Araştırma Makalesi
Research Article

Factors Affecting Platelet Count in Platelet-Rich Plasma

TROMBOSİTTEN ZENGİN PLAZMADA TROMBOSİT SAYISINI ETKİLEYEN FAKTÖRLER

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ABSTRACT

Introduction: The amount of platelet count in platelet-rich plasma (PRP) is very important for the concentration of growth factors, which play a major role in the effectiveness of PRP.

Aim: The aim of this study was to investigate the effects of demographic and clinical parameters on the number of platelets in PRP.

Materials And Methods: The data of 50 patients who received PRP in our stem cell center were scanned retrospectively in electronic environment. Demographic and clinical parameters (age, gender, body mass index, blood group, comorbidities, hypertension, coronary heart disease and diabetes, alcohol and cigarette usage) of the patients were recorded.

Results: The results of the correlation analysis performed to evaluate whether there was a relationship between age and BMI with regards to platelet count in PRP revealed no statistical significance ($p>0.05$). Analysis of Mann Whitney-U and Kruskal Wallis-H showed that there was a statistically significant difference in terms of platelet count, gender, presence of hypertension and coronary artery disease ($p<0.05$). It was observed that the mean platelet count of female patients was higher than that of male patients, and the presence of hypertension and coronary artery disease also caused a decrease in platelet count with a statistically significance ($p<0.05$).

Conclusion: According to the results of this study one can conclude that gender, presence of hypertension and coronary artery disease were correlated with platelet count of PRP. Additionaly the demographic and clinical findings such as age, BMI, blood group, diabetes mellitus, smoking and alcohol consumption had no significant effect on platelet count in PRP.

Keywords: PRP, platelet rich plasma, cell count, Platelet Derived Growth Factor

ÖZ

Giriş ve Amaç: Trombositten zengin plazmadaki (TZP) trombosit sayısı, TZP'nin etkinliğinde büyük rol oynayan büyüme faktörlerinin konsantrasyonu için çok önemlidir. Bu çalışmanın amacı, trombositten zengin plazmada demografik ve klinik parametrelerin trombosit sayısı üzerindeki etkilerini araştırmaktır.

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Gereç ve Yöntem: Kök hücre merkezimizde PRP uygulanan 50 hastanın verileri retrospektif olarak elektronik ortamda tarandı. Hastaların demografik ve klinik parametreleri (yaş, cinsiyet, vücut kitle indeksi, kan grubu, komorbiditeler, hipertansiyon, koroner kalp hastalığı ve diyabet, alkol ve sigara kullanımı) kaydedildi.

Bulgular: Yaş ile VKİ arasında TZP içerisindeki trombosit sayısı açısından bir ilişki olup olmadığını değerlendirmek için yapılan korelasyon analizi sonuçları istatistiksel olarak anlamlılık göstermedi ($p>0,05$). Mann Whitney-U ve Kruskal Wallis-H testi sonuçları trombosit sayısı ile cinsiyet, koroner arter hastalığı ve hipertansiyon arasında istatistiksel olarak anlamlı bir fark olduğunu gösterdi ($p>0,05$). Kadın hastaların ortalama trombosit sayısının erkek hastalara göre daha yüksek olduğu, hipertansiyon ve koroner arter hastalığı varlığının da trombosit sayısında istatistiksel olarak anlamlı bir azalmaya neden olduğu görüldü ($p<0,05$).

Sonuç: Bu çalışmada hipertansiyon, koroner arter hastalığı ve cinsiyetin TZP içerisindeki trombosit sayısına anlamlı derecede etkili olduğu bulunmuştur. Ayrıca yaş, VKİ, kan grubu, diabetes mellitus, sigara ve alkol tüketimi gibi demografik ve klinik bulguların trombosit sayısı üzerinde anlamlı bir etkisi gözlenmemiştir.

Anahtar Kelimeler: TZP, trombositten zengin plazma, hücre sayısı, Trombosit Türevli Büyüme Faktörü

Platelet-Rich Plasma (PRP) is defined as a portion of the plasma fraction derived from autogenous blood tissue with a platelet level above the above normal plasma. PRP contains high level of platelets, growth factors and coagulation factors. It was first used by heart surgeons in 1987 (1). After a long hiatus, in the 2000s, the use of PRP was introduced by maxillofacial surgeons (2). However, with the FDA approval in 2012, PRP has been widely used in many musculoskeletal problems (3).

Natural blood clot contains 95% red blood cells, 5% platelets and 1% white blood cells. On the other hand, PRP obtained from autogenous blood tissue by centrifugation process contains 4% red blood cells, 95% platelets, 1% white blood cells. PRP, which is thought to accelerate soft tissue and hard tissue healing, also contains high level of growth factors (4). The concentration of platelets is 3-5 times higher in PRP than in normal whole blood. The mean platelet count in whole blood ranges between 150,000/µl to 350,000/µl, but this number is over 1,000,000/µl in 5ml plasma, and the healing-enhancing effects are observed in this cutt-off value. The obtained PRP is used by injecting directly into the lesion area or mixing with graft materials (4, 5).

The growth factors secreted by PRP accelerates the healing process of bone tissue (6). Studies have shown that bone graft materials with PRP applications facilitate early bone regeneration as well as soft tissue healing. This process also increases mature trabecular bone density by 15-30% (7).

A large number of growth factors are secreted from platelets. Growth factors released from alpha granules modulate wound healing. Platelet Derived Growth Factor (PDGF) is one of the leading with its subsets. PDGFs (PDGFaa, PGGFbb and PDGFab) are the most well-known growth factors in wound healing (8). They stimulate the replication of mesenchymal stem cells, osteoblasts, endothelial cells and fibroblasts (8, 9). They have chemotactic effects on monocytes, neutrophils and fibroblasts, mesenchymal stem cells and osteoblasts (9). They have potent mitogenic effects on fibroblasts and smooth muscle cells in all phases of wound healing (angiogenesis, fibrous tissue formation and re-epithelialization) (10).

The human skin begins to collapse due to facial aging, sun rays, dermal or adipose tissue tension atrophy with forecoming years (11). Today, PRP is used by injecting

into the skin and dermis as mesotherapy (12, 13). Keratin and collagen tissues are structures that provide skin tension and flexibility. PRP injection into the skin tissue that has lost its tension, generates an increase in the number of keratin-synthesizing fibroblast and keratinocyte cells (14). In many cosmetic surgical procedures (facelift, reduction mammoplasty, abdominoplasty, etc.), PRP has been applied under the raised flaps and its positive effects have been reported, such as the need for drain use and compressive dressing, reducing postoperative pain and edema, and providing a shorter recovery period due to its positive effects on wound healing (15, 16).

Study Hypothesis

The main purpose of the use of PRP application is to give the target tissue much more platelets than can be carried by the blood circulation. Thus, the repair of the tissue begins rapidly and strongly and results in a shortened period of healing (14). The aim of this study was to investigate the effects of demographic and clinical parameters on platelet count in PRP.

MATERIALS AND METHODS

This research has been conducted in 50 individuals who were performed PRP between January 2019 and September 2021 in our stem cell center. The patient data were analyzed in a retrospective nature. The ethics committee approval has been granted at 14.01.2022. Informed consent was obtained from the patients.

The data of patients who received platelet-rich plasma between January 2019 and September 2021 in our stem cell center were scanned retrospectively in electronic environment. Demographic and clinical parameters (age, gender, body mass index, blood group), comorbidities (hypertension, coronary heart disease and diabetes), alcohol and cigarette usage of the patients were recorded from the hospital electronic database.

Preparation of Platelet-Rich Plasma

PRP isolation was carried out in line with the standards set by Acıbadem Labcell. In order to carry out the method, peripheral blood from the patients was taken into eight ACD tubes with the help of butterfly needle. The blood was centrifuged at 800G for 5 minutes without brake at a temperature range of 19 to 25 degrees Celsius in the Cell Processing Laboratory. Serum and buffy coat layers of decomposed peripheral blood obtained from centrifugation were transferred into 50 ml conical tubes with the help of a pipette in the Air-Flow cabinet. The obtained serum-buffy coat layers were centrifuged at 1200 G for 10 minutes at the same temperature. The waste formed on the centrifuged serum obtained as a result of centrifugation was removed with the help of a pipette and distributed into 6 vials as 2 ml. One of the vials was reserved for cell count and quality control. A sample was taken from the separated vial with the help of a 5 ml injector. The sample was stained with Sigma brand Trypan blue and platelet count was performed under microscope.

Statistical Analysis

Patient data collected within the scope of the study were analyzed with the IBM Statistical Package for the Social Sciences (SPSS) for Windows 23.0 (IBM Corp., Armonk, NY) package program. Frequency and percentage were given for categorical data, and median, minimum and maximum descriptive values for continuous data. The compatibility of continuous quantitative variables with the Gauss distribution was examined with the Kolmogorov-Smirnov test. For comparisons between groups, "Mann Whitney-U Test" was used for two groups and "Kruskal Wallis-H Test" was used for three groups. The relationship between continuous variables was evaluated with the "Spearman's Correlation Test". The results were considered statistically significant when the p value was less than 0.05.

RESULTS

A total of 50 cases were evaluated in this retrospective analysis. Majority of the patients were female 62% (n=31) and 38% (n=19) were male. The mean age of the subjects was 40 years (ranging between 21 – 66 years), and the body mass index was 24.7 kg/m2 (ranging between 18 – 34.2 kg/m2). The most frequent blood types were group A 40%(n=20) and group 0 28% (n=14). Rh positive individuals were in the majority 88% (n=44) study population (Table 1).

Table 1. Distribution of Demographic and Clinical Findings of Participants

Characteristics (N=50)	N (%) or Median (Min-Max)
Gender	
Female	31 (62.0)
Male	19 (38.0)
Age, years	40 (21-66)
BMI, kg/m²	24.7 (18.0-34.2)
Blood Group	
0	14 (28.0)
A	20 (40.0)
B	9 (18.0)
AB	7 (14.0)
Blood Type (Rh)	
Rh Positive	44 (88.0)
Rh Negative	6 (12.0)
Diabetes	2 (4.0)
Hypertension	4 (8.0)
Coronary Artery Disease	2 (4.0)
Smoking	9 (18.0)
Alcohol Consumption	10 (20.0)
Platelet count, x10³/ul	451.5 (12-7445)

When the comorbidities of the cases were examined, it was determined that 4% (n=2) of the patients had diabetes, 8% (n=4) of them had hypertension, and 4% (n=2) of them had coronary artery disease. The rate of smoking has been 18% (n=9) and alcohol consumption 20% (n=10) in the study population. The average cell count of the participants was 451.5x103 UL (minimum 12x103 /ul and maximum 7445 x103 /ul). It was determined that there was no statistically significant difference in the number of platelets according to the blood groups, diabetes, smoking and alcohol use of the patients ($p>0.05$).

The results of the correlation analysis performed to evaluate whether there was a relationship between age and BMI with regards to platelet count has been elaborated in Table 2. It was determined that there was no statistically significant relationship between platelet count, age and BMI ($p>0.05$).

Table 2. Evaluation of Platelet Count According to Demographic Findings

	Spearman's Correlation	Age	BMI
Platelet Count	Correlation Coefficient	-0.106	0.075
	P value	0.464	0.603

Table 3 shows the results of Mann Whitney-U and Kruskal Wallis-H tests were performed to evaluate whether there was a significant difference between demographic and clinical findings according to platelet count. When the table was examined, it was determined that there was a statistically significant difference in terms of platelet count, gender, presence of hypertension and coronary artery disease ($p<0.05$). It was observed that the mean platelet count of female patients was higher than that of male patients, and the presence of hypertension and coronary artery disease also caused a decrease in platelet count with a statistically significance ($p<0.05$).

It was determined that there was no statistically significant difference in the platelet count according to the blood groups, diabetes, smoking and alcohol use of the patients ($p>0.05$).

Table 3: Evaluation of Platelet Count According to Demographic and Clinical Findings

Characteristics (N=37)	Platelet Count, x10³/ul Median (Min-Max)	P value
Gender		**0.028**
Male	**276 (40-1064)**	
Female	**601 (12-7445)**	
Blood Type		0.408
0	169 (33-7445)	
A	523 (12-3877)	
B	534 (65-1251)	
AB	846 (40-2294)	
Blood Group (Rh)		0.132
Rh-Positive	499 (33-7445)	
Rh-Negative	177,5 (12-740)	
Diabetes Mellitus		0.322
No	451.5 (12-7445)	
Yes	317 (33-601)	
Hypertension		**0.042**
No	**499 (12-7445)**	
Yes	**85.5 (33-846)**	
Coronary Artery Disease		**<0.001**
No	**499 (12-7445)**	
Yes	**90.5 (77-104)**	
Smoking		0.631
No	439 (12-7445)	
Yes	601 (40-3285)	
Alcohol Consumption		0.942
No	499 (12-7445)	
Yes	248.5 (67-3285)	

DISCUSSION

Physicians were more focused on the bacterial cell number of PRP composition rather than platelets. They have utilized suspended particulate matter (SPM) to measure contamination load. The detection of platelets in PRP differs from bacterial load as it does not require a wide range, thus it is more difficult. WBCs and RBCs may interfere with the process and create an obstacle to identify the platelets. In previous studies, Lee and Tarassenko tried to elaborate the platelets count in PRP via optical method but failed to derive accurate outcomes as the RBC count range was lower than the average (30–40 × 104/μL in average) and the WBC count was not considered (18). Kitamura et al. have utilized spectrophotometry and evaluated P – PRP (pure PRP) as its accuracy was higher than L – PRP (Leukocyte- and platelet-rich plasma). The WBCs had more interference with the results due to their spherical and nucleated structure. They have shown that the ratio of platelet counts was 108.6 ± 22.0% in P – PRP, whereas it was 110.4 ± 64.0% in L-PRP preparations (19).

The terms "leukocyte-poor" or "pure PRP" are used to define PRP containing plasma and platelets with little-to-no leukocytes and no RBCs. Pure PRP is generated through laboratory cell separation (plasmapheresis), soft spin centrifugation systems, or more technical processing such as the generation of PRP from filtered leukocyte-reduced blood. Although a higher number of platelets were detected in many PRP products, high amount of white cell levels and most of these contained catabolic cytokine levels (20). Weibrich et al. indicated that the content of PRP can vary tremendously, depending on the system used for the preparation of PRP (21).

In an earlier study by Weinbrich et al., the effect of PRP preparation method and other factors on platelet count had been investigated. They elaborated that the platelet count significantly differed according to donor (median men 237,500/microL, women 272,000/microL). Another important finding of their study was the comparison of the methodology outcomes of blood bank PRP preparation (median men 1,302,000/microL, women 1,548,500/microL), and self-concentrated PRP (median men 944,000/microL, women 1,026,000/microL). They have stated that platelet concentration of the blood bank PRP correlated with the platelet count in donor whole blood, thus no significant correlation has been achieved between the platelet count of self-concentrated PRP and donor whole blood. The platelet concentration of the blood bank PRP correlated with the platelet count in the donor whole blood (Spearman's correlation coefficient r(s) = 0.73). However, there was no significant correlation between the platelet count of self-concentrated PRP and donor whole blood (r(s) = 0.22). They also found that gender had significant but irrelevant effects on platelet concentration, whereas age had no effect (22). Similar to Weinrich's data, no correlation existed between

age and cell count in our study. Additionally our research denoted that same outcome was applicable for BMI and platelet count.

During the evaluation of the differences in platelet count according to demographic and clinical findings, we did not determine statistical significances in terms of blood group, diabetes mellitus and alcohol consumption. However, gender, hypertension and coronary artery disease has significantly affected platelet count of PRP. Regarding these findings, one can state that most of the demographic and clinical findings did not generate a statistically significant difference in terms of platelet count, however some clinical findings could be stated as noteworthy. The platelet count has been numerically higher in blood group AB than in other blood groups, in Rh positive individuals than in negative subjects. One unexpected finding of this study was the higher cell count of smokers compared to non-smokers. However there were only 9 patients as smokers and this could be atrributed to the low number of subjects for statistical analysis.

Verma et al. found no significant associations between age, gender and platelet count and growth factors concentration (23). Their results were similar to Cho (24), Dragoo (25) and Arun (26). In our research, we have found that gender had significant effect while age results were consistent with these previous studies. Platelet count is a quality parameter that influences the regenerative potential of PRP, as well as the clinical outcome driving the physicians to achieve best possible composition.

The main limitation of this study could be attributed to its retrospective nature and relatively low number of sample size (n=50). PRP has wide clinical applications associated with its regenerative, antiaging and healing potential. These properties increase the importance of PRP quality, which is also affected by platelet count. In this study, although there were no statistically significant differences, these clinically significant results may guide further trials with larger sample size.

CONCLUSION

According to the results of this study one can conclude that presence of hypertension and coronary artery disease cause significant decrease in platelet count of PRP, and the mean platelet count of PRP was significantly higher in female patients than in male patients. Additionaly the demographic and clinical findings such as age, BMI, blood group, diabetes mellitus, smoking and alcohol consumption had no significant effect on platelet count in PRP.

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Competing interests

The authors declare that they have no competing interests.

Informed consent

Informed consent has been obtained from all the patients before the initiation of the study.

Abbreviations:

ACD: acid citrate dextrose
BMI: body mass index
CAD: Coronary Artery Disease
DM: diabetes mellitus
IL: interleukins
L – PRP: Leukocyte- and platelet-rich plasma
PDGF: Platelet Derived Growth Factor
PRP: platelet rich plasma
SPM: suspended particulate matter
SPSS: Statistical Package for the Social Sciences
SVF: stromal vascular fraction

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