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Short Communication

Flow Cytometric Cytological Analysis of the Drainage Fluid Following Laparoscopic Sleeve Gastrectomy

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Abstract

Background and aim: The tissue undergoes wound healing processes following the surgery, with various cell populations serving during different processes. The aim of the current study was to analyze the cell populations present in the drainage fluid collected from the patients who underwent laparoscopic sleeve gastrectomy (SG) and analyze cell subsets using a flow cytometry-based approach.

Materials and methods: The study subjects were patients who operated for laparoscopic SG, with a BMI of ≥35 kg/m2 and without any known comorbidities that might lead to intra- and postoperative complications. Draining fluid from the plastic chamber was collected in a 200 mL sterile plastic container for two days during the postoperative period. The monoclonal antibodies were utilized for the determination of cell sub-populations in the flow cytometry.

Results: The patient population consisted of 8 male and 8 female patients with a mean age of 38.6±14.2 years. The neutrophils were the predominant cell type in the postoperative period. The percentage of the monocytes and lymphocytes significantly decreased on postoperative day 2 ($p<0.01$ and $p<0.05$, respectively). T lymphocytes were the predominant lymphocyte population in the drain fluid samples during the postoperative period.

Conclusion: There is a strong presence of neutrophils on the surgery site, and the monocyte concentration significantly decreased on the postoperative second day. We suggest that the transformation of monocytes to the tissue macrophages during the wound healing process affected the monocyte ratio. T lymphocytes were the predominant population in the among the lymphocytes during the postoperative period.

INTRODUCTION

Sleeve gastrectomy (SG) is a procedure that involves the surgical removal of the gastric tissue in order to treat obesity and related comorbidities. With the increasing incidence of obesity, the number of metabolic surgery operations annually increases requiring a closer look into the intra- and postoperative stages [1].

In the early post-operative period of SG, an abdominal drain is inserted close to the site of resection in order to prevent morbidity causing factors such as the intra-abdominal collection of infectious or necrotic tissue, as well as to monitor any occurrence of post-operative bleeding. The optimum duration of the intra-abdominal drain is set to two-days postoperatively under normal circumstances in the clinical setting, and several studies report that the use of intra-abdominal drains are not related with decreased morbidity, and reoperation, and also might be resulting in increasing ratio of drain site-related infections [2,3].

Following surgical intervention, tissue goes through wound healing processes with the recruitment of different cell populations. The early phase of wound healing is known as inflammation, and different immune cells play a role in this course. It has long been known that neutrophils are the first cell cluster to increase after an inflammatory trigger, followed by the transport of monocytes to the site of injury [4,5]. Thus, daily evaluation of the collected fluid in terms of biochemical and cellular factors might shed light on the local processes ongoing in the post-operative period following abdominal surgery.

The aim of the current study was to analyze different cell populations present in the drainage fluid collected from the resection site in the postoperative period, in an attempt to provide a better understanding of the immune processes within this period. To our knowledge, this is the first report to present the evaluation of different cell types present in the drainage fluid in patients who underwent SG for the treatment of obesity.

MATERIALS AND METHODS

The data were collected following the principles of the Declaration of Helsinki. The patients were informed about the possible complications and technical details of the surgery; written informed consent was obtained from each patient. The inclusion criteria for the SG included patients with a BMI of ≥ 35 kg/m2 and without any known comorbidities that might lead to intra- and postoperative complications.

All patients were subjected to a detailed medical examination by a multidisciplinary team including a bariatric surgeon, endocrinologist, cardiologist, anesthesiologist, psychiatrist, urologist, gynecologist, and a dietician. An intensive workup of blood and urine tests, abdominal USG, chest X-ray, respiratory function testing, endoscopy of the upper GI tract, echocardiography, ECG, Doppler USG of the carotid and vertebral arteries along with psychiatric evaluation were performed for all of the patients before the surgery. A cervical smear of the uterus and breast USG were performed for all female patients and accompanied by mammography for the female patients older than 40 years of age in order to exclude the presence of any malignancy. The surgical procedure was performed as described earlier by the operating surgeon [6]. Briefly, the surgical procedure was performed laparoscopically by one surgeon, and the SG was performed following the placement of aa 33f bougie to the stomach. The SG procedure was performed using linear cutting stapler starting at the gastric greater curvature at a point located 2 cm from the pylorus. Stapler site was closed with 3/0 polydioxanone sulfate suture. The liver retractor was not used during the surgery.

Collection of draining fluid

Draining fluid from the incision site was collected in a plastic chamber, and emptied when full. The sampling of the drainage fluid was started on the post-operative first day at 8 am every morning.Samples were collected for two days in the postoperative period. Samples for the cellular analysis were collected in a 200 mL sterile plastic container and the cap was closed tightly. The samples were rushed to the laboratory of analysis in one hour's time in optimum conditions in a transport bag specific for specimen transportation.

Analysis of draining fluids

The processing of the fresh samples was started within one hour after their arrival at the laboratory in order to achieve the most possible number of alive cells in the sample. The leucocyte count was performed using a routine hematology analyzer (Sysmex XN-100, Sysmex Corp., Japan).

The following monoclonal antibodies were utilized: CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD14, CD16, CD19, CD20, CD21, CD22, CD38, CD45, CD56, HLA-DR. Neutrophiles and granulocytes were identified based on CD45 expression and side light scatter (SSC).

The samples were analyzed using flow cytometry on FACSCalibur (BD Biosciences, San Jose, CA). The acquired data were analyzed using CellQuest software (BD Biosciences, San Jose, CA). Data are expressed as a percentage of the specific cell populations in the sample.

Cells were identified as follows: T-helper cells: CD3+CD4+CD8- T cytotoxic cells: CD3+CD4-CD8+, mature B-cells: CD19+, CD20+, CD21+, CD22+, CD38+, HLA-DR+, pre-B phenotype: CD10+, CD19+, HLA DR+.

Monocytes were identified as being CD14 and CD16 positive, and neutrophils and were identified by side and forward scatter, with weak expression of CD 45 and CD14 negativity.

Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS-20 (Statistical Packages for Social Sciences-version 20). Data were presented in percentages. The significance of the differences between postoperative variables was tested using the Student's t-test. The correlations of the variables were tested using Pearson's correlation coefficiency test. Statistical significance was set as $p<0.05$.

RESULTS

Sixteen adult patients (8 M/8F) with a mean age of 38.6 ± 14.2 years underwent SG were enrolled in this study.

The ratios of the cell types were shown in Table 1 as mean ± SD. The neutrophils were the most common cell type in the postoperative period. The percentage of the monocytes and lymphocytes significantly decreased on postoperative day 2 ($p<0.01$ and $p<0.05$, respectively). T lymphocytes were the predominant lymphocyte population in the drain fluid samples during the postoperative period.

The ratio of the T-suppressor lymphocytes significantly decreased on postoperative day 2 ($p<0.05$), whereas the concentrations of mature B lymphocytes and neutrophils elevated significantly ($p<0.05$ and $p<0.05$ respectively).

There was a negative correlation between the ratios of T cytotoxic lymphocytes-total lymphocytes ($r= -0.38$, $p<0.05$), neutrophil-total lymphocytes ($r= -0.68$, $p<0.05$), T cytotoxic lymphocytes-mature B lymphocytes ($r= -0.56$, $p<0.05$), neutrophil-mature B lymphocytes ($r= -0.54$, $p<0.05$). We found a significant positive correlation between the neutrophil-total T lymphocytes ($r= 0.44$, $p<0.05$) and neutrophil-T cytotoxic lymphocytes ($r= 0.32$, $p<0.05$) (Table 2).

DISCUSSION

Abdominal surgery is a type of trauma resulting in the alterations in the immune system members in the injury site and also circulation [7]. Herein, we aimed to analyze the levels of these immune system cells in the drainage fluid following SG and observe the daily pattern of these cells in the specimens. Being placed locally in the incision site, we assumed that the alterations in the drainage fluid cell content might be reflecting the local immune response status of the tissue. Using FACS analysis of the drainage fluid samples, we have observed an initial pattern with the strong presence of CD45+ neutrophils during the first two post-operative days. Additionally, while we analyzed the characterization of the lymphocyte population, T lymphocytes were the predominant population in the postoperative period.

There are several studies showing altered expression of monocyte surface antigens following surgical procedures [8-10]. In our study, we observed a decreasing pattern in the macrophage population following the surgery in our patient group. We suggest that the decrease might be a result of the differentiation of monocytes into tissue macrophages, and present cellular surface markers in the study lack the performance of identifying these cells.

The neutrophil ratio was negatively correlated with total lymphocyte and mature B lymphocyte ratio, whereas positively

Table 1: Distribution of the immune cell types on the postoperative period in the drain fluid specimens.

Cell type	Post-op day 1 (%)	Post-op day 2 (%)	p value
Lymphocyte	4.6±0.32	2.8±0.44	**<0.05**
T cell	88.2±2.67	86.7±5.74	0.32
T helper	42.1±5.43	38.6±7.44	0.14
T supressor	42.6±7.6	38.7±5.4	**<0.05**
Mature B	1.8±0.46	2.2±0.32	**<0.05**
Monocyte	0.8±0.11	0.1±0.18	**<0.01**
Neutrophil	85.6±6.78	92.7±5.64	**<0.05**

Table 2: Correlation of the immune cell types on the postoperative period in the drain fluid specimens.

	Lymphocyte	T cell	T helper	T cytotoxic	Mature B	Monocyte
Lymphocyte						
T cell	-0.12					
T helper	-0.09	0.24				
T cytotoxic	**-0.38***	0.19	0.14			
Mature B	-0.11	-0.03	-0.08	**-0.56***		
Monocyte	-0.07	-0.04	-0.01	-0.13	0.06	
Neutrophil	**-0.68***	**0.44***	0.26	**0.32***	**-0.54***	-0.16

*p<0.05

correlated with T-cells in our study group. Also, neutrophils were reported to migrate to the inflamed tissue by the trigger of chemokines secreted by different inflammatory cells. By secreting cytokines such as TNF-alpha, neutrophil influx to the inflammatory site was accelerated even in the early phases of the tissue injury [11]. Relevant to this, the vast majority of the cells in drainage samples following the surgery consisted of the neutrophils in our patient group.

Among the lymphocyte population, T-cells were the predominant population when compared to the B-cell population. The percentage of the CD4+CD8- T-helper cell population decreased in all drain samples on the postoperative second day. The ratios of CD4-CD8+ T- cytotoxic cells were negatively correlated with the total lymphocyte, mature B-lymphocytes, and positively correlated with neutrophil ratios for all patients. Since these two groups of T-cells trigger the differentiation of B-cells into antibody-producing plasma cells, it is also evident that the ratios of T-cells are in a negative correlation with the ratio of mature-B cells, suggesting these cell types an exchanging pattern during the immunological processes following the tissue injury [12-14].

Despite the novelty of our study, we have to mention several limitations. Firstly, we did not perform an analysis of the peripheral ratios of immune system cells, therefore the correlation between the ratios in these samples is not possible. Secondly, we did not evaluate the other cell types that might be involved in the tissue healing processes. Thirdly, since the drain removal was performed on the postoperative second day, we could not have data on the healing process following this period. However, being similar in nature, studies in patients who underwent intraabdominal surgical procedures other than the SG, reported that T-cells were the predominant population among the lymphocytes in the first days of the postoperative period, and neutrophils started to increase immediately after surgery as a result of rapid inflammatory response [15,16].

CONCLUSION

In conclusion, to our knowledge, this is the first study that involves the cellular behavior of immune system cells in the incision site following SG. We report that there is a strong neutrophil presence on the surgery site, and the monocyte concentration significantly decreased on the postoperative second day, suggesting a transformation of these cells to the tissue macrophages during the wound healing process.

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