DOES THE DIFFERENCE IN LEUKOCYTE CONCENTRATION OF PRP AFFECT THE SHORT-TERM FOLLOW-UP RESULTS IN CASES DIAGNOSED WITH EARLY STAGE KNEE OSTEOARTHRITIS?

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DA LI RAZLIKA U KONCENTRACIJI LEUKOCITA PRP UTIČE NA REZULTATE KRATKOROČNOG PRAĆENJA U SLUČAJEVIMA KADA JE DIJAGNOSTIKOVAN OSTEOARTRITIS KOLENA U RANOJ FAZI?

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ABSTRACT

This prospective study was conducted for the clinical evaluation of pain severity and knee functionality following PRP injections with different leukocyte (WBC) concentrations applied to cases diagnosed with knee osteoarthritis. A total of 109 patients were included in the study. According to the leukocyte content the PRP injections were prepared as low-leukocyte content PRP (P-PRP) and high concentration leukocyte content PRP (L-PRP). Patients were divided into 2 groups. Group 1 (n=44) received low-leukocyte content PRP and Group II (n =65) received high-leukocyte content PRP. The patients were evaluated clinically with Visual Analog Scale (VAS) and Knee Society Score (KSS). The changes in the PLT levels of the L-PRP group after the procedure compared to the levels prior to the procedure were found to be statistically significantly greater than the changes in the P-PRP group. The mean VAS score of all the cases before treatment was 9.05±0.91 and this score decreased to 3.71±1.46 within 12 months. The increases in the mean Knee Society Score (KSS) values were determined as 16.92±1.97 within 6 months and 16.89±2.97 within 12 months in the P-PRP group and 19.71±1.24 within 6 months and 19.86±0.42 within 12 months in the L-PRP group. The most important aspect of this study is that, in contrast to many other studies, the results continued after the 6th month and were reported to be good in the 12th month. It was also recorded that L-PRP was clinically superior to P-PRP in the treatment of early stage knee osteoarthritis.

Keywords: *leukocyte, platelet rich plasma, knee osteoarthritisspleen*

SAŽETAK

Ovo prospektivno istraživanje sprovedeno je sa ciljem određivanja kliničke procene ozbiljnosti bola i funkcionalnosti kolena posle PRP injekcija sa različitim koncentracijama leukocita (VBC) primenjenih na slučajeve u kojima je dijagnostikovan osteoartritis kolena. U ispitivanje je uključeno 109 pacijenata PRP injekcije su pripremljene u odnosu na sadržaj leukocita kao sadržaj PRP sa niskim leukocitima (PR-PP) i visokom koncentracijom leukocita PRP (L-PRP). Pacijenti su razdvojeni u 2 grupe. Grupi 1 (n = 44) je dat PRP sa niskim sadržajem leukocita dok je II grupi (n = 65) dat PRP sa visokim sadržajem leukocita. Bolesnici su klinički procenjeni sa VASi KSS. Rezultati: Utvrđeno je da su promene nivoa PLT grupe L-PRP posle postupka u odnosu na stanje pre postupka statistički značajno veće od promena u P-PRP grupi. Prosečan rezultat VAS za sve slučajeve pre lečenja bio je 9,05±0,91 i ovaj rezultat je smanjen na 3,71±1,46 tokom 12 meseci. Povećanja srednjih vrednosti KSS određena su kao 16,92±1,97 za 6 meseci i 16,89±2,97 u 12 meseci u grupi P-PRP i 19,71±1,24 u 6 meseci i 19,86±0,42 na 12 meseci u L-PRP grupi. Najvažniji aspekt ove studije bio je da se, za razliku od mnogih drugih studija, ustanovi da se rezultati nastavljaju nakon šestog meseca i da su dobri u 12. mesecu, a zabeleženo je da je L-PRP klinički superiorniji od P-PRP u lečenju osteoartritisa kolena u ranoj fazi.

Ključne reči: *leukociti, plazma bogata trombocitima, osteoartritis kolena*

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INTRODUCTION

Current pharmacological or surgical treatment methods do not provide the desired level of good results for pathologies of the skeletal system, such as knee osteoarthritis which persists to be a clinical and societal problem (1–3). Therefore, the search for different treatment methods continues. One of these methods includes the activation of biological repair mechanisms so that damaged tissues can heal and repair themselves (1). In recent years, the application of platelet rich plasma (PRP) for biological repair has become more widely used. Platelets (PLT), as being a component of this compound, are known to contain a high amount of cytokines and growth factors which are very important in tissue healing and bone mineralisation (4). Therefore, local injections of PRP are used for biological repair in the field of orthopaedic surgery, as in all medical fields (5, 6). This method has been included in the treatment protocol of knee osteoarthritis (7). In some publications it has been reported that PRP with different content ratios is more advantageous therapy in clinical treatments (8).

However, there has been no study with a clear high level of evidence investigating the separate effects of the PLT and leukocyte (WBC) ratios in PLT concentrations included in PRP. To what extent the WBC ratio is responsible for the therapeutic positive or negative clinically observed effects has not been sufficiently clarified (9, 10).

This prospective study was conducted for the clinical evaluation of pain severity and knee functionality following PRP injections with different WBC concentrations applied to cases diagnosed with knee osteoarthritis. This research aims to obtain the data that could contribute to the literature regarding PRP applied with different concentrations of WBC content to patients with knee osteoarthritis.

MATERIAL AND METHOD

Approval for the study was granted by the University Ethics Committee with decision no 10840098-155. Informed consent was obtained from all patients. The inclusion criteria for this study were a diagnosis of primary knee osteoarthritis according to the American College of Rheumatology criteria (11) classified as Kellgren-Lawrence (12) Grade 1 and Grade 2, that had not responded to conservative treatment such as physiotheraphy and paracetamol, nonsteroidal anti-inflammatory drug treatment. A total of 164 patients in our clinic met these criteria. Patients were excluded if they had any patellofemoral or meniscal pathology determined by MRI in the knee where the injection was to be administered. As a result of the hemogram evaluation, patients with hematocrit <42% (n=9) or >52% (n=3) were excluded. Patients were also excluded if within the previous 2 weeks they had taken any nonsteroid anti-inflammatory drugs (n=18), or antithrombotic or anticoagulant drugs (n=16), if they were taking oral anti-diabetic drugs or if they had consumed alcohol or smoked within the previous 48 hours (n=6). One pregnant patient and one with an inflammatory rheumatological disease were also excluded. A total of 109 patients were included for evaluation in the study.

The PRP injections were prepared according to the leukocyte content as low-leukocyte content PRP (P-PRP) and high concentration leukocyte content PRP (L-PRP) (13). The classification according to the number of leukocytes in the PRP to be applied was made according to the Dohan Ehrenfest DM et al classification (10). The final PRP obtained from both commercial kits was prepared according to their recommended steps and guidelines. 0.5 ml of buffy coat sample was assigned for trombocyte analysis from each kit. A double-spin centrifugation process was performed at speed of 5000 rpm. Spinning time was 6 minutes for PRP preparation.

Three cycles of PRP were applied in patients who were divided into 2 groups. Group 1 (n=44) receieved low-leukocyte content PRP and Group II (n=65) received high-leukocyte content PRP. The second and third doses of the injections were applied at intervals of 10 days in both groups. During each PRP administration, the thrombocyte and white cell counts were determined from the blood samples taken from each patient. After preparation of the PRP, the thrombocyte and white cell counts were determined in the content of the PRP to be injected into the knee.

No topical or local anaesthetic pharmacological agent was applied during the injections. On the first day after injection administration, patients experiencing pain were recommended to use an ice compress and no physical rehabilitation program was recommended for any patient throughout the duration of the study.

The patients were evaluated clinically in respect of the severity of pain and knee functionality prior to the application of PRP and in 6 months and 12 months after the administration. In the determination of the severity of pain, Visual Analog Scale (VAS) scoring was used (14), and knee functionality was evaluated by the Knee Society Score (KSS) (15, 16).

Statistical analysis

The analysis of the data obtained in the study was conducted using the Statistical Package for the Social Sciences (SPSS) version 20.0 software. Descriptive statistics were shown as mean ± standard deviation (SD) and/or number (n) and percentage (%). As the research sample was formed from large volume samples (n>30), single and two sample hypotheses associated with independent parameters were formed using the Student's t-test. The Pearson Chi-square test was used to determine whether there was a correlation between variables in independent groups,where there was a conformity to specific theoretical possibility distribution of the sample results, whether the samples came from the same main mass and whether the rates of more than two main masses were equal. Homogeneity between groups, regardless of whether there was a difference between two or more groups or whether the difference between observed and expected frequencies was significant, was analysed on the

basis of the Yates Continuity Correction Test. The statistical analysis tests resulted in a yes/no response to the hypothesis (e.g., are there significant differences between the groups with $p<0.05$). A value of $p<0.01$ was considered to be highly statistically significant.

RESULTS

Evaluation of the demographic characteristics of the study participants

This study comprised 19 male and 90 female patients in the age range between 40 and 70. The demographic data of the cases and the findings of the radiological grading were reported on a standard form (Table 1).

No statistically significant difference was determined between the groups in respect of age, gender, or body mass index (BMI) ($p>0.05$).

Knee osteoarthritis was determined to be Grade 1 in 22 knee cases and Grade 2 in 87 knee cases. In 45 cases osteoarthritis was identified in the right knee, and in 64 cases in the left knee.

Evaluation according to the leukocyte and thrombocyte count of the PRP content

Following preparation of P-PRP, the mean platelet values before the 1st, 2nd and 3rd injections were found to be 971.86±292.15, 926.86±264.54 and 899.86±282.33 respectively and the WBC values were 5.02±4.65, 5.28±4.61 and 5.49±4.93. In the L-PRP group, the PLT values were determined as 1628.40±833.15, 1464.00±594.24 and 1578.60±796.32 respectively and the WBC values as 30.05±12.66, 27.14±10.36 and 28.16±10.75 (Table 2, Table 3).

Table1. Demographic characteristics of patients according to groups

		Total (n = 109)	P-PRP group (n = 44)	L-PRP group (n = 65)
		Mean ± SD	Mean ± SD	Mean ± SD
Age (years)		63.69±8.09	63.52±8.52	63.8±7.86
BMI (kg/m^2)		27.92±3.07	28.32±3.08	27.65±3.06
Track length (months)		13.76±2.46	13.07±2.25	14.23±2.50
		n (%)	n (%)	n (%)
Gender	Male	19 (17.4)	6 (13.6)	13 (20.09)
	Female	90 (82.6)	38 (86.4)	52 (80.0)
BMI level	Normal	14 (12.8)	4 (9.1)	10 (15.4)
	Overweight	68 (62.4)	28 (63.69)	40 (61.5)
	obese	27 (24.8)	12 (27.3)	15 (23.1)
Kellgren-Lawrence radiological criteria	Stage 1	17 (15.6)	6 (13.6)	11 (16.9)
	Stage 1	92 (84.4)	38 (86.4)	54 (83.1)

Table 2. Platelet counts before and after injection by groups

	P-PRP group			L-PRP group		
Platelet Count (10^3/mm^3)	Before procedure	After procedure	Difference	Before procedure	After procedure	Difference
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1st measurement	226.14±70.69	971.86±292.15	745.73±271.05	218.28±62.97	1628.40±833.15	1410.12±799.18
2nd measurement	224.82±67.95	926.86±264.54	702.05±248.86	206.65±54.63	1464.00±594.24	1257.35±573.19
3rd measurement	231.55±75.05	899.86±282.33	668.32±246.72	211.15±59.26	1578.60±796.32	1367.45±771.61

Table 3. Leukocyte counts before and after injection by groups

Leukocyte counts (10^3/mm^3)	P-PRP group			L-PRP group		
	Before procedure	After procedure	Difference	Before procedure	After procedure	Difference
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
1st measurement	5.90±1.66	5.02 ±4.65	-0.88±4.27	5.88±1.60	30.05±12.66	24.17±11.92
2nd measurement	5.90±1.40	5.28±4.61	-0.62±4.25	5.50 ±1.25	27.14±10,36	21.64±9.75
3rd measurement	5.99±1.50	5.49±4.93	-0.50±4.55	5.62±1.32	28.16±10.75	22.54±10.21

As a result of the statistical evaluation of the leukocyte counts in the P-PRP group, there was a decrease in the leukocyte measurements by mean 0.88±4.27 following the 1st PRP application compared to the mean prior to the procedure. The decrease in leukocyte measurements after the 2nd PRP was mean 0.62±4.25 and the reduction in leukocyte counts after the 3rd PRP injection was mean 0.50±4.55. The differences in all of these measurements before and after each injection were statistically significant ($p<0.01$). No statistically significant difference was determined between the leukocyte levels measured before the 1st, 2nd and 3rd injection ($p>0.05$). No statistically significant difference was determined among the leukocyte levels measured after the 1st, 2nd and 3rd injection ($p>0.05$).

As a result of the statistical evaluation of the leukocyte counts in the L-PRP group, there was an increase in the leukocyte measurements by mean 24.17±11.92 following the 1st PRP application compared to the mean prior to the procedure. The increase in leukocyte measurements after the 2nd PRP was mean 21.64±9.75 and the reduction in leukocyte counts after the 3rd PRP injection was mean 22.54±10.21. The differences in all of these measurements before and after each injection were statistically significant ($p<0.01$).

In the comparison of the two groups in respect of leukocyte count, no statististically significant difference was determined in the leukocyte level before the 1st application, the 2nd application and the 3rd application ($p>0.05$).

The PLT levels of the L-PRP group were found to be statistically significantly higher than those of the P-PRP group in the measurements taken after the 1st application, the 2nd application and the 3rd application ($p<0.01$).

The changes in the PLT levels of the L-PRP group after the procedure (compared to the levels before the procedure) at the 1st measurement, the 2nd measurement and the 3rd measurement were found to be statistically significantly greater than the changes in the P-PRP group ($p<0.01$)

Evaluation of the VAS scores

The mean VAS score of all the cases before the treatment was 9.05±0.91 and this score decreased to 3.71±1.46 within 12 months after the procedure. This tendency was observed to be similar in both L-PRP and P-PRP groups and in both the 6-month and 12-month follow-up examinations.

In the L-PRP patient group, the initial VAS score was mean 9.11±0.96 and this score decreased to 6.61±2.41 within 6 months after the procedure and to 3.58±1.54 within 12 months.

In the P-PRP patient group, the initial VAS score was mean 9.02±1.18 and this score decreased to 6.05±0.12 within 6 months after the procedure and to 3.79±1.46 within 12 months. (Table 4).

The reductions in the VAS values in both groups were determined to be statistically significant ($p<0.05$).

Evaluation of the KSS and Functional KSS scoring

In the L-PRP group, the KSS and functional KSS scores before the injection were determined to be 54.31±14.35 and 55.55±10.08 respectively and 77.83±10.69 and 74.22±10.62 in the 12-month follow-up examination. The increases in the KSS and functional KSS scores were found to be statistically significant ($p<0.05$).

In the P-PRP group, the KSS and functional KSS scores before the injection were determined to be 56.48±12.71 and 57.05±10.96 respectively and 78.98±10.52 and 73.86±13.93 in the 12-month follow-up examination. The increases in the KSS and functional KSS scores were found to be statistically significant ($p<0.05$).

The mean increases in the mean KSS measurements within 6 and 12 months after the injections compared to the mean values before the injections were 22.94±2.09 in Group I and 22.82±3.83 in Group II. The increases were determined to be statistically significant ($p<0.05$).

The increases in the mean KSS values were determined as 16.92±1.97 within 6 months and 16.89±2.97 within 12 months in the P-PRP group and 19.71±1.24 within 6 months

and 19.86±0.42 within 12 months in the L-PRP group. These increases were determined to be statistically significant ($p<0.05$). The changes in the KSS functional measurements within 12 months after the injection compared to the baseline values before the procedure showed a significant difference related to the WBC content ($p<0.05$) (Table 4).

Table4. Clinical results of the groups before and after injection

	P-PRP group			L-PRP group		
	Before injection	Last Control	Difference	Before injection	Last Control	Difference
Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Knee Society Scores	56.48±12.71	78.98±10.52	22.50±11.84	54.31±14.35	77.83±10.69	23.52±11.04
Knee Society Scores Functional	57.05±10.96	73.86±13.93	16.82±11.11	55.55±10.08	74.22±10.62	18.67±10.70
Visual Analog Scale	9.09±0.96	3.59±1.56	-5.50±1.78	9.02±0.87	3.78±1.40	-5.23±1.56

DISCUSSION

There has been wide acceptance in the scientific literature that more platelets are contained in PRP obtained after centrifuge processing from autologous full blood compared to normal blood. While the initial spin separates red blood cells from the buffy coat/plasma layer, it has been reported that more PLT can be separated in systems using a second spin. It has been emphasised that the final PLT values of PRP and the WBC concentration are affected by the number of centrifuges, the speed and the duration (17).

Some researchers have advocated that the WBC ratio should be kept at a low concentration in order to be able to obtain effective treatment by reducing the catabolic effect to a minimum (18). On the other hand, with the argument that WBCs as a source of enzymes and cytokines could be effective in the prevention of infection, it has also been stated that high concentrations of WBC should be contained in PRP (19, 20). In the current study, the mean platelet values in the prepared P-PRP before the 1st, 2nd and 3rd injection were 971.86±292.15, 926.86±264.54 and 899.86±282.33 respectively and the WBC values were found to be 5.02±4.65, 5.28±4.61 and 5.49±4.93. In the L-PRP group, the PLT values were determined as 1628.40±833.15, 1464.00±594.24 and 1578.60±796.32 respectively and the WBC values as 30.05±12.66, 27.14±10.36 and 28.16±10.75.

In the study by Yin W et al, which provided valuable data on knee osteoarthritis, pro-inflammatory cytokines at a high level in L-PRP were suggested to have a beneficial effect on growth factors in bone regeneration; therefore P-PRP and L-PRP groups were compared with molecular analyses. Histological examination of in-vivo effects was conducted by creating a calvareal defect in rats. It was observed that better histological results were obtained in the calvareal defects of the P-PRP group compared to the L-PRP group. The WBC content of L-PRP was shown to have created negative effects on bone regeneration by activating pro-inflammatory cytokines. It was concluded that P-PRP could be an effective treatment method in bone regeneration (21). In the current study, although no histological research was conducted , no statistically significant difference was determined in the clinial results between both types of PRP.

In the double-blind randomised controlled study by Duif et al, P-PRP was applied to degenerative lesions and patients were evaluated in respect of pain, functionality and quality of life. Knee arthroscopy for cartilage or meniscus degeneration was applied to a total of 58 patients, comprising 24 patients in the P-PRP group and 34 patients in the control group. At the end of 6 months, pain was significantly reduced in the group administered with P-PRP. However, it was emphasised that the same effect was not observed in 12 months. The data of the Lysholm score in the P-PRP group were reported to have significantly improved in the 6-month and 12-month period. In addition, the physical component scores of the Short Form (SF) -36 norm-based scale were reported to have significantly improved within 6 weeks and 6 months. However, it was stressed that there was no change in the results at the end of one year. It was concluded that the application of P-PRP could lead to a significant improvement in pain and knee functions over the period between 6 and 12 months (6). The results of the current study were similar to the clinical results of that study.

In the study by Patel S et al, 156 knees of 78 patients were divided into 3 groups. PRP preparates used in this clinical study had the PLT concentration 3 times higher than WBC filtered normal.

Group A comprised 52 knees administered with a single PRP injection dose; Group 2 comprised 50 knees administered with 2 doses of PRP; Group C comprised 46 knees administered with a single dose of isotonic sodium chloride (0.9% saline). Clinical evaluations were made using the WOMAC index before treatment, then in 6 weeks, 3 months and 6 months after the injections. The change in pain severity was examined by VAS. In the WOMAC scoring, a statistically significant improvement starting 2-3 weks after the treatment and lasting for up to 6 months was observed However, it was reported that this value showed a slight decrease at the end of 6 months. Similar improvements were observed in Group B, whereas the values in Group C were reported to worsen compared to the baseline. After comparison of the 3 groups, no improvement was reported in Group C compared to Groups A and B. In conclusion, it was emphasised that a single dose of PRP containing 10-fold PLT of WBC filtered normal was as effective as two injections of PRP in the symptomatic treatment of early stage osteoarthritis. In the results of that study, as in other clinical studies, the efficacy of the treatment was reported to start diminishing after 6 months (22). In the current study, no change was observed in the clinical results after 6 month post-treatment, and the treatment efficacy continued after 6 months.

In conclusion, the evidence from this study implies that within the mean follow-up period of 13.81±2.46 months there was an evident positive effect of PRP treatment on functional results and the elimination of pain in the treatment of early stage knee osteoarthritis. Clinically, in respect of pain severity and knee functionality, the KSS and KSS functional scores of the cases applied with L-PRP were observed to have increased more than those of the P-PRP group whereas the VAS values decreased. The most important aspect of this study is that in contrast to many other studies (6, 8-10, 13, 23), the results continued after the 6th month and were reported to be good in the 12th month and this was statistically significant. In addition, it was recorded that L-PRP was not only clinically superior to P-PRP, but also statistically significant in the treatment of early stage knee osteoarthritis ($p<0.01$).

CONFLICT OF INTERESTS

The authors state that there are no conflicts of interest regarding the publication of this article.

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