COMPARING ORAL LUMBROKINASE DLBS1033 AND BETAHISTINE MESYLATE IN BENIGN PAROXYSMAL POSITIONAL VERTIGO

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STUDY PROTOCOL, STATISTICAL ANALYSIS PLAN (SAP), AND INFORMED CONSENT FORM (ICF)

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1. STUDY PROTOCOL

1.1 Objectives

The research aims to determine the differences in:

- Decrease in IL-1β levels between benign paroxysmal positional vertigo (BPPV) patients receiving oral lumbrokinase DLBS1033 therapy and those receiving betahistine therapy.
- Decrease in TNF-α levels between BPPV patients receiving oral lumbrokinase DLBS1033 therapy and those receiving betahistine therapy.
- Decrease in VCAM-1 levels between BPPV patients receiving oral lumbrokinase DLBS1033 therapy and those receiving betahistine therapy.
- Quality of life between BPPV patients receiving oral lumbrokinase DLBS1033 therapy and those receiving betahistine therapy.
- Time to improvement of dizziness symptoms between BPPV patients receiving oral lumbrokinase DLBS1033 therapy and those receiving betahistine therapy.

1.2 Design and Methods

- Type of research: This study is a clinical trial with a double-blind randomized controlled trial design.
- Place and time of research: The study was conducted at the neurology polyclinic of Dr. Moewardi Regional General Hospital, Surakarta. Data collection began after obtaining ethics committee approval and continued until the required sample size was met, from April to August 2024.
- Research population: The actual research population consists of patients at the neurology polyclinic of Dr. Moewardi Regional General Hospital, Surakarta, who met the inclusion and exclusion criteria.
- Research subjects: Subject selection was carried out using purposive sampling.
    - Inclusion criteria: BPPV patients at the neurology polyclinic of Dr. Moewardi Regional General Hospital, aged 18-65 years, diagnosed by a neurologist or neurology polyclinic resident based on a history of worsening vertigo, nausea, vomiting, and imbalance with head movements, and findings of torsional nystagmus with latency, fatigability, and duration less than 60 seconds on Dix-

Hallpike maneuver; able to speak, read, and write Indonesian; and willing to cooperate as research subjects by signing the informed consent form.

- Exclusion criteria: Pregnant and breastfeeding patients; patients with neurological deficits indicative of central vertigo based on anamnesis and physical examination; patients with a history of ear infection, hearing impairment, tinnitus, and head trauma; patients with thyroid disorders, diabetes mellitus, genu osteoarthritis, history of neoplasm, history of heart disease and hypertension, history of ear/mastoid surgery, and currently experiencing other inflammatory and infectious conditions; patients with cognitive impairment based on attention and memory examination; patients participating in other clinical studies within 30 days before screening.
- Drop-out criteria: Patients who could not complete the entire research procedure; patients who did not follow procedures properly; patients who did not complete the DHI and VSS-SF questionnaires after receiving therapy.

- Sample size: The minimum sample size was calculated using Sopiyudin's sample size[1] formula with a 95% confidence interval (CI) and 5% confidence limits. Based on a total population of 751 peripheral vertigo patients treated at the neurology polyclinic of Dr. Moewardi Hospital in the last year, the calculation resulted in a minimum sample of 40 patients. An additional 10% was added to anticipate dropouts, making the total sample size 44 patients.
- Variable identification:
  - Independent variable: BPPV therapy.
  - Dependent variables: inflammation level, quality of life, time to improvement of dizziness symptoms in BPPV patients.
  - Confounding variables: age, body mass index, gender.
- Operational definition of research variables:
  - BPPV therapy: Administration of oral lumbrokinase (DLBS 1033, 2x490 mg daily) or betahistine (2x12 mg daily) for 14 days to diagnosed BPPV patients.
- Research instruments: Dizziness Handicap Inventory (DHI) and Vertigo Symptom Scale Short Form (VSS-SF) questionnaires, and biomarker examination (IL-1β, TNF-α, VCAM-1) using Human Quantikine R&D Systems kits on a Thermo Fisher Invitrogen auto-analyzer according to manufacturer specifications.
- Randomization protocol: The study protocol involved a two-block randomization to ensure balanced group assignments. The random sequences for the group allocation were generated by independent personnel of the direct study management and kept in sealed

envelopes. This allocation concealment ensured that neither the investigator team nor the participants knew their assigned treatment. Each package was coded with the subject identification number according to the generated random number and assigned to each eligible subject. These codes remained unrevealed until the study's completion.

1.3 Relevant Scientific Background

Over the past decade, researchers have explored new pathophysiological theories, proposing a connection between BPPV and oxidative stress, which triggers an inflammatory process explainable by ultrastructural mechanisms. Walther et al. demonstrated that human otoconia can exhibit varying degrees of ultrastructural degeneration, from minor to extensive changes.[2] Severe morphological alterations can manifest as fractures with substantial loss of calcium-rich otoconial material. High-grade disintegration of otoconia leads to complete dissolution and fragmentation of calcium-rich material into the endolymph, potentially causing BPPV symptoms. Calcium metabolism and oxidative stress, intertwined processes, closely involve inflammation. The endoplasmic reticulum, a primary calcium storage organelle, can increase calcium influx under stress, triggering a cascade of reactive oxygen species (ROS) formation in mitochondria.[3]

Further supporting this, Güçlütürk et al. investigated the potential role of inflammation in BPPV by studying the levels of IL-1β, IL-6, and TNF-α.[4] These pro-inflammatory mediators were significantly higher in BPPV patients and decreased with routine Brandt-Daroff repositioning maneuvers performed over 14 days. This suggests a possible inflammatory component in BPPV pathogenesis. Whether it is oxidative stress, otolith formation, migration into the semicircular canals, or the vertigo attacks in BPPV, a primary increase in inflammatory mediators can result.

These novel findings, elucidating the crucial role of inflammation in BPPV pathophysiology, offer new avenues for pharmacological treatment. Conventional medications like betahistine or flunarizine, often prescribed for BPPV, primarily offer symptomatic relief without addressing the underlying cause. Furthermore, not all patients are amenable to routine maneuver therapy, such as the Brandt-Daroff maneuver, a definitive BPPV treatment proven to reduce pro-inflammatory mediators when performed consistently for 14 days.[4]

In recent years, particularly since the COVID-19 pandemic, Traditional Indonesian Modern Medicines, or Obat Modern Asli Indonesia (OMAI)—natural and indigenous Indonesian drugs with scientific evidence of safety and efficacy—have seen rapid development.[5] Among OMAI with potential for BPPV pharmacotherapy are *Gingko biloba* and oral lumbrokinase DLBS1033, which contains *Lumbricus rubellus* or earthworm extract. The oral lumbrokinase DLBS1033 exhibits pleiotropic effects, effectively inhibiting the expression of inflammatory mediators implicated in BPPV pathophysiology, including NF-κβ, TNF-α, and VCAM-1.[6]

1.4 Informed Consent

The study includes a detailed Informed Consent form (attached in this document), which outlines essential information for prospective participants, including the research title, type, researchers' names and addresses, research location (Neurology Polyclinic and Clinical Pathology Laboratory of Dr. Moewardi Regional General Hospital Surakarta), study objectives, procedures (interviews, physical examination, blood sampling for biomarkers), participant selection criteria, voluntary participation, confidentiality, benefits (free blood sampling and biomarker analysis, access to results, transportation allowance of IDR 100,000 per visit for blood examination), and potential risks (pain at the blood sampling site).

2. STATISTICAL ANALYSIS PLAN (SAP)

2.1 Data Analysis

Statistical analysis was performed using SPSS for Windows version 25.0.

2.2 Specific Statistical Methods

- Normality test: The Kolmogorov-Smirnov test was used to check the normality of the data distribution for all variables. The Kolmogorov-Smirnov test was performed to verify the assumption of normal distribution for all variables, confirming that the data met this critical assumption for the subsequent parametric tests.
- Comparison between groups (day 7 and day 14):
    - Independent T-test: This test was used to determine significant differences between the oral lumbrokinase DLBS1033 group and the betahistine group for:
        - Mean DHI scores,
        - Mean VSS-SF scores,
        - Mean TNF-α levels,
        - Mean IL-1β levels,
        - Mean VCAM-1 levels.
- Analysis of symptom improvement time (multiple repeated ANOVA):
    - Given the significant statistical differences found with the independent T-test, further analysis regarding the time to symptom improvement in BPPV patients receiving oral lumbrokinase DLBS1033 was performed using multiple repeated ANOVA.
- Post-hoc analysis: Tukey B post-hoc test was used to compare the mean scores/levels between groups.

3. INFORMED CONSENT FORM (ICF)

Essential Information for Prospective Research Participants

Field	Details
Research Title	Comparing Oral Lumbrokinase DLBS1033 and Betahistine Mesylate in Benign Paroxysmal Positional Vertigo
Research Type	Clinical Trial
Researchers' Names	1. Stefanus Erdana Putra, MD. 2. Prof. Diah Kurnia Mirawati, MD., Ph.D. 3. Ratih Puspita Febrinasari, MD., Ph.D. 4. Subandi, MD., Ph.D.
Researchers' Address	Dr. Moewardi Regional General Hospital, Kolonel Sutarto Street Number 132, Jebres, Surakarta, Central Java, Indonesia, 57126
Research Location	Neurology Polyclinic and Clinical Pathology Laboratory, Dr. Moewardi Regional General Hospital, Surakarta

RESEARCH INFORMATION SHEET

Good morning/afternoon/evening, esteemed Participant,

We are lecturers and neurology residents from the Faculty of Medicine, Universitas Sebelas Maret, currently conducting research at Dr. Moewardi Regional General Hospital, Surakarta.

This research aims to determine the **differences in inflammation levels, quality of life, and dizziness symptom improvement time in patients with Benign Paroxysmal Positional Vertigo (BPPV)** who receive oral lumbrokinase DLBS1033 therapy compared to patients who receive betahistine therapy. This study will be conducted **during your treatment as a BPPV patient** at the Neurology Outpatient Clinic and Clinical Pathology Laboratory of Dr. Moewardi Regional General Hospital, Surakarta.

The researchers will conduct a brief **interview** to gather information on your current and past medical history and a **physical examination** of the research participants. Furthermore, researchers will also collect **blood samples** from research participants for biomarker examination, which will be conducted at the Clinical Pathology Laboratory of Dr. Moewardi Regional General Hospital, Surakarta.

You are invited to participate as a subject because you are a **BPPV patient who meets the inclusion criteria** for this study. Suppose you agree to participate in this research. In that case, you will be asked to **sign and write your identity and the date** on the confirmation consent form to participate as a research participant.

If you decide not to participate, this **will not affect your medical care**. Your participation in this study is **voluntary**. You have the **full right to withdraw** or cancel your participation anytime. All information in this study is **confidential**. The subjects' identities will be anonymized and used solely for research and data processing.

This research will be conducted during your visits or attendance at the hospital. By participating in this study, you can play an **essential role in providing scientific evidence** that assists clinicians in clinical decision-making regarding the application of oral lumbrokinase therapy as part of the causative therapy for BPPV patients. Thus, you indirectly help provide scientific knowledge to the public that can later be used in clinical decision-making. The results of this study are also expected to provide valuable information for advancing medical science and technology in Indonesia, especially in the field related to BPPV. The results of this study are novel in the medical field.

You will be exempt from blood sample collection fees and biomarker analysis fees for each blood test. The test results can be **communicated to you** as a research subject. As a subject, you have the full right to **access your data**. In addition, you will receive **transportation money of IDR 100,000 (one hundred thousand rupiah) per visit** for the examination each time you come for a blood test.

As a subject in this study, you will be involved in **venous blood collection**. This procedure may cause **pain at the blood collection site**. In some cases, this process may also cause **slight bruising or swelling**. So far, venous blood collection has reported no serious side effects. If there is bruising or hematoma at the former blood collection site, the researchers and Dr. Moewardi Regional General Hospital will manage it according to applicable clinical practice guidelines.

This research is a clinical trial. Post-blood collection management will be adjusted to the clinical practice guidelines at Dr. Moewardi Regional General Hospital. For patients, payment will be adjusted according to the payment/insurance scheme and patient care costs, and if other examinations are needed, all costs will be borne by the researchers.

No additional interventions or treatments will be given to you as a respondent. There are **no conflicts of interest**. This research is **self-funded** and comes from the funds of the researchers and research team members.

The research ethics committee has approved this research protocol. As a respondent, you can **contact the Health Research Ethics Committee of Dr. Moewardi Regional General Hospital, Surakarta**. The researchers will also submit a report to the Ethics Committee of Dr. Moewardi Regional General Hospital, Surakarta.

If there is anything you do not understand, please feel free to **ask us directly** or contact us at **(+62)85642134155 (Stefanus Erdana Putra)** or **(+62)271634634 (Health Research Ethics Committee of Dr. Moewardi Regional General Hospital)**. We thank you for your attention and assistance.

Sincerely,

1. Stefanus Erdana Putra, MD.
2. Prof. Diah Kurnia Mirawati, MD., Ph.D.
3. Ratih Puspita Febrinasari, MD., Ph.D.
4. Subandi, MD., Ph.D.

CONSENT FORM FOR PARTICIPATION IN RESEARCH

I, the undersigned, hereby declare my willingness to participate in the research titled "Comparison of Inflammation Levels, Quality of Life, and Dizziness Symptom Improvement Time in Benign Paroxysmal Positional Vertigo Patients Receiving Oral Lumbrokinase DLBS 1033 Therapy vs. Betahistine (A Study on Changes in IL-1β, TNF-α, and VCAM-1 Levels)" at Dr. Moewardi Regional General Hospital, Surakarta. I have been informed that the researchers have obtained permission to conduct this research and that it will not harm me during the research procedures.

Name :
…….

Address :
…….

Phone Number :
…….

This research will be coded, and my identity will remain confidential throughout the study. All data from physical and supporting examinations and the answers I provide are guaranteed to be confidential and will only be used for data processing purposes. I will not receive direct benefits from this research, but this research will provide information that can be used as data to present scientific evidence that helps clinicians in clinical decision-making regarding the application of oral lumbrokinase therapy as part of the causative therapy for BPPV patients, thus it is expected to provide valuable information for the advancement of medical science and technology in Indonesia, especially in the field related to neuro-oto-ophthalmology.

My participation in this research will require approximately 30 minutes during my visits to Dr. Moewardi Regional General Hospital, Surakarta.

This participation is voluntary, and I can withdraw as a respondent without any risk if any questions cause emotional discomfort or disturbance.

I have read this consent form and consciously agree to participate in this research.

Surakarta, …… 2024

Respondent, Witness,

……..… .....

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