

Peritoneal fluid leptin is associated with chronic pelvic pain but not infertility in endometriosis patients*

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BACKGROUND: Leptin influences the proinflammatory immune responses and has angiogenic activity *in vitro* and *in vivo*. The objective of this study was to evaluate the peritoneal fluid levels of leptin in patients with endometriosis and idiopathic infertility and compare them with a control group of tubal ligation/reanastomosis patients. **METHODS:** In this observational, prospective controlled study, peritoneal fluid from 108 women was obtained while they underwent laparoscopy for pelvic pain, infertility, tubal ligation or sterilization reversal. We measured the concentration of leptin in the peritoneal fluid and compared the levels among women who were divided into groups according to their post-surgical diagnosis. Sixty patients were diagnosed with endometriosis, 10 with idiopathic infertility and 38 had undergone tubal ligation or reanastomosis (control group). **RESULTS:** Peritoneal fluid leptin was significantly higher in endometriosis 14.62 ± 9.79 (mean \pm SD) ng/ml compared to idiopathic infertility [0.92 ± 1.57 ng/ml ($P = 0.0007$)] and to controls [0.78 ± 1.94 ng/ml ($P < 0.0001$)]. Leptin levels were positively correlated with the stage of endometriosis ($r = 0.45$; $P = 0.03$), and with pelvic pain in endometriosis patients ($r = 0.49$; $P = 0.001$). Peritoneal fluid leptin levels in patients with idiopathic infertility were comparable to controls. **CONCLUSIONS:** Higher levels of leptin were observed in peritoneal fluid of patients with endometriosis compared to those without the disease. These data suggest that the proinflammatory and neoangiogenic action of leptin may contribute to the pathogenesis of endometriosis. Moreover, leptin may play a role in endometriosis-associated pain.

Key words: chronic pelvic pain/endometriosis/idiopathic infertility/leptin

Introduction

Endometriosis is one of the most common benign gynaecological disorders and is present in >10% of reproductive age women in the USA. The exact aetiology of this enigmatic disease is unknown. Studies suggest that immune modulations involving cellular and humoral components are involved (Vinatier *et al.*, 1996; Koninckx *et al.*, 1998) where local and systemic abnormalities in immune responses have been described. Increased production of proinflammatory cytokines, such as interleukin (IL)-1, IL-8, tumour necrosis factor- α (TNF- α) (Arici *et al.*, 1997) and vascular endothelial growth factor (VEGF) has been consistently demonstrated in the peritoneal fluid of endometriosis patients (Bedaiwy *et al.*, 2002; Bedaiwy and Falcone 2003, 2004). Furthermore, angiogenesis involving VEGF is thought to be of fundamental importance in the early stages of endometriosis (Healy *et al.*, 1998).

Leptin is an adipocyte-derived protein belonging to the class of helical cytokines with a molecular weight of 16 kDa. It regulates food intake and energy expenditure and its plasma level correlates with the body fat mass (Halaas *et al.*, 1995). There is accumulating biological evidence that links leptin to endometriosis. First, leptin expression and secretion can be induced by certain cytokines such as TNF and IL-1 β and is considered to be an inflammatory mediator (Janik *et al.*, 1997; Mantzoros *et al.*, 1997; Zumbach *et al.*, 1997; Matarese, 2000). It also modulates CD4⁺ T-cell activities and cytokine production (Lord *et al.*, 1998). Second, leptin receptors have been localized in a wide variety of tissues including, but not limited to, T cells (Lord *et al.*, 1998), endometrium (Kitawaki *et al.*, 2000) and endothelial cells (Sierra-Honigmann *et al.*, 1998). Moreover, increased leptin expression in endometriosis cells has been shown to be associated with endometrial stromal cell proliferation and leptin gene up-regulation (Wu *et al.*, 2002).

However, it was recently reported that endometrial expression and localization of leptin and the leptin receptor in eutopic endometrium were similar between women with moderate/severe endometriosis and fertile controls (Lima-Couy *et al.*, 2004). Third, the neoangiogenic properties of leptin have been demonstrated both *in vivo* and *in vitro* (Bouloumie *et al.*, 1998; Sierra-Honigmann *et al.*, 1998). The close relationship of leptin to neoangiogenesis and cell-mediated immune responses suggest a possible role in the pathogenesis of endometriosis (Matarese *et al.*, 2000). Fourth, leptin concentration was shown to be increased in the serum and the peritoneal fluid of endometriosis patients compared with control women (Matarese *et al.*, 2000). Lastly, as leptin promotes angiogenesis and induces the expression of bcl-2, intercellular adhesion molecule 1 and matrix metalloproteinases, it could be one of the permissive factors that lead to the development of endometriosis in susceptible patients (La Cava *et al.*, 2004).

The main objectives of the study were: (i) to further investigate the relationship between leptin and pelvic endometriosis by measuring peritoneal fluid leptin concentrations in endometriosis patients and in women without pelvic pathology undergoing tubal ligation or reanastomosis and women with unexplained infertility; and (ii) to correlate peritoneal fluid leptin levels with the stage of the disease and the patient symptom profile in the endometriosis group.

Materials and methods

Patient enrolment

The Institutional Review Board of the Cleveland Clinic Foundation approved this study. Written informed consent was obtained from each patient. The study consisted of patients undergoing laparoscopy for chronic pelvic pain, infertility, tubal ligation, or sterilization reversal at a minimally invasive surgery unit in a tertiary care referral centre in the Midwest. Enrolment took place between 1999 and 2002. We excluded patients with blood-contaminated peritoneal fluid and divided the remaining patients into groups depending on their post-surgical diagnosis. The severity of endometriosis was graded according to the revised 4-stage American Fertility Society (1997) scoring system.

Preparation of the peritoneal fluid

Peritoneal fluid was aspirated from the peritoneal cavity through an abdominal port during laparoscopy. The cellular constituents of the

peritoneal fluid were removed by centrifugation at 300 g for 20 min. Peritoneal fluid supernatants were then collected and stored in aliquots at -70°C until the leptin concentrations were determined.

Detection of leptin in the peritoneal fluid

Levels of leptin were measured in the peritoneal fluid using commercially available, cytokine-specific, enzyme-linked immunosorbent assays (ELISA) (R & D Systems, Inc. Minneapolis, MN, USA). The frozen samples were thawed and analysed. Samples from each patient group were always measured in parallel and in duplicate to avoid inter-assay variance.

Statistical analysis

The demographic variables and peritoneal fluid measurements were compared across the patient groups with Kruskal–Wallis tests. Pair-wise comparisons between the groups were performed with the Wilcoxon's rank-sum test. Results of these analyses are reported using the mean \pm SD or the median and interquartile range where appropriate. Statistical computations were performed with SAS version 8.1 (SAS Institute, Cary, NC, USA), and statistical significance was assessed using two-tailed tests and an alpha level of $P < 0.05$.

Results

Patient demographics

Peritoneal fluid was obtained from 108 women: 60 with endometriosis, 10 with idiopathic infertility and 38 undergoing tubal ligation/reanastomosis. The age range was 18–44 years. Each group included patients in the proliferative and luteal phase of the menstrual cycle. Of the 60 patients with endometriosis, 34 had early disease (stage I and II) and 26 had advanced disease (stages III and IV). Mean \pm SD age of the study cohort was $33.0.2 \pm 5.5$ years, body mass index (BMI) was 24.19 ± 4.6 , and parity was 0.59 ± 0.9 . No significant differences were seen in age, parity and BMI between the three groups of patients (Table I).

Peritoneal fluid leptin

Peritoneal fluid levels of leptin were significantly higher in the endometriosis group than in the idiopathic infertility ($P = 0.0007$) and tubal ligation/reanastomosis groups ($P < 0.0001$), as well as the latter two groups combined ($P < 0.0001$). There

Table I. Leptin levels in the peritoneal fluid of three different study groups

Variable	Endometriosis	Idiopathic infertility	Tubal ligation/reanastomosis (L/R)	Overall <i>P</i> -value
Age (years)	33.0 \pm 5.5	31.3 \pm 3.6	33.6 \pm 5.9	0.59
Gravidity	1.0 \pm 1.3	1.0 \pm 1.7	2.8 \pm 2.8	0.15
Parity	0.6 \pm 0.9	0.3 \pm 0.6	1.6 \pm 1.1	0.09
Weight (kg)	65.2 \pm 12.6	83.9 \pm 40.2	72.1 \pm 14.7	0.47
Height (m)	1.6 \pm 0.1	1.6 \pm 0.0	1.6 \pm 0.1	0.18
Body mass index (kg/m ²)	24.2 \pm 4.6	25.39 \pm 18.21	25.6 \pm 4.1	0.18
Infertility (%)	64	100	23	< 0.0001 ^a
Pelvic pain (%)	56	0	0	< 0.0001 ^b
Leptin (ng/ml)	14.6 \pm 9.8	0.9 \pm 1.6	0.8 \pm 1.9	< 0.0001 ^c

Values are mean \pm SD unless otherwise stated.

^aEndometriosis versus idiopathic: $P < 0.05$; endometriosis versus tubal L/R: $P < 0.0001$; idiopathic versus tubal L/R: $P < 0.0001$.

^bEndometriosis versus idiopathic: $P < 0.0001$; endometriosis versus tubal L/R: $P < 0.0001$.

^cEndometriosis versus idiopathic: $P < 0.0001$; endometriosis versus tubal L/R: $P < 0.0001$.

P-values from Kruskal–Wallis (overall) and Wilcoxon rank-sum (between groups).

was no significant difference in the peritoneal fluid leptin levels between the follicular and luteal phases of the menstrual cycle in all patient groups. In the endometriosis group, the breakdown was: 36 in the follicular phase, 19 in the luteal phase and five patients without uterus. In the idiopathic infertility group, six patients were in the follicular phase and four in the luteal phase. In the tubal ligation/reanastomosis group, 23 patients were in the follicular phase and 15 were in the luteal phase. On correlating different parameters to the peritoneal fluid leptin values using Spearman correlation, peritoneal fluid leptin positively correlated with the stage of endometriosis ($P = 0.03$, $r = 0.4538$). It was also positively correlated with the presence of chronic pelvic pain as the leading presenting symptom as evaluated by the visual analogue scale ($P = 0.001$, $r = 0.4918$) but not when infertility was the main presenting symptom. None of the 35 patients with chronic pelvic pain and endometriosis presented with an infertility problem. Seventeen had completed their families at the time of surgery and 13 were not trying to conceive. Five patients had no uterus due to hysterectomy in a previous surgery. In the endometriosis and infertility group, 25 patients were included. All presented with a main complaint of infertility but five also had a secondary complaint of pain. However, these five patients did not change the conclusion since eliminating them showed a greater difference in the peritoneal fluid leptin levels between endometriosis patients with chronic pelvic pain versus those with infertility. Peritoneal fluid leptin levels in patients with idiopathic infertility was not different from those of patients undergoing tubal ligation or reversal.

Discussion

Our results indicate that peritoneal fluid leptin is significantly higher in endometriosis patients compared to those without the disease. This is in agreement with the earlier report by Matarese *et al.* (2000). They noted that not all affected patients had increased leptin concentrations. They also found that patients displaying peritoneal implants at all stages of endometriosis showed higher peritoneal fluid leptin concentrations than women in whom no implant was observed. In our study, leptin levels in the peritoneal fluid are positively correlated with the stage of the disease. This contradicts the initial observations (De Placido *et al.*, 2001; Mahutte *et al.*, 2003). This may be attributed to the fact that in their study, six of the nine women affected by advanced stage endometriosis had ovarian endometriomas but did not display any peritoneal lesions, unlike our series where the majority of patients with advanced stage disease had peritoneal implants. This suggests that endometriomas may behave differently from peritoneal implants.

The long signalling form of leptin receptor (Ob-Rb receptor) associated with leptin activity has been shown to be expressed in normal human endometrium (Kitawaki *et al.*, 2000). The exact role of this receptor in normal endometrium is unknown. However, the role of peritoneal fluid leptin during endometriosis may be to sustain and promote ectopic endometrial tissue growth and progression. Other angiogenic factors, such as VEGF (McLaren *et al.*, 1996) which correlates with the severity

of the disease (Shifren *et al.*, 1996) and IL-8 (Gazvani *et al.*, 1998), are both increased and correlated with the extent of active endometriosis (Iwabe *et al.*, 1998).

The present study was also designed to identify the relationship between peritoneal fluid leptin concentration in endometriosis patients and the spectrum of symptoms associated with the disease. We found that peritoneal fluid leptin was positively correlated with chronic pelvic pain but not with infertility in patients with endometriosis. The only previous study which addressed the relationship between leptin and infertility included 15 patients with unexplained infertility, eight with endometriosis and eight with polycystic ovary syndrome (PCOS). A significantly higher peritoneal fluid leptin concentration was found in patients with unexplained infertility and endometriosis compared to those with PCOS. The plasma leptin concentration did not differ between the three groups studied. This study postulated that leptin might be related to infertility (Gogacz *et al.*, 2001). In our study, this association could not be substantiated in the endometriosis group. Similarly, peritoneal fluid leptin levels in unexplained infertility patients were similar to those in the tubal ligation/reanastomosis group. This could be due to the small sample size in the former study. To the best of our knowledge, this is the first study that documents a positive correlation between peritoneal fluid leptin and chronic pelvic pain in endometriosis patients. It could be argued that peritoneal fluid leptin may play a role in the pathogenesis of endometriosis-associated pelvic pain.

In conclusion, we found higher levels of leptin in peritoneal fluid from patients with endometriosis compared to patients with idiopathic infertility and those undergoing tubal ligation/reanastomosis. In addition, peritoneal fluid leptin levels correlated positively with the stage of disease. Our findings suggest a potential role for leptin in the pathogenesis of this disease. Moreover, leptin may play a role in the pathophysiology of endometriosis-associated pain. There was no association between peritoneal fluid leptin levels and idiopathic infertility.

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