f]
Presence of synovial hypertrophy (≥2mm)[£]						
Baseline	[19/32(59.4%)][a]	[20/32(62.5%)][a]	[20/32(62.5%)][a]	0.96[c]		
12-Weeks	[14/26(53.8%)][a]	[15/26(57.7%)][a]	[19/25(76%)][a]	0.22[c]	[0.71(0.47-1.08)][d]; p=0.11[f]	[0.76(0.51-1.13)][d]; p=0.17[f]
Presence of meniscal protrusion (3mm)[€]						
Baseline	[15/32(46.9%)][a]	[17/32(53.1%)][a]	[16/32(50%)][a]	0.88[c]		
12-Weeks	[14/26(53.8)][a]	[17/26(65.4%)][a]	[15/25(60%)][a]	0.70[c]	[0.90(0.56-1.45)][d]; p=0.66[f]	[1.09(0.71-1.67)][d]; p=0.69[f]
Presence of infrapatellar bursitis (>2mm)[£]						
Baseline	[14/32(43.8%)][a]	[13/32(40.6%)][a]	[13/32(40.6%)][a]	0.96[c]		
12-Weeks	[8/26 (30.8%)][a]	[6/26 (23.1%)][a]	[14/25(56%)][a]	**0.04**[c]	[0.55(0.28-1.08)][d]; p=0.08[f]	**[0.41(0.19-0.90)][d]; p=0.03**[f]
Presence of Baker's cyst[¥]						
Baseline	[6/32(18.8%)][a]	[7/32(21.9%)][a]	[5/32(15.6%)][a]	0.81[c]		
12-Weeks	[4/26(15.4%)][a]	[3/26(11.5%)][a]	[5/25(20%)][a]	0.71[c]	[0.80(0.24-2.65)][d]; p=0.71[f]	[0.60(0.16-2.25)][d]; p=0.76[f]
Cartilage thickness (mm)[‡]						
Baseline [mean (SD)]	[1.54(0.43)][b]	[1.56(0.32)][b]	[1.53(0.33)][b]	0.96[c]		
12-Weeks [mean (SD)]	[1.49(0.48)][b]	[1.59(0.36)][b]	[1.42(0.33)][b]	0.33[c]	[-0.07(-0.30 to 0.16)][e]; p=0.51[f]	[-0.17(-0.36 to 0.03)][e]; p=0.08[f]

USG, ultrasonography; IA-PRP, Intra-articular platelet-rich plasma; IO+IA-PRP, Intra-articular intraosseous platelet-rich plasma; IA-NS, Intra-articular normal saline

[₡] Effusion – was considered as present if there was ≥4mm hypoechoic or anechoic intra-articular material that was compressible and displaceable, present in the suprapatellar recess (USG was done on knee in full extension)

[£] Synovial hypertrophy - was considered as present if there was ≥2mm abnormal hypoechoic intra-articular material that was poorly-compressible and non-displaceable, present in the suprapatellar recess (USG was done in knee on full extension)

[€] Meniscal Protrusion - was considered as present if there was protrusion of meniscal tissue out of the joint space >3mm from the joint line, present in medial joint space (USG was done on knee in full extension)

[£] Infrapatellar bursitis - was considered as present if there was an enlarged infrapatellar bursa (>2mm) on both transverse and longitudinal scans (USG was done on knee in 45° flexion)

[¥] Baker's cyst - was considered as present if there was a hypo-anechoic area in between the semimembranosus and medial gastrocnemius tendon (USG was done on popliteal region in prone position)

[‡] Cartilage thickness was measured at femoral condyle. Cartilage thickness was measured perpendicular to the surface at three points – at intercondylar notch, medial and lateral condyle (5 mm just medial or lateral from the top of the condyle), with the knee in maximum flexion position. The average cartilage thickness (mean value of the cartilage thickness) was calculated from thicknesses measured at 3-points.

[a] Counts and proportions are based on complete case data

[b] Cartilage thickness was measured at femoral condyle. It is presented as mean(SD)

[c] P-value between three groups (Group-I versus Group-II versus Group-III)

[d] Reported as risk ratio (95% Confidence Interval) with P value between two groups

[e] Mean differences (95% Confidence Interval) with P value between two groups

[f] P-value between two [(Group-I versus Group-III) or (Group-II versus Group-III)] groups

reduce/halt the bony and cartilage degradation process till 12-weeks. However, the study failed to demonstrate a significant reduction (compared to NS injection) in s-COMP levels or an increase in s-PIIANP levels following PRP injections. The PIIANP is a collagen type-II synthesis biomarker [19,20].

Musculoskeletal USG is one of the most convenient options for visualizing the articular and peri-articular soft tissue structures [23,24]. The findings from the USG knee have good construct validity [23,35] and inter-observer reliability [23,24]. In this study, we observed significant changes in the number of persons with synovial effusion and bursitis among persons who received IA+IO-PRP injections, implying that a single IA+IO-PRP injection might reduce/ prevent inflammatory changes in the knee joint at 12 weeks. However, we did not observe significant differences among the three groups in other aspects, like meniscal protrusion, baker's cyst, and cartilage thickness.

An important strength of this study was an RCT, which compared the efficacy of PRP injections with NS (placebo) injections. Quantitative assessments of inflammatory and bony biomarkers were done at frequent intervals. Fasting blood and urine samples for measuring biomarkers were collected strictly between 9-10 AM to avoid diurnal variation of OA biomarkers. This study was the first to evaluate biomarkers following IA- and IA+IO- PRP injections in the OA knee. All PRP injections were administered within one hour of preparation. The cells (leucocyte, platelet) and growth factors (PDGF-AB and TGF-β) from PRP were assessed quantitatively. The USG evaluations were performed before and end of the study. All injections were administered under a fluoroscope. All three groups were comparable in demographic variables and baseline characteristics.

The study had several limitations. First, each group had a significant dropout due to the breaking out of coronavirus disease (COVID-19) during the study period. Second, the study duration was limited to 12 weeks, focusing on the immediate changes in biomarkers. Third, the persons were not blinded to the intervention procedure, as it was challenging to blind those receiving IA+IO-PRP injections under GA. Fourth, persons recruited in Group-II received antibiotics, whereas the other two groups (Groups -I and -III) did not. However, there is no direct evidence that a single dose of antibiotic can cause pain relief, functional improvement, and changes in the levels of inflammatory and cartilage-turnover biomarkers at 6 and 12 weeks following injections.

Conclusion

Over the period (12 weeks) following PRP injections, both groups (Groups -I and -II) reported significant pain relief and improvement in knee functional activities. Compared to baseline parameters, at 12 weeks, both groups (Groups -I and -II) reported a significant reduction in inflammatory (ESR, CRP) and cartilage-degradation (u-CTX) biomarkers. On inter-group comparisons, persons who received IA+IO-PRP injections reported better clinical outcomes in pain relief, functional activities, and reduction of inflammatory activities, including USG knee findings. However, more RCTs with long duration and large populations are required to confirm the results and to investigate the persistence of the beneficial effects following PRP injections.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.injury.2022.11.036.

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