MMSL
Military Medical
Science Letters
Since 1925

ORIGINAL ARTICLE

THE ROLE OF CARTILAGE INTERMEDIATE LAYER PROTEIN2 (CILP2) IN EVALUATING THE EFFECT OF TREATMENTS (PLATELET-RICH PLASMA AND HYALURONIC ACID) ON PATIENTS WITH EARLY KNEE OSTEOARTHRITIS

Ali K. Albdeery, Ajil A. Alzamily ✉, Ihsan A. Alsalman

College of Medicine, University of AL-Qadisiyah, Iraq

Summary

Background: Cartilage intermediate layer protein 2 (CILP2) is a monomeric glycoprotein that is mostly expressed in the intermediate zone of articular cartilage and can be detected in the extracellular matrix (ECM). The level of CILP2 in serum and its quantity on the articular cartilage surface and throughout the articular cartilage indicates the potential utility of CILP2 for investigation as a biomarker for determining cartilage deterioration in joint disorders. The CILP2 measurement of changes in cartilage biomarkers may be an effective and sensitive tool for detecting the early development of knee osteoarthritis (KOA) in people at risk for KOA. Changes in CILP2 levels may be beneficial for the early diagnosis of KOA, as CILP2 appears to be linked to cartilage thickness reduction in people who are more likely to develop KOA. Platelet rich plasma (PRP) effectiveness of transforming growth factor, platelet-derived growth factor, and the vascular endothelial growth factor is thought to be related to their release. Because of their capacity to increase matrix formation, growth factors have been widely researched for OA and cartilage regeneration. Hyaluronic acid (HA) is a glycosaminoglycan polymer composed of N-acetyl glucosamine and D-glucuronic acid disaccharide molecules. Early embryonic development, inflammatory, wound repair, cell differentiation, and viscoelasticity are all affected by HA, and other biological processes The study was aimed to evaluate effecte of CILP2 levels after intraarticular injecting pure platelet-rich plasma and hyaluronic acid into patients with early knee osteoarthritis.
Materials and Methods: BT LAB kits were used to assess the serum CILP2 level. The experimental investigation included 18 control groups, 10 HA injections, and 21 pure PRP injections. age ranged from 35 to 75. The study was exluded all individuals with advanced osteoarthritis in the knee, COVID-19, diabetes, and autoimmune diseases. the time frame running from November 2021 until June 2022. Other variables in our research were age, gender, family history, use of antihypertensive medications or medications for other disorders, and body mass index (BMI).
Results: The findings of this study demonstrate elevated CILP2 levels in patients with early KOA before treatments injection compared with the healthy control. After treatments injection, the level of CILP2 was decreased compared with before injection.

✉ University of AL-Qadisiyah, College of Medicine, Iraq
🖳 ajil.alzamily@qu.edu.iq
☎ +9647810624784

Conclusions: CILP2 could be one of the prognostic signs of early KOA.

Key words: Osteoarthritis; CILP2; Joints; Arthritis, PRP; Hyaluronic acid

Introduction

One of the biggest synovial joints in the human body is the knee. It consists of bones such as (the distal femur, proximal tibia and patella), cartilage (articular cartilage and hyaline cartilage), ligaments and synovium, where this membrane lubricates and delivers the necessary nutrition to the avascular cartilage (1). Knee osteoarthritis (KOA) is a condition that progresses over time and is a primary cause of physical impairment (2). People who suffer from recurrent knee pain (such as slow walking and inability to stand) report more frequent knee pain than those with gradual onset knee osteoarthritis (3).

Excessive joint stresses, articular cartilage abnormalities, and subchondral bone deformities can cause KOA. High metabolic activity increases the activity of inflammatory reactions, which leads to the destruction of articular cartilage and subchondral bone (4). Chondrocytes release several inflammatory cytokines that act together to induce the creation of cartilage-degrading enzymes. Interleukin-1 (IL-1beta), tumour necrosis factor (TNF-alpha), and interleukin-6 are all important cytokines (IL-6) (5). Compared to established KOA, early KOA of the knee is characterized by indications and symptoms that may be sporadic and restricted. It at least predicts radiographic KOA (6). A high risk of developing early OA exists in several populations (7), Young people with meniscal tears are one example. Patients with trochlear dysplasia and patellar instability are another (8).

To confirm and characterize joint involvement, and to rule out pain and functional limitations, all patients with knee discomfort should be investigated.syndromes brought on by other reasons.alone or in combination, persistent knee discomfort with MRI results indicating that the first and primary symptom is OA. In the morning, after a period of inactivity, or especially in the evening, patients with early OA also feel stiff (9). stage of the illness using current conventional diagnostics, namely radiographic examination, when there are significant cartilage changes in the knee joint (e.g. shrinking of the articular surface in the knee and growth of osteophytes). Patients frequently have functional limitations and discomfort markers for cartilage by this stage. Cartilage intermediate layer protein 2 (CILP2) might be useful (10). KOA progression before symptoms of the significant structural disease is highlighted using conservative methods (11).

While cartilage variables may accelerate OA advancement in some people, bone or inflammation-related factors may accelerate OA progression in others (12). Both have changed the production and breakdown of components of the skeletal matrix and are involved in metabolic changes in tissues linked with the joints in various phases of incident OA (13). Several molecular indicators of cartilage turnover have previously been useful in determining patients of joint danger degeneration (14). The radiographically measured knee OA degree is strongly linked with cartilage oligomeric Matrix protein (COMP) serum concentrations, which binds Collagen II (15). As evaluated by magnetic resonance imaging, COMP levels in the blood and the carboxy-terminal neoepitope of type II collagen produced by collagenase are linked to knee joint deterioration and osteoarthritis. In addition, C-peptide of type II collagen, commonly utilized as a urine biomarker for joint cartilage degeneration, was higher in OA patients than in controls (16). Early incidence and development of knee OA are predicted by a collagenase-generated peptide of the human type II collagen test (17). in a mouse OA model, serum levels of CILP2 were lower (18).

CILP2 is a monomeric glycoprotein in the extracellular matrix (ECM) primarily expressed in the articular cartilage's intermediate zone (19). The RNA and protein expression of CILP2 in cartilage and non-cartilaginous tissues is poorly understood. CILP2 has a restricted mRNA dispersion at the surface. During the mouse articular cartilage formation, confined to the articular cartilage's middle zone with the maturation of meniscal cartilage, we demonstrate that CILP2 is a glycoprotein that undergoes proteolytic processing in the same way the protein CILP1 is found in articular cartilage in humans. Ultrastructure investigations revealed that CILP2 might have a role in linked Collagen VI-containing superstructures. Importantly, CILP2 transcriptional activation in a mouse

experimentally induced was increased in mechanically caused osteoarthritis. Down-regulated, implying a role for CILP2 depletion in the arthritis pathophysiology; Furthermore, CILP1 and CILP2 are expressed in skeletal muscle for the first time, indicating that the CILPs may play new functions in no cartilaginous tissue ECM formation and function (18).

The decrease of CILP2 in the cartilage of people with osteoarthritis and the expression of CILP2 on the surface of the articular cartilage and throughout the articular cartilage suggest that CILP2 may be useful for investigation as a biological marker for determining cartilage degradation in joint disorders. In injured cartilage, the C-terminal polypeptide of CILP2 was recently susceptible to the degradation of a specific metalloprotease (20). Finally, it can be concluded that for people who have an increased risk of developing osteoarthritis in the knees, measuring changes in cartilage biomarker CILP2 may be an effective and sensitive tool for detecting the early development of knee osteoarthritis. A previous knee injury can expect increased meniscus thickness (21).

Current therapy for osteoarthritis includes non-steroid anti-inflammatory medications, corticosteroids, viscosupplementation, glucosamine, and chondroitin sulfate (22). These drugs have an asymptomatic impact on OA, and therapy options that will affect cartilage deterioration and the illness are being explored. This field involves looking at cytokines, metalloproteinase inhibitors, growth factors, nitric oxide, and gene therapy (23). This study will include two treatments, the most used for osteoarthritis of the knee joint: pure-platelet-rich plasma and hyaluronic acid (HA).

Methods

Sample collection: In the period between November 2021 to June 2022, interventional examinations were conducted on patients diagnosed with osteoarthritis of the knee, and their number was (31) patients, based on the type of therapy divided into two groups that we gave them. The first group consisted of (10) patients, the hyaluronic acid treatment group, where they were examined before and after the injection for (6) weeks. The second group consisted of (21) patients, the pure platelet-rich plasma treatment group, and they were also examined before and after the injection. The average age of the participants in this study was between (35-75) years. They were selected and diagnosed by doctors in private clinics in Al-Diwaniyah city.

Study Design

Interventional study was used to study the effect of intervention treatment on patients with KOA and compare it with control group in non-randomized clinical trials in medical clinics at Al-Diwaniyah Governorate.

Inclusion criteria: People diagnosed with early osteoarthritis of the knee.

Exclusion Criteria: All patients with diabetes mellitus, autoimmune disease, advanced knee osteoarthritis and COVID-19 were excluded from this research. Due to the dysregulation of the immune system, the levels of cytokines are abnormal in these diseases and thus affects the diagnostic role of (CILP2).

Blood collection: This process was done twice on each patient. Once before injecting the treatment and once after the therapy for six weeks. In each blood draw, about (5) ml of blood is collected from each patient who participated in the study by vein puncture. After that, put into a gel tube, leaving the blood at room temperature stable min. The blood was separated by centrifugation at 6300 g. Then was, the serum Transfer to a labelled Eppendorf tube and stored at -80°C until use. all experience Serum is needed. As for the patients who used the pure platelet-rich plasma treatment, (10) ml was withdrawn from the vein of each patient and the plasma was extracted from it.

Cytokine Human CILP2: his level in the serum was estimated using BT LAB kits.

Assay Procedure

1 Comply with all instructions for preparing the reagents, standards, and samples. Before use, bring all reagents to room temperature. The experiment is carried out at room temperature.

2   Find out how many strips are needed for the test. To use, place the strips in the frames. The strips should be kept between 2 and 8°C.

3   Fill a standard well with 50 l of standard. It should be noted that a standard well already contains biotinylated antibodies because they are present in the standard solution.

4   Add 50 ml of streptavidin-HRP to the sample wells and standard wells after adding 40 l of sample and 10 l of anti-CILP2 antibody to the test wells (Not blank control well). Mix thoroughly. Apply a sealant to the plate. At 37°C, incubate for 60 minutes.

5   Take off the sealant and use a wash buffer to wash the plate five times. For each wash, soak wells in 300 μl of wash buffer for 30 to 60 seconds. Aspirate or decant each well for automatic washing, then use wash buffer to run each cycle five times. Place paper towels or another absorbent material nearby to blot the plate.

6   Fill each well with 50 l of substrate solution A and 50 l of substrate solution B. Plate should be incubated for 10 minutes at 37 °C in the dark.

7   Fill each well with 50 l of Stop Solution. The blue colour will instantly turn yellow.

8   Within 10 minutes of applying the stop solution, measure the optical density (OD value) of each well using a microplate reader set to 450 nm.

Results

Based on the patient number it appears that females are more liable to develop early knee osteoarthritis (KOA) problem more than males. Looking at patients' age, it has been noticed that the percentage of a male at age 30-39 was significantly ($P<0.001$) higher than females, while at age 50-59 both males and females showed a higher percent more than other age groups and it was significantly ($P<0.001$) higher in female more than male. While the lowest percentage was noticed in the 70-79 years age group. For BMI it has been noticed that there was no consistent relationship between age and BMI. However, patients at age 70-79 showed a sort of increase in their BMI more than other age groups, also some had a significant increase ($P<0.01$-$P<0.001$) in BMI for a female more than males and vice versa (Table 1).

Table 1. Demographic parameters of studied patients.

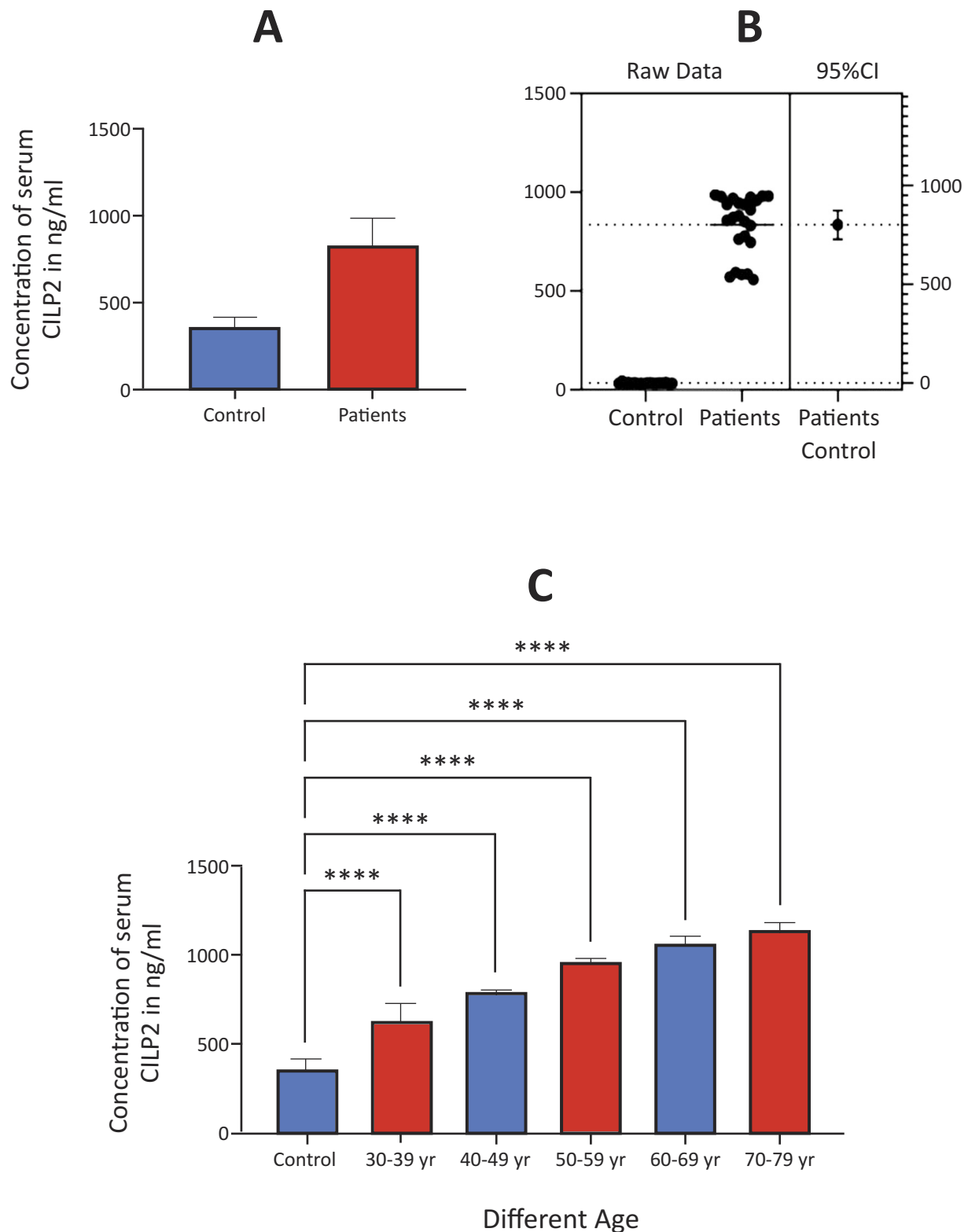|	Control		Patients		P
	Male	Female	Male	Female	
Gender Number	15	3	11	20	P<0.0001
	83%	17%	35%	65%	
Age					
30-39 year	N (7) 47%	N (3) 100%	N (4) 36%	N (1) 5%	P<0.001
40-49 year	N (3) 20%	/	N (1) 9%	N (2) 10%	NS
50-59 year	N (5) 33%	/	N (4) 36%	N (9) 45%	P<0.001
60-69 year	/	/	N (1) 9%	N (6) 30%	P<0.0001
70-79 year	/	/	N (1) 9%	N (2) 10%	NS
BMI					
30-39 year	26 ± 3	23.8 ± 2	29.7 ± 2	22.59 ± 2	NS
40-49 year	29.4 ± 2	/	28.34 ± 1	33.9 ± 1	P<0.001
50-59 year	26.6 ± 3	/	30.88 ± 1	26.7 ± 2	P<0.001
60-69 year	/	/	30.85 ± 1	30.03 ± 2	NS
70-79 year	/	/	34.01 ± 1	30.7 ± 1	P<0.01

A

B

C

Figure 1. Estimation of serum CILP2. (A) a comparison between control and patients' group, (B) an estimation plot that illustrates the presence of a significant increase in the level of CILP2 in the patients' group as compared to control, (C) A comparison between control and patients among different age groups showed the presence of a significant increase (P<0.0001) in the patients' group more than control in all age subgroups.
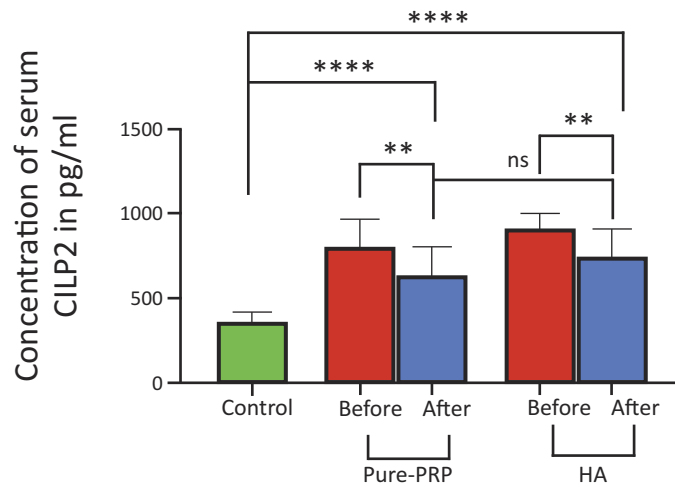
Figure 2. Estimation of CILP2 serum concentration after the injection of Pure-PRP and Hyaluronic acid (HA). A significant decrease (P<0.05-P<0.0001) in the serum concentration of CILP2 as compared to before injection. However, the concentration was significantly (P<0.0001) higher than the control.
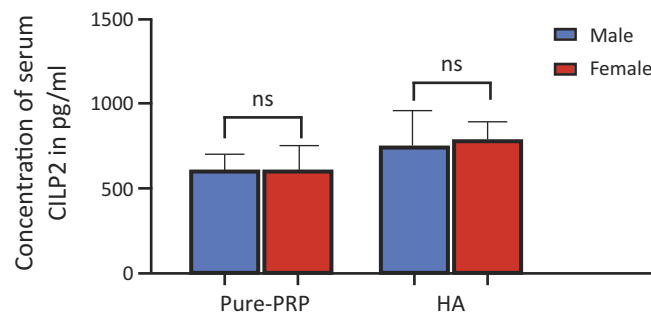

Figure 3. Estimation of CILP2 serum concentration after the injection of Pure-PRP and Hyaluronic acid (HA). No significant difference was found between males and females after the injection.
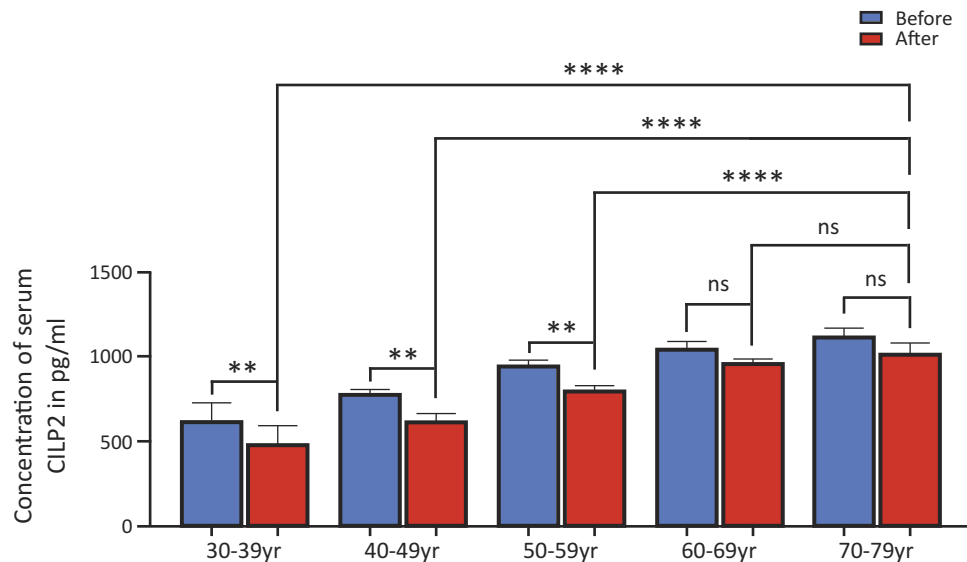

Figure 4. Estimation of CILP2 serum concentration after the injection of Pure-PRP and Hyaluronic acid (HA) among the different age groups. A significant decrease in the serum concentration of CILP2 after the injection for all age groups. However, the concentration was significantly higher in the (70-79) age group than in other groups indicating a slow improvement for older patients.
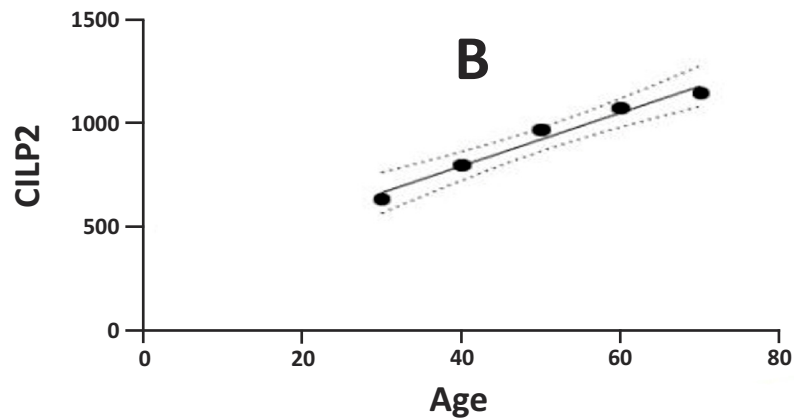
Figure 5. Simple linear regression shows a strong correlation between CILP2 level and age (r=0.9, P<0.05).

Discussion

Knee osteoarthritis (KOA) is the most common cause of physical impairment in the elderly (24). Knee-related disability Pain, comorbidity, pathophysiology, socio-demographic, psychological, and social variables are likely to have a role in osteoarthritis (25). OA can affect any joint. However, the knee is the most usually affected. Platelet-rich plasma therapy is one of the latest methods used to treat knee osteoarthritis (26). It is characterized by being easy to prepare by separating it from the patient's blood, and there are no side effects resulting from its use as a treatment (27). The platelet-rich plasma contains growth factors that help in the differentiation and regeneration of bone and cartilage cells in the knee joint and stimulates its repair (28).

Hyaluronic acid is also one of the well-known treatments for the roughness of the knee joint. It reduces the pain level, facilitates the joint's movement, and provides the possibility to re-form the cartilage and bone cells in the joint (29). Changes in CILP2 levels may be beneficial for the early diagnosis of knee OA, as CILP2 appears to be linked to cartilage thickness reduction in people who are more likely to develop knee OA (30).

This study consists of two main groups. The first group is the patients injected in the knee joint with pure-platelet-rich plasma treatment. The second group is the patients for whom the knee joint is injected with hyaluronic acid treatment.

In this study, an elevated serum CILP2 level was observed in patients with early osteoarthritis of the knee. The findings are consistent with prior research (21). The study found a significantly reduced total cartilage thickness, with the tibiofemoral and lateral cartilage bodies experiencing the highest volume losses. The thickness of the cartilage of volleyball players over 40 years of age was reduced on MRI scans obtained initially and after a follow-up period of 2 years. In volleyball players, there were higher levels of CILP2. Interestingly, these levels seemed to correlate with reduced cartilage thickness in people more likely to develop knee osteoarthritis.

And consistent with (31), CILP2 levels in the blood appear to be linked to cartilage loss in those at a higher risk of developing knee osteoarthritis.

When the first group of patients was injected with pure platelet-rich plasma, a decrease in the level of CILP2 was observed in the serum after six weeks. The improvement of the cartilage condition could explain this due to the structural effect of the treatment. The findings are consistent with (32). Because of its three recognized biological features, pure-PRP is a therapy for cartilage injuries and symptom relief. Pure-PRP, for starters, has an anabolic impact on chondrocytes, MSCs, and synoviocytes, leading to increased cell proliferation, cartilaginous ECM buildup, and HA production. Second, platelet rich plasma (PRP) might be used as a bioactive cell scaffold to fill gaps and aid cartilage repair. Finally, pure-PRP can reduce inflammation and improve OA symptoms with a clinically acceptable safety profile.

Also, when using hyaluronic acid (HA) as a treatment after injecting it into the knee that suffers from early osteoarthritis, we noticed a decrease in the level of CILP2 in the blood. HA treatment can explain this effect, which reduces pain and inflammatory cytokines and stimulates repair processes. This is consistent with the study (33). Hyaluronic acid (HA) injections into the joints are meant to improve the synovial fluid's viscoelastic characteristics and reverse the pro-inflammatory pathways brought on by OA.

Estimation of CILP2 serum concentration after the injection of Pure-PRP and Hyaluronic acid (HA). No significant difference was found between males and females after the injection. This means that this study needs a longer period to collect more samples through which the difference in response between the two sexes can be found contradicts the study (34) KOA becomes more common as people age, and women have greater rates than males, especially beyond 50 years old.

Estimation of CILP2 serum concentration after the injection of Pure-PRP and Hyaluronic acid (HA) among the different age groups. A significant decrease in the serum concentration of CILP2 after the injection for all age groups. However, the concentration was significantly higher in the (70-79) age group than in other groups indicating a slow improvement for older patients. This is consistent with the study (35–37). As fewer live, active cells respond to the growth factors generated by pure-PRP, older age and high grades of KOA are likely to have worse responses to pure-PRP and HA injection.

The evaluation of CILP2 in the serum of patients with early KOA has been observed between different sexes between different age groups, and a significant difference was found ($P < 0.05$-$P < 0.01$) between males and females in all age groups. The level in women was higher than in males; this study corresponds to(38–40). Women are more likely to develop KOA than men as they age, especially after age 50 years. This explains the rise in CILP2 level as a function of disease progression.

No clear difference was found between pure-PRP and HA injections. These results are consistent with the results of studies (41–43). There is no difference in the effect of HA and pure-PRP injections on patients with early knee osteoarthritis. This contradicts studies (44–46) that PRP injections are more effective and beneficial in treating early osteoarthritis of the knee joint than hyaluronic acid. The experimental studies on the use of biological therapy has increasing advanced with the introduction of cell-based or cell free therapy, such as, stem cells, cell secretome, and cell exosomes (47-49).

Conclusions

CILP2 could be one of the prognostic signs of early KOA.

Recommendations

This study could recommend the following:

1 Studying the relationship between more cytokines, hyaluronic acid and pure platelet-rich plasma treatments and their effect on osteoarthritis of the knee.

2 Comparing the effect of more injected treatments such as stem cells, corticosteroids, and others with the effect of injecting hyaluronic acid and pure platelet-rich plasma.

3 Study of the genetic pool of (CILP2) in patients with early KOA to provide the genetic prognosis.

4 The use of synovial fluid instead of blood in the detection and diagnosis of early knee osteoarthritis.

Conflict of Interest

No conflicts of interest exist between the authors and the publishing of this work.

Adherence to Ethical Standards

The ethical committee approved the study at the University of Al-Qadisiyah. The registration number (UoQ/CoM/11-100 in 08.03.2022).

References

1. Agency for Healthcare Research and Quality. Treatment of Osteoarthritis of the Knee : An Update Review. Comp Eff Rev. 2017;(190):2.
2. Cross M, Smith E, Hoy D, et al. The global burden of hip and knee osteoarthritis: Estimates from the Global Burden of Disease 2010 study. Ann Rheum Dis. 2014;73(7):1323–1330.
3. Driban JB, Price LL, Eaton CB, et al. Individuals with accelerated knee osteoarthritis have greater pain and functional impairments than individuals with common knee osteoarthritis: Data from the osteoarthritis. Osteoarthr Cartil. 2015;23(2015):326–327.
4. Primorac D, Molnar V, Rod E, et al. Knee Osteoarthritis : A Review of Pathogenesis and. Genes (Basel). 2020;11(8):854–889.
5. Tehranzadeh J, Booya F, Root J. Cartilage metabolism in osteoarthritis and the influence of viscosupplementation and steroid: A review. Acta radiol. 2005;46(3):288–296.
6. Case R, Thomas E, Clarke E, et al. Prodromal symptoms in knee osteoarthritis: a nested case–control study using data from the Osteoarthritis Initiative. Osteoarthr Cartil. 2015;23(7):1083–1089.
7. Luyten FP, Denti M, Filardo G, et al. Definition and classification of early osteoarthritis of the knee. Knee Surgery, Sport Traumatol Arthrosc. 2012;20(3):401–406.
8. Arendt EA, Dahm DL, Dejour D, et al. Patellofemoral joint: from instability to arthritis. Instr Course Lect. 2014;63:355–368.
9. Hensor EMA, Dube B, Kingsbury SR, et al. Toward a clinical definition of early osteoarthritis: onset of patient-reported knee pain begins on stairs. Data from the osteoarthritis initiative. Arthritis Care Res (Hoboken). 2015;67(1):40–47.
10. Kraus VB, Burnett B, Coindreau J, et al. Application of biomarkers in the development of drugs intended for the treatment of osteoarthritis. Osteoarthr Cartil. 2011;19(5):515–542.
11. Guermazi A, Roemer FW, Burstein D, et al. Why radiography should no longer be considered a surrogate outcome measure for longitudinal assessment of cartilage in knee osteoarthritis. Arthritis Res Ther. 2011;13(6).
12. Buttgereit F, Burmester GR, Bijlsma JWJ. Non-surgical management of knee osteoarthritis: Where are we now and where do we need to go? RMD Open. 2015;1(1):1–5.
13. Cibere J, Zhang H, Garnero P, et al. Association of biomarkers with pre-radiographically defined and radiographically defined knee osteoarthritis in a population-based study. Arthritis Rheum. 2009;60(5):1372–1380.
14. Kraus VB, Collins JE, Hargrove D, et al. Predictive validity of biochemical biomarkers in knee osteoarthritis: Data from the FNIH OA Biomarkers Consortium. Ann Rheum Dis. 2017;76(1):186–195.
15. Vilím V, Vytášek R, Olejárová M, et al. Serum cartilage oligomeric matrix protein reflects the presence of clinically diagnosed synovitis in patients with knee osteoarthritis. Osteoarthr Cartil. 2001;9(7):612–618.
16. King KB, Lindsey CT, Dunn TC, et al. A study of the relationship between molecular biomarkers of joint degeneration and the magnetic resonance-measured characteristics of cartilage in 16 symptomatic knees. Magn Reson Imaging. 2004;22(8):1117–1123.
17. Robin Poole A, Ha N, Bourdon S, et al. Ability of a urine assay of type II collagen cleavage by collagenases to detect early onset and progression of articular cartilage degeneration: Results from a population-based cohort study. J Rheumatol. 2016;43(10):1864–1870.
18. Bernardo BC, Belluoccio D, Rowley L, et al. Cartilage intermediate layer protein 2 (CILP-2) is expressed in articular and meniscal cartilage and down-regulated in experimental osteoarthritis. J Biol Chem. 2011;286(43):37758–37767.
19. Lorenzo P, Bayliss MT, Heinegårdt D. A novel cartilage protein (CILP) present in the mid-zone of human articular cartilage increases with age. J Biol Chem. 1998;273(36):23463–23468.

20. Zhen EY, Brittain IJ, Laska DA, et al. Characterization of metalloprotease cleavage products of human articular cartilage. Arthritis Rheum. 2008;58(8):2420–2431.
21. Boeth H, Raffalt PC, MacMahon A, et al. Association between changes in molecular biomarkers of cartilage matrix turnover and changes in knee articular cartilage: a longitudinal pilot study. J Exp Orthop. 2019;6(1):1–10.
22. Anandacoomarasamy A, March L. Current evidence for osteoarthritis treatments. Ther Adv Musculoskelet Dis. 2010;2(1):17–28.
23. Fajardo M, Di Cesare PE. Disease-modifying therapies for osteoarthritis: Current status. Drugs and Aging. 2005;22(2):141–161.
24. Skou ST, Pedersen BK, Abbott JH, et al. Physical activity and exercise therapy benefit more than just symptoms and impairments in people with hip and knee osteoarthritis. J Orthop Sport Phys Ther. 2018;48(6):439–447.
25. Mills SEE, Nicolson KP, Smith BH. Chronic pain: a review of its epidemiology and associated factors in population-based studies. Br J Anaesth. 2019;123(2):273–283.
26. Di Martino A, Di Matteo B, Papio T, et al. Platelet-Rich Plasma Versus Hyaluronic Acid Injections for the Treatment of Knee Osteoarthritis: Results at 5 Years of a Double-Blind, Randomized Controlled Trial. Am J Sports Med. 2019;47(2):347–354.
27. Scott S, Roberts M, Chung E. Platelet-Rich Plasma and Treatment of Erectile Dysfunction: Critical Review of Literature and Global Trends in Platelet-Rich Plasma Clinics. Sex Med Rev [Internet]. 2019;7(2):306–312. Available from: https://doi.org/10.1016/j.sxmr.2018.12.006
28. Liu X, Yang Y, Niu X, et al. An in situ photocrosslinkable platelet rich plasma – Complexed hydrogel glue with growth factor controlled release ability to promote cartilage defect repair. Acta Biomater [Internet]. 2017;62:179–187. Available from: http://dx.doi.org/10.1016/j.actbio.2017.05.023
29. Vasvani S, Kulkarni P, Rawtani D. Hyaluronic acid: A review on its biology, aspects of drug delivery, route of administrations and a special emphasis on its approved marketed products and recent clinical studies. Int J Biol Macromol [Internet]. 2020;151:1012–1029. Available from: https://doi.org/10.1016/j.ijbiomac.2019.11.066
30. Rodríguez-Merchán EC, Moreno-García A. Biomarkers in Osteoarthritis: Their Role in Predicting the Progression of the Disease and Their Ability to Assess the Efficacy of Existing Treatment. Controv Orthop Surg Low Limb. 2021;185–195.
31. Sebastian A, Murugesh DK, Mendez ME, et al. Global gene expression analysis identifies age-related differences in knee joint transcriptome during the development of post-traumatic osteoarthritis in mice. Int J Mol Sci. 2020;21(1):364.
32. Xie X, Zhang C, Tuan RS. Biology of platelet-rich plasma and its clinical application in cartilage repair. Arthritis Res Ther. 2014;16(1):1–15.
33. Miller LE, Fredericson M, Altman RD. Hyaluronic acid injections or oral nonsteroidal anti-inflammatory drugs for knee osteoarthritis: systematic review and meta-analysis of randomized trials. Orthop J Sport Med. 2020;8(1):2325967119897909.
34. Urwin M, Symmons D, Allison T, et al. Estimating the burden of musculoskeletal disorders in the community: the comparative prevalence of symptoms at different anatomical sites, and the relation to social deprivation. Ann Rheum Dis. 1998;57(11):649–655.
35. Akhlaque U, Ayaz S Bin, Akhtar N. Efficacy of intra-articular autologous platelet rich plasma injection in primary knee osteoarthritis; a quasi-experimental study. J Pak Med Assoc. 2020;1–10.
36. O'Donnell C, Migliore E, Grandi FC, et al. Platelet-rich plasma (PRP) from older males with knee osteoarthritis depresses chondrocyte metabolism and upregulates inflammation. J Orthop Res. 2019;37(8):1760–1770.
37. Testa G, Giardina SMC, Culmone A, et al. Intra-articular injections in knee osteoarthritis: A review of literature. J Funct Morphol Kinesiol. 2021;6(1):15.
38. Shamsi M, Safari A, Soroush A, et al. The survey of knee osteoarthritis in the population over age 50 visited in the health bus in Kermanshah, Iran. J Aging Res. 2021;2021.
39. Tschon M, Contartese D, Pagani S, et al. Gender and sex are key determinants in osteoarthritis not only confounding variables. A systematic review of clinical data. J Clin Med. 2021;10(14):3178.
40. Kour N, Gupta S, Arora S. A survey of knee osteoarthritis assessment based on gait. Arch Comput Methods Eng. 2021;28(2):345–385.
41. Filardo G, Previtali D, Napoli F, et al. PRP injections for the treatment of knee osteoarthritis: a meta-analysis of randomized controlled trials. Cartilage. 2021;13(1_suppl):364-375.

42. Xu Z, He Z, Shu L, et al. Intra-articular platelet-rich plasma combined with hyaluronic acid injection for knee osteoarthritis is superior to platelet-rich plasma or hyaluronic acid alone in inhibiting inflammation and improving pain and function. Arthrosc J Arthrosc Relat Surg. 2021;37(3):903–915.

43. Raeissadat SA, Rayegani SM, Hassanabadi H, et al. Knee osteoarthritis injection choices: platelet-rich plasma (PRP) versus hyaluronic acid (a one-year randomized clinical trial). Clin Med Insights Arthritis Musculoskelet Disord. 2015;8:CMAMD-S17894.

44. Nie L, Zhao K, Ruan J, et al. Effectiveness of platelet-rich plasma in the treatment of knee osteoarthritis: A meta-analysis of randomized controlled clinical trials. Orthop J Sport Med. 2021;9(3):2325967120973284.

45. Costa LAV, Lenza M, Irrgang JJ, et al. How Does Platelet-Rich Plasma Compare Clinically to Other Therapies in the Treatment of Knee Osteoarthritis? A Systematic Review and Meta-analysis. Am J Sports Med. 2022;03635465211062243.

46. Rawat PK, Agarwal R, Ahuja Y, et al. Intra-articular platelet rich plasma in osteoarthritis knee. Int J Orthop. 2021;7(4):614–617.

47. Merkhan MM, Shephard MT, Forsyth NR. Physoxia alters human mesenchymal stem cell secretome. Journal of Tissue Engineering. 2021 Oct;12:20417314211056132. https://doi.org/10.1177/20417314211056132

48. Chen L, Merkhan MM, Forsyth NR, et al. Chorionic and amniotic membrane-derived stem cells have distinct, and gestational diabetes mellitus independent, proliferative, differentiation, and immunomodulatory capacities. Stem Cell Res. 2019;40:101537. doi: 10.1016/j.scr.2019.101537. Epub 2019 Aug 13. PMID: 31422237.

49. Keshtkar S, Kaviani M, Soleimanian S, et al. Stem Cell-Derived Exosome as Potential Therapeutics for Microbial Diseases. Frontiers in Microbiology. 2021;12.