Original Article

Serum Interleukine 35 in Iraqi female patients with newly diagnosed of rheumatoid arthritis

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Summary:

Background: Rheumatoid arthritis (RA) characterized by local and systemic effects of inflammation has a wide range of biochemical markers implicated directly or indirectly to its pathogenesis.

Objective: To evaluate interlukine 35 (IL-35) in Iraqi females with newly diagnosed RA and to assess its contribution in the disease process.

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Patients and method: Serum of (55 Females) of newly diagnosed RA and 23 healthy Females were used to estimate their interlukine levels.

Results: Females of RA showed a significant increase in the levels of interleukine 35(IL-35) and in the levels of High Sensitivity C-Reactive Protein (hs CRP). While there were no significant changes in the level of alkaline phosphatase (ALP) and calcium (Ca). The correlation between IL-35 and each of hs CRP, ALP and Ca (-ve) were non-significant (r = -0.066, -0.04271 and -0.103) respectively.

Conclusion : This is the first study to show the elevation of serum levels of IL-35 in Iraqi female patients with newly diagnosed RA which indicates that IL- 35 may be a good biomarker for early RA.

Key Words: Rheumatoid arthritis (RA), Interleukin 35 (IL-35), High sensitivity C- reactive protein (hs CRP), Calcium (Ca), alkaline phosphatase (ALP).

Introduction:

Rheumatoid arthritis (RA) is a systemic autoimmune disease primarily affecting the joints. Chronic inflammatory processes lead to cartilage damage and bone erosions, resulting in destruction of the total joint architecture. (1-3) Rheumatoid arthritis is caused by a number of proinflammatory molecules released by macrophages (4). These include reactive oxygen species and eicosanoids such as prostaglandins, leukotriene>s and cytokines (5). Interleukin 35 has been identified as a novel immunosuppressive autoinflammatory cytokine, which plays a critical role in the inhibition of inflammation and autoimmune disease (6). It is a new member of the IL-12 family and is a hetrodimer composed of P35 subunit of IL-12 and the IL- 27 B(EBI3) subunit (7,8). Secretion of IL-35 has been confirmed only in non- stimulated mouse Tregs and in stimulated human Tregs (9). C-Reactive protein named for its capacity to precipitate the Somatic C- polysaccharide of streptococcus pneumonia and it is the acute phase of protein synthesized in response to injury (10). Alkaline phosphatase are a group of enzymes that are present in many different tissues , the two major ALP isoforms in humen serum produced in bone and liver , are difficult to distinguish because they are both encoded by the tissue non specific ALP gene , both isoforms differ only in their patterns of post translational glycosylation.(11,12) Calcium is the most plentiful mineral in the human body. The majority of calcium in bones and teeth. A small percentage of Ca is found in blood and other fluid (13).

Patients and methods:

Different samples collected from two groups. The first was of 55 female patients with newly diagnosed RA by testing the elevation in ESR, positive RF and X-rays of their joints at Baghdad Teaching Hospital who were not taking any medication, while the second group was of 23 healthy persons control. Both groups are matching in their ages. Five milliliters of venous blood collected into plastic tubes from each subject in the study after a 12 hr fast, left at room temperature for 15 min, then centrifuged at 3000 rpm for 15 min. Serum divided into aliquots for subsequent measurement of IL-35, hs CRP, ALP and Ca. IL-35, hs CRP are determined by Elisa Kit, while ALP and Ca by a colorimeter.

Statistical analysis : Data expressed as (mean+ SD) statistical significance which were determined by unpaired student's t-test and followed by person's correlation (r). P values equal or lower than 0.05 were considered as statisticcally significant.

Results:

A significant $p \leq 0.000$ increase in the level of IL- 35 and hs CRP were observed in RA female patients compared with healthy control while the levels of ALP and Ca show non-significant changes as shown in Table(1).

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Table (1) concentration of IL-35, hs CRP, ALP and Ca in serum of Female patients with RA and Female control

Group	No.	IL- 35 (Pg/ml) mean± SD	hs CRP (mg/L) mean ±SD	ALP (U/L) Mean ± SD	Ca (mmol/L) mean±SD
patients of RA	55	38.37 ± 5.42	586.0 ±76.61	76.5 ± 9.17	2.13 ± 0.1
Control	23	22.7 ± 2.26	75.8 ±0.78	79.8 ±5.88	2.14 ± 0.15
P value	0.000 S	0.000 S	0.000 S	0.30 NS	0.89 NS

S: significant
NS:Non significant
IL-35: Interlukine 35
hs CRP: High sensitivity c-reactive protein ALP: alkaline phosphatase
Ca:Calcium

Table (2) Values of r indicating the correlation between serum IL-35 against hs CRP, Alp and Ca.

	IL-35
hsCRP	-0.066
ALP	-0.042
Ca	-0.103

IL-35:Inlterlukine35
hs CRP: High sensitivity c-reactive protein ALP: alkaline phosphatase
Ca: Calcium

Discussion:

High significant increase in the concentration of IL-35 in serum of RA female patients which is a novel immunosuprressive cytokine, belonging to the IL-12 Sub family members appears to play a key role in the suppressive function of Treg against conventional Tcells (14). Ectopic expression of IL-35 confers regulatary activity to naive Tcells. Furthermore IL-35 suppresses Tcell proliferation in vitro (8, 14) IL-35 consist of EBi 3 and p35 are both expressed in human . Treg cells and the molecular required for contact independent Tcell suppression (15). The type of Tcell response depends on the type of cytokine production in the inflammatory environment. Both DCs (Dendritic cells) and iTr (35) types of Treg cell produce IL- 35 to suppress Th1, Th17 and Th2 responses and in RA pathological outo – antigen signalling by DCs and other antigen presenting cells leads to outo – antigen induced Tcell responses.(16,17) High significant increase in hs CRP in RA female patients because it is an acute phase protein whose serum concentration reflects ongoing inflammation. The elevations in hs CRP are seen in all chronic inflammatory states RA.(18,19) In RA patients measurement of CRP and ESR may be more helpful in assessing disease activity than measuring either alone (20). Table (2) Shows a negative significant correlation between IL-35 and hs CRP, ALP and Ca. There was negative non-significant (-ve) correlation between Serum IL- 35 and hsCRP (r = -0.066) also negative significant correlation (-ve) between the serum lL- 35 and ALP and Ca (r = -0.042) and (r = -0.103) respectively.

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