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Serum YKL-40 levels as a novel marker of inflammation in patients with endometriosis

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Summary

Purpose: To establish serum YKL-40 concentrations in patients with endometriosis compared to age-matched healthy subjects. *Materials and Methods:* This was a cross-sectional clinical study conducted in a tertiary care center. Demographics and serum YKL levels were determined and noted in a total of 63 cases (33 endometriosis patients, 30 healthy controls). Measurement of YKL-40 levels was made using a YKL-40 enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol. *Results:* The mean serum YKL-40 levels of the patient group was 106.0 ± 15.9 (range 23.44 to 382.55) years, while the mean serum YKL-40 levels of the controls was 52.2 ± 7.0 (range 22.35 to 160.0) years ($p = 0.003$). *Conclusions:* This is the first study evaluating serum YKL-40 levels in endometriosis. The present results indicate that YKL-40 levels were increased in patients with endometriosis compared to controls. The authors propose that circulating YKL-40 levels could be a novel biomarker for diagnosis and follow-up of endometriosis.

Key words: Endometriosis; Serum; YKL-40 levels; Marker.

Introduction

Endometriosis is a gynecological disease that affects approximately 2%-48% of women during their reproductive years [1]. It is characterized by the presence and proliferation of endometrial glands and stroma outside the uterus. The patients mainly complain of pelvic pain, dysmenorrhea, and infertility. Laparoscopy offers the most specific and sensitive technique for evaluating and monitoring endometriosis. However, microscopic or occult endometriosis may be misdiagnosed due to the inability to visualize the lesions. Moreover, invasive procedures have been linked to the accidental development of endometriosis [2]. Furthermore, persistence or recurrence of endometriosis mostly occurs after regular therapy. Therefore, the benefits of non-invasive, biochemical diagnostic markers, especially in serum, for detection of endometriosis are evident. To date, however, no molecules are shown to be competent for the diagnosis of endometriosis.

YKL-40 is a 40 kDa heparin- and chitin-binding glycoprotein also known as human cartilage glycoprotein 39 (HCgp39), 38-kDa heparin-binding glycoprotein or chitinase-3-like protein 1 (CHI3L1) [3]. The abbreviation YKL-40 is based on the one letter code for the first three N-terminal amino acids, tyrosine (Y), lysine (K) and leucine (L) and the apparent molecular weight of YKL-40 [4]. YKL-40 is normally expressed by a number of different cell types such as chondrocytes, synoviocytes, vascular smooth muscle cells, macrophages, and neutrophils. It has been recognized as a growth factor capable of stimulating connective tissue cell growth and endothelial cell migration, while inhibiting mammary epithelial cell differentiation [5].

Growing evidence has indicated that expression levels of YKL-40 are elevated in multiple human diseases including type 2 diabetes, obesity and insulin resistance in children, Alzheimers' diseases, and heart failure [6]. Elevated YKL-40 levels were observed in a vast array of inflammatory diseases that contain bacterial infections, rheumatoid arthritis, osteoarthritis, hepatitis, asthma and chronic obstructive pulmonary diseases, neuroinflammation, and bowel lesions [7]. In the chronic inflammatory diseases, YKL-40 is supposed to mediate infiltration, differentiation, and maturation of macrophages, the primary leukocytes in response to inflammation [8].

The present study was designed to establish serum YKL-40 concentrations in patients with endometriosis compared to age-matched healthy subjects. To the authors' knowledge, this is the first study that evaluates serum YKL-40 levels in endometriosis patients. Potential markers could lead to novel diagnostic, therapeutic, and prognostic methods for the management of endometriosis and may improve our understanding of the pathogenic mechanism of endometriosis.

Materials and Methods

Study design

This study was approved by the local Institutional Review Board. Written informed consent was obtained from all subjects. A total of 63 women of reproductive age with regular menstrual

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Table 1. — *Demographic and clinical variables of the study group. Data are expressed as mean ± SD or number (percentage).*

	Patient group (n = 33)	Control group (n = 30)	*p* value
Age	32.06 ± 0.86	31.53 ± 1.43	0.087
BMI	26.21 ± 2.29	25.63 ± 2.68	0.358
CA-125 level (U/ml)	59.88 ± 5.79	19.6 ± 3.54	< 0.001*
Dysmenorrhea	25 (76%)	9 (30%)	< 0.001*
Chronic pelvic pain	22 (66.7%)	5 (16.7%)	< 0.001*
Marital status (Married/single)	22/11	13/17	0.108

cycles (25-32 days) admitted to the present tertiary center between February and September 2013 constituted the study group. Of these participants, 33 patients with endometriosis (patient group) and 30 fertile women (control group) without endometriosis were included in the study. The diagnosis of endometriosis was confirmed histopathologically in all cases in patient group. The decision of the operation was verified by high CA-125 levels combined with at least four-months of ultrasound examination.

Serum YKL-40 analysis

YKL-40 levels were determined using a YKL-40 enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer's protocol. Absorbance was measured using a microplate reader.

Statistical analyses

Data were analyzed using the Statistical Package for Social Sciences 19.0 for Windows. Parametric tests were applied to data of normal distribution and non-parametric tests were applied to data of questionably normal distribution. Independent-samples t-test and Mann–Whiney U-test were used to compare independent groups. Data are expressed as mean ± SD or median (interquartile range), as appropriate. All differences associated with a chance probability of 0.05 or less were considered statistically significant.

Results

A total of 63 cases consisting of 33 endometriosis patients and 30 healthy controls met the eligibility criteria for the study. The mean age of the patient group was 32.06 ± 0.86 (range 30 to 34) years, while the mean age of the controls was 31.53 ± 1.43 (range 26 to 34) years (p = 0.087). The mean body mass index (BMI) of the patient group was 26.21 ± 2.29 (range 20.3 to 31.9) kg/m^2, while the mean BMI of the controls was 25.63 ± 2.68 (range 21.2 to 32.3) kg/m^2 (p = 0.358). The mean CA-125 level of the controls was 59.88 ± 5.79 (range 49.63 to 75.08) U/ml, while the mean CA-125 level of the controls was 19.6 ± 3.54 (range 10.16 to 28.17) U/Ml (p < 0.001). Dysmenorrhea and chronic pelvic pain were found to be more frequent in endometriosis group (p < 0.001). Demographic and clinical variables of the groups are shown in Table 1.

The mean serum YKL-40 levels of the patient group was 106.0 ± 15.9 (range 23.44 to 382.55) years, while the mean serum YKL-40 levels of the controls was 52.2±7.0 (range 22.35 to 160.0) years (p = 0.003, Figure 1).

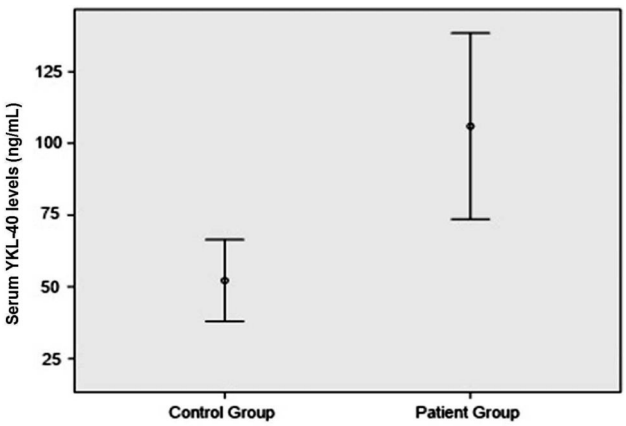

Figure 1. — Figure comparing serum YKL-40 values in patients with endometriosis and controls. Serum YKL-40 was significantly higher in patients with endometriosis.

Discussion

In the present study, the authors attempted to demonstrate whether there was a relationship in serum YKL-40 concentrations between patients with endometriosis and age-matched healthy subjects. This is an original and unique study for evaluating serum YKL-40 levels in endometriosis and the authors found elevated YKL-40 levels in patients with endometriosis compared with control subjects.

Endometriosis is a multifactorial disease and angiogenesis plays an important role in pathophysiology [9]. The angiogenic potentials of both the endometrium and the peritoneal environment influence lesion establishment [10]. Indeed, endometriotic lesions require an adequate blood supply to survive in their ectopic sites. In normal eutopic (intrauterine) endometrium, it has been suggested that vessel elongation, rather than branch point sprouting, is the primary mechanism for rapid vessel growth during the proliferative phase [11]. The precise mechanism in endometriosis lesions has not been evaluated up to now. Recruitment of new capillaries from existing, adjacent peritoneal microvessels was postulated [10]. Derivation of new blood vessels from circulating endothelial progenitor cells, the so-called "vasculogenesis," also seems to be important in the pathogenesis of endometriosis [12]. The initiation, extent, and suppression of angiogenesis are highly dependent on the balance between pro- and anti-angiogenic factors. Hanahan and Folkman described this phenomenon as the angiogenic switch in tumors [13]. Recently, molecules with anti-angiogenic potential appear to be suppressed in patients with endometriosis compared to controls. For example, interferon gamma-induced protein 10 concentrations are reduced in peritoneal fluid and adiponectin levels are reduced both in the peritoneal fluid and serum of endometriosis patients compared to controls [14]. Vascular endothelial growth factor (VEGF) is one of the most prominent and well-studied proangiogenic growth factors in endometriosis and it is widely believed that VEGF is the main stimulus for angiogenesis and increased

vessel permeability in this disease [15]. Significantly increased VEGF levels have been detected in the peritoneal fluid and lesions of endometriosis patients compared to controls [16]. Similarly, YKL-40 was recently identified as a potent angiogenic factor capable of inducing endothelial cell angiogenesis in breast cancer [17]. In the tumor microenvironment, a significant amount of angiogenic factors are secreted from tumor cells and activate adjacent vascular endothelial cells to induce angiogenic responses [18]. YKL-40 and VEGF are believed to be mainly derived from tumor cells and both have strong angiogenic activities in tumor development.

Dupont *et al.* suggested that YKL-40 may be a novel marker for the detection of early stage ovarian cancer compared to CA 125 and CA 15-3 [19]. The usefulness of YKL-40 in the management of recurrent ovarian cancer has been reported. Patients with high plasma YKL-40 at the time of relapse had significantly shorter survival rates than patients with normal levels the plasma YKL-40 was demonstrated to be an independent prognostic factor [20]. Therefore, an elevated level of serum YKL-40 can serve as a novel marker for the detection of endometrial cancer, and identify a high risk subset of patients at risk for poor clinical outcomes. To date, only one study has reported the expression and distribution of the YKL-40 in peritoneal endometriosis [21]. The results of this study yielded that YKL-40 was associated with the severity of peritoneal endometriosis. However, it is not clear whether the expression of YKL-40 was caused by the proliferation and turnover of ectopic endometrial tissue or extracellular matrix remodeling and fibrosis. In the present study, serum YKL-40 concentrations in patients with endometriosis were significantly higher than age-matched healthy subjects. The authors think that inflammatory process during endometriosis results in elevated YKL-40 levels. The exact mechanism through which YKL-40 levels are elevated remain to be elucidated via further in vitro and in vivo trials.

Limitations of the present study are relatively small size of this series and lack of definite criteria for selection of patients. In addition, some details of history and factors that may influence the outcome may not be completely documented. Due to these restrictions, associations should be interpreted with caution. However, the authors hope that this study will pioneer further studies on this method.

Conclusion

The present data demonstrate that YKL-40 is an effective tool for identification of women with and without endometriosis. The authors anticipate that further studies of the proteins identified herein will expand our understanding of the nature of endometriosis and assist in the eventual development of new diagnostic and therapeutic approaches for endometriosis.

References

[1] Sangi-Haghpeykar H., Poindexter A.N.: "Epidemiology of endometriosis among parous women". *Obstet. Gynecol.,* 1995, *85*, 983.

[2] Gurates B., Bulun S.E.: "Endometriosis the ultimate hormonal disease". *Semin. Reprod. Med.*, 2003, *21*, 125.

[3] Rehli M., Krause S.W., Andreesen R.: "Molecular characterization of the gene for human cartilage gp-39 (CHI3L1), a member of the chitinase protein family and marker for late stages of macrophage differentiation". *Genomics*, 1997, *43*, 221.

[4] Hauschka P.V., Mann K.G., Price P., Termine J.D.: "Report of the Ad Hoc Committee on Nomenclature and Standards for Bone Proteins and Growth Factors". *J Bone Miner. Res.*, 1986, *1*, 485.

[5] Scully S., Yan W., Bentley B., Cao Q.J., Shao R.: "Inhibitory activity of YKL-40 in mammary epithelial cell differentiation and polarization induced by lactogenic hormones: a role in mammary tissue involution". *PLoS One*, 2011, *6*, e25819. doi: 10.1371/journal.pone.0025819. Epub 2011 Oct 3.

[6] Harutyunyan M., Christiansen M., Johansen, J.S., Køber L., Torp-Petersen C., Kastrup J.: "The inflammatory biomarker YKL-40 as a new prognostic marker for all-cause mortality in patients with heart failure". *Immunobiology*, 2012, *217*, 652.

[7] Nielsen K.R., Steffensen R., Boegsted M., Baech J., Lundbye-Christensen S., Hetland M.L., *et al.*: "Promoter polymorphisms in the chitinase 3-like 1 gene influence the serum concentration of YKL-40 in Danish patients with rheumatoid arthritis and in healthy subjects". *Arth. Res. Therapy*, 2011, *13*, R109.

[8] Rehli M., Niller H.H., Ammon C., Langmann S., Schwarzfischer L., Andreesen R., Krause S.W.: "Transcriptional regulation of CHI3L1, a marker gene for latest ages of macrophage differentiation". *J. Biol. Chem.*, 2003, *278*, 44058.

[9] McLaren J.: "Vascular endothelial growth factor and endometriotic angiogenesis". *Hum. Reprod. Update*, 2000, *6*, 45.

[10] Rocha A.L., Reis F.M., Taylor R.N.: "Angiogenesis and endometriosis". *Obstet. Gynecol. Int.*, 2013, *2013*, 859619. doi: 10.1155/2013/ 859619. Epub 2013 May 26.

[11] Gambino L.S., Wreford N.G., Bertram J.F., Dockery P., Lederman F., Rogers P.A.: "Angiogenesis occurs by vessel elongation in proliferative phase human endometrium". *Hum. Reprod.*, 2002, *17*, 1199.

[12] Laschke M.W., Giebels C., Menger M.D.: "Vasculogenesis: a new piece of the endometriosis puzzle". *Hum. Reprod. Update*, 2011, *17*, 628.

[13] Hanahan D., Folkman J.: "Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis". *Cell*, 1996, *86*, 353.

[14] Takemura Y., Osuga Y., Harada M., Hirata T., Koga K., Morimoto C., *et al.*: "Serum adiponectin concentrations are decreased in women with endometriosis". *Hum, Reprod*., 2005, *20*, 3510.

[15] Taylor R.N., Lebovic D.I., Mueller M.D.: "Angiogenic factors in endometriosis". *Ann. N. Y. Acad. Sci.*, 2002, *955*, 89.

[16] Donnez J., Smoes P., Gillerot S., Casanas-Roux F., Nisolle M.: "Vascular endothelial growth factor (VEGF) in endometriosis". *Hum. Reprod.*, 1998, *13*, 1686.

[17] Shao R., Hamel K., Petersen L., Cao Q.J., Arenas R.B., Bigelow C., *et al.*: "YKL-40, a secreted glycoprotein, promotes tumor angiogenesis". *Oncogene*, 2009, *28*, 4456.

[18] Hanahan D., Weinberg R.A.: "Hallmarks of cancer: the next generation". *Cell*, 2011, *144*, 646.

[19] Dupont J., Tanwar M.K., Thaler H.T., Fleisher M., Kauff N., Hensley M.L., *et al.*: "Early detection and prognosis of ovarian cancer using serum YKL-40". *J. Clin. Oncol.*, 2004, *22*, 3330.

[20] Dehn H., Hogdall E.V., Johansen J.S., Jorgensen M., Price P.A., Engelholm S.A., Hogdall C.K.: "Plasma YKL-40, as a prognostic tumor marker in recurrent ovarian cancer". *Acta Obstet. Gynecol. Scand.*, 2003, *82*, 287.

[21] Kim P.J., Hong D.G., Park J.Y., Cho Y.L., Park I.S., Lee Y.S.: "Immunohistochemical expression of YKL-40 in peritoneal endometriosis". *Gynecol. Endocrinol.*, 2010, *26*, 58.

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