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Original Article

Can the addition of ultrasound-guided genicular nerve block using 5% dextrose water augment the effect of autologous platelet rich plasma in treating elderly patients with knee osteoarthritis?

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ABSTRACT

Background: To investigate whether the addition of injecting 5% dextrose water to the genicular nerves can augment the effect of platelet rich plasma (PRP) injections in treating elderly patients with moderate degree of knee osteoarthritis (OA).
Methods: Forty-six elderly patients with grade 3 on the Kellgren and Lawrence knee OA grading system were divided into control and genicular nerve block groups. Both groups received 4 milliliters (mL) of PRP injected into the knee joint and 2 mL of PRP to the pes anserinus complex. In the nerve block group, 2 mL of 5% dextrose water was also injected to each genicular nerve. In the control group, normal saline was used to inject the genicular nerves. Proteomic technique of 2-dimensional electrophoresis was used to detect the changes in synovial fluid (SF) protein concentrations. Lequesne Functional Index was used to evaluate knee functional status.
Results: After 3 monthly PRP injections, concentrations of SF total protein, and proteins associated with inflammation decreased. Protein concentrations associated with chelation increased. In the nerve block group, improvements in pain and in the functional status of the knee joint lasted up to a period of 6 months. At 1 month and 3 months after the injections, Lequesne Functional Index was less than 7 (a value of 7 or higher indicates knee OA).
Conclusion: The concept of combining 5% dextrose water injection to the genicular nerves, and PRP injection into the knee joint and to the pes anserinus complex can be a feasible non-operative conservative treatment option for elderly patients with knee OA.

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At a glance commentary

Scientific background on the subject

For elderly patients with at least moderate degree of knee osteoarthritis, multiple monthly autologous platelet rich plasma injections combined with 5% dextrose water injection to the genicular nerves can be an effective non-operative treatment option.

What this study adds to the field

This study has contributed to the field of platelet rich plasma treatment as to how many injections are needed and whether simultaneous genicular nerve blocks are beneficial for patients with moderate to severe degrees of knee osteoarthritis. Synovial fluid proteomics are also thoroughly investigated in this study.

Knee osteoarthritis (OA) is the most common cause of pain in elderly patients [1]. These symptoms are often caused by the increased amount of synovial fluid (SF) gathered in the knee bursas, causing supra-patellar bursitis, and pes anserine bursitis [2,3]. The characteristic of knee OA is the progressive degeneration of articular cartilage, and chondrocytes producing inflammatory mediators that are released into the SF [4]. SF proteomic analysis can help us understand the status and the disease progression of knee OA. Elderly patients with moderate to severe degrees of knee OA are also affected by mal-alignment of the knee caused by narrowed medial compartment. The narrowed medial compartment can cause protrusion of the meniscus, and medial collateral ligament (MCL) sprain [5].

Autologous platelet-rich plasma (PRP) has been shown to be effective in treating patients with early stages of knee OA. The effect has been shown to be better than hyaluronic acid (HA) [6]. Growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF-β) are stored in platelet α-granules, and take part in the repair of articular cartilage, tendons, and ligaments [7]. When treating elderly patients with moderate to severe knee OA, injecting PRP solely into the knee joint may not be enough as pes anserine bursitis, MCL sprain, and meniscus injuries can all cause pain. Genicular nerves supplying the knee joint can be hypersensitive and inflamed due to continuous pain stimulation from these soft tissue structures. Prolotherapy has been shown to be effective in treating neuronal inflammation, using 5% dextrose water as the preferred injectant [8,9]. No study has investigated on whether the addition of prolotherapy can augment the effect of PRP in treating elderly patients with moderate to severe degrees of knee OA.

Proteomics, and functional pain scale of Lequesne Index will be used in this study to measure the SF protein concentrations and evaluate the changes in knee joint status before, during, and after the completion of PRP injections plus genicular nerve blocks. The SF total protein concentrations, and the changes in individual SF protein concentrations will be calculated. Individual proteins showing significant changes in concentrations will be further validated using Western Immunoblotting. Our previous study has shown that simultaneous intra-articular (IA) and pes anserinus complex PRP injections is more effective than performing IA PRP injection alone when treating elderly patients with moderate degree of knee OA [3]. Up on the conclusion of this study, we hypothesize that the injection of 5% dextrose water to block the genicular nerves can further augment the effectiveness of PRP, offering prolonged improvements in knee join pain and functional status. The crucial results obtained in this study can help us establish the most feasible injection technique for elderly patients who prefer the conservative non-surgical method in treating their knee OA.

Methods

This is a single-blinded, case-controlled study that was conducted in a tertiary hospital rehabilitation outpatient clinic. A total of 46 elderly patients with an average age of greater than 65 years of age and diagnosed with grade 3 on the Kellgren and Lawrence system (moderate degree of knee OA) for the classification of knee OA were recruited. Patients were divided into a control group ($N = 20$) and a nerve block group ($N = 26$). All the recruited patients signed the informed consent before participating in this study. This study was approved by the Institutional Review Board (IRB) of Chang Gung Medical Foundation.

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The inclusion criteria were:

1. Elderly patients who prefer the non-surgical option to treat their knee OA.
2. Patient had history of taking oral analgesics, knee joint steroid or HA injections or received physical modality treatments but without any obvious reduction in SF volumes and knee pain.
3. Protruded medial meniscus causing MCL sprain and the thickness of supra-patellar bursa was measured to be >2 mm (mm) as confirmed by musculoskeletal ultrasound. The ultrasound transducer was placed gently on top of the bursa. Therefore, the thickness of the bursa was not interfered by the pressure of transducer placement.
4. Increased hypoechoic changes at the pes anserinus complex, indicating bursitis and inflammation of the tendons, also confirmed by musculoskeletal ultrasound.

Patients with platelet counts less than 150,000 per microliter or taking anti-coagulant medications were excluded from this study. Patients were considered as “dropouts” when they have failed to comply to the monthly injections and regular outpatient clinic follow-ups after the completion of 3 monthly PRP injections. The "REGEN" environmental chamber for storage of platelet concentrate kit was used for PRP preparation (REGENLAB, RegenACR-C Classic, Switzerland). After harvesting 10 mL of blood from the patient, it was then injected into the PRP test tube chamber. Centrifugation was then performed for 15 min under the speed of 3400 rounds per minute (rpm). After centrifugation, the test tube was gently shaken for about 10 times. Approximately 6 milliliters (mL) of PRP supernatant was aspirated and was ready to be used for injection.

In both groups, 4 mL of PRP was injected into the knee joint, and 2 mL of PRP was injected to the pes anserinus complex (accurate injection under ultrasound-guidance can bath the pes anserine bursa, MCL, tendons (gracilis, sartorius, and semitendinosus), and the medial meniscus [3]. In the nerve block group, 6 mL of 5% dextrose water was also injected to the genicular nerves (2 mL to each nerve) that are located at the superior lateral, superior medial, and inferior medial aspects of the knee condyles [Figs. 1 and 2] [8]. In the control group, the same volume of 0.9% normal saline solution was injected to the genicular nerves.

Musculoskeletal ultrasound was used as an accurate guiding tool to place the needle correctly into the suprapatellar bursa site for SF aspiration, and intra-articular and pes anserinus complex PRP injections. Genicular nerves are often not clearly visible under ultrasound examination. When performing genicular nerve blocks, ultrasound is used to find the arteries that are located adjacent to the genicular nerves first. Using ultrasound-guidance, dextrose water is injected adjacent to the arteries, bathing the genicular nerves in a perineural fashion [8]. Injection was performed monthly for a total of 3 months. The T3300 Tablet Ultrasound Imaging System with a bandwidth of 4–15 MHz linear transducer was used (BenQ Medical Technology Corporation). Accurate placement of the needle into the bursa is a must to avoid unnecessary poking that may cause blood contamination in the SF. The standard lateral approach with the knee slightly flexed was applied for the aspiration and injection procedures. The same physician with board certified sonologist license interpreted the ultrasound images, and performed all the aspiration and injection procedures to avoid experimental bias.

Lequesne Index

The Lequesne Functional Index for degenerative knee joint was used to evaluate the functional status and the extent of knee pain before, during, and after PRP/nerve block injections. The Lequesne Functional Index was evaluated by the same experimenter to avoid inter-tester variability. An index value of greater than 7 is indicative of knee degeneration [10].

Calculation of synovial fluid total and individual protein concentrations

The Bradford method was applied for total SF protein calculations [11]. The proteomic technique of 2-dimensional electrophoresis (2-DE) was used to detect significant increases and decreases in SF protein spot intensities before and after PRP/nerve block injections. Steps of protein concentration calculations, 2-DE, and protein identification using mass spectrometry (MS) are thoroughly mentioned in our previously published article [12]. In short, the MS-compatible PlusOne silver staining kit (GE Healthcare BioSciences) was used in this study to stain the 2-DE gels. Spots were excised using a One-Touch Plus spot-picker with 3-mm diameter spot cutter (Web Scientific Limited, Crewe, UK) and placed into 96-well plates (Genomic Solutions, Huntingdon, UK). In-gel digestion was carried out using a ProGest robotic digestion system (Genomic Solutions). Gel pieces were de-stained in a solution containing 15 mM of potassium ferrocyanide/50 mM sodium thiosulphate

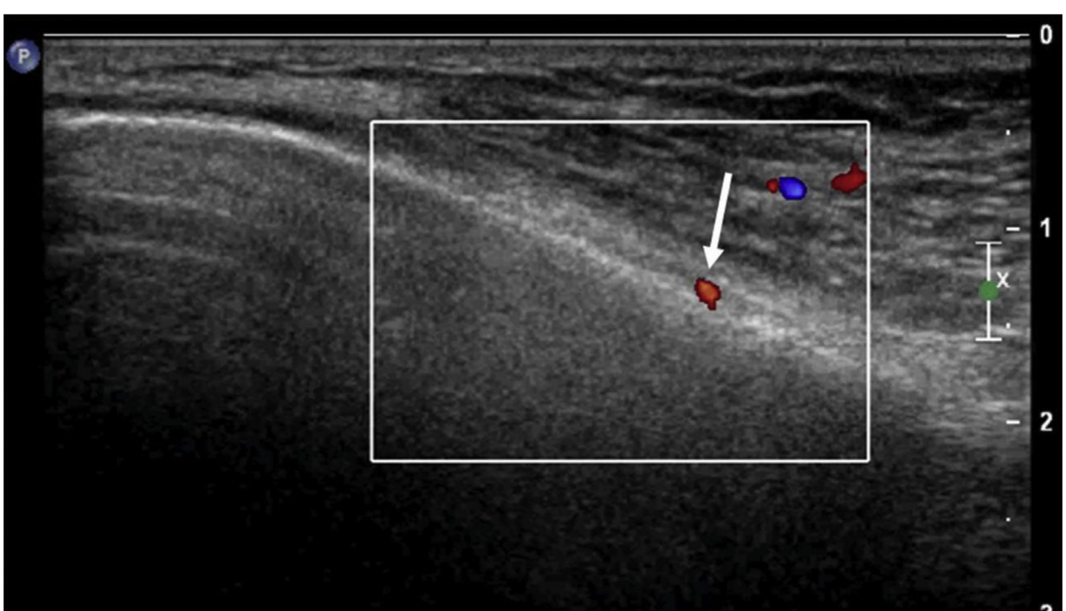

Fig. 1 Musculoskeletal ultrasound image of the genicular artery (arrow) at the superior lateral supracondylar area of the femur. The genicular nerve is located just adjacent to the genicular artery.

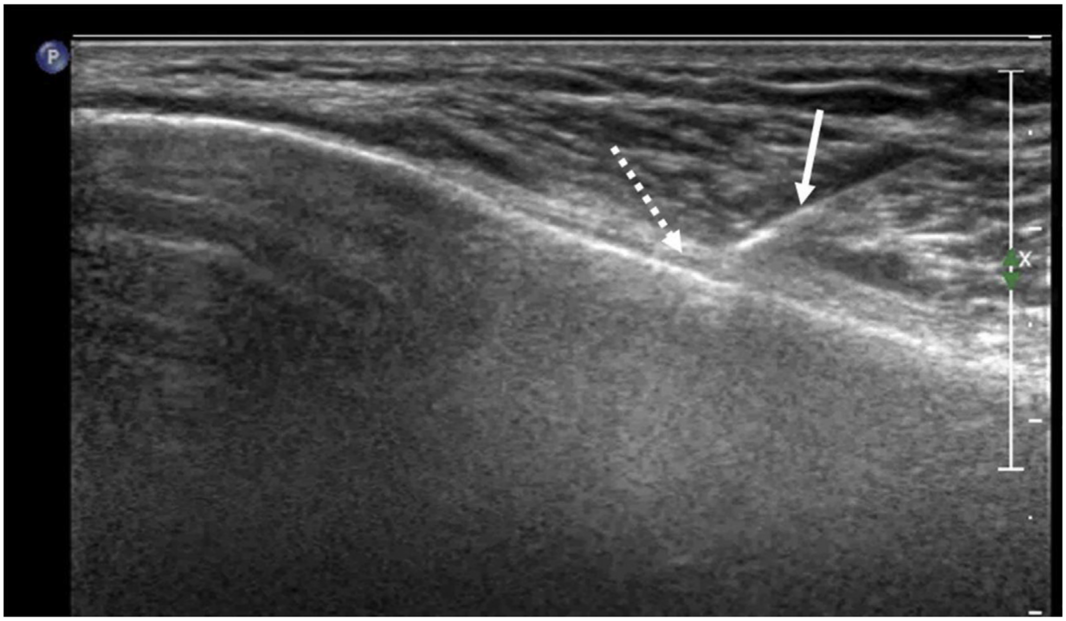

Fig. 2 Musculoskeletal ultrasound image showing how genicular nerve injection is done. Arrow indicates the injection needle. The needle approaches the target in a cephalad to caudad direction. The injection target is located adjacent to the artery (dotted arrow). The injectant then baths the surrounding soft tissue of the artery, indicating perineural injection to the genicular nerve.

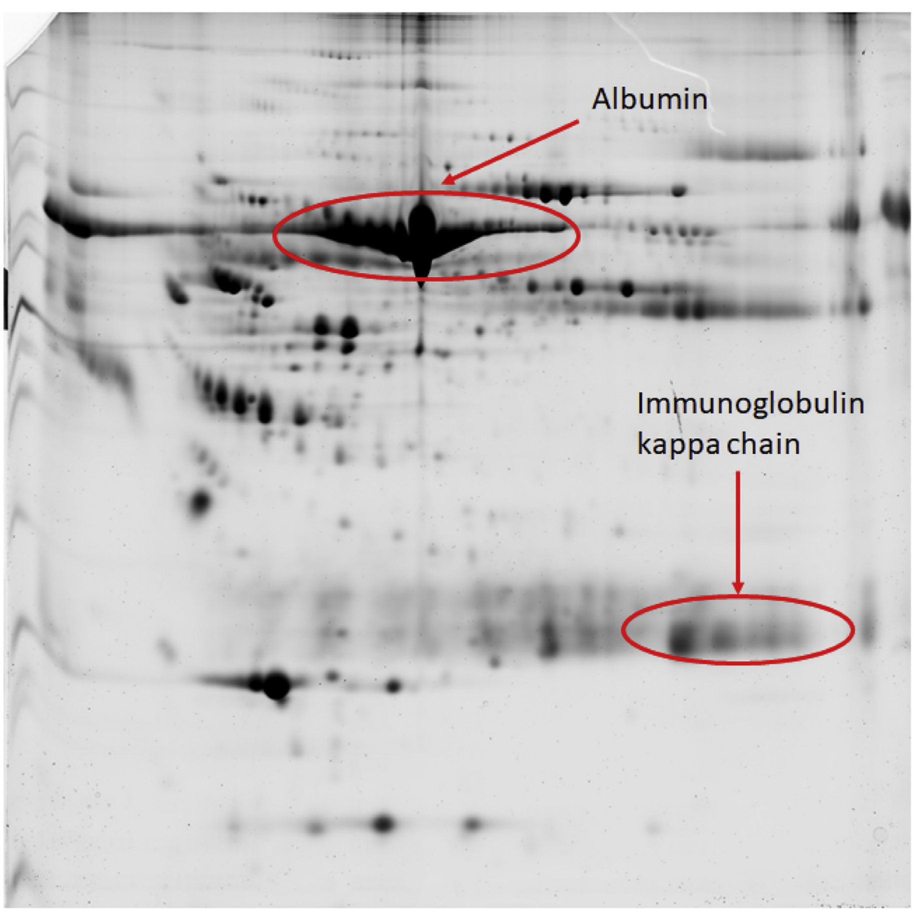

Fig. 3 2-DE gel image of synovial fluid proteins before PRP injections.

followed by three washes with ddH_2O. Cysteine residues were reduced with DTT and derivatized with iodoacetic acid. Each gel piece was then dehydrated with ACN and dried at 60 °C followed by rehydration with a minimum volume of digestion buffer, containing modified trypsin in 25 mM of ammonium hydrogen carbonate (Promega, Madison, WI), and incubated for 8 h at 37 °C. Protein names and accession numbers were determined according to the SwissProt and TrEMBL databases.

Western immunoblotting

Western immunoblotting method was used to validate the protein spots that revealed at least 2-fold changes in concentrations after 2-DE analyses. Polyclonal and monoclonal antibodies were purchased from the ABCAM company (ABCAM, Cambridge, MA, USA) to detect the proteins of immunoglobulin kappa chain (IGKC), haptoglobin (HPT), apolipoprotein A-I (APOA1), matrix metalloproteinase-9 (MMP9), serpin B13 (SERPINB13), cartilage matrix protein (MATN1), transthyretin (TTR), and complement 5 (C5). The steps required in Western immunoblotting can be observed in our previously published article [12].

Statistical analysis

Repeated measures ANOVA and pairwise comparisons with Bonferroni correction for post hoc tests was used to compare the SF total protein and individual protein concentrations, and changes in Lequesne Functional Index values before, during, and after PRP plus dextrose water nerve blocks or normal saline injections. Protein concentrations were calculated, and the Lequesne index evaluated at the time periods of 1st, 2nd, 3rd PRP and nerve blocks or normal saline injections, and the follow up time periods of 1, 3 and 6 months. The independent sample t-test was used to compare the differences of LeQuesne Functional Index, average SF total protein concentrations, and average number of protein spots on 2-DE gels between the nerve block and the control groups during each PRP injection, and follow up periods. The Mann–Whitney U test was used to compare the difference in age between patients. The Statistical Program for Social Sciences (SPSS) program (SPSS Inc., Chicago, IL) was used for data calculations. Values were presented as means ± standard deviations, and with $p < 0.05$ considered as statistically significant.

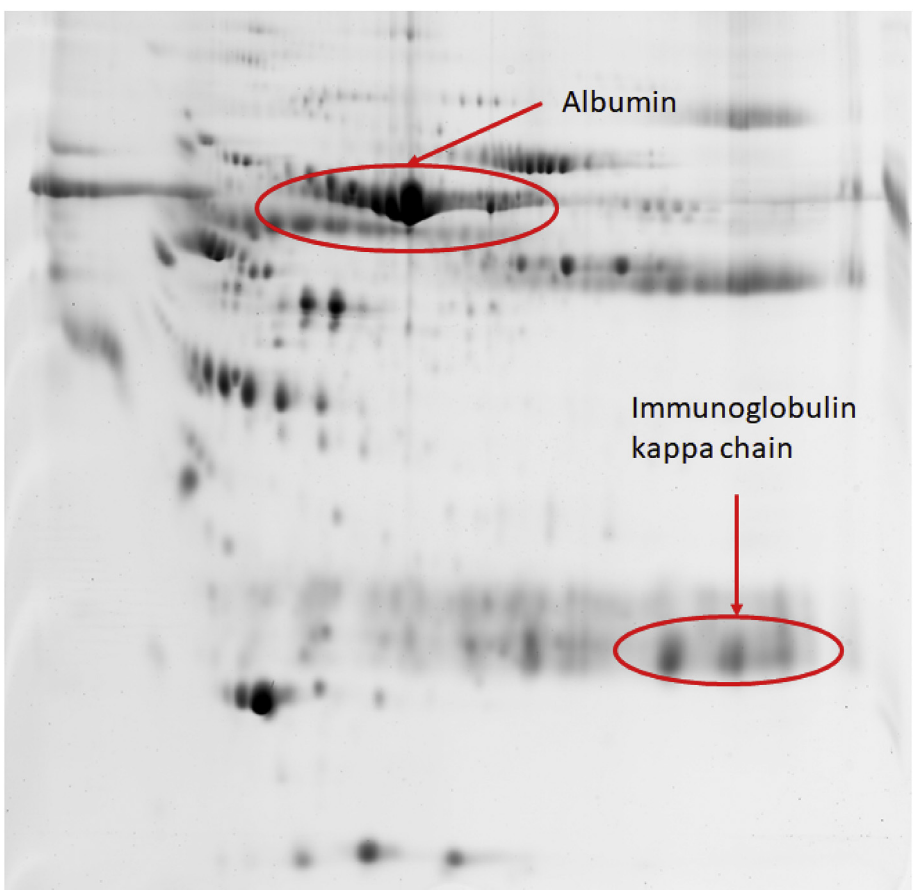

Fig. 4 2-DE gel image of synovial fluid proteins after the completion of 3 monthly PRP injections. Since synovial fluid total concentrations are decreased after PRP injections, 2-DE gel images indicated significant decreases in albumin and immunoglobulin protein spot intensities.

Results

The average platelet concentration factor was measured to be 1.93 ± 0.46 times as compared with the baseline whole blood after centrifugation. The mean age of the 26 recruited patients in the nerve block group (>65 years of age, 14 female and 12 male patients) was 70 ± 5.8 years and the mean age of the 20 recruited patients in the control group (>65 years of age, 12 female and 8 male patients) was 69 ± 3.17 years. The age distribution in these 46 patients did not reveal any statistical differences as confirmed by Mann–Whitney U test. No patients dropped out of this study.

In both groups, 3 monthly PRP injections resulted in significant concentration changes in SF total protein and individual protein concentrations. SF total protein concentrations, and the number of protein spots on 2-DE gel images decreased significantly after the completion of 3 monthly PRP injections [Figs. 3 and 4]. In-between group comparisons did not reveal any significant differences in terms of protein spot numbers on 2-DE gels between the two groups [Table 1]. After 2-DE analyses, the 4 SF proteins that revealed the most significant decreases in concentrations were immunoglobulin kappa chain (IGKC), haptoglobin (HPT), apolipoprotein A-I (APOA1), and matrix metalloproteinase-9 (MMP9). The 4 SF proteins that revealed the most significant increases in concentrations were SERPINB13 (SERPIN), cartilage matrix protein (MATN1), transthyretin (TTR), and complement (C5). The changes in the concentrations of these proteins were at least 2-fold. These proteins were further validated by Western immunoblotting [Fig. 5 reveals the images of the individual protein spots identified by 2-DE, Fig. 6 reveals increased protein band intensities of TTR, Fig. 7 reveals decreased protein band intensities of IGKC, and Table 2 mentions the names of these proteins confirmed by MS].

In terms of average Lequesne Functional Index, significant decreases were observed starting at the time of 3rd injection (patients have received 2 monthly PRP and 5% dextrose water genicular nerve blocks) and lasting up to a period of 6 months in the nerve block group. Significantly, Lequesne Functional Index improved to a value of less than 7 at 1 month and 3 months after the injections (index value greater than 7 is indicative of knee OA). In terms of in-between group comparisons, Lequesne Functional Index values were significantly

Table 1 Differences in LeQuesne index, synovial fluid total protein concentrations, and number of protein spots before, during and after PRP/nerve block injections.

Measured parameters	Experimental time periods					
	Time of 1st PRP injection	Time of 2nd PRP injection	Time of 3rd PRP injection	1 month after 3 monthly PRP injections	3 months after 3 monthly PRP injections	6 months after 3 monthly PRP injections
Nerve block group Lequesne Functional Index	15.38 ± 2.96	14.61 ± 2.25	6.14 ± 1.17$^{+,\#}$	4.88 ± 2.08$^{+,\#}$	5.14 ± 1.75$^{+,\#}$	6.68 ± 1.36$^{+,\#}$
Control group Lequesne Functional Index	15.22 ± 3.71	14.55 ± 3.64	11.43 ± 1.54	7.38 ± 2.41*	9.38 ± 2.26	10.47 ± 2.33
Nerve block group Average SF total protein concentration in μg/μL	35.17 ± 4.77	36.22 ± 3.23	34.04 ± 2.44	26.51 ± 2.51*	26.83 ± 2.32*	27.42 ± 3.69
Control group Average SF total protein concentration in μg/μL	33.78 ± 3.66	32.57 ± 2.86	30.55 ± 2.67	25.33 ± 3.11*	26.17 ± 3.53*	28.71 ± 3.98
Nerve block group Average number of protein spots on 2-DE gels	501.33 ± 41.53	488.41 ± 42.63	459.87 ± 45.96	350.15 ± 37.17*	299.37 ± 38.22*	333.34 ± 26.97*
Control group Average number of protein spots on 2-DE gels	518.74 ± 55.17	533.27 ± 47.56	498.59 ± 38.77	344.68 ± 35.52*	355.49 ± 40.11*	360.37 ± 45.18*

*Significant statistical differences at the time of follow up periods (received 3 monthly PRP injections) as compared with the time when patients only received 1 PRP injection (analyzed using the repeated measures ANOVA and pairwise comparisons with Bonferroni correction for post hoc tests, $p < 0.05$).

$^{+}$Significant statistical differences at the time of 3rd PRP injection (received 2 monthly PRP injections) and the follow up periods as compared with the time when patients only received 1 PRP injection (analyzed using the repeated measures ANOVA and pairwise comparisons with Bonferroni correction for post hoc tests, $p < 0.05$).

$^{\#}$Significant statistical differences after comparisons between nerve block and control groups (analyzed using the independent sample t-test, $p < 0.05$).

Values expressed as mean ± standard deviations (SD).

Abbreviations: SF: Synovial fluid; PRP: Platelet rich plasma; NS: Normal saline.

lower in the nerve block group as compared with the control group during the follow up periods [Table 1].

Discussion

This study thoroughly investigated the proteomic changes of the SF before, in between, and after the completion of 3 monthly PRP injections plus 0.9% normal saline (control group) or 5% dextrose water genicular nerve blocks. Changes in pain and functional status of knee joint were also thoroughly investigated. Our previous study has shown that simultaneous IA and pes anserinus complex PRP injections are needed in effectively treating elderly patients with moderate to severe knee OA. Injecting PRP solely into the knee joint is not adequate in these elderly patients [3]. This study documented that the addition of 5% dextrose water to the genicular nerves can further augment the effect of PRP in treating elderly patients with knee OA as compared with the controls of injecting normal saline. Therefore, the improvements in LeQuesne index values observed in this study is the end result of combined treatment effect of IA and pes anserinus complex PRP injections plus genicular nerve blocks using dextrose water. Without these injection treatments, the natural disease progression course of moderate to severe knee OA should only get worse with time, resulting in further deterioration of knee function.

Musculoskeletal ultrasound can be used as an effective tool in guiding the needle to the target site that is to be injected [13]. Bathing the MCL and medial meniscus areas can ensure tissue repair and regeneration of these tissues, resulting in less pain during standing and walking [14]. The 2-DE technique can detect hundreds of protein spots simultaneously in one gel image [15]. Albumin and immunoglobulin are the abundant proteins that constitute about 60–80 percent of the SF total protein concentration [12]. As a result, significant decrease in SF immunoglobulin kappa chain protein (IGKC) concentration may be the major cause of decreased total protein concentration after PRP injections.

Inflammation of the synovial membrane is an on-going process in knee OA as cartilage destruction is observed and IGKC is highly associated with inflammation [16]. Haptoglobin (HPT) is an acute phase glycoprotein and its increased concentration is closely correlated with inflammation [17]. It has been shown that IGKC, and albumin protein concentrations are increased in knee OA [18]. Dysregulation of the SF lipid profile is the cause of inflammation in knee OA patients. Apolipoprotein A-1 (APOA1) protein is plasma derived and is associated with the lipid transport. The pro-inflammatory property of APOA1 is correlated with the expression of matrix metalloproteinase-9 (MMP9) [19]. MMP9 induces inflammation in OA by decreasing the expression of chondrocyte proteins, causing knee pain, and the loss of joint movements [20]. As a result, decreases in SF

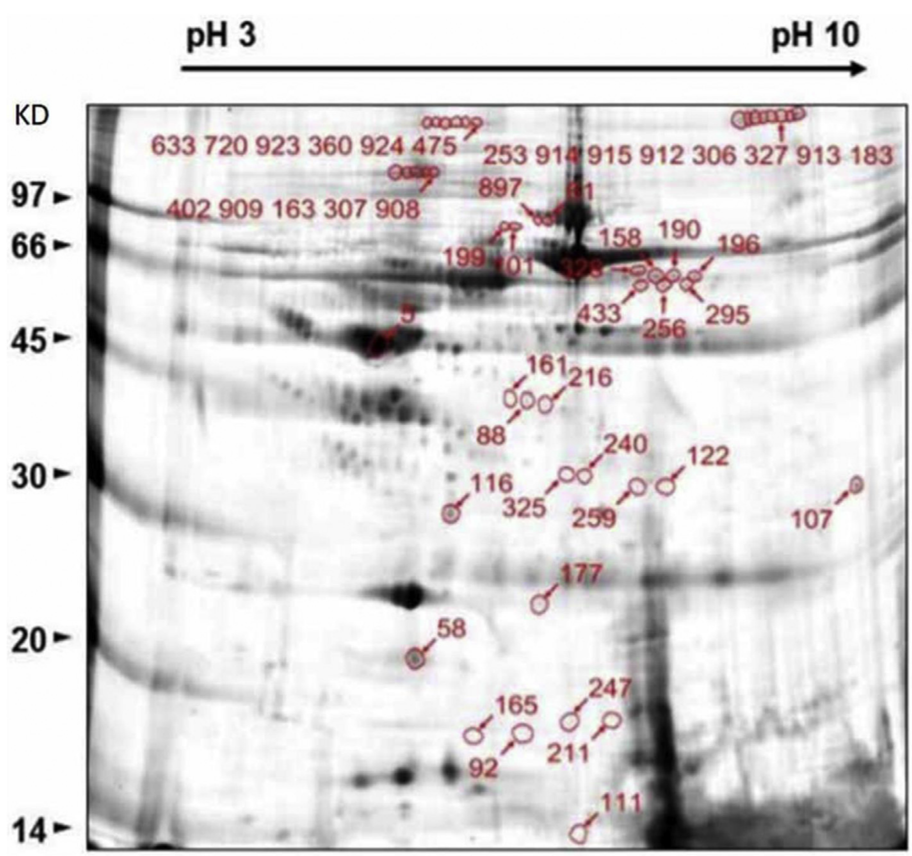

Fig. 5 SYPRO Ruby stained 2-DE gel image of the SF sample. Each number indicates an SF protein spot. Molecular weights (MW) are expressed in kilodaltons (KD).

IGKC, HPT, APOA1 and MMP9 concentrations after PRP injections highly indicate the attenuation of inflammatory responses.

Amyloid deposition is a risk factor of developing OA, and its concentration is positively correlated to the severity of knee OA. Transthyretin (TTR) is synthesized in the choroid plexus and liver, and is detected on aged cartilage surface. TTR can chelate amyloid and avoid amyloid deposition in the knee joints [21]. As a result, increases in SF TTR concentrations after PRP injections can suggest less risk in developing OA. Cartilage matrix protein (MATN1) is an extracellular matrix protein that contributes to a healthy cartilage microenvironment. In MATN1 knock-out mice, chondrocyte hypertrophy is observed, and results in cartilage damage [22]. The complement system is associated with the ability of phagocytic cells to clear pathogens. Thus, increased complement C5 (C5) concentration suggests better clearance of pathogens in knee OA patients [23]. Serpin B13 (SERPIN) is also known as plasminogen activator inhibitor-1 (PAI-1) protein. It has inverse correlation with immunoglobulin. As a result, increased expression of the serpin gene indicates suppression of the inflammatory process that occurs in knee OA [24].

Although similar SF proteomic pictures were observed in both groups, but the changes in the degree of pain and the functional status of the knee joints were significantly different. Perhaps the most meaningful finding in this study is that the average Lequesne Functional Index improved to a value of less than 7 at 1 month and 3 months after the completion of PRP plus dextrose water genicular nerve blocks. This was not observed in the control group when normal saline was injected. Lequesne Functional Index of less than 7 suggests that the functional status of the knee joints has improved significantly as an index value of 7 is indicative of knee OA [10]. The proposed mechanism is the dextrose mediated inhibition of the transient receptor potential cation channel subfamily V member 1 (TRPV1) receptors and neurogenic inflammation [25]. In OA patients with grade 3 on the Kellgren and Lawrence system, knee joints are often troubled with pes anserine bursitis, and medial joint space narrowing. These can contribute to chronic pain, weakness, and deteriorated functional status of the knee joint. Chronic knee pain can cause hypersensitivity and inflammation of the surrounding genicular nerves, activating the TRPV1 receptors. Ligaments, tendons and joints have TRPV1 – sensitive C pain fiber innervations. TRPV1 activation will lead to

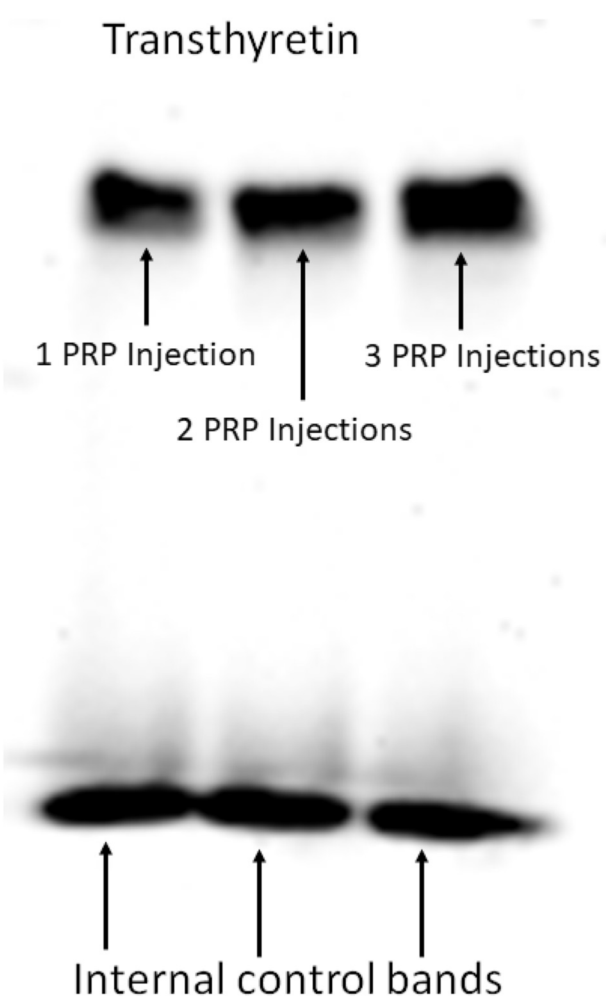

Fig. 6 Western immunoblotting of transthyretin protein. Increased band intensities (indicating increased concentrations) can be observed after each PRP injection.

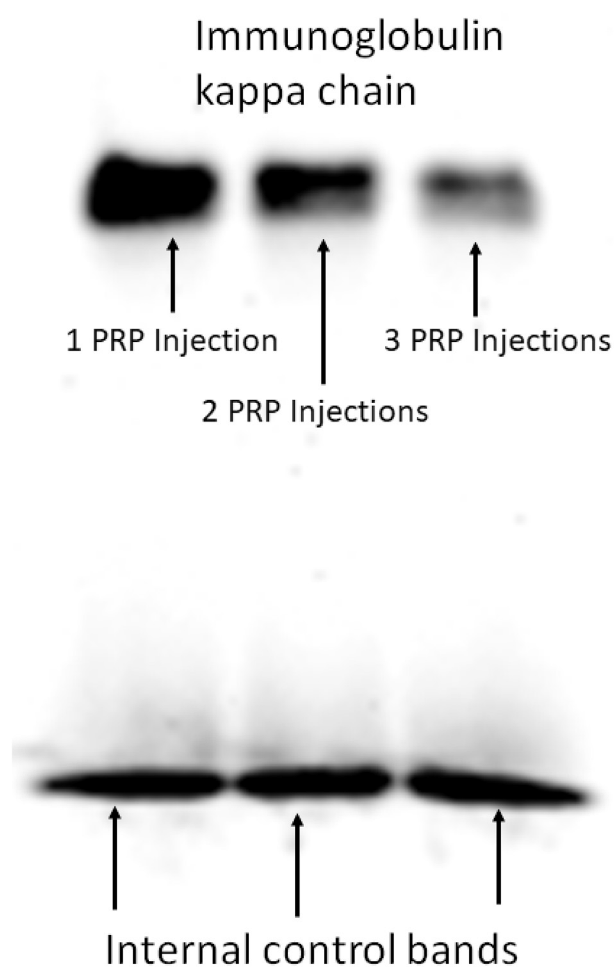

Fig. 7 Western immunoblotting of immunoglobulin kappa chain protein. Decreased band intensities (indicating decreased concentrations) can be observed after each PRP injection.

Table 2 Synovial fluid protein names confirmed by mass spectrometry.

Spot name[a]	Description	Protein ID	Mr (kDa)[b]	pI[b]	Changes in %
APOA1	Apolipoprotein A-1	P02647	30.00	5.56	↓ 277
MMP9	Matrix metalloproteinase-9	P14780	92.00	5.70	↓ 214
IGKC	Immunoglobulin kappa chain	Q6P5S8	25.77	5.94	↓ 395
HPT	Haptoglobin	P00738	43.35	6.13	↓ 227
TTR	Transthyretin	A6XMH1	14.96	5.49	↑ 308
MATN1	Cartilage matrix protein	P21941	54.00	8.31	↑ 244
C5	Complement 5	P01031	73.29	5.01	↑ 207
SERPIN	SERPIN B13	F8WE70	18.89	5.20	↑ 211

↑: Percentage increase in protein concentration after patient has received 3 monthly PRP injections.
↓: Percentage decrease in protein concentration after patient has received 3 monthly PRP injections.
[a] Protein name and accession number according to the SwissProt and TrEMBL databases.
[b] Predicted molecular weights in kilodaltons and p *I* (isoelectric point) according to protein sequence and Swiss-2DPAGE databases.

deep pain, and burning sensations of the knee joint [26]. Therefore, desensitization of the genicular nerves can be a feasible concept in treating patients with moderate to severe knee OA. Genicular nerves are located at the superior medial, inferior medial, and superior lateral condylar regions. Five percent dextrose solution has been shown to be the most ideal injectant in desensitizing the hypersensitive nerves. Nerve block using 5% dextrose water to bath the genicular nerves has been shown to offer effective pain reduction lasting from 4 h to several weeks [9]. The injection is perineural and 5% dextrose water has an osmolality similar to the human physiological condition and is not harmful to nerves [9]. In the control group, 0.9% normal saline was the injectant used and has not been reported to have any influence on TRPV1 receptors.

As a result, the addition of 3 monthly perineural genicular nerve blocks by injecting 5% dextrose water can further augment the treatment effectiveness of PRP, offering significant improvements in knee pain and functional status lasting up to a period of 6 months. There were shortcomings in this study. For instance, other injectants such as steroids may also be applied to the genicular nerves to see if improvements in pain and function can also be achieved.

Conclusion

This study covered proteomics and functional status evaluations for elderly patients with grade 3 on the Kellgren and Lawrence grading system for knee osteoarthritis. After 3 monthly platelet rich plasma injections, synovial fluid total protein concentrations, and proteins associated with inflammation (eg/immunoglobulin) were significantly decreased. Synovial fluid protein concentrations associated with chelation (eg/transthyretin) increased. The application of perineural genicular nerve blocks by injecting 5% dextrose water further augments the treatment effectiveness of platelet rich plasma, offering long-lasting pain and functional improvements of the knee joints lasting up to a period of 6 months. At 1 and 3 months post PRP plus genicular nerve blocks, the average Lequesne Functional Index improved to a value of less than 7, suggesting significant improvement in knee function as an index value of greater than 7 indicates the likelihood of knee osteoarthritis. As a result, the strategy of combining 3 monthly genicular nerve blocks using 5% dextrose water with platelet rich plasma injections into the knee joint and the pes anserinus complex can be an effective non-surgical conservative treatment option in treating elderly patients with moderate to severe degrees of knee osteoarthritis.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

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REFERENCES

[1] Kon E, Mandelbaum B, Buda R, Filardo G, Delcogliano M, Timoncini A, et al. Platelet-rich plasma intra-articular injection versus hyaluronic acid viscosupplementation as treatments for cartilage pathology: from early degeneration to osteoarthritis. Arthroscopy 2011;27:1490–501.
[2] Yagi S, Sata M. Rupture of pes anserine bursa in a patient with pes anserine pain syndrome due to osteoarthritis. J Med Invest 2019;66:211–2.
[3] Chen CPC, Chen JL, Hsu CC, Pei YC, Chang WH, Lu HC. Injecting autologous platelet rich plasma solely into the knee joint is not adequate in treating geriatric patients with moderate to severe knee osteoarthritis. Exp Gerontol 2019;119:1–6.
[4] Stohr H, Molday LL, Molday RS, Weber BH, Biedermann B, Reichenbach A, et al. Membrane-associated guanylate kinase proteins MPP4 and MPP5 associate with Veli3 at distinct intercellular junctions of the neurosensory retina. J Comp Neurol 2005;481:31–41.

[5] Uysal F, Akbal A, Gokmen F, Adam G, Resorlu M. Prevalence of pes anserine bursitis in symptomatic osteoarthritis patients: an ultrasonographic prospective study. Clin Rheumatol 2015;34:529–33.

[6] Laudy AB, Bakker EW, Rekers M, Moen MH. Efficacy of platelet-rich plasma injections in osteoarthritis of the knee: a systematic review and meta-analysis. Br J Sports Med 2015;49:657–72.

[7] Khoshbin A, Leroux T, Wasserstein D, Marks P, Theodoropoulos J, Ogilvie-Harris D, et al. The efficacy of platelet-rich plasma in the treatment of symptomatic knee osteoarthritis: a systematic review with quantitative synthesis. Arthroscopy 2013;29:2037–48.

[8] Kim DH, Choi SS, Yoon SH, Lee SH, Seo DK, Lee IG, et al. Ultrasound-guided genicular nerve block for knee osteoarthritis: a double-blind, randomized controlled trial of local anesthetic alone or in combination with corticosteroid. Pain Physician 2018;21:41–52.

[9] Wu YT, Ke MJ, Ho TY, Li TY, Shen YP, Chen LC. Randomized double-blinded clinical trial of 5% dextrose versus triamcinolone injection for carpal tunnel syndrome patients. Ann Neurol 2018;84:601–10.

[10] da Silva FS, de Melo FE, do Amaral MM, Caldas VV, Pinheiro IL, Abreu BJ, et al. Efficacy of simple integrated group rehabilitation program for patients with knee osteoarthritis: single-blind randomized controlled trial. J Rehabil Res Dev 2015;52:309–22.

[11] Olson BJSC. Assays for determination of protein concentration. Curr Protoc Pharmacol 2016;73. A.3a.1–32.

[12] Chen CP, Hsu CC, Pei YC, Chen RL, Zhou S, Shen HC, et al. Changes of synovial fluid protein concentrations in supra-patellar bursitis patients after the injection of different molecular weights of hyaluronic acid. Exp Gerontol 2014;52:30–5.

[13] Chen CPC, Cheng CH, Hsu CC, Lin HC, Tsai YR, Chen JL. The influence of platelet rich plasma on synovial fluid volumes, protein concentrations, and severity of pain in patients with knee osteoarthritis. Exp Gerontol 2017;93:68–72.

[14] Bagwell MS, Wilk KE, Colberg RE, Dugas JR. The use of serial platelet rich plasma injections with early rehabilitation to expedite grade III medial collateral ligament injury in a professional athlete: a case report. Int J Sports Phys Ther 2018;13:520–5.

[15] Gorg A, Weiss W, Dunn MJ. Current two-dimensional electrophoresis technology for proteomics. Proteomics 2004;4:3665–85.

[16] Chang X, Cui Y, Zong M, Zhao Y, Yan X, Chen Y, et al. Identification of proteins with increased expression in rheumatoid arthritis synovial tissues. J Rheumatol 2009;36:872–80.

[17] Park HJ, Oh MK, Kim NH, Cho ML, Kim IS. Identification of a specific haptoglobin C-terminal fragment in arthritic synovial fluid and its effect on interleukin-6 expression. Immunology 2013;140:133–41.

[18] Izai M, Miyazaki S, Murai R, Morioka Y, Hayashi H, Nishiura M, et al. Prorenin-renin axis in synovial fluid in patients with rheumatoid arthritis and osteoarthritis. Endocrinol Jpn 1992;39:259–67.

[19] de Seny D, Cobraiville G, Charlier E, Neuville S, Lutteri L, Le Goff C, et al. Apolipoprotein-A1 as a damage-associated molecular patterns protein in osteoarthritis: ex vivo and in vitro pro-inflammatory properties. PLoS One 2015;10:e0122904.

[20] Lu S, Xiao X, Cheng M. Matrine inhibits IL-1beta-induced expression of matrix metalloproteinases by suppressing the activation of MAPK and NF-kappaB in human chondrocytes in vitro. Int J Clin Exp Pathol 2015;8:4764–72.

[21] Akasaki Y, Reixach N, Matsuzaki T, Alvarez-Garcia O, Olmer M, Iwamoto Y, et al. Transthyretin deposition in articular cartilage: a novel mechanism in the pathogenesis of osteoarthritis. Arthritis Rheumatol 2015;67:2097–107.

[22] Yang X, Trehan SK, Guan Y, Sun C, Moore DC, Jayasuriya CT, et al. Matrilin-3 inhibits chondrocyte hypertrophy as a bone morphogenetic protein-2 antagonist. J Biol Chem 2014;289:34768–79.

[23] Wang Q, Rozelle AL, Lepus CM, Scanzello CR, Song JJ, Larsen DM, et al. Identification of a central role for complement in osteoarthritis. Nat Med 2011;17:1674–9.

[24] Comblain F, Dubuc JE, Lambert C, Sanchez C, Lesponne I, Serisier S, et al. Identification of targets of a new nutritional mixture for osteoarthritis management composed by curcuminoids extract, hydrolyzed collagen and green tea extract. PLoS One 2016;11:e0156902.

[25] Morgan M, Nencini S, Thai J, Ivanusic JJ. TRPV1 activation alters the function of Adelta and C fiber sensory neurons that innervate bone. Bone 2019;123:168–75.

[26] Arsenault P, Chiche D, Brown W, Miller J, Treister R, Leff R, et al. NEO6860, modality-selective TRPV1 antagonist: a randomized, controlled, proof-of-concept trial in patients with osteoarthritis knee pain. Pain Rep 2018;3:e696.