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Research Article

Correlation of Calprotectin with Galectin-3, Adropin and CTLA-4 in Iraqi Rheumatoid Arthritis Patients

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ABSTRACT

Background
Rheumatoid arthritis (RA) is a common autoimmune infection described by enduring inflammation and intersections destruction

Aim of the work
The current research was planned to measure the serum level of calprotectin (Calpro) in cases with RA & its correlation with the galectin-3 (GAL3), adropin (AD) and cytotoxic T lymphocyte as-sociated antigen-4 (CTLA-4).

Methods
The study concerned on 44 Iraqi patients of RA in compared with 40 healthy control (HC) matched for sex, age and ethnic background, erythrocyte sedimentation rate (ESR) was measured, while serum Calpro, GAL3, AD and CTLA-4 levels were estimated by means of sandwich ELISA test using commercially available kits.

Results
Serum Calpro, GAL3, CTLA4, was importantly greater (P<0.001) while vitamin D (VD) and AD was significantly lower (P<0.001) in RA patients when compared to control.
There was a significant positive association among Calpro and both of (GAL3 and CTLA4). A important negative relationship among Calpro and AD were observed

Conclusion
The present results explained that serum Calpro is a measurable, useful specific indicator of infection commotion and joint impairment in RA patients.

Key words: Calprotectin, Galectin-3, Adropin, Rheumatoid Arthritis

Introduction

Rheumatoid Arthritis (RA) is an auto immune infection connecting to prolonged synovial irritation & damage of the cartilage & bone, mainly upsetting the minor interactions of the hands & feet which causing a diverse amounts of malformation and practical incompetence [1, 2]. Several joints in the body are subsequently affected. RA patients are having amplified danger for cardiovascular syndromes, together with atrial fibrillation and stroke, and mortality, in addition to other autoimmune diseases [3].

Calpro may be beneficial medical impassioned indicator, throughout the previous 30 years, there was high attention in calprotectin. It was establish in the synovial material in RA cases, exactly in the inside stratum together to the cartilage-pannus joint. The locations where pannus come through the gristle are the main places of gristle damage & bone corrosions in RA [4].

It has been proposed that rheumatoid irritation is facilitated favorably by initiated proinflammatory Th1 cells [5]. The levels of serum calpro have been revealed to be a useful indicator of infection action &interaction irritation but not prognostic of the result of cases with RA, in young RA, calpro seems to be higher to conservative indicators for checking pathological action [6].

Numerous outlines of sign have associated GAL3 as a pro inflammatory intermediary in RA. Synovial sore and fundamental interaction impairment was lesser in GAL3 mice with antigen- encouraged arthritis compared to wild-type controls [7]. Ohshima et al. [6] described that galectin-3 in the plasma was greater than previously in patients with established RA related with HC; they conveyed GAL3 binding protein were communicated in specific at places of gristle & bone damage in RA interactions [8].

Adropin seems to contribute in the conservation of energy homeostasis and insulin reply, narrowly linked to the growth and development of atherogenesis [9]. Slight AD values leads to endothelial damage and dysfunction [10].

CTLA-4 is an intracellular protein that translocates to cell surface through recruitment of enzymes such as GTPase ADP ribosylation factor-1 and phospholipase D [11]. The CD28-CD80/CD86 stimulatory pathway is necessary in improving & enhancing T cell stimulation & interleukins manufacture in RA. CTLA-4-Ig, was confirmed for the dealing of vigorous RA via the US Food &Drug Management in 2005 [12].

The paper designed to estimate the function of Calpro as an inflammatory indicator in patients with RA according to the active scores of disease to assess the possibility of introducing Calpro in the diagnosis and monitoring of patients with RA and to found the correlation of Calpro level with GAL3, AD and CTLA-4 patients with RA.

MATERIAL AND METHODS

The study was carried out in Al-Fallujah teaching hospital at Al-Anbar Governorate/ Iraq, from September 2017- April 2018. A total of 44 RA patients (29female and 15 aged 47-66 year), the identification of RA was made on the basis of the recommended criteria by WHO. Forty age and sex matched (27female and 13 aged 44-65 year) healthy individuals served as controls who attended for routine health check up at the hospital. None of the healthy control was taking any medication or nutritional complement; they were carefully chosen after in depth physical examination and laboratory tests.

Samples collection: 5 ml. venous blood samples were collected in plain tubes, the samples were permitted to coagulate for 60 minutes subsequent the samples were centrifuged for 0.5 hour at 4000 rpm. Then serum was kept instantly at -20C until use.

Erythrocyte sedimentation rate (ESR) was determined in blood using the westergren method, Rheumatoid factor (RF) was measured by turbidimetry assessable technique. Serum concentrations of Calpro, GAL3, AD and CTLA-4 were determined by ELISA using a commercial kit manufactured by Mybiosource Company. Micro ELISA system (washer & reader) (Thermo, Germany) and incubator (Gallenkamp, U.K.) were used in ELISA determination.

Weight was estimated by uniform beam mass measures without footwear and with only light clothes. Height was estimted with the subjects shoeless and vertical with the feet together. BMI is calculated by divided weight in kilogram per height in meters squared and is self-regulating of sex and age.

Statistical Analysis

All results are exppresed as mean ± S.D. & dissimilarities among means were evaluated by the Student t test. Variances were deliberated important at $P < 0.05$. The results analysed by SPSS programe (version 22). The statistical importance, direction and strength of right connection among two quantitative parameters, one of which existence a non-normally distributed variable, was measured by Spearman's rank linear relationship coefficient, & a probability (P) value less than the 0.05 was counted statistically important.

RESULTS

Table no.1shows the average ages of the RA and control subjects were (56.00±5.80) and (55.00±6.70) years, respectively, (P =0.140). The mean BMI values of two groups were in the common range and the mean value in RA group was higher (26.3 ± 4.90) than in healthy group (25.2 ± 4.70) kg/m^2although the diversity was statistically non-important (P= 0.090). Also important elevated (P<0.001) of ESR and RF levels in patients with RA as compared to control group as shown in table no.1, (figures 1-Aand 1-B) respectively.

Serum Calpro, GAL3, and CTLA-4 levels were significantly higher (P<0.001) in patients with RA when compared to control group as shown in table no.1, (figures 1-C, 1-D and 1-E) respectively.

The level of serum AD was significantly lower (P<0.001) in patients with RA compared to control group as shown in table no.1, (figure 1-F). The normal levels of Calpro, GAL3, and CTLA-4 were (8.66 ± 3.5 ng/ml), (4.63±2.42 ng/ml) and (1486.88±929.19 pg/ml) elevated in the RA patients who recorded (33.74 ± 9.47 ng/ml), (10.57±4.89 ng/ml) and (4110.95±1276.96 pg/ml) respectively.

The mean serum levels of AD was significantly higher (P<0.001) among healthy controls (4175.65 ±1149.32) compared to RA patients (1327.01 ±502.35) pg/ml.

Table (1): Evaluation of different biomarkers among RA cases and HC

Parameters		Mean ± S.D	T-Test	P-value
Age years	cases	56.00 ± 5.80	1.231	0.120
	controls	55.00 ± 6.70		
BMI Kg/m^2	cases	26.3 ± 4.90	0.953	0.142
	controls	25.2 ± 4.70		
W-H R	cases	0.93 ± 0.07	0.653	0.190
	controls	0.92 ± 0.05		
ESR mm/h	cases	44.75 ± 7.82	23.426	<0.001
	controls	14.53 ± 2.44		
RF IU/ml	cases	59.02 ± 15.63	15.738	<0.001
	controls	18.53 ± 5.62		
Calprong/ml	cases	33.74 ± 9.47	15.791	<0.001
	controls	8.66 ± 3.5		
GAL-3 ng/ml	cases	10.57 ± 4.89	7.044	<0.001
	controls	4.63 ± 2.42		
AD pg/ml	cases	1327.01 ± 502.35	-14.951	<0.001
	controls	4175.65 ± 1149.32		
CTLA-4pg/ml	cases	4110.95 ± 1276.96	10.699	<0.001
	controls	1486.88 ± 929.19		

The Pearson correlation test showed a strong positive association among serum Calpro with ESR, GAL-3 and CTLA-4, as shown in table no.2 (figures 2-A, 2-B and 2-C). In additional, a important negative correlations between serums Calpro with AD, as shown in table no.2 (figure 2-D)

Table 2. Pearson correlation coefficients of serum Calpro with studied parameters

Parameters	Calpro(ng/ml)	P-value
Age (years)	r = 0.32	0.06
BMI (kg/m^2)	r = 0.18	0.23
W-H R	r = 0.155	0.32
ESR (mm/h)	r = 0.861	< 0.05
RF (IU/ml)	r = 0.896	< 0.05
GAL-3 (ng/ml)	r = 0.841	< 0.01
AD (pg/ml)	r = -0.860	< 0.01
CTLA-4(pg/ml)	r = 0.800	< 0.01

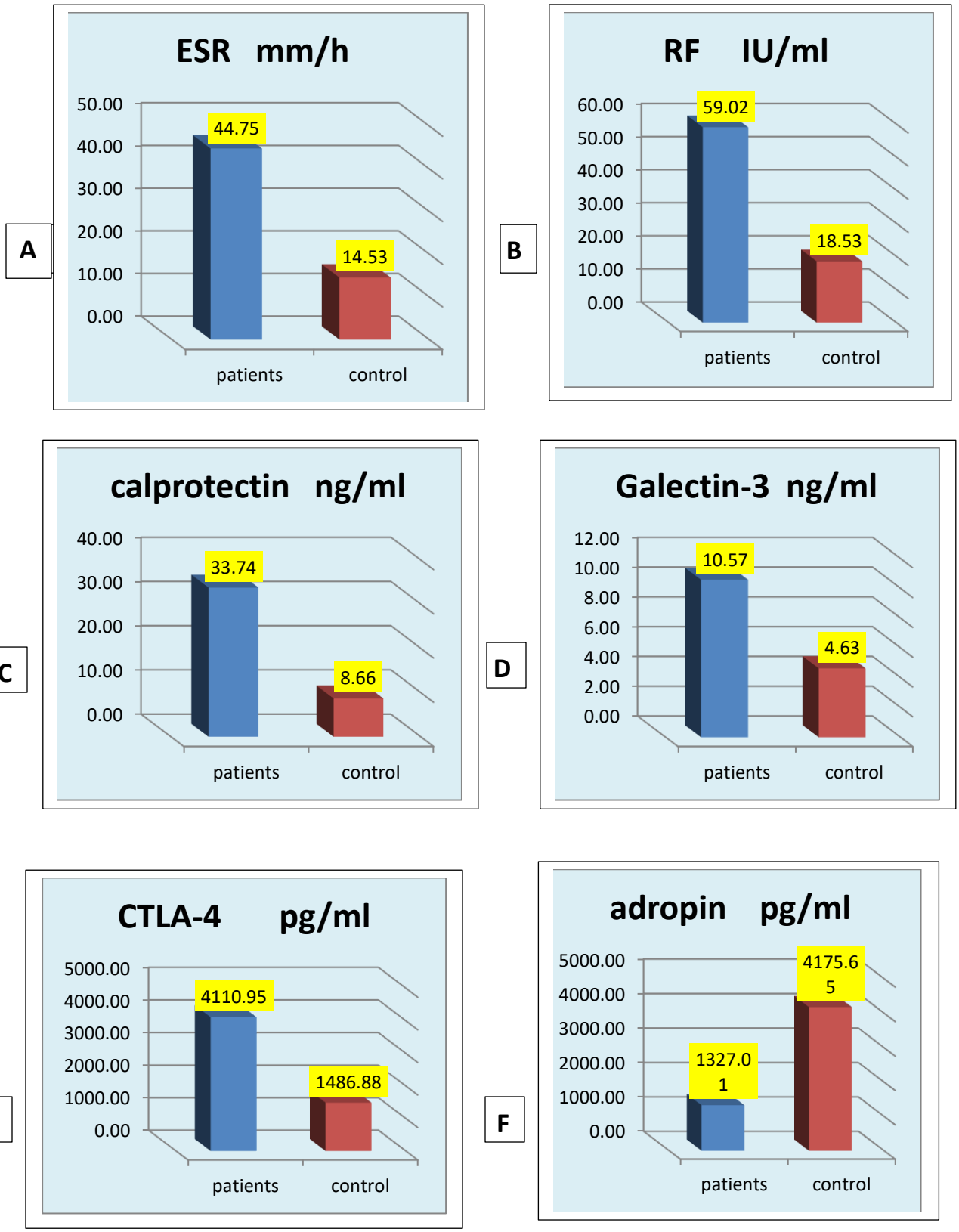

Fig. (1): Comparison between biochemical parameters in RA patients and controls

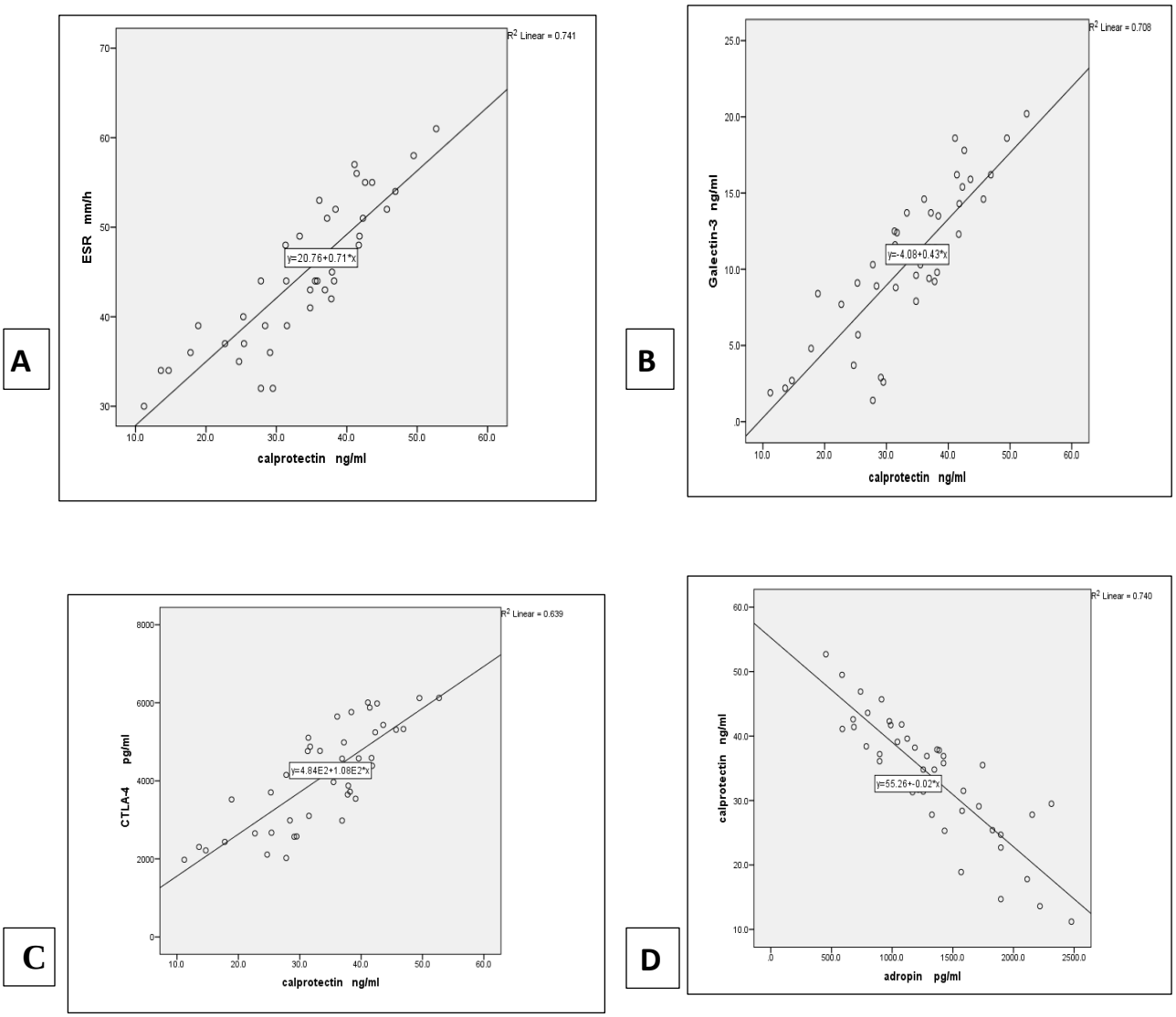

Fig. (2): The association between serum Calpro with ESR, GAL-3, CTLA-4 and AD

DISCUSSION

RA is a prolonged infection that has an adverse action on health through pain, action constraint and emotional stress.

In the present study, Calpro, GAL-3 and CTLA-4 were expressively augmented in the cases with RA compared to control.

Results also demonstrate there were important increases in ESR and RF levels in RA patients as compared to HC. This result is similar with previous study [13].

The study clearly proved that patients RA disease had significantly lower AD level than the healthy control groups.

Inciarte-Mundo *et al*. recently established that calpro improved ranges infection action than CRP or ESR in cases with RA taking tocilizumab, a biological mediator with an affected influence on acute phase reactants (as a result of its obstruction of interleukin-6 [14]. The communication of monocytic calpro with initiated endothelium leads to it's secreting [6], this could description for the high calpro levelss in the body liquids for RA patients.

The control of serum calpro concentrations may happen in altered immune & immune pathological responses, particularly in severe sore or Th1-facilitated reactions. In certain aspects, the susceptibility &changing aspects of calpro appear to

overwhelmed convential inflammation parameters like CRP [15].

Extracellular calprotectin improves the transendothelial movement for inflammatory cells. Calprotectin is existing at great levels in the cytosol of lymphocytes; also calprotectin is translocated for the tissue and cytoskeletal structures upon activation [16]. Calprotectin is extremely expressed via synovial macrophages, and then might production amplifying proinflammatory cytokine reactions in RA [17].

Because calpro is a small molecular weight protein of only 36.5 kDa, it caneasily diffuse into the blood circulation from the inflamed joints [18]. As a result, calpro might well play major role in the harshness of inflammatory action in the RA joints, and hence has the potential to remain a biomarker of inflammation for RA.

Serum calpro concentrations can benefit stratify disease action in patients with RA getting biologics [19, 20]. Calprotectin concentrations possibly extra precisely distinguish disease action in cases with RA treated with TNF inhibitors (TNFi.) than the acute-phase response (CRP level and ESR) [19].

Clinical and experimental sign that GAL3 shows an acute function in RA growth, e.g., by controlling RA synovial fibroblast roles. Thus, GAL3 encourages individual pro inflammatory interleukins and chemokine communication profiles in RA synovial fibroblasts compared to dermal fibroblasts [21].

Absences of Gal3 on dendritic cells support slower IL10 manufacture & augmented IFN-γ by allogeneic T cells [22].

Higher levels of pro-inflammatory cytokines are showed in elevating the harshness of RA via stimulating oxidative stress & sore [23].

Adropin is a recently recognized protein that its defensive role on endothelial cells has been exposed in the past, and has been denoted to as a new controller of these cells [24].

Low AD levels are related by endothelial dysfunction, additionally, AD may have defensive properties on the endothelial job [25].

AD insufficiency is related by decreased nitric oxide bioavailability in the endothelium [26].

RA has been observed as a prolonged inflammatory disease, as a result AD as a possible anti-inflammatory protein show an central role in the inhibition of RA [27].

The correlations of Calpro with age, BMI and W-H R may well not be establish in the present smaller study, where most patients were over-weighted at start, which could affect the correlations.

This study identified the strong positive relationship among serum Calpro values & each of markers of inflammation (ESR, RF) and GAL3, CTLA-4denoting its role as a parameter of inflammation.

We established an important association among CRP concentrations & serum calpro concentrations. Identical results were conveyed via Cerezo *et al*., [28] and Adel et al [29], who reported important association among serum calpro and CRP levels.

Present paper indicated that serum calpro concentrations have a important association with RF concentrations, & this consistent with Garcia *et al* data [30]. Inconsistent data were issued via Adel *et al*., [29], they detected insignificant associations among serum calpro and RF concentrations; this inconsistency may be refer to their select of joining cases in a inactive rheumatoid status in their research.

CTLA4 is an essential co stimulatory particle, which can prevent IL-2 manufacture & expression of IL-2 receptors & prevent the job of cytotoxic T cells [31]. Its critical functions in keeping immune balance are validated via the growth of antagonistic & serious auto immune infections in animal models missing CTLA4 [32].

A strong negative association was found among serum Calpro levels and AD. Hence, serum adropin should be utilized as a biomarker for assessing the danger of increasing RA.

The accurate role of AD in RA mechanism still vague. Inflammation shows a vital role in the increase of RA. Adropin significantly lowered the mRNA expression stages of TNF-α & IL-6 in the pancreas tissue of diabetic rats [33]. Circulating adropin concentration was adversely associated with TNF-α concentration in female with poly cystic ovarian syndrome [34]. Therefore, adropin may play a protective role in RA improvement through anti-inflammatory effects. The current research presents some determination. Such as, the size of sample was not adequately big to complete ultimate decisions. More researches by great populations are thus necessary. However, further follow-up studies involving large samples are required to elucidate the accurate role of these cytokines in disease development and in maintaining persistent inflammation

In conclusion, serum concentrations of Calpro, Gal-3 and CATL-4 may be used as diagnostic tests in RA. The study found important positive association among these markers and harshness of RA. Positive correlation of these markers with other well-

established RA markers point to the significance of Calpro, Gal-3 and CATL-4 as serum makers in predicting the result of RA. Serum adropin concentrations are negatively connected with Calpro. Adropin may be involved in the patho genesis of RA development.

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