European Journal of Obstetrics & Gynecology and Reproductive Biology 200 (2016) 1–5

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Soluble tumor necrosis factor-alpha receptors in the serum of endometriosis patients

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ARTICLE INFO

Article history:
Received 10 November 2015
Received in revised form 7 February 2016
Accepted 11 February 2016

Keywords:
Endometriosis
Non invasive diagnosis
Soluble TNF receptors
Serum

ABSTRACT

Introduction: We examine serum levels sTNFR-I and sTNFR-II in endometriosis patients, and their role as biomarkers of endometriosis.
Material and methods: Women were diagnosed with endometriosis during laparoscopy to investigate pelvic pain and/or infertility ($N = 62$). Control group included women with pelvic pain and/or infertility, whose laparoscopy showed no abnormalities ($N = 55$). Serum concentrations of sTNFR-I and sTNFR-II were measured using Bioplex Protein Array system. Non-parametric statistics were used.
Results: Endometriosis patients had significantly higher levels of sTNFR-I than controls (257.46 pg/ml, IQR = 2.37–1048.92 versus 130.39 pg/ml, IQR = 0.99–361.1 respectively, P value = 0.01). For TNFR-II, difference between women with (232 pg/ml, IQR = 0.0–624.4), and women without (132.93 pg/ml, IQR = 0.0–312.81) endometriosis was not significant (P value = 0.05). Early stage endometriosis patients had significantly higher level of sTNFR-I (559.13, IQR = 1.82–1289.86) and sTNFR-II (248.8, IQR = 0–644.65) than control women (P value is 0.01 for TNFR-I and 0.04 for TNFR-II). Levels of sTNFR-I and sTNFR-II were comparable for advanced endometriosis and controls, and between early and advanced endometriosis. As a biomarker for all- stage endometriosis, sTNFR-I produces AUC of 0.62, sensitivity of 61%, and specificity of 47.3%, at a cutoff of 81.87 pg/ml. For early stage disease, sTNFR-I yields AUC of 0.68, sensitivity of 60.7%, specificity of 75%, at a cutoff of 351.22 pg/ml.
Conclusion: sTNFR-I is significantly higher in serum of endometriosis patients than controls. As an endometriosis biomarker, sTNFR-I achieves better performance for early stage disease.

Introduction

Endometriosis affects 5–10% of reproductive age women, and 30–50% of women with pelvic pain and/or infertility [1]. Endometriosis is associated with chronic pelvic pain and infertility, with subsequent poor quality of life. Current treatments are inadequate with unacceptable side effects. Consequently, endometriosis related health care costs are high. Increased risk of ovarian cancer further exacerbates sufferings of endometriosis patients [2,3].

Currently, the gold standard for diagnosis of endometriosis is laparoscopy [4]. Laparoscopy is an invasive procedure with inherent risks. Risk of death as a result of this procedure is 0.1 per 1000. Risk of bowel, bladder or vessel injury is 2.4% [5]. Due to invasive nature of laparoscopy, endometriosis detection is often delayed on an average of 8.5 years [6]. Moreover, laparoscopic diagnosis of endometriosis shows a high rate of inter-observer variability, and correlation with histological confirmation is not perfect [7,8].

Immune system is aberrantly activated in endometriosis patients. Peritoneal macrophages show overproduction of cytokines, and growth factors. Immune cell mediators paradoxically enhance endometriosis through stimulation of endometrial cell proliferation and adhesion (IL-6, and IL-1), increased angiogenesis

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http://dx.doi.org/10.1016/j.ejogrb.2016.02.025
0301-2115/

Table 1
Clinical characteristics of women with and without endometriosis.

	Endometriosis group $N=62$	Control group $N=55$	P value
Age (years)	30.8 ± 3.9	31.4 ± 3.3	0.5
BMI	25.2 ± 2.8	24.6 ± 2.3	0.4
Proliferative phase	$N=26$	$N=29$	N/A
Secretory phase	$N=36$	$N=26$	N/A
Early stage disease (stages I and II)	$N=25$	N/A	N/A
Advanced stage disease (stages III and IV)	$N=37$	N/A	N/A

(VEGF, IL-8), attraction of more immune cells (IL-8, RANTES and MCP-1), and up-regulating aromatase enzyme (PG-E2) [9]. Local immunological changes are reflected at the systemic level as well [10,11].

Tumor necrosis factor-alpha (TNF-α) is a major pro-inflammatory cytokine that is implicated in endometriosis pathogenesis [11]. TNF-α stimulates endometrial cell proliferation, and adhesion to peritoneal m cells, regulates matrix metalloprotienases (MMPs), and stimulates angiogenesis [11,12].

TNF-α, works through two types of transmembrane receptors (TNFRs): TNFR1 and TNFR2, which are encoded by two different genes. Soluble forms of TNF-α receptors (sTNFR) exist from cleavage of the extracellular domain of the transmembrane receptors. There are two types of soluble receptors: sTNFR-I and sTNFR-II, both of which function mainly to antagonize the effects of TNF-α through sequestering the active ligand away from the transmembrane receptors [13].

Few studies examined the role of sTNFR in endometriosis [14,15]. None of these studies evaluated these soluble receptors as disease biomarkers. In the present study, we examine serum levels of TNFR-I and TNFR-II in endometriosis patients and control women, as well as their potential as disease biomarkers.

Material and methods

Study subjects

Sixty two cases of endometriosis, and 55 control women were recruited. Women were diagnosed with endometriosis during laparoscopic evaluation of pelvic pain and/or infertility at the Gynecologic Endoscopy Unit, Lubeck University. Laparoscopies were performed in proliferative and secretory phases of the cycle by experienced endometriosis surgeons. Endometriosis diagnosis depended on laparoscopic visualization of lesions and was confirmed by pathological examination. Extent of endometriosis was scored according to the revised American Fertility Society (rAFS) classification. Control group included women with pelvic pain and/or infertility, in whom laparoscopy revealed no pelvic abnormalities. In both groups, menstrual cycle phase was assigned based on patient's menstrual history and histological evaluation of endometrial biopsy. Exclusion criteria included irregular menses, having any pelvic or systemic inflammatory condition or infections, use of hormonal medications, or pregnancy within the last three months. The institutional review board at Lubeck and Assiut Universities approved the study, and all enrolled cases gave their written consent.

Blood sample withdrawal and serum preparation

Venous blood samples (5–10 ml) was taken preoperatively from all study participants. Blood samples were centrifuged at 3000 rpm for 10 min at 4 C, and clear serum was stored at −80 C in aliquots.

Soluble TNF receptor measurement in serum of endometriosis patients and control women

Concentrations of sTNFR-I and sTNFR-II were measured in serum of endometriosis patients and controls using the Bioplex Protein Array system (Bio-Rad, Hercules, CA, USA), as described before [10,16]. Sensitivity of assay is <10 pg/ml. Intra- and inter-assay coefficient of variation is <10%. The range of detection for target cytokine receptors is 2–32,000 pg/ml.

Statistical analysis

As data was not normally distributed, non parametric statistics were used. Serum levels of sTNFR-I or sTNFR-II in different groups were expressed as median ± interquartile range (IQR), from 25th to 75th percentiles. Mann–Whitney test was used for group comparison. Receiver Operator Characteristic ROC curve was constructed for serum sTNFR-I and TNFR-II as endometriosis biomarkers, and to identify particular cutoff points. All statistical analysis was done using SPSS software, version 21.

Results

Patients' characteristics

We recruited 62 endometriosis cases and 55 control women. Clinical characteristics of study subjects are in Table 1.

Serum levels of sTNFR-I and sTNFR-II in endometriosis patients and control women

Women with endometriosis had significantly higher serum levels of sTNFR-I than control women (257.46 pg/ml, IQR = 2.37–1048.92 versus 130.39 pg/ml, IQR = 0.99–361.1 respectively, P value = 0.01). For TNFR-II, the difference between women with (232 pg/ml, IQR = 0.0–624.4), and women without (132.93 pg/ml, IQR = 0.0–312.81) endometriosis was not statistically significant (P value = 0.05), as identified in Table 2.

Serum levels of sTNFR-I and sTNFR-II in early (stages I and II) and advanced (stages III and IV) stage endometriosis

There was no significant difference between early or advanced endometriosis groups regarding their serum levels of sTNFR-I or sTNFR-II. However, Early stage endometriosis patients contained

Table 2
Serum levels of sTNFR-I and sTNFR-II in endometriosis patients and control women.

Soluble receptor	Endometriosis ($N=62$)	Control ($N=55$)	Significance
sTNFR-I	257.46 (2.37–1048.92)	130.39 (0.99–361.1)	0.014
sTNFR-II	232 (0.0–624.4)	132.93 (0.0–312.81)	0.055