Research Article

The Role of Serum Fucose, Protein Bound Fucose and Other Biomarkers in Patients with Rheumatoid Arthritis

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Abstract

Background: Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that principally attacks synovial joints. **Aims**: To evaluate the status of serum fucose and protein bound fucose, anti-CCP, TNF-α and RF in serum of RA patients. **Subjects and methods**: The study has included 30 patients with early RA (with disease duration <1 year) (23 females+ 7 males) with age range (30-61) years and 30 patients with late RA (with disease duration >1 year) diagnosed according to the 2010 EULAR criteria (25 females, 5 males) with age range (30-60) and 28 healthy control individuals (22 females,6 males) with age range (30-55), who were age and sex matching with patients. **Results:** Levels of total serum fucose and protein bound fucose significantly decrease in RA patients than healthy control, while anti-CCP, TNF-α, RF, CRP and ESR significantly increase in RA patients than healthy control. Levels of total serum fucose, protein bound fucose & ACCP were not significant between three stages of activity in late & early RA patients. The level TNF-α & RF were significant only in very active cases in late RA, while not significantly in all cases of early RA. Positive correlation was found between ACCP & ESR in RA patients & early RA patients. Positive correlation found between TSF & PBF, negative correlation found between TNF-α & ESR in late RA patients. Positive correlation found between ACCP & ESR, CRP & ESR in early RA patients. **Conclusions:** This study illustrate that total serum fucose and protein bound fucose aid to diagnosis RA patient. Also Anti-CCP is a good & specific marker for diagnosis of RA,it is a good prognostic and as index test of severity.

Keywords: *Rheumatoid arthritis, fucose, protein bound fucose, Anti-CCP & tumor necrosis factor*

INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic inflammation of the synovial tissue and proliferation of the synovial membrane leading to damage of the articular structures. It mainly affects synovial joints, producing symmetrical arthritis, joint pain and stiffness.[1] The American College of Rheumatology (ACR) classification criteria and the European League Against Rheumatism (EULAR) publish diagnostic guidelines which help in diagnosis.[2] RA is associated with decreased physical function, disability, underemployment, and overall diminished quality of life, RA associated with reduced life expectancy by an average of 5 to 15 years.[3] It is a major cause of chronic disability and handicap.[4] There is a prominent immunological dysfunction in the joints and many other tissues by accumulation of chronic inflammatory cells including T and B- lymphocytes, monocytes and macrophages.[5]

Fucose (6-deoxy-L-galactose) is a monosaccharide that is a commonly produced by mammalian cells,[6] that body requires it for optimal function of cell-to-cell communication.[7,8] Fucose found on glycoproteins and glycolipids in vertebrates.[9] Two structural features distinguish fucose from other six-carbon sugars present

in mammals. These include the lack of a hydroxyl group on the carbon at the 6-position (C-6) and the L-configuration.[10] Fucose frequently exists as a terminal modification of glycan structures, specific terminal glycan modifications, including fucosylation, can confer unique functional properties to oligosaccharides and are often regulated during ontogeny and cellular differentiation.[11]

Serum protein bound carbohydrates (fucose) have been examined by numerous investigators, the transfer of a fucose residue to oligosaccharides and proteins which called fucosylation, is regulated by many kinds of molecules.[9] Fucose bound protein is able to differentiate active from inactive phase of RA.[12]

The expressions of terminal sugar in synovial and plasma fibronectins were studied in relation to rheumatoid arthritis (RA) progression defined according to the early and late (established) RA patients. In the early RA group, the synovial fibronectin reactivation was lowest. In the established (late groups) fucosylation significantly increased. Moreover, the expression of alpha1-6-linked fucose was found to be related to disease activity. Such alterations may be applicable as a stage-specific marker for diagnosis and therapy of RA patients.[13] Levels of fucose are low in those with rheumatoid arthritis, and supplementation is showing promise as a harmless but surprisingly effective treatment. What is particularly interesting is the lower a person's level of fucose (as well as galactose, another essential sugar); the more advanced the disease.[14] The RF antibody is present in about 75% - 80 % of RA patients, but its specificity is limited since RF is also found in patients with other autoimmune diseases, and to a certain extent in the healthy elderly individuals.[15]

In spite of RF has relatively low specificity, its consider as the major laboratory hallmark of RA and used as diagnostic marker for RA,[16] it is not clear whether RF is directly related to the symptoms of RA, although RF is found significantly more often in cases of aggressive joint inflammation. Since the presence of RF is one of the American College of Rheumatology criteria for RA in 1988.[2] Combined routine determination of IgM-RF, IgG-RF and IgA-RF are recommended for an improved sensitivity, for diagnostic specificity and for predictive value.[17]

A rheumatoid factor is widely considered a key predictor of progressive disease, with seronegative individuals generally displaying relatively mild symptoms. High titer of RF is associated with more sever disease and poor prognosis.[18] The history of anti-citrullinated protein antibodies started exactly four decades ago when the APF (anti-perinuclear factor) antibodies were described in1964.[19] With the discovery of filaggrin (a naturally citrullinated protein from epidermis) is the common antigen targeted by both the APF and the AKA (anti-keratin antibodies).[20]

Citrulline antibody directed against a circular peptide called citrulline is a non-standard amino acid.[21] There is no direct role of anti-CCP antibodies in the patho-physiology of RA.[22] In anti-CCP positive patients, the locally produced anti-CCP antibodies will form immune complexes with these citrullinated proteins; the immune complexes will subsequently cause activation of inflammatory cells and production of pro-inflammatory cytokines.[23] The cytokines promote infiltration of more inflammatory cells into the synovium that will eventually die and give rise to the production of more citrullinated proteins. In this manner, anti-CCP antibodies might contribute in the perpetuation of the inflammations and the chronicity of the disease.[23]

Serum Tumor Necrosis Factor –Alpha (TNF-α) is a potent cytokine that exerts diverse effects by stimulating a variety of cells. It is produced mainly by monocytes-macrophages series, but also by B cells and fibroblasts. Newly synthesized TNF-α is inserted into the cell membrane and subsequently released through the cleavage of its membrane-anchoring domain by a serine metalloproteinase. Thus, TNF- a secretion might be suppressed by inhibitors of this enzyme. Perhaps the best studied aspect of TNF-α is its ability to promote inflammation. It is an autocrine stimulator as well as a potent paracrine inducer of other inflammatory cytokines, including IL-1, IL-6, IL-8, and GM-CSF.[24]

Also, it promotes inflammation by stimulating fibroblasts to express adhesion molecules, such as intercellular adhesion molecule 1, these adhesion molecules interact with their respective ligands on the surface of leukocytes, resulting in increased transport of leukocytes into inflammatory sites, including the joints in patients with rheumatoid arthritis.[25]

The current study was conducted aiming to: 1) Evaluate the status of serum fucose and protein bound fucose, anti-cyclic citrullinated peptide antibody, TNF-α and rheumatoid factor in serum of RA patients and compare them with control; 2) Determine their role in patho-biochemistry of RA; 3) Assess their roles as biomarkers

for diagnosis and prognosis of the diseases and their role in association with disease activity; 4) Explore the correlation of these measured parameters to each other during the disease process.

PATIENTS AND METHODS

Three study group were investigated. This study was included on 90 subjects were investigated:

First group: Patients with early rheumatoid arthritis less than one year, No=30 (female =7) & (male =23), range of age (30-61) years.

Second group: Patients with late rheumatoid arthritis more than one year, No=30 (female =5) & (male =25), range of age (30-60) years.

Third group: Healthy control group who had no history or clinical evidence of RA or any other chronic disease, No=28 (female =6) & (male =22), range of age (30-55) years.

This study performed during the period from January 2016 to May 2016. These subjects were selected from patients attending the outpatient clinic in Al-Yarmouk Teaching Hospital – Rheumatology & Rehabilitation Consultation Unit. The tests were done in Al – Yaromouk Teaching Hospital Laboratories.

Spectrophotometer instrument was used for determination total serum fucose & protien bound fucose. ELISA technique was used for determination of anti-CCP, TNF-α, RF Screen. Statistical analyses were done using SPSS. The data were expressed as mean±SD. The comparison of P-value significance (Sig.) in any test was: S= Significant difference ($p<0.05$). HS= High Significant difference ($p<0.01$). NS = Non-significant difference ($p>0.05$). All the statistical analyses were done by using Pentium-4 computer through the Statistical Package for the Social Sciences (SPSS) program (version-18) and Excel application "PASW".

RESULTS

Serum level of total serum fucose: Figure (1) show the level (M±SD) of TSF expressed as mg/ dL in sera of Rheumatoid Arthritis patients (3.44±1.52mg /dl) in comparison with healthy control (10.89±2.72 mg /dl). There was highly significant decrease in serum level (M±SD) of TSF in RA patients when compared to normal healthy (P = 0.0001). The sensitivity = 98.3% and specificity=100% in RA patients calculated by ROC curve [26].

In addition, Figure (1) show a highly significant decrease in serum level of TSF in both late & early RA patients that expressed as mg/dL (M±SD) (3.28 ±1.77) & (3.61 ±1.22) respectively, when compared to healthy control (10.89 ±2.72) (P = 0.0001), however, no significant difference between early & late RA patients was found.

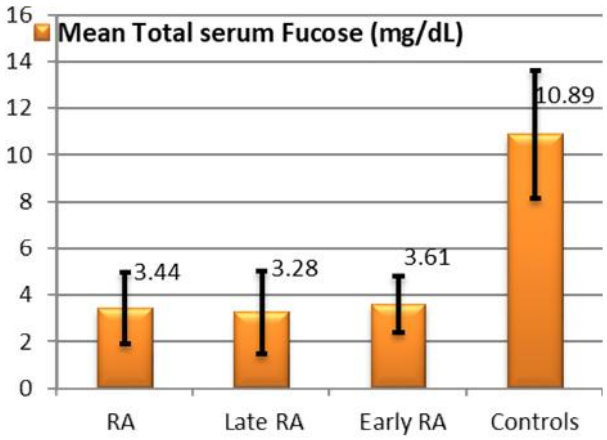

Figure 1: The comparison of the mean of Total serum Fucose (mg /dl) between Rheumatoid Arthritis, late, early Rheumatoid Arthritis patients with healthy controls.

2- Serum level of protein bound fucose (PBF): Figure (2) show the level (M±SD) of PBF expressed as mg /dl in sera of Rheumatoid Arthritis patients (1.93±1.94) in comparison with healthy control (5.07±0.84). There was highly significant decrease in serum level (M±SD) of PBF in RA patients when compared to healthy control (P=0.0001) the sensitivity = 78.3% and specificity=100% in RA patients.

In addition, figure (2) show a highly significant decrease in serum level (M±SD) of PBF in both late & early RA patients (1.80±1.89) & (2.06±2.02) respectively when compared to healthy control (5.07±0.84), (P=0.0001), however, there was no significant difference between late & early RA patients. 3- Serum level of ACCP antibody: Figure (3) show the level (M±SD) of ACCP expressed as (RU /ml) in sera of RA patients (39.62±30.54) in comparison with healthy control (0.58±0.44). There was

highly significant increase of ACCP in RA patients when compared to healthy control (P=0.0001).

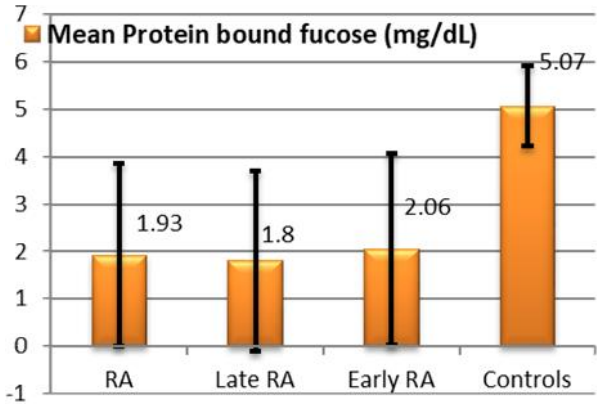

Figure 2: The comparison of the mean of serum level of Protein bound Fucose (mg/dl) between Rheumatoid Arthritis, late, early Rheumatoid Arthritis patients with healthy controls.

Table 1 also represent the ranges expressed as (RU / ml) of serum ACCP in RA patients (1.00-93.00) & healthy control (0.01-1.5). The sensitivity = 96.7% and specificity=100% in RA patients.

Figure (3) also shows the level (M±SD) of ACCP expressed as (RU /ml) in sera of late RA patients (44.7±30.03), early RA patients (34.5±30.69) in comparison with healthy control (0.58±0.44). There was highly significant increase in serum level (M±SD) of ACCP in both late & early RA patients when compared to healthy control (P=0.0001). However, no significant change in serum level of ACCP was noticed between late and early RA patients.

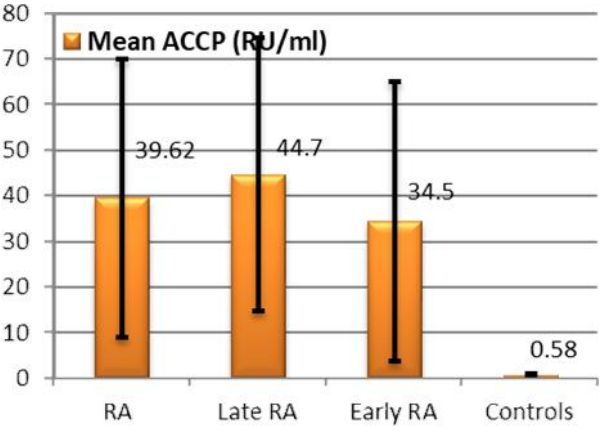

Figure 3: The comparison of the mean serum level ACCP (RU/ml) between Rheumatoid Arthritis, late, early Rheumatoid Arthritis patients with healthy controls.

4- Serum level of Tumor Necrosis Factor- α: Figure (4) show the level (M±SD) of TNF-α expressed as (pg/ml) in sera of Rheumatoid Arthritis patients (56.10±24.33) in comparison with healthy control (20.16±8.13). There was highly significant increase of TNF-α in RA patients when compared to healthy control (P = 0.0001). The sensitivity = 78.3% and specificity=100% in RA patients.

Figure (4) show the level (M±SD) of TNF-α expressed as (pg/ml) in sera of late RA patients (58.97±25.64), early RA patients (53.22±23.01) in comparison with healthy control (20.16±8.13). There was highly significant increase of TNF-α in both late & early RA patients when compared to healthy control (P = 0.0001). However, no significant difference between late & early RA patients.

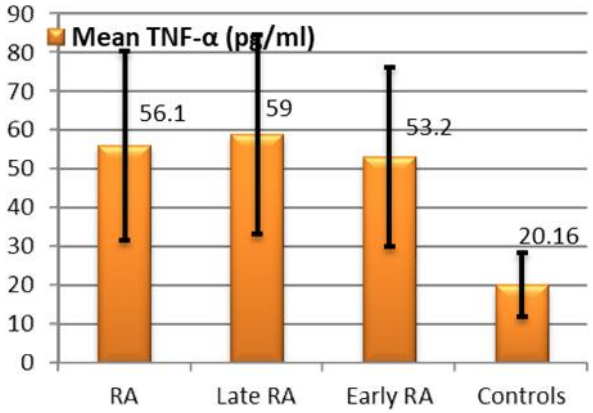

Figure 4: The comparison of the mean serum level of TNF-α (pg/ml) between Rheumatoid Arthritis, late, early Rheumatoid Arthritis patients with healthy controls.

DISCUSSION

The Role of Total serum fucose (TSF) in RA disease:

This study showed that, the mean level of total serum fucose concentrations was low in patients with RA disease (late and early RA) compared to healthy control. These results are agreeing with Thompson et al.,[14] that shows the levels of fucose are low in those with rheumatoid arthritis, also Westwood et al.,[19] who found L-fucose was significantly decreased compared to healthy controls.

The current study shows decrease serum level of fucose in late & early RA patients but not enough to use to distinguish between both stages of RA, not agree with Przybysz et al.,[13] who found the alterations in concentration of fucose may be applicable as a stage-specific marker especially for diagnosis and therapy of RA patients. The higher expression of terminal sugars could be associated with repair and adaptation processes in longstanding disease.

High sensitivity (98.3%) and specificity (100%) of TSF in RA patients, in which the sensitivity refers to the

proportion of people with disease who have positive results, while specificity refers to the proportion of people without disease who have negative results, both used to quantify how good & reliable the test is.

Also this study showed the difference of activity in late & early RA patients in which TSF value was not significant between inactive & moderate activity, inactive & very active cases and between moderate activity & very active cases in both late & early RA patients, The present study is not agree with Kamel at al.,[15] who found serum L-fucose was significantly related with disease activity and used as an additional parameter and an indicator for the disease activity in the follow up of patients with RA, also not in agreement with Przybysz et al.,[13] who found the expression of alpha1-6-linked fucose was related to disease activity and this seems to be due to the small number of patients in this study which also done in single center.

In this study decrease level of protein bound fucose in late & early RA patients it's not enough to distinguish between both stages of RA these results are not agree with recent study by Houssien et al.,[17] who found fucosyl determinant present of these in the serum and synovial fluid of RA patients might be applicable as a stage-specific marker in the diagnosis and therapy of RA patients. Also, this study showed the high sensitivity (78.3%) & specificity (100%) of PBF in RA patients these results give a good diagnostic value.

Also, it showed the activity of both late & early RA patients, PBF value was not significant between inactive & moderate activity, inactive & very active cases and between moderate activity & very active cases in both late & early RA patients.

These results were not agreeing with previous study of Shetlar,[12] which found that the protein bound fucose was able to differentiate active from inactive phase of RA. The decrease levels of fucose production from liver have been attributed to tissue destruction and reflecting a process of protein synthesis in which L-Fucose is the immune-dominant sugar on glycoproteins, and its presence may increase the antigenic response.[27] Thus, the accumulation of L-fucose is probably an important process in the synthesis of glycoproteins.[28,29]

This study showed a significant increase in the mean level of ACCP in RA, late and early RA patients when compared to healthy control as shown in Figure.(3)

These results are in agreement with Iraqi study by Alsaffar et al.,[27] which showed the mean level of ACCP was higher in both late & early RA patients than healthy control, and considered ACCP a highly sensitive (96.7%) and highly specific (100%) in Iraqi RA patients.

The level of ACPA in late & early RA patients is not significantly changed between inactive & moderate activity, inactive & very active and between moderate activity & very active in both late & early RA patients these are in agreement with Chioato et al.,[30] which found anti-CCP antibodies not affected disease activity as defined by the DAS28 score but the treatment has an effect on the level of ACCP in which the mean level of ACCP was higher before treatment than after treatment.

The results of this study were in agreement with multiple studies which shows that the level of TNF in serum, synovial fluid and tissue from RA patients are higher than those measured in healthy individuals (27,28,31,32)

Also, this study shows the sensitivity (78.3%) and specificity (100%) of TNF-α in RA patients in whom TNF was not detected in the serum of normal subjects or in synovial fluid of patients with osteoarthritis.[27]

The level of TNF-α is not significantly affected in active & moderate activity, inactive & very active cases while significantly affected in moderate activity & very active cases, and these finding are in agreement with Petrovic-Rackov et al.,[33] which found a significantly higher serum level of TNF in RA patients with high disease activity according DAS 28 index.

The level of TNF-α is not significantly affected in active & moderate activity, inactive & very active cases and moderate activity & very active cases, which it is in agreement with [33] which found the high levels of TNF-alpha not affected by disease duration, stages & disease activity.

Conclusions

This study illustrates that total serum fucose and protein bound fucose aid to diagnosis RA patient but weren't enough to distinguish between late & early RA and between the three stages of disease activity in late and early RA, also Anti-CCP is a good & specific marker for diagnosis of RA, it is related with higher probability of radiological joint damage (irreversible), so it is a good prognostic and as index test of severity. The presence of TNF-α may be contributed to the sensitivity of RA, so used anti TNF-α as therapeutic agents to decrease disease

activity and improve quality of life in a majority of RA patients.

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