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KINETICS OF TNF-α AND IL-6 IN OBESE PATIENTS AFTER BARIATRIC SURGERY

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ABSTRACT

Changing diet and lifestyle has led to an impressive increase in the incidence of obesity in recent decades. Obesity in humans is a serious health issue per se, but also through the association with related disorders, e.g., type II diabetes, hypertension and metabolic disturbances. At the same time it is associated with increased levels of inflammatory cytokines secreted by adipocytes who are responsible for inducing inflammatory status. The aim of our study was to assess the of kinetics of plasma levels of pro-inflammatory cytokines (IL-6 and TNF-α) and some seric markers of the metabolic syndrome (total cholesterol, triglycerides, glycaemia) in patients with malignant obesity, before and after laparoscopic sleeve gastrectomy. The results of our study confirm the benefic role of bariatric surgery. The technique may improve the chronic disease and the state of inflammation associated with obesity.

KEY WORDS: *pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), morbid obesity, bariatric surgery.*

INTRODUCTION

In today's world, obesity became a pandemic. The World Health Organization estimates that in the next 20 years obesity will become the first morbidity worldwide (WHO). Between 1960-2000, the prevalence of obesity has doubled, increasing from 13,3% to 30,9%. The prevalence of malignant obesity (BMI≥40), has increased during the last decade of the XX^{th} century from 2.9% to 4,7% (Rojano-Rodriguez, 2013). The adipose tissue secrets pro-inflammatory adipokines witch associates obesity with systemic and chronic low-grade inflammation state (Vilarrasa, 2007). Out of the adipokines, tumor necrosis factor-α (TNF-α) and interleukine-6 (IL-6) have a pro-inflammatory effect opposite to adiponectin witch has an anti-inflammatory, antidiabetic, cardioprotective and antitumoral effect (Divella, 2016).

Currently, bariatric surgery is considered to be the most effective treatment in obesity (Frikke-Schmidt, 2017).

Our study sought to evaluate the serum levels of TNF-α and IL-6 in patients with malignant obesity before and after longitudinal sleeve gastrectomy. A second goal was the post surgery assessment of possible correlations between kinetics of pro-inflammatory cytokines and that of other metabolic factors (total cholesterol, triglycerides, glycaemia), markers of comorbidities present in the studied subjects (cardiovascular diseases, type 2 diabetes).

MATERIALS AND METHODS

The study included 35 patients (mean age 40 years) with morbid obesity. The criterion for the patients' eligibility for the study was a BMI of >40 kg/m2 and a lack of symptoms associated with acute or chronic inflammation.

Morbidly obese patients admitted to the Surgery Clinic II of the County Hospital Timisoara for bariatric surgery were considered. Obese patients with associated pathology were selected: 15 cases with hypertension, 10 with type 2 diabetes and 10 with hypercholesterolemia. In parallel, biological samples were collected from obesity-free patients who were operated in the clinic for appendicitis and diverticulitis. All patients provided a written consent before the study. Also the study was approved by the ethics committee of Victor Babes University of Medicine and Pharmacy.

Blood samples were collected in tubes with EDTA as anticoagulant. The data was collected at two time points: before treatment (baseline) and six month after surgery. The samples were mixed and centrifuged immediately at 3000g at 4 °C for 20 min. After centrifugation, the samples were stored at −80 °C until they were used. IL-6 and TNF-α concentrations were assessed by enzyme-linked immuno sorbent assay (ELISA).

The obtained values were statistically analyzed using the student test. Values were expressed using mean and standard deviation (mean ± std). We considered statistically significant a p value of less than 0.05.

RESULTS AND DISCUSSIONS

By comparing serum levels of IL-6 and TNF-α between the control group and obese patients with associated comorbidities, we showed significantly higher seric values of the two markers compared to the control group. The IL-6 values for the control group were 2,156 ± 0,307 pg / ml, and in obese patients we recorded the following levels: hypertension (5.04 ± 0.34 pg / ml), type 2 diabetes (5.73 ± 0.465 pg / ml) and hypercholesterolemia (4.421 ± 0.481pg / ml) (fig. 1).

Regarding the expression of TNF-α, we showed significantly increased serum values in obese patients with comorbidities (hypertension - 28,663 ± 0,808 pg / ml, type 2 diabetes - 36,379 ± 1,213 pg / ml, hypercholesterolemia - 21,48 ± 1,34 pg / Ml) compared to the control group (4.549 ± 0.420 μg / ml) (fig. 2).

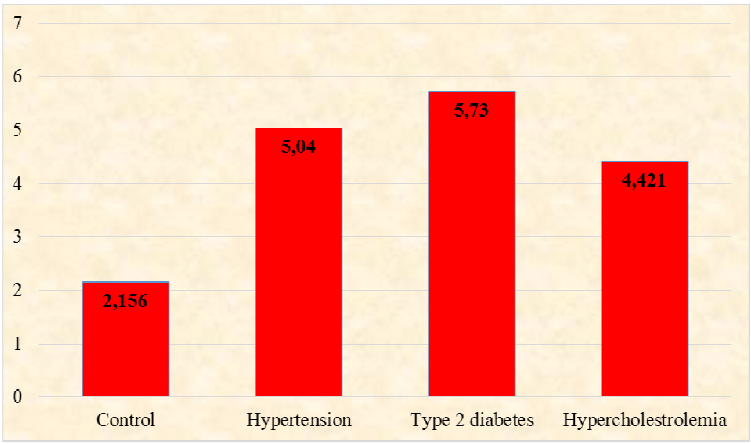

FIGURE 1. IL-6 serum levels (pg/ml)

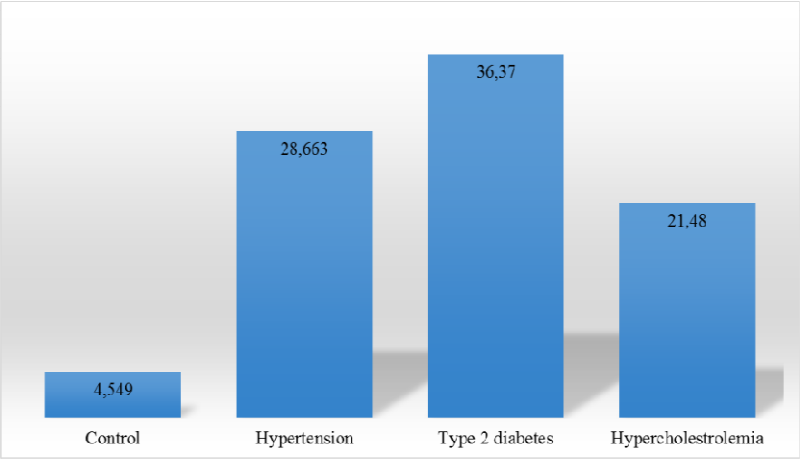

FIGURE 2. TNF-α serum levels (pg/ml)

TABLE 1. Serum values of IL-6 before (A) and after (B) bariatric surgery (mean±std)

Pathology (n=number of patients)	IL6 serum value (pg/ml)		p
HYPERTENSION n=15	A	5,04±0,34	≤ 0,001
	B	4,147±0,682	
HYPERCHOLESTEROLAEMIA n=10	A	4,421±0,481	0,06
	B	3,802±1,127	
TYPE 2 DIABETES n=10	A	5,73±0,465	≤ 0,001
	B	4,135±0,306	

Comparative analysis of serum levels of IL6 in patients with morbid obesity before and after bariatric surgery revealed the decrease in cytokine levels in all cases studied. Statistically significant values were observed in patients with associated hypertension and type 2 diabetes (Table 1). Analysis of TNF-α expression revealed statistically significant differences between serum values of obese patients before and after surgery for all three associated pathologies (Table 2).

TABLE 2. Serum values of TNF-α before (A) and after (B) bariatric surgery (mean±std)

Pathology (n=number of patients)	TNF-α serum value(pg/ml)		p
HYPERTENSION n=15	A	28,663±0,808	≤ 0,001
	B	21,159± 1,122	
HYPERCHOLESTEROLAEMIA n=10	A	21,48± 1,34	0,0007
	B	18,388±2,12	
TYPE 2 DIABETES n=10	A	36,379±1,213	0,0001
	B	31,341±4,45	

The development of obesity and associated metabolic disorder is closely related to the increase in levels of pro-inflammatory cytokines such as IL-1, IL-6, TNF-α. Multiple studies report that their plasma concentration is correlated with the amount and distribution of fatty tissue, inducing the synthesis of acute phase proteins (CRP) and inflammatory status. Thus, most studies describe increased serum levels of IL-6 and TNF-α before surgery and decreased levels to varying degrees after bariatric surgery (Popko *et.al*, 2010; Fain, 2006; Viana *et al*., 2013; Kardassi *et al*., 2014). However, there are studies that report unchanged levels of proinflammatory cytokines after surgery (Catalan *et al*., 2007) or even post surgery increases in TNF-α (Appachi *et al*., 2011).

The literature is almost unanimous in appreciating that after bariatric surgery more or less significant decreases in serum levels of IL-6 and TNF-α can be observed, which improves the inflammatory status of patients with malignant obesity.

Numerous studies looked for differences between surgical methods and post-surgery kinetics of pro-inflammatory cytokines. The conclusion of most of the studies is that there are no significant differences between the kinetics of inflammatory markers based on the type of surgery: Roux-en-Y gastric bypass, laparoscopic adjustable gastric banding and laparoscopic sleeve gastrectomy; all three surgical treatment modalities lead to a significant decrease in BMI, blood pressure and inflammatory serum markers at 3.6 and 12 months after the intervention (Viana *et al,* 2013). All three surgical treatment methods determine a significant decrease of BMI, blood pressure and inflammatory seric markers at 3,6 and 12 months after the intervention (Viana *et al*, 2013; Fenske *et al.,* 2013).

A meta-analysis study reported a 27% decrease in plasma levels of IL-6 after bariatric surgery, but argued that post surgery changes in TNF-α levels were not significant (Rao, 2012). Another study reports unmodified levels of IL-6 and TNF-α after surgery and suggests that the postoperative decrease in CRP levels occurs without the intervention of IL-6 and TNF-α, and the decrease in CRP could be interpreted as a marker of decrease in cardio- vascular risk in

obese patients (Laimer *et al*., 2002). Supporting this hypothesis comes another study on patients with morbid obesity that are subjected to hypocaloric diet and balneal treatment. The study concludes that decreasing CRP in the absence of TNF-α and IL-6 changes does not improve the function of adipocytes and only the removal of a large amount of fat will result in this (Rosc *et al*., 2015).

In the same context, a comparative study of inflammatory markers performed before and after bariatric surgery reports post-surgery significant decreases in IL-6 in subcutaneous adipose tissue (SAT) but not in visceral tissue (VAT). In terms of TNF-α, expression levels have a weak tendency to decrease and this only in SAT; the conclusion of the study is that only CRP can be considered a reliable marker in improving post-surgery inflammatory status, not IL-6 or TNF-α (Pardina *et al.,* 2012).

Adipose tissue is the major storage site for cholesterol and triglycerides. This explains why the increase in adiposity is associated with dyslipidemia. Inflammation increases the synthesis and release of adipokins, thus contributing to the development of adiposities and their metabolic consequences (Bays *et al.,* 2014). Adiposities include, among other things, the elevation of serum levels of TNF-α and IL-6, which in most studies is associated with increased risk of developing cardiovascular disease (Heneghan *et al*., 2011). Studies are unanimous in assessing the effects of bariatric surgery on the lipid profile in subjects with malignant obesity; significant decrease in serum levels of TG, total cholesterol and elevated levels of HDL cholesterol are reported (Bays *et al.,* 2014; Shuai *et al*., 2015; Jacobson *et al*., 2014). Improving the postoperative lipid profile occurs in parallel with the decrease in TGF-α and IL-6 levels (Appachi & Kashyap, 2013; Pardina *et al.,* 2012; Miller *et al.,* 2011; Julve *et al.,* 2014).

Insulin-resistance (IR) is an important factor in the pathogenesis of type 2 diabetes. Cytokines released by adipose tissue are involved in initiating and promoting pro-inflammatory status, contributing to the onset of IR (Timar *et al.,* 2014). TNF-α mediates apoptosis, IR, lipolysis, inhibition of insulin-stimulated glucose transport and inhibition of insulin receptor autophosphorylation (Bluher, 2009; Cildir *et al.,* 2013).

A study conducted on plasma levels of CRP in obese patients shows that before surgery there is a significant linear relationship of pro-inflammatory citokines with body weight and BMI; at 6 months after surgery they noted a significant decrease in proinflammatory cytokine levels, weight and glycemic levels (Rojano-Rodriguez *et al*., 2014). The authors speculate that lowering levels of pro-inflammatory cytokines could be a marker of lowering incidence and reduced risk of cardiovascular disease in the obese.

The metabolic role of IL-6 remains controversial because multiple functions are attributed to it, including specific tissue effects in glucose metabolism and insulin-signaling (Piya *et al.*, 2013). Several studies report elevated plasma levels of IL-6 positively associated with metabolic syndrome, IR and diabetes (Ferreira-Hermosillo *et al.,* 2015; Sindhu *et al.,* 2015). Solá believes that obesity correlates with inflammatory status due to increased cytokine synthesis, but that the inflammatory status does not correlate with metabolic syndrome (Solá *et al*., 2009).

Another study correlates obesity, inflammatory markers and type 2 diabetes with visceral adipocyte diameter; the findings show elevated levels of TNF-α in non-diabetic obese versus control, elevated IL-6 in both groups (diabetic and non-diabetic obese) compared to the control, higher adipocyte diameter in diabetic obese than in non-diabetic subjects and a

significantly positive correlation between adipocyte size and inflammatory markers (TNF-α and IL-6) (Bahceci *et al*., 2007).

It is recognized today that adipokines synthesized by fatty tissue act as endocrine and paracrine messengers to regulate appetite / satiety balance, fatty tissue distribution, inflammation, blood pressure, homeostasis and endothelial function (Bluher, 2014; Lehr *et al.*, 2012; van de Voorde *et al.,* 2013). In this context, elevated serum levels of TNF-α and IL-6 in the obese patients are markers of a chronic inflammatory status that increase the risk of developing diabetes and atherosclerosis.

In a study of obese patients with renal dysfunction, a significant improvement in blood pressure, glycemic levels, dyslipidemia and inflammatory status is reported 6 months after surgery; the authors assume that inflammation is the key to the hormone regulation mechanism involved in appetite / satiety balance and that bariatric surgery improves the regulation of inflammatory response (Neff *et al.,* 2013).

Another prospective survey, performed 12 months after bariatric surgery, concludes that weight loss favored by the surgical act is associated with decreasing blood pressure levels and improved inflammatory status (Fenske *et al.,* 2013). Rubin associates obesity with increased inflammatory status due to increased levels of IL-6, TNF-α and blood pressure, triglycerides and HDL cholesterol as associated risk factors for atherosclerosis (Rubin *et al*., 2010).

Regarding the correlation between obesity, serum levels of inflammatory markers and the age of subjects, the literature is unanimous in assessing the presence of inflammatory status in obese subjects, regardless of age. Thus, several studies report increased levels of TNF-α and IL-6 in obese children. Moreover, the authors have found positive correlations of inflammatory markers with BMI, triglycerides and blood pressure suggesting that considered together, all of these factors contribute to the onset of obesity in children (Shin *et al*., 2008, Yan *et al.,* 2009). A research on adolescents with severe obesity undergoing surgery, suggests that bariatric surgery significantly decreases serum levels of IL-6 and TNF-α and estimates that this will lead to a major decrease in risk factors for the development of type 2 diabetes, blood pressure and cardiovascular diseases (Kelly *et al.,* 2016).

CONCLUSIONS

Bariatric surgery represents currently the only effective method of treatment in malignant obesity. It determines body mass decrease, reduces the inflammatory status and glycaemia, improves the lipidic profile and blood pressure. Regardless of the surgical technique, the main advantage is that it reduces the risk of occurrence and progression of some serious comorbidities like type 2 diabetes, dyslipidemia, hypertension, atherosclerosis and other cardiovascular disease.

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