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ORIGINAL ARTICLE

Fibronectin plasma levels in gynecological cancers

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Summary

Purpose: *Fibronectin (FBN) is involved in the motility and migration of malignant cells. The purpose of this study was to investigate FBN plasma levels in gynecological cancers patients and in healthy women.*

Methods: *The study took place between 1998 and 2003. One hundred women with histologically diagnosed cancer of gynecological organs (cervix, ovary, endometrium, breast) formed the study group (group A), whereas the control group (group B) consisted of 100 healthy women. FBN plasma levels were measured with the radial immunodiffusion method.*

Results: *The average age of group A patients was 42.08 years (range 33-77), and of group B it was 41.1 years (range 32-65). Both groups were compared with the Student's-t test. The median plasma value of FBN in all gynecological malignancies was 258.4 mg/l (standard deviation/SD 163.9, p=0.0066, t-statistics: 2.768, t_{95}: 1.984, 95% CI: 225.4-290.9). The plasma levels were significantly elevated when compared to the control group (median=213 mg/l). The distribution of values showed a statistically important "tail" in high plasma levels (FBN >400 mg/l). Plasma levels of FBN were more increased in breast and cervical malignancies when compared to ovarian and endometrial cancers.*

Conclusion: *FBN plasma levels were significantly increased in the total of group A patients, but not significantly increased in the endometrial and ovarian subgroup. Whether or not FBN could reliably be a marker for gynecological cancers should be confirmed in studies with larger number of patients.*

Key words: fibronectin plasma levels, gynecological malignancies

Introduction

FBN is a glycoprotein which plays an important role in the cell motility and adhesion. It was first described in 1940 as a "cold insoluble globulin of plasma". In 1948 Morrison described the existence of this cold insoluble globulin in a fibrinogen molecule which did not interact with thrombin. In 1975 Ruoslathi and Vaheri also found a large multidomain glycoprotein on a fibroblast surface, which was named "surface fibronectin" [1].

There are two different forms of FBN, the soluble form (plasma FBN) and the insoluble form (cellular FBN). The soluble form exists in the amniotic fluid, in the cerebrospinal fluid, in the allantoid, in the arthric fluid and in the basal membranes of the cells. Cellular FBN is found in the fibroblasts, liver cells and endothelial cells.

The FBN gene is located in chromosomes 2 or 11. Probably, there are two different epitopes for the cellular and plasma FBN.

FBN molecule consists of 17 amino acids, which form 3 different types of homologous repeating modules, types I, II, III. The aminoterminal and the carboxyterminal region of the molecule is made from 12 type I modules, which mainly bind on fibrin and collagen molecules. Type II module binds mainly on collagen, whereas type III module is characterized by the existence of the integrin binding tripeptide Arg-Gly-Asp (RGD). Integrins are a category of transmembrane heterodimeric receptors, which allow cell adhesion, growth and migration [2,3].

Concerning the association between FBN and gynecological malignancies it has been reported that there might be a positive relationship between the extracellular molecule and these malignancies, so that

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FBN plasma levels could serve as a potential diagnostic marker.

Different factors are involved in the cancer cell migration, such as integrins as well as the epidermal growth factor (EGF) in cases of cervical cancer [4-11]. In addition A-beta estradiol, estriol, progesterone, medroxyprogesterone acetate, danazol, EGF, transforming growth factor-a (TGF-a) and TGF-b may play a role in endometrial cancer cell motility and peritoneal tumor cell invasion [12-16]. Finally, elevated FBN plasma levels are reported in cases of breast cancer [17,18].

The aim of the present study was to investigate the relationship between plasma FBN levels and gynecological cancers, due to its involvement in the motility and migration of malignant cells.

Methods

The study took place in the 2nd Department of Obstetrics and Gynecology of the University of Athens between 1998 and 2003. One hundred women with histologically confirmed cancer of gynecological organs (20 cases with cervix cancer, 20 cases with ovarian cancer, 20 cases with endometrial cancer and 40 cases with breast cancer) were included in group A. Group B (controls) consisted of 100 healthy women.

The FBN plasma levels were measured preoperatively with the radial immunodiffusion method (NANORID™,The Binding Site LTD, UK). This method is based on the radial immunodiffusion of the investigated substance which takes place on a substrate with agarose and special monoclonal antibodies for the tested substance. The method involves antigen diffusing radially from a cylindrical well through agarose gel containing an appropriate monospecific antibody. Antigen-antibody complexes are formed like a precipitating ring (20-24° C; incubation time 96 h). The ring size will increase until equilibrium is reached between the formation and breakdown of these complexes. This point is termed "completion". At this stage a linear relationship exists between the square of the ring diameter and the antigen concentration.

Each blood test for estimation of the FBN plasma levels was made twice. The results of both groups were compared using the Student's t-test. A p-value<0.05 was considered as statistically significant.

Results

The average age of group A patients was 42.08 years (range 33-65), whereas of group B it was 41.1 years (range 32-67). The median value of FBN in all gynecological malignancies was 258.4 mg/l (SD: 163.9; t-statistics: 2.768; t_{95}: 1.984; 95% CI: 225.4-290.9; p: 0.0066). The median level of FBN in the control group B was 213 mg/l. Plasma levels of the women with malignancies were significantly elevated when compared to the control group (Table 1, Figure 1). The distribution of values showed a statistically important "tail" in high plasma levels (FBN >400 mg/l). When analysing FBN plasma levels separately for each malignancy (Table 1), and designing the opposite histograms (Figure 2) some interesting results emerged. Plasma levels of FBN were quite more increased in breast and cervical malignancies when compared with ovarian and endometrial cancers. Characteristically, in breast cancer not only the median value of FBN was elevated, but the distribution was shifted to values up to 900 mg/l. On the contrary, the maximal value of FBN in endometrial cancer was 400 mg/l and in ovarian 490 mg/l. Furthermore, in the subgroups of endometrial and ovarian cancers no statistically significant difference was observed. For ovarian malignancies the

Table 1. Analysis of fibronectin plasma values of the whole studied group and of each malignancy separately

	MD (mg/l)	*SD*	*Degrees of freedom*	*t-statistics*	*p-value*	t_{95}	*95 % CI*
Group A (n=100)	258.4	0.0066	99	2.768		1.984	225.4-290.9
Cervical cancer (n=20)	266.65	188.3	19	1.272	0.218	2.043	178.4-354.7
Endometrial cancer (n=20)	217.9	129.0	19	0.171	0.866	2.093	157.5-278.3
Ovarian cancer (n=20)	228.3	90.1	19	0.778	0.446	2.043	186.3-271.3
Breast cancer (n=40)	289.3	186.5	39	2.589	0.0067	2.022	229.7-348.9

MD: median, SD: standard deviation, t-statistics: Student's t-distribution, t_{95}: two-sided Student's factor for 95% CI, CI: confidence interval

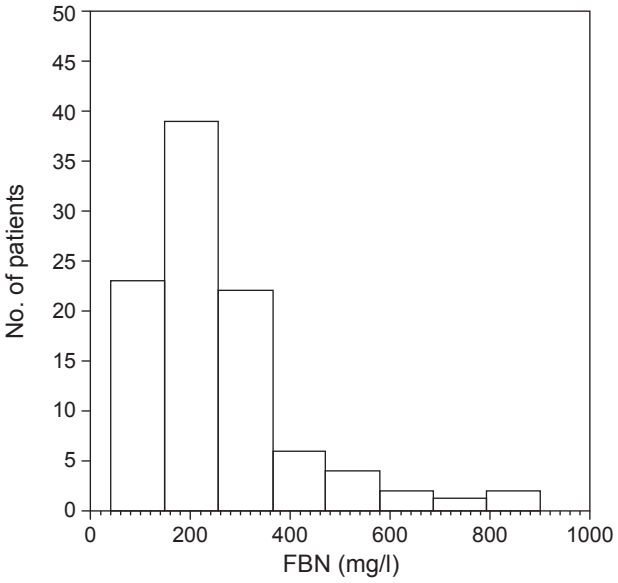

Figure 1. Distribution of median values of fibronectin (FBN) plasma levels in patients with gynecological malignancies.

average plasma FBN level was 228.3 mg/l (SD: 90.8; t-statistics: 0.778; p=0.446; t_{95}: 2.043; 95% CI: 186.3-271.3), whereas for endometrial cancer the average was 217.9 mg/l (SD: 129.0; t-statistics: 0.171; p=0.866; t_{95}: 2.093; 95% CI: 157.5-278.3) also not statistically significant compared with the control group. The subgroup with breast cancer displayed statistically significant different FBN plasma levels compared with the control group. The median value was 289.2 mg/l (SD: 186.5; t-statistics: 2.589; p=0.0067; t_{95}: 2.022; 95% CI: 229.7-348.9). In cervical cancer the median value of plasma FBN level was 266.5 mg/l (SD: 188.3; t-statistics: 1.272; p=0.218; t_{95}: 2.043; 95% CI: 178.4-354.7), without statistical difference compared to the control group.

Discussion

The association of increased FBN plasma levels with gynecological cancers has been investigated by a

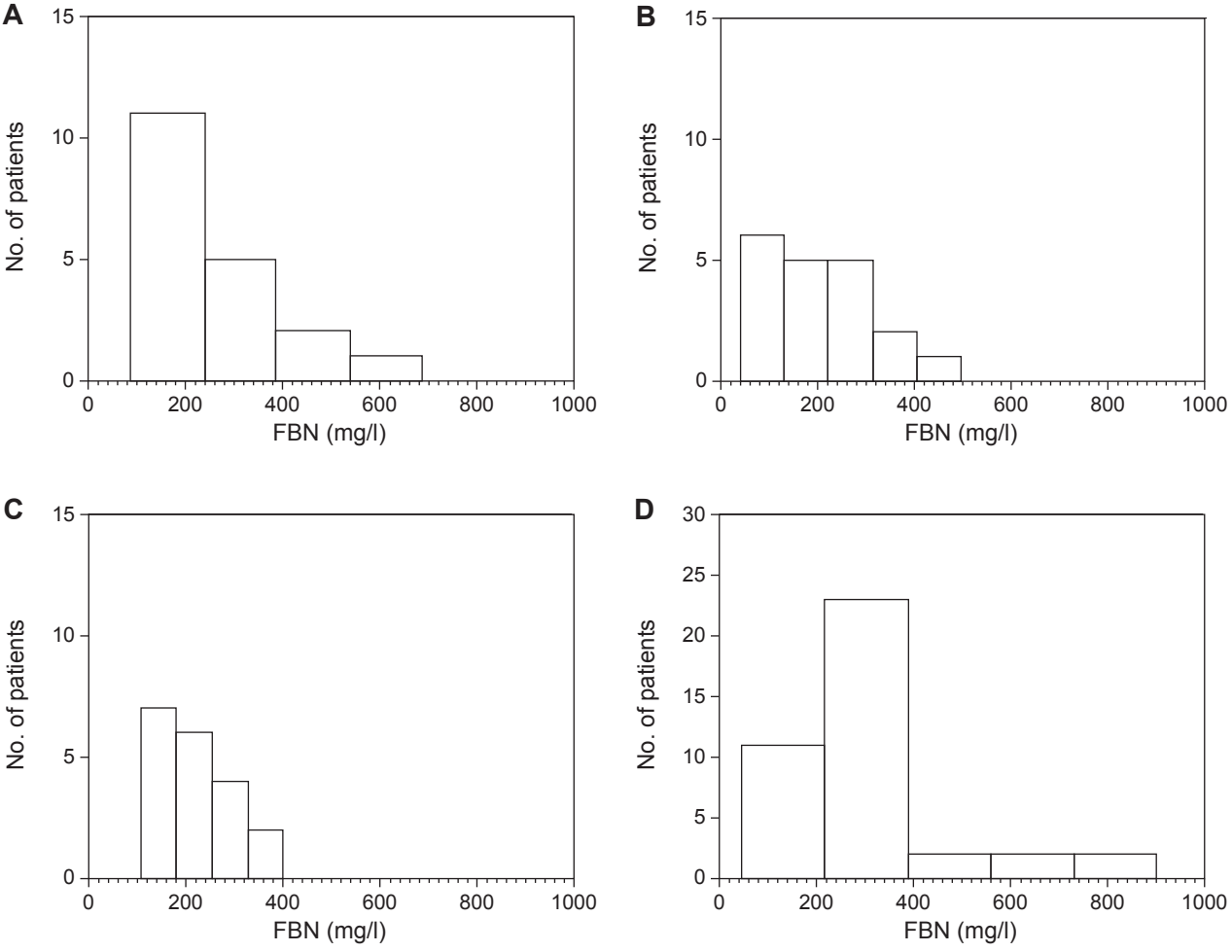

Figure 2. Distribution of median values of fibronectin (FBN) plasma levels in patients with: **A**) cervical, **B**) ovarian, **C**) endometrial and **D**) breast malignancies.

number of authors till today. It has been reported that there might be a statistically positive association between this extracellular molecule and the gynecological malignancies, so that FBN plasma levels could serve as a diagnostic marker for the above mentioned malignancies.

Ricciardelli and Rodgers [3] described elevated FBN levels in women with ovarian cancer. Furthermore, Tan et al. [12] found that the increased cancer cell migration of endometrial adenocarcinoma could be associated with FBN. Concerning the pathogenesis of the ovarian cancer cell migration, it seems that cancer cells migrate into the peritoneum through the integrins, which interact with proteins of the extracellular matrix, e.g. FBN. According to the results of the present study in women with ovarian cancer the FBN levels were increased although not significantly. Possibly the elevated values could be proven statistically significant in studies with larger numbers of patients.

Concerning the association between FBN and cervical cancer, it has been supported that the integrins as well as the EGF are involved in the disease progression. Yang et al. [4], Goldberg et al. [5], and Moro et al. [6] reported that the integrins and EGF promote the interaction between cancer cells and extracellular proteins, e.g. FBN. The result is an increased motility and migration of malignant cells. This has also been supported by Bill et al. [7] and Pichard et al. [8], who investigated the role of EGF and its receptor (EGFR) in the pathogenesis of cancer cell migration. In addition, Mitra et al. [9] described a decreased cancer cell migration after administration of antibodies against the integrin alpha-5 beta-1, which is the most frequent FBN receptor and binds to the RGD FBN tripeptide. Relatively recently, Hauck et al. [10] reported that FAK (focal adhesion kinase) inhibitors decrease cancer cell migration. FAK is located in the cancer cells and plays an important role in the interaction between malignant cells and extracellular proteins, like FBN.

Our data support the current evidence concerning the possible role of extracellular matrix proteins, like FBN, in cancer cell motility and invasion. In women with cervical cancer the FBN plasma levels were higher than in the control group.

The relationship between endometrial cancer and adhesion molecules, like FBN, has been studied by a number of investigators who think that many different hormones and other factors are involved in the development of cancer cell migration in association with extracellular substances. A-beta estradiol, estriol, progesterone, medroxyprogesterone acetate, danazol, EGF, TGF-a and TGF-b are involved in the migration and peritoneal tumor cells invasion. Ueda et al. [11] have described a decreased endometrial cancer cell interaction with FBN with the use of medroxypregesterone acetate and danazol resulting in cancer cell migration and invasion inhibition. On the contrary, the tumor cell interaction with FBN was increased after the use of EGF and TGF-a. Estriol, progesterone and TGF-b seem to have no effect on the motility and migration of tumor cells. Tan et al. [12] and Wunsche et al. [13] have also described the same effects on cell migration and invasion. The decreasing effect of progesterone on cancer cell migration and invasion seems to be induced by the decreased levels of adhesion extracellular molecules, like integrins alpha-3, beta-1, beta-3, cadherin-6 and FBN.

Recent studies of the human genome suggest that progesterone inhibits the effect of nuclear factor kB (NF-kB) which is characterized by a strong antiproliferative activity. Davies et al. [16] claim that the effect of progesterone on some genes results in the production of other proteins (A2O and ABIN-2) which inhibit the ability of NF-kB transcription. Of note, genes like TRAP-1 and SMAD-4 that encode anti-inflammatory proteins are activated by progesterone, whereas genes encoding pro-inflammatory proteins are inhibited by progesterone according to the studies of Davies et al. [14,15].

In our study women with endometrial and ovarian cancer were not found to have significantly elevated levels of plasma FBN. Based on our results it appears that FBN could be ignored as a potential marker for endometrial and ovarian malignancies. However, studies with larger number of patients are required in order to statistically confirm this result. In contrast, the levels of plasma FBN were statistically increased in breast cancer cases compared to the control group. In their reports Boccardo et al. [17] and Ruelland et al [18] also described increased levels of FBN in women with breast cancer.

As mentioned before in the total group of malignancies the FBN plasma levels were significantly elevated compared with the control group. FBN seems to be strongly associated with cancer cell migration in cases of gynecological malignancies. Concerning the results of our study, FBN plasma levels could represent a new potential diagnostic marker. Further investigations with larger patient groups should answer the question whether FBN could reliably be a marker for gynecological cancers.

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