Prognostic Significance of Inflammatory Cytokines in Obese Patients with Endometrial Cancer Undergoing Robotic Versus Laparotomy Procedures

Dimitrov T1*, Gorchev G1, Tomov S1, Sarfraz A1, Kameliya1, Tsvetanova1, Tantchev L1, Malkodanski I2 and Ivanova I1

1Department of Gynecological Oncology, Medical University of Pleven, Bulgaria
2Department of Gynecological Oncology, Florida Hospital Cancer Institute, USA

Abstract

Several biological mechanisms mediate the association between obesity and endometrial neoplastic risk. The increased body mass index (BMI) with >30 kg/m2 is also associated with the long-term maintaining of a high level of inflammatory processes, increased pro-inflammatory cytokines, and acute-phase inflammatory proteins. In the practice of gynecologic surgeons, there is growing interest in monitoring a group of cytokines as independent prognostic factors (such as markers of aggressiveness and opportunity for better therapeutic influence on the neoplastic process). We report on the monitoring of cytokine profiles of interleukin (IL)-8, tumor necrosis factor (TNF)-α and their association with C-reactive protein (CRP) in the serum of patients with stage I endometrial cancer treated with robotic-assisted laparoscopic hysterectomy (R) and traditional laparotomy/open (O) surgical procedures. The patients’ age ranged from 46-81 years. Compared to the pre-surgical specimens, the levels of IL-8, TNF-α and CRP were substantially higher in post-surgical specimens, particularly at 3rd hour. Regardless of the pre- or post-surgical time points, the levels of these cytokines/mediators have been significantly higher in morbidly obese patients with BMI >40 kg/m2. It is conclude that the inflammatory cytokines and acute phase proteins contribute to the prognostic assessment in the neoplastic disease, particularly in morbidly obese endometrial cancer patients. Immune-sparing role of robotic-assisted surgery as compared to open surgery seems to be a better treatment option for overweight women with early-stage endometrial cancer.

Keywords: Endometrial cancer; Obesity; Inflammatory cytokines; Robotic surgery; Prognosis

Introduction

Endometrial cancer is the most common form of gynecological malignancies. The main characteristics of Type I endometrial cancer patients are: endometrioid histology, low-grade, ~85% distribution, estrogen-dependent, obese body habitus, peri-menopausal age, risk factors (diabetes, polycystic ovary syndrome, nulliparity, and late menopause), genetic mutations (PTEN activation, KRAS mutation, and microsatellite instability) with relatively better prognosis [1]. Malignant neoplasms of the uterine mainly affect the endometrium and their increased incidence is frequently associated with triggering the pathogenetic mechanisms such as endocrine disorders, hypokinesia (reduced locomotor activity, obesity, hormone therapy), etc [2]. Overweight women with body mass index (BMI) >30 kg/m2 face three-fold higher risk of developing endometrial cancer as compared to the normal weight women [3]. In the post-menopausal period, obesity leads to conversion of androgens, increase in serum bioavailability of estrogens that are not balanced by progesterone, which in turn, promotes the mitogenic activity of endometrial cells [4-6]. Obesity is also associated with maintaining a continuous high level of inflammatory processes, thereby increasing the levels of pro-inflammatory cytokines and acute-phase inflammatory proteins [7-9]. Surgery is a controlled injury, and in the practice of gynecologic surgeons, there is growing interest for potential role(s) of a group of cytokines that may serve as independent prognostic factors. These cytokines may potentially be identified as markers of the disease aggressiveness and provide opportunity for therapeutic influence on the neoplastic process because cytokines are immunological products, which are signs of biological balance and can potentially alter the programmable cell death [10,11]. Hence, comprehensive assessment at immunological level of the distortions in intracellular metabolism forms the basis for a differential approach to some of the predictions concerning the individual
Material and Methods

At our primary single institution, 97 women diagnosed with stage I endometrial cancer for this study. The patients were divided into two groups based on the individual diagnostic characteristics and their BMI, and surgical approach/procedures performed (i.e., O vs. R). During the course of the pre-operative clinical diagnosis, 68 patients were identified with an extremely high BMI (>40 kg/m²). The surgical management of early-stage endometrial cancer patients were accomplished through our standard operative procedures optimized for the traditional laparotomy/open (O) and the newer state-of-the-art minimally invasive (MIS) approach, i.e., robotic-assisted laparoscopic surgery (R). All surgeons who participated in the study were competent in both surgical procedures. Patients’ demographics, clinico-pathological, and peri-operatives data were contemporaneously abstracted from the medical records as an ongoing quality initiative. This use of clinical specimen and corresponding retrospective data used in this study was approved by the primary institutional review board. For dynamic monitoring, patients blood samples were collected pre-operatively, and at the 3rd hour, post-operatively. Serum specimens thus obtained were stored at -20°C before use. The levels of IL-8, TNF-α, and CRP were measured utilizing the respective human ELISA assay kits (Diaclone SAS, Besancone Cedex, France) accordingly to the manufacturer’s protocols. The samples were read at 450 nm optical density on an ELISA Microplate Reader (Model 2100 Stat Fax, Awareness Technology, Inc, Ramsey, MN, USA).

Results

The patients’ age ranged from 46-81 years (n=97). The comparative characteristics of the dynamic monitoring of serum levels of IL-8 in the two surgical groups of the patients are presented in (Figure 1). It is evident that compared to the pre-surgical, the levels of IL-8 in the specimens collected at the 3rd hour post-operatively are much higher in O group (in red) and remained higher even after day-3, post-operatively. In contrast, the R group (in blue) of patients was found to have relatively lower levels of IL-8 in serum specimens collected at all time-points (Figure 1). (Figure 2) summarizes the data on IL-8 levels (at 3rd hour, post-operatively) in accordance with BMI of the study patients and the type of surgical procedures performed. In the lower BMI group of patients (left panel), the IL-8 levels were found to be nearly 2-fold higher in O group specimens as compared to the R group. Interestingly, this phenomenon was even more obvious in the patients with BMI >40 kg/m² where IL-8 levels were higher in the O group of samples as compared to the R group (Figure 2). Furthermore, morbidly higher BMI of patients was clearly associated with elevated levels of IL-8 (about 3 to 4-fold) regardless of the surgical approach/interventions (Figure 2). Next, in view of the multifactorial significance of TNF-α, the dynamic serum level of TNF-α was measured in these specimens. As shown in Figure 3, TNF-α level was relatively higher in the open surgery group of patients at all the time-points (in red) as compared to the robotic group of patients (in blue). Notably, the R group of specimens at all time-points exhibited minimum deviations as compared to the O group. Also, a slight reduction is observed in the levels of TNF-α (close to baseline values) after the 3rd day following the R surgery, which lacked in the O group, (Figure 3). In fact, three of the women operated with O technique, TNF-α continued to rise on the 3rd day after the intervention. These patients also have had clinically proven post-operative complications (infection, dehiscence, and intestinal obstruction). The results on the measurement of TNF-α levels (at 3rd hour, post-operatively) in accordance with BMI of the study patients and the type of surgical procedures performed are shown in (Figure 4). In the lower BMI group of patients (left panel), the TNF-α levels

Figure 1: Measurement of IL-8 level in the serum of women with stage I endometrial cancer treated with robotic-assisted laparoscopic (R) and conventional laparotomy/open (O) surgical techniques.

Figure 2: Cumulative levels of IL-8 in the BMI stratified specimens collected at 3rd hour post-operatively from women with stage I endometrial carcinoma, operated with robotic-assisted laparoscopic (R) and conventional laparotomy/open (O) surgical approaches.

Figure 3: Measurement of TNF-α level in the serum of women with stage I endometrial cancer treated with robotic-assisted laparoscopic (R) and conventional laparotomy/open (O) surgical techniques.

Figure 4: Cumulative levels of TNF-α in the BMI stratified specimens collected at 3rd hour post-operatively from women with stage I endometrial carcinoma, operated with robotic-assisted laparoscopic (R) and conventional laparotomy/open (O) surgical approaches.
were higher (more than 5-fold) in O group specimens as compared to the R group. Likewise, the TNF-α levels were consistently higher in both surgical groups in patients with BMI >40 kg/m². The level TNF-α was more than double in the open group as compared to the robotics group of morbidly obese patients (Figure 4). We then measured the CRP level in the patients’ specimens collected at the 3rd hour post-operatively in both surgical groups. Individual patients’ CRP levels are shown in Figure 5. Based on the comparative analyses, the average values in robotic technique was CRP = 7.906 mg/L (which turns out to be within the normal range of <10 mg/L). However, a significant increase in the average CRP level was observed in the open surgery group (Δ CRP = 19.886 mg/L), suggesting the persistence of an inflammatory response, (Figure 5).

### Discussion

This study demonstrates that profiling of immunological cytokines (such as IL-8, TNF-α, and CRP) contributes to the prognostic assessment in neoplastic endometrial cancer. Our data also demonstrates the immune-sparing role of the MIS (robotic-assisted laparoscopic hysterecctomy) as a better treatment option for overweight women with stage I endometrial cancer as compared to the traditional laparotomy/open surgical approach. Endometrial cancer is the frequent gynecologic malignancies, and most patients present with early-stage disease. The common symptoms of this disease are: vaginal bleeding after menopause, bleeding between periods, an abnormal, watery or blood-tinged discharge from the vagina, and pelvic pain, etc. Hence, the disease prognosis and timely detection is critical in the successful management of such patients. Current standard treatment of patients with endometrial cancer is surgery (i.e., hysterectomy, salpingo-oophorectomy, and/or lymphadenectomy, preferably MIS), followed by chemotherapy, radiation and/or hormone therapy (depending on the various clinico-pathological and peri-operative factors). In addition to the above noted facts, tumor markers are being increasingly recognized as important tools for early diagnosis, prognosis, therapy response and monitoring for patients with endometrial cancer. It has been widely recognized that IL-8 affects the movement of leukocytes and macrophages (chemotaxis, migration, respiratory burst) by promoting antimicrobial activity. The extent of the inflammatory state of the body is also caused by the level of proinflammatory cytokine such as IL-8, which is associated with the angiogenic effect on thymic endothelial cells that is responsible for cell survival rate [12-14]. Our data clearly suggest that post-operatively IL-8 levels are up-regulated in patients with early-state endometrial cancer, particularly in much higher levels in the morbidly obese cases.

Another role in the progress of inflammatory immune processes, cellular apoptosis, and malignancies is attributable to TNF-α. Along with other key interleukins (such as IL-6, IL-8, and IL-10, etc.), this cytokine is also involved in both the acute and chronic inflammatory processes. Various immune epithelial and tumor cells take part in the production of TNF-α, which is accumulated intracellularly, forming a non-covalent trimeric bond, as it expresses on the cell surface. When bound on the cell surface, TNF-α may form lysis with the tumor or virally-infected cell. The separation of the membrane-bound TNF-α leads to feedback about the activation or delay in the production of TNF-α itself and of other biologically active cytokines [15,16]. Our observations on the measurement of TNF-α support the fact that post-operatively this cytokine is also up-regulated endometrial cancer, particularly in much higher levels in the morbidly obese and those treated with open surgical procedure. The activation of TNF-α triggers the activation or suppression of a number of inflammatory processes, vascular permeability, hemorrhagic and necrotic deviations, tumor-associated vascularisation, anti-tumor treatment and further spread of the neoplastic process.

The increase in IL-8 and TNF-α entails an increase in CRP levels, which is synthesized by the liver in response to inflammatory, infectious, neoplastic, traumatic or autoimmune processes. CRP is a pentameric glycoprotein, commonly known as acute-phase protein. This protein is often used as apoptosis and necrosis marker (with normal value in blood plasma of <10 mg/L). It binds with lipo-phosphatidylcholine of the cell membranes, with further activation of the complement cascade, phagocytosis, fibrinogen synthesis and closing of the pathogenetic circle while maintaining the inflammatory process [13,16,17]. As noted above, the CRP levels were significantly upregulated across the board in endometrial cancer patients treated with open laparotomy surgical group (post-operatively). This CRP increase was associated with the increase in IL-8 and TNF-α levels, particularly at 3-hours, post-operatively. The MIS is a method to reduce the morbidity of surgery and has been shown in general to reduce blood loss, complications, post-operative pain and hospital length-of-stay compared with traditional laparotomy approach [18]. In order...
to treat the disease, surgeons balance complications and invasiveness with clinical outcomes in order to determine which techniques are best. The widespread adoption of robotic surgery (most updated form of MIS) for gynecologic indications during the past decade have been encouraging. Its development and utilization addresses many of the limitations of traditional laparoscopy instruments by restoring dexterity and intuitive instrument movement, 3-D vision, ergonomics, and autonomy.

**Conclusion**

We thus conclude that the inflammatory cytokines and acute phase proteins contribute to the prognostic assessment in the case of neoplastic diseases, particularly in morbidly obese endometrial cancer. Furthermore, the immune-sparing role of robotic-assisted surgery as compared to open surgery is demonstrated as a better treatment option for overweight women with early-stage endometrial cancer. Our data re-confirms that MIS (robotic surgery) reduces the so called “cytotoxic burst”, which presupposes the decrease of post-operative risk of hospitalization, complications, and potentially intensive care encounters.

**References**