STUDY PROTOCOL OUTLINE

NCT NUMBER : PENDING

TITLE : Effectiveness of Platelet Rich Plasma, Conditioned Medium From Umbilical Cord Mesenchymal Stem Cell Culture (MSCs) Secretome and hyaluronic acid for the Treatment of Knee Osteoarthritis

DATE of DOCUMENT : OCTOBER 1, 2022

ABBREVIATIONS

COMP	: *Cartilage Olligometric Matrix Protein*
FAS	: Full Analysed Set
HA	: Hyaluronic Acid
HIV	: Human Immunodeficiency Virus
OA	: Osteoarthritis
PRP	: Platelet Rich Plasma
RSMH	: RSUP Mohammad Hoesin
UC MSCs	: Umbilical Cord Mesenchymal Stem Cell Culture (MSCs)
USG	: Ultrasonography
VAS	: *Visual Analog Scale*
WOMAC	: *The Western Ontario and McMaster Universities Index*

1. Abstract

Osteoarthritis (OA) is defined as progressive destruction of the articular cartilage with subchondral bone thickening, osteophyte formation, and mild chronic inflammation of the synovium. The prevalence of osteoarthritis in the world is relatively high and is one of the most common musculoskeletal diseases. Based on the RSMH Rheumatology Division annual report, osteoarthritis is the most common disease treated in the rheumatology outpatient clinic. There has been an increase in the number of osteoarthritis patients from 2019 to 2021.

Inflammation in OA is caused by proinflammatory cytokines that can stimulate the expression of catabolic enzymes and induce the transformation of subchondral bone and articular cartilage. During the process of the articular cartilage matrix turnover, cartilage matrix fragments will be released, and the degradation products of another cartilage metabolism will be released into the synovial fluid and blood serum. One of the released macromolecules is COMP. Thus COMP is a degradation product of articular cartilage and can be a diagnostic and prognostic marker in serum for the diagnosis of knee OA. One of the treatment techniques for osteoarthritis (OA) is intra-articular injection. However, existing intra-articular injection treatment is not optimal, and long-term use of painkillers can cause side effects. So, currently, many studies are examining the latest treatments that can produce better and longer-lasting functional results, such as Platelet Rich Plasma and Mesenchymal Stem Cells Secretome or Secretome. In addition, many preclinical to clinical studies and experiments have reported the effectiveness of Platelet Rich Plasma, Mesenchymal Stem Cells Secretome and hyaluronan separately for improving musculoskeletal diseases, one of which is osteoarthritis. In this study, the two therapeutic modalities will be compared together so that the effectiveness of administration in knee OA patients will be determined by knowing the changes in the degree of pain (WOMAC and VAS) and the condition of the joint cartilage with changes in the COMP marker concentration.

Method:

The design of this study was experimental with a clinical trial design, randomized and open-label.

Expected results:

It will be known how the effectiveness of giving secretome and hyaluronic acid in patients with knee osteoarthritis. The doctor used the results of this study to consider the administration of secretome and hyaluronan in patients with knee osteoarthritis.

Keywords:

Knee Osteoarthritis (OA), Secretome, Hyaluronic Acid, Intra-articular Injection

2. Brief Summary

The clinical trial will be carried out at the Dr. Moh. Hoesin Central Hospital, Palembang and planned from October 2022 to March 2023.

In this, prospective, Open-label study, patients with mild to moderate symptomatic knee osteoarthritis will be randomized to receive either a series of **platelet-rich plasma** or hyaluronic acid or Conditioned Medium From Umbilical Cord Mesenchymal Stem Cell Culture (MSCs) Secretome injections under ultrasound guidance. Each of the groups were given 5 times injection 3 cc **platelet-rich plasma** or 2 cc hyaluronic acid or 2cc CM UC MSCs (secretome) at intervals 1 weeks.

Clinical data in the form of subjective outcome measures will be collected pre-treatment and 6 months after injection.

3. Study Design

Study Type :	Interventional (Clinical Trial)
Actual Enrollment :	45 participants
Allocation:	Randomized
Intervention Model:	Parallel Assignment

Masking:	None (Open Label)
Primary Purpose:	Treatment
Official Title:	Comparative Effectiveness of **Platelet Rich Plasma,** Conditioned Medium From Umbilical Cord Mesenchymal Stem Cell Culture (MSCs) Secretome and hyaluronic acid for the Treatment of **Knee Osteoarthritis**
Study Start Date :	March 2021
Actual Primary Completion Date :	March 2022
Actual Study Completion Date :	October 2022

4. Arms and Interventions

Arm	Intervention/treatment
Experimental: Hyaluronic Acid (HA) Hyaluronic acid administered 5 times as an intra-articular injection under ultrasound guidance as a series of one weekly injections to the affected knee. 1 weekly injections are of low molecular weight hyaluronan in a 2mL injection.	Biological: Hyaluronic Acid
Experimental: **Platelet-rich Plasma** (PRP)	Biological: **Platelet-rich Plasma** (PRP)

Arm	Intervention/treatment
Platelet-rich plasma administered 5 times as an intra-articular injection under ultrasound guidance as a series of one weekly injections to the affected knee. 1 weekly injections are of leukocyte poor, buffer/additive free, singe spin, **platelet-rich plasma** averaging 3mL in volume.	
Experimental: UC MSCs Secretome UC-MSCs Secretome administered 5 times as an intra-articular injection under ultrasound guidance as a series of one weekly injections to the affected knee. 1 weekly injections are Conditioned Medium From Umbilical Cord Mesenchymal Stem Cell Culture (MSCs) Secretome averaging 2 mL in volume.	Biological: Conditioned Medium From Umbilical Cord Mesenchymal Stem Cell Culture (MSCs) Secretome

5. Outcome Measures

Primary Outcome Measures :

1. Visual Analog Scale (VAS) [Time Frame: Pre-Treatment]
   To asses pain score, score 1(good)-10(worst)
2. Visual Analog Scale (VAS) [Time Frame: 3 month after injection]
   To asses pain score, score 1(good)-10(worst)
3. Visual Analog Scale (VAS) [Time Frame: 6 month after injection]
   To asses pain score, score 1(good)-10(worst)
4. Western Ontario and McMaster Universities Osteoarthritis Index [Time Frame: Pre-Treatment]

To evaluate the condition of patients with osteoarthritis, including pain, stiffness, and physical functioning of the joints. The WOMAC is a self administered questionnaire consisting of 24 items divided into subscales including pain (0-20), stiffness (0-8) and physical function (0-68). Higher scores indicate worse pain, stiffness and functional limitations.

5. Western Ontario and McMaster Universities Osteoarthritis Index [Time Frame: 3 month after injection]

   To evaluate the condition of patients with osteoarthritis, including pain, stiffness, and physical functioning of the joints. The WOMAC is a self administered questionnaire consisting of 24 items divided into subscales including pain (0-20), stiffness (0-8) and physical function (0-68). Higher scores indicate worse pain, stiffness and functional limitations.

6. Western Ontario and McMaster Universities Osteoarthritis Index [Time Frame: 6 month after injection]

   To evaluate the condition of patients with osteoarthritis, including pain, stiffness, and physical functioning of the joints. The WOMAC is a self administered questionnaire consisting of 24 items divided into subscales including pain (0-20), stiffness (0-8) and physical function (0-68). Higher scores indicate worse pain, stiffness and functional limitations.

Secondary Outcome Measures

1. Laboratory Asessment [Time Frame: Pre-Treatment]

   COMP

2. Laboratory Asessment [Time Frame: 3 monts after injection]

   COMP

6. Eligibility Criteria

Ages Eligible for Study:	30 Years to 60 Years (Adult, Older Adult)
Sexes Eligible for Study:	All
Accepts Healthy Volunteers:	No

Criteria

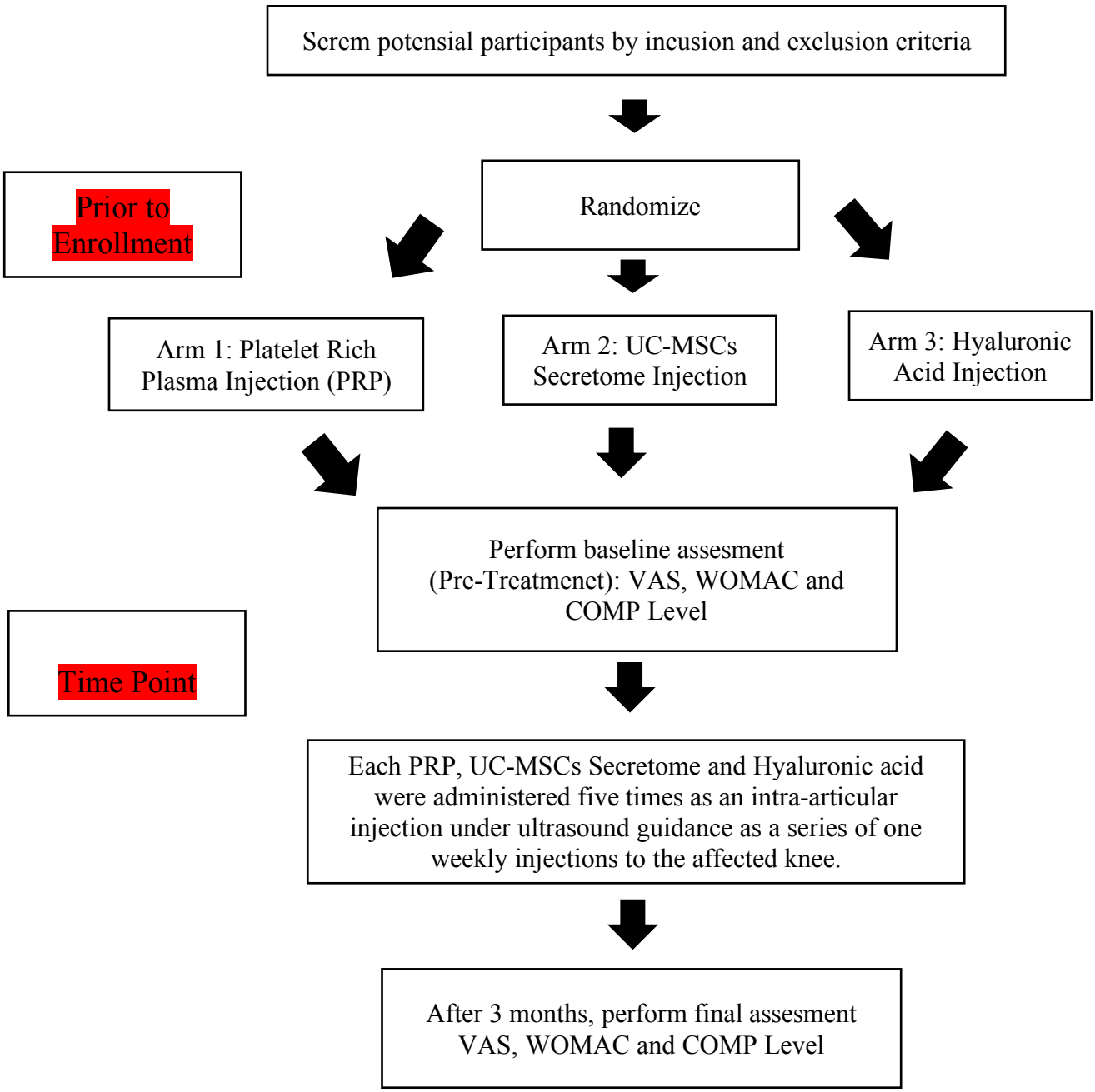
Inclusion Criteria:

- Patients aged 30-60 years
- Suffering from grade 2 and 3 OA was identified by two observers who differed accordingly Kellgren-Lawrence research scale
- Patient with knee pain that had been continuing for at least 12 months with no relief using anti-inflammatory medications and that deteriorated with weight-bearin
- Patients can understand the nature of the study and written informed consent is given to patients

Exclusion Criteria:

- Age > 60 years
- Acute Knee Osteoathritis Effusions
- Patients are not willing to obey the study protocol
- There are signs of infection local or general infection or positive serology for HIV, hepatitis and syphilis
- There is a congenital disease that causes significant deformity of the knee can interfere with cell applications and interpret results
- Articular injection of the knee by any drug during the previous 3 months
- Participate in any clinical trial or treatment 30 days before the study
- Other conditions may, according to medical criteria, not support participation in this research (The recent history of knee trauma, Autoimmune rheumatic diseases, Uncontrolled systemic diseases such as diabetes or hypertension, patient with Immunosuppressive or anticoagulant treatment and cancer.

7. Flow Diagram (*Randomized Contrroled Trial)*



8. Safety Monitoring Plan

Complications are rare but may include injection site pain, septic arthritis, hemarthrosis, and allergic reactions. This study will minimize this by ensuring the correct injection

location using an ultrasound (USG); disinfectant in the injection area, and the injection will be carried out by a Rheumatology Consultant Internal Medicine Specialist.

In case of complications related to intra-articular injections, research subjects can come for a consultation to RSMH with medical expenses using the subject's health insurance or borne by the research team if the subject does not have health insurance.

9. Analysis Plan

The design of this study was experimental with a clinical trial design, randomized and open-label. The full analysis set (FAS) will include all subjects who were randomized to study treatment. Analyses performed on the FAS will take into account subjects allocation to treatment groups as randomized. Clinical data in the form of subjective outcome measures will be collected pre-treatment and 6 months after injection.

10. Literatured Cited

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(*Mohon diisi atau tempelkan stiker jika ada*)

RM 017B.8 (Revisi IV)

INFORMED CONSENT

I am (Dr. dr. Radiyati Umi Partan, SpPD-KR, M.Kes.) a researcher from (Internal Medicine Program, FK UNSRI/RSMH Palembang), with this request you to participate voluntarily in the research entitled "(Effectiveness of Platelet-Rich Plasma (PRP), Mesenchymal Stem Cells Secretome (Secretome), and Hyaluronic Acid (Umarone) in Knee Osteoarthritis Patients) with several explanations as follows:

1. The purpose of this study was to obtain a new therapeutic method by injection/injecting drugs into the knee joint. Those who participate in this study will receive the latest therapeutic method with intra-articular injection (knee joint injection) with the latest therapeutic modality for free. In addition, this research will be able to determine a more effective therapeutic modality.
2. You were involved in the research because it met the criteria required by the researcher. Your involvement in this research is voluntary.
3. If you disagree with this method, you can choose another method, namely resigning, or you may not participate in this research. For that, you will not be penalised
4. This study will last 24 weeks, where five injections will be given at an interval of 1 week. Patients will be evaluated for pain until the 24th week.
5. You will not be given a replacement/compensation for lost time/inconvenience during the research because this research is voluntary. However, the patient will be monitored and given treatment for side effects.
6. After completing the research, you will be given information about research results in general through education and direct delivery of results.
7. You will get information about your health condition during data collection/sampling at the beginning and end of the study.
8. You will be informed if side effects or unexpected findings are found during this study.
9. You will also be informed of other data related to your situation that may be found during sampling/data collection unless the findings are not yet valid.
10. (Platelet-Rich Plasma (PRP)) The procedure is to take 30 ml of blood and then process it in the RSMH Clinical Pathology laboratory and inject it into the painful knee; this method may cause a little pain and discomfort that the subject may experience from actions received during the study. Pain during injection was minimised with a local anaesthetic spray, and there was little chance of harm to the subject (or other people, including his family) as a result of participating in the study.

11. This procedure was carried out five times with an interval of 1 week. Evaluation (VAS and WOMAC) was carried out every week for the first five weeks, then the 12th and 24th weeks. Side effects of administration were carried out throughout the study.
12. (Mesenchymal Stem Cells Secretome (Secretome□)) The procedure is to take five ml of blood, then Mesenchymal Stem Cells Secretome (Secretome□) is injected into the affected knee; this method may cause slight pain and discomfort, which may be experienced by the subject from the actions received during the study. Pain during injection was minimised with a local anaesthetic spray, and there was little chance of harm to the subject (or other people, including his family) as a result of participating in the study. This procedure was carried out five times with an interval of 1 week. Evaluation (VAS and WOMAC) was carried out every week for the first five weeks, then the 12th and 24th weeks. Side effects of administration were carried out throughout the study.
13. (Hyaluronan (Umarone□)) The procedure is to take five ml of blood, and then Hyaluronan (Umarone□) is injected into the affected knee; this method may cause less pain and discomfort that the subject may experience. Received during the study. Pain during injection was minimised with a local anaesthetic spray, and there was little chance of harm to the subject (or other people, including his family) as a result of participating in the study. This procedure was carried out five times with an interval of 1 week. Evaluation (VAS and WOMAC) was carried out every week for the first five weeks, then the 12th and 24th weeks. Side effects of administration were carried out throughout the study.
14. The benefit you get from your participation is that clinical improvement is expected after treatment. Those who participate in this study will receive the latest therapeutic method with intra-articular injection (knee joint injection) with the latest therapeutic modality for free. In addition, this research will be able to determine a more effective therapeutic modality.
15. The research was conducted with the hope that it can provide benefits to patients, the wider community and the development of science, in this case, the treatment of knee osteoarthritis.
16. After completing this research, you can continue treatment/advanced health services at the Rheumatology Poly RSMH Palembang with health insurance (BPJS) or pay if the general patient follows the applicable provisions at RSMH.
17. After receiving treatment or medical treatment as a result of research, you must wait until the treatment or health action is legally approved.

18. You can use analgesics (painkillers) or other non-pharmacological measures while waiting for legal approval.
19. You will be informed if new information is obtained from this research or other sources.
20. All data in this study will be stored by the researcher and the research team in files and research reports. Data will be kept confidential by using a numeric code system and the initials of the subject's name. Patient data will be stored and kept confidential by the researcher.
21. All the information you provide in this research will not be disseminated, so its confidentiality will be guaranteed.
22. This research received funding from UNSRI and RSMH, but the funders could not access the research data without permission from the researcher. Researchers have confirmed that there is no conflict of interest in this case
23. The researcher and seven members of this study.
24. During the study, researchers will be responsible for the occurrence of risks that can occur, such as septic arthritis and allergic reactions. This study will minimise this by ensuring the correct injection location using an ultrasound (USG) disinfectant in the injection area, and the injection will be carried out by a Rheumatology Consultant Internal Medicine Specialist.
25. If there are other risks from this research, you can get health services in the form of free treatment. For example, if the study has side effects, the patient will be treated directly by a specialist rheumatology consultant. Treatment can range from outpatient to inpatient, free of charge.
26. If there is a disability or death due to this research, the researcher considers compensation if the cause is directly due to the drug given.
27. The above follows the existing provisions.
28. This research has received ethical approval from the KEPK, which provides a letter of ethical approval.
29. You will be provided with information if there is a violation of the implementation of this research protocol. If there is a violation, the lead researcher will give a warning.
30. You will get an explanation of the research design and the treatment that will be carried out until the research is completed.
31. All important information will be disclosed during the research, and you have the right to withdraw data/information during the research.

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(*Mohon diisi atau tempelkan stiker jika ada*)

32. The researcher will keep all examination results confidential and will not be disclosed except with your permission.
33. Research will use your medical records and laboratory results only if you give permission.
34. This study uses your blood sample. Researchers will only use the sample according to the purpose of this study, and if there is any remaining sample, it will be destroyed so that it is not misused.
35. This study involved you (a woman of patient age), and you have the right to continue to follow this study or withdraw if the study has side effects.
36. This study involved you (pregnant/breastfeeding women), and you have the right to continue to follow this study or withdraw if the study has side effects.
37. Research involves you as a disaster victim for research purposes and is not related to humanitarian assistance that others may provide.
38. This research was not conducted online and did not use online or digital tools.

I hope you are willing to be a respondent in this study, where you will fill out a questionnaire related to the research. Then, after you have read the aims and objectives of the research above, I ask you to fill in your name and signature below.
I agree to participate in this research.

Name : _____

Signature : _____

Thank you for your willingness to participate in this research.

Yours faithfully

Witness

Researcher

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Dr. dr. Radiyati Umi Partan, SpPD, K-R, M.Kes