Bezafibrate Plus Berberine in Mixed Dyslipidemia

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INTRODUCTION

Food and beverages have radically changed in recent decades, with an increase in their caloric content, production, distribution and availability at low cost. This has modified the lifestyle, which by having these comforts has led the world to a sedentary lifestyle. These factors, as well as the genetic ones, have influenced the increase in health risks as well as metabolic diseases, where cardiovascular diseases, as well as disorders of lipid and glucose metabolism wreak havoc on the health of those who have these alterations.[1,2]

Among the risk factors that can be modified are dyslipidemias, which constitute a group of diseases related to an alteration in the lipid profile. Its classification depends on the value that is altered in relation to total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), as well as low-density lipoprotein cholesterol (C- LDL). The reference values to consider an alteration in lipid metabolism based on its concentration is for TC ≥ 200 mg / dL, in a profile in which only this value is altered, it is called isolated hypercholesterolemia, when a TG value is presented. ≥ 150 mg / dL is considered hypertriglyceridemia and if we have an alteration in both TC ≥ 200 mg / dL and TG ≥ 150 mg / dL it is considered as mixed dyslipidemia. Mixed dyslipidemia is considered to have a higher potential health risk than isolated dyslipidemia, these patients may develop complications related to hypercholesterolemia such as chronic renal failure, loss of sight, atherosclerosis or related to hypertriglyceridemia such as pancreatitis and fatty liver , or the above at the same time. It is necessary to mention that atherosclerosis is a condition that can trigger cardiovascular diseases and is present in mixed dyslipidemia.[2]

The National Health and Nutrition Survey (ENSANUT) 2006 reported a prevalence of hypercholesterolemia of 26.5% of the surveyed population, in turn 18.2% was diagnosed with mixed dyslipidemia,[1] on the other hand, ENSANUT 2012 reported that 25.8% of the Mexican population suffers from hypercholesterolemia. Therefore, the diagnosis and treatment of dyslipidemias is considered a priority for health systems.[3]

For the treatment of this disease, the guidelines establish changes in diet as well as in lifestyle, as a basic action. However, in some patients these measures do not work, which makes it necessary to start a pharmacological intervention with a lipid-lowering agent, which can be a monotherapy or a combination therapy.

Combined therapies with lipid-lowering drugs for the treatment of mixed dyslipidemia are still under investigation, it has been shown that in some patients or groups of patients the available therapeutic options are not the best option given the monetary cost they represent and the adverse events that can complicate the health of patients, which is why it is necessary to find combined therapies with a low presence of adverse events that help patients meet the therapeutic objectives that are TC values <200 mg / dL and TG <150 mg / dL.[4,5]

From the group of lipid-lowering drugs, bezafibrate has been used for the treatment of mixed dyslipidemia as a therapeutic option due to its main action as a TG reducer with a low presence of adverse events; Its mechanism of action is as an agonist of the peroxisome proliferator activated receptor (PPAR) alpha (α), beta (β) and gamma (γ), it forms a temporary bond with the retinoid X receptor (RXR) at the cellular level. The cellular machinery increases the catabolism of lipoproteins (LP) in the blood, which is why the concentration of TG, as well as LDL-C and very low-density lipoproteins (VLDL) decreases, with an increase in levels and the quality of C-HDL.[6,7]

The use of natural extracts for the treatment of diseases and the recovery of health has accompanied humanity since its origins, today in the cultures of the world the use of these extracts in an empirical way prevails and the Mexican culture is not the exception. In Asian culture there is an extract of natural origin that has been used for the treatment of various diseases where dyslipidemia is one of them, this extract is obtained from plants of the genus Berberis bulgaris, the extract from the rhizome and stem of the plants of this genus is known as berberine (BBR). The combination of BBR plus bezafibrate could offer a pharmacological treatment alternative for patients with mixed dyslipidemia.

BACKGROUND

A study by the Ministry of Health on the health of Mexicans determined that conditions such as hyperglycemia, overweight and obesity, poor dietary habits, HA, alcohol or drug consumption, low glomerular filtration, smoking, elevated TC (≥ 200 mg / dl), malnutrition and low physical activity, are the 10 main risk factors to the health of Mexicans and these factors can act synergistically in the development of cardiovascular disease. Given that TC ≥ 200 mg / dL, it is one of the 2 components of mixed dyslipidemia, treating those who suffer from it early is of the utmost importance since this can avoid the increased risk of cardiovascular disease. Diagnosis is by determining the plasma concentration of TC and TG, treatment consists of general nutrition and exercise recommendations, as well as lipid-lowering drugs in many cases.[8,9,10]

When non-pharmacological measures are insufficient, it is necessary to use lipid-lowering drugs.[10] Of these, statins are the first-line drug, they are effective in reducing TC levels, in high doses they are effective in lowering TG, however When TG values ≥ 442.5 mg / dl occur, it is no longer a first-line therapy.[11] This is why combined therapies are used for the treatment of mixed dyslipidemia.

Combined therapy for the management of mixed dyslipidemia, the combination of statins and fibrates leads the drug treatment guidelines, however this combination presents a muscle involvement between 0.3-33% and its association increases the probability of presenting myopathy that can increase the presence of rhabdomyolysis.[12]

Plants and natural extracts have been used to treat diseases since the dawn of mankind. In recent times, this trend has increased and today the search for natural therapeutic options that are effective for the treatment and recovery of health continues, where BBR has shown to be an extract that can be used for the treatment of mixed dyslipidemia.[13]

A meta-analysis on the use of BBR in which 11 clinical trials were included in which a daily dose of 500 to 1500 mg of BBR was administered, results of this

work showed a significant reduction in CT and TG values, without modifying the rest lipid profile.[14]

In a clinical trial carried out in patients with hypercholesterolemia, it is shown that the administration of BBR at a dose of 500 mg every 12 h orally, allows changes in TC in 18% ($p < 0.001$), TG in 28% ($p < 0.001$) and LDL-C by 20% ($p < 0.001$), although HDL-C values remained unchanged.[15]

Pérez Rubio et al., 9 conducted a clinical trial in which BBR 500 mg V.O. every 8 h for 12 weeks in a population with metabolic syndrome, reported a significant decrease based on statistical tests in the values of TC (187.0 ± 33.3 vs 175.6 ± 27.0 mg / dL) and TG (215.9 ± 64.7 vs 131.1 ± 51.2 mg / dL), however, HDL-C and LDL-C did not change.

From the group of lipid-lowering drugs, bezafibrate has been a drug of choice for the treatment of hypertriglyceridemia and in combination with some other lipid-lowering drug for the treatment of mixed dyslipidemia.[16]

The safety and efficacy of bezafibrate make it a useful drug for the treatment of dyslipidemia, it is well tolerated and can be combined to achieve better control of mixed dyslipidemia. In turn, a meta-analysis of the use of bezafibrate in relation to the lipid profile, showed its effect in reducing the concentration of TC and TG and an increase in HDL-C levels, as well as a cardioprotective effect, which helped in reducing coronary heart disease by up to 28%.[17]

A clinical trial where bezafibrate was compared against placebo with a duration of 5 years in which 400 mg of bezafibrate were administered per day, showed a reduction in TG of up to 20.6 mg / dL without showing significant changes on TC, LDL-C, They also report an increase of up to 17 mg / dL of HDL-C.[18]

In the Mexican population, another clinical trial was carried out in patients with mixed dyslipidemia, to which bezafibrate was administered at a dose of 400 mg V.O. every 24 h for 12 weeks, it was reported that after treatment and when comparing with baseline values, TG were significantly reduced by 24.4% in the group that was administered bezafibrate ($p < 0.05$), however, the rest of the lipid profile such as CT, HDL-C and LDL-C did not show a significant statistical

difference.[16] This is the first study in which BBR was used in combination with bezafibrate as a therapeutic option for the treatment of mixed dyslipidemia.

HYPOTHESIS

The effect of the administration of the combination of BBR plus bezafibrate modifies the lipid profile in patients with mixed dyslipidemia.

OBJECTIVES

General

To assess the effect of the administration of the combination of BBR plus bezafibrate on the lipid profile in patients with mixed dyslipidemia.

Specific objectives

Determine the concentration of CT, TG, HDL-C, LDL-C, and VLDL-C before and after the intervention with BBR, bezafibrate and BBR plus bezafibrate.

Secondary objectives

To determine the effect of the administration of BBR, bezafibrate and BBR plus bezafibrate on body weight, height, body mass index (BMI), waist circumference, blood pressure, fasting glucose, creatinine, uric acid.

Describe the tolerability of the administration of BBR, bezafibrate and BBR plus bezafibrate.

METHODOLOGY

To assess the effect of the administration of the combination of BBR plus bezafibrate on the lipid profile in patients with mixed dyslipidemia.

Specific objectives

Determine the concentration of CT, TG, HDL-C, LDL-C, and VLDL-C before and after the intervention with BBR, bezafibrate and BBR plus bezafibrate.

Study design

Single randomized, parallel group, double-blind clinical trial. Resident population of the Guadalajara Metropolitan Area between 30 and 60 years of age, of any sex, with a diagnosis of mixed dyslipidemia.

Study site

Institute of Experimental and Clinical Therapeutics (INTEC) located in the Department of Physiology, University Center for Health Sciences, University of Guadalajara.

The sample size was calculated according to the formula for clinical trials, 61 in which the following values were taken into account for its calculation: The Zα corresponds to the value of statistical confidence, which was prefixed to a value of Z1-β of 95%, for a type I error of 5% expressed as a value of 0.05 with two tails. For this confidence, the value of Z was 1.96.

Z1-β that expresses the statistical power or power and was prefixed to the value of Z-β of 80%, for a type II error of 20%, which is equivalent to a value of 0.20. For this statistical power and this type II error, the typified Z score was 0.842.

The expression δ refers to the standard deviation in the basal TG concentrations obtained from the BBR studies, which was 35 mg / dL.62 d is the expected TG difference of 44.2 mg / dl.36

The result was n = 10 for each intervention group, in order to ensure sufficient statistical power, the sample size was increased by 20% due to possible losses, giving n = 12 patients per group.

Inclusion criteria

The study population was selected based on the fulfillment of the following criteria:

Men and women, age completed: 30 to 60 years residents of the Guadalajara metropolitan area, ability to communicate and comply with the established requirements.

Diagnosis of mixed dyslipidemia (TG ≥ 150 mg / dL and TC ≥ 200 mg / dL), No drug treatment on lipid profile, signing of the consent letter under information.

People with suspected or confirmed pregnancy, lactating or puerperium, TC ≥ 400 mg / dL were not included, TG ≥ 500 mg / dL, BMI ≥ 39.9 kg / m2, DM2, Kidney and / or liver disease, Intolerance to bezafibrate or BBR, PAS / DBP ≥140 / 90 mmHg, consumption of drugs with known action on lipid profile, glucose metabolism, BP or body weight.

Elimination criteria. The withdrawal of informed consent, loss of follow-up, presence of a serious adverse event, adherence to treatment <80% were considered as a reason for exclusion, but not so in the final statistical analysis. Presence of any non-inclusion criteria during the intervention.

Variables

Independent variable

Pharmacological intervention:

Administration of 1500 mg of BBR homologated with 400 mg of placebo (calcined magnesia)

Administration of 400 mg of approved bezafibrate with 1500 mg of placebo (calcined magnesia)

Administration of 1500 mg of BBR with 400 mg of bezafibrate

Dependent variables

Primary: lipid profile (TC, TG, LDL-C, HDL-C, VDL)

Secondary: weight, height, BMI, WC, BP, glucose, creatinine and uric acid, as well as tolerability

Control variables

Diet: They were given general nutrition recommendations based on the provisions of the dietary and physical activity recommendations of the National Academy of Medicine of Mexico, as shown in ANNEX document.

Exercise: They were asked to continue with the daily physical activity they already did and not to increase it.

The patients who came voluntarily for clinical evaluation generally had some cardiovascular risk factor such as dyslipidemia, smoking, etc.

Groups, intervention allocation and blinding

Three groups were formed randomly which were integrated in the following way.

The first group of 12 patients with mixed dyslipidemia received O.V. one 500 mg BBR capsule and one 500 mg placebo capsule at breakfast, one 500 mg BBR capsule at lunch, and one BBR capsule and one 500 mg placebo capsule at night.

The second group of 12 patients with mixed dyslipidemia received one 200 mg bezafibrate capsule in the morning and one 500 mg placebo capsule, one 500 mg placebo capsule with food, and one 200 mg bezafibrate capsule and one of 500 mg placebo in the evening.

The third group of 12 patients with mixed dyslipidemia received 200 mg of Bezafibrate in the morning with 500 mg of BBR, a 500 mg capsule of BBR in the meal and finally a 200 mg capsule of bezafibrate and a 500 mg of BBR at night.

During the visit at the beginning of the intervention period, all participants who met the inclusion criteria and agreed to participate in the study were asked to select a sealed envelope of 36; 12 sachets from group 1, 12 from group 2 and 12 from group 3, which were mixed. At the time of selection, they were all equal with a number corresponding to each intervention group. Once scrambled, they were arranged in a box and the patient took one at random.

The opening of the envelope selected by each participant allowed to know a numerical code that identified the intervention that the participant received. Blinding was guaranteed in which the participant was unaware of the type of

treatment received, as well as the investigator. At the end of the study when the data were completed, the treatment codes were opened for statistical analysis, this without the person in charge of carrying out the statistical analysis knowing the treatment that the participants received. Participants who left the study without having completed the procedures, but who received at least one dose of the intervention, were taken into account for statistical analysis.

Clinical determinations

All the anthropometric and vital signs determinations as well as the procedures for the interrogation of the physical examination and the integration of the corresponding clinical history were carried out using accepted universal methods and in accordance with the General Health Law in its regulations on the presentation of care services medical.

The physical examination as well as the clinical questioning were performed again on all the occasions required within the development of the study or when adverse events were suspected, which were also directly recorded in the attachment diary of each participant.

Age: defined as the number of years elapsed since the patient's date of birth Gender: considered as the phenotypic sex of the participant at the time of birth and registered as female or male

Weight: taken in kilograms (kg), the participant was placed in an upright position with his feet resting on the electrodes of the TANITA bc100 scale, this model has the ability to determine the user's body composition thanks to the bioelectric impedance system. The participant was placed in a standing position dressed in light clothing, without shoes and with bare feet, with the bladder evacuated before the measurement.

Height: it was measured in centimeters (cm) using the stadiometer integrated into the TANITA bc100 scale, it was taken standing up, with the knees fully extended, upright posture, the gaze at right angles to the vertical and the lower edge of the orbit in the same horizontal plane as the external auditory canal.

BMI: is the relationship between weight in kilograms and that of height in meters squared. This reading is provided automatically by the TANITA scale with the Quetelet formula (kg/m^2).

BP: was determined by OMRONMR digital sphygmomanometer in mmHg. With the participant sitting upright to form a right angle with both feet resting on the floor without crossing the legs and after 15 min of rest, a cuff was adjusted 3 cm above the elbow crease of the left arm. 3 shots were taken with a time difference of 3 minutes between each of them to obtain the average value of BP.

WC: A fiberglass tape was used to obtain the measurement in cm, based on the ISSAC method for anthropometric determinations, when visualizing an imaginary line that started from the armpit hollow to the iliac crest, based on the point middle between the last rib and the top of the iliac crest (hip). At this point the WC was taken.
All clinical determinations were carried out by medical and nutritional personnel in charge of this project.

Laboratory determinations

For the collection of all blood samples in the visits to the research center, all participants were summoned at 8 a.m., the essential requirements in each of the visits were: 1) fasting state of 12 h, 2) They were recommended to consume low-fat foods during the three days prior to the collection, 3) adequate intake of fluids, 4) not to attend in a state of wakefulness during the night preceding the collection of the samples.

Collection of blood samples for clinical chemistry analysis was performed by simple venipuncture in the anterior ulnar region at the height of the elbow crease, the procedure used to collect blood samples, as well as the volume of the It was established based on the activities required in each of the work visits. The total volume of blood required to collect during the entire field work was 10 ml per patient. The determinations of lipids, as well as glucose were carried out using colorimetric-enzymatic techniques as described below, as well as the expected intra- and inter-assay CVs based on the information provided by the

manufacturer (Biosystems) for each of the reagents and we use mg / dL as the unit of measure for all determinations.

TC: The serum concentrations of TC were determined by the method based on the use of three enzymes, TG: The concentrations of TG in blood were measured by a method based on the enzymatic hydrolysis of serum TG to glycerol and FFA by the action of LPL, HDL-C: Serum HDL-C concentrations were measured using a method based on a modification of the classical precipitation procedure using optimized amounts of polyvinyl sulfonic acid, LDL-C: The Fridewald formula LDL-C = TC - (HDL-C + TG / 5) in mg / dL was used to calculate this value, VLDL: The TG / 5 ratio was used to calculate this value.

Glucose: it was determined using the glucose oxidase technique. The glucose in the sample is oxidized to glucuronic acid and hydrogen peroxide (H2O2) in the presence of glucose oxidase, CR: it was quantified by the Jaffé method, by means of which a reaction with picrate occurs in an alkaline medium, causing a complex colored, UA: It was measured by means of a technique that involves two reactions. All measurements were made automatically in the ERBA Mannheim XI 100 equipment, the reagents used were appropriate for each test and specific for each one.

STATISTIC ANALYSIS PLAN

The data obtained were expressed in measures of central tendency and dispersion for quantitative variables (mean and standard deviation) and for qualitative variables (frequencies and percentages). Statistical significance (p-value) was determined by:

When performing the normality test (Shapiro-Wilk) of the variables of the 3 groups, it was found that, among others, the TG did not present a normal distribution, therefore, for the interpretation of the results, non-parametric tests were used.

Kruskal-Wallis test to identify differences between the three intervention groups in relation to the quantitative variables of the included participants.

Wilcoxon rank test to identify differences between the baseline and final evaluation of each intervention group in relation to the quantitative variables of the included participants.

Mann-Whitney U test to identify differences between two intervention groups in relation to the quantitative variables of the included participants.

X2 test or Fisher's exact test to identify differences between two intervention groups in relation to the qualitative variables of the included participants.

The preset level to consider the probability obtained from any of the statistical methods used was $p \leq 0.05$.

All variables were analyzed with the SPSS v22 program for windows.

Recruitment

15 detection campaigns were carried out in different points of the metropolitan area of Guadalajara, in which 290 people were attended, who were asked if they suffered or were referred to suffer from any cardiovascular disease, dyslipidemia, diabetes, prediabetes, etc. They were determined if they had obesity by calculating the BMI and the WC was determined; A capillary glucose test was also taken, for which a One touch ultra-glucometer was used to discard, by way of scrutiny, any alteration related to glucose metabolism.

Of these patients, 60 reported having a dyslipidemia reference of their own or of a direct relative and were invited to the INTEC located at the University Center for Health Sciences of the University of Guadalajara to carry out a more complete scrutiny.

Scrutiny

Each patient was summoned to INTEC at 08:00 am with a 12-hour fast. The visit during the scrutiny period, like the rest of the scheduled visits, took place at INTEC from 8 a.m. During the first visit, a complete clinical record was integrated, for which a clinical interrogation and comprehensive physical examination were carried out, all the clinical characteristics required for the evaluation of the selection criteria for admission were corroborated. 5 ml of

venous blood were taken by venipuncture with an 18 Gy catheter in the brachial vein to determine the lipid profile (CT, TG, HDL-C) and glucose.

The 60 patients who were attended with the intention of knowing whether or not they suffered from mixed dyslipidemia, after carrying out the laboratory procedures, the results showed that 21 patients did not suffer from mixed dyslipidemia, so they did not continue as part of the study, however they were offered nutritional care by nutrition staff. Of the 39 who met the criteria for mixed dyslipidemia, 3 decided not to participate in the study and 36 agreed to participate; They were explained in detail each of the aspects of the project, the consent letter was read and explained under detailed information and they were asked to come the next day or the third day to begin their inclusion in the study and thus have a scheduled visit cycle for each one.

Intervention period

Through the indicated laboratory tests, each of the biochemical selection criteria was identified and documented. The patient who met the inclusion criteria was assigned to one of the intervention groups.

Visit 1. Participants who met the inclusion criteria, signed the consent letter and agreed to participate in the study, were invited to extract one of the sealed envelopes that contained the assignment code to any of the three interventions. A treatment adherence diary was provided to the participant. In the diary, adherence to treatment was recorded day by day and the participant recorded each taking of the intervention, as well as the discomfort that she might feel daily. The indication to take each of the capsules was: “take one capsule from bottle 1 and one from bottle 2 and ingest them with liquid in the first bite of breakfast, one from bottle 3 with the first bite of food and one from bottle 4 and another from jar 5 with the first bite of dinner only Once the first visit was concluded, each patient was asked to come within the next 30 ± 3 days and was asked to keep the attachment diary, as well as the bottles to count the capsules consumed, it is in this visit in which were given the food recommendations that appear in the food guidance guide of the Ministry of Health.[63]

Visits 2 and 3. They were carried out on days 30 and 60 ± 3 days after starting treatment. During these visits, the treatment adherence diary was reviewed, the capsule consumption record in the adherence diary and the number of capsules that were in each of the bottles were counted, and likewise, the patient was questioned to identify the possible presence of adverse events and were recorded in the file of each participant. The doubts that each patient might have were solved, they were reiterated about any doubts that the participant might have at the time, the bottles were collected, the capsules contained in each of them were quantified, the happy faces marked in the Attachment diary and finally the patient was given treatment for one more month. An appointment was made in 90 ± 3 days.

End of the intervention period 90 ± 3 days after the start of the intervention period. The clinical and laboratory measurements were taken again as at the beginning of the project. Adherence to treatment was evaluated

ETHICAL ASPECTS

The study is classified according to the Regulation of the General Health Law on Research for Health, in its Third Title, Chapter I, Article 64 as a study with greater than minimal risk, for which it required signing of consent under information of each participant (see ANNEX).

The procedures adhered to the ethical norms, the Regulations of the General Health Law in Research Matters and the principles emanating from the l8th Medical Assembly of Helsinki, Finland in 1964 and the modifications made by said Assembly in Tokyo Japan in 1975, in Venice, Italy in 1983, in Hong Kong in 1989 and the 48th General Assembly of Somerset West, Republic of South Africa in 1996, 59th General Assembly, Seoul, Korea, October 2008 64th General Assembly, Fortaleza, Brazil, October 2013 where the medical research (clinical research). The study qualified for research in humans.[19]

- In accordance with the guidelines of good clinical practice, all participants in the study were identified only by initials and number in the electronic database. The data was kept confidential, as were the results of clinical and laboratory tests, to ensure privacy. The results of this study are presented in this work, without indicating information that could reveal the identity of the participants.[65]

The present work was approved by the Bioethics Committee of the Department of Physiology of the University Center for Health Sciences, with the record number DF / CB059 / 13, as well as by the Clinical Trials where the record number NCT 02548832 was obtained.

FINANCING

It was funded in parallel with other studies

CONFLICT OF INTEREST

The project does not present conflicts of interest, it is an original research generated by the researchers involved in this project and it was not sponsored by the pharmaceutical industry. The interest of the present investigation is entirely the search for new knowledge in the field of therapeutic options for the treatment of mixed dyslipidemia.

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Annex I

LETTER OF CONSENT UNDER WRITTEN INFORMATION

NCT 02548832

This informed consent form may contain words that you do not understand. Please ask the study doctor or research team staff to explain any words that you do not understand clearly. Do not sign this form unless you have received satisfactory answers to all of your questions.

Purpose and description of the study

Cholesterol and triglycerides are fats that are normally found in the blood, however when their concentrations are higher than normal they become a health risk, since they increase the possibility of different diseases, including heart problems. . When a person has cholesterol or triglyceride levels higher than normal, the diagnosis of dyslipidemia is established. For the treatment of people with dyslipidemia, it is usually started with measures of diet and exercise, however, despite these measures, some patients require adding one or two medications to the treatment in order to normalize the values of fats in the blood. .

The drugs that will be studied for the treatment of mixed dyslipidemia are: 1) Berberine, which is a natural substance of Asian origin that has been shown to improve blood fats. 2) Bezafibrate, which is one of the first choice drugs to lower triglycerides and cholesterol. 3) The combination of berberine + bezafibrate.

This study will last 90 days, from the first visit to the last, with an average of 5 visits (appointments to the research center). The study will involve 32 people diagnosed with dyslipidemia who will be randomly assigned to one of the following 3 treatments:

- Berberine 1500 mg orally
- Bezafibrate 400 orally
- Combination of berberine (1500 mg) plus bezafibrate (400 mg)

You and the rest of the study participants will take the medicine as directed, and it will be provided free of charge by the investigator in charge of your evaluation in sufficient quantity for you to take in the interval between your appointments. Since this study is blind, you will not know which treatment group it belongs to, however this information will be accessible if necessary.

Procedures

If you agree to participate, the following will occur:

Procedures that are routine in the care of patients in this service:

You will go to the Research Unit fasting from 12 noon to 8:00 am.

a) Clinical Procedures: you will be given a medical history that includes a complete physical examination with the measurement of your weight and blood pressure.

b) Laboratory procedures: we will take a blood sample from one of your arms, approximately 7ml equivalent to approximately 2 teaspoons of your blood, to perform some laboratory studies.

The laboratory studies that we will perform include measurement of your glucose, creatinine, uric acid, and a lipid (fat) profile. For your safety and hygiene, all the material used in this study is sterile and disposable, and at the end of the planned analyzes, the rest of the sample will be destroyed.

The purpose of conducting laboratory and clinical studies is to learn more about your general health conditions. It will take us approximately 20 minutes to perform these clinical and laboratory tests. We will give you the results of your lab studies within 3 days.

In case of meeting the criteria to enter the study and once this letter of consent is signed, another day will be called to the Research Unit on a 12-hour fast at 8:00 a.m. where the following specific procedures will be performed:

Specific procedures of this investigation:

During the second visit, which will take place no more than 7 days after your first visit, a blood sample (4 ml) will be taken again to **assess your lipid (blood fat) profile.**

On this second visit, you will be given the medicine, which you will take 2 capsules in the morning, one in the afternoon and two at night. The medicine will be provided to you free of charge and in sufficient quantity.

After this visit, you will be asked to come in three more times (every 30 days) to assess your health, in addition to assessing your weight, waist, and blood pressure.

The blood tests will be carried out at the beginning and will be repeated until the 90th day (3 months) according to the appointment calendar that will be given to you. At all appointments to the Research Unit, you will be questioned about your health.

The results of the final laboratory tests will be delivered to you and explained to you by the medical staff in charge of this project. You will be given a clinical summary that includes recommendations for continuing your treatment.

The knowledge that results from this research will help to know and propose treatment options for glucose intolerance.

Profits

You can get the benefit of knowing your metabolic profile, that is, the amount of sugar in your blood, uric acid, fats in your blood such as cholesterol and triglycerides (among others), as well as knowing the functioning of your liver and kidneys.

You will receive professional guidance on what steps you should take to lower your risk of heart disease or diabetes. Nutrition recommendations will be offered to you in the hope that it will help you improve your disease control.

Possible risks and annoyances

<u>Discomforts or risks associated with clinical evaluation procedures:</u>

Weight, height, waist, and blood pressure measurements are noninvasive clinical studies that do not cause pain, discomfort, or risk.

Discomforts or risks associated with laboratory procedures:

Discomfort during blood sampling is minimal. Sometimes the procedure to take a blood sample can cause a little pain or a slight discomfort, it is possible that a small bruise may form.

Discomforts or risks associated with the administration of berberine:

Berberine has been shown to be safe, however it can temporarily produce gastrointestinal symptoms such as gas, diarrhea or abdominal pain.

Discomforts or risks associated with the administration of bezafibrate:

Bezafibrate is generally well tolerated. Adverse effects may occur in 5-10% of patients. The main complaints are gastrointestinal such as loss of appetite, a feeling of fullness in the stomach and nausea. Other side effects that appear infrequently can include fatigue, headache, and increases in certain liver enzymes.

If you experience any complications from your participation in this study, you will receive the necessary treatment and follow-up. Any complication will be attended by specialized personnel of this Unit.

Participation or withdrawal

Your participation in this study is completely voluntary. If you decide not to participate, you will continue to receive the medical care provided by the Research Unit. If you do not want to participate in the study, your decision will not affect your right to obtain the health or other services you receive.

For the purposes of this research we will only use the information that you have provided us from the moment you agreed to participate until the moment you let us know that you no longer wish to participate, so if during the study you decide to withdraw, you can do so. without this representing an alteration in your medical care.

However, you should only enter this study if your current plan is to continue until the end of the study (3 months).

You can ponder your decision to agree to participate or not to participate in the research project and your answer can be provided at a subsequent appointment.

You are free to ask any member of the research team as many questions as you like.

If you wish to suspend your participation, please contact those responsible for the study: Dr. en C. Manuel González Ortiz (33 36764255), LN. Karina G. Pérez Rubio (33 10918297) LN. Juan Miguel León Martínez (33 15515550) Cardiovascular Research Unit (36173499)

Some of the doctors responsible for the study will make a final visit to ensure your health.

The study doctor may also discontinue your participation at any time without your consent for any of the following reasons:

- If you do not follow the instructions for your participation in the study
- If the treatment appears to be harmful or intolerable to you
- If the study is canceled

Contact staff for questions and clarifications

If you have questions or want to talk to someone about this research study, you can communicate from 8:00 a.m. to 4:00 p.m., Monday through Friday, with Dr. Manuel González Ortiz, who is the researcher responsible for the study, at the aforementioned telephones.

In the event of an emergency arising from the study, you can dial the previous phone numbers with Dr. Manuel González Ortiz, at any time of the day and on any day of the week.

Compensation

Your participation in this study is completely voluntary, both the beginning of the study and its follow-up will be done only with your consent. Your contribution to our work will be compensated with the benefits mentioned above in the benefits section, which do not include monetary or in-kind compensation.

Privacy and confidentiality

The information that you provide us that could be used to identify you (such as your name, telephone number and address) will be kept confidential and separately as well as your answers to the questionnaires and the results of your clinical tests, to guarantee your Privacy.

The team of researchers, the people involved in your health care, and your family doctor will know that you are participating in this study. However, no one else will have access to the information that you provide us during your participation in this study, unless you wish to do so. We will only provide your information if it is necessary to protect your rights or well-being (for example, if you suffer physical harm or need emergency care), or if required by law.

When the results of this study are published or presented at conferences, for example, no information that could reveal your identity will be released. Your identity will be protected and hidden. To protect your identity, we will assign you a password that we will use to identify your data, and we will use that password instead of your name in our databases.


Did you understand the proposed information? YES NOT

Patient statement

By signing this consent, I acknowledge that I have been informed about the study methods and the administration of the treatments, the disadvantages and benefits and adverse events that may occur due to the procedures and medications.

I certify that I have had sufficient time to read and understand the above information. I also acknowledge that all the technical language used in the description of the research study was explained to me to satisfaction and that I received answers to all my questions.

I confirm that I have received a copy of this consent form. I understand that I am free to withdraw from the study at any time, without losing any benefits or suffering any penalty. I freely and without reservation give my consent to participate as a patient in this study.

COMPETITOR

Participant signature _____ Signature Date _____

Participant name: _____

Participant's address: _____

Telephone numbers to locate it: _____

WITNESS 1

Signature of witness 1 Signature Date

Witness 1 Name: _____

Address of witness 1: _____

Relationship with the Partic pant: _____

WITNESS 2

Witness 2 Signature: Signature Date

Witness 2 Name:

Address of witness 2:

Relationship with the Participant:

Investigator statement

I or my authorized representative have discussed with the participant or their authorized legal representative, the nature and purpose of this study, as well as the possible risks and benefits of their participation. I believe that the participant and / or their legal representative have received the complete information in understandable and appropriate language and that the explanation was understood.

Investigator's signature: Signature Date

Investigator's name:

Participant identification code: