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Relationship Between Biomarker of Human Calprotectin with Rheumatoid Arthritis

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Abstract:

The etiology of RA, a chronic inflammatory joint disease, is complex and involves the immune system. One of the main components of neutrophils and polymorphonuclear granulocytes, which have been linked to the pathophysiology of autoimmune inflammatory illnesses like RA, is calprotectin. Comparing the human calprotectin biomarker in rheumatoid arthritis patients to that of the healthy group was the goal of the study. Female patients in this study were collected from rheumatoid arthritis clinics in Karbala city. Blood samples were taken from about 50 healthy women in the control group and 80 patients who had RF disease. The information regarding patients and samples was collected, and statistical analyses were carried out between the control samples and human calprotectin levels in Rheumatoid arthritis patients, showing a highly substantial rise in CLP levels ($P < 0.001$). When comparing women with rheumatoid arthritis to those in the control group, there was also a significant rise ($p < 0.001$) in the CLP level by age and before or after menopause. According to statistical analysis, the new diagnostic group's serum human CLP level (102.76 ±0.72 ng/ml) was substantially greater ($p < 0.001$) than the treated group's. Furthermore, compared to the overweight and normal weight groups, the obese group's blood level of human CLP was considerably ($p<0.001$) greater. Furthermore. According to our findings, serum human CLP in women having RA had a negative correlation with estradiol-17b and a positive correlation with RF and ACPAs.

Keywords: RA, calprotectin, autoimmune inflammatory illnesses.

Introduction

RA is an inflammatory disease of the joint that is chronic and has a complicated pathophysiology driven by the immune system (1-3). High levels of inflammation early in the illness's progression model course must be understood to treat RA patients as best as possible (4,5). Numerous traits of such a biomarker are present in the circulating amount of calprotectin (6). One of the main components of neutrophils and polymorphonuclear granulocytes, which have been linked to the pathophysiology of autoimmune inflammatory disorders, including RA, is calprotectin (7,8).

Calprotectin is a neutrophilic cytosolic protein that makes up 40–60% of the cytosolic protein. It is also a multifunctional protein of the S100 that is present in monocytes and macrophages (9,10). Calprotectin is a multigene subfamily of cytoplasmic EU-binding proteins that functions as a pro-inflammatory mediator and is produced right after neutrophil activation (11,12). Therefore, elevated levels of calprotectin caught the interest of scientists as a new inflammatory marker of RA

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(13,14). Now, it is known that calprotectin can respond to inflammation as quickly as 2 hours upon the induction of inflammation (15,16). Research has highlighted possible links between calprotectin and disease activity across several rheumatic joint disorders (17,18). Furthermore, calprotectin levels were shown to favorably correlate with RA patients' radiological indications of joint degeneration (19,20). Calprotectin has been detected in synovial macrophages and fibroblast-like synoviocytes, and its levels are elevated in serum and synovial fluid SF in RA (21,22). However, there was no significant correlation between SF calprotectin and plasma calprotectin in reactive arthritis patients. Calprotectin concentration was also associated with white blood cell (WBC) numbers in blood and SF (23, 24). Our knowledge of the role of calprotectin in diffusion in patients with and without the autoantibodies used to categorize RA, IgM RF, and ACPA, which may help create customized treatment, is still lacking, despite the abundance of prior information in this area (25,26). Therefore, we set out to measure the calprotectin levels in serum and SF in patients with both RA and knee synovitis.

Material and Methods

Subject Population (patients and controls)

Inclusion criteria

This research scrutiny encompassed a sum of 130 females aged between thirty (30) to sixty-nine (69) years old, and written consent was obtained. The subject population was divided into two groups: an eighty (80) female patient group, RF positive, and fifty (50) ladies in the control group who seemed to be in good health. The female patients were sourced from a private rheumatoid arthritis clinic located in Karbala. The patients' thorough clinical history and examination served as the basis for the specialized doctor's consultation. Age, BMI, pre and post menopause, new diagnosis and treatment, and length of illness were used to further partition the patient group into subcohorts.

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 samples collection

A 5 ml disposable syringe was used to take blood samples from the vein. To prepare the serum, the samples were poured into a gel tube without an anticoagulant. Blood was centrifuged for 20 minutes at 2000–3000 RPM after being allowed to clot at room temperature for ten minutes. After that, the serum was separated and kept frozen at -80 °C until the research laboratory analysis was finished.

Result

Human Calprotectin.

The human calprotectin level of the female patient group (92.08±1.25 ng/ml) was significantly higher (P value <0.001) than that of the control group (37.80±0.43 ng/ml), according to the results in Figure 1.

Comparison of the Biomarker Human calprotectin level in women patients and the control group according to age

The levels of human calprotectin in four patient and control groups are shown in Figure 2. According to the results, the third patient group (those aged 50–59) had a significantly higher blood level of human calprotectin (97.32±0.70 ng/ml) than the control group (37.04±0.50 ng/ml) ($p<0.001$). However, compared to the control groups (39.43±1.49, 37.15±0.63, and 37.75±0.44 ng/ml), the three patient groups (30–39, 40–49, and 60–69 years) demonstrated negligible increases ($p<0.05$) in serum human calprotectin (77.01±0.99,

88.08±0.61, and 105.87±0.49 ng/ml). When comparing patients by age group, the results reveal a substantial increase (p<0.001) between the fourth groups.

Comparison of biomarker Human Calprotectin in the women patients group and control group according to pre- and post-menopausal status:

Calprotectin in 2 groups of patients and a control, as shown in Figure 3. The results indicate the presence of a highly significant increase (p<0.001) in serum human calprotectin in two groups of pre and post-menopausal patients, 82.54±0.38 and 101.59±0.33 ng/ml, respectively, compared to control groups (38.15 ±0.05and 36.85±0.02 ng/ml), respectively, but insignificant (p value > 0.05) between pre and post-menopausal patients with rheumatoid arthritis.

Comparison between biomarker Calprotectin according to diagnosis (new diagnosis and treated).

The serum human calprotectin level of the new diagnostic group (102.76 ±0.72 ng/ml) was significantly higher (P value <0.001) than that of the treated group (83.79 ± 1.06 ng/ml), according to the results in Figure 4.

Comparison between biomarker CLP according to body mass index (BMI)

Figure 5 demonstrates that the serum human calprotectin level was substantially greater (p<0.001) in the obese group at 102.72 ± 0.73 ng/ml than in the overweight and normal weight groups (89.17± 0.66 and 77.07± 1.00 ng/ml, respectively), and that the serum human calprotectin Compared to the normal weight group (77.07±1.00 ng/ml), the overweight group's level was substantially higher (p<0.001) at 89.17± 0.66 ng/ml.

Comparison between biomarker CLP according to the duration of disease:

The serum human calprotectin level in the one-year group (104.82±0.58) was significantly higher (p<0.001) than in the two groups (1–10 and 11–19 years) (93.84±0.73 and 80.01±1.03), respectively. Additionally, there was a significant increase (p<0.001) in the 1–10 year group (93.84±0.73) ng/ml compared to the 11–19-year group (80.01±1.03) ng/ml.

Correlation between Biomarker CLP and RF, ACCP, and estradiol in rheumatoid arthritis: Correlation coefficient relationship between CLP and RF

Figure 7 shows a significantly positive correlation between human calprotectin and RF (r= 0.845) in rheumatoid arthritis.

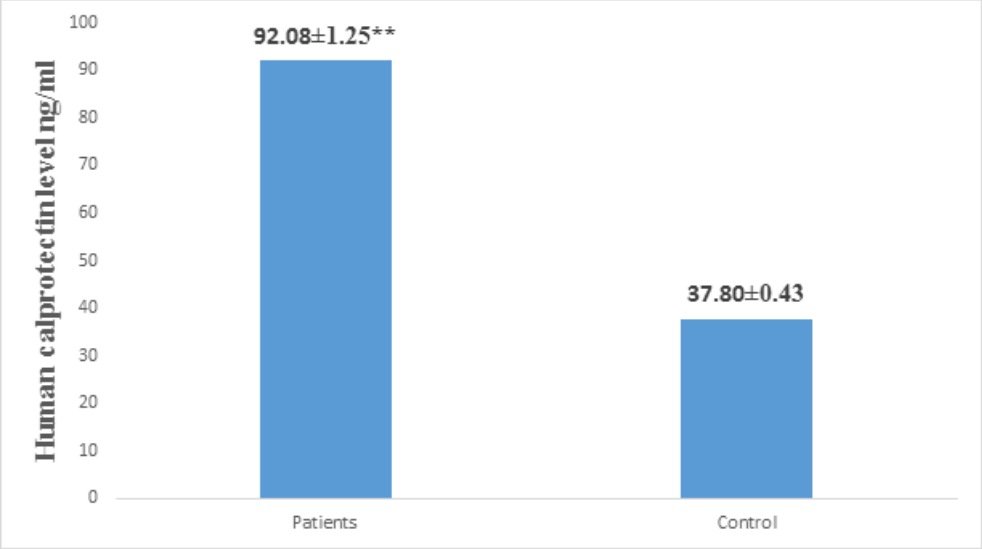

Figure 1: Human Calprotectin in the patient group compared with the control group.

** refer to high significant (p value < 0.001) patients= 80, control n=50

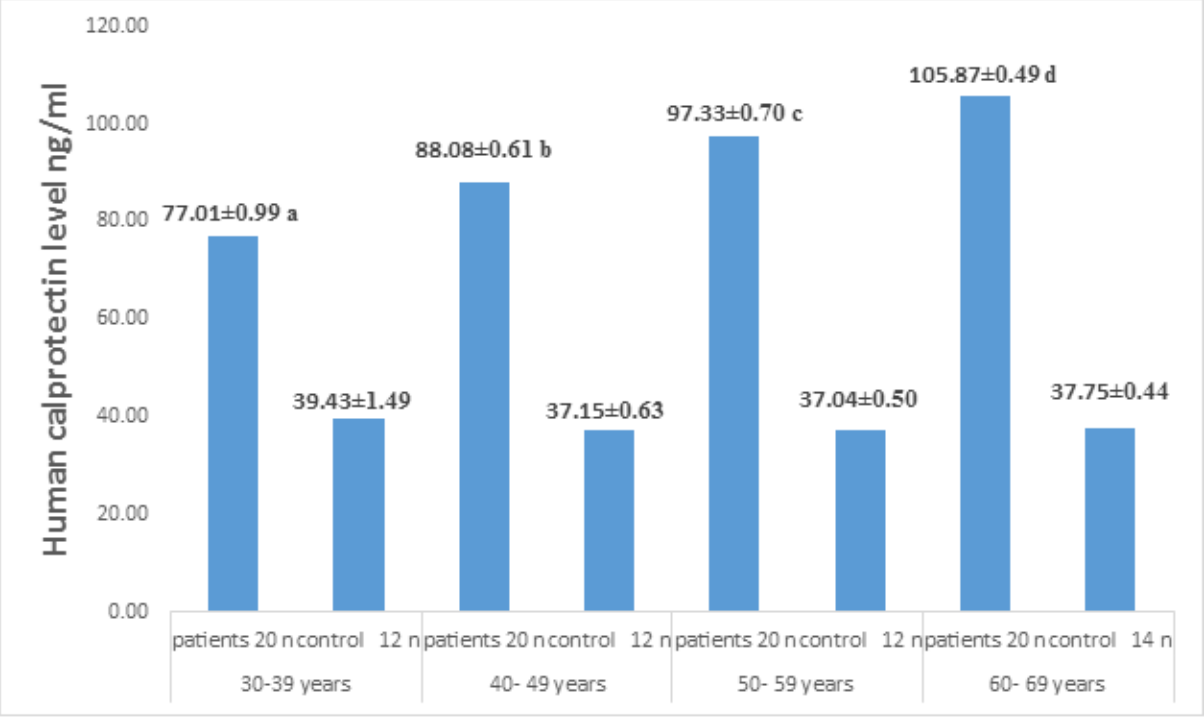

Figure 2: Human Calprotectin Level (ng/ml) in patient groups by age compared to control groups.

** High significant (p value < 0.001) is used.
Insignificant (p-value>0.05) is referred to.
Significant differences (p value < 0.001) are shown by differential letters.

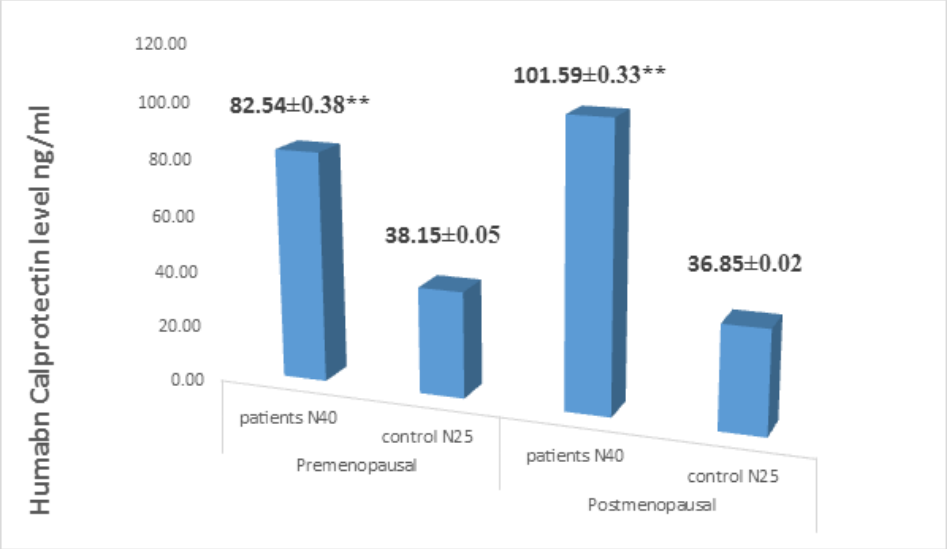

Figure 3: Human calprotectin levels (ng/ml) in premenopausal and postmenopausal groups are compared.

** High significant (p value < 0.001) is used.
Insignificant (p-value> 0.05) is referred to.

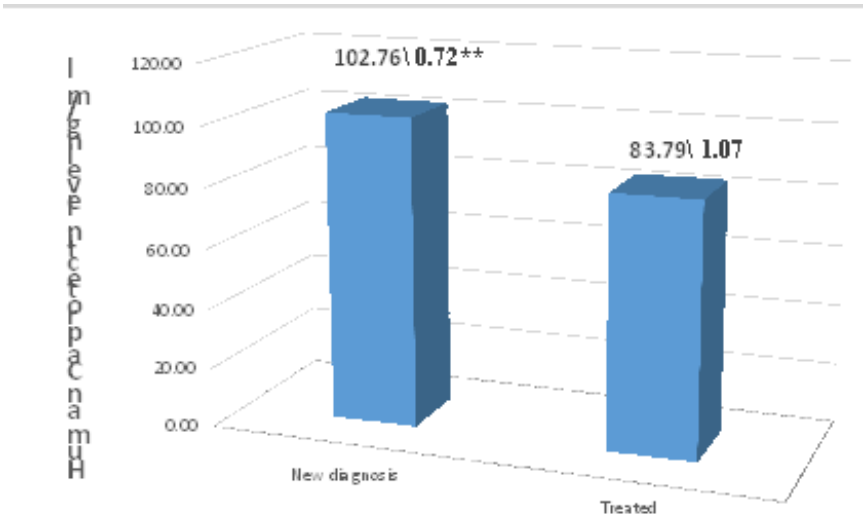

Figure 4: The new diagnostic group's human calprotectin level (ng/ml) was compared to the treated group's.
** High significance (p value < 0.001) is indicated.

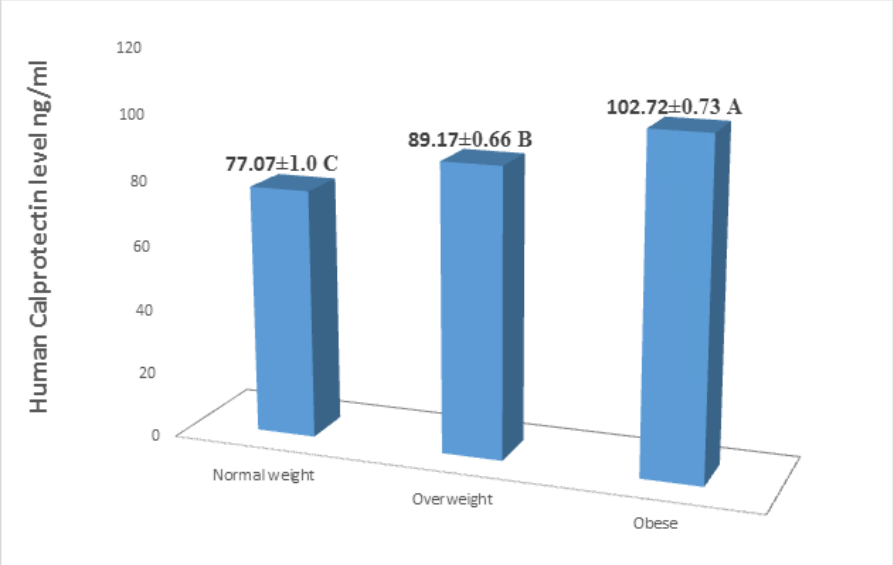

Figure 5: Level of Human Calprotectin (ng/ml) in obese, overweight, and normal weight groups.

Significant differences (p value < 0.001) are shown by differential letters.
20 is normal weight, 25 is overweight, and 35 is obese.

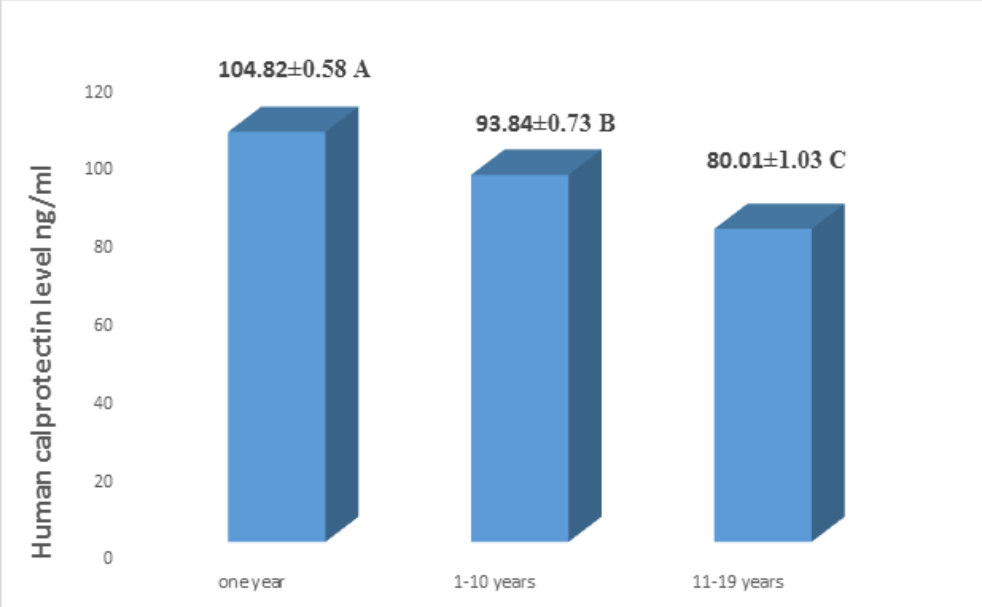

Figure 6: Level of Human Calprotectin (ng/ml) in three groups (one, 1-10, and 11-19 years).

Differential letters refer to significant differences (p-value < 0.001).
One year (25), 1-10 years (25), 11-19 years (30)

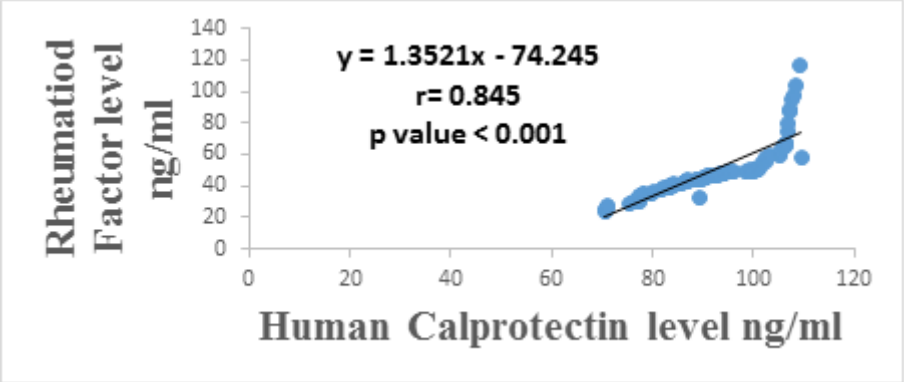

Figure (7) Correlation coefficient relationship between Human calprotectin and RF in rheumatoid arthritis

Correlation coefficient relationship between CLP and ACPAs

Figure 8 shows a significantly positive correlation between human calprotectin and ACPAs (r= 0.931) in rheumatoid arthritis.

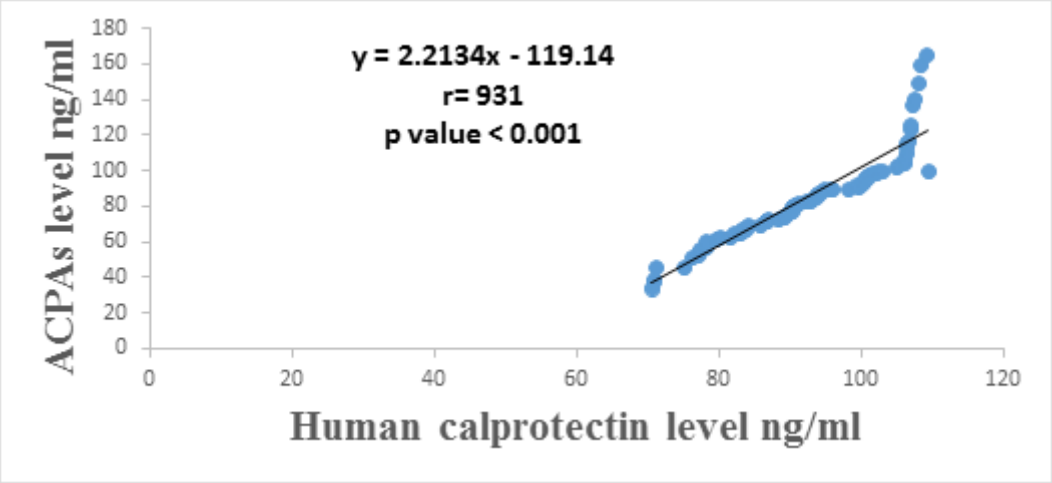

Figure 8: Correlation coefficient relationship between Human calprotectin and ACPAs in rheumatoid arthritis.

Correlation coefficient relationship between CLP and Estradiol-17 B

Figure 9 shows a significantly negative correlation between human calprotectin and estradiol-17 B (r= -0.993) in rheumatoid arthritis.

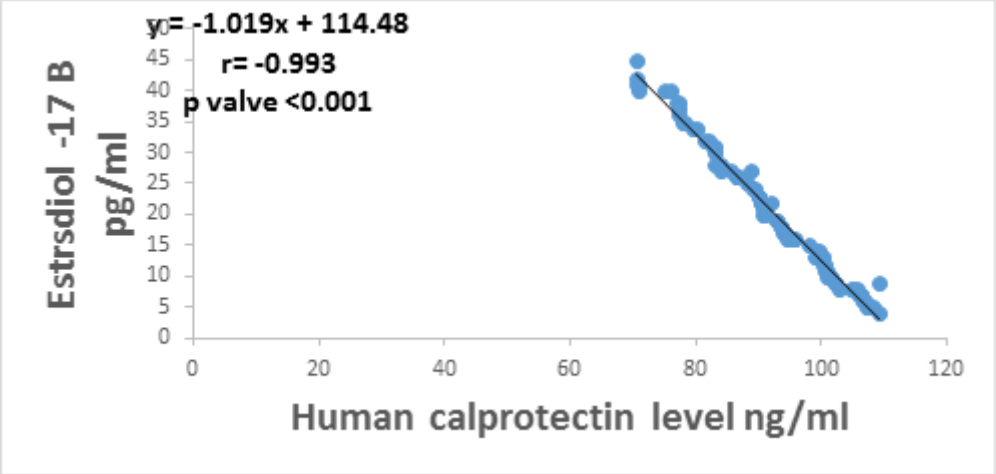

Figure 9: Correlation coefficient relationship between Human calprotectin and estradiol-17 B in rheumatoid arthritis.

Discussion

Calprotectin

The current study found that the mean serum level of calprotectin was a highly significant increase of the serum calprotectin in RA patients compared with control groups ($p<0.001$), which is consistent with many of the findings of (27), which found that the mean serum levels of calprotectin were elevated in RA patients compared to healthy controls ($p<0.001$), as shown in Figure (1). These findings also aligned with our findings, which showed that the mean serum levels of calprotectin in RA patients were significantly higher than in control groups ($p<0.0001$). This is even more consistent with (28).

According to Figure 2, the older age group's calprotectin level was substantially higher than the other groups'. Inflammation-related biological processes include the release of calprotectin into the extracellular space and its interaction with one or more signaling molecules. It has been shown that in RA, calprotectin interacts with RAGE to enhance the production of pro-inflammatory cytokines by activating nuclear factor (NF)-κB and p38 MAPK by macrophages, even if the precise nature of this connection is unclear. The rise we observed can be explained by the fact that this process is more pronounced in older experiment groups (29,30).

The results of Figure 4, which demonstrated a decrease in the level of serum CLP in patients after therapy compared with fresh diagnosis of the disease, were consistent with the findings of those who found a substantial decline in median CLP after 12 months of treatment with MTX in RA patients. Similarly, after three months of standard treatment, (31) showed that those with newly diagnosed RA had lower levels of serum CLP. Additionally, it was shown that RA patients who have been using MTX for a long time had a much lower level of CLP than those who are just beginning to use MTX (32) suggests that MTX could affect myeloid cells in a particular way, which could explain the drop in serum CLP levels.

According to Figure 5, obese patients had significantly higher levels of calprotectin than other groups. In line with earlier findings by (33), we demonstrated elevated calprotectin levels in both obesity and obesity-related type 2 diabetes, leading to the recent description of CLP as a novel obesity marker.

When the gene expression levels of the calprotectin subunits S100A8 and S100A9 in visceral adipose tissue (VAT) are examined, it can be concluded that adipokines derived from adipose tissue have a variety of functions in the body and may be a connection between obesity and the development of molecular events (34).

Correlation of Calprotectin with RF, ACPAs, and Estradiol 17 B

In current results, serum human calprotectin shows a positive correlation with RF (Figure 7) and ACPAs (Figure 8) and a negative correlation with estradiol-17 B (Figure 9) in women with rheumatoid arthritis. The current findings concur with those of prior studies. The notion that CLP plays a role in the pathophysiology of RA is supported by (35), who discovered a strong positive correlation between serum CLP levels and RF and anti-CCP titers. Serum CLP and HAQ were found to be significantly correlated in another investigation, which was consistent with another study by (36). As a result, CLP might be regarded as a biomarker of RA prognosis and disease severity.

According to other studies by (37), there was an independent correlation between CLP and joint radiographic deterioration in RA. In RA patients, CLP has been shown to predict a 10-year radiographic progression. According to (36), there is no correlation between joint radiographic damage and CLP levels.

Conflict of interest: NIL

Funding: NIL

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