ROLE OF THE PRESERVATION OF VEGETATIVE INNERVATION OF THE SMALL PELVIS IN THE SURGICAL TREATMENT OF DEEP INFILTRATING ENDOMETRIOSIS, AND FUNCTION OF SNCG (Γ-SYNUCLEIN) OF DISEASE FORMATION

PhD thesis

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1. Introduction

1.1. Pathogenesis of endometriosis

The topic of the pathogenesis of endometriosis is a question of abundant reasearch even today. In order to explain the origin of the disease many hypotheses have been brought to light, of which Sampson's theory of retrograde menstruation is the most widely known explaining peritoneal endometriosis. Furthermore, to understand different phenotypes of the disease other, non-endometirial hypotheses were born in the last decades, such as the metaplasia, the induction theories, the impact of Muller remnants, the metastatis, the problems with embryonal development as a cause and also the stem cell theory wich all are aiming to explain the pathogenesis of endometriosis.

Based ont he research on the pathogenesis of endometriosis genetic predisposition, environmental factors and eating habits can all have on impact on the development of the disease. Changes in the hormonal environment of the small pelvis leading to hyperestrogenaemia and progesterone resistance, elevation of the white blood cell count in the peritoneal fluid containing citokines and growth factors as well, increased oxidative stress, the abundance of proangiogenic molecules and the dominance of remodelling, and also, the changes of the immune system all can participate in the appearance of the disease.

1.2. The γ-synuclein

The γ-synuclein (SNCG, breast cancer-specific protein-1) is a member of the synuclein protein family. It is a 13kDa molecular weight oncogenic and chaperone protein that contains 123 aminoacids. Under physiological circumstances it is present in the retina, the ophactory epithelium, the peripherial nervous system, the primary sensory neurones, the sympathetic and motoneurones, the cardiac and skeletal muscles, the pancreas, the liver and the endometrium. Its physiological function in unknown.

According to the literature, due to its impact on cellular proliferation, hormone-dependent signalling, angiogenesis and remodelling it might have a role in the pathogenesis of different, gynecological and non-gynecological malignancies.

Since these processes listed previously might play a role in the pathogenesis of endometriosis as well, scientists have proposed the impact of SNCG on the pathogenesis of endometriosis, too. In human ovarian endometriosis histological samples SNCG expression was shown to be higher compared to its expression in eutopic endometrium. In an endometriosis animal model, after the administration of a γ-synuclein-inhibitor endometriosis leasions shrunk and their vascularisation tended to decrease as well. However, the data ont he relationship between SNCG and the pathogenesis of endometriosis is limited for now.

1.3. Treatment of endometriosis

Treatment of endometriosis in individual, the choice of therapy is based on the age of the patient, her desire to concieve and her symptoms. Since up till now the definitive diagnosis of endometriosis requires surgery and the accuraccy of imaging modalities is limited, in western Europe and in the US an average of 8 to 12 years pass between the first symptom and the definitive diagnosis. This leads to rather advanced disease at the time of surgery in rather high percentage of patients. Besides the general complications linked to surgery, the chances of nerve infiltration by endometriosis and also, iatrogenic nerve damage due to surgery – both leading to autonomic dysfunction – need to be taking under consideration when consulting the patient. In order to decrease the chances of complications and to optimize the their postoperative quality of life, nerve-sparing surgical approach has been introduced.

The goal of nerve-sparing surgery is to preserve the superior hypogastric plexus, the hypogastric nerves and the inferior hypogastric plexus, by which postoperative vegetative functions such as passing stool, urination and sexual functions should be preserved as much as possible. By extending our knowledge on the localization and function of autonomic nerves and plexuses and lowering the radicality of our surgeries out goal in therapy is not only the treatment of pain and infertility but ensuring the best possible postoperative vegetative function and quality of life of our patients.

2. Objective

1. Based on the previous data on the biological effect of SNCG we proposed to examine the role of γ-synuclein on the pathogenesis and progression of endometriosis. Our goal was to ascertain the presence and concentration of SNCG in the peritoneal fluid and plasma of patients with endometriosis and healthy controls.

2. Furthermore, we proposed to examine the postoperative vegetative bladder function of the first 50 patients who underwent nerve-sparing laparoscopic surgery for deep infiltrating endometriosis.

3. Matherials and methods

3.1. Patients

Between January 2016 and December 2016, 45 patients awaiting laparoscopies were enrolled in our study. Based on the intraoperative findings patients were divided into three groups: the control group which did not show any sign of endometriosis during surgery, and patients with endometriosis were divided into two groups based on their rAFS scores (minimal-mild and moderate-severe endometriosis groups). Preoperative hormonal treatment (oral contraceptives, dienogest, GnRH analogues) and presence of fibroids were our exclusion criteria.

For the protection of our human subjects, the study protocol was approved by the Institutional Ethical and Review Board of Semmelweis University in Budapest, Hungary. Informed consent was obtained from all of the patients before they were entered into this study (No: 143/2008).

3.2. Detection of SNCG in the peritoneal fluid and plasma of our patients

In our study we collected plasma and peritoneal fluid of patients. Blood samples were obtained right before surgery, before the administration of any anaestethic drugs. Blood samples were centrifugated at 1811xg for 10 minutes at 4°C. Plasma samples were then stored at -80°C in our biobank. During the collection of peritoneal fluid special care was taken to avoid blood contamination. Our samples were centrifugated at 200xg for 10 minutes at room temperature and were also stored -80°C in our biobank. SNCG concentration of the samples were determined using ELISA (SEA939Hu, Cloud-Clone Corp., Houston, Texas, USA).

3.3. Statistical analysis

Based on the results of Shapiro-Wilk test γ-synuclein showed normal distribution in the peritoneal fluid and not normal distribution in the plasma samples. Mann-Whitney U test was

used to compare SNCG levels. Results were considered statistically significant when p value < 0.05. For statistical examination Statistica 8.0 software was used.

3.4. Retrospective analysis of vegetative bladder function

Between March 31st 2004 and March 31st 2015, 50 nerve-sparing laparoscopic bowel resections were performed at our hospital. The retrospetive analysis on the vegetative bladder function was obtained on these patients. All surgeries and follow-up was performed by the surgeon himself. Anamnestic and surgical data was obtained using the IT system at our hospital.

4. Results

All of the patients who participated in our study were of reproductive age. Their age was 31±9.5, 33±6 and 33±4 years in control, minimal-mild and moderate-severe endometriosis groups. Regarding age, anamnestic data and BMI the three gropus of patients were comparable. The indication for surgery was infertility, ckronic pelvis pain, primary amenorrhoea and bleeding disorder. Statistical power was 0.98. Anamnestic data and symptoms of the patients is shown on Table 1.

Table 1. Demographic and clinical data of the patients (BMI: body mass index).

	Control	**Stage I–II**	**Stage III–IV**	**p value**
Age (years, median)	33.5 (25–36)	33 (27–37.5)	32 (30–37)	0.919
BMI (kg/m^2, median)	22.3 (21.6–27)	21.8 (20–24)	22.3 (21–23.9)	0.482
Gravidity n (%)				
0 n (%)	8 (53)	10 (67)	10 (67)	0.68
1 or more n (%)	7 (47)	5 (33)	5 (33)	0.68
Previous surgery n (%)				
Laparoscopy n (%)	3 (20)	3 (20)	4 (27)	0.88
Laparotomy n (%)	3 (20)	2 (13)	2 (13)	0.84
Gynaecological symptoms				
Infertility n (%)	2 (13)	8 (53)	4 (27)	0.055
Abnormal uterine bleeding n (%)	4 (27)	1 (7)	0 (0)	0.054
Pelvic pain n (%)	7 (47)	11 (73)	14 (93)	0.018
Classification of endometriotic lesions				
No endometriosis n (%)	15 (100)	0 (0)	0 (0)	
Superficial n (%)	0 (0)	12 (80)	15 (100)	<0.0001
Endometrioma n (%)	0 (0)	1 (6.7)	11 (73.3)	
Deep infiltrating endometriosis n (%)	0 (0)	4 (26.7)	12 (80)	

Concentration of γ-synuclein was statistically significantly higher in the peritoneal fluid of patients with endometriosis compared to controls (p=0.04). SNCG levels in the peritoneal fluid were 1.2-fold higher in case of endometriosis compared to patients free from the disease. Figure 1. shows the γ-synuclein levels in the peritoneal fluid in the study groups.

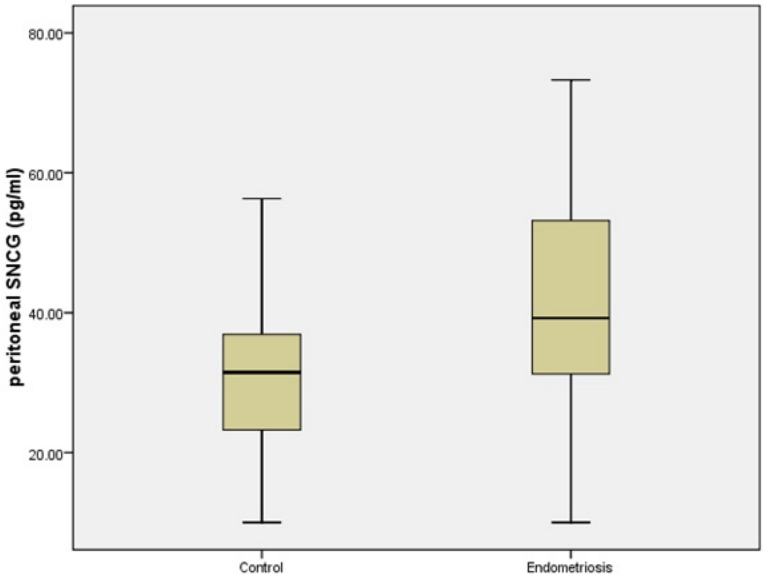

Figure 1. SNCG levels in the peritoneal fluid of control group and patients with endometriosis

Regarding the plasma SNCG levels no significal difference was shown between the study groups. γ-synuclein was present in 40% (6 patients) of the control, 26.7% (4 patients) of stage I-II and 13.5% (2 patients) of stage III-IV endometriosis groups. Figure 2. shows SNCG levels in the plasma in the study groups.

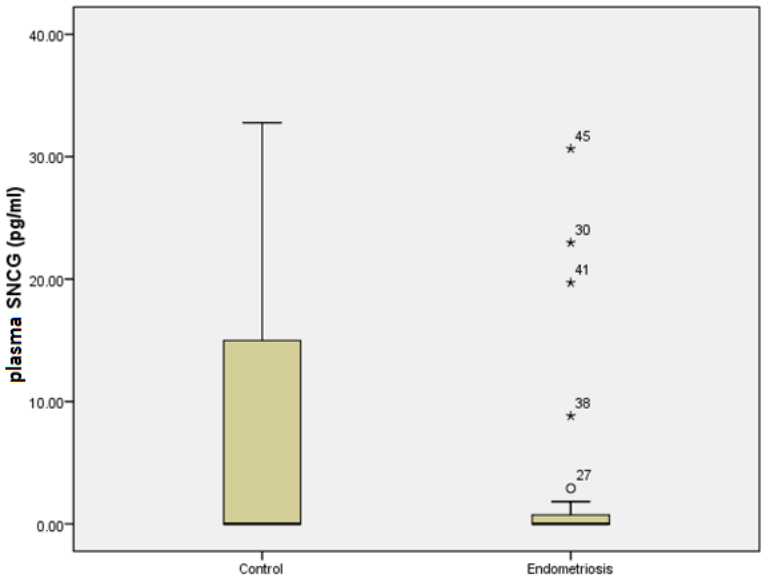

Figure 2. Plasma SNCG levels in the control gruop and in patients with endometriosis

When examining the peritoneal fluid SNCG levels we discovered that γ-synuclein was not only present in patients with endometriosis but in 100% of the control group as well. SNCG concentrations in the peritoneal fluid and plasma of different study groups are shown on Table 2.

Table 2. Revised American Fertility Society (rAFS) scores and gamma synuclein (SNCG) levels of the disease-free patients and the endometriosis patients, and the SNCG levels of the different endometriosis phenotypes [p^a = p value between the controls and all of the endometriosis patients, p^b = p value between the stage I–II and stage III–IV endometriosis patients, and p^c = p value between the superficial + ovarian endometrioma (OMA) and deep infiltrating endometriosis (DIE) cases].

	Control	**All endometriosis cases**	**Stage I–II**	**Stage III–IV**	**Superficial + OMA**	**DIE**	**p value**
rAFS (median)	0 (0–0)	10 (7–41)	7 (4–8)	41.5 (26–61)	7 (4–10)	36.5 (17–61)	p^a=0.0018 p^b=0.1031 p^c‹0.0001
SNCG in peritoneal fluid (pg/ml)	31.5 (26–36.9)	39 (31–53)	36.9 (30.4–53)	39 (31.5–53.5)	43.8 (31.7–55.4)	34.3 (31–45)	p^a=0.04 p^b=0.66 p^c=0.94
SNCG in serum (pg/ml)	10.7 (21.6–27)	0 (0–0.4)	0 (0–0.7)	0 (0–0)	0 (0–0)	0 (0–8.8)	p^a=0.086 p^b=0.97 p^c=0.46

Anamnestic data ont he first 50 nerve-sparing bowel resection is shown in Table 3, intraoperative data on Table 4.

Table 4. Anamnestic and demographic data of the first 50 patients operated by nerve-sparing bowel resection between 2004 and 2015, at Semmelweis University Ist Department of Obstetrics and Gynecology

	No of cases (n)	**%**	**median±SD**
Age (years, median)	-	-	31,5 (23-43)
Pregnancy			
0	40	80	ϟ 0 (0-1)
1	10	20	
Infertility n (%)			
yes	30	60	-
no	20	40	-
Previous surgeries n (%)			
no	15	30	
1	25	50	ϟ 0 (0-3)
2 or more	10	20	

Table 5. Intraoperative data of the first 50 patients operated by nerve-sparing bowel resection between 2004 and 2015, at Semmelweis University Ist Department of Obstetrics and Gynecology

Localization	**No of cases (n)**	**%**
Sigma	8	16
Sigma + rectum	19	38
Rectum	25	50
Sigma + ileum	6	12
Sigma + appendix	2	4
Ileum	1	2
Coecum	2	4
Vagina	6	12

According to our retrospective analysis we detected temporary bladder dysfunction in 6 patients (12%). Patients who experienced vegetative dysfunction required self-catheterisation for maximum 7 days, after one week spontaneous voiding was successful in all patients. Due to nerve-sparing approach we detected no permamnent bladder-disfunction.

5. Conclusions

We are the first to present that SNCG expression in the peritoneal fluid of patients with endometriosis is statistically significantly higher than in the disease-free control group ($p=0.04$). Concentrations of γ-synuclein in the peritoneal fluid of the endometriosis groups was 1.2-fold higher compared to the control group. However, regarding plasma SNCG levels we did not detect any significant differences between patients with endometriosis and controls

Previous data on SNCG expression was performed in a local manner, on histological samples in endometriosis cases and in malignant tumours as well. No information on the detection of γ-synuclein concentrations in peritoneal fluid or plasma samples are available in the literature neither in benign nor in malignant diseases. Hovewer, our results are in line with previously published data on elevated expression of SNCG in ectopic endometrium.

Based on our results we suppose that elevated SNCG expression in the peritoneal fluid of patiens with endometriosis plays a role in the pathogenesis and progression of the disease. According to previous data γ-synuclein has an impact on disease formation via multiple pathways, since its elevated expression inhibits BUBR1 serin/threonine mitotic checkpoint kinase, leads to induction and elevated expression of MMP2 and MMP9 enzymes and it enhances expression of ERα, too. As a result SNCG has an impact on mitotic activity, stimulates remodelling, hormone-dependent signalling and neoangiogenesis by which it can play a role in the pathogenesis and progression of endometriosis. According to previous research on the pathogenesis of endometriosis, many factors (such as proinflammatory citokines) interfere with disease formation and progression in a local manner. Our results suppose that γ-synuclein acts locally as well. Our study shows that SNCG expression is elevated in the peritoneal fluid of patients with endometriosis while no changes were detected of its expression in the plasma. According to these results γ-synuclein acts locally on the pathogenesis of the disease.

Based on the previous data of the literature in physiological circumstances SNCG is present in the retina, the ophactory epithelium, the peripherial nervous system, the primary sensory neurones, the sympathetic and motoneurones, the cardiac and skeletal muscles, the pancreas, the liver and the endometrium. In studies on ovarian histological samples authors have found no expression of γ-synuclein in healthy ovarian tissue but appearance of the protein in praecancerous and malignant conditions. In contrast, according to our results SNCG was present in the peritoneal fluid in physiological circumstances as well, we detected a 100%

positivity in the peritoneal fluid of endometriosis-free control patients. Even though our results seem to contradict previous results in the literature, comparison of these has its limitations due to the differences of the techniques used (immunhistochemistry versus ELISA). Moreover, the exact source of SNCG in the peritoneal fluid in our healthy patients is not known. Hovewer, our results raise questions regarding the physiological function of γ-synuclein. Its biological function needs to be further investigated.

Definitive diagnosis and (temporary) curative treatment of endometriosis is only possible via surgery. Diagnosis of the disease is still a challange since the sensitivity and specificity of imaging methods is limited. As a consequence, between the first symptom of the patient till definitive diagnosis of the disease a decade might pass. Due to these circumstances at time of surgery we are facing advanced disease in many cases which can lead to severe complications regarding the patients postoperative quality of life. Beyond the well known, ordinary complications of surgical interventions postoperative autonomic function of the patients tend to have increased focus since it has an impact on their long-term quality of life. Even though endometriosis with nerve-infiltration can lead to vegetative dysfunction by itself, radical surgery can lead to iatrogenic nerve damage which can worsen autonomic functions as well. In order to preserve vegetative innervation nerve-sparing surgical approach has been introduced.

In our study we collected the postoperative data of patients who underwent nerve-sparing laparoscopic bowel resection between March 31st 2004 and March 31st 2015 at our hospital. During this time period we performed 50 bowel resections using nerve-sparing approach. Temporary bladder dysfunction was detected in 6 cases (12%) of our patients, all of which resolved spontaneously in maximum 7 days. No patient required permanent self-cathererisation in our study group. Our results are comparable with the data seen in the literature and confirm the importance and usefulness of nerve-sparing approach in order to decrease the postoperative morbidity and to optimize quality of life of our patients. Table 5 summarizes the exprerience and results of the founding teams of nerve-sparing surgery and the results of our team concerning postoperative bladder dysfunction.

Table 5. Need for self-catheterisation after nerve-sparing surgery

Authors	**No of patients**	**No of patients requiring self-catheterisation (n) and their proportion compared to the whole study group (%)**	**Average time frame while self-catheterisation was required (days)**
Volpi et al.	24	7 (29.2%)	18
Possover et al.	91	0 (0%)	-
Kavallaris et al.	16	8 (18.5%)	no data available
Ceccaroni et al.	61	no data available	39.8
Bokor et al.	50	6 (12%)	≤ 7

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