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Research article

# PRP as a modulator of inflammation in FLS of RA patients by regulation of galectins and TGF-β1

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INTRODUCTION

An autoimmune and inflammatory condition known as rheumatoid arthritis is characterized by joint inflammation and an aggressive fibroblast-like synoviocytes. (FLS) One of the most significant immunological regulators are the galectins. Platelet-rich plasma are probably effective in immunomodulation. The aim of the present work is to investigate the role of platelet rich plasma (PRP) as a modulation of inflammation, which affects the expression of galectins and TGF-β in FLS from Rheumatoid arthritis (RA) patients.

*Methods:* Human FLS cells from RA patients' synovial fluid were cultured in DMEM-F12 medium, characterized by flowcytometry, treated with PRP alone, TNF-α+PRP, SF + PRP, TNF-α alone, and untreated control groups. Expression of Galectin-1, Galectin-3, Galectin-9, and TGF-β1 genes was assessed by Real-Time PCR.

*Results:* In SF + PRP, TNF + PRP, and PRP groups, the gene expression of Galectin-3 was considerably reduced ($P > 0.05$). Galectin-1 and TGF-β1 expression levels were also lowered ($P > 0.05$) in the TNF + PRP groups. Galectin-9 expression increased significantly in the PRP group ($P > 0.05$). Galectin-3 expression was markedly and extensively reduced in multiple study groups after treatment of FLS cells with 10 % PRP. Galectin-3 expression was considerably reduced when FLS were exposed to TNF- and synovial fluid in conjunction with PRP to simulate localized body inflammation.

*Conclusion:* Our results showed that PRP may be useful in lowering FLS-induced inflammation in RA patients' joints, particularly when Galectin-3 is involved. In the future, inflammatory illnesses like RA may be treated locally using PRP or its derivatives, which will have a larger immune modulation role and more likely pathways.

## 1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects about 1 % of the adult population and it's incidence is three times higher in women compare to men [1,2]. The disease is characterized by synovium inflammation, bone destruction and

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extra-articular manifestations such as rheumatoid nodules, pulmonary involvement and cardiovascular disease [3]. Several risk factors may be contributed in development of RA including genetics and environmental factors. Among the environmental factors, smoking has most vigorous link with RA. Cigarette smoking increases the risk of RA development and undesirably affects the clinical period. It is relating to the production of RF and anti-citrullinated protein autoantibodies (ACPA) [4]. There are currently two types of therapies for rheumatoid arthritis, including; Disease–modifying anti-rheumatic drugs (DMARDs) and Non-steroidal anti–inflammatory drugs (NSAIDs). TNF-α inhibitors, anti-TNF-α antibodies, and an IL-1 receptor antagonists, are the biological DMARDs agents, but their usage were limited due to the side effects such as serious infections [4,5]. In RA, the synovial membrane is targeted in inflammatory reactions thus the accumulation of activated T-cells, B-cells, plasma cells, macrophages, dendritic cells are seen which ultimately leads to destruction of bones and articular cartilage by producing inflammatory factors [6].

platelet rich plasma (PRP) is a prominent source of growth factors concentrates, cytokines, proteins and ions that play an important role in cell adhesion, migration, proliferation, pain relief, diminish inflammation and tissue regeneration [7]. Activated platelet can release many platelet-derived growth factors. The benefits of using PRP includes low cost and low side effects with simple method of usage [8]. Many studies have shown the effect of PRP on reducing pain and inflammation in osteoarthritis (OA). However, it has been studied less about the role of PRP in RA. The effect of PRP on chondrocytes has been studied in vitro, but it's effect on non-chondrocytic cells, such as fibroblast like sinoviocytes (FLS) cells has been less marked [9]. Examining the impact of PRP as a therapeutic agent in rheumatoid arthritis patients' FLS cell culture media and its correlation with TGF-β1 cytokine and galectin ligand production is one of the study's goals. Galectins are significant ligands for immune regulation at the level of non-immune cells, including fibroblasts, mesenchymal stem cells, and FLS. As a result, it is likely that changes in these ligand levels in FLS's supportive microenvironment, in conjunction with the regulatory cytokine TGF-β1, will affect the pathophysiology and immunology of RA patients. Put differently, immunological modulation and disease improvement can result from microenvironmental interaction between stimulants or therapeutic substances and immune cells, such as FLS, which are crucial in RA. Two potential cell lines are involved in rheumatoid arthritis disease: the unregulated immune cells and the cell matrix or supporting microenvironment. It serves as therapy as well.

In this study, treatment of human RA-FLS cells with PRP were performed to determine the effect of PRP on cell inflammation, inflammatory cytokines and immune regulators such as Galectin-1, Galectin-3, Galectin-9 and TGFβ Related factors were measured by Real-Time Polymerase Chain Reaction (RT-PCR). These findings will prepare substantial clue for the potential and contingency of using PRP in treatment of RA.

## 2. Material and methods

**Patients.** 10 R A patients with active disease, who were referred to the rheumatology specialist's office, were randomly selected and synovial fluid samples were taken. Based on the rheumatologist's diagnosis, the patients met the criteria of the American College of Rheumatology. With full informed consent, inoculations were taken from the patients and the study protocol was approved by the medical ethics committee of the Shahid Sadougi University of Medical Sciences, Yazd, with the reference number IR.SSU.MEDICINE.REC.1396.17.

Isolation of synovial fibroblasts. Primary RA synovial fibroblasts were isolated from the synovial fluid of RA patients. The synovial fluid was centrifuged ((Eppendorf, Germany)) at 800g for 10 min, cell pellets were suspended in Dulbecco, s modified Eagle, s medium (DMEM)/F12 (Gibco, USA) supplemented with 10 % fetal bovine serum (FBS) (Gibco, USA), penicillin, streptomycin, plated in 25 cm2 flasks and incubated for 24 h at 37 °C in 5 % CO2. The non-adherent cells were discarded and the adherent cells were cultured in fresh medium. When the cell layers were confluent, they were trypsinized and subcultured. Cells were used after third passage. Non-Adherent cells were washed out and the medium was refreshed every 3 days. The remaining adherent cells were cultured for 2 additional weeks in a flask before trypsinization, and then passaged to new culture flasks [10]. The study groups included a control group without intervention and 4 intervention groups. The first group was the control, culture of FLS cells alone and without any intervention. The second group of FLS cells exposed to synovial fluid were affected by PRP in the first 24 h and then in the next 48 h. The third group was exposed to TNF-α in the first 24 h and PRP in the next 48 h. The fourth group was exposed only to TNF-α for 48 h and the fifth group was exposed only to PRP for 48 h. Based on some research designs, FLSs were activated using TNF-α (10 ng/ml) [11],synovial fluid (SF)diluted 1: 8 in culture medium [6], and 10 % PRP concentration [12,13].

Flow cytometry for characterization of FLS The cells were assessed by Flow cytometry by specific positive marker and negative marker.Flow cytometry (Becton Dickinson, USA) analyses of CD-90, CD14, CD68, CD11b, and HLA-DR were performed on the third passage of FLS. FLS cells from third passage were used to study after confirmation of markers by flow cytometry. The single cells were resuspended in phosphate-buffered saline and stained with the following phycoerythrin (PE) - labeled¬ antibodies: CD11b, Fluorescein Isothiocyanate (FITC) - labeled antibodies: CD-90(Thy-1), CD14, CD68, and PerCP labeled antibodies: HLA-DR.

PRP Preparation: Ten healthy volunteers aged 25–35 years of age participated in this study. PRP was prepared following the protocol of the double spinning method as previously reported [14].10 ml of whole blood was initially centrifuged at 220 g for 10 min to separate the PRP and platelet-poor plasma (PPP) portions from the red blood cell fraction. The second cycle of centrifugation followed at 330 g for 15 min to separate the PRP from PPP. Approximately 5 ml of PRP preparation was obtained from the two cycles of centrifugation. PRP was activated with CaCl2 to release growth factors before each examination [15]. The donor's platelet counts in the venous blood were 2–2.5 $\times$ $10^5$ platelet/μl. The concentrated platelet counts were 1–1.5 $\times$ $10^6$ platelet/μl [16].

RNA Extraction. Cells isolated from the synovial fluid were plated in 12 well plate with 2 $\times$ $10^4$ cell per well, then TNF-$\alpha$, synovial fluid and PRP were added to treat cells. Cells were harvested after treatment by addition 380 $\mu$ l TRIzol (GeneALL, South Korea) to each well. Then incubated for 5 min at room temperature and centrifuged at 12000 $\times$ g for 10 min at 4 °C and transferred the supernatant to a fresh tube. Then added 76 $\mu$ l chloroform (sigma-USA), shacked vigorously for 15 s and stored for 2 min at room temperature. Then

centrifuged at 12,000 × g for 15 min at 4 °C, then transferred the liquid phase to a fresh tube. Then added 190 μl isopropyl alcohol and gently mixed the solution, 3 ~ 5 times. Samples were incubated for 10 min at room temperature. Centrifuged at 12,000 × g for 10 min at 4 °C, and the supernatant were discarded. 380 μl of 75 % ethanol was added to wash the RNA pellet. Centrifuged at 7500 × g for 5 min, carefully discard the supernatant, ethanol, and air-dry the RNA pellet for 5 min. Dissolved RNA in DEPC- treated water by incubated for 10 ~ 15 min at 56 °C.

**Gene Expression Evaluation.** Total RNAs were extracted using TRIzol and were reverse transcribed to cDNA using BIOFACT Reverse Transcription Kit (BIOFACT, Korea). RNA concentration was determined using a Nanodrop (Thermo fisher scientific,USA). Quantitative real-time polymerase chain reaction (PCR) analysis was performed on cDNA using SYBR Green RT-PCR Kit on Real-Time PCR system. mRNA expression of Galectin-1, Galectin-3, Galectin-9 and TGF-β1 was evaluated. The specific primer sequences are listed in Table 1. Data were analyzed according to the comparative Ct method and were normalized by GAPDH expression in each sample.

**Statistical analysis**. Sampling of patients was done randomly and with the referral and approval of a rheumatologist. All the experiments were performed at least in triplicate. The software of GraphPad prism (version 6.01, USA) was utilized for statistical analysis. Results were present as means ± standard deviation (SD). Kulmogrouf Smirnov test was done for normal distribution of data and then parametric test; One-way analysis of variance select and do data analysis. ANOVA was used to compare differences between experimental groups. Statistical difference is significant as $P < 0.05$. GAPDH gene was used as an internal control gene to normalize the results. The normalized value of this ratio was calculated using the $2^{-\Delta\Delta CT}$ method for each sample, and the relative expression of each gene compared to the control was reported.

## 3. Results

### 3.1. Culture and identification of FLS cells

FLS cells from synovial fluid samples of RA patients were cultured in a DMEM- F12 medium, and the cells were evaluated and studied at passage 3. The cells were examined at 40× magnification with an inverted microscope (Fig. 1). Flow cytometry analysis showed that more than 87 % of the cells are in FLS cell index marker CD-90+, and other unrelated markers were below 1 %, which included $CD14^{-}$, $CD68^{-}$, CD11b-, HLA-DR- and Therefore, they are considered negative and it is a confirmation of FLS cells (Fig. 2).

### 3.2. Platelet - rich plasma

To evaluate the anti-inflammatory role of PRP in the expression of immune mediators, we investigated the effect of PRP on the expression of Galectin-1, Galectin-3, Galectin-9 and TGF-β1 genes in FLS cells derived from RA patients, as shown in Fig. 3. FLS cells were cultured for 48 h in exposure to PRP in different groups.

### 3.3. Real Time-PCR

#### 3.3.1. Galectin-1

Treating FLS cells with SF + PRP led to a decrease in galectin-1 expression (0.79 ± 0.143) compared with untreated controls (1.0± 0.12, $p = 0.3$). Treatment of FLS cells with TNF-α + PRP led to a significant decrease in galectin-1 expression (0.77 ± 0.149) compared with untreated controls. (1.0 ± 0.12, $p = 0.016$*). PRP treatment group of FLS cells led to significantly increase in galectin-1 expression (1.09 ± 0.1) compared with SF + PRP (0.79± 0.143) and TNF + PRP (0.77± 0.149) groups. These results are displayed in Fig. 3 (Part A) of relative expression.

#### 3.3.2. Galectin-3

The expression of galectin-3 has decreased significantly compared to the control in all study groups except TNF group. Treatment of FLS cells with SF + PRP led to a significant decrease in galectin-3 expression (0.52 ± 0.17) compared with untreated controls (1.21± 0.2, $P < 0.0001$). Also, TNF-α+PRP treatment of FLS cells led to a significant decrease in galectin-3 expression in this group (0.64 ± 0.05) compared with untreated controls (1.21± 0.2, $P = 0.0003$). Treating FLS cells with PRP singly, led to a significant decrease in galectin-3 expression (0.69± 0.16) compared with untreated controls (1.21± 0.2, $P = 0.0013$). Only the TNF-α group showed increased expression of galectin-3 compared to the control group, which was not significant. These results are displayed in Fig. 3 (Part B) of relative expression.

**Table 1**
The sequence of primers in GAPDH, Galectin-1, Galectin-3, Galectin-9 and TGF-$\beta_1$.

| | Forward primer sequences (5′-3′) | Reverse primer sequences (3′-5′) |
|---|---|---|
| GAPDH | CAAGAGCACAAGAGGAAGAGAGAG | TCTACATGGCAACTGTGAGGAG |
| Galectin-1 | CTCCTGACGCTAAGAGCTTCG | CCAGGCTGGAAGGGAAAGAC |
| Galectin-3 | CTGCTGGGCCACTGATTGT | TTGTTCTCATTGAAGCGTGGGTTA |
| Galectin-9 | GGACGGACTTCAGATCACTGT | CCATCTTCAAACCGAGGGTTG |
| TGF-$\beta_1$ | ACCAACTATTGCTTCAGCTCCAC | ACTTCCAGCCGAGGTCCTT |

**Fig. 1.** Fibroblast-like synoviocytes cultured in DMEM-F12 media extracted from synovial fluid of RA patients. In one way, adherent cells form a monolayer culture at the bottom of the flask. The cells have a fibroblast morphology and a spindle-shaped appearance. They are shown in passage three at 40× magnification with an inverted microscope.

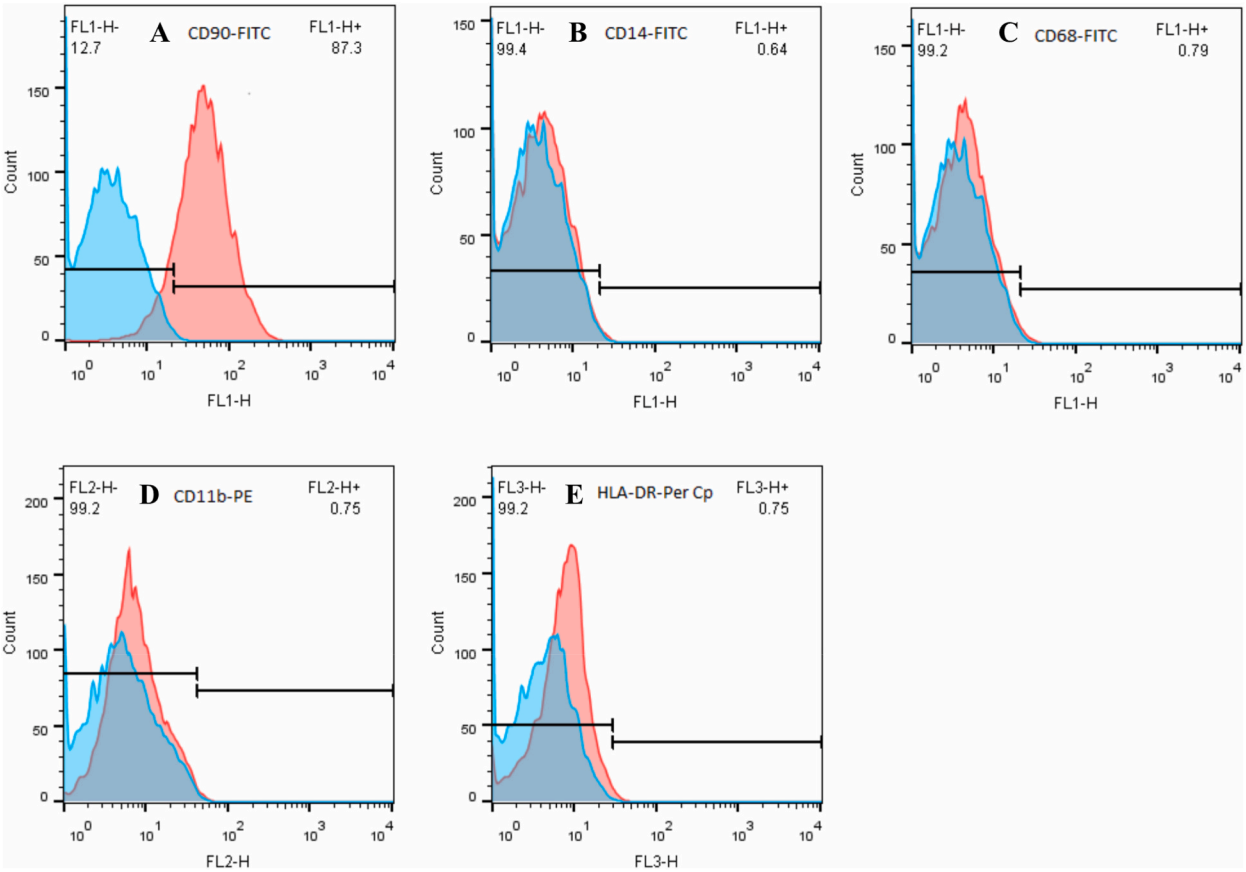


**Fig. 2.** Flow cytometry analysis of the surface markers and confirmation of the FLSs
The percentage of, (A) CD90, (B)CD14, (C) CD68, (D) CD11b and(E) HLA-DR markers expression is indicated. The expression of CD14, CD68 and CD90 on FLS cells was determined using FITC- conjugated Ab detected by FL-1H channel of flowcytometr and the expression of CD11b was determined using PE-conjugated Ab detected by FL-2H channel of flowcytometr and the expression of HLA-DR was determined using PerCP-conjugated Ab detected by FL-3H channel of flowcytometr. The results demonstrated that the CD90 marker is highly expressed on FLS cells and other markers below 1 %, consider negative and confirmed these cells.

### *3.3.3. Galectin-9*

Treating FLS cells with PRP singly, led to a significant increase in galectin-9 expression (1.21 ± 0.2) compared with untreated controls (0.72± 0.16, P = 0048). PRP treatment singly group of FLS cells led to significantly increase in galectin-9 expression (1.21 ± 0.2) compared with TNF + PRP (0.43± 0.07 P = 0003) and SF + PRP (0.76± 0.16 P = 0.001) groups. Use of TNF-α with PRP in treatment of FLS cells led to a decrease in galectin-9 expression (0.43 ± 0.078) in compared with untreated controls (0.72 ± 0.16, P = 0.052). Treatment of FLS cells with SF + PRP led to an increase in galectin-9 expression (0.76± 0.16) compared with untreated controls

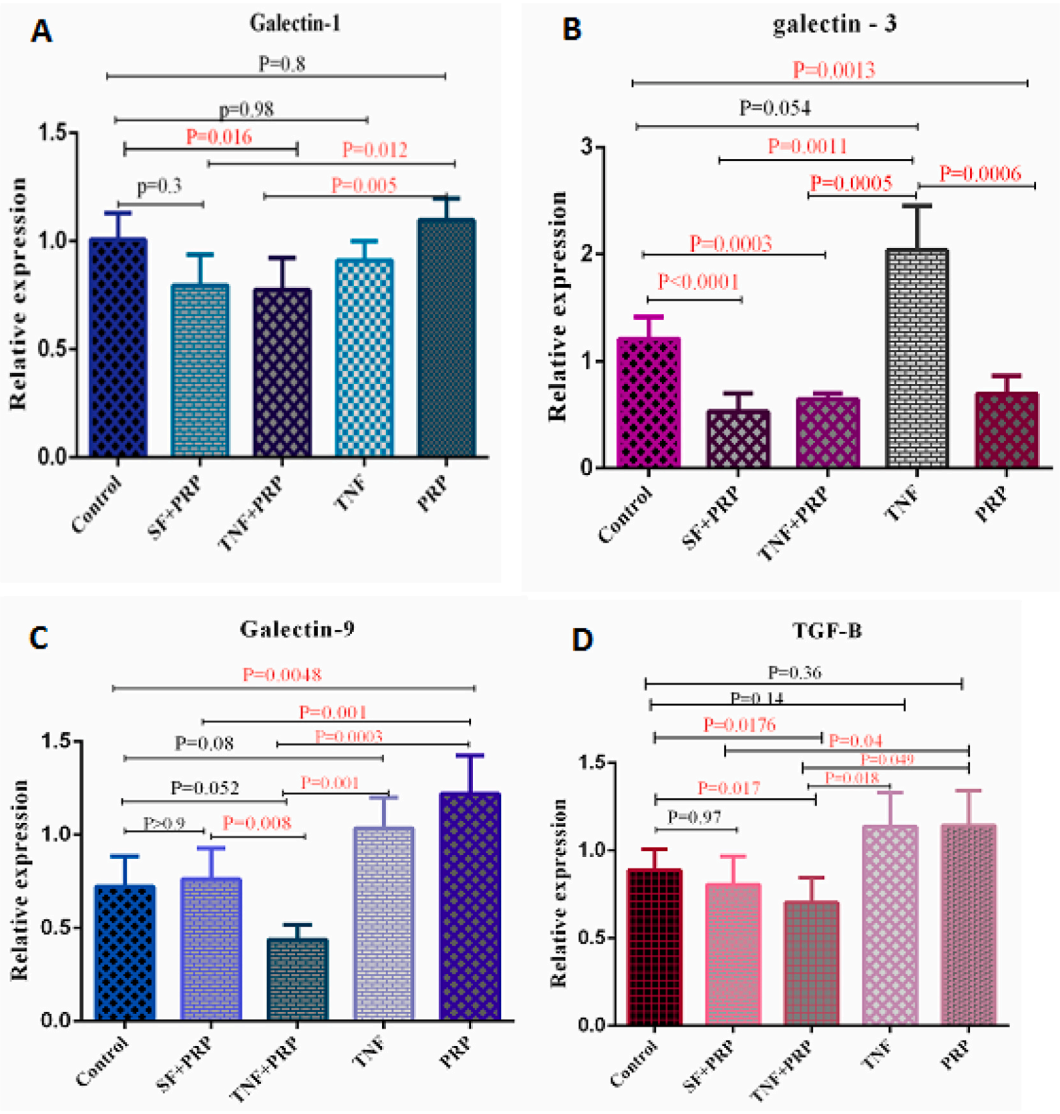


**Fig. 3.** The gene expression of galectin-1, 3, 9 and TGF-$\beta_1$. The effect of platelet-rich plasma on gene expression of human rheumatoid arthritis' FLS cells has been shown. Gene expression of (A) galectin-1, (B) galectin-3, (C) galectin-9 and (D) TGF-β was measured by RT-PCR. Expression of each gene was calculated relative to the expression of housekeeping gene GAPDH. Data are presented as the mean ± standard deviation of the mean of triplicate samples. Relative expression was calculated using the $2^{-\Delta\Delta CT}$ method for each sample, and expression of each gene compared to the control. One-way ANOVA was used to compare differences between experimental groups. P-value of <0.05 considered significant.

(0.72 ± 0.16, P > 0.99). In the SF + PRP group, a significant increase in galectin-9 expression (0.76± 0.16) was observed compared to the TNF + PRP group (0.43± 0.07 P = 0.008). In the case of the TNF group, a significant increase in galectin 9 expression (1.03± 0.16) was observed compared to the TNF-PRP group (0.43± 0.07 P = 0.0013). These results are displayed in Fig. 3 (Part C) of relative expression.

### *3.3.4. TGF-β1*

TNF + PRP treatment of FLSs led to a significance decrease in TGF-β expression in this group (0.7 ± 0.14) compared with untreated controls (0.88± 0.11, p = 0.017).PRP treatment group of FLS cells led to significantly increase in TGF-β expression (1.14 ± 0.2) compared with SF + PRP (0.8± 0.16 p = 0.04) and TNF + PRP (0.7± 0.14 p = 0.049) groups. Treating FLS cells with PRP singly, led to an increase in TGF-β expression (1.14 ± 0.2) compared with untreated controls but it was not significant (0.88± 0.11, P = 0.36). The FLS treated with TNF showed a significant increase in the expression of TGF-β (1.13 ± 0.19) compared to the TNF + PRP group (0.7± 0.14 p = 0.018).The FLS treated with TNF + PRP showed a significant decrease in TGF-β expression (0.7 ± 0.14) compared with untreated controls (0.88± 0.11, P = 0.017). These results are displayed in Fig. 3 (Part D) of relative expression.

## 4. Discussion

Many studies on the mechanism and biological effects of PRP in the treatment of various musculoskeletal and joint disorders have shown that the use of PRP causes improvement in various clinical cases [17]. PRP has played a role in various medical fields such as orthopedics and inflammatory joints due to its role in wound healing and tissue regeneration [18]. Several studies have shown that PRP can have therapeutic effects in orthopedics and improve damaged inflammatory joints due to growth factors [19]. PRP has been widely used in various diseases, including arthritis, and less attention has been paid to RA. The present study investigated the expression of immunomodulatory and anti-inflammatory genes in human RA-FLS cells with the potential to induce repair when exposed to PRP in vitro.

It was observed that exposure to PRP in human synoviocytes decreased the expression of inflammatory cytokines and increased immunomodulatory cytokines. PRP contains a high concentration of PDGF, TGF-β1, IGF, VEGF, and epidermal growth factor and has been the focus of researchers [20]. The results of the present study showed that the expression of inflammatory indicators such as galectin-3 was suppressed after exposure to PRP while the TNF-α exposure group don't, so PRP can probably be useful for the treatment of RA [21]. According to the results of Ohshima et al.'s study, Galactin-3 is involved in the inflammation and activation of synovial fibroblasts, and the accumulation of Galactin-3 is increased by TNF-α, and our study also shows this increase in Galactin-3 in association and synergism with TNF-α [22].In addition, the expression of IL-6, GM-CSF, IL-8 as inflammatory factors and MMP-3 as an invasive factor in synovial fibroblasts increases under the stimulating role of galectin-3. Therefore, galectin-3 promotes Inflammatory and invasive phenotype is important in synovial fibroblasts [23]. Galectin-3 is considered as an indicator of disease activity. In our study, the desired effect of PRP is a significant reduction of inflammatory galectin-3. This reduction of galectin-3 can be associated with improvement of joint destruction and reduction of inflammation and disease activity [22].

Galectin-9 as a regulatory molecule, elevated in serum of RA patients and also expressed by the fibroblasts and binds to the T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) [23,24]. Through this interaction, Galectin-9 negatively regulates $CD4^+$ T cell and induce apoptosis signals in TH1 and TH17 cells [25]. About half of the immune cells that infiltrate through the synovial tissues are $CD4^+$ T cells that contribute in the generation of ectopic germinal centers where B cells proliferate and produce antibodies [4] TH17 cells also play a prominent role in the pathogenesis of RA. They differentiate through induction of IL-1, IL-6, IL-23, and TGF-β1 which are the main cytokines of fibroblasts cells and produced in the inflammatory conditions of joints [26].Galectin-9 also reported can induced apoptosis of hyperproliferative RA FLS and play role in the alleviate of RA [27]. As mentioned, IL-6 is produced through the stimulation of galectin-3. Based on the results of our study, treating FLS cells with PRP decrease production of Galectin-3 and increase production of Galectin-9, which maybe can lead to decreased cell differentiation of TH1 and TH17 as the prominent cells in the pathology of RA. In a recent study, Fujita et al. have investigated the correlation between circulating Galectin-9 and ACPA titers, and showed that high levels of circulating Galectin-9 correlates with the ACPA titers and other inflammatory markers of rheumatoid like MMP-3 and ESR. These results point to that, Galectin-9 could be a marker of disease activity under the status of ACPA. In addition, an increase has seen in the serum level of Galectin-9 in patients with progressive joint damage [24]. We also saw a significant increase in expression of Galectin-9 by synovial fibroblasts of RA patients due to treatment with PRP. Expression of TIM-3 by osteoclasts has been determined already; and Galectin-9 involves in preventing osteoclastogenesis through interaction with TIM-3 on osteoclasts [28]. Similar to our study, the effects of TNF-α on astrocyte cells have shown an increase in galectin-9, and they have emphasized the regulatory role of galectin-9 and apoptosis of encephalitogenic T cells [29]. PRP is probably an effective treatment by reducing inflammatory cytokines in limiting joint damage and reducing RA disease activity [30,31]. The present results also indicated that PRP treatment increased the gene expression of anti-inflammatory factors such as Galectin-1, Galectin-9 and TGF-β 1 in PRP-treated FLS cells. TGF-β1 is a multifunctional cytokine that play a key role in regulating immune response [32]. In an animal study, the effect of treating chondrocytes with PRP was investigated and an increase in expression of TGF-β1 was seen [17] Sullivan et al. showed that treatment of fibroblasts with TNF-α resulted in a significant increase in TGF-β1 protein expression [33]. Possible mechanisms of TNF-α effect in increasing TGF-β include increased half-life of TGF-β 1mRNA. TNF-α can activates the ERK-specific mitogen activated protein kinas pathway leading to increased TGF-β 1 production in fibroblasts primarily through post-transcriptional mechanisms involving TGF-β 1 transcriptional stability. Our results similarly show an increase in TGF-β 1in the group exposed to TNF-α alone. Inflammatory environment of rheumatoid synovial promotes an aggressive phenotype in FLS cells. Therefore, the suppressive effect of PRP depends on the presence of other secreted factors in vitro. Decreases in gene expression of TGF-β 1 and galectin-1 in SF + PRP and TNF + PRP groups, may be interpreted by increase inflammation in microenvironment that cells were cultured in it. By adding synovial fluid (containing inflammatory factors) to cultured FLS cells, inflammation of the environment was increased, and it is likely the concentration of PRP (10 %) has not been sufficient to increase the anti-inflammatory factors. Therefore, maybe increasing the concentration or exposure time of FLS cells with PRP can overcome the environmental inflammation and increase the expression of anti-inflammatory factors and immune modulation. Based on animal model study, tong et al. demonstrated that treatment with PRP suppressed inflammatory factors like IL-1β, IL-6, IL-17A, TNF-α and IFN-γ and increased TGF-β1, VEGF, PDGF and ICAM-1 in vitro and suggested that the efficacy and feasibility of PRP treatment was notable in RA and avascular necrosis [17]. The study of Lippross et al. showed that PRP can attenuate arthritic changes and reduce inflammatory mediators in synovial membrane and cartilage in RA [34]. Also, Ich Hur et al. demonstrated that PRP significantly reduced inflammatory cytokines (IL-6, MMP-3, and MCP-1) in the presence of IL-1β. Overall, our results are consistent with those of other studies demonstrating a protective effect of PRP. In concordance of studies discussed above, suppressing galectin-3 expression by PRP treatment on FLS cells can ameliorated the inflammation in microenvironment of FLS, suggesting the effectiveness of PRP in attenuating of inflammation in RA. There were some limitations in this study. The release of growth factors from PRP depends on the activation of platelets, and varies from one individual to others. These might have influenced the results. Therefore, further experiments with large sample size, higher concentration and increased treatment time

are required to confirm the effects of PRP in RA.

## 5. Conclusion

In conclusion, 10 % PRP concentration stimulated increases in the expression of anti-inflammatory cytokines such Galectin-1, Galectin-9, and TGF-β1 in FLS cells even without the development of inflammation using TNF-α and SF. PRP significantly decreased the expression of Galectin-3 in FLS cells with or without stimulate by TNF-α and SF and in PRP treated FLS cells. In vitro, TNF-α and SF could affect FLS cells' cytokine profiles. This study's findings suggest that PRP treatment of FLS cells has a positive impact on the inflammatory cytokines that are produced during RA. The use of PRP, its mode of action, and analysis of other potential molecules implicated in immune regulation in rheumatoid arthritis all require a great deal of investigation. In general and according to the relatively good results on immune modulation through the effect on the FLS cells of the rheumatoid environment by decreasing the type 3 inflammatory galectins and increasing the inhibitory galectins 9 and also increasing the TGF-β1 suppressive cytokine, it can be Future work will use different fractions or concentrations of PRP in clinical and patient studies in combination with other new immunosuppressive therapies. Also, investigating the simultaneous effects of these cells with B and T lymphocytes can be a point of hope for the treatment of autoimmune and inflammatory diseases. Overall, the findings of this study indicated that PRP can be a promising immunomodulator for inflammatory fibroblast-like synoviocyte cells in rheumatoid arthritis.

Limitation of study One of the limitations of our study, which was also mentioned in the article at the suggestion of the respected referees, was the small number of patients with joint fluid, and on the other hand, due to budget and time, we could not use more samples.

Conflict of interests The authors declare that they have no conflict of interests.

Data avalibility statement: Data will be made available on request by email to me:immuno.2006@yahoo.com.

## CRediT authorship contribution statement

**Shourangiz Piramoon:** Writing - review & editing, Writing - original draft, Visualization, Software, Project administration, Investigation, Formal analysis, Data curation. **Mohammad Taher Tahoori:** Writing - review & editing, Writing - original draft, Validation, Supervision, Software, Methodology, Investigation, Conceptualization. **Mohammad Bagher Owlia:** Visualization, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Mohammad Reza Royaei:** Writing - review & editing, Writing - original draft, Visualization, Software, Investigation, Formal analysis.

## 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.

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