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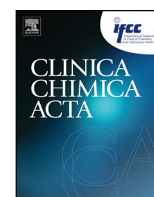

Increased plasma soluble CD40 ligand concentration in pelvic inflammatory disease

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ABSTRACT

Background: The role of soluble CD40 ligand (sCD40L) in pelvic inflammatory disease (PID) remains unclear. We sought to determine whether sCD40L was an efficient serum marker as with WBC and CRP in PID patients.
Methods: Enzyme-linked immunosorbent assay was used to measure the plasma levels of sCD40L before and after routine protocol treatments in sixty-four PID patients and seventy healthy controls.
Results: The level of plasma sCD40L (pg/ml) was significantly elevated in PID patients (1632.83 ± 270.91) compared to that in normal controls (700.33 ± 58.77; $p = 0.001$) and decreased significantly as compared to that in the same patients (928.77 ± 177.25; $p = 0.0001$) after they received treatment. The concentration of sCD40L was significantly correlated with the level of plasma C-reactive protein (CRP) in the blood (r = 0.202, $p = 0.01$, n = 134). When the cutoff level of plasma sCD40L levels was determined to be 1612.26 pg/ml based on ROC, the sensitivity, specificity, and the area under the curve of plasma sCD40L level for predicting PID were 0.26, 0.97, and 0.58 (95% confidence interval: 0.48–0.68), respectively, while the adjusted odds ratio (AOR) with their 95% CI of plasma sCD40L for PID risk was 7.09 (95% CI = 1.14–43.87, $p = 0.03$).
Conclusions: The expression of plasma sCD40L was increased in patients with PID and detection of plasma sCD40L could be useful for the diagnosis of PID.

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1. Introduction

Pelvic inflammatory disease (PID) is caused by micro-organisms [1] and is related to serious long term consequences including infertility, chronic pelvic pain, and ectopic pregnancy [2–4]. Acute PID is hard to diagnose because of a wide variation in the clinical pictures. Clinical diagnosis alone has only 87% sensitivity and 50% specificity [5]. Therefore, to develop non-invasive adjunctive strategies capable of detecting and controlling pelvic inflammatory disease is strongly recommended.

CD 40 ligand (CD40L, formally known as CD154) is a 39 kD transmembrane glycoprotein that belongs to tumor necrosis factor α (TNF-α) family and is expressed on the surface of monocytes, macrophages, T cells, B cells, platelets, and endothelial cells [6–8]. The CD40L receptor, CD40, is a 49 kD membrane glycoprotein of tumor necrosis factor receptor (TNFR) superfamily and is found on monocytes, macrophages, neutrophils, B cells, T cells, mast cells, dendritic cells, platelets, endothelial cells, smooth muscle cells, and fibroblasts [6,7]. It was reported that CD40 can initiate macrophage antimicrobial activity by rerouting intracellular pathogens to lysosomal compartment to induce fusion between pathogen containing vacuoles and lysosome, this autophagy-dependent fusion resulted in antimicrobial activity against pathogen invasion [9]. The interaction between CD40L and its receptor CD40 has been implicated to mediate inflammatory processes through the induction of various proinflammatory cytokines [6,7] and matrix metalloproteinase (MMP) [10,11] as well as the activation and recruitment of leukocytes [12]. Some of the induced proinflammatory cytokines, including interleukin 1β (IL-1β), tumor necrosis factor α (TNF-α), and interferon-r (IFN-r), subsequently augment the production of CD40L and CD40, which contribute to the expansion of inflammatory response [6,12]. The increased expression of CD40L has been reported to enhance the production and activity of monocytes and pro-inflammatory cytokines, such as IL-1, IL-6, IL-8, and TNF-α, and monocyte chemoattractant protein-1 (MCP-1) to mediate inflammatory response [6,13,14]. Interrupting the signaling pathway of CD40–CD40L

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by the administration of anti-CD40L antibody was reported to decrease the risk of inflammatory disease in numerous animal models, including collagen-induce arthritis (CIA) [15], encephalomyelitis [16], thyroiditis [16], and inflammatory bowel disease [17]. We suggest that CD40L plays an important role for controlling the severity of inflammation.

A cleavage of CD40L from the surface of cells produces a truncated soluble form of CD40L [18,19]. Soluble CD40L (sCD40L) is detectable in the blood and preserves biologic activity contributing to the mediation of inflammatory process [7,8,18–21]. The increased concentration of sCD40L in the blood has been reported in numerous inflammatory-related diseases, including HIV-associated neuroinflammation [20], Behcet disease [21], psoriasis [22], inflammatory bowel disease [23], and pancreatitis [4]. The activation and migration of leukocytes as well as the production of proinflammatory cytokines and MMPs were reported to play a pivotal role in pelvic inflammatory disease [24–31]. We hypothesized that CD40–sCD40L binding could facilitate the amplification of pelvic inflammatory response through the generation of cytokines and MMPs and the activation of leukocytes [7,8,18–21]. Also, the soluble form of CD40L could mediate inflammatory process in pelvic inflammatory disease, and plasma sCD40L level may be related to the severity of PID. However, to date there has been no study on the role of plasma sCD40L in patients with PID. Therefore, we estimated the expressions of sCD40L in plasma of patients with PID to establish a non-invasive strategy on its significance in the diagnosis and treatment of PID.

2. Materials and methods

2.1. Subjects and specimen collection

This was a hospital-based case–control study. We cooperated with gynecologists at the Chung Shan Medical University Hospital in Taichung, Taiwan. Sixty-four women, who were diagnosed as having PID by gynecologists (Wang PH, Tee YT, and Lin LY) according to the characteristic criteria of the national guidelines for pelvic inflammatory disease [32,33], between April, 2006 and August, 2011 were recruited as a case group. Seventy healthy women, who visited the Department of Obstetrics and Gynecology or the Department of Family Medicine for health examinations such as cervical Papanicolaou smear and breast examination, were randomly selected to match the sixty-four PID patients with regard to demographical and clinical data such as age matched to within five years, race, ethnicity, resident area, cigarette smoking, and alcohol drinking status. The control group was as similar as possible to the case group, but did not have pelvic inflammatory disease. The diagnosis of PID should conform to all the minimal criteria of the Centers for Disease Control and Prevention (CDC) including lower abdominal pain or pelvic pain of no other origin with one of the following criteria: uterine tenderness or adnexal tenderness or cervical motion tenderness. In order to maximize specificity and reduce the chance of delayed or missed diagnosis, in addition to the criteria mentioned above, the patients should have at least one of the following minor criteria: oral temperature >38.3 °C, abnormal vaginal or cervical mucopurulent discharge, an abundance of white blood cells (WBCs) on microscopic inspection of vaginal secretions, elevated C-reactive protein (CRP), elevated erythrocyte sedimentations, or laboratory documentation for *Neisseria gonorrhoeae* or *Chlamydia trachomatis*. Women, who were pregnant, breastfeeding, and taking oral pills or antibiotics for other forms of inflammatory diseases, or who had systemic diseases or cancers which originated from any organs such as cervix and ovary or who had undergone a gynecologic operation within 2 months prior to admission, were excluded from the study. Whole blood specimens were collected from the seventy healthy controls and sixty-four PID patients before and after they received treatment based on the routine protocols suggested by the CDC. The recommended parenteral regimens were cefotetan or cefoxitin plus doxycycline, or clindamycin plus gentamicin. All PID patients were admitted to the ward units in the Department of Obstetrics and Gynecology, Chung Shan Medical University Hospital. They were given antibiotics intravenously for at least 3 days, or for another 24 h after they were afebrile. Thereafter, oral antibiotics were given until day 14 of treatment. The pre-treatment blood samples were obtained before PID patients received treatment protocols, and post-treatment blood samples were obtained one week after treatment commenced. All blood samples analyzed for nonspecific inflammatory markers, such as WBC and C-reactive protein (CRP) [34,35], as well as plasma were analyzed for the expressions of sCD40L. Both the technician who measured the levels of sCD40L as well as the clinical laboratory staff who measured WBC, neutrophil counts, lymphocytes, and CRP were blinded to this study. The blood samples obtained for the measurement of sCD40L were placed in tubes containing EDTA and were immediately centrifuged and stored at −80 °C. The study was performed with the approval of the Chung Shan University Hospital Institutional Review Board and written informed consent was obtained from each patient.

2.2. Sample size and statistical power

We focused on the effect size (mean difference) of soluble CD40L expression between groups. Based on data from Erturan et al. [22], assuming α value was set at 0.05, our sample size had at least 80% power to detect a mean difference of 350 pg/ml based on the standard deviation was 700 pg/ml of sCD40L and the alternative hypothesis was two-sided [22].

2.3. Measurements of sCD40L level by ELISA

The sCD40L level in the plasma samples was analyzed by human sCD40L ELISA kits (R&D Systems, Abingdon, UK). From each plasma sample, 100 μl was directly transferred to the microtest strip wells of the ELISA plate and then assayed according to the manufacturer's instructions. The absorbance was measured at 495 nm in a microtest plate spectrophotometer, and the plasma level of sCD40L was quantified with a calibration curve using human sCD40L as a standard [23].

2.4. Statistical analysis

Experimental results are presented as the mean ± SE. An independent t-test was used to compare the differences of plasma levels of sCD40L, WBC and C-reactive protein (CRP) between the healthy women and PID patients before they received the treatment protocols. A paired-sample t test was used to test the difference of these parameters between pre-treatment and post-treatment plasma. Spearman correlation analysis was used to estimate the correlations between plasma level of sCD40L and inflammatory marker C-reactive protein.

The adjusted odds ratio (AOR) and their 95% confidence intervals (CIs) of sCD40L, WBC, CRP and PID risk were estimated by utilizing multiple logistic regression models after adjusting for confounding.

p value <0.05 was considered significant. The data were analyzed on SAS statistical software (Version 9.1, 2005; SAS Institute Inc., Cary, NC) and SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) statistical software.

3. Results

Except for age ($p = 0.01$), there was no significant difference in the distribution of demographic characteristics between PID patients and healthy controls. Blood cell count regarding WBC was significantly increased in patients with PID (11,391.71 ± 650.15) before they received treatment compared with those in healthy controls (7103.14 ± 302.85, $p = 0.0001$) and those in PID patients after they received treatment (6462.65 ± 278.37, $p = 0.01$). Also, plasma CRP-level was significantly increased in patients with PID (6.76 ± 0.80) before they received treatment compared with those in healthy controls (0.38 ± 0.03, $p =$

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