Study of Resistin and YKL-40 in Rheumatoid Arthritis.

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Abstract: The objective of this study was to investigate the association between serum and synovial fluid levels of both resistin and YKL-40 with markers of inflammation, disease activity and radiographic joint damage and to determine if they have a role in the pathogenesis of RA. This study was conducted on 30 patients with RA and 15 healthy controls with acute post traumatic knee effusion. Serum and synovial fluid levels of both resistin and YKL-40 were measured in patients and controls using ELISA technique. Plain x-ray of hands, wrists and feet were done for all patients and assessed according to Larsen score. Serum levels of resistin and YKL-40 were significantly higher in RA patients than controls and in active RA than in non active patients. Also, their levels significantly correlated with CRP, ESR, RF, disease activity parameters and Larsen score. Synovial YKL-40 levels showed significant correlation with CRP, ESR, RF, disease activity parameters and Larsen score. On the other hand, resistin synovial levels significantly correlated with CRP, ESR, RF and synovial leucocytic count. As a conclusion, serum and synovial resistin and YKL-40 provided new and direct information on local disease activity in rheumatoid arthritis. Our data supported the hypothesis that resistin and YKL-40 are involved in the pathogenesis of RA. Also, serum resistin and YKL-40 could be considered as a prognostic marker in RA as they predict radiographic progression of joint damage. The identified properties of resistin and YKL-40 make each of them a novel and interesting therapeutic target in rheumatoid arthritis. [Journal of American Science 2010;6(10):1004-1012]. (ISSN: 1545-1003).

Key words: resistin, YKL-40, rheumatoid arthritis

1. Introduction:

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by the development of synovitis, which damages cartilage, bone, ligaments and tendons (Naredo et al., 2005). Efforts have been undertaken for the last two decades to find out reliable biomarkers of disease prognosis in early RA patients and biomarkers of disease activity to be used during different treatment regimens and follow up of RA patients (Johansen,2006).

White adipose tissue, which is now considered to be a frank endocrine organ, produces a large number of molecules that share the functional and structural properties of cytokines and are therefore called adipocytokines (Otero et al., 2006). Resistin is a newly described adipocytokine which was initially linked to insulin resistance by impairing insulin action and causing insulin resistance (Steppan and Lazar, 2004). More and more evidence indicated that resistin may also be involved in the inflammatory process **(**Kaser et al., 2003**).** Recent studies have shown the regulation of proinflammatory cytokine expression by resistin (Bokarewa et al., 2005). Other evidence linking resistin to inflammation is that plasma resistin levels were found associated with many inflammatory markers in some pathophysiological conditions (Stejskal et al., 2003). Moreover, intra-articular injection of recombinant resistin into the knee joint of healthy mouse caused arthritis accompanied by leukocytic infiltration and hyperplasia of the synovium (Bokarewa et al., 2005).

YKL-40 is a human cartilage glycoprotein-39 (HC gp-39) which is related in amino acid sequence to the chitinase protein family. YKL-40 is a major secretory protein of human chondrocytes and synoviocytes (Johansen et al., 1996). Their levels are low in normal human cartilage but are increased in both inflammatory and degenerative joint disease and therefore, YKL-40 may be a marker of cartilage turnover and synovitis (Harvey et al., 1998).

YKL-40 was detectable in synovial fluid from RA patients with concentrations from a few hundred nanograms to more than 5 micrograms per ml (Volck et al., 2001). Johansen (2006) suggested that YKL-40 may play a fundamental role in the pathophysiology of RA and that immunological tolerance of the protein may control disease activity in RA patients. YKL-40 stimulates the proliferation rate of synovial cells and chondrocytes *in vitro* (De Ceuninck et al., 2001 & Recklies et al., 2002) indicating an autocrine function. YKL-40 is suggested to be a differentiation marker in chondrocytes (Imabayashi et al., 2003) and may protect the cells from undergoing apoptosis. Also, it has been reported that YKL-40 has a role as an autoantigen in rheumatoid arthritis- with the capacity

to induce a T-cell-mediated autoimmune response (Volck et al., 1998).

The aim of this study was to investigate the association between serum and synovial fluid levels of both resistin and YKL-40 with markers of inflammation, disease activity and radiographic progression and to determine if they have a role in the pathogenesis of RA.

2. Patients and Methods

Thirty patients with rheumatoid arthritis diagnosed according to the American College of Rheumatology revised criteria (Arnet et al., 1988) were included in this study. They were collected from the Outpatient Clinic of Physical Medicine & Rehabilitation Department, Tanta University Hospitals. Laboratory investigations were performed in Clinical Pathology Department, Tanta University Hospitals. The 30 patients were 24 females and 6 males and their ages ranged from 20 to 60 years with a mean value of 44.5 ± 6.4 years. The disease duration ranged from 2 to 15 years with a mean value of 6.88 ± 0.83 years.

Regarding to therapy, all patients were receiving methotrexate and non-steroidal anti-inflammatory drugs (NSAIDs), 5 of them were receiving also hydroxychloroquene.

No patients had received intra-articular glucocorticoid therapy within the last 4 weeks. Also, RA patients with current infection or with cardiac, hepatic, renal, malignancy or other diseases which might affect the parameters to be investigated were excluded from the study. Also, diabetic patients were excluded.

Fifteen healthy individuals with acute post-traumatic knee effusion matched for age and sex were served as controls.

All the patients were subjected to the following:

1. Full history taking.
2. Thorough clinical examination with stress on:
   * Locomotor system examination.
   * Assessment of disease activity according to Mallya and Mac (1981).

3. Laboratory investigations:

**Complete blood count (CBC):*

It was done on coulter counter Sysmex and blood film was stained by leishman's stain for differential leucocytic count.

**Erythrocyte sedimentation rate (ESR)* with Westergren method.

**Rheumatoid factor (RF)* concentration by a turbidimetric method suing SYNCHRON Rheumatoid Factor Reagent.

*C-reactive protein (CRP) was assayed by nephelometry (Behringwerke, Marburg, Germany).

*Synovial leucocytic count.

*Serum and synovial resistin and YKL-40 were dermined by ELISA technique.

Sampling:

Blood samples were drawn from the cubital vein in patients and controls at the day of clinical examination after overnight fasting. Blood samples were collected and 0.5 cc of blood was added to a tube containing 50µ of di-potassium salt of anhydrous ethylendiamine tetra-acetic acid (EDTA) (10% conc.) for complete blood count then about 3 cc of blood samples allowed to clot at room temperature for 30 min and then centrifuged at 4°C at 1500 rpm for 10 min. Serum samples were either analysed immediately or stored at -70°C for later analysis.

Synovial fluid was obtained by arthrocentesis of the knee joint under aseptic technique from fifteen of our RA cases and from all controls. The synovial fluid was withdrawn using a 1.2 mm gauge heparinized needle and collected in two tubes; 2 mL in an anticoagulant tube (sodium heparin or liquid EDTA) for microscopic examination; and about 5 mL into a plain (no anticoagulant) tube for ELISA. Immediately after aspiration, the synovial fluid samples in the plain tubes were centrifuged at 3000 rpm for 10 min to remove cells and debris, and the supernatant was stored at -70^{o}C until analysis. While Synovial fluid, in an anticoagulant tube, was counted for leucocytes using the heamocytometer.

Resistin and YKL-40 measurement:

Serum and synovial YKL-40 were assayed by a commercial two-site sandwich immunoassay in a microtitre stripwell format (Chondrex, Metra Biosystems, Inc, USA) using streptavidin- coated microplate wells, a biotinylated-Fab monoclonal capture antibody, and an alkaline phosphatase–labeled polyclonal detection antibody. The sensitivity of the ELISA was 10 ng/ml.

Resistin levels in serum and synovial fluid were measured by enzyme-linked immunosorbent assay (ELISA) kits according to the protocol provided by the manufacturer (human resistin ELISA kit, Biovendor Laboratory Medicine, Inc., Brno, Czech Republic). Briefly, matched samples of serum and synovial fluid were diluted 1/10 in BSA-PBS and introduced into the parallel strips coated with capture polyclonal anti-resistin Abs. Biotin-labeled anti-resistin Abs, streptavidin-HRP conjugate, and corresponding substrate were used for color development. The obtained absorbance values were

compared with serial dilution of recombinant human resistin. Lowest detectable level was 1 ng/ml.

4. Radiographic assessment:

Post ero-anterior radiographs of hands, wrists and feet were examined for all patients and joint destruction was classified by comparison with standard reference films according to Larsen score (Larsen et al., 1977).

Statistical analysis:

The statistical analysis was performed with SPSS software system (SPSS Inc., Chicago, IL; version 12.0). Results were given as means and standard deviation. Comparison between groups was performed by student's t test. Correlation study was done using Spearman's rank correlation coefficient (r). A value of $p < 0.05$ was considered significant, while $p < 0.001$ was highly significant.

3-Results:

This study was conducted on 30 RA patients and 15 individuals with acute post-traumatic knee effusion matched for age and sex were served as controls. The patients were 6 males (20%) and 24 females (80%), their ages ranged from 22 to 62 years with a mean value of 44.5±6.4 years. The duration of the disease ranged from 2 to 15 years with a mean value of 6.88 ± 0.83 years. The controls were 3 males (20%) and 12 females (80%). Their ages ranged from 32 to 49 years with a mean value of 46.8±8.4 years (table, 1). The basic demographic clinical and laboratory data of RA patients were presented in table (1).

In this study, the serum level of resistin in RA patients ranged from 3.9 to 13.6 ng/ml with a mean of 7.96±2.46 ng/ml and that of synovial fluid ranged from 27.4 to 51.2 ng/ml with a mean of 36.59±6.2 ng/ml. While in the control group the serum level of resistin ranged from 1.2 to 4.9 ng/ml with a mean of 3.61±1.24 ng/ml and that of synovial fluid ranged from 4.6 to 12.9 ng/ml with a mean of 9.13± 1.8 ng/ml (table 1).

Resistin levels in serum and synovial fluid were significantly higher in RA patients than controls. Also, resistin levels of RA patients were significantly higher in synovial fluid than in serum (table, 1). Moreover, no significant difference was found between males and females regarding serum and synovial resistin levels.

Resistin levels in serum and synovial fluid showed statistically significant difference between patients with active and inactive disease (table, 2). Serum resistin levels were significantly correlated with clinical and laboratory parameters of disease activity and also, with RF and CRP as shown in table (3). On the other hand, non significant correlation was found between serum resistin levels and age and duration of the disease (table 3).

The levels of synovial fluid resistin were not influenced by age, disease duration or parameters of disease activity, but significantly correlated with ESR, CRP, RF and synovial leucocytic count as shown in table (3). Also, significant positive correlation was found between serum and synovial resistin (table 3). No significant difference in serum and synovial resistin concentrations were found between males and females.

In this study also, the serum level of YKL-40 in RA patients ranged from 90 to 332 ng/ml with a mean of 117.08±43.83 ng/ml and that of synovial fluid ranged from 485 to 3598 ng/ml with a mean of 1949.66±839.22 ng/ml. While in the control group the serum level of YKL-40 ranged from 25 to 89 ng/ml with a mean of 42.6 ±18.9 ng/ml and that of synovial fluid ranged from 112 to 392 ng/ml with a mean of 309.4 ± 97.9 ng/ml (table 1).

YKL-40 levels were significantly higher in synovial fluid than in serum in both patients and controls ($p< 0.001$). Moreover, YKL-40 levels were significantly higher in patients than in controls regarding serum and synovial fluid ($p < 0.001$) (table,1). No significant difference in serum and synovial YKL-40 concentrations were found between males and females. Also, statistically significant difference was found between patients with active and inactive disease regarding serum and synovial YKL-40 ($p < 0.001$) as shown in table (2).

Statistically significant correlation was found between both serum & synovial YKL-40 and clinical & laboratory parameters of disease activity. Also, significant correlation was found between serum & synovial YKL-40 with RF and CRP. Moreover, YKL-40 in serum was correlated significantly with synovial YKL-40 (table, 3). On the other hand non significant correlation was found between serum & synovial YKL-40 and age & duration of the disease (table 4).

Radiographic findings:

Significant positive correlation was found between serum resistin levels and Larsen score for radiological joint damage, but the correlation was non significant regarding synovial resistin levels as shown in table (3). The concentration of serum and synovial YKL-40 was significantly correlated with radiographic grading of Larsen score (table, 4).

Table (1): Basic demographic, clinical and laboratory data of rheumatoid arthritis patients and controls

Parameter	RA patients (n=30)	Controls (n=15)	t	P
	Mean ± SD	Mean ± SD		
Age (years)	44.5 ± 6.4	46.8 ± 8.4	0.897	NS
Sex: Male: female	6:24	3:12		
Disease duration (years)	6.8 ± 0.83			
Morning stiffness (min)	126.84 ± 6.79			
Grip strength(mmHg)	112.1 ± 14.13			
RAI	18.5 ± 1.8			
Pain scale (VAS)	6.9 ± 0.53			
Hb (g/dl)	9.8 ± 0.92			
ESR 1st hour (mm/h)	60.0 ± 18.7	11.4 ± 1.6	5.3	HS
Disease activity grade	3.2 ± 0.4			
CRP (mg/L)	39.2 ± 11.75	1.28 ± 0.15	6.7	HS
RF (IU/mL)	200 ± 92.6			
Serum resistin (ng/ml)	7.96±2.46	3.61±1.24	5.8	HS
Synovial resistin (ng/ml)	36.59±6.2*	9.13± 1.8	6.9	HS
Serum YKL-40 (ng/ml)	117.08 ±43.83	42.6±18.9	4.1	HS
Synovial YKL-40 (ng/ml)	1949.66 ±839**	309.4 ±97.9	5.4	HS

-RAI: Ritchie articular index. -VAS: visual analogue scale.

-*Significant difference between serum and synovial resistin ($p < 0.001$).

-**Significant difference between serum and synovial YKL-40 ($p < 0.001$).

Table (2): Comparison between serum & synovial resistin and YKL-40 in patients with active and inactive disease

Parameter	Active RA (n=12)	Inactive RA (n =18)	t	p
	Mean ± SD	Mean ± SD		
Serum resistin (ng/ml)	9.3 ± 2.01	7.6 ± 2.06	4.7	HS
Synovial resistin(ng/ml)	39.8 ± 6.9	30.4 ± 7.2	5.9	HS
Serum YKL-40(ng/ml)	154.9±32.7	99.2±17.05	6.4	HS
Synovial YKL-40(ng/ml)	1994.9±432.4	1112.9±96.08	4.7	HS

Table (3): Correlation of serum & synovial resistin with some clinical and laboratory parameters

Parameter	Resistin			
	Serum		Synovial	
	r	p	r	p
Age (years)	0.16	NS	0.14	NS
Disease duration (years)	0.15	NS	0.13	NS
Morning stiffness (min)	0.36	S	0.22	NS
Grip strength (mmHg)	-0.35	S	-0.12	NS
RAI	0.57	HS	0.15	NS
Pain scale (VAS)	0.57	HS	0.21	NS
Hb (g/dl)	-0.69	HS	-0.23	NS
ESR (mm/h)	0.35	S	0.37	S
Disease activity grade	0.63	HS	0.18	NS
CRP (mg/L)	0.58	S	0.44	HS
RF (IU/mL)	0.37	S	0.39	S
Serum resistin(ng/ml)	-	-	0.68	HS
Synovial resistin(ng/ml)	0.49	HS	-	-
Synovial leucocytic count	-	-	0.53	HS
Larsen score	0.34	S	0.16	NS

Table (4): Correlation of serum & synovial YKL-40 with some clinical and laboratory parameters.

Parameter	YKL-40			
	Serum		Synovial	
	r	p	r	p
Age (years)	0.15	NS	0.13	NS
Disease duration (years)	0.18	NS	0.11	NS
Morning stiffness (min)	0.51	S	0.53	HS
Grip strength (mmHg)	-0.34	S	-0.36	S
RAI	0.62	HS	0.61	HS
Pain scale (VAS)	0.59	HS	0.61	HS
Hb (g/dl)	-0.47	HS	-0.49	HS
ESR (mm/h)	0.33	S	0.30	S
Disease activity grade	0.54	HS	0.57	HS
CRP (mg/L)	0.35	S	0.75	HS
RF (IU/mL)	0.34	S	0.38	S
Serum YKL-40 (ng/ml)	-	-	0.72	HS
Synovial YKL-40 (ng/ml)	0.69	HS	-	-
Larsen score	0.52	HS	0.76	HS

4- Discussion:

Chronic active inflammation of the rheumatoid joint often leads to irreversible destruction of articular cartilage and subchondral bone. Measurements of acute-phase response, serum CRP and ESR, are considered to be suitable biochemical markers for long-term monitoring of disease activity in RA. However, differences exist between clinical disease activity and the level of ESR or serum CRP. Normal values of ESR and CRP are found in patients with apparently clinically active disease (Emery and Luqmani, 1993) and the development of bone erosions in the hands from patients with early RA can occur independently of clinical symptoms and the acute–phase response (Otterness, 1994). Additional biochemical markers are therefore needed. Among these markers are resistin and YKL-40.

Resistin is an adipokine and a novel cytokine with a proinflammatory properties in human. The fact that resistin is abundantly expressed in bone marrow cells and, in particular, in leukocytes and macrophages, and that molecules of resistin like molecules (RELM) family are found in inflamed tissues suggests that resistin can play a role in the inflammatory process (Migita et al., 2006). Several studies have suggested that YKL-40 might be a useful new marker of severity for inflammatory and degenerative joint diseases and it may allow treating arthritis before its symptoms become painfully obvious (Johansen et al., 2001).

The aim of this study was to investigate the association between serum and synovial fluid levels of both resistin and YKL-40 with markers of inflammation, disease activity and radiographic joint damage and to determine if they have a role in the pathogenesis of RA

Serum & synovial resistin:

In this study we found that the levels of serum and synovial resistin were significantly higher in RA patients than in controls and there is no significant difference between males and females. Furthermore, serum resistin levels showed a significant correlation with clinical and laboratory parameters of disease activity, with inflammatory markers as CRP and ESR, with RF and with Larsen score. On the other hand, it showed no correlation with age and duration of disease. These findings are in accordance with that of Forsblad d'Elia et al., (2008) who found that serum resistin was significantly correlated with CRP and Larsen score in their RA patients and concluded that resistin was associated with increased inflammation and joint destruction. Also, these findings are in agreement with Gonzalez-Gray et al.(2008) and Migita et al. (2006) who reported that serum levels of resistin in their RA patients were significantly higher than controls and that serum resistin levels significantly correlated with CRP, ESR and disease activity scores, but not with age, sex or disease duration. Also, Senolt et al. (2007) found that serum resistin levels significantly correlated with CRP and disease activity scores but not with age and sex. Rho et al., (2009) concluded that concentrations of adipocytokines are increased in patients with RA and may modulate radiographic joint damage.

This study showed that the synovial resistin levels were significantly higher than in the sera of our RA patients and significantly correlated with CRP, ESR and RF but not correlated with age,

disease duration, disease activity parameters or Larsen score. These findings were confirmed by the results of Schäffler et al. (2003) and Bokarewa et al. (2005) who found that the synovial resistin levels were significantly higher than that in the sera of their RA patients and significantly correlated with CRP and ESR. The lower levels of resistin in the circulation compared with the that in the synovium was explained by Bokarewa et al. (2005) by increasing local production and/or a preferential accumulation of this molecule at the site of inflammation. This study also showed that serum and synovial resistin levels were significantly higher in active than in non active rheumatoid patients.

This study also found significant positive correlation between serum and synovial resistin levels. Also, the synovial resistin levels correlated significantly with synovial leucocytic count which is known to indicate the severity of intra-articular inflammatory process, a finding which is coincided with that of Bokarewa et al (2005). In their study on resistin levels in synovial fluid and serum in RA. Senolt et al. (2007) suggested that serum resistin levels could be more relevant to systemic inflammation and/or disease activity, while synovial fluid resistin reflects the particular inflammatory process of the affected joint.

Serum & synovial YKL-40:

This study found that serum and synovial YKL-40 were significantly higher in RA patients than in controls. This finding is in agreement with Knudsen et al. (2009) and Knudsen et al. (2008) who found at the beginning of their study that all their RA patients had significantly elevated serumYKL-40 compared to healthy controls. Also, Volck et al. (2001) found that the synovial fluid level of YKL-40 was higher in RA knees compared to normal knee joints. They also stated that this observation may indicate that the chondrocytes of the arthritic cartilage contribute to the high level of YKL-40 in synovial fluid from patients with RA. Also, they hypothesized that YKL-40 may play a role in cartilage remodeling in arthritic joints. Also, in this study, no significant difference in serum and synovial YKL-40 concentrations was found between males and females. This is in consistent with Johansen et al. (1996) who found no difference in serum concentration of YKL-40 between sex and different age groups.

YKL-40 levels were significantly higher in synovial fluid of our RA patients than in serum and a significant positive correlation exists between levels of YKL-40 in serum and synovial fluid. These results are in agreement with Harvy et al (1998) and Conrozier et al. (2000) who reported that YKL-40 local production by joints was a 10-15 fold higher in synovial fluid than in serum and suggested that the increased serum levels derived from arthritic joints. Also, Volck et al. (2001) concluded that a relationship exists between YKL-40 in serum and synovial fluid. This finding could be explained as YKL-40 in synovial fluid is derived from cells in the inflamed synovium, chondrocytes and synovial fluid neutrophils, and the joint derived YKL-40 influences serum YKL-40 (Conrozier et al., 2000).

In this study we found that serum and synovial YKL-40 were influenced by the disease activity as evaluated by clinical and laboratory markers of disease activity. There was statistically significant increase in serum and synovial YKL-40 in active RA patients in comparison to inactive patients. This finding coincided with Johansen et al. (2001) who confirmed that serum YKL-40 was increased in 54% of RA patients with active disease. Also, Syversen et al., (2010) found association between YKL-40 and DAS28 in their RA patients. In our study, a statistically significant correlation was found between serum &synovial YKL-40 and clinical and laboratory parameters of disease activity. This coincided with Volck et al. (2001) who also found significant correlation between serum & synovial fluid concentrations of YKL-40 in RA patients with ESR & CRP. This finding also was found by Johansen et al. (2001), Vos et al. (2000) and Matsumoto & Tsurumoto (2001). Johansen et al. (1999) stated that approximately 70% of their RA patients with elevated serum YKL-40 had also high ESR or serum CRP indicating that ESR, serum CRP and YKL-40 levels reflect inflammation of RA patients differently. Also, Syversen et al., (2010) stated that YKL-40 is a marker of joint inflammation in RA.

Volck et al. (2001) stated that CRP and ESR, the gold standards of biochemical assessment of disease activity in RA, are produced in the liver by hepatocytes and thus an indirect measure of joint inflammation. By contrast, serum YKL-40 is more direct measure of joint inflammation as it is locally released in the arthritic joint.

The results of this study showed no correlation between serum & synovial YKL-40 and age & disease duration. This coincided with the finding of Vos et al. (2000) and Peltomaa et al. (2001) who found no relation of serum YKL-40 and age & duration of disease in their RA patients. On the other hand, we found significant positive correlation between serum & synovial YKL-40 and RF. This is in agreement with Vos et al. (2000).

This study showed that serum and synovial YKL-40 were significantly correlated with radiographic progression according to Larsen score. This finding is in agreement with Matsumoto et al.(2001), Peltomaa et al. (2001) and den Broeder et al.(2002) who stated that serum YKL-40 correlated with Larsen score, the number of bone erosions and Sharp score and RA patients with bone erosions had higher serum YKL-40 than patients without erosions. Two longitudinal studies of 1-3 years of patients with early RA have shown that the mean serum YKL-40 levels during the study periods were related with the progression in Larsen score and developed more bone erosions compared to patients with normal serum YKL-40 (Johansen et al., 1999). So, serum YKL-40 had a predictive value for the risk of radiographic progression of joint damage in patients with early RA. On the contrary Syversen et al.,(2010) concluded that YKL-40 is not a predictor of progressive joint destruction in RA but only marker of joint inflammation and disease activity.

5. Conclusion:

Serum and synovial resistin and YKL-40 provided new and direct information on local disease activity in rheumatoid arthritis. Our data supported the hypothesis that resistin and YKL-40 are involved in the pathogenesis of RA. Also, serum resistin and YKL-40 could be considered as a prognostic marker in RA as they predict radiographic progression of joint damage. The identified properties of resistin and YKL-40 make each of them a novel and interesting therapeutic target in rheumatoid arthritis.

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8/1/2010