Facts related to the collection of biological samples in the National Health Examination Survey – Portuguese component of the European Health Examination Survey

Marta Barreto da Silva[1], Vânia Francisco[1], Paula Rasteiro[2], Eduardo Sousa[2],Astrid Vicente[1], Mafalda Bourbon[1], Fátima Martins[1], Maria Teresa Seixas[1], Aida Fernandes[2], Álvaro Beleza[2], Francisco Mendonça[3], Ana Paula Gil[1], Carlos Matias Dias[1]

1. Instituto Nacional de Saúde Dr. Ricardo Jorge; 2. Laboratório de Saúde Pública Dra. Laura Ayres; 3. Administração Regional de Saúde do Algarve

Introduction

The Health Examination Survey (HES) – The Portuguese Component of the European Health Examination Survey (EHES) consists on the diagnosis of the health status of the Portuguese resident population, through the description of its health determinants (using clinical, biochemical and genetic approaches) and through the use of health care services, providing an important contribution to health planning and research in Portugal. It is a cross-sectional observational study, which follows the international recommendations for the first European Health Examination Survey (EHES), currently in preparation with the collaboration of the National Health Institute Doutor Ricardo Jorge . This survey includes an interview, physical and clinical measurements and collection of biological samples. The EHES gets its data via the national HES. This joint approach of national HES/EHES is advantageous to allow comparability of data between the regions participating European countries, and ensure the highest quality of the data.
The emphasis of the national HES resides in the fact that, for the same individuals, data will be collected for several different items, allowing the improvement of the actual knowledge of population health. An additional advantage is the creation of a database of biological materials and interview data which will be available to researchers and health professionals.
In this context, a pilot study was conducted in the municipality of S. Bras de Alportel in collaboration with the Regional Health Administration of the Algarve (Figure 1).

Figura 1. Map of the Algarve Region, indicating S. Brás de Alportel.

Objectives

Main Objectives

1. Collect health data and information about risk factors related to the Portuguese population, using the methodology recommended by the EHES;
2. Create a biobank of biological samples (serum, plasma and DNA) that will allow the development of future studies in public health.

Secondary Objectives

1. Test the feasibility of collecting blood samples under this specific scientific protocol;
2. Develop the logistics for the collection and transportation of biological samples;
3. Guarantee the quality of the biological samples from collection to storage or examination.

Materials and Methods

Participants

The invitation of the participants was initially made by letter two weeks before the scheduled date for the collection of the data, explaining the purpose of the study and requesting participation of the contacted person (with a letter of invitation leaflet). After the notice, a second mailing was done, stating the place, day and hour for the interview and physical examination. From the second week on , all participants were contacted by telephone by a member of the Health Centre of S. Brás de Alportel, to ensure maximum participation and planning dates for possible reschedule dates, based on availability of participants.

In this study participated 221 individuals (95 men and 126 women) aged 26 to 91 years. All participants were given a brief description of the objectives of the study, all of whom signed a consent form.

Blood Collection

The blood sample collection involved a total of 20.5 ml of whole blood from each participant, distributed by the following tubes: Vacutainer SST ™ (for serum separation, 8.5ml) Vacutainer Fluoride (for measurement of blood glucose 2.0 ml) Vacutainer K3EDTA (for separation of plasma, DNA extraction and complete blood count, 2x4ml + 1x2ml), according to the sampling scheme shown in Figure 2. Only duly accredited laboratory technicians and phlebotomists were allowed to perform blood collection. The team received training on the collection of blood samples using the Vacutainer ® system, as well as on matters concerning safety and health and welfare of participants.

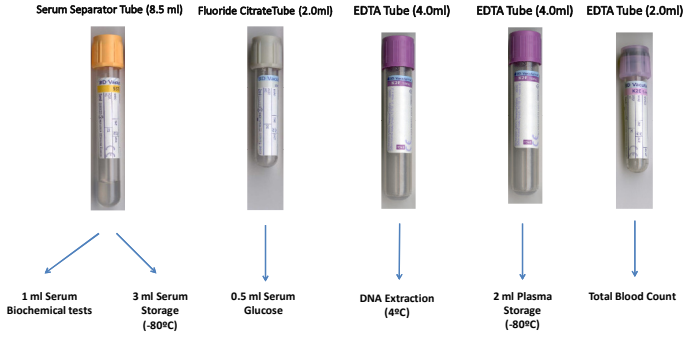

Figure 2. Sampling scheme.

Sample Processing

The serum (8.5 ml), fluoride (2 ml) and EDTA tubes for the separation of plasma (4 ml) were centrifuged at 2000g for 10 minutes. Blood samples and their record sheets were placed in a refrigerated transport box specific for this purpose. Biological samples were collected and transported from the Health Center of St. Brás de Alportel to the Regional Laboratory of Public Health Dr. Laura Ayres (Faro) where they were collected by a carrier, arriving at INSA at 10:00 the next day. The exceptions to this procedure were specified on the record sheets.

Biochemical and Hematological Tests

The samples were processed and transported to the INSA in Lisbon, where they were analyzed for total cholesterol, LDL, HDL, glucose, triglycerides, creatinine, ALT, AST, γ-GT, CRP and iron. A Complete Blood Count was also performed.

Results

Of the 221 participants, we were able to collect blood from 219, representing a success rate of 99.5%. From the participants who had a blood sample collected, we were able to collect all the tubes (5) to 185 (83.7%) of them. From 22 (10%) participants, 4 tubes were collected. This fact is related to the absence of sampling the CBC tube on Friday and holyday eves, given that the CBC test is very sensitive to the collection time. These two classes together represent 93.7% of the total samples, which is consistent with other studies of the same type. A partial sample was collected to 13 (6.4%) participants. We were unable to collect blood from only 1 (0.5%) participant (Figure 3). Almost all samples were collected in good conditions. Only one sample corresponding to 0.5% of total samples underwent hemolysis. We processed an average of 9 samples per day, ranging from a minimum of 3 and a maximum of 15 samples per day.

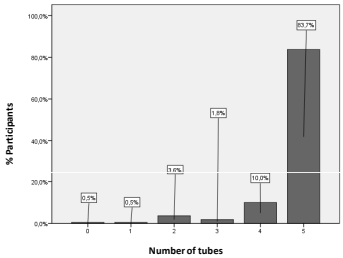

Figure 3. Number of tubes collected to each participant.

From the Serum tube, 1 ml was delivered to the clinical chemistry laboratory INSA for biochemical determinations. The biochemical tests were performed on all samples (N = 219). The rest of the serum was distributed in aliquots of 300µl each. The number of serum aliquots per sample varies between 0 and 13, with an average of 8.6 aliquots per sample. In total, 1897 serum aliquots were created, which are stored at -80 ° C.

The plasma obtained from the EDTA tube was distributed in aliquots of 300µl each. On average, 4.4 plasma aliquots per sample were created, ranging from 0 and 7 aliquots per sample. In total, 959 aliquots of plasma were created, which are stored at -80 ° C.

A CBC was performed for 103 individuals (49.3% of the total samples). The reasons why the CBC was not performed on the remaining 118, is related to the fact that samples taken on Friday only arrived at INSA on Saturday (51.7%) and to the poor transport conditions (48.3%). The quality of the blood count is closely correlated with the collection time, with temperature and with its packaging conditions during transport.

DNA was extracted from 4 ml of blood. DNA was obtained from 95% of the participants (N = 210). No tube for DNA extraction was collected for the remaining 5% (N = 19). The average concentration of DNA obtained for all samples is 310 ng / µl. The DNA obtained is of excellent quality, easily amplified by PCR as can be seen in Figure 4.

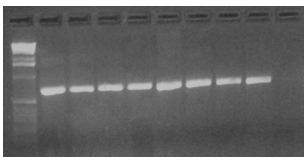

Figure 4. PCR amplificated DNA

All data relating to blood collection were transferred to a database at INSA, including code, type and quantity of sample. The location of your freezer, as well as the location of samples within the respective boxes will be introduced in the management information system laboratory (LIMS), so that any individual sample can be easily located at the freezer / rack / box / tube.

Conclusions

In this study a biobank was created, which consists on a representative sample of the population of S. Brás de Alportel, allowing future investigations.

We conclude that it is possible to collect the amount of blood foreseen under the scientific protocol without causing discomfort to the participants. This is corroborated by the fact that the success rate and quality of blood collection is quite high, comparable to similar studies.

Experience shows that some aspects of sample transport should be improved in future studies. For the CBC the possibility of using a portable device that allows you to make determinations on the same sampling point is under consideration .

FCT Fundação para a Ciência e a Tecnologia