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Assessing rheumatoid arthritis patients' blood levels of the human 14-3-3 protein antibodies

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Abstract:

Rheumatoid arthritis (RA) sufferers may have several problems. Common and significant complications, particularly in rheumatoid arthritis (RA), include synovitis, joint deformity, and structural bone loss. This study aimed to compare the biomarker 14-3-3 protein in patients with rheumatoid arthritis and a healthy group. Female patients were collected from rheumatoid arthritis clinics in Karbala city. Blood samples were taken from 50 healthy women as a control group and 80 patients with RF disease. Information about patients and samples was collected, and Statistical analysis showed that the amount of 14-3-3 protein in rheumatoid arthritis patients was significantly higher ($p < 0.001$) than in the control group. There was also a significant increase ($p < 0.001$) in the amount of 14-3-3 protein, based on age and whether before or after menopause, compared to individuals with rheumatoid arthritis in the control group. Statistical analysis revealed that the new diagnostic group's blood level of human 14-3-3 eta protein (16.74 ± 0.30 ng/ml) was considerably greater ($p < 0.005$) than that of the treated group. Furthermore, a statistically significant ($p<0.001$) rise in the human 14-3-3 serum level was observed in the group that was obese as opposed to the groups that were overweight and normal weight, respectively. Furthermore, the 1-year-old group's blood level of human 14-3-3 eta protein was statistically significantly ($p<0.001$) higher than that of the 1–10 and 11–19-year-old groups, respectively. Also, serum human 14-3-3 eta protein was positively correlated with RF and ACPAs and negatively correlated with estradiol-17b in women with RA.

Keywords: Rheumatoid arthritis, RF, ACPAs, estradiol-17b.

Introduction

The number of Americans affected by rheumatoid arthritis exceeds 15 million. It is a chronic inflammatory autoimmune disease that affects women more than men (1,2). Inflammatory process in the synovial fluid is mostly mediated by neutrophils, mast cells, lymphocytes, synovial tissue cells, and platelet microparticles (3,4). Joint deformity, synovitis, and structural bone loss result from this. Severe joint dysfunction may result from this inflammation, which can also cause excruciating joint pain and edema (5,6).

One of the most notable symptoms of RA is severe fatigue, which is significantly more profound and distinct than the typical fatigue experienced by people who do not have RA (7,8). These characteristics include diminished social participation, lack of sleep, decreased physical activity, which is crucial to general well-being, and a sense of loss of control over the prognosis (9,10).

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Methods

Subject Population (patients and controls)

Inclusion criteria

A total number of one hundred and thirty subject females (130) were included in this study; their ages range from 30-69 years old. The subject population was divided into two groups: eighty (80) females as the patient group who suffer from RF and fifty (50) females as the control group. Female patients were gathered from a private rheumatoid arthritis clinic in Karbala. A professional doctor evaluates the patients, considering their medical history. Age, BMI, pre and after menopause, new diagnosis and treatment, and length of illness were used to categorize the patient group.

Exclusion criteria

The study excluded women who were taking thyroid hormones or antithyroid drugs, as well as people who suffer from long-term conditions such as Cushing's syndrome, diabetes, hyperprolactinemia, renal or liver disease, or high blood pressure. Additionally excluded were women who had hormonal treatment, such as using oral contraceptives.

Blood sample collection

A 5 ml disposable syringe was used to draw blood samples from the vein. To make the serum, the samples were moved into a gel tube without any anticoagulant. The blood was centrifuged for 20 minutes at 2000–3000 RPM after being allowed to coagulate for ten minutes at a temperature of 25 ^{0}C. It was placed. isolated, and they are usually stored at -80°C until parameters are measured.

Results

Human protein 14-3-3.

The Human protein 14-3-3, when compared to the control group with patients, the level (12.60±0.48 ng/ml) was substantially higher (P value <0.001). (1.79± 0.03 ng/ml), according to the results in Figure 1.

Comparison of the Biomarker Human protein 14-3-3level in patients and the control group according to age

Figure (2) displays the levels of Human protein 14-3-3in four patient and control groups. In comparison to the control groups (1.92± 0.12, 1.75± 0.05, 1.77± 0.04, and 1.76±0.03 ng/ml), The findings show a substantial rise (p<0.001) in serum human 14-3-3 eta protein in the following age groups: 30–39, 40–49, 50–59, and 60–69 years (patients 6.87±0.33, 10.98±0.21, 14.55±0.28, and 17.98±0.23 ng/ml, respectively). The findings show a substantial rise (p<0.001) across the four categories when patients are compared by age.

Comparison of biomarkers of Human protein 14-3-3 in women patients and the control group according to pre- and post-menopausal status

Figure (3) displays the levels of Human protein 14-3-3 in two patient and control groups. The findings demonstrate that two groups of rheumatoid arthritis patients had significantly higher blood levels of human 14-3-3 eta protein before and after menopause (p<0.001). The control groups' levels were 1.80±0.05 and 1.76±0.02 ng/ml, whereas the patients' levels were 8.93±0.38 and 16.26±0.33 ng/ml, respectively. Nevertheless, there was no statistically significant difference between the two groups (p value > 0.05).

Comparison between the biomarker Human 14-3-3 eta protein according to diagnosis (new diagnosis and treated) in the rheumatoid arthritis group.

Figure 4 revealed that the new diagnostic group's serum human 14-3-3 eta protein level (16.74±0.30 ng/ml) was significantly higher (P value <0.005) than that of the treated group (9.38±0.39 ng/ml).

Comparison between the biomarker Human 14-3-3 eta protein according to body mass index (BMI) in female patients.

Figure (5) shows that the serum level of human 14-3-3 eta protein was significantly higher (p<0.001) in the obese group (16.72±0.30) ng/ml than in the

overweight and normal weight groups (11.4±0.24 and 6.89±.34 ng/ml, respectively) and in the overweight group (11.4±0.24) ng/ml than in the normal weight group (6.89±0.34).

Comparison between the biomarker Human 14-3-3 eta protein according to the duration of the disease

The one-year group's serum level of human 14-3-3 protein (17.612±0.24) was substantially greater ($p<0.001$) than that of the two groups (1–10 and 11–19 years) (13.13±0.28 and 7.98±0.37), respectively, as shown in Figure 6. Furthermore, compared to the 11–19 year group (7.98±0.37), the one–to–10–year group's blood level of human 14-3-3 eta protein (13.13±0.28) was considerably greater ($p<0.001$).

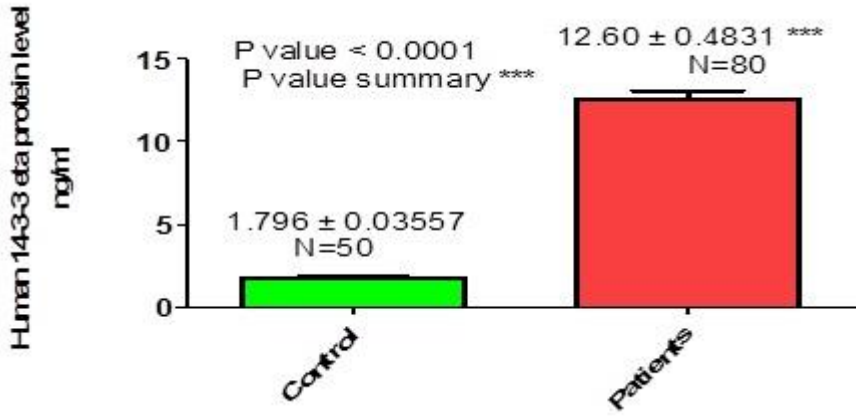

Figure 1: Human 14-3-3 protein in the patient group compared with the control group.

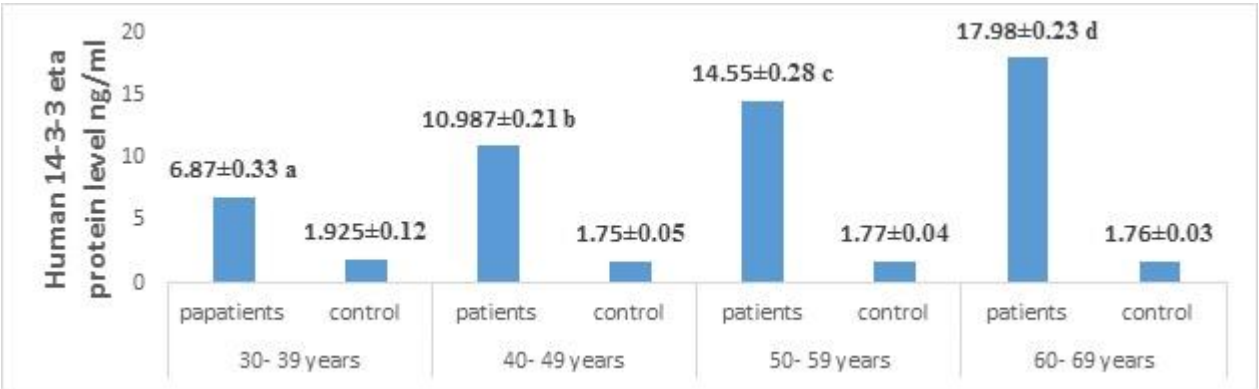

Figure 2: Human protein 14-3-3 levels (ng/ml) in patient and control groups by age.

** Refer to highly significant (p value < 0.001).

Differential letters refer to significant differences (p value < 0.001).

Figure 3: Level of Human 14-3-3 eta protein (ng/ml) in in premenopausal group compared with the postmenopausal group.

** Refer to highly significant (p value < 0.001).

Refer to insignificant (p value > 0.05).

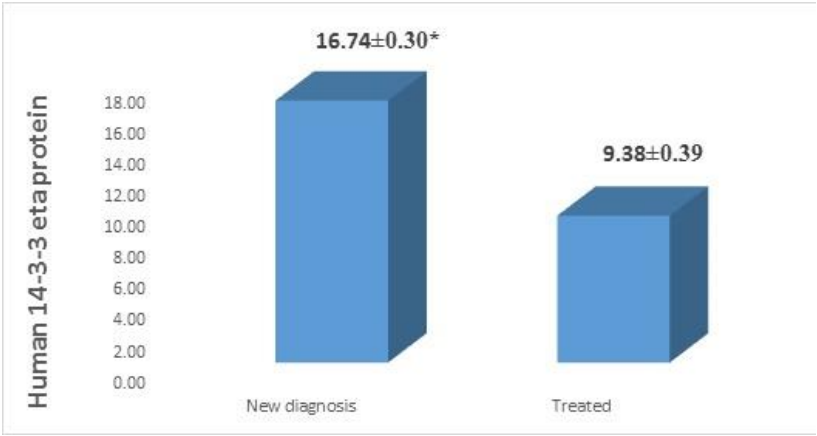

Figure 4: Human 14-3-3 eta protein levels ng/ml in the groups with new diagnoses and those receiving treatment.

* Refer to significant (p value < 0.005).

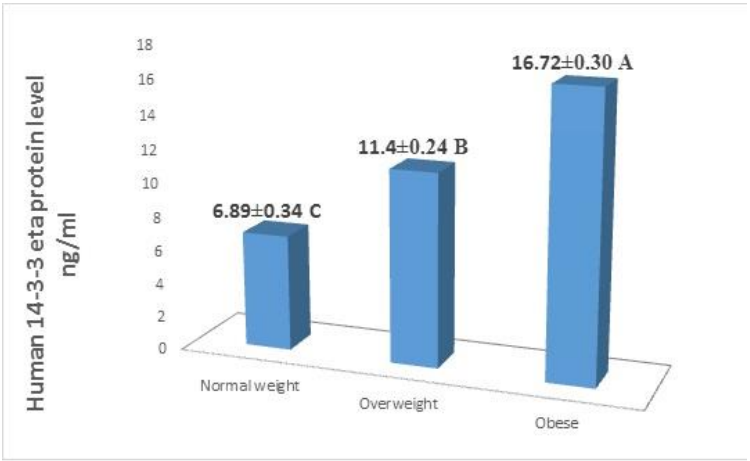

Figure (5) Level of Human 14-3-3 eta protein (ng/ml) in obese, overweight, and normal weight groups.

Differential letters refer to significant differences (p value < 0.001).

Normal weight n=20, overweight n=25, obese =35

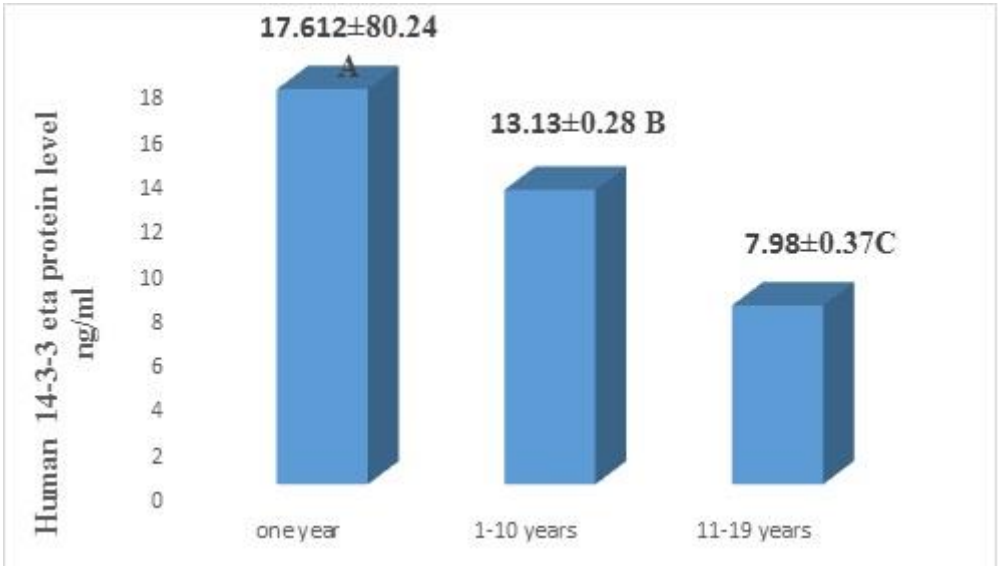

Figure 6: Three groups (one, one to ten, and eleven to nineteen years old) had different levels of human 14-3-3 eta protein (ng/ml).

Significant differences (p value < 0.001) are shown by differential letters.
One year (25), 1-10 years (25), 11-19 years (30)

Correlation between the Biomarker of Human 14-3-3 eta protein with RF, ACCP, and estradiol in rheumatoid arthritis

Correlation coefficient relationship between Human 14-3-3 protein with rheumatoid arthritis

Figure (7) In rheumatoid arthritis, demonstrates a strong positive connection between RF (r=0.87) and Human 14-3-3 eta protein.

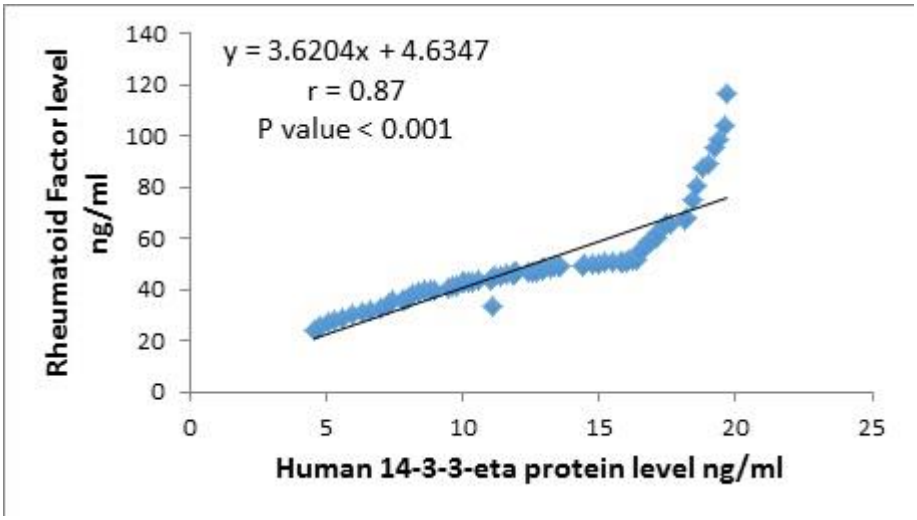

Figure (7) Correlation coefficient relationship between Human 14-3-3 protein and rheumatoid arthritis

Correlation coefficient relationship between Human 14-3-3 eta protein and ACPAs.

Figure (8) shows a significant positive correlation between Human 14-3-3 eta protein and ACPAs (r= 0.946) in rheumatoid arthritis.

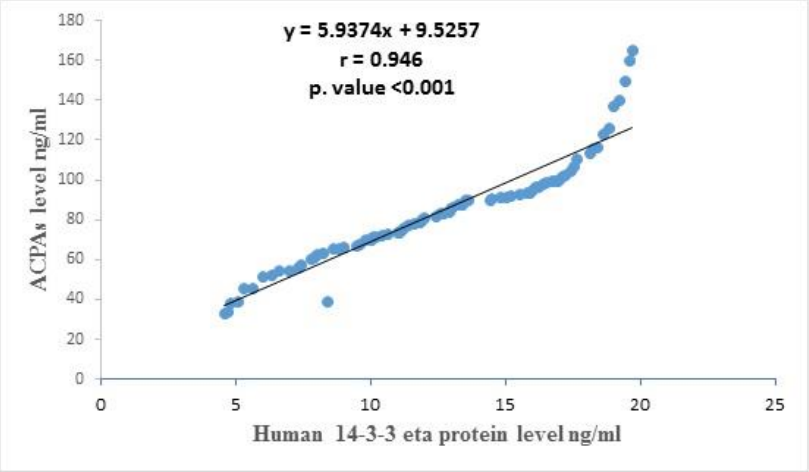

Figure (8) Correlation coefficient relationship between Human 14-3-3 eta protein and ACPAs in rheumatoid arthritis

Correlation coefficient relationship between Human 14-3-3 eta protein and estradiol-17 B.

Figure (9) shows a significantly strong negative correlation between Human 14-3-3 eta protein and estradiol-17 B (r= -0.995) in rheumatoid arthritis.

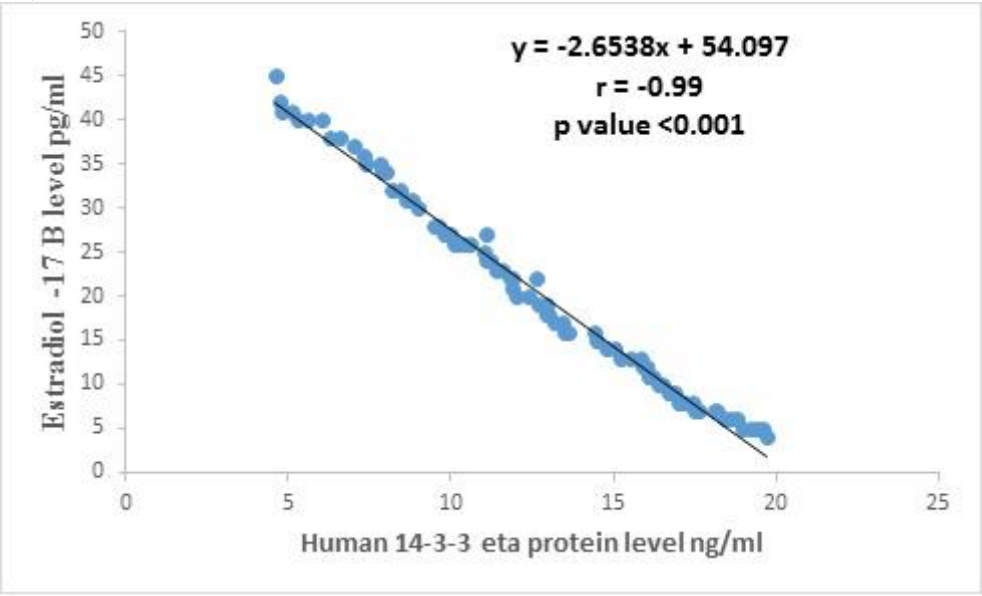

Figure (9) Correlation coefficient relationship between Human 14-3-3 eta protein and estradiol-17 B in rheumatoid arthritis

Discussion

Human 14-3-3 ETA

The current study's findings demonstrated that RA patients had considerably greater blood levels of the 14-3-3 eta proteins Figure 1 than did healthy controls ($P<0.001$). Serum14-3-3η has the same discriminative performance as RF and ACPA, according to this study, which increases its diagnostic potential. (11) The study discovered that those who tested seronegative for RF and ACPA had higher serum14-3-3η protein levels, which may boost the sensitivity of diagnosing RA (12). demonstrated that the 14-3-3 protein in the blood can increase the number of RA patients identified. shown that the combination of serum14-3-3η protein, rheumatoid factor, and anti-cyclic citrullinated peptide antibody improved the diagnostic capture of RA patients (13-15).

In previous research, serum 14-3-3 levels were measured in healthy controls and patients with RA, osteoarthritis, and other rheumatic illnesses. They discovered that compared to those with other autoimmune disorders, OA patients, or healthy controls, RA patients had much greater amounts of it (16,17). The results of the current study (as illustrated in Figure 2) may be explained by the correlation between protein eta and the peak of incidence, which is between the ages of 50 and 60 (18). They may also be related to joint degeneration in older age by activating a signaling cascade of pro-inflammatory cytokines and MMPs expression with the progression of disease activity and joint destruction, and erosion (19,20).

Numerous factors, including MMP-1 and MMP-9, as well as innate immunological stimulation for signaling, including MMPK/ERK, SAPK/JNK, and JAK/STAT, may be linked to the induction of 14-3-3 eta protein. This might result in an increase in inflammatory markers, including IL-1β and tumor necrosis factor (21, 22).

The present study, as indicated in Figure 3, indicated a significant increase in 14-3-3 protein in post menopause women than pre menopause a negative regulation between phosphoserine/ threonine binding 14-3-3 with estrogen receptors-α; therefore, highly level of 14-3-3 eta protein in post-menopausal women with a low level of estrogen. Therefore, the eta protein may overlap with estrogen function by pathophysiological pathways, including RA and cancer (23,24).

Figure 4 from our investigation demonstrates a considerable rise in the blood level of human 14-3-3 eta protein in newly diagnosed diseases as compared to those that have been treated. Our findings contradict those of prior studies on (25). Both early and established rheumatoid arthritis were found to have a similar rise in serum 14-3-3η levels. This was accounted for by the activation of extracellular regulated kinase 1,2, which causes additional joint injury by producing matrix metalloproteinase, IL-6, interleukin 1 (IL-1), and receptor activator of nuclear factor κB ligand (26-28).

Because no prior research has shown a correlation between 14-3-3 eta protein and BMI, the explanation for the high level of protein eta in obese females may be related to high BMI, which causes high protein expression with induction of pro-inflammatory cytokines like IL-1, IL-6, and TNF as well as factors that degrade bone like RANKL and MMPs and highly activate immune cells like T or B lymphocytes (29-31). Figure 5 showed a significant increase in 14-3-3 eta protein in obese patients compared to those who were overweight or of normal weight (32).

Association between rheumatoid arthritis and the human 14-3-3 protein

Our results show that serum human 14-3-3 protein is positively correlated with RF (Figure 7) and ACPAs (Figure 8) and negatively correlated with estradiol-17 B (Figure 9) in women with rheumatoid arthritis.

Numerous studies have found a favorable correlation between blood 14-3-3η, RF, and ACPA levels in RA patients. Additionally, they have shown a correlation between RF and ACPA levels and the expression of 14-3-3η (27, 30).

Conflict of interest: NIL

Funding: NIL

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