

YKL-40 and fibronectin levels in patients with placental invasion anomaly

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Abstract

Aim: To investigate the level of YKL-40 and fibronectin in patients with total placenta previa and to evaluate the presence of placental invasion anomaly such as accreta.

Materials and Methods: A total of 60 patients were included in this prospective study. The patients were classified according to the placental localization as assessed through ultrasound. The study group consisted of 33 patients diagnosed with placenta previa or accreta with previous cesarean section. These patients were also subdivided into two groups according to the histopathological examination results as invasion-positive group and invasion-negative group. The control group consisted of 27 patients who were admitted to the gynecology clinic with previous cesarean deliveries and had normal placental localization. Serum YKL-40 and fibronectin levels were measured in two groups via human chitinase-3-like protein 1 (Ykl-40/CHI3L1) and fibronectin enzyme-linked immunosorbent assay kits.

Results: Mean serum levels for YKL-40 and fibronectin were similar between the study and control groups. In the subgroup analysis according to invasion anomaly, the level of YKL-40 in invasion-positive group (n=11) was higher than invasion-negative group (n=22), indicating a statistically significant difference.

Conclusion: Outcomes of this research indicates that YKL-40 can be used as a marker for identifying placental invasion anomalies.

Keywords: Invasion anomaly; fibronectin; placenta accreta; placenta previa; YKL-40

INTRODUCTION

Placenta previa (PP) is defined as the localization of the placenta that extends over the internal cervical os. Its incidence is about 1 in 200 pregnancies at term, varying worldwide (1). Placenta accreta (PA) is identified as abnormal adherence of the placenta to the myometrium with preserved decidua (2). Placenta increta is identified as the myometrial invasion of the trophoblasts, while placenta percreta is defined as abnormal infiltration of placental tissue through its surrounding pelvic structures (e.g. the bladder) (3). The terminology of "placenta accreta" is usually referred to the broad spectrum of situations that includes accreta; increta; and percreta. It also refers to the term of "morbidly adherent placenta" (MAP) (4). The prevalence of PA is about 1 in 300 pregnancies (5). However, the incidence of PP and PA has increased with higher cesarean delivery rates in recent years (5). It has been shown that there is a dose-response dependent

association between the number of prior cesarean births and subsequent PP and PA (6). Other well-known factors that predispose risk for PP include increasing maternal parity, increased age at delivery, use of cigarette/cocaine, multiple gestations, and previous history of PP (2). All of these factors been closely related with endometrial/myometrial damage and ischemic conditions of the endometrial tissue. In addition, these implications may result in abnormal placental implantation at the lower segment of the uterus. Decidual vascularization defects due to inflammation and atrophic changes are also considered to be related to the PP (7, 8).

YKL-40 (also known as chitinase 3-like-1 is a chitinase-like glycoprotein that can be found in the serum of blood and many diverse types of human tissues (9). YKL-40 is from the family 18 of glycosyl hydrolases, but Ykl-40 has not got enzymatic properties (9). YKL-40 is mainly secreted by cancer cells, macrophages, neutrophils, vascular smooth

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muscle cells, and endothelial, fetal, and stem cells (10-12). Although its role is yet to be fully elucidated, recent researches have suggested that YKL-40 may participate in inflammatory process, angiogenesis, cell proliferation and differentiation, and remodeling of the extracellular matrix (13). In addition, macrophages have been shown to play a central role in the processes of implantation, placentation and placental development (14).

Fibronectin is a high-molecular-weight glycoprotein found in plasma and is associated with extracellular matrices and cell surfaces (15). It has many diverse functions, including cell adhesion and migration, cell differentiation, enhancement of macrophage phagocytosis, invasion of tumor cells, and wound healing (16). Various cell types produce fibronectin including monocytes, fibroblasts, vascular endothelial cells, and endometrial stromal cells (16,17). Since fibronectin has been shown in vitro to participate in cell adhesion, studies investigating physiological properties of fibronectin have become popular. Previous studies on cell invasion during embryogenesis have suggested that fibronectin is a major component of certain cell migratory pathways, suggesting some insight into how trophoblast invasion can be controlled by fibronectin in the decidual tissue (15). MAP is strongly related with intraparenchymal bleeding in placental tissue, villous changes of maternal vascular under perfusion, and a lymphoplasmacytic infiltrate at the site of implantation (18). As the possible contribution of the basal chronic inflammatory infiltrate to MAP should be investigated in future studies (19), we, in the current study, aimed to investigate the level of YKL-40 and fibronectin in women with total PP and MAP.

MATERIALS and METHODS

This study was conducted in prospective nature at a tertiary clinic between July 2014 and July 2015. The study was approved by the institutional Ethics Committee (Date: 25/09/2014; No. 177) and was conducted in accordance with the principles of 1964 Helsinki Declaration. A written signed informed consent was retrieved from each participant.

A total of 60 women were included in this prospective study. Those with multiple pregnancies, pregnancies with diabetes mellitus, hypertension, preeclampsia, inflammatory diseases, and other comorbidities were excluded from the study. All patients were classified according to the placental localization as assessed by ultrasound:

- i) The study group consisted of 33 patients who were diagnosed with PP or PA with previous cesarean delivery. These patients were also subdivided into two groups according to the histopathological examination results as invasion-positive group (placenta accreta, increta, percreta) and invasion-negative group (only PP).
- ii) The control group consisted of 27 volunteer patients who were admitted to the gynecology clinic with previous cesarean deliveries and had normal placental localization (i.e., placenta anterior, posterior and fundus).

Blood samples were collected from both study and control groups immediately before the operation and were centrifuged at 3,000 rpm for 10 min and stored at -80°C. Operative procedures and postoperative pathological results were recorded (i.e., cesarean section, cesarean hysterectomy, whether there is accreta). Age, gravidity, parity, age of gestation, applied surgery, and pre- and postoperative blood samples were recorded.

Determination of serum YKL-40 and fibronectin concentrations

Serum YKL-40 concentrations were measured using the YKL-40/CHI3L1 enzyme-linked immunosorbent assay (ELISA) kits (Biotech, Shanghai, China). The coefficient of variations in assay was <10%. The assay range was 1 ng/mL to 400 ng/mL.

Serum fibronectin concentrations were measured via human fibronectin ELISA kits (Biotech, Shanghai, China). The coefficient of variations in assay was <10%. The assay range was 20 ng/mL to 400 ng/mL.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 23.0 statistical software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean \pm standard deviation (SD), and median (min-max) values. The Mann-Whitney U and t-test's been used to compare and analyze statistically significant differences between the groups. A p value was at the level of < .05 .

RESULTS

Of a total of 33 patients in the study group, cesarean hysterectomy were performed in 11 patients who had invasion anomaly, while the remaining patients (n=22) underwent cesarean section and optional tubal ligation. According to the histopathological examination findings, two patients had percreta, three patients had increta, and six patients had accreta. A total of 27 patients in the control group underwent only cesarean section and optional tubal ligation. Bladder rupture occurred in two patients in the study group in whom cesarean hysterectomy was performed. In control group, bladder rupture occurred only in one patient. The mean ages of the patients with PP and control patients were 31.33 ± 5.43 years and 30.20 ± 5.09 years, respectively. There was no significant difference in demographic features such as gravida and parity between the groups. According to the gestational age at delivery, the mean gestational age in the previa group was lower than the control group (36.63 ± 1.16 weeks versus 38.17 ± 0.66 weeks, respectively), indicating a statistically significant difference between the groups ($p=0.0001$). In addition, the mean birth weight in the previa group was lower than the control group (2960.30 ± 371.74 g versus 3230.80 ± 348.55 g, respectively), indicating a statistically significant difference ($p=0.007$). However, no significant differences were observed in the preoperative hematological parameters such as hemoglobin, hematocrit, and platelet levels between the study and

control groups. On the other hand, we found a significant difference in the postoperative hematological parameters such as hemoglobin and hematocrit values between the two groups, indicating lower postoperative hemoglobin and hematocrit levels in the previa group compared to the control group ($p=0.001$ and $p=0.004$, respectively) (Table 1).

Table 1. Comparison of demographic features and laboratory findings between study and control group

Characteristics	Study group (n:33, mean± Std)	Control group (n:27, mean± Std)	P value
Gravida	3.8 ±1.3	3.6 ±1.4	0.40
Parity	2.2 ±1.1	2.3 ±1.1	0.80
Gestational age (week)	36.6 ±1.1	38.1 ±0.6	0.0001
Birth weight(gr)	2960 ±371.7	3230 ±348.5	0.007
Preoperative Hb	11.2 ±1.21	11.6 ±1.23	0.19
Preoperative Htc	34.6 ±3.9	35.3 ±3.5	0.49
Postoperative Hb	9.1 ±1.3	10.3 ±1.1	0.001
Postoperative Htc	28.4 ±4.2	31.4 ±3.1	0.004
Preoperative PLT	225.3 ±75.8	240.5 ±73.9	0.44
Postoperative PLT	184.4 ±78.0	200.8 ±56.2	0.37

Std: Standard deviation; Hb: Hemoglobine; Htc: Heamatocrite
PLT: Platelet

Table 2. Comparison of serum fibronectin and Ykl-40 levels between groups

Characteristics	Study group (n:33, mean± Std)	Control group (n:27, mean± Std)	P value
Fibronectin (ng/ml)	224.3 ± 51.7	216.7 ± 33.6	0.57
Ykl-40 (ng/ml)	212.1 ± 64.5	205.5 ± 38.2	0.98

Table 3. Fibronectin and Ykl-40 values of study group according to invasion anomaly

Characteristics	Invasion positive (n:11, mean ±std)	Invasion negative (n:22, mean ± std)	P value
Fibronectin (ng/ml)	239.91 ±74.00	216.183 ±4.78	0.32
Ykl-40 (ng/ml)	238.03±75.57	190.89 ±40.88	0.02*

The mean serum levels for YKL-40 and fibronectin were similar between the study and control groups (Table 2). However, in the subgroup analysis according to invasion anomaly, the level of fibronectin in invasion-positive group (n=11) was higher than invasion-negative group (n=22), although it did not reach statistical significance ($p=0.327$). In addition, the level of YKL-40 in invasion-positive group was higher than invasion-negative group, indicating a statistically significant difference ($p=0.022$). Although the difference of fibronectin was not statistically significant, the level of fibronectin was higher in invasion-positive group and YKL-40 values were significantly high in invasion-positive group, suggesting the presence of invasion and inflammation (Table 3).

DISCUSSION

MAP refers to abnormal placental implantation with the placental adherence to the myometrium, preventing separation of the placenta from the uterine wall at the cesarean section (19, 20). It consists of the pathological entities of PA (75%), placenta increta (18%), and placenta percreta (7%) (4). Moreover, myometrial adhesion may serve as a risk factor for the subsequent development of PA (20). The incidence of MAP has increased nearly ten-fold over the past five decades (21). MAP frequently requires a surgical intervention of the placenta to excise the adherent placental tissue from the uterine wall. However, hysterectomy may be inevitable in inseparable cases. Therefore, MAP is considered the major indication of hysterectomy in cesarean operations (22).

The definite pathological mechanism underlying MAP are not understood enough, yet (19). One of the most important etiological reason for MAP and PP is defective decidualization at the implantation region. However, there is a limited number of studies which reviewed pathological findings at the basal plate of the placenta in patients with MAP. In previous small-scale researches, abnormal basal plate findings in patients with MAP were determined as uteroplacental vascular abnormalities, decidual hemosiderosis/infarction and acute/chronic inflammation (3,18). The largest and extensive systematic study showed that MAP was associated with chronic inflammation, bleeding and maternal defective vascular perfusion (19). The existence of a chronic inflammatory infiltration in MAP may be the representative of an abnormal immune response at the maternal fetal interface. This chronic inflammatory condition may be caused by a reaction to the advancing trophoblastic tissue. Moreover, this may have an act in managing its invasiveness (4).

Ultrasonography and magnetic resonance imaging (MRI) can be useful in predicting and minimizing complications of PP and MAP. In addition, serum and genetic markers were proposed to be predictors for placenta accreta such as Troponin I and *pro-B-type natriuretic peptide* (pro-BNP) (23). More complex molecules have been analyzed to identify cases with placental invasion anomalies such as cell-free beta-human chorionic gonadotropin (b-HCG) messenger ribonucleic acid (mRNA) in the maternal plasma and considered useful for the prenatal diagnosis of PA, particularly to select those with PA requiring hysterectomy (24). In addition to the sonographic and MRI findings, certain types of serum inflammation and invasion markers can be utilized to confirm the diagnosis in patients with suspected placental invasion anomalies. In the literature, fibronectin and YKL-40 levels have never been studied in cases with placental invasion anomaly.

YKL-40, which is secreted from inflammatory and cancer cells, has been considered to play a key role in cell proliferation, differentiation, angiogenesis, and inflammation. In a study, serum YKL-40 levels were shown to significantly increase in cases with endometrial cancer

(25). Zou et al. (26) found YKL-40 to be associated with poor clinical outcomes, showing a statistically significant difference for tumor stages and grades in women with ovarian cancer. In addition, Fan et al. (27) reported that YKL-40 seemed to be effective in the diagnosis and follow-up of endometrial cancer. In another study investigating the immunoreactivity of YKL-40 in normal, hyperplastic, and neoplastic endometrium, the authors demonstrated that myometrial invasion was present in advanced endometrial cancer cases with lymphovascular space invasion (28). According to our study results, serum YKL-40 levels were similar between the study and control groups, although, in the previa group, YKL-40 levels were significantly higher in invasion-positive patients than invasion-negative patients. Therefore, we can conclude that YKL-40 may indicate the invasiveness of the placental tissue.

Furthermore, increased YKL-40 levels in endothelial dysfunction can be attributed to endothelial damage. Endothelial dysfunction is known a critical factor in several conditions such as hypertension, obesity, atherosclerosis, and diabetes mellitus, and YKL-40 has been shown to significantly increase in pregnant women with gestational diabetes mellitus compared to those without and in preeclamptic women (13).

Preeclampsia is known to be associated with the maternal inflammatory response. YKL-40 is an inflammatory marker for both acute and chronic inflammatory states and vascular processes. Seol et al. (29) reported that circulating Ykl-40 increased in preeclamptic patients and levels of YKL-40 were associated with disease severity. In our study, we found increased YKL-40 levels in invasion-positive group, which can be attributed to the inflammatory process due to MAP.

In addition, fibronectin has been shown to play a key role in cell adhesion, growth, migration and differentiation, wound healing, and embryonic development (14). It is synthesized in the endothelial cells and increased levels may suggest endothelial damage. Preeclampsia is characterized by poor placental perfusion and other pathological alterations in the placenta (i.e., defective trophoblast invasion), which forms the basis of endothelial plasma markers such as fibronectin. Similarly, Bodova et al. (30) found that there was an association between preeclampsia and increased plasma fibronectin levels, compared to normal pregnancies. In addition, low-grade inflammatory response has been shown in the peritoneal cavity of patients with endometriosis, which results in accumulation of the peritoneal fluid fibronectin to the inflammatory tissues and endometriosis implants (17). Defective trophoblast invasion and inflammation which play a role in MAP may also lead to increased fibronectin levels (4,19). In the literature, there is no study regarding PP and fibronectin values. In our study, we found that fibronectin values were similar between the previa and control groups; however, fibronectin was higher in invasion-positive group than invasion-negative group, although it couldn't reach statistical significance.

LIMITATIONS

Nonetheless, there are some limitations to this study. Small sample size and relatively small number of women with MAP can be considered as the main limitations. Therefore, further large-scale studies are required to evaluate the discriminative role of YKL-40 and fibronectin in MAP.

CONCLUSION

In conclusion, YKL-40 significantly increased in women with MAP, compared to women without MAP, likely reflecting the inflammation and invasion of MAP in our research. Therefore, YKL-40 level which is inflammation and invasion marker was found to be higher in invasion-positive group. This report is the first prospective study evaluating the YKL-40 and fibronectin levels in placenta previa and placental invasion anomalies. However, further extensive studies are needed to verify these reported outcomes.

Competing Interests: The authors declare that they have no competing interest.

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