Concentrations of follicle stimulating hormone are increased in ovarian tumor fluid: implications for the management of ovarian cancer

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Summary

Purpose of investigation. Significant progress has been made in recent years in the understanding of the mechanisms postulated by the gonadotropin theory of ovarian carcinogenesis. In the present study we compare FSH concentrations between serum and fluid from cysts or the rectouterine pouch of patients with epithelial tumors and non-neoplastic lesions. *Methods*. We enrolled 277 patients. They were divided into five groups: I (n = 44) - ovarian cancer patients, II (n = 16) - borderline tumors, III (n = 40) - benign epithelial cystadenomas, IV (n = 137) - non-neoplastic lesions and V (n = 22) - admitted for "second-look" laparoscopy. *Results*. There were any significant differences between FSH concentrations in serum and tumor fluid in patients with ovarian cancer (36.46 vs 28.11 mIU/ml) and borderline epithelial tumors (31.5 vs 22.7 mIU/ml). For benign cystadenomas the respective concentrations were 28.96 mIU/ml in serum and 6.93 mIU/ml in tumor fluid in these groups $p < 0{,}0000001$. The same highly significant differences were found in non-neoplastic lesions (24.97 vs 4.77 mIU/ml), $p < 0{,}0000001$. Patients who underwent "second-look" laparoscopy demonstrated significant differences ($p < 0.05$) as FSH concentration in serum and peritoneal fluid when neoplastic cells were not disclosed, but the difference was not significant ($p = 0.752$) when fluid from the rectouterine pouch was positive for carcinoma cells. *Conclusions*. The results of our study can reflect an ineffective tumor: blood barrier and easy diffusion of gonadotropins into the tumor tissue. Local reduction of FSH levels through administration of GnRH analogs may in some clinical situations produce clear therapeutic benefits for the management of ovarian malignancies.

Key words: Ovarian cancer; Etiopathogenesis; Gonadotropins; GnRH analogs; Treatment.

Introduction

Ovarian cancer is notable for the highest mortality among gynecological tumors [1]. A mean survival time of three years in women with advanced ovarian cancer is currently all that can be achieved with the traditional approach consisting of surgical resection of the tumor followed by chemotherapy (platinum-paclitaxel) [2]. Clearly, investigations must continue for more effective and less toxic methods of treatment based on what is known about the etiopathogenesis of this neoplasm. Significant progress has been made in recent years in the understanding of mechanisms postulated by the gonadotropin theory of ovarian carcinogenesis [3-7]. Evidence is accumulating that gonadotropins (FSH, LH) participate in the neoplastic transformation of the normal ovarian epithelium [8-10]. Gonadotropin receptors have been revealed in several ovarian cancer cell lines [11-13] and it has conclusively been demonstrated that overexpression of the FSH receptor (FSHR) markedly increases the risk of cancer by enhancing the proliferation of preneoplastic ovarian surface epithelial cells [14]. Receptor and non-receptor mechanisms by which gonadotropins act as hormonal promoters of carcinogenesis in the ovary are much better understood today [3-5, 7].

Schiffenbauer *et al*. [15] found an association between neoangiogenesis which is profoundly important in carcinogenesis and gondotropin stimulation. According to these researchers, gonadotropin-related tumor growth can be attributed to the activation of vascular endothelial growth factor (VEGF) by gonadotropins. We have previously reported that gonadotropin concentrations are significantly higher in the fluid from malignant tumors than from benign or non-neoplastic tumors [16, 17].

Indirect proof for the involvement of gonadotropins in the pathogenesis of ovarian cancer arises from the fact that ovarian cancer is virtually absent in the prepubertal period as long as the gonadostat is operational and only germ-cell tumors are observed in this period. By suppressing the pulse generator in the hypothalamic arcuate nucleus, the gonadostat inhibits pulsatory release of the gonadotropin-releasing hormone (GnRH) and gonadotropins and in consequence reduces their concentrations. Our previous studies showed a tendency to premature menopause in patients with BRCA 1 gene mutation. We believe that hypergonadotropic activity in these patients may predispose them to ovarian cancer at a younger age [19].

Understandably, the gonadotropin theory of ovarian cancer has paved the way to the use of GnRH analogs in ovarian cancer patients aimed at reducing gonadotropin levels [20-23].

The present work was undertaken to compare FSH concentrations in serum and fluid from the cyst or rectouter-

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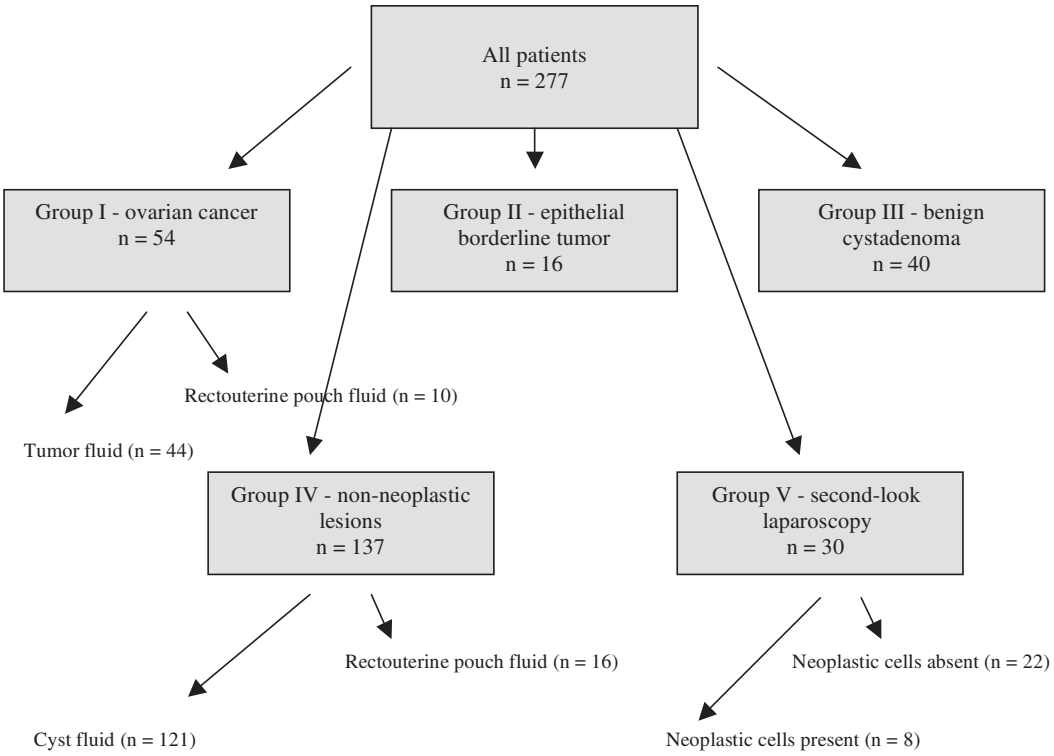

Figure 1. — Patient groups and material collected.

ine pouch of patients with epithelial tumors and non-neoplastic lesions of the ovary. We also hoped to gather information on the effectiveness of vascular endothelium as a barrier preventing diffusion of FSH from the blood to tumor tissue.

Material and Methods

We enrolled 277 patients treated because of a cyst, tumor or fluid in the rectouterine pouch at the Chair and Department of Gynecological Surgery and Oncology of Adults and Adolescents, Pomeranian Medical University. Some of the patients were previously operated on for ovarian cancer and appeared for "second-look" laparoscopy. Blood was sampled prior to the procedure. Fluid was obtained from the tumor, cyst, or rectouterine pouch during laparoscopy, laparotomy, or culdocentesis. Definitive diagnosis was based on the results of histopathology or cytology and the patients were allocated to one of the following groups: I (n = 54) ovarian cancer (44 cases when tumor fluid was obtained and 10 cases of solid tumors when fluid could only be obtained from the rectouterine pouch); II (n = 16) borderline tumors; III (n = 40) benign epithelial cystadenomas; IV (n = 137) non-neoplastic lesions including 121 patients with cysts and 16 patients with fluid in the rectouterine pouch; V (n = 30) "second-look" laparoscopy (22 cases without neoplastic lesions and 8 cases with carcinomatous cells in the aspirate).

FSH concentrations were measured in serum and fluid using commercial MEIA test kits from Abbott and the AxSYM robotnic analyzer. FSH binds to the anti-bFSH coated microparticles forming an antibody-antigen complex. An aliquot of the reaction mixture containing the antibody-antigen complex bound to the microparticles is transferred to the matrix cell. The microparticles bind irreversibly to the glass fiber matrix. The matrix cell is washed to remove unbound material. Then anti-αFSH subunit specific alkaline phosphatase conjugate is dispensed into the matrix cell and binds with the antibody-antigen complex. The matrix cell is once again washed to remove unbound material. The substrate, 4-methylumbelliferyl phosphate, is added to the matrix cell and the fluorescent product is measured by the MEIA optical assembly. Sensitivity of the FSH AxSYM assay was determined as 0.01 mIU/ml and represents the lowest measurable concentration of FSH that can be distinguished from zero.

Histopathologic and cytologic diagnosis was established at the Department of Genetics and Pathomorphology, Pomeranian Medical University.

Statistics. Distribution of the results deviated from normal according to the Shapiro-Wilk W test. Consequently, means were compared using the non-parametric Mann-Whitney U-test for two variables and the level of significance was taken as $p < 0.05$.

Results

We failed to find any significant differences between FSH concentrations in serum (36.46 mIU/ml) and tumor fluid (28.11 mIU/ml), as well as between serum (24.41 mIU/ml) and peritoneal fluid (19.99 mIU/ml) in patients with ovarian cancer (group I). In borderline epithelial tumors (group II), concentrations of FSH in serum and tumor fluid were 31.5 and 22.47 mIU/ml (n.s.), respectively. For benign cystadenomas (group III), the respective concentrations were 28.96 mIU/ml and 6.93

Table 1. — *FSH concentrations in serum and fluid of patients with epithelial ovarian tumors (groups I, II, and III).*

GROUP		FSH in serum [mIU/ml]		FSH in fluid [mIU/ml]		p
		Mean	Median	Mean	Median	
I	W n = 54	34.2 [1.32-117.86]	30.44 [26.91-41.5]	26.6 [1.12-78.39]	17.16 [20.09-33.12]	NS [p = 0.139]
	tumor fluid n = 44	36.43 [1.32-117.86]	36.025 [27.78-45.09]	28.11 [1.12-78.39]	16.73 [20.45-35.76]	NS [p = 0.223]
	rectouterine pouch fluid n = 10	24.41 [7.87-54.21]	24.05 [14.16-34.65]	19.99 [3.86-55.3]	17.92 [8.54-31.45]	NS [p = 0.352]
II	n = 16	31.5 [2.07-96.66]	11.38 [14.01-49.01]	22.47 [0.05-99.86]	4.51 [4.99-39.95]	NS [p = 0.132]
III	n = 40	28.96 [2-106.31]	14.18 [19.79-38.14]	6.93 [0.01-64]	1.85 [2.61-11.25]	p < 0.000001

W - whole group, analysis of tumor fluid and rectouterine pouch fluid together; group I - cancers, group II - borderline tumors, group III - cystadenomas.

Table 2. — *FSH concentrations in serum and fluid of patients with non-neoplastic ovarian pathologies (group IV).*

GROUP		FSH in serum [mIU/ml]		FSH in fluid [mIU/ml]		p
		Mean	Median	Mean	Median	
IV	W n = 137	24.97 [0.1-126.99]	7.25 [19.45-30.48]	4.72 [0.01-95.93]	1.97 [2.74-6.69]	p < 0.0000001
	cyst fluid n = 121	24.53 [0.1-124.28]	7.92 [18.81-30.24]	3.75 [0.01-78]	1.55 [2.17-5.33]	p < 0.0000001
	rectouterine pouch fluid n = 16	28.31 [0.61-126.99]	6.21 [7.13-49.51]	12.04 [0.95-95.93]	4.04 [-0.5-24.63]	p < 0.05

W - whole group, analysis of cyst fluid and rectouterine pouch fluid together.

Table 3. — *FSH concentrations in serum and rectouterine pouch fluid of ovarian cancer patients undergoing second-look laparoscopy (group V).*

GROUP		FSH in serum [mIU/ml]		FSH in fluid [mIU/ml]		p
		Mean	Median	Mean	Median	
V	W n = 30	50.75 [0.98-105.94]	51.99 [38.54-62.96]	27.69 [0.14-79.2]	15.48 [17.24-38.14]	p < 0.005
	S-L(-) n = 22	52.79 [0.98-105.94]	65.17 [37.82-67.77]	23.73 [0.14-79.2]	6.63 [11.51-35.95]	p < 0.005
	S-L(+) n = 8	45.14 [2.68-91.92]	41.62 [19.26-71.01]	38.59 [1.71-70.49]	41.91 [-15.25-61.92]	NS p < 0.752

W - whole group V with presence and absence of neoplastic cells in rectouterine pouch fluid; S-L(-) - neoplastic cells absent in rectouterine pouch fluid; S-L(+) - neoplastic cells present in rectouterine pouch fluid.

($p < 0.0000001$; Table 1). The same highly significant difference was found for FSH concentrations in serum and fluid of patients with non-neoplastic lesions (24.97 vs 4.72 mIU/ml; $p < 0.0000001$; Table 2). In the subgroup of patients with ovarian cysts, FSH concentrations in serum and cyst fluid were 24.53 and 3.75 mIU/ml, respectively ($p < 0.0000001$) mIU/ml). When the pathology was limited to the presence of fluid in the rectouterine pouch, FSH concentrations in serum and fluid were 28.31 and 12.04 mIU/ml), respectively ($p < 0.05$). Finally, patients who underwent second-look laparoscopy demonstrated significant differences ($p < 0.005$) as to FSH concentrations in serum and peritoneal fluid when neoplastic cells were not disclosed. The difference was not significant ($p = 0.752$) in cases testing positive for carcinomatous cells (Table 3).

Discussion

The hormonal model of tumorigenesis dating back to the 1940s [24] has lost nothing of its validity and remains the object of much discussion in the literature [3-7]. In support of this statement is the long list of direct and indirect proof for the involvement of gonadotropins in proliferative processes of ovarian surface epithelial cells [4, 5, 9, 15-17]. Mandai *et al*. [25] demonstrated the presence of gonadotropin receptors (LH/hCG) on ovarian cancer cells and Choi *et al*. [14] showed in addition that overexpression of FSHR in ovarian epithelium is accompanied by enhanced proliferation and induction of oncogenesis. Parrot *et al*. [11] reported that the gonadotropin receptor (OCC1) is present in some cell lines only which are also notable for a proliferative reaction in response to gonadotropins in vitro.

The discovery by Kakar *et al*. [26] and Irmer *et al*. [27]

that 80% of epithelial ovarian tumors carry receptors specific for gonadoliberin with properties identical to pituitary receptors has paved the way for therapeutic applications of gonadoliberin analogs in ovarian cancer and enabled clinical practice to benefit from what is known about the role of gonadotropins in proliferative processes of ovarian cancer cells. Clinical trials with gonadoliberin analogs began in the end of 1980s and continuously provide new insights [28-30]. Hasan *et al*. [30] used tamoxifen with goserelin in ovarian cancer patients not responding to chemotherapy and observed a therapeutic effect in 50% of cases (CR 3.8%, PR 7.7%, SD 38.5%). A similar observation was made by Zidan *et al*. [22] in patients with relapse of advanced ovarian cancer. Gonadoliberin analogs produced full remission lasting eight months in one out of 15 patients (6.8%), partial remission of 14 months in one patient (6.8%), and stabilization of symptoms for 7.5 months in three patients (20%). Similar findings were reported by Pasceviciute *et al*. [29]. Keeping in mind that the response to second and third chemotherapy cycles is observed in just 15% and less than 10% of cases, respectively, hormonal therapy with its low toxicity is certainly an important alternative in selected cases [29]. We have previously reported that the best therapeutic effect can be expected when therapy is supplemented with GnRH analogs [31].

Two aspects have been distinguished in the mechanism of action of GnRH analogs: firstly, they reduce the levels of gonadotropins, and secondly they act directly on receptors and suppress in various ways the proliferation of neoplastic cells [32-36]. In spite of structural similarities between GnRH receptors in the pituitary gonadotrope and tumor cells, the mechanism of signaling differs markedly [36]. Binding of ligand to the receptor on tumor cells (G1 protein ai) activates phosphotyrosine phosphatase (PTP) leading to dephosphorylation of the EGF receptor and suppression of neoplastic growth induced by EGF [37]. In some cell lines, antiproliferative activity is synonymous with induction of apoptosis [38]. It has further been shown that triptorelin, an analog of GnRH I, activates the JNK/c-jun pathway thereby suppressing neoplastic growth [39].

We have found that concentrations of FSH in serum and epithelial tumor (malignant or borderline) fluid do not differ significantly. No significant differences were noted when FSH concentrations in serum were compared with concentrations in peritoneal fluid obtained during the first cytoreductive surgery or during the second-look laparoscopy which disclosed the presence of carcinomatous cells. We take this finding as indirect evidence for an ineffective barrier allowing FSH to diffuse from the blood and accumulate in the tumor or peritoneal cavity. On the other hand, the barrier appears to be more tight in cystadenomas and non-neoplastic cysts, resulting in significantly ($p < 0.0000001$) lower concentrations of FSH in cyst fluid. The difference was also significant ($p < 0.005$) when second-look laparoscopy proved negative for carcinomatous cells.

Our present results consistently show that FSH concentrations in the fluid surrounding proliferating cells (ovarian cancer, borderline tumors, relapse) do not differ significantly from serum concentrations. As neoplastic cells are not capable of synthesizing gonadotropins, FSH seems to pass to fluid through an ineffective barrier separating neoplastic cells from the blood. In view of overexpression of gonadotropin receptors on tumor cells [11, 12], induction of proliferation processes and promotion of tumor growth is facilitated in this situation [10, 13]. Of particular interest is the observation that FSH passes freely into the peritoneal fluid. This diffusion opens the way to further tumor growth if cytoreductive surgery is unsuccessful in removing neoplastic cells from the peritoneum, and gonadotropin levels in serum remain unreduced. We believe that the use of GnRH analogs is theoretically well founded if aimed at reducing accumulation of gonadotropins in tumor tissue, an objective no less important than direct inhibition of receptor-mediated antiproliferative activity. Elimination of gonadotropins from the tumor means that all adverse effects of their action would disappear concomitantly.

It has been demonstrated that gonadotropins support cell proliferation by activating transcription of protein kinase Cα [40]. In OVCAR-3 cells, gonadotropins stimulate secretion of estradiol which subsequently induces cell growth [41]. By activating proteolysis and promoting invasiveness of tumor cells mediated by protein kinases (PKA, P13K), gonadotropins favor metastasis. FSH promotes the activity of several genes responsible for metabolic processes and cell proliferation [9]. It has been suggested that neoplastic spread in the peritoneum is the result of greater adhesiveness of ovarian cancer cells induced by gonadotropins [42].

We tend to relate the ineffective barrier in malignant and borderline tumors to neoangiogenesis which is necessary for uncontrolled proliferation, invasiveness, and metastases [43, 44]. Angiogenesis in tumors takes place in stages resembling the physiologic process but the structure of tumor vessels is different: the vascular wall is formed by endothelial cells only, the basal membrane is much thinner, and the composition of the extracellular matrix is altered. In consequence, and because of increased synthesis and secretion of VEGF [43, 44], permeability of tumor vessels is increased. Elevated concentrations of VEGF have been reported in serum and fluid from epithelial ovarian tumors, suggesting its role in tumor growth [45] and its potential use as a tumor marker [46]. Increased expression of VEGF-C and VEGF-2 is associated with aggressiveness of the tumor and its ability to spread rapidly [47].

In conclusion, similarly high levels of FSH in serum, tumor fluid, and peritoneal cavity fluid from patients with malignant epithelial neoplasms of the ovary reflect an ineffective tumor: blood barrier and easy diffusion of gonadotropins into the tumor tissue. Local reduction of FSH levels through administration of GnRH analogs may in some clinical situations produce clear therapeutic benefits for the management of ovarian malignancies.

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