Medical Unit: Subdirección de Enseñanza e Investigación C.M.N. "November 20"

IDENTIFICATION OF BIOMARKERS DERIVED FROM ADIPOSE TISSUE WITH POTENTIAL UTILITY IN THE DIAGNOSIS AND PROGNOSIS OF CARDIOVASCULAR RISK OF OBESE PATIENT

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Background

Obesity is a nutritional disorder that is considered a risk factor for several metabolic abnormalities, such as insulin resistance, Diabetes Mellitus type 2 (DM2), NAFLD, endothelial dysfunction, cardiovascular risk and is associated with high morbidity and mortality.

According to the panel of experts from the National Heart and Lung Institute of the United States, "obesity is a chronic disease that results from the interaction of the genotype and the environment." In 1997, the World Health Organization defined obesity as "an excess of fat that accumulates in such an amount that health can be adversely affected." In Mexico, obesity represents a priority problem for public health, since its high frequency places the country in second place of prevalence worldwide.

A scale to classify obesity is the body mass index (BMI), in which the obese subject is one with a BMI ≥ 30kg / m2 and subdivided into 3 classes (Table 1).

Table 1. Body Weight Classification According to the BMI in Adults

	BMI
Normal	18.5-24.9 kg / m2
Obese Grade 1	30-34.9 kg / m2
Obese Grade 2	35.0 -39.9 kg / m2
Obese Grade 3	≥40 kg / m2

Morbid Obesity

Morbid obesity refers to an extreme degree of obesity, which affects basic physiological functions and is related to specific risks (Table 2). It has been estimated that a morbid obese man reduces his life expectancy by up to 22% compared to an individual of normal weight.

Table 2. Impact on the long-term health of morbid obesity
Decreased life expectancy
Dyslipidemia
Decrease in the quality of life of economic and social opportunities
Obstructive sleep apnea
Cardiovascular diseases
Gastroesophageal reflux disease
DM2
Cancer
Depression
Osteoarthritis
Hypertension
Kidney failure

Bariatric Surgery

Bariatric surgery not only induces weight loss, but also significantly improves the covariabilities associated with morbid obesity in those patients accepted. Clinical trials and observational studies have shown that bariatric surgery can prolong survival in patients with extreme obesity. In fact, the National Institutes of Health of the United States recommend bariatric surgery that should be presented with BMI> 40 kg / m2 or> 35 kg / m2 concomitant related to obesity.

Surgeries to reduce food intake reduce the amount of oral intake through a bag that limits the passage of food. It has also been proposed that a) it would induce a sense of emergence or suppression of appetite as a mechanism in weight loss; b) compress the vagus nerve; c) decreased the secretion of gastrointestinal hormones, such as ghrelin or peptide YY. The surgeries that are included in this type are the adjustable gastric band and sleeve gastrectomy.

Hybrid procedures are represented by the gastric Roux-en-Y derivation (DYRG), which involves reducing the size of the stomach by 15 ml by resecting the upper stomach portion and anastomosing it to the small intestine. The stomach remains viable but the

food is derived from the small intestine and the remaining stomach has a lower capacity; the duodenum does not absorb the macronutrients responsible for post-prandial hormonal responses.

Appetite reduction can be partially explained by the YY principle and the glucagon-like principle1-3 The duodenum bypass also contributes to decrease the absorption of iron and calcium, requiring its supplementation4. The biliopancreatic diversion (BPD) involves the resection of the upper 2/3 of the stomach, leaving a stomach capacity of 250 ml, disconnecting half of the jejunum and ileum from the alimentary tract and reconnecting it to the terminal ileum. With the delay in the action of the digestive enzymes within the alimentary tract, most of the fat and a significant fraction of the proteins is excreted as a result of the decrease in its ingestion. There is a dramatic loss in weight, but certain micronutrients are not absorbed, so supplements are required. There is a modification of DBP, called "switch" DBP, which consists of a sleeve gastrectomy with a less dramatic intestinal derivation. The difference lies in the preservation of the pylorus, which leads to fewer complications.
Postoperative mortality at 30 days ranges from 0.1% to 2% 5-7 and depends on the complexity of the operation, the co-morbidities of the patient, the experience of the surgeon and the hospital institution. The patient with morbid obesity can be complicated early by thromboembolism (1%), respiratory failure (<1%) hemorrhage (1%), peritonitis (1%) and / or wound infection (2%). Laparoscopy has been useful in reducing these rates. On the other hand, late complications include obstruction, ulcerations, hypoglycemia, steatorrhea, and bacterial overgrowth. Dietary modification and antibiotics can help control the severity of these side effects.

Bariatric Surgery - Metabolic and Cardiovascular Benefits
Bariatric surgery offers sustained weight loss. On average, time loses 50% of the weight of the 5 years, although this depends on the type, aggressiveness and complexity of the surgery.
Effect on DM2. Up to 50% -60% of patients with DM2 are known as morbidly obese. The most relevant clinical impact of surgically induced weight loss is the ability to completely reverse DM2 in a large percentage of patients. In a long-term study of surgical and conventional therapy, DM2 was reversed in 21% of the control group and in 72% of the study group after 2 years of follow-up.55 At 10 years of follow-up, DM2 it could be reversed in 13% of the control group and in 36% of the group with surgery. The odds ratio on recovery of DM2 with surgery at 2 years was 8.42 (95% confidence interval, 5.68 to 12.5) and at 10 years it was 3.45 (95% interval)

JUSTIFICATION

Bariatric surgery induces a significant reduction in the co-morbidities associated with obesity, such as DM2, dyslipidemia, liver disease, arterial hypertension, obstructive sleep apnea and cardiovascular risk. However, this does not happen in all obese patients, even when there is a reduction in weight.
It is known that adipose tissue participates actively in the synthesis of cytokines and its constitutive role in metabolic phenotypes has been suggested. It is possible that the

intrinsic mechanisms of adipose tissue participate in several benefits observed in morbidly obese patients who undergo anti-obesity surgery. Therefore, this study explores the participation of adipose tissue, as an active component, that can define metabolic phenotypes linked to the modification of cardiovascular risk after bariatric surgery.

HYPOTHESIS

- H1 Metabolic phenotypes based on basic mechanisms of adipose tissue are associated to the cardiovascular risk modification after bariatric surgery.

- H0. Metabolic phenotypes based on basic mechanisms of adipose tissue are NOT associated to the cardiovascular risk modification after bariatric surgery.

GENERAL OBJETIVE

To evaluate the association of the cardiovascular benefit of the obese patient after bariatric surgery with the basic mechanisms of adipose tissue (metabolic profile)

SPECIFIC OBJECTIVES

a) Characterize the basal (pre-surgical) metabolic profile of adipose tissue.
b) Evaluate post-surgical modification of cardiovascular prognosis
c) Determine the association of the cardiovascular prognosis modification (risk subgroups) with the basal metabolic profile.

DESIGN

This is an OBSERVATIONAL study, of a prospective cohort follow-up. We will study morbidly obese patients who undergo bariatric surgery (this is not a proposal of the present protocol, it is routinely done as a treatment for morbid obesity in the CMN "20 de Noviembre", ISSSTE, and it would also be carried out although the patient does not participate in this study). It was considered that the independent variable is the change in cardiovascular risk after bariatric surgery and the dependent variable is the basal metabolic profile of adipose tissue. This order of variables was decided by methodological strategy, since it will be more convenient to determine and stratify based on the change in cardiovascular risk, than based on the basal metabolic profile of adipose tissue.

$$n = \frac{(Z^{\alpha/2})^2 \quad (p(1-p))}{d^2}$$

where:

n = sample size. $Z^{a/2}$ = Z-value of the alpha error with a confidence of 95%, assigning alpha = 0.05 p = Expected population prevalence for the event under study (according to previous reports). d =difference between the expected population prevalence value and the acceptable error. Considering the average difference in the modification of the basic mechanisms in adipose tissue associated with metabolic risk in similar studies

$$n = \frac{(1.96)^2 (0.20 (1-0.20))}{(0.13)^2} \quad n = \frac{0.614}{0.0169} = 36.3$$

Additionally, for reasons of comparison and standardization of results, a C group would be considered: 10 non-obese patients undergoing non-bariatric surgery.

Definition of the observation units.

Patients with morbid obesity subjected to bariatric surgery, with significant modification of cardiovascular risk in the postoperative period.

Definition of the control group

Due to the observational design of the before-after evaluation (of bariatric surgery), the morbidly obese patient in the pre-surgical baseline is his own reference ("control") for the postoperative period. It is also considered non-obese subjects subjected to abdominal surgery eg. Hernioplasty, fundoplication etc. to know the "nomal" metabolic profile of adipose tissue.

Inclusion criteria

- Men and women.
- Over 18 years.
- With morbid obesity and candidates for bariatric surgery, under the routine of the treating service.
- Signature of acceptance of your participation, through informed consent.

Exclusion criteria.

- Medication with potential effect on fatty tissue or cardiovascular risk in the last month.
- Serious infections in the last month.
- Clinically unstable conditions.

Elimination criteria.

- Desire not to continue in the study
- Samples or insufficient information for an adequate analysis.

Definition of variables and units of measurement.

Definition of variables and units of measurement.			
Variable	**Dependent / Independent**	**Type**	**Units of measurement**
Total body fat, visceral and subcutaneous	Dependent	Discontinuous Quantitative	Percentage
Serum markers of inflammation PCR and IL-6	Dependent	Quantitative continuous	µUI / dL
Serum adipokines: leptin and adiponectin	Dependent	Quantitative continuous	µUI / dL
Differentiation and hypertrophy of adipocytes and angiogenesis	Dependent	Qualitative dichotomous	Present or absent
Pro-inflammatory markers at tissue level	Dependent	Qualitative dichotomous	Present or absent
Oxidative stress and tissue endoplasmic reticulum	Dependent	Qualitative dichotomous	Present or absent
Insulin sensitivity	Dependent	Qualitative dichotomous	Sensitive or not sensitive
HOMA	Dependent	Qualitative dichotomous	Higher or lower than 15
Prognostic scales mortality due to independent cardiovascular cause	Independent	Quantitative discontinuous	Own rating
Flow mediaded dilatation	Independent	Quantitative discontinuous	Percentage
Carotid intima media thickness	Independent	Quantitative discontinuous	Millimeters

Selection of sources, methods, techniques and procedures for collecting information

With the acceptance of the Institutional Ethics, Research and Biosafety Committees, patients will be invited and included according to the selection criteria.

Prior signature of informed consent, will include patients with morbid obesity, candidates for bariatric surgery and without additional modifiers of adipose tissue metabolism. Patients will be chosen in accordance with the criteria of the Bariatric Surgery Service, without modifying the routine procedures performed in this service.
At the same time, a group of non-obese patients (obesity comparison group) scheduled and undergoing non-bariatric abdominal surgery (hernioplasty, cholecystectomy, appendectomy, etc.) will be included.In all groups, total body fat, subcutaneous and visceral fat will be determined non-invasively by computed tomography, with the support of the Department of Imaging (Dr. Julita Orozco and Dr. Mario Osorio) as described below:

TOMOGRAPHIC MEASUREMENT OF SUBCUTANEOUS AND VISCERAL GREASE

Subcutaneous fat:
The thickness of adipose tissue will be measured, in the abdominal thickest site, at the level of the umbilical scar, with references from the skin to the muscular layer, either in vertical measurement (antero-posterior) or in lateral measurement.

Visceral fat:
Epicardial fat: The thickness of the adipose tissue will be measured
epicardial level, in the thickest site.

Preperitoneal fat:
The thickness of adipose tissue will be measured,
level of the umbilical scar; from the muscular layer (vertical measurement or ant-post) to the first visceral structure.

Perirenal fat:
The thickness of adipose tissue will be measured
perirenal, in the thickest site.

During the surgical act, a sample of 1-2 cubic cm of visceral and subcutaneous adipose tissue will be taken, in all groups, considering all aspects of patient Ethics and Biosafety described in the respective sections of this project. In patients with laboratorial evidence and image of progression to NAFLD, a sample of 1-2 cubic cm of liver tissue will be taken, in accordance with international guidelines for diagnosis and treatment, and with the study protocol established by the service. It will be guaranteed that the taking of biopsies will not affect the course of the surgery. The biological samples acquired will be properly identified, contained and stored for a maximum period of 1 year. Analysis and determinations will be made according to good laboratory practices. Subsequently, they will be treated and disposed of in appropriate bags for disposal. To comply with the provisions of section 6 of NOM-087-ECOL-SSA1-2002 Biological-Infectious Waste.
To study the characteristics of each metabolic profile, the following variables will be analyzed:

a) Adipose tissue (visceral and subcutaneous) where the degree of differentiation and hypertrophy of adipocytes and angiogenesis will be determined by morphometric methods of histopathology; as well as markers of inflammation, oxidative stress, endoplasmic reticulum stress and adipose tissue dysfunction through immunohistochemistry and in vitro tests on isolated adipocytes.
b) Liver tissue the degree of steatosis, steatohepatitis and fibrogenesis, considered as clinical progression of metabolic damage, will be determined.
c) Serum level, inflammation markers (TNFa, CRP and IL-6) and adipokines (leptin, adiponectin, resistin) will be determined by ELISA.

Finally, in all groups, follow-up variables related to post-surgical modification of cardiovascular risk will be determined; through:
Prognostic scales of cardiovascular mortality: They will be determined by Drs. Moises Ortíz and Juan Suárez Cuenca, based on probability calculation with variables such as age, gender, co-morbidities, etc. using Framingham or SCORE scales. Place: Clinical Research Division, CMN "November 20", ISSSTE.
Determination of serum markers of endothelial dysfunction and ankle / arm index. They will be determined by Dr. Juan Suárez Cuenca and associated researchers, using commercially available ELISA kits and direct determination of systolic pressure in the ankle and arm, with the help of Doppler scan type Doptone, respectively. Place: Biomedical Research Laboratory and Clinical Research Division, CMN "November 20", ISSSTE.
Determination of subclinical atherogenesis (thickness of the carotid intima media). It will be determined by the Department of Imaging, with the support of Dr. Toriz. The Doppler transducer will be placed in the neck and the common carotid artery will be located, then the thickness of the intimal vascular mean will be determined 1 cm from the carotid bifurcation. The determination will be made by two observers to estimate the inter-observer correlation. Place: Department of Imaging, CMN "November 20", ISSSTE.

The determination of these variables of cardiovascular risk modification will be determined basally and periodically at 3, 6 and 9 months after bariatric surgery, in all groups.

Definition of the processing plan and presentation of the information
Data analysis will be done through descriptive statistics applied to demographic variables, and inferential statistics, which will include difference of averages, correlation, strength of association and relative risk, as well as multivariate logistic regression analysis to evaluate independent associations. With the variables most associated with the modification of cardiovascular risk, a metabolic profile will be established as a predictive model of the greatest cardiovascular benefit after bariatric surgery. Statistical significance will be considered with $p < 0.05$

ETHICAL CONSIDERATIONS

This study is considered as minimal risk. Bariatric surgery (this procedure is not a proposal of the present protocol, it is routinely done as a treatment for morbid obesity in the CMN "20 de Noviembre", ISSSTE and would also be done if the patient does not participate in the study) will be performed according to the routine procedures of the Department of Bariatric Surgery. The project complies with the guidelines and recommendation of the Declaration of Helsinki. The protocol will be submitted to the Ethics and Institutional Research Committee. All patients will be asked to read and sign informed consent.

BIOSECURITY CONSIDERATIONS.

Bariatric surgery procedures are not part of this investigation. In any case, it is emphasized that this project does not alter the surgical procedure and routine biosecurity measures, which are based on the best clinical practice and international recommendations (Neff KJ, le Roux CW, Bariatric surgery: a best practice article. Pathol 2013; 66: 90-8). Sampling during surgery will be done in accordance with the Regulation of the General Health Law on Health Research, respecting aspects of taking the appropriate measures to avoid any risk or damage to research subjects (includes adequate preoperative assessment). , biosecurity measures typical of an operating room and sampling by an experienced surgeon). In any case, the privacy of the individual subject of the investigation will be protected, and it will be sought to limit the likelihood that the subject will suffer any harm as a result of the study.

The biological samples acquired will be properly identified, contained and stored for a maximum period of 1 year. Your analysis and determinations will be made according to good laboratory practices. Subsequently, they will be treated and disposed of in appropriate bags for disposal. To comply with the provisions of section 6 of NOM-087-ECOL-SSA1-2002 Biological-Infectious Waste.

The research is considered to be of minimal risk, since it is a common procedure with obtaining a minimum sample of visceral and subcutaneous adipose tissue. Likewise, the principal investigator will suspend the investigation upon noticing any risk or damage to the health of the subject in whom the investigation is carried out. All participants will read, understand and sign an informed consent.

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