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Impact of sleeve operation on C-peptide in more obese patients

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Abstract: ***Aim:*** The main aim of our education is to assess the effect of sleeve operation for more obese patients (BMI > 35 kg/m^2) on C-peptide in serum pre- and post (6 months) laparoscopic sleeve gastrectomysurgery (LSG). ***Methods:*** Fifty obese patients (BMI > 35 kg/m^2) admitted to Gastrointestinal surgery center at Mansoura university in Egypt and twenty persons (as control group) were enrolled in this prospective study. The patients were submitted to LSG and were monitored for weight loss (BMI change) and improvement; Lipid profile, liver enzymes and C-peptide levels at 6 months follow-up post-operation. ***Results:*** There was a highly significant difference between BMI before and after operation. Also, a significant decline in C-peptide levels compared to preoperative levels (P<0.001). ***Conclusion:*** LSG is an effective surgical procedure to achieve a significant weight loss and control of C-peptide levels, which reflects a good healthy life for obese patients.

keywords: Obesity, BMI, C-peptide and LSG.

1.Introduction

Obesity (a person with a body mass index [BMI ≥ 35 kg/m^2) is a health issue in the world. It is associated with increased risk of diabetes mellitus **[1].** The incidence of obesity keeps growing, and by the year 2025 if the current trend continues around 40% of the US population will be obese **[2].** Obesity is measured by body mass index (BMI) **[3].** Obesity is considered the main risk factor for type-2 diabetes mellitus (DM) and a number of human cancers **[4].** Bariatric surgery including LSG has proven to be successful in treating not just obesity but also obesity-related diseases **[5].** Complication and mortality rates after Laparoscopic sleeve gastrectomy (LSG) appear to be lower in comparison to other forms of bariatric surgery **[6].** The LSG is currently the most frequently performed procedure in the United States and it gained worldwide popularity as a standalone procedure among the in the armamentarium of the all bariatric surgeon **[7].** In Recent years, using of LSG operations for obesity has been increased **[8].** During LSG approach, a tubular gastric pouch was created by inserting a bougie along the lesser curvature of the stomach **[9].** The gastric pouch size usually varies from 60 to 120 ml, but we can create different volumes of the SG when the size of the bougie is changing **[10].** The prospective advantages of the LSG are that it does not require placement of an external body, confers immediate restriction of caloric intake, and can be done in less time than needed for bypass procedures **[11].** The passage of the food through the pouch into the distal stomach and the rest of the gut are mechanically delayed, leading to reduction of energy intake and finally weight loss **[12].** LSG led to long-term weight loss **[13].** Weight loss after bariatric surgeries is related to restrictions of nutrient intake and hormonal modifications **[14]** Suggested mechanisms of LSG involve alterations in gastric emptying **[15]** and changes in serum ghrelin (satiety hormones) resulting in increased satiety **[16].** C-peptide is a short 31 amino-acid polypeptide peptide which links the

insulin's alpha-chain to its beta-chain in the proinsulin molecule **[17].** The central glycine in C-peptide allows the correct positioning of the alpha and beta chains for insulin to obtain its tertiary structure **[18]** C-peptide is an enzymatic cleavage product that arises from proinsulin from pancreatic beta cells which is produced in equimolar amounts to endogenous insulin **[19].** Measuring C-peptide levels is a useful indicator of beta cell function. There is substantial evidence that C-peptide plays a putative role in the development of microvascular abnormalities associated with diabetes **[20].** Patients with T2DM, their body makes insulin but doesn't use it well which can cause higher C-peptide levels than normal **[21].** LSG is performed for obesity and obesity-associated T2DM leading to decrease of high levels of C-peptide **[22].**

Materials and methods:

Sample collections

This cohort study is performed on 70 samples: 20 samples out of 70 are control group and 50 morbidly obese patients were admitted to Gastrointestinal surgery center at Mansoura university in Egypt to LSG operation. The patients were followed up for 6 months.

Data collection

Data were collected prospectively and entered into a database. All patients were evaluated with routine laboratory investigations in the form of complete blood count ALT and AST, lipid profile, BMI, etc…

Sample collection and storage

Serum: Use a serum separator tube and let samples to clot for 2 hours at RT or overnight at 4^{o}C before centrifugation for 20 minutes at approximately 1,000×g. Afterwards, save samples in aliquot at -20^{o}C to use it later.

Quantitative in vitro determination of high density lipoprotein cholesterol (HDL-C) in human serum or plasma on photometric systems

Procedure:

Bring samples and reagents to RT. The blank tube contains 1000 µL from reagent 1 while the sample tube contains the same plus 10 µL from the sample. Incubate at 37°C for 5 min. And then read absorbance (A1).then from the reagent 2 to the tubes. Mix, incubate at 37°C for 5 min. And then read absorbance (A2).

= [(A2-A1) sample or calibrator] - [(A2-A1) blank]

Quantitative determination of total cholesterol in human serum Or plasma

Procedure:

Bring the Reagent to RT. Pipette into labeled test tubes:the blank sample contain 1.00 ml of the reagent while the slandered contains 1.00 ml reagent added to 10 µL slandered solution. The last tube contains 1.00 reagent and 10 µL sample

Incubate the tubes for 5 minutes at 37ºC or ten minutes at RT (16-25ºC). Measure the absorbance (A) at 546 nm of the Sample and Standard against the Blank. The color is stable for at least 2 hours.

Quantitative determination of triglycerides in human serum and plasma

Procedure:

Bring the Reagent to RT. Pipette into labeled test tubes:the blank sample contain 1.00 ml of the reagent while the slandered contains 1.00 ml reagent added to 10 µL slandered solution. The last tube contains 1.00 reagent and 10 µL sample

The tubes are incubated for 5 minutes at 37ºC or for 10 minutes at RT. Measure the absorbance (A) at 546 nm of the Sample and Standard against the Blank. The color is stable for at least 1 hour.

The Quantitative Determination of Circulating C-Peptide Concentrations in Human Serum.

Procedure:

Dispense 50µL of C-Peptide controls, standards, and specimens into the correct wells. Softly mix for ten seconds. Dispense 100µL of enzyme conjugate reagent into every well. Mix softly for 30 seconds. Incubate at RT for 60 minutes. Empty the content of the plate into a waste container to remove the incubation mixture. Rinse the microtiter plate with 1 x washing buffer for 4times and 1 times distilled water. To remove all residual water Droplets, strike the microtiter plate onto absorbent paper

roughly. Dispense 100µL of TMB substrate reagent into wvery well. Mix for 10 seconds softly. Incubate for 20 minutes in the dark at RT. Finally add 100µL of Stop Solution to stop the reaction to every well. Mix softly for 10 seconds till the blue color completely changes to yellow. Read the optical density within 15 minutes at 450nm with a microtiter plate Reader.

Statistical analysis

Descriptive results were expressed as mean ± SD (standard deviation) and range or number (percentage) of patients with a condition. The differences in continuous variables were assessed using student t-test or ANOVA and chi-square (X2) test for variable categories. The p-values were two-sided and statistical

Result and Discussion

Analysis of BMI in the cases group Pre –and post-Laparoscopic Sleeve Gastrectomy.

The result in **Table (1) .**Showed that the mean BMI decreased from 49.198 ±7.75 kg/m2 preoperatively to 42.921± 5.448 kg/m2 at the 6 month follow-up examinations

There was very high remarkable difference between cases group (preoperative) and

cases group (postoperative); P < 0.001. significance was considered when p < 0.05. All statistical analyses were performed using Statistical software package (SPSS 22.0 for Microsoft Windows, SPSS Inc.

Table (1): Analysis of BMI in the cases group before and after operation

	Groups		**P**
	Cases group (Preoperative) (N=50)	**Casesgroup (Postoperative) (N=50)**	
BMI(Kg/m^2)	49.198 ±7.75	42.921± 5.448	<0.001*

Table (2) shows that there is a remarkable difference between cases (preoperative) and cases (postoperative). Also, there is a significant decline in postoperative cases group in comparison to postoperative level with a high significant difference (P<0.001). As it decrease from preoperative level (4.93 + 2.72) to Postoperative level (2.83 + 1.15).

Table (2): Analysis of C-peptide in the cases group before and after operation.

Parameters	**Cases Groups (N=50)**		**P-value**
	(Preoperatie)	(Postoperativ)	
C-peptide (ng/ml) (Mean ± SD)	4.93 ± 2.72	2.83 ± 1.15	<0.001 *

Mean serum levels of lipid profile in the cases group pre- and post-laparoscopic sleeve gastrectomy.

Table (3) illustrates the Comparison between cases group (preoperative) and cases group (postoperative) regarding the lipid profile.

The blood cholesterol, triglycerides and LDL levels were highly significantly decreased at the six month follow-up examinations (P<0.001) post operation.

Total Cholesterol (mg/dl): There is a remarkable difference between cases (preoperative) and cases (postoperative). Also, it decreased in postoperative cases group comparing to preoperative.

Regarding triacylglycerol TGs (mg/dl): There is a remarkable difference between cases (preoperative) and cases (postoperative). Also, it decreased in postoperative cases group comparing to preoperative.

high-density lipoprotein (HDL) (mg/dl): There is an obvious difference between cases (preoperative) and cases (postoperative). HDL levels increased following surgery with a significant difference (P=0.019) between preoperative cases (36.55±7.02) and postoperative cases (40.198±7.795).

Parameters	**Cases groups**		P
	Pre-LSG (N=50)	Post-LSG (N=50)	
Cholesterol (mg/dl)	204.58 ± 37.08	144.56 ± 30.50	< 0.001*
TGs (mg/dl)	167.9±41.25	110.68± 34.63	< 0.001*
HDL (mg/dl)	36.55 ± 7.02	40.198 ± 7.795	0.019* 0.001*
LDL (mg/dl)	134.33 ± 34.7	82.77 ± 27.17	< 0.001*

low-density lipoprotein (LDL) (mg/dl): There is an obvious difference between cases (preoperative) and cases (postoperative). Also, it decreased in postoperative cases group comparing to the preoperative. LDL levels were

highly significantly decreased at 6 months (P<0.001).followingsurge

Analysis of glycemic profile in the cases group before and after operation

Table (4) illustrates the Comparison between cases group (preoperative) and cases group (postoperative) regarding to the glycemic profile; fasting blood glucose (FBG) and Glycated HB (HbA1c).

The changes in fasting blood glucose (FBG) in comparison to the mean preoperative levels were highly significant reduction (P<0.001), also HbA1c levels showed a significant fall at 6 months post-LSG (p=0.005).

FBG (mg/dL): There is a highly remarkable difference between cases (preoperative) and cases (postoperative). Also, there is highly significant decrease in postoperative (P<0.001) comparing to preoperative group. As the preoperative mean FBG was 98.58 ± 14.497 and the postoperative mean was 91.32 ± 10.425

Table (4): Analysis of glycemic profile in the cases group before and after operation

	Groups		P
	Cases group (Preoperative) (N=50)	Cases group(Postoperative) (N=50)	
FBS(mg/d)	98.58 ± 14.49	91.32 ± 10.425	< 0.001*
HbAIC(%)	6.7221.042	6.09 ± 0.628	0.005*

Table (5) illustrates the Comparison between cases group (preoperative) and cases group (postoperative) Liver function tests,

AST (IU/l): There is a remarkable difference between cases group (preoperative) and cases group (postoperative) (p<0.001). Also, it decreased in postoperative cases group comparing to preoperative.

ALT (IU/l): There is a remarkable difference between cases group (preoperative) and cases group (postoperative) (p< 0.0638). Also, it decreased in postoperative cases group comparing to preoperative.

ALB and Bil: There is no significant difference between cases (preoperative) and cases (postoperative). A slight increase has been recorded in cases group (postoperative) compared to cases group (preoperative)

Table (6) illustrates the Comparison between cases group (preoperative) and cases group (postoperative) RBC,WBC and Hemoglobin

Parameters	Groups		P value
	Pre-LSG group (N=50)	Post-LSG group (N=20)	
AST (U/l)	39.7 ± 23.87	32.48 ± 15.63	<0.001*
ALT (U/l)	32.16± 14.809	27.48 ± 9.607	0.0638
ALB(g/dl)	4.182 ± 0.3685	4.272 ± 0.3453	0.2106
Bil(mg/dl)	0.6028 ± 0.2235	0.6632 ± 0.1723	0.1333

RBC and WBC : There is no significant difference between cases (preoperative) and cases (postoperative). Hemoglobin(gm %)There is a highly remarkable difference between cases (preoperative) and cases (postoperative). Also, there is highly significant decrease in postoperative (P<0.001) comparing to preoperative group

Table (13) illustrates the Comparison between the control group and post-LSG group regarding to the glycemic profile; fasting blood glucose (FBG) and Glycated HB (HbA1c).

Parameter [a]	**Groups**			Test of significance
	Pre - LSG (N=5) (Mean ± SD)	Post - LSG (N=5) (Mea± SD)	Control group (N=20) (Mean ± SD)	
RBCs (x 10^6/ µl)	4.537 ± 0.446	3.899 ± 0.317	4.55 ± 0.377	0.111
Hemoglobin (gm%)	12.42 ± 1.145	11.39 ± 0.744	11.54 ± 2.54	<0.001*
WBCs	6.424 ± 1.347	6.173 ± 1.55	6.125 ± 1.414	0.717

Glucose monitoring profile

Regarding to FBG (mg/dL): There is no significant difference between control group and postoperative cases group. Also, there is no significant increase in postoperative (P=0.559) comparing to the control group. As the postoperative mean FBG was 91.32 ± 10.425and the control group mean was 89.80±6.72

Regarding to HbA1c (%): There is no significant difference between control group and postoperative cases group. Also, there is no significant increase in postoperative (P<0.392) comparing to the control group. As the postoperative mean HbA1c was 6.09 ±0.63 and the control group mean was 5.775 ± 0.5

Table (13): Comparison of the glycemic When we compare the lipid profile (CHO, TGs, HDL and LDL) values for two groups, we observed no significant difference was obtained between groups (P= 0.624, P= 0.805, P=0.799 and P=0.576) for CHO, TGs, HDL, and LDL. (table14). These results were reflected the values were close to each other in the post-LSG group and control one since it was (144.56 ± 30.50 vs 163 ± 19.74 mg/dl) for CHO, (110.68± 34.63 vs 107 ± 25.28 mg/dl) for TGs, (40.198 ± 7.795 vs 39.32 ± 2.74 mg/dl) for HDL and (82.77 ± 27.17 vs 102.32 ± 16.49 mg/dl) for LDL

profile between the control group and post-LSG group

Parameters	Groups		P
	Cases group (N=50)	Control group(N=20)	
FBS(mg/dl)	91.32±10 .425	89.8 ± 6.72	0.5590. 001*
HbAIC (%)	6.090.63	5.775 ± 0.51	0.392

Lipid monitoring profile

Table (14): Comparison of the lipid profile between the control group and Post-LSG group.

Parameter	Groups		Pvalue
	Post-LSG group (N=50)	Control group (N=20)	
Cholesterol(mg/dl)	144.56 ± 30.50	163 ± 19.74	0.624
TGs (mg/dl)	110.68± 34.63	107 ± 25.28	0.805
HDL (mg/dl)	40.198 ± 7.795	39.32 ±2.74	0.799
LDL (mg/dl)	82.77 ± 27.17	102.32±16.49	0.576

Table (15) illustrates the Comparison between the control group and post- LSG group regarding obesity related hormones C-peptide .

On the other hand, with regard to the c-peptide hormone, the results that we obtained through the study showed that the levels of this hormone decreased to the normal level (2.83 ±1.156 P =0.347) and very similar to the control group (2.33 ± 1.15) when compared together with no significant difference between two groups (P=**0.347**)

Table (15): Comparison of the serum levels of hormons between the control group and post-LSG group.

Parameter	Groups		P
	Post-LSG group (N=50)	Control group (N=20)	
C-peptide(ng/ml)	**2.83 ±1.156**	**2.33 ± 1.15**	**0.347**

Discussion

The laparoscopic sleeve gastrectomy is gaining importance as a single-stage procedure for treating morbid obesity, which was proven to be an effective option resulting in a remarkable weight loss [23]. The present study shows a significant decline in the BMI after surgery (P < 0.001), a result that agrees with previous findings conducted by Elbanna et al [24] who found a significant decrease in the BMI of patients before (53.8±8 with range 40–7

5 kg/m2) and after (47.34±4.4 with range, 37–56.7 kg/m2) 1 year of operation with a p-value less than 0.0001. Our results show also that cholesterol and triglycerides levels were highly significantly decreased at the 6 months follow-up examinations (P<0.001). Szczygielska et al [25] showed that the levels of triglycerides and total cholesterol were greater in obese persons in comparison with normal weight subjects. However, Ruiz-Tovar et al., [26] found that Laparoscopic sleeve gastrectomy was being explored as a viable surgical option for reduction of serum triglycerides and increase HDL, but with little effect on LDL and total cholesterol. Our results show further that HDL levels increased following surgery with a significant difference (P=0.019) between preoperative cases (36.55±7.02) and postoperative cases (40.198±7.795), while the LDL levels were highly significantly decreased at 6 months (P<0.001) following surgery. This result agrees with findings reported by Al Khalifa et al [27] who showed that all the parameters except for serum HDL level which showed an obvious reduction in obese persons.

LSG induces marked changes in the GI tract, which could be responsible for the altered hormonal responses [28, 29]. In the present work, we found that at follow-up after laparoscopic sleeve gastrectomy, the fasting levels of C-peptide were significantly declined in comparison to preoperation baseline levels (P<0.001). Our result agrees with that reported by Chen et al [30] who found that the decrease of Fasting C- Peptide may be included in the decline in free triiodothyronine and TSH after LSG. This finding agrees also with that of Lee et al [31] who found levels of C-peptide were a useful predictor of success of the treatment. Our finding disagrees with the result of Min et al [32] who showed a non-significant reduction in fasting C-peptide.When we compare between the control group and post-LSG group regarding to the glycemic profile; we found the changes in fasting blood glucose (FBG) and HbA1c in postoperative cases group comparing to the control group levels were no significant increase in postoperative (P<0.559).

As the postoperative mean FBG was 91.32 ± 10.425 and the control group mean was 89.8±6.72. As the postoperative mean HbA1c was 6.09 ± 0.63 and the control group mean was 5.775 ± 0.51. While a study conducted by Kimura et al.,[33] found that there was no significant difference in fasting plasma glucose (121.2±32.2 vs 116.6±25.0mg/dl), HbA1c (5.9±0.6 vs 6.4±1.9%), HDL-cholesterol (37.1±10.3 vs 39.0±9.0 mg/dl), LDL-cholesterol (111.1±38.1 vs 91.3±33.0mg/dl) between the obesity and non-obesity group un like our results.

Various studies have demonstrated improvements in lipid profiles following LSG. In our study when we compare between the control group and post- LSG group regarding total Cholesterol, TGs, HDL and LDL. We found that there is no significant difference between control group and postoperative cases group for total Cholesterol and LDL, as the cholesterol decreased in postoperative cases group (144.56 ± 30.50) compared to the control group (163 ± 19.74), LDL it decreased in postoperative cases group (82.77 ± 27.17) compared to the control group (102.32 ± 16.49).For TGs,there is no significant difference between control group and postoperative cases group (P=0.805). Also, it increased in postoperative cases group (110.68±34.63) comparing to the control group (107±25.28).

For HDL,there is a difference between control group and postoperative cases group but not significant HDL levels were increased in postoperative cases group (40.198±7.795) com paring to the control group (39.32± 2.74) but no significant difference (P=0.799). Regarding to C-peptie, there is an increase in postoperative cases group compared to control group but no significant difference between two groups (P=0.347).

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