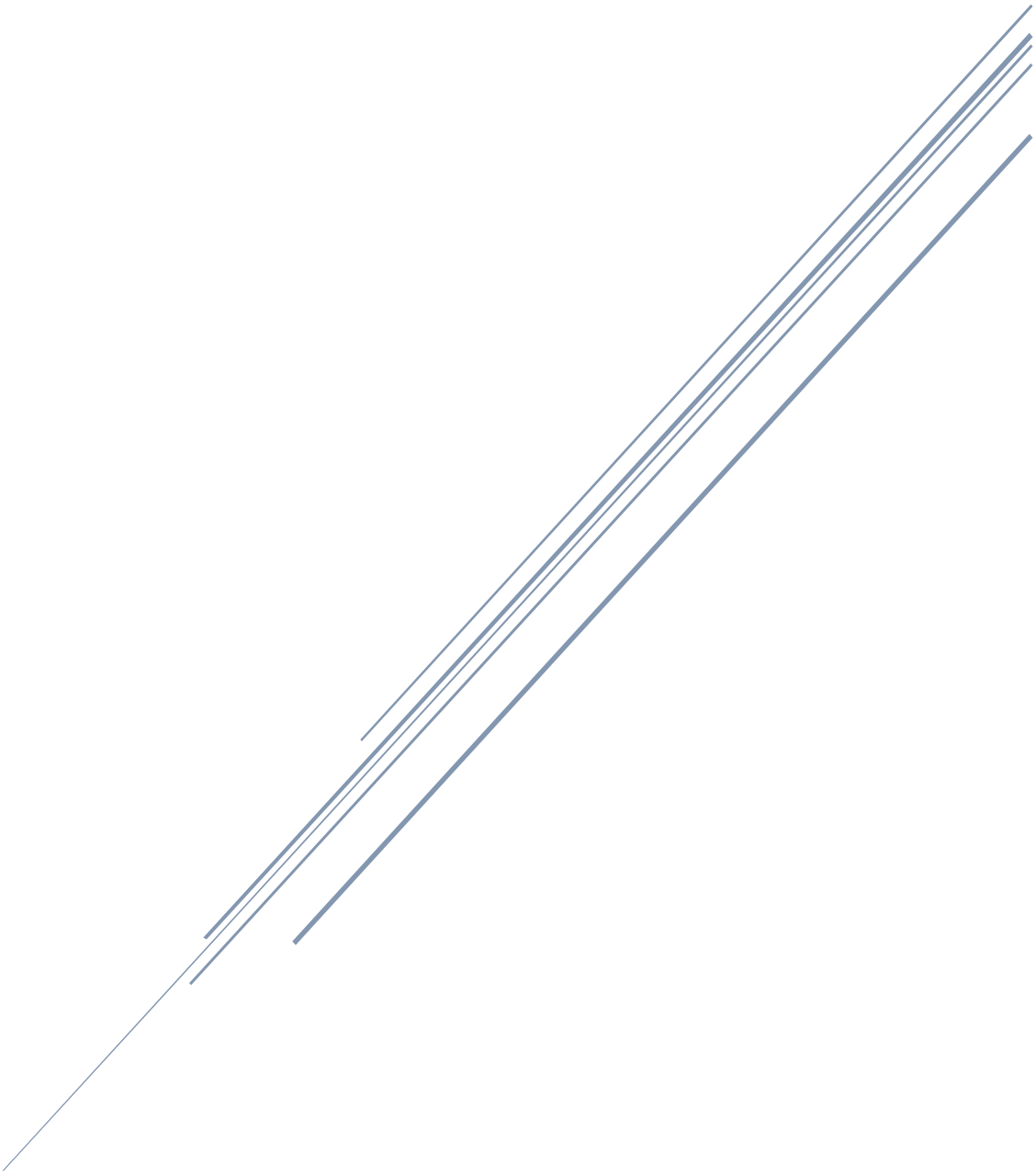
Official Title: Effect of Monazite Sands in Patients with Osteoarthritis of the Knee

Ethical committee Protocol No: 3.160.891

Study protocol



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1. Type of study

A randomized, blind-control clinical trial with paired samples will be conducted. Patients who meet the diagnostic criteria proposed by NICE (2014) with approximately 150 patients who agree to participate in the study and sign the Free and Informed Consent Term (Appendix A) will be elected.

2. Experimental Protocol

The study will be carried out on the beaches of Areia Preta de Guarapari (source of monazitic sands) and on the beach of Itapoã in Vila Velha (sand without radiation). Selected patients will have their knees fully covered with beach sand, properly analyzed for radioactivity and temperature, for 30 minutes on 2 mornings a week for a period of 1 (one) year.

Patients will be enrolled in relation to age, gender, weight, height and body mass index (BMI). An investigation / monitoring will be performed regarding the current use of medications for disease control and associated comorbidities.

3. The size of the sample

The sample size of the patients was calculated considering the necessary amount of subjects to detect difference of at least 3 points in the pain perception through the visual analgesic scale (scale from 0 to 10), considering a significance level of 1% and test power of 95% in two-tailed hypothesis test, as well as the value of 4 for standard deviation (MATA et al., 2015). The sample was calculated in 126 patients and considering a sample loss around 20%, the number of subjects was 150, with 75 subjects in each sample (BENAZZI, FIGUEIREDO, BANASSI, 2010).

4. Ethical issues

The ethical issues of submission to the Human Research Ethics Committee will be met.

The team responsible for patient follow-up and data collection will be properly trained and composed of specialists from different areas, including an Orthopedist and a Pharmacist, and academics from the Medicine, Nursing, Physiotherapy and Pharmacy Courses at Vila Velha University.

5. Inclusion criteria

Will be included in the study:

● Patients who meet the NICE (2014) diagnostic criteria for Primary Knee Osteoarthritis.

● Age between 30 and 95 years.

6. Exclusion Criteria

Patients with:

● History of previous knee surgery.

● History of fracture in the knee region.

● History of any secondary OA associated with any cystic arthropathy.

● Any treatment for the knee with methotrexate.

● History of hemorrhagic disorders.

● History of any infiltration of the knee during the previous 6 months (eg corticosteroids and hyaluronic acid).

● Morbid obesity.

● History of severe psychiatric disorders.

● History of skin cancer.

7. Removal criteria

Patients will be removed when:

● Undergoing knee surgery during the course of the research.

● Subjected to joint infiltration with corticoids or any other type and medication of intra-articular use.

● Heat intolerant or other discomfort during exposure to sand.

For evaluation and follow-up of the volunteers will be carried out in parallel a work of pharmaceutical attention with monitoring of the use and frequency of medications, especially the use of analgesics and / or anti-inflammatories.

8. Recruitment of volunteers

The volunteers will be recruited at the basic health units of the municipality of Vila Velha for internal dissemination and with the assistance of the Community Teaching Service Interaction Program (PISEC) of the Medicine Course of the Vila Velha University to disseminate the project to pre-formed groups example: HIPERDIA or group of elderly).

9. Intervention

Patients selected for the study will have their knee (s) affected by OA fully submerged in the beach sand 2 (two) times per week for 30 (thirty) minutes each session at the same location and at the same time of day.

The natural gamma (□) radiation doses of the monazite sands will be monitored in both groups through equipment that counts and accumulates the records for the desired time. In addition, the radiation measurements □ will be associated with the atmospheric and climatic measurements of each group. An interview with the patients will be performed initially (Appendix B), blood collected for analysis and applied to the Visual Analogue Scale (Annex C) and WOMAC (Annex D). The questionnaires as well as the blood collection for laboratory analysis will be repeated at the beginning of the study (day zero) and after 1, 2, 3, 6, 9 and 12 months for later analysis and comparison of the data.

10. Values evaluated

10.1 Primary outcome Measure:

Change pain perception through the visual analgesic scale of the volunteers (VAS)

10.2 Secondary Outcome Measures:

- Change total score (0 - 96) the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)- (lower scores indicate lower levels of symptoms or physical disability)
- Reduction of Nonspecific inflammatory markers: evaluation of nonspecific inflammatory markers (HSV and CRP) and specific (IL-1β, IL-6, IL-8, IL-17, TNF-α and TGF-β)
- Reduction of Self-medication to treat pain

11. Collection of biological material

Volunteers blood samples will be taken at time zero, after 1, 2, 3, 6, 9 and 12 months, after agreement and signature of the ICF. Blood collection will be performed by a specialist in the laboratory of clinical analyzes at predetermined dates immediately after exposure of the patients to the sand. The samples will be transported in an isothermal box with ice to the Vila Velha University laboratory, where they will be immediately frozen at -80ºC until analysis.

12. Biochemical analyzes

12.1- Non-specific markers

The biochemical analyzes in the blood collected from the volunteers will be performed in the laboratory of clinical analysis to be contracted. The following tests will be performed: Hemogram, C-reactive Protein and erythrocyte sedimentation rate.

12.2- Specific markers

For the study of osteoarthritis, IL-1β, IL-6, IL-8, IL-17, TNF-α and TGF-β are considered as classic biomarkers. These cytokines will be quantified in serum samples obtained from patients by the Cytometric Bead Array (CBA) technique (BD Bioscincts Pharmigen, San Diego, CA, USA). The CBA assay allows multiple cytokines present in serum, plasma or even culture supernatants to be separated and quantified by cytometry using a single fluorophore. Samples after being labeled will be read by Flow Cytometry using the BD FacsCalibur cytometer, the results obtained will be analyzed by FCAP Array Software V3.0 (BD Bioscinces Pharmigen, San Diego, CA, USA). The results obtained after analysis will be expressed in pg / mL. The tests and analyzes will be carried out in partnership with the Laboratory of Cellular and Molecular Immunology of the Infectious Diseases Nucleus (NDI), located at the Federal University of Espírito Santo (UFES).

13. Clinical evaluation and follow-up of volunteers

The clinical evaluation and application of the questionnaires in the patients will be made by an expert advised of undergraduate students properly trained for the application of the questionnaires and of potential alarm signals.

13.1 Visual Analog Scale

It consists of a graduated scale of 0 to 10 with regular intervals where zero means absence of pain and 10 is the maximum pain already experienced by the patient.

13.2 WOMAC

It consists of a questionnaire for specific assessment of the quality of life of patients with knee osteoarthritis. It is divided into three domains pain, stiffness and daily activities from 24 questions in which the patient has 5 response options (none, little, moderate, intense and very intense) and from the answers obtained has a score dividing the by the number of items in each of the domains.

13.3 Pharmaceutical Care

It consists of evaluating the patients' pharmacological therapy, being able to collect data on the health situation, use of medications and dosages administered, as well as adverse reactions and possible drug interactions.

14. Statistical analysis

Statistical analysis will be performed in the SPSS program (Statistical Package for the Social Sciences, version 13.0). For the analysis, descriptive statistics will be performed, with measures of central tendency (mean, median and standard deviation), for variables that follow the normal distribution. Qualitative variables will be expressed in frequency and percentage. The difference will be analyzed by the chi-square test or Fischer test for the categorical variables and the Mann-Whitney and Student tests for the resulting variables. A statistically significant difference will be considered when $p < 0.05$.

REFERENCES

- ● Benazzi LEB, Figueredo ACL, Banassi DG. Avaliação do usuário sobre o atendimento oftalmológico oferecido pelo SUS em um centro urbano no sul do Brasil. Ciências e Saúde Coletiva, 2010 v.15, n.3, p.861-868.
- Kang HR, Jung CY, Lee SD, Kim KH, Kim KS, Kim EJ. Efficacy and safety of electrical moxibustion for knee osteoarthritis: study protocol for a randomized controlled trial. Trials. 2018 Mar 5;19(1):159. doi: 10.1186/s13063-018-2514-x.
- Srivastava S, Saksena AK, Khattri S, Kumar S, Dagur RS. Curcuma longa extract reduces inflammatory and oxidative stress biomarkers in osteoarthritis of knee: a four-month, double-blind, randomized, placebo-controlled trial. Inflammopharmacology. 2016 Dec;24(6):377-388. Epub 2016 Oct 19.
- Mata J, Cabrera S, Sanchís P, Valentí P, Hernández P, Fortuny R, Lirola S,Aguilar JL. Electro-acupuncture for treatment of knee pain from osteoarthritis and the possible endocrinology changes: a study protocol for a randomized controlled trial. Trials. 2015 Jun 3;16:248. doi: 10.1186/s13063-015-0766-2.
- Svarstad, B L, Chewning, B A, Sleath, B L, Claesson, C. The Brief Medication Questionnaire: a tool for screening patient adherence and barriers to adherence. Patient Educ Couns. 1999, 37 (2):113-24.
- Bellamy N, Buchanan W, Goldsmith C, Campbell J, Stitt L (1988) Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. J Rheumatol 15(12):1833–1840.
- FERNANDES, Marcus Ivanovith. Tradução e validação do questionário de qualidade de vida específico para osteoartrose WOMAC (Western Ontario and McMaster Universities) para a língua portuguesa. 2001. 103 f. Dissertação (Mestrado em Ciências) - Escola Paulista de Medicina, Universidade Federal de São Paulo. São Paulo, 2003.
- Aitken RC. Measurement of feelings using visual analogue scales. Proceedings of the Royal Society of Medicine. 1969;62(10):989-993.