MALABSORPTION AND GUT MICROBIOTA PROFILE CHANGES AFTER LAPAROSCOPIC MINI-GASTRIC BYPASS VS ROUX-EN-Y GASTRIC BYPASS:

A PROSPECTIVE MULTICENTER COMPARATIVE STUDY

23/01/2018

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Background:

Bariatric surgery is the best choice to treat morbid obesity and its severe co-morbidities (1, 2, 3).In 1997, Robert Rutledge proposed a new procedure, the mini gastric bypass (MGB), a variation of the Roux-en-Y gastric bypass (RYGB) with a single anastomosis (3). MGB seems to provide similar results as concern weight loss and control of metabolic co-morbidities while presenting the benefit of being more easily laparoscopic performed and revisable (4, 5).However, this operation is still controversial because of biliary reflux and possibly increasing the risk of esophageal cancer in absence of validated steps of the procedure (volume of the gastric pouch, type gastro-jejunal anastomosis, distance from Treitz) (6).

The biliopancreatic limb length may explain the results of superior excess weight reduction as well as the higher incidence of hemoglobin deficiency after 5 year follow-up comparing MGB vs RYGB (7). This malabsorption could results in severe malnutrition defined as a %EBMIL > 100 % and an albumin level < 30 g/l (8).

In 2005 Rutledge observe malnutrition in 31/2,401 patients (5), Noun et al. reported 4 casesof malnutrition out of 1,000 patients of MGB (9), Lee et al.

(10) reported a revision rate of 1.7% at 9 years follow-up in their series of 1,322 patients andthemost common cause of revision was malnutrition in nine cases. In addition, Wang et al. (11) operated 423 MGB and reported anemia as a late complication in 28% of patients, and most cases were microcytic, the reason for the high incidence was reported to be likely due to the duodenal bypass with malabsorption of iron. However all data published on the mal-absorptive effects of MGB are retrospective so, until now, there is no prospective comparative study to assess the malaborbitive efficacy of MGB vs RYGB. Recently an experimental study carried out in a rat model evaluated the malabsorption of MGB versus RYGB suggesting a higher protein malabsorption induced by MGB (not compensated by the intestinal adaptation) then RYGB (12).

Recent works have indicated that the gut microbiota may mediate some of the beneficial effects of bariatric surgery, and changes in the composition and diversity of the gut microbiota have been observed in the short and long term after RYGB in humans (13, 14), as well as after vertical sleeve gastrectomy and RYGB in mice (15). However, there are no investigations of these changes after MGB.

AIM OF WORK:

To evaluate in patient undergoing MGB or RYGB the effect of the treatment on enteric mal-absorption and microbiota profile in the first year after surgery.

Primary end point:

-To evaluate and compare RYGB *vs* MGB bile acids levels and microbiota profile shift at 6 and 12 months after surgery and its impact on metabolic and nutritional status after surgery.

Secondary end point:

-To measure GLP-1 plasma level (pg/mL) before and 12 months after surgery in MGB vs RYGB patients.

Other parameters:

1. To evaluate at 0 – 6 and 12 months after surgery the enteric mal-absorption profile in terms of lipid (Triglycerides, cholesterol, HDL and LDL), proteins (hemoglobin, albumin and ferritin), carbohydrates and micronutrients (minerals and vitamins) and nutritional status in subjects undergoing MGB *vs* RYGB.

2. To compare the effect of RYGB vs MGB surgery on total weight loss percent, excess weight loss percent (%EWL), excess BMI loss percent, major co-morbidities control and quality of life.

3. To evaluate the endoscopic /histological esophageal mucosal lesions secondary to bile reflux at 12 months in MGB vs RYGB patients

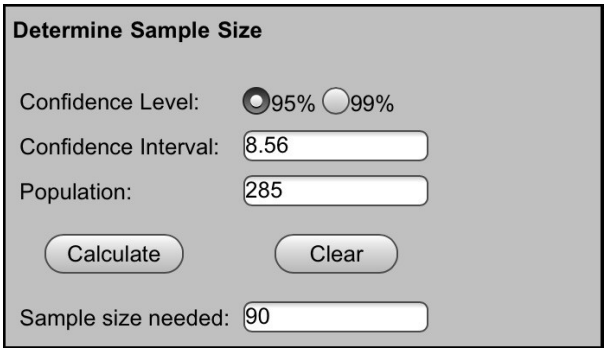
4. to evaluate energy expenditure at 0 and 12 months by Indirect calorimetry

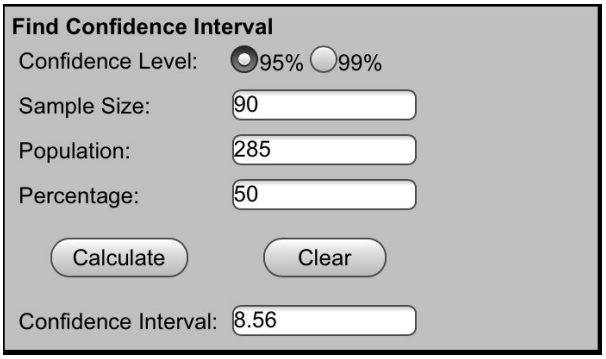
Type of study:

Multicentric Prospective pilot comparative study.

Patients:

We had to evaluate Sample Size using bariatric patients population who underwent MGB or RYGB during 12 months (the follow up period of this study) in the three centres involved in the study, because of there aren't others high methodological level studies or randomized trials or methnalysis about this argument (Primary end points and secondary and points). Our population is 285 patients: to obtain a confidence level of 95 %, an effect size of 0.5, Sample size is 90 patients (45 MGB and 45 RYGB) with an interval confidence of 8.56.





Tab 1

Tab 2

The sample size will be 90 cases (45 MGB and 45 RYGB) considering at least 20% drop-out at 1 year. (Tab 1-2)

Inclusion Criteria:

Obese patients with age 18-65 years old, BMI 40-55 kg/m ^{2}, non smokers, and candidates to primary MGB or RYGB without any concomitant surgeries except

hiatal hernia repair. Patients will be informed on the differences of the two procedures and the enrollment in the two study groups will be on the basis of patient choice.

Exclusion Criteria:

- Age ≤ 18 or ≥ 65 years old,
- BMI≤ 40 or ≥55
- Smokers
- Any required associated surgical procedure except hiatal hernia repair
- Conversion to open surgery
- Postoperative complications requiring reoperation
- No compliance to the follow up schedule.
- Histological positivity to Helicobacter pylori previous or current
- Patients with different intraoperative measurement of the bowel distal to Treitz Ligament (tolerance plus or minus 10%).
- Free PPI 4 weeks before 6^{th} month (after surgery)
- Corticosteroids, vitamin E, fish oil treatment in the previous two months
- Anti or pre- biotics treatment in the previous 2 months
- Chronic gastrointestinal diseases or syndromes (e.g., IBD, IBS)
- Previous bariatric surgery (intragastric balloon excluded).
- Previous resective bowel surgery

- Previous pancreatic surgery
- Previous hepato bilio pancreatic surgery
- Gallbladder gallstones

Methods:

Pre-and post-operative Assessment:

All patients will sign an informed consent (Sicob guidelines 2016). Study protocol will be approved by the Ethical Committee.

All patients will undergo routine blood laboratory investigations before surgery, 6 months and 12 months after surgery including: serum hemoglobin, glucose, HbA1c, cholesterol, triglycerides, LDL, HDL, T3, T4, TSH, GLP-1. Assessment of the nutritional status will be performed before 6- and 12 months after bariatric surgery by blood testing for serum iron, calcium, zinc and vitamins A, D, B12 and cortisol level.

A standardized meal test will be performed before, 6 and 12 months after bariatric surgery with 250 ml of liquid meal Oxepa®, (375 calories, 55.5% fat,

28% carbohydrate, 16.5% protein). Blood samples will be collected at baseline, 30, 60, 90 and 120 minutes after meal test in order to assess the concentration of the circulating lipids, GLP-1, bile acids (C4 precursor of bile acids) and FGF 19 (Fibroblast Growth Factor 19) levels. A stool sample will be collected at 0 – 6 – 12 months (It' s necessary to indicate date and time of defecation) stored at -80°C for targeted gut microbiota metagenomic (MG) study, lipids and proteins levels assessment. Every sample will be made by two parts: one for metagenomics evaluation and the other one for lipids and proteins levels assessment. Before fecal sampling, patients will be asked to fill out a three days food diary. All patients with vitamin D deficiency (< 20 ng/mL) will be supplemented before surgery with 10,000 IU of Vitamin $_3$Ddaily (16). Every value of nutritional status shoud be annotated in Winfood software. The selection criteria, work up, informed consent and schedule of the multidisciplinary follow up will follow the Guidelines of Italian Society for Obesity Surgery (SICOB) adopted in September 2016 (www.sicob.org).

Intra-operatively:

Standardization of the techniques will be guaranteed:

1. RYGB group
2. MGB group

All patients will have intraoperative measurement of the whole length of bowel from Treitz ligament to the ileocecal junction (expected range 6-8 m).The common limb will be therefore about 2/3 of total small bowel length.

Roux en Y gastric bypass

The steps of the standard double loop RYGB technique will be followed (17). The gastric pouch will be created 7 cm from the gastro-esophageal junction to obtain a volume of 30-40 ml, and the length of the alimentary limb will be 150 cm and 3.5-4 cm gastro-jejunostomy will be performed by linear stapler. The length of the biliopancreatic limb will be from 65 to 75 cm beyond the ligament of Treitz. The lengths of both limbs should carefully measured with a graduated instrument. The mesenteric defects will be closed.

Mini-gastric Bypass

The gastric pouch will be performed starting below the incisura angularis (transverse resection 4 cm) on the lesser curvature (18).Then the stomach will be transected against a 36 Fr bougie up to the gastro-esophageal junction Then 1/3 of the small bowel will be excluded (approximately 200cms) and 3.5-4 cm gastro-jejunostomy will be performed by linear stapler.

Postoperative diet

After surgery and under the supervision of the registered dietitian, the diet will be transitioned from liquid diet for 2 weeks to pureed proteic diet for 1 week then soft solid proteic diet for 2 weeks then maintenance (regular food). All patients should have daily vitamin and mineral supplementation up one year after surgery following the chart below:

Vitamin A	0,8 mg
Vitamin B	11,65 mg
Vitamin B	22,1 mg
Vitamin B	324 mg
Vitamin B	62,1 mg
Vitamin B12	0,00375 mg
Vitamin C	120 mg
Vitamin D3	0,0075 mg
Vitamin E	12 mg
Vitamin K1	0,0375 mg
Folic Acid	0,2 mg
Pantothenic Acid	9 mg
Iron	21 mg
Magnesium	187,5 mg
Selenium	0,055 mg
Zinc	10 mg

Diet progression after bariatric surgery

1. Liquid Proteic Diet (for 2 weeks)

Types of food	Calories/day
Reduced fat milk Lactose-free: 200ml	Protein: 52.32g (31.29%)

Fruit juice: 200ml Light white yogurt: 125ml Vegetable clear broth: 400ml Oral protein supplementation: 400ml	Fat: 14.52g (19.54%) Carbohydrate: 86.6g (51.58%) Kcal: 669

2. Pureed Proteic Diet (for 1 week)

Types of food	Calories/day
Reduced fat milk Lactose-free: 200ml Light white yogurt: 125ml Vegetable clear broth: 400ml Oral protein supplementation: 400ml Pureed lean beef, chicken or turkey, fish (or baby food meats): 80g Pureed fruits (or baby food fruit): 100g Soft cheese: 50g Extra virgin oil: 10g	Protein: 62.4g (25.69%) Fat: 43.26g (40.07%) Carbohydrate: 85.52g (35.21%) Kcal: 971.65

3. Soft Solid Proteic Diet (for 2 weeks)

Types of food	Calories/day
Reduced fat milk Lactose-free: 200ml Light white yogurt: 125ml Oral protein supplementation: 400ml Chopped lean meats (or Lean ground beef or turkey, or Deli-sliced turkey, breast, chicken, ham): 50g Tuna fish, salmon or white fish: 50g Pureed fruits (or baby food fruit) or canned fruits: 100g Mashed or well-cooked vegetables: 100g Extra virgin oil: 10g	Protein: 74.4g (30.41%) Fat: 32.66g (30.04%) Carbohydrate: 99.4g (40.60%) Kcal: 978.65

4. Manteinance (Regular Food)

Types of food	Calories/day

Reduced fat milk Lactose-free: 200ml Biscuits: 30g Oral protein supplementation: 200ml Light white yogurt: 125ml Lean red meat (or tender chicken, turkey and beef: 70g Fish: 70g Raw fruits without skins: 100g Raw and cooked vegetables: 100g Toasted breads or Low fat crackers: 30g Extra virgin oil: 20g	Protein 78.9gm (27.69%) Fat 42.24gm (33.35%) Carbohydrate: 116.6 gm (40.9%) Kcal 1116.64

Postoperative follow up:

Total weight loss percent, excess weight loss percent (%EWL), excess BMI loss percent, co-morbidities control and quality of life, evaluated by The TSD-OC (SIO obesity-related disability) test (19). It is a questionnaire developed by the Italian Society of Obesity (SIO); it is made of 36 items divided into 7 sections: pain, rigidity, activities of daily living, housework, outdoor activities, occupational activities, and social life. Each question is answered using a visual

analogue scale, with a score ranging from 0 (absence of difficulty) to 10 (the highest degree of disability) for each item.

Data recording and variables analysis:

Basic demographic data are recorded as well as detailed information on history, preoperative data, surgical procedure, intra-operative findings, and postoperative course. The statistical analysis will be performed on the pooled groups. All statistical analyses will be performed using IBM SPSS v.20 software. P value of less than 0.05 will be considered statistically significant.

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