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Original Article

Correlation of plasma osteopontin and neutrophil gelatinase-associated lipocalin levels with the severity and clinical outcome of pelvic inflammatory disease

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ABSTRACT

Objective: To investigate the correlation of two important inflammatory biomarkers, plasma osteopontin and neutrophil gelatinase-associated lipocalin (NGAL), with the severity and outcome of pelvic inflammatory disease (PID).
Materials and methods: Sixty-one patients with PID, including 25 patients with tubo-ovarian abscess (TOA), were consecutively recruited. Their blood samples were tested for the concentrations of plasma osteopontin and NGAL using enzyme-linked immunosorbent assay. The associations of these biomarkers with TOA, length of hospitalization, and incidence of surgery were also analyzed.
Results: Plasma osteopontin level was significantly increased in PID patients with TOA compared to PID patients without TOA (median 107.77 ng/mL vs. 72.39 ng/mL, $p = 0.004$). However, there was no significant difference for plasma NGAL. If the cutoff level of plasma osteopontin was set at 81.1 ng/mL, there was a 76.0% sensitivity and a 24.0% false negative rate in predicting TOA in PID patients. Plasma osteopontin significantly correlated with length of hospital stay ($r = 0.467$, $p < 0.001$), and this correlation was better than that of NGAL. However, neither biomarker was associated with incidence of surgery.
Conclusion: Plasma osteopontin has a better correlation with TOA and length of hospitalization compared to NGAL. If plasma osteopontin level falls below 81.1 ng/mL, PID patients will have about a 20% chance of developing TOA. Incorporating plasma osteopontin, but not NGAL, will allow for an adjuvant diagnostic biomarker for TOA and predictor of length of hospital stay.

Introduction

Osteopontin, a phosphorylated acidic glycoprotein first identified in bone, is involved in bone morphogenesis [1]. It is secreted by osteoclasts, osteoblasts, and kidney and endothelial cells, and by activated immune cells, such as T cells and macrophages [2,3]. It may regulate inflammatory cell mobilization and is strikingly upregulated at sites of inflammation and tissue remodeling, acting as a pro- and anti-inflammatory cytokine [4–6]. Neutrophil gelatinase-associated lipocalin (NGAL or lipocalin 2) was first isolated from specific granules of human polymorphonuclear neutrophils [7]. It is a 25-kDa secretory glycoprotein and belongs to a large family of lipocalins that comprise a group of more than 20 small secreted proteins defined on the basis of their three-

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dimensional structure [8]. Innate immune cells produce and secrete NGAL in bacterial infection before NGAL limits bacterial growth by sequestering the iron-laden siderophore [9].

Pelvic inflammatory disease (PID) is an infection of the female reproductive system that subsequently induces neutrophil and macrophage activation to fight against pathogens [10,11]. It is composed of a spectrum of inflammatory disorders of the upper female genital tract involving the uterus, fallopian tubes, and ovaries, and can result in an ectopic pregnancy and chronic pelvic pain. Furthermore, scarring and adhesion formation that accompanies healing of damaged oviducts may lead to female infertility of tubal factor. Because of its wide variation in the symptoms and signs, ranging from subtle or mild symptom to severe abdominal pain with abscess, PID is difficult to diagnose and may require admission and even surgery. Women with tubal factor infertility apparently induced by past episodes of PID often give no history of PID [12]. Early diagnosis and treatment may prevent the progression of PID to the more severe forms, such as tubo-ovarian abscess (TOA), and decrease the incidence of tubal factor infertility.

To date, there has been no report comparing plasma osteopontin and NGAL expressions between PID patients and TOA patients. No studies have investigated their implication in the clinical outcome of PID. This study hypothesizes that the levels of plasma osteopontin and NGAL are correlated with the severity of PID. Thus, the purposes of this study are to compare the plasma levels of NGAL and osteopontin between patients with PID and those with TOA, and to investigate the correlation of these biomarkers with the clinical outcomes of PID patients in terms of need for surgery and length of hospital stay.

Materials and methods

Participants and sample collection

This hospital-based study consecutively recruited 61 PID patients who received treatment at the Department of Obstetrics and Gynecology of Chung Shan Medical University Hospital, Taichung, Taiwan between April 2006 and September 2007. Among them, 25 were diagnosed as having TOA. Diagnosis of PID was based on the characteristic criteria set by the Centers for Disease Control and Prevention (CDC) for PID. Patients with pelvic or lower abdominal pain of no known origin and either uterine/adnexal tenderness or cervical motion tenderness were diagnosed as PID. By contrast, TOA was defined by the presence of a palpable adnexal mass and abscess in a tubo-ovarian mass on pelvic sonography in PID patients.

All PID patients were admitted to the wards of the Gynecology Department from the emergency room, where computed tomography scanning was done to rule out appendicitis and pelvic tumors other than TOA. Women who were pregnant, breastfeeding, taking antibiotics, suffering from major comorbidities such as heart disease and hypertension, suspected of having tumors originating from any organ except TOA, or had undergone gynecologic surgery within 3 weeks prior to admission, were excluded from the study. All PID patients in the wards received treatment based on the protocols suggested by the CDC. They received the same regimens of antibiotics, which were given intravenously for at least 3 days, or for an additional 24 hours after they were afebrile. Oral antibiotics were given thereafter until Day 14 of treatment.

If clinical signs or symptoms such as pelvic pain or fever did not improve or became exacerbated, if signs of TOA rupture or misdiagnosis of adnexal malignant tumor were suspected, or if medical treatment failed, surgery was performed to confirm the diagnosis and as definitive treatment.

The mean age and SD of patients with PID and TOA were 34.1 ± 13.1 years and 37.2 ± 11.6 years ($p = 0.353$), respectively. Sixty-one blood samples were collected prior to treatment and were analyzed for plasma concentrations of osteopontin and NGAL. The results were then compared between PID patients and TOA patients. The parameters were measured by clinical laboratory staff members who were blinded to the study. The hospital's Institutional Review Board approved the study and all of the participants provided written informed consent.

Measurements of plasma osteopontin and NGAL

Plasma osteopontin and NGAL levels were analyzed with human osteopontin enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Abingdon, UK) and human NGAL ELISA kits (R&D Systems), respectively. From each plasma sample, 50–100 μL was directly transferred to the microtest strip wells of the ELISA plate and then assayed according to the manufacturer's instructions. Absorbance was measured at 450 nm in a microtest plate spectrophotometer, and osteopontin and NGAL levels were separately quantified with a calibration curve using human osteopontin and NGAL as standards, respectively.

Statistical analysis

The Mann–Whitney *U* test was used to test the statistical significance of differences in plasma levels of osteopontin and NGAL between 36 PID patients and 25 TOA patients before they received treatment. The biomarkers were also compared between PID patients who received surgery and those who did not. Spearman's rank correlation analysis was used to correlate these biomarkers with length of hospital stay.

Results

Among the 61 PID patients, 25 patients had TOA. No sepsis or organ failure cases were found in these patients. The basic characteristics of patients with PID or TOA are shown in Table 1. The level of plasma osteopontin was significantly increased in PID patients with TOA compared to PID patients without TOA (median 107.77 ng/mL vs. 72.39 ng/mL, $p = 0.004$; Table 1). However, there was no significant difference for plasma NGAL (median 22.78 ng/mL vs. 16.83 ng/mL, $p = 0.681$; Table 1). If 81.1 ng/mL was set as a cutoff level of plasma osteopontin for differentiating TOA patients from other PID patients, sensitivity and the specificity would be 76.0% and 63.9%, respectively, using a receiver operating characteristic curve (Table 2).

Table 1
Basic characteristics and levels of plasma osteopontin and neutrophil gelatinase-associated lipocalin (NGAL) in patients with pelvic inflammatory disease (PID) or tubo-ovarian abscess (TOA).[a]

Variables	Patients with PID ($n = 36$)	Patients with TOA ($n = 25$)	*p*
Osteopontin (ng/mL)	72.39 (43.19–362.59)	107.77 (43.75–415.52)	0.004[b]
NGAL (ng/mL)	16.83 (7.61–161.39)	22.78 (6.49–386.74)	0.681
Age (y), mean ± SD	34.1 ± 13.1	37.2 ± 11.6	0.353
Socioeconomic status	Middle	Middle	
Resident area	Mid-Taiwan	Mid-Taiwan	
Ethnicity	Taiwanese	Taiwanese	
Regular cigarette smoking	None	None	
Regular alcohol drinking	None	None	

[a] Statistical analysis: Mann–Whitney *U* test. A *p* value <0.05 was considered significant.
[b] $p < 0.05$.