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Clinical Significance of Prolactin Level in the Assessment of Rheumatoid Arthritis Disease Activity and Severity

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Abstract

Aim: To determine the level of prolactin in the sera of rheumatoid arthritis (RA) patients and to correlate this level with clinically and radiologically evaluated disease activity and severity parameters.

Methods: The study included 30 patients with RA and 20 healthy individuals as controls. All the patients were subjected to clinical and musculoskeletal examination with the assessment of disease activity using the DAS 28 score and disease severity using the Health Assessment Questionnaire (HAQ) and the modified Duke Severity of Illness score (DUSOI). Larsen score was used to assess radiological severity. Laboratory investigations included measurement of serum prolactin (sPRL) using the Enzyme Linked Immunosorbent Technique (ELISA) technique, a complete blood count (CBC), kidney function tests, measurement of the rheumatoid Factor (RF), antibodies to cyclic citrullinated peptides (anti-CCP), the erythrocyte sedimentation rate (ESR) and the C-Reactive protein (CRP).

Results: RA patients showed a significantly higher median prolactin level when compared to the control group ($p<0.001$). The median prolactin level was significantly increased in the positive RF and positive anti-CCP antibodies patients compared to negative cases ($p= 0.027$ and $p= 0.011$ respectively). The median prolactin level increased significantly in higher X-ray grades ($p= 0.040$). The prolactin level showed significant positive correlations with the ESR ($p=0.048$), DAS28 ($p < 0.001$), the short HAQ ($p <0.001$) and the X- ray grading ($p=0.042$). The prolactin level showed an excellent AUC (AUC=0.926). At a cut-off value of 10.7 ng/mL, its sensitivity was 80%, specificity was 85%, the PPV was 78%, NPV was 92.7%, and the accuracy was 86.5% regarding the discrimination between RA cases and healthy subjects.

Conclusion: sPRL level is suggested to be a marker for RA disease activity and severity being significantly higher in RA patients compared to healthy subjects, being significantly correlating with the disease activity and severity parameters as well as having a striking 80% sensitivity and 85% specificity for diagnosis of RA.

Keywords: Rheumatoid arthritis, serum prolactin, autoimmunity,DAS28, disease activity, severit.

1. Introduction

In chronic rheumatoid arthritis (RA), immune cells accumulate and synovial fibroblasts are activated inside the synovial membrane, leading to cartilage degradation and joint deformations [1].

Lactotrophic cells in the pituitary gland generate prolactin (PRL), a polypeptide hormone that is primarily controlled by dopamine neurons in the brain [2]. Extra-pituitary locations, such as immune cells, such as lymphocytes, also release it [3].

Anti-antigen tolerance among developing B-cells is disrupted by PRL, which stimulates the immune system by enhancing the proliferation of lymphocytes and the production of cytokines, immunoglobulins, and autoantibodies (2).

Patients with RA had higher levels of serum PRL than controls in certain studies, although these findings haven't been confirmed in all of them yet. Higher secretion of PRL or increased synthesis of PRL by immune cells like macrophages might support a link between PRL levels and disease progression (4).

Immune competence is maintained by PRL, which plays a function in autoimmune rheumatic disorders' development (5).

Because of the growing prevalence of autoimmune diseases, researchers are scrambling to come up with new treatment modalities (6). The goal of our study is to measure the level of PRL in RA patients' sera and correlate this level with clinically and radiologically evaluated disease activity and severity parameters.

2. Patients and Methods

2.1. Patients

This is a case –control study carried out on 30 RA female patients in the childbearing age. Patients were recruited from the inpatients' unit and the outpatients' clinics of the Rheumatology, Rehabilitation and Physical Medicine department, Benha university hospitals. All the patients fulfilled the 2010 classification criteria of the American College of Rheumatology/European League against Rheumatism (ACR/EULAR) for RA [(7)].

2.2. Exclusion criteria

- All pregnant, lactating, and post-menopausal females,
- Females using hormonal contraception,
- Females having other autoimmune rheumatic diseases.

A control group of 20 apparently healthy female subjects of matched ages were included from the staff personnel and relatives of patients.

This study was approved by the Research Ethical Committee of the Faculty of Medicine at Benha University, Egypt on 16\7\2019. A written consent was obtained from all participants.

2.3. Methods

All the patients were subjected to the following:A Detailed history taking and full clinical and musculoskeletal examination with the assessment of:

- RA disease activity using the DAS 28 score ***(8),***
- Functional status using the Health assessment Questionnaire (HAQ) ***(9)*** ,
- The modified Duke Severity of Illness score (DUSOI) ***(10).***

Laboratory investigations:

Ten ml of venous blood were withdrawn from each participant under complete aseptic conditions for:

- A complete blood count (CBC), kidney function tests,

- The measurement of the rheumatoid factor (RF), antibodies to cyclic citrullinated peptides (anti-CCP), the erythrocyte sedimentation rate (ESR), and the C - reactive protein (CRP).
- Measurement of serum prolactin (PRL) using the Enzyme Linked Immunosorbent Technique (ELISA) technique [(11)]. The ELISA kit was supplied by the Immunospec Corporation/ USA. The sample was put in a serum separating tubes for 30 minutes till clotting then centrifuged. The separated serum was used for PRL measurement. The results were expressed in ng/ml according to the manufacturer's instruction.

Radiological evaluation:

Plain radiographs for both hands and wrists were obtained. Assessment of joint damage was using the Larsen's score [(12)].

2.4. Statistical Analysis

The collected data was revised, coded, tabulated, and introduced to a PC using a Statistical package for Social Science (**IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.).** Data were presented and suitable analysis was done according to the type of data obtained for each parameter. Shapiro test was done to test the normality of data distribution. The mean and standard deviation (± SD) were used to describe parametric data, while the median and range were used to describe non-parametric numerical data. The frequency and percentage were used to describe non-numerical data.

3. Results

This study was conducted on fifty participants, who were divided into two groups, table [(1):]

Group I: 30 RA female patients (100%), whose ages ranged from 21 to 45 years and duration of disease ranged from 1-15 years.

Group II: 20 age and sex matched healthy female volunteers (100%) as a control group, whose ages ranged from 17 to 31 years.

- Patients' data, clinical assessments, and radiologic findings in RA patients are shown in table [(2)]. Laboratory data are shown in table [(3).]
- The median serum prolactin (sPRL) level of RA patients was highly statistically significantly increased ($p<0.001$) compared to the controls (13.9 vs 6.6 ng/mL respectively); figure [(1).]
- The median sPRL was significantly higher in positive RF (17.3 ng/mL) and positive anti-CCP antibodies (24 ng/mL) patients compared to negative patients ($p=0.027$ and $p=0.011$ respectively)
- Table [(4)] shows correlations of sPRL level with RA patient's ages, disease duration and clinical assessment data.
- Serum prolactin level showed an excellent AUC (AUC=0.926). At a cut-off value of 10.7 ng/mL, its sensitivity was 80%, specificity was 85%, the PPV was 78%, NPV was 92.7%, and the accuracy was 86.5% or discrimination between RA patients and healthy subjects, (table 5 and figure 2).

Table (1) Comparison between the studied groups regarding age distribution.

	Control N=20 mean±SD	**RA N=30 mean±SD**	***p***
Age (years)	36.3±5.8	35.4±5.7	0.626

SD, standard deviation; student t test was used for numerical parameters.

Table (2) Patients' data, clinical assessments, and radiologic findings in RA patients.

Variables		**RA N=30**	
Age (years)	**mean±SD**	35.4	5.7
Disease duration (years)	**Median, range**	6	1-15
Disease Activity score 28	**Median, range**	4.8	2.2-6.9
Disease Activity grades (N and %)	**Mild**	4	13.3
	Moderate	12	40
	Severe	10	33
DUSOI	**Median, range**	37.5	6.25-75
Larsen's score (N and %)	**Normal**	9	30
	Grade 1	10	33.3
	Grade 2	3	10
	Grade 3	8	26.7

DUSOI= Duke Severity of Illness Checklist,

Table (3) Laboratory data of RA patients.

Parameters			**RA (N=30)**	
ESR (mm/1st h)		**Median, range**	60	19-180
CRP (mg/l)		**Median, range**	6.9	3-12
RF	**Negative**	**N, (%)**	7	23.3%
	Positive	**N, (%)**	23	76.7%
Anti- CCP Abs	**Negative**	**N, (%)**	6	20%
	Positive	**N, (%)**	24	80%
Serum prolactin (ng/mL)		**Median, range**	13.9	8.1-44.5

ESR= erythrocyte sedimentation rate, CRP= C-reactive protein, RF= rheumatoid factor,
Anti- CCP Abs= anti-citrullinated protein antibodies

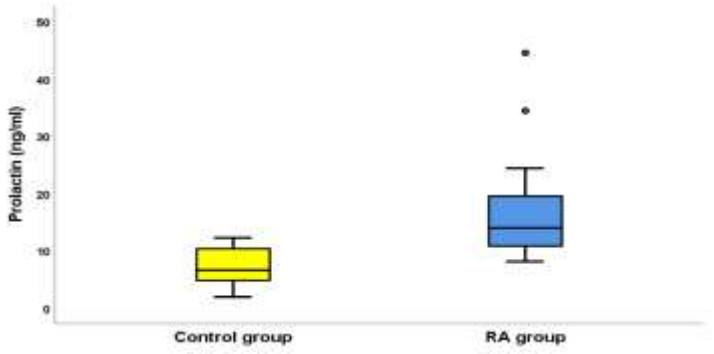

Fig. (1) Median serum prolactin level in RA patients and controls.

Man-Whitney test was used for comparison, p <0.001**: highly significant.

Table (4) Correlations of serum prolactin level with ages, disease durations and clinical assessment data in the RA group.

	Prolactin	
	r	**P**
Age/years	0.044	0.816
disease duration/years	0.120	0.528
ESR mm/1st hr	0.360	**0.048***
CRP mg/l	0.285	0.127
RF	0.410	**0.024***
Anti-CCP antibodies	0.472	**0.008***
DAS 28	0.962	**<0.001***
Short HAQ	0.621	**<0.001***
DUSOI	0.934	**<0.001***
X-Ray grading	0.365	**0.042***

r = Correlation coefficient, ESR= erythrocyte sedimentation rate, CRP= C-reactive protein, RF= rheumatoid factor, Anti-CCP Abs= anti-citrullinated protein antibodies, DAS-28= 28-joint disease activity score HAQ= health assessment questionnaire, DUSOI= Duke Severity of Illness Checklist, P*= significant (<0.05).

Table (5) Validity of prolactin levels for discrimination between RA cases and the control group.

	Prolactin
AUC	0.926
Cut off	10.7
Sensitivity (%)	80
Specificity (%)	85
PPV (%)	78
NPV (%)	92.7
Accuracy (%)	86.5

AUC= Area under curve, ROC= Receiver operating curve, PPV= Positive predictive value, NPV= Negative predictive value.

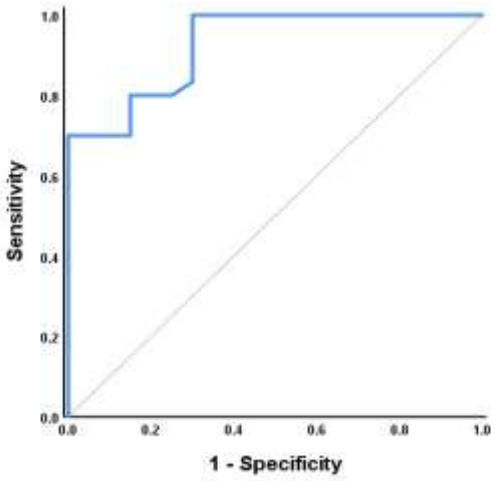

Fig. (2) ROC curve of prolactin level for discrimination between RA cases and the control group.

4. Discussion

In addition to the growing synovitis of the afflicted joints and the infiltration of the synovial fluid by immune cells such as T cells, B cells, and monocytes, RA is a chronic autoimmune disease accompanied with neovascularization. Bone erosion and cartilage degeneration may also be brought on by synovial lining hyperplasia (pannus), the outcome of macrophage and fibroblast-like synoviocyte proliferation [13].

Prolactin is a protein hormone produced by the anterior pituitary gland's lactotroph cells. Mammary gland functions include milk production stimulation and breast tissue growth [14]. Other extrapituitary locations, such as neurons, ovary, placenta, prostate, skin cells, endothelium, bone marrow adipose tissue, spleen, and immunological cells including lymphocytes, release PRL as well. [3].

A number of autoimmune disorders have been linked to high levels of prolactin in the bloodstream, and these findings show that prolactin has an important role in inflammatory and immunological processes [15].

PRL and RA have been linked in many research investigations. In RA pathogenesis, the human PRL gene was identified on chromosome 6 next to the HLA area, which is located on chromosome 5. The fact that RA patients' sera had higher levels of PRL suggested that it may have a function in the condition. The correlation between PRL concentrations and disease activity may be explained by the release of PRL by immune cells such as macrophages [4].

Prolactin levels in RA patients were measured and correlated with clinical and radiographic evaluations of disease activity and severity.

In contrast to the healthy controls, RA patients had significantly higher PRL levels in our study.

In patients with positive RF and anti CCP findings, we found a substantial rise in PRL levels compared to those with negative results. In addition, the levels of PRL were linked to higher X-Ray Larsen scores. PRL levels and disease activity markers (ESR, DAS, d short HAQ) were shown to have significant positive relationships with the Larsen score as a disease severity indicator.

Wu et al. (2019) and Joda (2019) both reported substantial outcomes in their respective patient groups [3, 16].

Based on the function of prolactin in lymphocyte stimulation, antibody production, inhibition of apoptosis, and modulation of B-cell tolerance, this was concluded [17]. Proinflammatory cytokines and cartilage-degrading enzymes may be produced by fibroblast-like synovial cells stimulated by PRL [5].

A positive connection was established between PRL levels and disease activity indices (ESR, DAS, and short HAQ) with positive RF and anti CCP, which is consistent with previous studies by Fojtikova et al., 2010, Fayez et al., 2015 and Iqbal et al., 2017. (1,18&6). It was then proposed that Prolactin may promote autoimmunity by activating the immune system by disrupting the normal process of immunological tolerance among growing B cells toward self-antigen; this idea was later supported by the findings of the study.

Comparatively, Haga et al. (2014) discovered in 307 treated RA patients that the serum PRL levels did not correlate with disease activity, serology or demographic characteristics, which might be ascribed to the varied techniques employed to measure disease activity in these RA patients [19].

Along with the positive link with highly +ve RF and anti CCP as unfavourable prognostic variables, we found a strong +ve relationship between PRL levels and Larsen's score on the illness severity side. Iqbal et al. 2017 findings mirrored those of Fojtikova and Fayez et al., 2010 and Fayez and Iqbal, 2015 (1,18&6).. Using the hypothesis of PRL's capacity to regulate immunological homeostasis, this may be explained. Immune cells, chondrocytes, and synovial fibroblasts have been shown to contain PRL receptors on their surfaces, and this has been confirmed. IL-12, tumour necrosis factor alpha, and interferon alpha are all proinflammatory cytokines that are affected by PRL in these immune cells. The anti-apoptotic properties of PRL are also responsible for the enhanced persistence of both auto-reactive B cells and T

cells.

In contrast to our findings, Haga et al., 2014 found no differences in Serum-PRL levels between RF-positive and -negative individuals, or between those with and without anti-CCP antibodies [19].

Adan's idea from 2013 formed the basis for their explanation of their findings. Later researchers administered prolactin to rats' knee joints and discovered that PRL protected condrocytes from apoptosis and pannus development due to inflammation [20].

A more significant link was found between high levels of hyperprolactinemia and disease activity in RA patients in Tang et al., 2011. Furthermore, they authorised the use of bromocriptin (a prolactin-lowering drug) to treat individuals with hyperprolactinemia, which resulted in a decrease in disease activity ratings [4].

A few discrepancies in the data from our patients and Haga et al., 2014 [19].

We had higher female ratio (100% VS 68%), higher Anti CCP positivity (80% VS 67%) and higher disease activity scores with lower age group patients.

However, the difference in mean Prolactin levels between RF and anti-CCP positive patients and their negative counterparts was not statistically significant (Karthiga, 2015). [21].

Menopause patients were not included in our study, and our research was further hampered by the lack of male participants due to the limited sample size (30 patients). Prolactin levels in synovial fluid were another practise restriction.

5. Conclusion

sPRL level is suggested to be a marker for RA disease activity and severity because it is significantly higher in RA patients compared to healthy subjects, is significantly correlating with disease activity and severity parameters, and has a striking 80 percent sensitivity and 85 percent specificity for the diagnosis of RA.

At the very least, individuals with hyperprolactinemic RA should seek therapy with PRL targeting.

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