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ORIGINAL ARTICLE

ANTI-INFLAMMATORY ACTIVITY OF PLATELET-RICH PLASMA TREATMENT IN THE INFLAMMATION MANAGEMENT OF KNEE OSTEOARTHRITIS: EXPERIMENTAL STUDY

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Summary

Background: The level of pro-inflammatory Cluster of Differentiation 68 (CD 68) could be beneficial for examination as a biomarker for identifying cartilage or knee tissue degradation in joint problems. Because Cluster of Differentiation 68 appears to be linked to cell damage in the injury area, its measurement may be an effective and sensitive tool for detecting the early development of knee osteoarthritis (KOA) in people at risk for knee osteoarthritis.
Aim of the study: The study aimed to evaluate which type of PRP (Pure-PRP and leukocyte-PRP) are suitable for patients with KOA via assessing the levels of serum CD 68 concentration.
Materials and Methods: Serum Cluster of Differentiation 68 level was computed using ELISA kits. The experimental study comprises 21 pure-platelet-rich plasma (P-PRP) injections,11 leukocytes platelet-rich plasma (L-PRP) injections, and 16 control groups. Ranged from 35- 75 years old. All patients with diabetes mellitus, autoimmune disease, and severe knee osteoarthritis were excluded from this study. The period of the study was between November 2021 to June 2022. This study assessed other factors such as age, sex, family inheritance, and body mass index (BMI). The level of CD 68 was measured in the serum before and after the injection for six weeks.
Results: The level of the study showed CD 68 elevated before injection in patients with knee osteoarthritis. A significant decrease of CD 68 ($P< 0.01$-$P<0.001$) in the serum concentration after injection as compared to before injection. However, the concentration was significantly higher than the control.
Conclusions: In conclusion, both P-PRP and L-PRP demonstrated anti-inflammatory properties. Patients in both groups experienced a significant decrease in CD 68 serum levels, however, the P-PRP was more effective than the L-PRP.

Key words: platelet-rich plasma (PRP); Osteoarthritis (OA); Cluster of Differentiation 68 (CD 68); Pro-inflammatory

Introduction

One of the most intricate joints in the human body is the knee. The femur (thigh bone) and tibia (shin bone) are joined at the knee joint (tibia). The patella is the bone that covers the top of the knee, while the fibula is the smaller

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bone that runs alongside the tibia. (1). The sophisticated dynamic mechanics of the knee joint depend on the joint's mechanical stability and the exchange of dynamic feedback between the joints and the central nervous system. The sense of proprioception can provide information regarding the movement and position of the knee joint. It plays a crucial role in controlling muscle activity and is necessary to maintain the knee joint's dynamic stability (2). Multiple matrix macromolecules constitute the extracellular matrix (ECM). When the ECM components are arranged in an ordered form, they display a wide range of structural, biochemical, and functional diversity, endowing the matrix with various physical, biomechanical, and biochemical properties (3).

Knee Osteoarthritis (KOA)

The knee is the joint where osteoarthritis (OA) is most common (4). Early KOA results in an imbalance in the inflammatory mediators of the joint, which in turn induces cartilage deterioration, extracellular matrix degeneration, systemic inflammation, chondrocyte death, osteophyte formation, and bone remodelling. These signs and symptoms are all directly related to KOA (5). Joint degeneration brought on by KOA is the result of an imbalance between the breakdown and repair of joint tissue. A combination of cellular changes and biomechanical forces within the joint cause several secondary alterations to occur at once (6).

Platelet Rich Plasma (PRP)

PRP is an autologous blood-based product with bioactive compounds that help repair and regenerate tissue (7). New research reveals that PRP, in addition, to its basic function in hemostasis, may also have a regenerative impact on some bodily tissues (8). Studies have shown that platelet-rich plasma provides some relief from symptoms in patients with early KOA of the knee and is at least as effective as some intra-articular medications for reducing symptoms of the disease (9, 10). To promote tissue regeneration, utilize this minimally invasive method. Alpha granules are found in platelets, and approximately 70% of their growth factors are produced during the first 10 minutes after activation by mechanical or chemical methods and released almost entirely within the first hour (11). These growth factors activate some of the cells involved in tissue repair and regeneration of bone and cartilage (12). There is no risk of an immune response or transmission of infectious disease because the plasma is autologous (extracted from the product of the same patient) (13). There is no standard technique for preparing PRP (14). In general, the separation technique depends on the number of centrifugation cycles and the duration of apheresis (15).

Growth factors

Megakaryocytes are the source of platelets (16). A restricted, site-specific response is produced by the growth factors found in activated platelet–granules (17). Approximately 70% of the growth factors in the α-granule are produced in the first 10 minutes after activation (18). It has been demonstrated that these growth factors, together with coagulation factors, cytokines, chemokines, and other platelet-stored proteins, promote the release of chondrocyte cartilaginous matrix and inhibit the catabolic effects of pro-inflammatory cytokines (19). The main growth factors and growth factor families from PRP used in treating OA are tissue growth factor (TGF), insulin-like growth factor-1 (IGF-1), bone morphogenetic proteins (BMP), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), and hepatocyte growth factor (HGF) (20). TGF- is one of the key elements in cartilage regeneration due to its function in the proliferation and differentiation of chondrocytes (21). TGF-β induces chondrogenic differentiation of mesenchymal stem cells (MSCs) (20). IGF-1 is crucial for the function of cartilage because it promotes chondrocyte division and the production of extracellular matrix (22). BMP assists in cartilage cell emigration (23). FGF plays an important role in cartilage repair (24). PDGF plays a role in all cells that originate from the mesoderm and helps in the regeneration of articular cartilage by encouraging the proliferation of chondrocytes (25). Vascular endothelial growth factor (VEGF) induces vascularisation; stimulation of vascular endothelial cells (26).

The influence of white blood cell concentration is a significant concern when using platelet-rich plasma. Leukocytes are essential sources of healing-related cytokines and enzymes, especially for avoiding infections, according to proponents of L-PRP (27). Others contend that the presence of white blood cells in platelet-rich plasma results in an increase in pro-inflammatory cytokines and enzymes such as matrix metalloproteinases (MMPs), which can have a diametrically opposite effect (28).

Cluster of Differentiation

Name is used to describe proteins that are present on cell surfaces. The identification of cell phenotypes is made possible by the varied numbers that are allocated to each distinct surface molecule. The identification of cell phenotypes is facilitated by the surface expression of a particular CD molecule (29). In terms of physiology, CD antigens can work in various ways, frequently as cell-important receptors or ligands (the substance that activates a receptor). Typically, a signal cascade is initiated, affecting the cell's activity. Some CD antigens have activities other than cell signalling, including cell adhesion, cell activation, and cell inhibition (30). In immunophenotyping, the CD antigens are frequently used as cell markers, allowing cells to be differentiated based on the molecules found on their surface. These markers are frequently employed to link cells with specific immunological capabilities (31). In osteoclasts, circulating macrophages, and tissue macrophages, and other monocyte-derived cells, the protein known as the cluster of differentiation 68 (CD 68) is highly expressed (e.g., Kupffer cells, microglia) (32). In the bone marrow, macrophage/granulocyte-macrophage colony-stimulating factors promote the expression of CD 68 and other macrophage-specific genes in common myeloid progenitors during differentiation between lymphoid and myeloid lineages (33). Several non-hematopoietic cell types, such as human umbilical cord mesenchymal stem cells, fibroblasts, endothelial cells, and several tumour cell lines, as well as intimal smooth muscle cells of human arteries, showed low levels of CD 68 expression (34). The family of lysosomal-associated, mucin-like membrane proteins, to which CD 68 belongs, is closely linked (35). CD 68 is mostly found in the membranes of lysosomes, but a small amount is also on the outside of the cell (36). CD 68 is a scavenger receptor for oxidized low-density lipoprotein and may be involved in cell-to-cell communication, even though its biological function has not been fully figured out (37). The study aimed to evaluate the efficacy of injected P-PRP and L-PRP for patients with KOA. Which could be better in medication for inflammation and also might be reduced the symptom of KOA via measuring the level of serum CD 68 concentration. The study aimed to evaluate which type of PRP (Pure-PRP and leukocyte-PRP) are suitable for patients with KOA via assessing the levels of serum CD 68 concentration.

Materials and Methods

A non-randomized experimental study was conducted in the specialized clinics for joint diseases and fractures in the governorates of Al-Diwaniyah and Babylon in Iraq during the period from November 2021 to June 2022. The study included 32 patients (18 females, 14 males) who were diagnosed with KOA by the consultant. They were divided into two groups: The first group included 21 patients who have received a single injection of P-PRP therapy, and the second group included 11 patients who have received a single injection of L-PRP therapy. Samples were taken from patients before knee injection and six weeks after knee injection. In addition, 16 healthy subjects were used in the study as a control, as shown in Figure 1. All participants were subject to formal informed consent about the use of their samples in the research study. No missing patients in the current study.

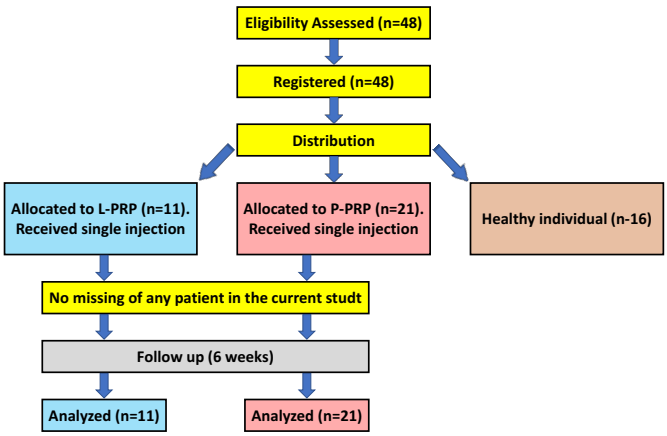

Figure 1. Workflow diagram of the current study; the study includes a total number of study participants(n=48), divided into two groups: The first groups are healthy individuals (n=16) and the second group of patients with knee osteoarthritis (KOA) are divided into two subgroups, P-PRP injection(n=21) and L-PRP injection(n=11). After a six-week patient reassessment. No dropping of any patients in the current study.

Blood collection

By puncturing a vein, five millilitres of the patient's entire blood were withdrawn. After that, the blood was placed in a gel tube and kept stable at room temperature for 10 to 15 minutes. The blood was then separated using a centrifuge for 5 minutes at a speed of 11000 RPM. The serum was then put into an Eppendorf tube and kept at (-80°C) until it was needed (38).

Inclusion Criteria

Patients with KOA without other chronic diseases, and candidates for PRP therapy due to low or unresponsive to other therapy. Age 35 to 70 years old.

Exclusion criteria

The research excluded obese individuals, severe knee OA, diabetes mellitus patients and other chronic diseases.

Cluster of differentiation-68

Serum CD 68 level was computed using Elabscience® kits as exemplified as shown in Figure 2 below:

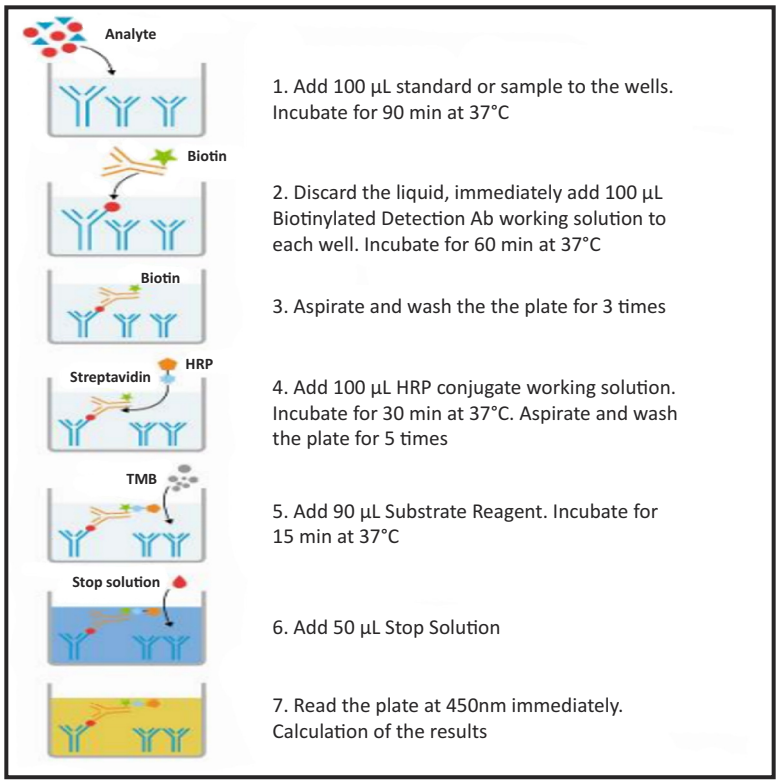

Figure 2. Method for measuring CD 68 using the ELISA technique.

Statistical Analysis

The results were analyzed using GraphPad Prism (9.2.1, USA) and Microsoft Excel version 19. Presented as mean ± standard deviation. T-test, one-way ANOVA and correlation analysis were used to measure the degree of significance.

Results

Demographic characteristics

The proportion of males between the ages of 35 and 39 was up to 50%, whereas the percentage of females between the ages of 50 and 59 was 61%, which was significantly (P<0.0001) higher than the percentage of males (22%). Between ages 60 and 70, the female prevalence rate was 28%, which was significantly (P<0.05) higher than the male prevalence rate of 14%. There was found to be no consistent correlation between age and Body Mass Index (BMI) (Table 1).

Table 1. Demographic table. Based on the patient number it appears that females are more liable to get Osteoarthritis problem more than males.

		Control		Patients		P value
		Male (n=11)	Female (n=5)	Male (n=14)	Female (n=18)	
Age n(%)						
Age range	35-39	6(55%)	2(40%)	7(50%)	/	/
	40-49	2(18%)	2(40%)	2(14%)	2(11%)	NS
	50-59	3(27%)	1(20%)	3(22%)	11(61%)	P<0.0001
	60-70	/	/	2(14%)	5(28%)	P<0.05
BMI						
Age range	35-39	20-36.3	27.3-28.7	18-25	/	NS
	40-49	23.3-27.1	26.1-35.5	22.8-24.3	22-24.3	P<0.001
	50-59	24-37	27.1	25-25.3	20.2-38.6	P<0.001
	60-70	/	/	28.7-29.4	25.5-30.9	Ns

Assessment of serum CD 68 concentration before injection

The level of CD 68 in the serum was significantly higher than in control subjects Figure 3A, a comparison of the levels of CD 68 in the patient group and the control group, shown as an estimation plot, which demonstrates the presence of a significant rise in the level of CD 68 in the patient group Figure 3B).

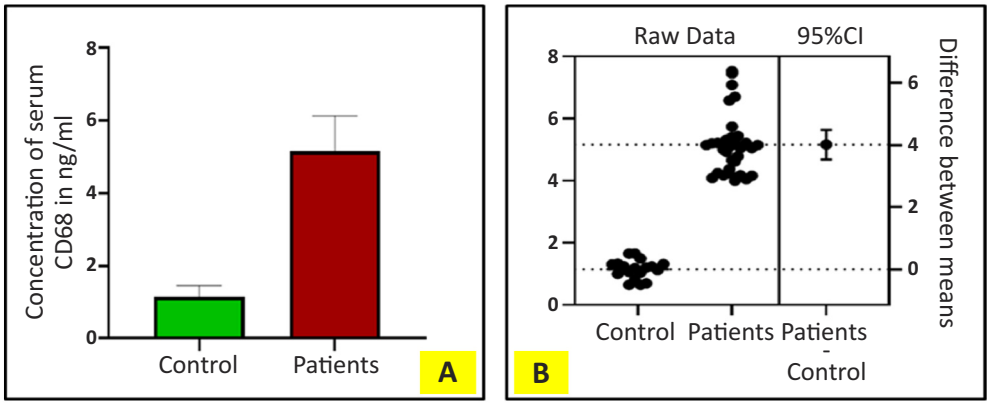

Figure 3. Estimation of serum CD 68. (A) a comparison between the control and patients group, (B) an estimation plot that illustrates the presence of a significant increase in the level of CD 68 in the patient group as compared to the control.

Evaluation of serum CD 68 concentration after injection

After receiving injections of P-PRP and L-PRP, an estimation of the CD 68 serum concentration was performed resulting in a discernible lessening ($p<0.01$-$p<0.001$) in the concentration of CD 68 in the serum after injection. On the other hand, the concentration was considerably greater in patients as compared to the control group in the case of L-PRP patients, however, there was no significant ($0<0.001$) difference in the case of P-PRP patients (Figure 4).

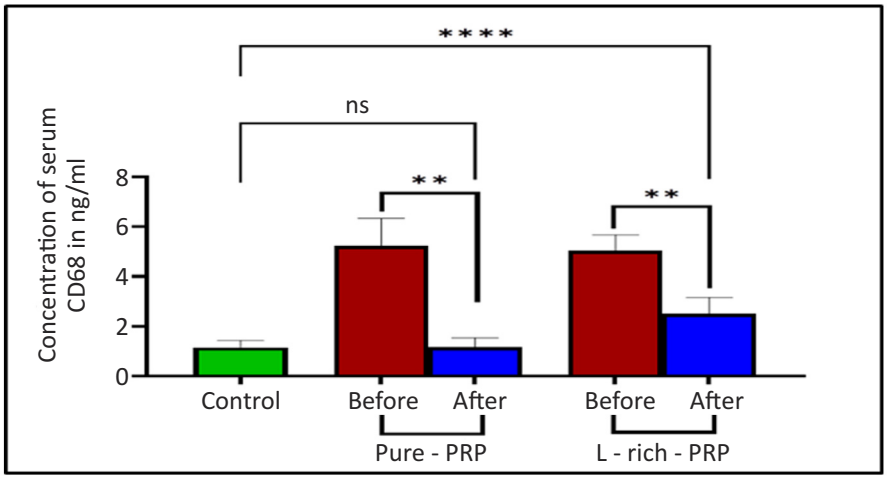

Figure 4. Estimation of CD 68 serum concentration after the injection of P-PRP and L-PRP. A significant decrease ($P<0.01$-$P<0.001$) in the serum concentration of CD 68 as compared to before injection. However, the concentration was significantly ($P<0.001$) higher in patients than in control for L-PRP patients, while no significant difference for P-PRP patients.

Evaluation of serum CD 68 concentration in both gender after injection

Assessment of the level of CD 68 in serum concentration in patients with knee osteoarthritis after the injection of P-PRP and L-PRP revealed that there is no significant difference between male and female patients (Figure 5).

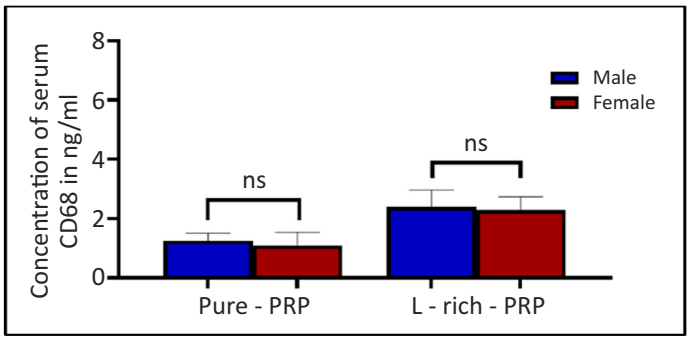

Figure 5. Estimation of CD 68 serum concentration after the injection of P-PRP and L-PRP. No significant difference finds between males and females after the injection.

Estimation of serum level for the marker concerning age

The relationship between the marker and age for all age groups is shown as a simple linear regression, showing no correlation between it and age (Figure 6).

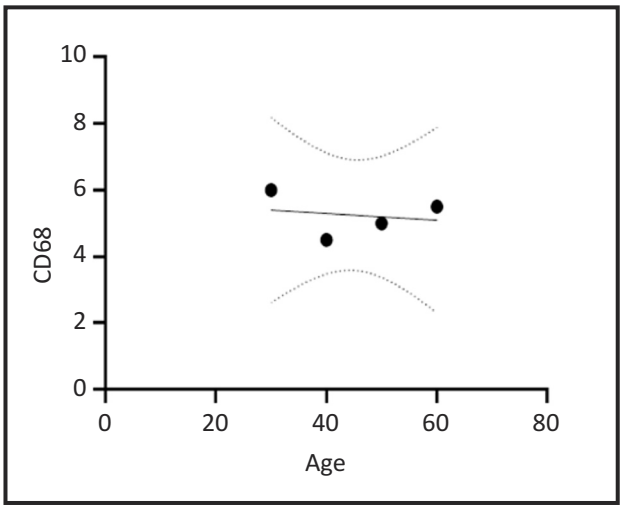

Figure 6. simple linear regression shows no correlation between CD 68 level and age (r=0.1).

Discussion

The knee joint is one of the largest joints in the human body (39), and one of the most susceptible to injuries and degenerative changes. Due to its localisation between the femur and tibia, it plays a vital role in everyday activities such as walking, kneeling or rising from a seated position (40). Platelet-rich plasma includes growth factors that promote the development and regeneration of bone and cartilage cells in the knee joint and their repair (41, 42). Sanchez *et al*., 2008, compared the response of an autologous preparation of PRP injected into the joint works with another drug for treating KOA. They found that the PRP injections led to much less pain and a better physical function subscale than the other group (43). Duymus *et al*., 2017, researchers compared the effectiveness of three treatments for KOA: intra-articular injections of PRP, Hyaluronic acid HA, and ozone(O_3) gas. PRP was more effective than either HA or ozone injections at giving patients at least 12 months without pain (44). Filardo *et al*., 2012, Some patients experienced slight discomfort and effusion following injections, especially in the PRP group, where post-injective pain was considerably higher (P = 0.039). This self-limiting reaction did not affect the outcome (45). we examine the efficacy of PRP therapy alone, without any surgical procedures, anti-inflammatory medication, or physiotherapy routine connected with the treatment of knee osteoarthritis.

Due to the potential impact of anti-inflammatory drugs on the effect of PRP on joint inflammation, they were not used concurrently with PRP therapy (46). Changes in CD 68 levels in the serum of patients with osteoarthritis may be useful in the early diagnosis of early knee osteoarthritis (47). Antigen CD 68 has been identified as a macrophage-associated antigen (48), owing to its widespread presence in mononuclear phagocytes, including circulation monocytes (49), bone, macrophage, spleen, liver, and lung (50). Since the presence of a CD 68 inside the cell is about 80-85%, it can reflect the damage to the affected area (51).

The primary finding of this research was that intra-articular injection of platelet-rich plasma (PRP) led to an improvement in knee osteoarthritis during a follow-up period of six weeks. These analyses would have been crucial for identifying the parameters linked with pain reduction, whether resulting from an anti-inflammatory impact or cartilage tissue regeneration. Two key groups make up this study, patients in the first group received P-PRP injections into the knee joint. The second group is the patients for whom the knee joint is injected with L-PRP, in addition to a group of healthy individuals. This study demonstrated that, depending on the number of patients, women are more prone to knee osteoarthritis than men. In general, a person's susceptibility to disease increases as their age increases. Due to the small sample size, it was noticed in this study that the association between BMI and age is unstable. Moreover, the limitation of the study could be due to variation in the response which is primarily linked to the surrounding milieu (52, 53) which modulated cellular behaviour (54, 55) based on localized tissue oxygen level (55, 56) or or presence of sufficient vitamins and minerals (57, 58).

The level of CD 68 in the serum of patients with knee OA is greater than in healthy individuals. The measurement of CD 68 serum concentration following the injection of P-PRP and L-PRP for all age groups revealed a substantial drop in CD 68 blood concentration compared to before injection. Following injection, there was no discernible difference between males and females. showing no association between age and CD 68 levels.

Conclusion

In conclusion, both P-PRP and L-PRP methods demonstrated anti-inflammatory properties. Patients in both groups in the current study (P-PRP and L-PRP) have shown a significant decrease in the CD 68 levels. However, the injection with P-PRP was more effective than the L-PRP. This study has a couple of limitations, the first one was studying the possibility of using a placebo effect for the control individuals. The second limitation was performing a cartilage biopsy for patients and subjects who participated in our study because they disagree.

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Competing Interests

There are no conflicts of interest associated with the publishing of this paper.

Adherence to Ethical Standards

The ethical committee approved the study at the University of Al-Qadisiyah (registration code CMUQ 11/1000 on 8/3/2021).

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