Can Osteopontin Be Considered a Biomarker for Endometriosis?

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Endometriosis is a multifactorial disease that is manifested by infertility and pelvic pain. The purpose of the s*tudy was to evaluate the effect of progesterone treatment on the serum level of osteopontin, a multipotent cytokine, in patients with endometriosis. The study was prospective and we evaluated osteopontin levels that were measured in the serum of 40 patients with endometriosis and 12 healthy women using a standardized Enzyme-Linked Immunosorbent Assay (ELISA) kit. Osteopontin seric levels were lower in endometriosis patients and increased after progesterone treatment. Because of the large dispersion of data even in the control group, we find the association between osteopontin and endometriosis questionable.*

Keywords: endometriosis, osteopontin biomarker, progesterone, endometriosis treatment

Endometriosis is a debilitating disease, even though it often leaves no visible signs. Despite of its devastating effects on the women's body, endometriosis can be a master of disguise in terms of symptoms. Endometriosis is thought to be a cumulative factor disease, triggered by genetic factors-polygenically inherited disease, immunological, hormonal and environmental factors [1,2]. Thus, endometriosis could be defined as a multifactorial illness. The only certain way to diagnose endometriosis is by laparoscopy, and the golden standard is represented by histological confirmation [3,4]. Treatment for endometriosis ought to include a series of objectives mainly the pelvic pain and infertility. This can be done by aiming the prevention or delaying endometriosis, through surgical or medical therapy [2,4,5].

Oldber et al. firstly discovered in 1986 a sialoprotein that forms a strong bond to hydroxyapatite and decided to call it osteopontin (OPN), from the latin *pons,* by this suggesting its role as a binder between cells and the mineral in the matrix via Arg-Gly-Asp sequence [6]. Since its discovery, studies have shown the presence of OPN in other tissues except bone, like kidney, ovary, uterus, lactating breast [7]. Besides its role of cell-adhesion protein, OPN was reported to act as a cytokine and, also as a cell differentiation antigen [8,9]. The pathophysiologic substrate is based on chronic inflammation, in which, it has been proven that OPN has an important role [10-12]. Consequently it was hypothesized that OPN could play a role in the development of endometriosis, through its contribution in cell migration, attachment and invasion [8,13].

Also, chronic inflammation was showed to be associated with progesterone resistance in patients with endometriosis [14]. It is well known that outside of pregnancy, in the follicular phase of the menstrual cycle, the low level of progesterone is causing the endometrial tissue to break down and to install menstruation. This phenomena is caused mainly through an increase in local proinflammatory cytokines, chemokines and MMPs [15,16]. Among this proinflammatory cytokines lies OPN, and it has been showed that progesterone and OPN play intricate roles in the pathogenesis of endometriosis. Some studies investigated the regulation of OPN by progesterone, because OPN is induced by progesterone in mice [17], and the periodicity of endometrial expression of OPN is progesterone-influenced [18].

Our study tracked the OPN levels in the serum of women with endometriosis, in direct correlation with progesterone treatment and surgical cure and in comparison with the OPN serum levels of healthy women.

Experimental part

Material and methods

This is a prospective, case-control study that tried to establish the underlying cumulative risk factors of endometriosis. The study group included 52 patients aged between 21 and 42 years old were included in the study. The inclusion criteria were: adult age (18+ years), a clearly defined diagnosis of endometriosis-caused infertility. The study did not include women with body mass index >30, neoplasms, autoimmune diseases, diabetes mellitus, infectious disease, pregnancy in evolution, under anti-inflammatory or hormonal treatment for other diseases, depression and treatment for depressive disorders, Cushing syndrome, Turner syndrome.

Every patient enro--lled in the study signed a written informed consent. A form of the consent was approved by the Commission for Medical Ethics of the University of Medicine Grigore T.Popa Iasi.

The blood was collected from the patients in three essential moments: M1= the day of the diagnosis; M2= 6 months after diagnosis, in the day of the surgery, and M3= 6 months after surgery. The patients were as follows: patients investigated in M1 were all without treatment (40 patients without treatment and 12 patients control group). Patients investigated in M2, were divided as following: 24 patients without treatment prior to surgery, 16 patients that followed treatment with 0.075mg desogestrel daily for six months. In M3, 6 months after surgery, the patients were divided again in 3 groups: 15 patients that did not followed a treatment, 25 patients that followed treatment after surgery with 0.075 mg desogestrel, daily for six months

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(16 of them continued the treatment with desogestrel and 9 of them started the treatment with desogestrel immediately after surgery). The patients were monitored monthly both by clinical and sonographic examination, by evaluating the dimension, homogeneity, shape, the presence of horizontal level, the vascularization of the cysts, its position relative to the uterus and the contralateral ovary. All the patients were examined by trans-vaginal ultrasonography using Doppler mode every three months.

Six mL of blood were collected in Clot Accelerator Tubes from every person included in the study. The blood samples were centrifuged on 4500 rpm, 5 min. The serum was collected, aliquoted and frozen within one hour after the sample collection, and kept at -20ÚC until analyzed. Serum OPN was quantified using a Human Osteopontin ELISA kit (RAB0436, Sigma-Aldrich, USA) according to the manufacturer's instructions. We choose the 4ºC, overnight incubation procedure and used undiluted serum samples and a Heidolph Titramax 1000 Plate Shacker set at 1.5 cycles/s. The final absorbance was read at 450 nm using a Bio Rad spectrophotometer. Results were calculated by the software by plotting the mean absorbance of the samples on a standard curve generated with standard concentration solution provided by the kit producer.

Statistical analysis

The data was processed using the statistical functions SPSS 18.0 at a significance level of 95%.

Significance tests used were: the χ^2 test, the t-Student test, the $F_{(ANOVA)}$ test and the *Pearson* (r) correlation coefficient.

Results and discussions

At M1, OPN levels varied between <74 to 879.24 pg/mL in the patients with endometriosis and from <74 to 1084.30 pg/mL in the control group, recording a lower mean level in patients with endometriosis (220.15 vs 337.22 pg/mL; p=0.373).

At M2, OPN varied between <74 and 569.39 pg/mL in untreated patients with endometriosis and between <74 and 1740.50 pg/mL in the subgroup of patients with endometriosis that followed treatment. The analysis of these results showed that the medium level of OPN is significantly higher in patients with endometriosis that underwent treatment (455.13 vs 186.72 pg/mL; p=0.05).

At M3, the OPN level ranged between <74 la 222.20 pg/mL in patients with the illness that didn't followed treatment and varied between <74 la 5706.30 pg/mL in the subgroup of patients that followed treatment, recording a medium level significantly higher in patients with endometriosis that followed treatment (1178.13 vs 114.25 pg/mL; p=0.001) (table 1).

The management of endometriosis can be considered in two different manners, separately or in association: the surgical cure and the medical therapy of endometriosis.

The first step of our study was to detect the serum level of OPN in all the women included in the study. We compared the level of OPN between these two groups in M1, and obtained results that showed values, with a mean level of OPN in the endometriosis group of 220.15 pg/mL and 337.22 pg/mL in the control group.

The second step was to divide the patients with endometriosis intro two subgroups, and test the level of OPN in the moment prior to surgery, 6 months after diagnosis. We observed that in the subgroup that followed treatment for 6 months with 0.075mg desogestrel daily, the mean level of OPN was 2.43 times higher than in the subgroup without treatment. Also, we observed that compared to the control group the subgroup with untreated endometriosis have a low level of OPN, and in the subgroup that followed treatment with progesterone, the mean level of OPN is higher. Thus, OPN seems to be highly influenced by progesterone.

The third step was to test the OPN level of patients that underwent surgery, 6 months after the intervention. We subdivided them in two groups, the first one including the ones that followed treatment with 0.075 mg desogestrel once a day after the intervention, and the second subgroup that included the ones who did not follow a medical treatment. We tested the women in the subgroup without

Table 1

DESCRIPTIVE STATISTICAL CHARACTERISTICS OF OPN

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max	p F_{ANOVA} test
					Lower Bound	Upper Bound			
M1									
endometriosis group	40	220.15	263.89	87.96	17.31	422.99	<74	897.24	**0.373**
control group	12	337.22	309.69	89.40	140.46	533.99	<74	1084.30	
M2									
without treatment	24	186.72	160.83	37.91	106.74	266.70	<74	569.39	**0.050**
with treatment	16	455.13	523.21	165.45	80.85	829.42	<74	1740.50	
M3									
without treatment	15	114.25	62.22	27.83	36.98	191.51	<74	222.20	**0.001**
with treatment	25	1178.13	857.00	656.55	674.36	1730.61	<74	5706.30	

any medical treatment to see if surgery alone can have a major influence on the biomarker OPN serum levels and found out that, 6 months after surgery, the mean value of OPN was 114.25 pg/mL, with a range of <74 to 222.20 pg/mL. We can see that in the subgroup without treatment that underwent surgery and was tested 6 months after the intervention, compared with the group without treatment tested in the moment before surgery, the level of OPN decreased but this drop was almost insignificant. Comparing these results with the control group and the endometriosis group, we can see that all these results are alike, with very low variations between them.

Our study showed that progesterone treatment of the patients with endometriosis, either before or after surgical treatment, increased the serum levels of osteopontin. The patients that were treated only by surgical means had a low level of OPN 6 months after the surgery (M3) comparative with those that followed treatment with oral desogestrel after the intervention. In this last subgroup, the mean level of OPN was 10.31 times higher than in the subgroup without progesterone administration and significantly higher than the mean OPN level of the control group.

These results are somehow curious, because OPN levels seem to be more influenced by the progesterone than by the illness itself.

The medical therapy of endometriosis includes different products, all having the same background effect. However, these products have a series of particularities that distinguish one from another. For example, the major disadvantage for the use of medical treatment in spite of the surgical one is represented by the inability to obtain a pregnancy as long as the women are following it, most of these drugs being used as effective contraceptives [2,4,19].

In terms of surgical treatment, for the intervention to be successful, it is necessary to be preceded by extensive imaging investigation [20]. More than that, the variability of the endometriosis lesions can mislead the surgeon or can be hardly recognizable, especially if we talk about deep lesions or peritoneal small lesions. That is why, we could say that the surgical treatment of endometriosis is operator dependent and its success varies depending on the surgeon‘s skills and experience. Therefore, surgery often fails to remove all the present endometriosis, and by this failing to improve pain and other symptoms in a series of patients [2,21].

Moszynski et al., in 2013, conducted a study on OPN and found out that the median OPN level was significantly lower in the serum of women with endometriosis than in women with other types of benign ovarian pathology, with a range between 4.82-106 pg/mL [22]. These results are consistent with the ones we obtained and, contrary to what Cho et al. obtained in their study in 2009, that showed higher levels of OPN in patients with endometriosis than those without the disease [1].

In the literature, there are plenty of sources that described the role of OPN in several autoimmune diseases such as multiple sclerosis [23] Crohn‘s disease [24] and some types of cancer [10,25]. Endometriosis itself is underlined by a chronic inflammatory process, which means that the immune system plays a role in its development [26].

Conclusions

Our results showed OPN does not seem to be useful as a biomarker in the investigation of endometriosis, irrespective of its expression in endometrial tissue. We are basing our assertions on two facts: firstly, after the surgery, moment in which the patient is at least hypothetically cured, the OPN levels did not seem to suffer major variations compared with the control group and secondly, OPN levels seems to be highly influenced by the treatment with progesterone (desogestrel). It is known that endometriosis is partially caused by progesterone resistance and the loss of progesterone signaling in the endometrial tissues. Further studies should continue to investigate the exact mechanism by which progesterone resistance that is found in endometriosis and the serum levels of progesterone are affecting the levels of OPN, and what are the implications of these intricate connections.

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