The effect of laparoscopic greater curvature plication on peripheral blood lymphocyte subsets (CD4+, CD8+ T cells) in morbidly obese patients

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Abstract

Background: Bariatric surgery is the only and effective treatment for morbid obesity and it can also improve the obesity-related comorbidities. However, the effect of bariatric surgery on immune status is still unclear. In our study we investigated the relationship between surgical weight loss and peripheral blood lymphocyte percentages.

Methods: Morbidly obese patients (n=20, age range 25-50 years, body mass index [BMI] range (37-45kg/m^2) who had undergone laparoscopic greater curvature plication LGCP (were enrolled in a prospective study to determine the percentages of their peripheral blood T cells (CD4+, and CD8+ T cells) before and (4 months) postoperatively using flow cytometry. The data were expressed as the percentage of total lymphocytes±the standard error of the mean.

Results: A decrease in the BMI occurred at 4months postoperatively with loss of weight of (31.20±1.2%). Preoperative BMI was 44.71±4.3 (range 37-45kg/m^2) and postoperative BMI was 31.80±1.1(range 24-33kg /m^2). The mean percentage of CD4+ T lymphocytes preoperatively was 38.2±1.5 and postoperatively was 29.3±2.6 p <0.05 (which is statistically significant) so there is postoperative decrease in the percentage of CD4+T lymphocytes, the mean percentage of CD8+ T lymphocytes preoperatively was 17.3±1.8 and postoperatively was 9.5±1.7p<0.05 (which is statistically significant) so there is postoperative decrease in the percentage of CD8+ T lymphocytes.

Conclusion: This study found that weight loss after LGCP in morbidly obese patients showed attenuated activation of circulating immune cells (decrease in CD4+ T helper cells percentage and decrease in CD8+ killer T cells percentage) and more regulation of chronic inflammation in morbidly obese patients.

Key words: Bariatric surgery, LGCP, morbid obesity, immunity.

Introduction:

Obesity is a world wide health problem with several comorbidities including respiratory diseases, cardiovascular diseases, gall stones, osteoarthritis and reproductive disorders.[1] Furthermore, obesity is associated with decreased immunocompetence.[2] Several studies revealed increased incidence of infections and many types of cancers in obese individuals.[3,4]

Bariatric surgery is the only effective treatment for morbid obesity and it also can improve the obesity-related comorbidities.[5] In obese patients bariatric surgery was also associated with a decrease in overall mortality.[6,7]

In obesity low grade inflammation is the product of the activated innate immune system, with activated tissue based innate immune cells and circulating immune cells.[8,9] Recent studies revealed the presence of multiple leucocyte subsets like mast cells and T-cells in adipose tissue which regulate inflammation.[10-13]

It was also found recently that obesity reduces thymopoiesis and reduces immune surveillance.[14]

Since 2006, laparoscopic greater curvature

plication (LGCP) technique has been evaluated to eliminate AGB, VSG associated complications by restriction without gastric stapling resection and without an implant used.

The aim of our study was to detect the effect of laparoscopic greater curvature plication on peripheral blood lymphocytes subsets (CD4+ and CD8+ T cells) in morbidly obese patients.

Patient and methods:

This prospective study included 20 patients underwent laparoscopic greater curvature plication (LGCP) to assess the effect of LGCP on excess weight loss and the percentages of their peripheral blood T cells (CD4+ and CD8+ T cells) before and four months postoperatively using flow cytometry.

The data were expressed as the percentage of total lymphocytes ± the standard error of the mean. From June 2010 to July 2012 in El Fayoum University Hospital we used the National Institute of Health's (NIH) inclusion criteria for bariatric surgery (patients with a body mass index >40kg/m^2 or BMI over 35kg/m^2 with at least one comorbidity). We took an informed consent from all our patients. The study included twenty patients; 15 (75%) female patients aged between (25-46years) and 5 (25%) male patients aged between (38-50 years). Patients were considered clinically obese with a near BMI 44.71±4.3 (37-45kg/m^2), mean age 39.5±9.5 (25-50years). The outcome included loss of weight in short time, change of BMI and the percentage of peripheral blood T cells (CD4+ and CD8+ T cells) before and after surgery.

Patients had full history taking, especially for family history of similar condition, BMI, age, social habits of smoking, alcohol consumption, present medical history of any drug intake especially steroids, salicylic acid and non-steroidal anti-inflammatory drugs (NSAIDs)…etc. Their past history of any deep venous thrombosis (DVT), any post surgical morbidities in the abdomen, any current clinical disease in the abdomen (e.g hernia, post surgical scarring…,etc) was also recorded.

The results of their preoperative laboratory tests (including complete blood count (CBC), blood sugar, T3. T4, TSH liver, kidney functions, their coagulation profile and of their pre-operative abdominopelvic ultrasound were noted. Upper GIT endoscopy was done preoperatively for all patients to exclude gastritis, pulmonary function tests, ECG and anaesthetic consultation were done for all patients preoperatively. Fifteen of the patients were smokers, with no alcohol consumption. Their imaging studies revealed non cancerous abdominopelvic ultrasound and chest x-ray. Their preoperative laboratory tests revealed mild anaemia in 6 patients. We excluded patients who showed unfitness for general anaesthesia and major abdominal surgery, patients who were sweet or alcohol addicts, also patients with end stage obesity comorbid diseases as advanced diabetes, advanced atherosclerosis, patients with psychological instability, patients with fear of operations, drug addiction and patients with lack of motivation to weight loss.

Surgical procedure:

LGCP procedure was done under general anaesthesia, and started by division of the greater curvature blood supply using the Harmonic scalpel distally till the pylorus and then proximally till the angle of His. Then the stomach was folded into itself over a 32- Fr bougie applying a first row of extramucosal stitches of 2-0 vicryl. This row guided another row created with extramucosal running suture lines of 2-0 prolene. Methylene blue was injected intraoperatively to check for leakage. The patients started eating 10 days post-operatively. Follow up were at 4 months after surgery. **Figures(1-4)**.

Immune cell preparation and flow cytometry analysis:

Whole blood from all subjects were collected in acid citrate dextrose (ACD). Simultaneous collections were made in tubes containing potassium-EDTA for total lymphocyte count and allowed determination of absolute counts. Evaluation of CD4 and CD8 lymphocyte counts and subsets were assessed by three-color immunofluorescence flow cytometry using a MAbs panel to the desired cell surface proteins including

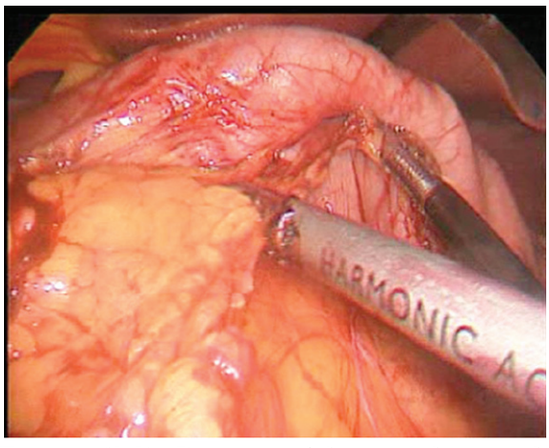
fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated MAb to CD4 and CD8 (Beckman Coulter Electronics, Hialeah, FL). All antibodies were prepared according to the manufacturer's directions and then were incubated with 100p,L of whole blood for 5 min at 25°C before red cell lysis and fixation using Immunoprep reagents and Q-prep equipment (Beckman Coulter), as directed by the manufacturer. Cells were then stored at 4°C for up to 24 h before analysis by flow cytometer (EPICS XL; Beckman Coulter).

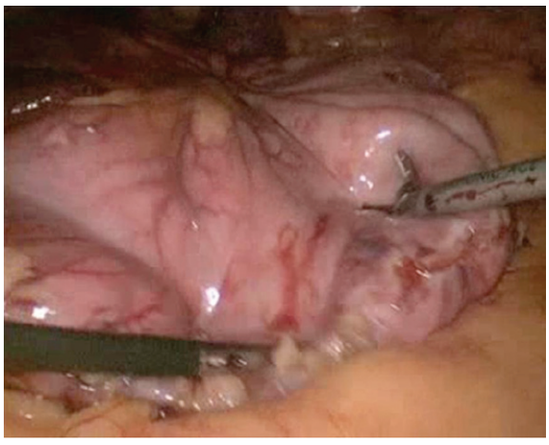
Statistical analysis:

Differences between means and the effects of treatments were determined by one-way ANOVA using Tukey's test, $P<0.05$ was considered statistically significant.

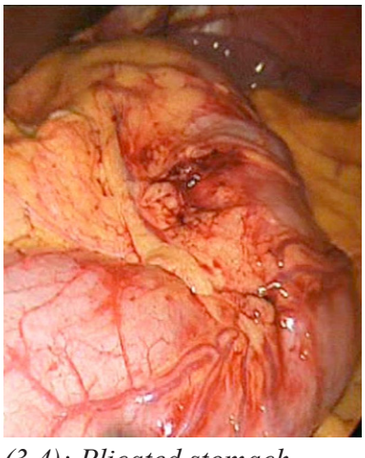
Results:

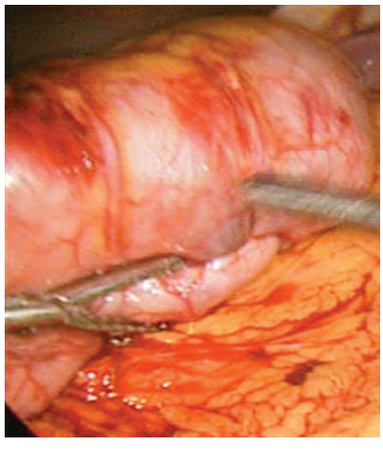
The mean operative time was 65.6±10.2 (60-120) miutes. There were no conversions. Postoperative complications were 5% in the form of nausea and vomiting treated within 12 days, no intraoperative complications. Mean hospital stay was 8.2±3.5 range (7-12) days, loss of weight was (31.20±1.2%); preoperative BMI was 44.71±4.3 (range 37- 45kg/m^2) and postoperative BMI was 31.80±1.1 (range 24-33kg/m^2). The mean percentage of CD4+ T lymphocytes preoperatively was 38.2±1.5 and postoperatively was 29.3±2.6 $p<0.05$ so there is postoperative decrease in the percentage of CD4+ T lymphocytes. The mean percentage of CD8+T lymphocytes preoperatively was 17.3±1.8 and postoperatively was 9.5±1.7 $p<0.05$ so there was postoperative decrease in the percentage of CD8+ T lymphocytes. **Figures(5,6)**.



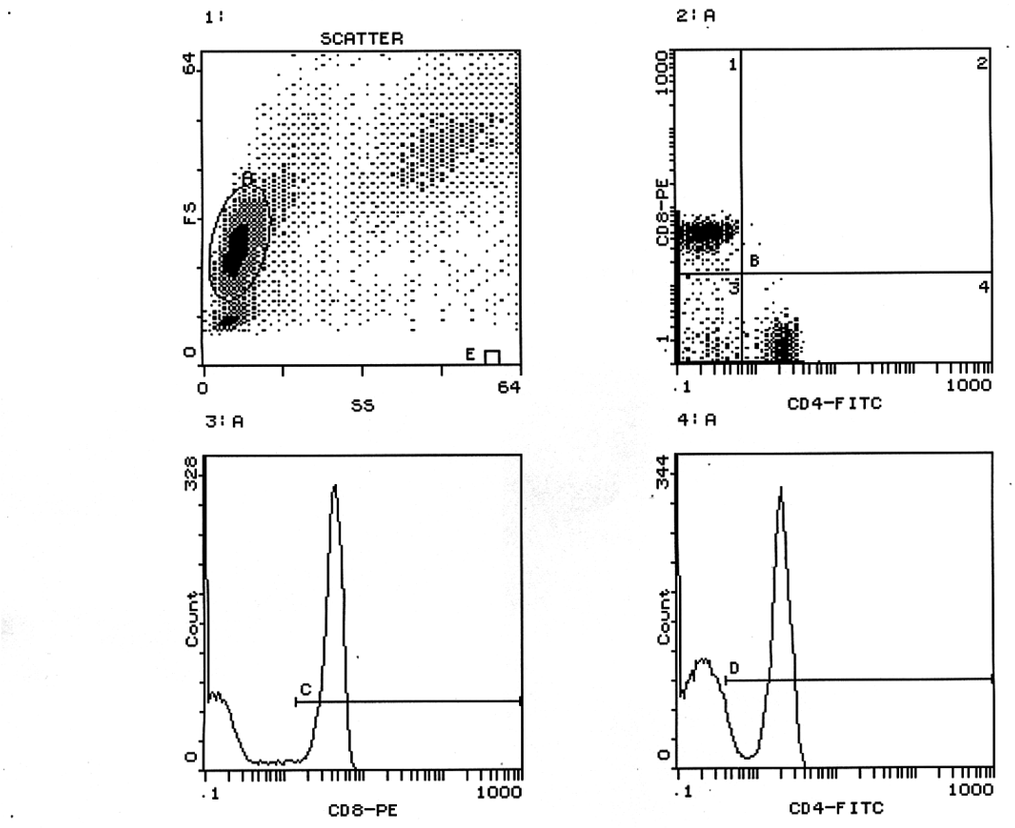


Figures (1,2): Division of the vascular supply of the greater curvature of the stomach.





Figures (3,4): Plicated stomach.

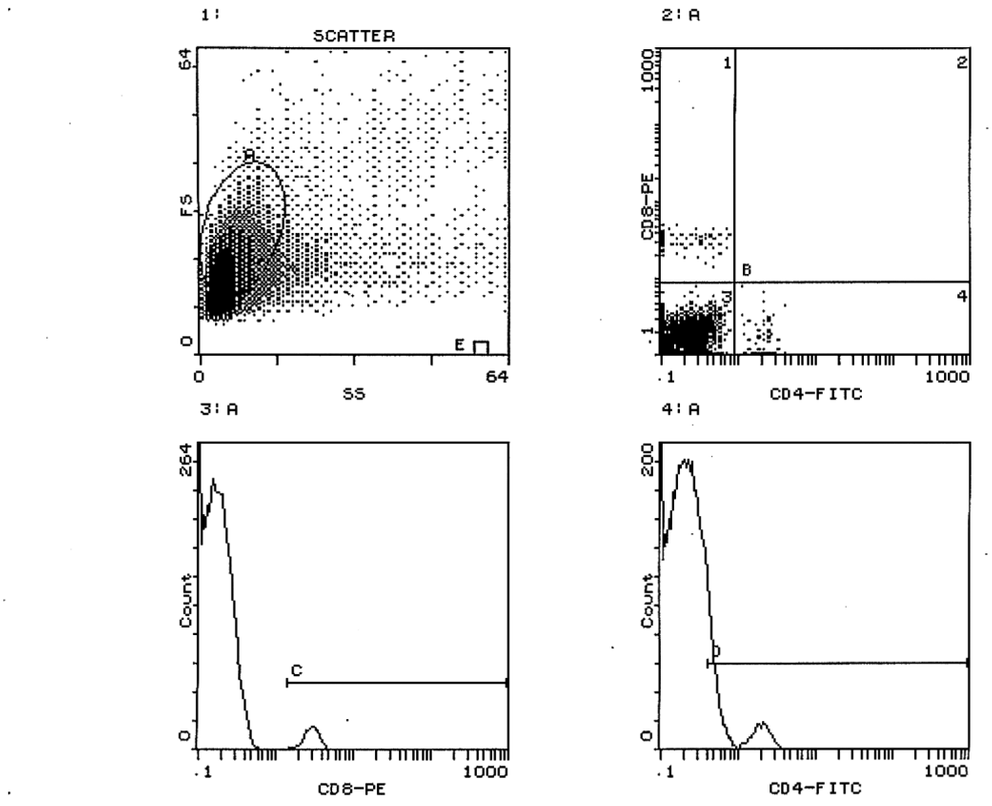


Stats: Not Normalized, Listgating: Disabled

Hist	Region	ID	%	Count	MnI X	MnI Y
1	A	A	58.3	58282	6.9	23.8
	E	E	0.00	0	****	****
2	B1	B	34.5	20110	0.183	4.50
	B2	B	0.13	74	1.35	3.84
	B3	B	28.1	16398	0.179	0.177
	B4	B	37.2	21700	2.21	0.130

Hist	Region	ID	%	Count	MnI X
3	C	C	34.6	20148	4.50
4	D	D	41.2	24027	2.04

Figure (5): Representative flow cytometry for morbidly obese patient CD4+ & CD8+ T cells percentages preoperatively.



Stats: Not Normalized, Listgating: Disabled

Hist	Region	ID	%	Count	MnI X	MnI Y
1	A	A	65.7	65658	6.0	18.7
	E	E	0.00	0	****	****
2	B1	B	2.04	1337	0.265	3.14
	B2	B	0.00	3	7.01	1.86
	B3	B	95.9	62971	0.175	0.149
	B4	B	2.05	1347	2.17	0.189

Hist	Region	ID	%	Count	MnI X
3	C	C	1.97	1294	3.20
4	D	D	8.06	5294	0.946

Figure (6): Representative flow cytometry for morbidly obese patient CD4+ & CD8+ T cells percentages four months postoperatively.

Table (1): Patients characteristics.

Data		P- value
Age (years)	39.5 ±9.5	
Gender	15 (75%) females 5 (25%) males	
BMI (preoperative) (kg/m2)	44.71 ± 4.3	
BMI (postoperative) (kg/m2)	31.80 ± 1.1	
Weight loss (%)	31.20 ± 1.2%	
Operative time (minutes)	65.6 ± 10.2	
Hospital stay (days)	8.2 ± 3.5	
CD4+T lymphocytes percentage (preoperatively).	38.2 ± 1.5	<0.05
CD4+T lymphocytes percentage (postoperatively).	29.3 ± 2.6	
CD8+T lymphocytes percentage (preoperatively).	17.3 ± 1.8	<0.05
CD8+T lymphocytes percentage (postoperatively).	9.5 ± 1.7	

Data are expressed as mean values ± SD (standard deviation).

Discussion:

CD4+T lymphocytes and CD8+ T lymphocytes are types of white blood cells known as lymphocytes which have markers on the surface known as CD4, CD8. CD4 cells are commonly known as T-helper cells, they help to detect and fight off bacterial and viral infection. CD8 cells detect and try to fight off infections caused by viruses or diseases such as cancer and are known as killer T cells.

Morbid obesity is characterized by infiltration of adipose tissue with macrophages.[15] This study of morbidly obese patients showed that dietary energy restriction and weight loss reduced CD4+ T cells percentage $p<0.05$, reduced CD8+ T cells percentage $p<0.05$ and this is considered statistically significant compared to other studies which did not show change in CD4+ T cells and CD8+ T cells percentages.[16,17]

Data on the effect of loss of weight post bariatric surgery on inflammation and immune cells are emerging, suggesting the reduction in the circulating inflammatory markers.[18] Other study showed also that immune cell activation can be regulated by acute energy restriction.[19] The mechanism by which weight loss and energy restriction in obesity attenuate proinflammatory activation of immune cells are unclear.

A study of gastric bypass showed a decrease in T lymphocytes CD95 and CD69 expression indicative of reduction of T lymphocytes activation.[20] Our study builds on the data of decreased CD4+ T cells and decreased CD8+ T cells that there is attenuated activation of circulating immune cells post weight loss by laparoscopic greater curvature plication (LGCP).

In our study LGCP showed satisfactory weight loss like other studies,[21,22] there were no major complications compared to other studies which showed leakage[22] and bleeding.[23] Limitations of this study include lack of a control group undergoing dietary energy restriction without weight loss.

In summary, this study found that weight loss after LGCP in morbidly obese patients showed attenuated activation of circulating immune cells (decrease in CD4+T helper

cells percentage and decrease in CD8+killer T cells percentage) and more regulation of chronic inflammation in morbidly obese patients.

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