Higher serum levels of tumour necrosis factor and its soluble receptors are associated with ovarian tumours

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Abstract

Introduction: Tumour necrosis factor (TNF) and its soluble receptors type 1 (sTNF-R1) and type 2 (sTNF-R2) have been suggested as key mediators between apoptosis and cancer cell progression. The aim was to examine concentrations of the parameters in the serum of women with ovarian tumour and in the fluid from ovarian cysts of women with serous cystadenoma.

Material and methods: The study included 125 women with ovarian tumours. As a control, sera were obtained from 70 healthy female volunteers. Concentrations of TNF, sTNF-R1 and sTNF-R2 were measured by enzyme-linked immunosorbent assay (ELISA).

Results: Significant increases of TNF, sTNF-R1 and sTNF-R2 were found in the serum of women with ovarian tumour in comparison to the control ($p < 0.0001$). The highest levels of all studied parameters were observed in women with ovarian cancer. In the ovarian cyst fluid the concentrations of the evaluated parameters increased significantly as compared to the serum ($p < 0.0001$).

Conclusions: Our data showed changes in regulatory mechanisms of apoptosis in women with ovarian tumours which are associated with increased concentrations of all studied factors. Serum estimated TNF and especially sTNF-R may be used as complementary diagnostic markers in patients with ovarian tumours.

Key words: tumour necrosis factor, soluble receptor of tumour necrosis factor, ovarian tumours.

Introduction

Ovarian cancer is one of the most lethal gynaecological cancers and a high mortality rate makes this disease a major health problem for women. Approximately 70% of patients with ovarian cancer are diagnosed at advanced stages (International Federation of Gynecology and Obstetrics – FIGO stage III and IV) and the 5-year survival rate for this group is only 10% to 40% [1]. Nevertheless, the still unclear aetiology of ovarian cancer and unclear understanding of the nature of its precursor lesions are the main reasons for the slow development of effective early detection markers and in consequence ineffective targeted therapy.

Recent studies have noted impairments of apoptosis in malignant cells including ovarian cancer cells [2]. Factors of the tumour necrosis factor (TNF) superfamily play an important role in the control of apoptosis. One of the major systems involved in such processes is TNF and its receptors.

The TNF, a protein consisting of 157 amino acids, is synthesized as a membrane-anchored 26-kDa precursor (pro-TNF) that is cleaved to the

secreted 17-kDa form [3]. In response to TNF, cell activation is mediated via two TNF receptors (TNF-R): tumour necrosis receptor type 1 (TNF-1R1) and tumour necrosis receptor type 2 (TNF-R2) [4]. TNF-R1, with 55 kDa molecular weight, is the main receptor mediating the cellular effects of TNF in most cell type [5]. It belongs to the death receptor family and contains the death domain (DD), that shares the capability of cell activation to independent metabolic pathways. TNF-R2, which is identified as a 75 kDa protein, does not possess a DD, although it can mediate a cell death signal, which may be indirect through TNF-R1 [6]. Both receptors are ubiquitously expressed, whereas TNF-R2 is mainly expressed on immune cells [7]. The binding of homotrimeric TNF to either receptor can activate three important signalling pathways: TNF receptor associated death domain (TRADD); nuclear factor κB (NF-κB), a major cell survival signal; and c-Jun N-terminal kinase (JNK) signalling cascades [8].

The membrane-bound receptors can be cleaved and released as a soluble form from the cell surface following proteolysis as the soluble TNF-R1 (sTNF-R1) and the soluble TNF-R2 (sTNF-R2). Increased concentrations of TNF and its soluble receptors in serum and other biological fluids are associated with different diseases, including malignant diseases [9]. For this reason, the aim of the study was to evaluate the selected mechanisms which control apoptosis, through measurement of the concentrations of TNF, sTNF-R1 and sTNF-R2 in the pre-treatment serum of affected women and in ovarian cyst fluid of women with serous cystadenoma.

Material and methods

Patients and clinical samples

The study group consisted of 125 women aged 21 to 62 years (mean age: 46.8 ±11.7 years) with newly diagnosed ovarian tumour. This group included 50 women aged 21-62 years (mean age: 44.2 ±11.5 years) with ovarian serous cystadenoma, 35 women aged 24 to 64 years (mean age: 43.9 ±7.2 years) with mature cystic teratoma, and 40 women aged 29 to 59 years (mean age: 45.5 ±10.4 years) with ovarian serous cystadenocarcinoma in stage Ia. The tumour assessment was based on results of clinical symptoms, gynaecological and histological examinations and laboratory analysis, including CA125 level. They did not have any sign of endometriosis previously. The tumours were graded according to World Health Organization criteria and staging was done by implementing the criteria of FIGO. All women were patients of the Department of Gynaecology and Obstetrics of the 6th Hospital in Katowice, Poland. The control group consisted of 70 healthy female volunteers aged 21-62 years (mean age: 46.9 ±9.2 years), who had no evidence of pathological disorders or any inflammations in the reproductive system. Not all women smoked and none had received hormonal or anti-inflammatory treatment during the 3 months preceding material collection or surgery. Characteristics of the study population are shown in Table I.

The analysed material was venous peripheral blood obtained by venipuncture from all studied women and the ovarian cyst fluid of women with serous cystadenoma. In women with ovarian tumour, the blood was taken after establishing the diagnosis, directly prior to the operation, and the ovarian cyst fluid was obtained during the surgery. In the control group, the blood was taken when the women were admitted for follow-ups. Blood and fluid was centrifuged at 600 × g for 10 min and next samples were aliquoted and stored at –70°C until the time for measurement.

Cytokine assay

The concentrations of TNF, TNF-R1 and TNF-R2 were determined by enzyme-linked immunosorbent assay (ELISA) using the commercial kit Bender MedSystems (Vienna, Austria). All determinations were performed in duplicate, according to the instructions. The sensitivity of the kits was 5.8 pg/ml for TNF, 80 pg/ml for sTNF-R1 and 0.15 pg/ml for sTNF-R2. CA125 level in serum of all studied women

Table I. Baseline characteristics of women with ovarian tumours and control group

Variable	Ovarian tumour	Control group
Number of women	125	70
Age [years]	21-62 (46.8 ±11.7)	21-62 (46.9 ±9.2)
BMI [kg/m^2]	18-28 (25.7 ±3.9)	21-32 (26.1 ±3.6)
Menstrual status:		
Pre-menopausal	68	40
Post-menopausal	57	30
Menstrual phase:		
Follicular phase	68	40
Luteal phase		
Concentration of CA125 [U/ml]	> 35	< 35

Values are means ± SD. BMI – body mass index

was measured using a commercial *Microparticle Enzyme Immunosorbent Assay* (MEIA) kit (Abbott Diagnostics, Wiesbaden, Germany). The upper reference limit for CA125 was used according to the manufacturer's recommendation – below 35 U/ml.

The Ethical Committee of the Medical University of Silesia according to the Declaration of Helsinki approved this study.

Statistical analysis

Results of TNF, sTNF-R1 and sTNF-R2 are presented as mean ± standard deviation and were examined for normality of distribution by the Shapiro-Wilk test. Parametric data were analysed using a Student's *t*-test. For nonparametric data, Fisher's exact test was used to indicate statistical significance because it analyses the variance relationship both within and among the groups. The data of CA125 level are presented as the number of women with higher or lower concentration than 35 U/ml. Correlations were tested by Spearman's rank correlation test and presented as the correlation coefficient (r). A $p < 0.05$ was considered statistically significant. All analyses were performed with Statistica for Windows 8.0 software.

Results

Concentrations of TNF, sTNF-R1 and sTNF-R2 in the serum of women with ovarian tumours and the control group and also in the ovarian cyst fluid are shown in Table II.

TNF

Mean serum TNF level of women with ovarian tumours was significantly higher compared to controls. Along with higher tumour stage the concentration of TNF increased. The highest studied parameter levels were observed in serum of women with ovarian cancer. Differences between all groups were statistically significant: $p < 0.01$ between women with ovarian serous cystadenoma and mature cystic teratoma; $p < 0.0001$ between women with ovarian serous cystadenoma and ovarian cancer; $p < 0.0001$ between mature cystic teratoma and ovarian cancer. Additionally, concentration of TNF was significantly lower in serum compared to ovarian cyst fluid ($p < 0.0001$).

sTNF-R1

Serum sTNF-R1 level was significantly higher in women with ovarian tumours compared to controls ($p < 0.0001$). Along with higher tumour stage the concentration of receptor increased, but the difference between sTNF-R1 level in women with ovarian serous cystadenoma and mature cystic teratoma was not statistically significant. The highest concentration of sTNF-R1 was observed in serum of women with ovarian cancer, and it was significantly higher compared to other studied groups ($p < 0.0001$). Increased sTNF-R1 in ovarian cyst fluid in comparison with serum of women with ovarian serous cystadenoma ($p < 0.0001$) was observed.

sTNF-R2

Serum sTNF-R2 level was significantly higher in women with ovarian tumours compared to controls ($p < 0.0001$). Along with higher tumour stage the concentration of receptor also increased, but the difference between sTNF-R2 level in women with ovarian serous cystadenoma and mature cystic teratoma was not statistically significant. The highest concentration of sTNF-R2 was observed in serum of women with ovarian cancer, and it was significantly higher compared to other studied groups ($p < 0.0001$). Increased sTNF-R2 in ovarian cyst fluid

Table II. Serum levels of TNF, TNF-R1 and TNF-R2 of women with ovarian tumors and in ovarian cyst fluid of women with serous cystadenoma

Group	*n*	TNF [pg/ml]		TNF-R1 [ng/ml]		TNF-R2 [ng/ml]	
		Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Serum:							
Ovarian tumors	125	15.57-48.90	24.90 ±7.91*	1.01-2.60	1.71 ±0.53*	2.40-5.30	3.51 ±0.74*
Serous cystadenoma	50	15.57-25.18	18.59 ±2.70*,†,‡,§	1.01-1.53	1.25 ±0.15*,‡,§,‖	2.46-3.73	3.08 ±0.33*,‡,**
Mature teratoma	35	16.45-25.11	22.94 ±2.30*,‡	1.38-2.45	1.82 ±0.32*,‡	2.40-3.88	3.19 ±0.56*,‡
Serous cystadenocarcinoma	40	24.72-48.93	34.12 ±6.10*	1.99-2.63	2.33 ±0.19*	3.17-5.35	4.28 ±0.63*
Control	70	< 5.8	< 5.8	0.59-0.68	0.62 ±0.04	1.10-2.12	1.80 ±0.41
Ovarian cyst fluid:							
Serous cystadenoma	50	40.29-60.55	50.97 ±5.79	2.79-4.25	3.58 ±0.43	4.1-6.33	5.34 ±0.61

*Values are means ± SD; *p < 0.0001 compared to control group, †p < 0.01 in mpared to mature teratoma, ‡p < 0.0001 compared to ovarian cancer, §p < 0.0001 compared to ovarian cyst fluid of serous cystadenoma, ‖p < 0.0001 compared to mature teratoma, **p < 0.001 compared to ovarian cyst fluid of serous cystadenoma*

in comparison with serum of women with ovarian serous cystadenoma ($p < 0.0001$) was observed.

A non-statistically significant correlation between levels of studied parameters in serum and in fluid from ovarian cyst was observed. Additionally, a statistically significant positive correlation was found between the concentration of TNF and sTNF-R1 ($r = 0.75$, $p < 0.0001$) and the concentration of TNF and sTNF-R2 ($r = 0.77$, $p < 0.0001$).

In addition we analysed the relationship between TNF and CA125. Positive significant correlations between TNF and CA125 ($r = 0.747$, $p < 0.0001$), between sTNF-R1 and CA125 ($r = 0.842$, $p < 0.0001$) and between sTNF-R2 and CA125 ($r = 0.662$, $p < 0.001$) were observed.

Discussion

Ovarian cancer is generally diagnosed at advanced stages of the disease. Moreover, the fact that the survival of the patients with stages I–II of the disease ranges from 60% to 90% suggests the potential for a high treatment rate with earlier detection of the disease. That fact is associated with finding specific markers of ovarian tumours. Strategies for identification of biomarkers for non-invasive tests, such as ELISA assays, have been in use for decades. Unfortunately, neither aetiology nor pathogenesis of ovarian tumours is still completely known even though there is intensive research of the disease. A better knowledge of the disease has led to the study of newer biological or molecular prognostic factors that may complement or even replace some of the less objective convectional parameters.

It is known that the TNF-TNF-R system plays an important role in inflammation, angiogenesis, programmed cell death, and proliferation, which are all crucial components in malignant transformations. The complicated roles of TNF in cancer development have emerged. Its anticancer property is mainly through inducing cancer death. However, TNF also stimulates proliferation, survival, migration and angiogenesis in most tumours that are resistant to TNF-induced cytotoxicity, resulting in tumour promotion. TNF is a double-edged sword that could be either pro- or anti-tumourigenic. Moreover, soluble forms of TNF-R1 and TNF-R2 are derived from the extracellular binding domains of each receptor and they regulate the activity of TNF by competing with its receptors. Some research supports laboratory results and strongly suggests that TNF and its soluble receptors could be useful in cancer detection and staging or predicting prognosis [10].

In this study, our results did not show the presence of TNF in the serum of the control. However, increased concentration of it was associated with the clinical extent of the tumours. The highest TNF level was observed in the serum of women with ovarian cancer. We also evaluated the concentration of TNF in the ovarian cyst fluid of women with ovarian serous cystadenoma in comparison to the serum. Significantly higher levels of this parameter may indicate a local generated immune response and intensity of cancerogenesis. Moreover, it could be the reason for destruction of the peritoneal-like epithelium, which builds the cyst.

Studies have indicated significant changes of TNF level in women with ovarian tumours but the results are still ambiguous. The majority of them observed significant increases in TNF level in the serum of women with ovarian cancer [11-14]. Their investigation first of all related to epithelial ovarian cancer. Dobrzycka *et al*. [11] apart from increased TNF level also found a correlation with tumour stage and with reduced mean survival time. On the other hand, Kutteh *et al*. [15] observed the highest TNF level in women with papillary serous cystadenocarcinoma and the lowest in women with mucinous cystadenomas. Naylor *et al*. [16] reported higher expression of TNF in ovarian cancer cells, which was dependent on the stage of progression of disease. This may confirm our results of higher level of TNF in ovarian cyst fluid and the suggested local immune response against cancer cells. However, other studies did not detect TNF in the serum of women with ovarian cancer, in women with non-malignant ovarian cysts or in bioptates of ovarian fibrosarcoma, which indicated that the lower TNF secretion by immune cells might suppress the influence on lymphocyte T proliferation [17, 18].

In our study, we additionally examined the concentration of sTNF receptors in the serum of affected women. In controls sTNF-R1 and sTNF-R2 levels were in the range of physiological values. At the same time, in the group of women with ovarian tumour concentrations of both receptors significantly exceeded the range of physiological values. Our results indicated the highest sTNF-R1 and sTNF-R2 in women with ovarian cancer and the lowest in women with ovarian serous cystadenoma. This suggested that both sTNF-R1 and sTNF-R2 levels can be useful as laboratory markers for differential diagnostics of ovarian tumours. We also affirmed that in women with ovarian tumour, the secretion of sTNF-R2 in the serum was higher than sTNF-R1. Moreover, a strong positive correlation between TNF or its receptors and CA125 might suggest that CA125, being a tumour marker, is closely related to the cytokine system. TNF correlated with severity of cancer, indicating its superiority as a marker for further, larger studies, but the measurement of complementary serum markers, such as TNF, can improve the use of marker screening for ovarian tumour. Studies suggested that other tumour markers have also been implicated in pathogenesis of ovarian tumours, but their diagnostic use is limited [19].

This observation was consistent with the results of other authors [20-23]. In addition, the ovarian cancer cells had the capacity for spontaneous secretion of more sTNF-R1, but less receptor type 2 [24]. Additionally, serum TNF-R1 level correlated with morphological ultrasound score and CA125 [22]. The observations made in Burger's study [25] showed a relationship between levels of CA125, sTNF-R1 and sTNF-R2 and risk of progression of epithelial ovarian malignancies. Among patients with low or high CA125 levels, those with high sTNF-R1 levels and low sTNF-R2 levels had the lowest risk, patients with low-low or high-high sTNF-R1 and sTNF-R2 levels, respectively, had an intermediate risk, and patients with low sTNF-R1 levels and high sTNF-R2 levels had the highest risk of progression [25].

The present study demonstrated higher concentration of sTNF-R1 and sTNF-R2 in the fluid than in the serum of affected women and suggested intensity of the local immune response. The higher receptor levels might be associated with augmented secretion of soluble receptors by the malignant cells. It leads to a decrease in count of membrane type receptors on the cell surface and prevents interaction between TNF and its membrane receptors [26] and, in consequence, leads to inhibition of apoptosis induction. In that way, malignant cells can protect themselves from the immune reaction. Analysis of cytokine levels of ovarian cyst fluid may be a pure way to study cytokine expression to gain more insight into tumour-host interaction. Studies indicate that higher IL-6 or IL-8 levels in cyst fluid are correlated with malignancy and are observed in malignant cyst fluids. Additionally, T helper 1 (Th1) subtype and Th2 cytokine expression depends on the type of tumours [27]. In our results, higher levels of TNF and its receptors in cyst fluid than in serum confirmed that the immunosuppressive state created by ovarian tumour is reflected in the cystic fluid within the tumour.

An interesting observation was made in our analysis of the correlation between TNF and its receptor levels in the serum of women with ovarian tumours. A significant positive correlation between TNF and TNF-R1, as well as TNF and TNF-R2, which we observed, may suggest the ovarian cancer cells' capacities for autocrine TNF secretion with contemporary shedding of both receptors from the cell surface. Similar results were reported by Langkopf *et al.* [28], who suggest that higher levels of TNF and its receptors in biological fluids are the ovarian cancer cells' protection against cytotoxic TNF properties. Given the suggested pro-apoptotic role of TNF, elevated concentrations of TNF-R may antagonize TNF in the environment, and consequently attenuate the apoptotic process of inflexed ovarian cancer cells. It has been suggested that the failure to eliminate ovarian cancer cells, possibly due to abnormalities of the immune system, especially impairment of the TNF-sTNF-R pathway, may be conducive to the development of cancer. Moreover, the mechanisms regulating sTNF-R in women with ovarian cancer are unknown. Because of competitive TNF-sTNF-R binding, soluble receptors serve as TNF antagonist. It has been suggested that an increase in circulating sTNF-R level in women with ovarian cancer may promote survival of ovarian cancer cells by antagonizing TNF, a pro-apoptotic cytokine.

Impairments of the TNF-TNF-R system are observed in other gynaecological diseases, especially in endometriosis and endometriosis-associated infertility [29, 30]. Increased serum and peritoneal fluid TNF levels have been implicated in the pathophysiology of endometriosis. Because of this, higher TNF may indicate both conditions – endometriosis and ovarian tumours. However, in women with endometriosis, TNF acts generally as a pro-inflammatory cytokine rather than as a pro-apoptotic factor. It can increase the permeability of local blood vessels, thereby causing inflammatory exudation into the peritoneal cavity and aggravation of peritoneal inflammation, and in consequence reduces fertility [30].

In conclusion, among women with ovarian tumours, impairment of apoptosis appears to occur, which demonstrates the increase in concentrations of all the studied parameters. Serum TNF, sTNF-R1 and sTNF-R2 levels are more elevated in patients with ovarian cancer. Higher concentrations of them in the ovarian cyst fluid than in serum suggest local suppression of the immune response, and intensity of apoptosis impairment. Serum TNF, sTNF-R1 and sTNF-R2 are all biochemical factors which may be implicated in the pathophysiology of ovarian cancer. Their levels, especially sTNF-R1 and sTNF-R2, might be used as complementary diagnostic markers in patients with ovarian tumours, and may gradually improve early diagnostics for women with ovarian cancer.

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