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ORIGINAL ARTICLE

Significance of serum levels of angiopoietin-2 and its relationship to Doppler ultrasonographic findings in rheumatoid arthritis patients

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KEYWORDS

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Abstract *Background:* Angiopoietin-2 (Ang-2) is connected to angiogenesis in synovial regions, but the significance of its levels in patients with rheumatoid arthritis (RA) is still unclear.

Aim of the work: To evaluate the significance of serum levels of Ang-2 in patients with RA. Also, to determine Ang-2 relationship to the findings of joints Doppler ultrasonographic findings.

Patients and methods: This study included 40 patients with RA, and 25 matched healthy controls. All patients were subjected to assessment of pain using visual analogue scale (VAS), assessment of personal activity using the Health Assessment Questionnaire (HAQ) score, and calculation of disease activity score (DAS 28). Laboratory assays of complete blood count (CBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) titre, and measurement of serum levels of Ang-2 by ELISA. Doppler ultrasonography (US) assessment for eight joints, with calculation of synovial thickness and total signal score (TSS), was done.

Results: Serum Ang-2 levels were significantly higher among patients (3191.3 ± 594.9 pg/ml) than controls (1771.7 ± 103.1 pg/ml) ($p < 0.001$). Serum Ang-2 levels were significantly correlated with ESR, CRP, DAS28, and duration of morning stiffness ($p < 0.001$, $p < 0.001$, $p < 0.001$, and $p = 0.025$, respectively). There was a significant correlation between serum Ang-2 levels and findings of US, regarding joint synovial thickness, and TSS ($p < 0.001$, for both).

Conclusion: Patients with RA had significantly higher levels of serum Ang-2 versus controls. In those patients, serum Ang-2 levels were significantly correlated with disease activity markers (ESR,

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CRP), DAS28, and duration of morning stiffness. Moreover, these levels were significantly correlated with synovial thickness, and TSS. The role of Ang-2 in RA pathogenesis might open the door to the development of new therapeutic strategies, particularly which target angiogenesis.

1. Introduction

Rheumatoid arthritis (RA) is a disease characterized by the chronic inflammation of joint synovial tissues. Inflammatory synovial tissue is called pannus. At such sites, many newly formed vessels are observed [1,2]. Angiogenesis is the formation of new capillaries from pre-existing blood vessels. Angiogenesis has been associated with inflammation and chronic inflammatory diseases, including RA [3,4].

Angiopoietin-2 (Ang-2), which is 478 amino acids in length with a molecular weight of 70 KDa, is a glycoprotein, and an angiogenic factor, plays an important role in the angiogenesis of pannus [5]. It is expressed in endothelial cells, stored in vesicles, and is rapidly released in response to specific stimuli at sites of vascular remodeling [6]. Ang-2 acts by binding to endothelium specific receptor Tyrosine kinase-2 (Tie-2) and the Ang/Tie system tightly controls the endothelial phenotype during angiogenesis and vascular inflammation in a unique fashion [7].

Doppler ultrasonography (US), which directly visualizes the synovial-membrane vessels, provides very early information on changes in synovitis activity during the course of inflammatory joint disease [8,9]. Doppler US can assess the synovial pannus and vascular tissues along with the detection of low-velocity blood flow at the microvascular level [10,11]. US is far more sensitive than physical examination for detecting rheumatoid synovitis. Also, it has similar sensitivity to magnetic resonance imaging, but is both far easier to use and considerably less expensive [12,13].

Therefore, the aim of the current study was to estimate serum levels of Ang-2 in patients with RA. Also, to correlate these levels with various clinical and Doppler US parameters.

2. Patients and methods

This study included forty RA patients [36 (90%) were females, and 4 (10%) were males], and their mean age was 43.9 ± 6.6 years. In addition, twenty five, age and sex matched healthy volunteers, were included. They included 20 (80%) females, and 5 (20%) males, and their mean age was 44.2 ± 6.8 years.

All RA patients were attendants of the Rheumatology inpatient or outpatient Department, Faculty of Medicine, Menoufiya University Hospital, in the period from September 2012 to May 2013.

Diagnosis of RA was made according to the American Rheumatism Association (ARA) criteria of American College of Rheumatology [14]. Patients with other suspected or known collagenic disease, liver disease, or renal disease were excluded from this study. The study was approved by our ethics committee of the faculty of medicine, and an informed consent was taken from all subjects.

All patients and controls were subjected to full history taking especially for duration of morning stiffness, fatigue and HAQ score assessment; and clinical examination, particularly for number of swollen joints, number of tender joints, and patient's assessment of pain using visual analogue scale (VAS). Moreover, disease activity score (DAS 28), was calculated with assessment of swollen and tender joints using 28-Joint counts [15].

Laboratory investigations included assays of complete blood count (CBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) titre, and serum levels of Ang-2.

2.1. Sample collection and assay

7 ml of venous blood samples were collected from all subjects under complete aseptic condition by clean venipuncture then dispensed into three tubes: 3 ml into plain tubes for chemical analysis of the previously mentioned parameters, 2 ml was transferred into EDTA tubes for complete blood count and 1.6 ml of blood was transferred to a tube with 0.4 ml citrate for ESR measurement. The samples in plane tubes, let to stand to clot and serum was separated in aliquots after centrifugation and stored at −70° until analysis of the following materials. CRP was determined using immunoassay [16]. RF concentration was assessed using RF Latex which was a slide agglutination test for the qualitative and semiquantitative detection of RF in human serum. Latex particles coated with human gammaglobulin were agglutinated when mixed with samples which contain RF [17]. ESR was determined according to Westergren method, and CBC was determined using a pentra-80 automated blood counter (ABX-France, Montpellier, France).

2.2. Ang-2 sample assay

It was assessed by The RayBio-Human Ang-2 Enzyme-Linked Immunosorbent Assay (ELISA) kit, which was in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human Ang-2 in the serum. This assay employed an antibody-specific for human Ang-2 coated on a 96-well plate. Standards and samples were pipetted into the wells and Ang-2 present in a sample was bound to the wells by the immobilized antibody. The wells were washed and biotinylated anti-human Ang-2 antibody was added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin was pipetted to the wells. The wells were again washed, a TMB substrate solution is added to the wells and colour develops in proportion to the amount of Ang-2 bound. The Stop solution changes the colour from blue to yellow, and the intensity of the colour is measured at 450 nm.