Project Research under EpiReumaPt study

1. Project identification

a. Regional variation and determinants of vitamin D status in Portugal

b. Principal Investigator:

José António Pereira da Silva

Rheumatology Department, Centro Hospitalar e Universitário de Coimbra, Portugal

c. Co-Investigators

1. **Tânia Santiago,** Rheumatology Department, Centro Hospitalar e Universitário de Coimbra, Portugal
2. **Andréa Marques,** Rheumatology Department, Centro Hospitalar e Universitário de Coimbra, Portugal
3. **Maria de Fátima Pina,** Faculdade de Medicina da Universidade do Porto, Portugal
4. **Jorge Santos,** Clinical Pathology Laboratory, Centro Hospitalar e Universitário de Coimbra, Portugal
5. **Loreto Carmona,** Instituto de Salud Musculoesqueletica - InMusc

2. Department(s), Laboratory(ies), Institution(s) where the study will be developed

- Rheumatology Unit, Centro Hospitalar e Universitário de Coimbra, Portugal
- Clinical Pathology Laboratory, Centro Hospitalar e Universitário de Coimbra, Portugal

3. Summary

Research has helped clarify the major role of vitamin D in the maintenance of calcium homeostasis and bone health. However, a number of evidence gaps exist. Vitamin D deficiency is highly prevalent and is now recognized as a worldwide health problem. It has been observed to varying degrees in many different countries, regardless of geographical location. However, the epidemiology and geographical distribution of vitamin D insufficiency in Portugal is not known.

This study will allow to clarify some of the gaps in the following areas: the prevalence of vitamin D insufficiency and deficiency in Portugal, the major demographic, clinical and environmental predictors of Vitamin D status in our country, the association of circulating 25-hydroxyvitamin D concentrations with bone health outcomes in the general Portuguese population, with emphasis on falls, bone mineral density and osteoporotic fracture, at individual and geographical levels.

With this knowledge we hope to contribute a better understanding of the role of Vitamin D and ultimately assist in the development of a preventive strategy in the situations of deficiency of this vitamin.

4. State of Art

Vitamin D insufficiency is a worldwide health problem that affects not only musculoskeletal health but also a wide range of acute and chronic diseases. Vitamin D is produced endogenously when the skin is exposed to sunlight, or obtained exogenously from nutrients, in small amounts, or supplements. Exposure to sunlight is the main source of this vitamin. Solar UV radiation penetrates the skin and converts 7-dehydrocholesterol into previtamin D_3, which is rapidly transformed into vitamin D_3 (1). Vitamin D from diet and from skin is hydroxylated in the liver to 25-hydroxyvitamin D [25(OH)D], the main determinant of vitamin D status. It is then metabolized in the kidneys in its active form, 1,25-dihydroxyvitamin D, in a tightly regulated process controlled by circulating parathyroid hormone, calcium and phosphorus levels, among others.

The role of vitamin D in bon metabolism has been recognised for along time and its deficiency has been clearly related to a higher risk of osteoporosis and osteomalacia(2). This is especially due to the role of Vitamin D in promoting the active absorption of calcium in the intestine. (3).

Through the ensuing hypocalcemia, Vitamin D deficiency increases the production of parathormone and, thus, bone resorption.

More recently, several diseases, such as type 1 and type 2 diabetes, multiple sclerosis, autoimmune diseases, and infectious diseases have been associated with the insufficiency of Vitamin D(2). Researchers have also identified vitamin D insufficiency as a possible risk factor for cardiovascular diseases, either directly or through a relation with other cardiovascular risk factors, including, hypertension and obesity (1, 4-6).

The status of Vitamin D is determined by measuring the serum concentration of the major circulating form of vitamin D, 25(OH)D. This is the most widely accepted indicator of vitamin D status and reflects combined contributions from cutaneous synthesis, and dietary intake including fortified foods and supplemental sources of vitamin D. Serum 25(OH)D has a half-life of approximately two to three weeks, and varies over a wide range. In contrast, the active form of vitamin D, 1,25-$(OH)_2$D, has a short circulating half-life and is tightly regulated over a narrow range by parathyroid hormone, calcium and phosphate. Serum 1,25-$(OH)_2$D is not a good measure of vitamin D status since a decrease in the circulation may not occur until vitamin D deficiency is severe (7).

Serum 25(OH)D levels fluctuate during the year according to sun exposure and tend to show a gradual decline with age, although absolute levels do vary across studies, according to geographical location, dietary vitamin D intake and the assay method used (8). As people get older, the skin's ability to synthesize vitamin D declines (9), while intestinal vitamin D absorption also becomes less efficient (10). These changes may be compound by lower exposure to sun, as a result of diminished physical activity, and reduced dietary vitamin D intake.

Recent studies also demonstrated that levels of vitamin D are different between and within countries, regardless of latitude (2, 11). However, most of the studies have focused on countries situated at northern latitudes. It is less clear what the extent of vitamin D insufficiency is in southern European countries and whether there are geographical differences within countries.

The growing suggestions about the high prevalence of vitamin D deficiency in the general population and its potential impact on bone health and other health outcomes, highlight the

need deepen our knowledge about the epidemiology of Vitamin D status in Portugal and their health correlates.

5. Aims

Primary objective:

To establish the epidemiology of vitamin D status in Portugal including its relation with age and gender.

Secondary objectives

1) To explore geographic distribution of vitamin D status in Portugal and its correlations with socioeconomic, environmental and clinical factors;

2) To study the association between vitamin D status and the epidemiology of osteoporotic factures per geographical region;

3) To establish a clinic-based algorithm to identify individuals at high risk of vitamin D deficiency.

Research questions

- What are the mean Vitamin D levels in the Portuguese population, and how does it vary by gender and age strata?
- What is the prevalence of Vitamin D deficiency and insufficiency in the Portuguese Population, and by age and gender strata?
- How do these parameters vary across geographical regions in Portugal?
- Is there an association—direct or indirect—between vitamin D levels and:
    a. Gender and age
    b. Occupation
    c. Body mass index
    a. Rural versus urban residence areas
    d. Consumption of dairy products (per region)
    e. Hours of sunshine per geographical region
    f. Environmental factors, per region : smoking, drinking, coffee, medication, and others.
    g. Comorbidities: rheumatic diseases, others. (at individual level)

h. Period of the year
i. Bone mineral density (at individual level)
j. Occurrence of falls (at individual levels)
k. Occurrence of osteoporotic fractures (at individual level and per region)
l. Albumin, calcium, phosphorus and magnesium levels (at individual level)

* All analyses will be adjusted for significant individual/geographic confounders.

6. Study design

This proposal will use the data from EpiReumaPT – Epidemiologic Study of the Rheumatic Diseases in Portugal. This cross-sectional population health survey includes non-institutionalized adults in Portuguese Mainland and Islands (Madeira and Azores). The sample is representative of the Portuguese population. The sample size was stratified by region and dimension of the location (< 2,000; 2,000-9,999; 10,000-19,999; 20,000-99,999; and 100,000 inhabitants). The number of surveyed persons in each geopgraphy was proportional to the actual distribution of the population. Because there is no reliable list of households in Portugal, a random selection of points in the map of each location was performed. At each household, the individual whose birthday was closest to the day of the visit was selected for the interview and, if available, was immediately invited to answer the questionnaire. A total of 10 661 interviews and 3 886 appointments with blood colection were performed (More details about the process are given in reference (12)).

7. Population

This study will include all 3 886 participants that attended a clinical appointment in the context of EpiReumaPT and accepted to provide a blood sample for immediate analysis and biobanking. All participants who have a frozen serum sample stored in the biobank will be included in the study. No exclusion criteria will be applied.

8. Detail Description (Methodology)

This will be a cross-sectional nation-wide study, using the sociedemografic, medical and laboratory data as well as blood samples collected during the EpiReumaPT study.

The meteorological data of duration of sunshine (hours) will be obtained from meteorological stations from Instituto Português do Mar e da Atmosfera and/or from satellite images provided by the International Research Institute for Climate and Society. Serum 25(OH)D will be analyzed in Clinical Pathology Laboratory of Coimbra University Hospital by a trained biochemist using the method ADVIA CENTAUR - 25 OH Vitamin D Total ®. This method includes a chemiluminescent assay that utilizes a specific antibody as a competitive binder and does not require sample extraction. With the availability of commercial assays (e.g., immunoassays), large numbers of samples can be processed rapidly (13, 14). The levels of albumin, calcium, phosphorus and magnesium will also be evaluated in Clinical Pathology Laboratory of Centro Hospitalar e Universitário de Coimbra. For all these lab analysis, 200 microliters of serum from each subject will be required from the biobank.

We will include the following variables:

1. Registered Sociodemographic data from EpiReuma.pt:

a. Age
b. Gender
c. Date of data/blood collection
d. Ethnicity
e. Marital status
f. Residence (rural, urban) – Postal code
g. Occupation
h. Education

2. Registered medical data from EpiReuma.pt – all of the mentioned below if available:

a. Weight
b. Height
c. Body mass index (Kg/m^2)
d. Exposures: smoking, drinking
e. Type of rheumatic diseases
f. Comorbidities
g. Medication (such as, glucocorticoids, antiepileptic, biphosponates, others)
h. Risk factors for osteoporotic fractures included in Frax tool

i. Number of falls over the previous year

j. History of fractures

3. Complementary evaluations from EpiReuma.pt (when available)

a. X ray: lumbar and thoracic spine

b. Bone mineral density

c. Any other lab results available of relevance (creatinine, alkaline phosphatase, other)

4. Data Serum measurement – performed as part of this proposal

a. 25(OH)D level

b. Albumin, calcium, phosphorus, magnesium levels

5. National register data – per region

a) Meteorological data

   a. Duration of sunshine (hours/year/season)

   b. Average temperature and rain

b) Chemical qualities of drinking water – calcium, acidity

c) Consumer habits – dairy products, tobacco, alcohol, Vitamin D

d) Prevalence of osteoporotic hip fracture per region, stratified by age and gender

9. Statistical analysis

The mean vitamin D levels and frequency of Vit D status will be estimated with 95% confidence intervals adjusted by sampling design. Descriptive data will be summarised with mean ± SD, or as frequencies-absolute and relative. Pending on previous confirmation of normal distribution, differences in VIt D levels between any two groups will be assessed by Student's *t*-test, and among three or more groups by analysis of variance (ANOVA) followed by Scheffé's test. Stepwise multiple linear regression analysis will be used to analyse the associations between determinants and vitamin D levels and logistic regression will be used to analyse the association of determinants with Vitamin D status. A *p*-value lower than 0.05 will be considered statistically significant. All the analyses will be performed with the Statistical

software R version 2.14.1 (Project for Statistical Computing, (15) and WinBUGS14 (WinBUGS14, Cambridge, UK)(16) using R2WinBUGS package to connect both tools.

Geographical information systems will be used to georeference the data, calculate the municipality neighbourhood matrix and map the results.

A Spatial Hierarchical Bayesian model will be used to account for correlation within municipality and to separate the effect of individuals and regional predictors on individual's outcomes. The outcome can be affect by the regional and neighbourhood effect but many studies only take into account the within regional effect and we propose to use an approach that take into to account also the between-area dependency and not assumed that the geographical units are statistically independent as previously has been done.

10. Time line

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<tr><td>2014</td><td></td><td colspan="7"></td><td>Receive clinical data from Epireumapt plus clean data</td><td>Lab analysis</td><td colspan="2">Analyze the data</td></tr>
<tr><td>2015</td><td colspan="2">Analyze the data</td><td>Publication of the data</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
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11.Budget

A. Transportation of blood samples:	
From Lisbon (IMM) to Coimbra (CHUC)	**100**

B. Lab analysis		
(25-OH) Vitamin D: 7.2 euros/per subject (3886 subjects)		**29.979**
	TOTAL	**€ 30.079**

12. Sponsors

This study have already received a financial support of € 1000, from Sociedade Portuguesa de Reumatologia (Fundo de apoio à investigação e formação reumatológicas). Siemen's healthcare international will provide almost all of the kits for determination of 25-OH Vitamin D level.

The Rheumatology association of Coimbra will fund the remaining of the costs.

13. Publications

The results of this study will origin several publications in international and peer review journals. By request to research team everyone can have access to the data.

14. Strategy of data protection

All participants signed an informed consent to participate in EpiReumaPT and to provide blood samples for biobank. This project was approved by the National Ethics Committee from the Ordem dos Médicos, and the Comissão Nacional de Protecção de Dados, the Portuguese data protection authority (in accordance with the Portuguese law number 67/98, October 26th regarding protection of personal data). The study was conducted in accordance with the applicable laws and regulations including, but not limited to, the Guideline for Good Clinical Practice and the ethical principles stated in the Declaration of Helsinki (amended in Edinburgh). A specific application will be made to the will be made to the Ethics Committee of Biobanco IMM, where the samples are stored, regarding the use of samples for the purposes of this study.

We will have access to personal data of participants, only to their study number.

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