A Hospital Based Case Control Study on Serum Prolactin Levels and Its Correlation with Anti CCP Levels in Recently Diagnosed Rheumatoid Arthritis in Chennai, Tamil Nadu

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ABSTRACT

BACKGROUND

Prolactin, an anterior pituitary hormone, exhibits immunoregulatory properties. It up regulates Th1 cytokines expression; that plays a crucial role in autoimmune disorders. In autoimmune diseases like rheumatoid arthritis (RA), altered serum prolactin level causes exacerbation. Hence, administration of drugs like bromocriptine, cabergoline in rheumatoid arthritis patients with elevated serum prolactin cause disease remission. In RA patient, an antibody by name ACPA - Anti citrullinated protein antibodies are released which binds with citrullinated proteins. Anti cyclic citrullinated peptides (Anti-CCP) is 90 % specific for rheumatoid arthritis. Hence, this study was done to determine the status of serum prolactin and correlate serum prolactin with anti CCP in recently diagnosed rheumatoid arthritis patients.

METHODS

Serum prolactin and anti-cyclic citrullinated peptide antibody levels were measured using enzyme linked immunosorbent assay (ELISA) technique in 55 recently diagnosed rheumatoid arthritis patients, compared with 27 age and sex matched apparently healthy controls. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software version 20. Tests of significance using unpaired student's t-test at 5 % significance was used to compare the serum prolactin between cases and controls. Pearson's correlation co-efficient to measure the linear relation between serum prolactin concentration and anti CCP were carried out.

RESULTS

87 % of study population was females, with majority in the age group of 31 to 40 years. The mean serum prolactin concentration in cases was 33.531 ± 17.92 ng/mL and in controls was 14.4 ± 5.9 ng/mL. The 95 % confidence interval for mean of serum prolactin concentration in cases were 50.84 to 16.22 ng/mL, controls were 22.54 to 6.26 ng/mL. The p value was significant, less than 0.001. Pearson correlation between prolactin and anti CCP had no significance; p – value 0.760.

CONCLUSIONS

Serum prolactin concentration in rheumatoid arthritis patients was higher. These results identify the use of dopamine agonist as an adjuvant treatment in remission of rheumatoid arthritis. There was no correlation between serum prolactin and anti CCP levels among RA patients.

KEYWORDS

Rheumatoid Arthritis, Prolactin, Cytokines, Anti Cyclic Citrullinated Peptide, Hormone

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BACKGROUND

Rheumatoid arthritis is an autoimmune disease affecting 1 to 2 % of general population worldwide with increased female preponderance due to hormone. Since early 19th century, various debates are ongoing in role of pituitary hormones that to prolactin in immune modulation.[1] In the time of stress, prolactin is needed to balance the effect of glucocorticoids and other inflammatory mediators. Prolactin up regulates the expression of Th1 cytokines involved in autoimmune diseases.[2,3] In few case reports, administration of bromocriptine and cabergoline in rheumatoid arthritis patients with increased prolactin levels causes disease remission was shown.[4]

Prolactin is a polypeptide hormone secreted in anterior pituitary gland by lactotroph cells. Its synthesis is regulated by both dopamine and hypothalamic hormones. Prolactin is a pleiotropic hormone, apart from its widely known role in lactation and reproduction also has a crucial role in immune regulation as a potent cytokine. Multiple studies worldwide conducted are unable to provide consistent results due to its own limitations.

Anti CCP levels of 40 to 59.9 U/L is considered positive. In undifferentiated arthritis, anti CCP concentration tells us about the future development into RA. In RA patients, anti CCP is an erosive disease marker. Aggressive and erosive RA is common in anti CCP positive individuals. In order to explore more regarding this area, this study aims to determine the status of serum prolactin in patients with recently diagnosed rheumatoid arthritis and to compare serum prolactin levels with apparently healthy individuals and also correlate serum prolactin concentration levels among cases with the serology status - rheumatoid factor, anti CCP antibodies and inflammatory status - Erythrocyte sedimentation rate (ESR).

METHODS

This case control study was conducted in Institute of Biochemistry, Madras Medical College, Chennai; from January 2014 to September 2014. This study was carried out after obtaining ethical committee approval from Madras Medical College Institutional Ethics Committee, held on 11.12.2013.

Inclusion Criteria

The cases were recently diagnosed rheumatoid arthritis patients in the rheumatology outpatient department (OPD) of Rajiv Gandhi Govt. General Hospital, Chennai according to ACR criteria 2010. Patients with morning stiffness of joints more than 1 hour, with symmetrical joint involvement presenting with pain, swelling, tenderness, age more than 20 years, clinically diagnosed as RA were included in the study. The controls were age and sex matched apparently healthy individuals. Total cases & controls were 55 and 27 in number respectively with mean age of 41.51 ± 11.7 years.

Exclusion Criteria

1. RA patients already on treatment.
2. Women on treatment for PCOS, pregnancy and lactation, infertile individuals and women on oral contraceptive pills.
3. Patients on treatment for non-communicable diseases like diabetes mellitus, hypothyroidism (TSH > 5µIU/L), renal failure, hypertension, any chronic illness and other autoimmune diseases.
4. Pituitary microadenoma and macroadenoma and individuals with signs and symptoms of hyperprolactinaemia.
5. Patients on chemotherapy, hormone replacement therapy and on any one of the following drugs namely- antipsychotics, H2 blockers, antidepressants, dopamine agonist, isoniazid, and calcium channel blockers.
6. Patients with recent chest wall trauma were excluded based upon the clinical history and routine laboratory investigations.

The disease activity of cases was noted using the disease activity score (DAS).

Investigations Carried Out

Peripheral venous blood 5 mL was collected after obtaining informed consent. Serum was separated.

- Prolactin, anti-citrullinated peptide antibody, thyroid stimulating hormone (TSH) were measured using sandwich method ELISA.
- Rheumatoid factor was detected using latex agglutination method.
- ESR was measured using conventional Westergren's method.
- Renal function test and uric acid levels were tested using fully automated clinical chemistry analyser.
- Complete blood count and haemoglobin levels were measured using 3-part cell counter.

Prolactin & Anti CCP Measurement

Prolactin was measured using sandwich ELISA method - Monoclonal prolactin antibody coated microtitre plate was used. Prolactin in serum gets sandwiched between coated antibody and HRP labelled anti-prolactin antibody. After incubation, TMB-substrate was added that reacts with HRP coated bounded antibody which is indicated by a yellow colour formation and absorbance was read at 490 nm in ELISA reader.

Anti-citrullinated peptide was measured using sandwich ELISA method – Recombinant citrullinated rat fillagrin antibody was used and absorbance was read at 450 nm in ELISA reader.

Statistical Analysis

Using SPSS software version 20, statistical analysis was analysed, and the following tests were carried out. Test of significance was done for serum prolactin using unpaired

students t-test at 5 % significance. For linear relationship between serum prolactin and anti CCP; Pearson's correlation co-efficient was used. To compare more than 2 variables in a group', one way analysis of variance (ANOVA) was used.

Ethics

Only volunteers consenting to the study were included. The study involved only a minimal risk of sample collection for biochemical analysis. This study reports a section of results from the parent study, which was approved by the Institutional Ethics Committee of Madras Medical College and Rajiv Gandhi Government General Hospital (ECR/270/Inst./TN/2013-No. 09122013).

RESULTS

Baseline Characteristics of Population

55 cases and 27 age and sex matched apparently healthy controls were enrolled in the study. The mean age of the study population was 41.51 ± 11.7 years. 87.3 % of the study population were females and majority of the study population fall between 31 to 50 years. 78.75 of the study population among females were in postmenopausal stage. 60 % of cases had high disease activity and 40 % of the cases had moderate disease activity based upon their DAS score. The mean serum urea level (Enzymatic method) of the study population was 24.57 ± 4.7 mg/dL. The mean serum creatinine (Jaffes method) concentration among study population was 0.9 ± 0.2 mg/dL. The mean serum TSH among study population (Sandwich ELISA) concentration was 2.5 ± 2.2 µIU/mL.42 % of cases were positive for rheumatoid factor.

The mean ESR among cases was 38.6 ± 27.1 and among controls was 12.1 ± 4.4. The mean serum prolactin levels between cases was 33.531 ± 17.92 ng/mL and control was 14.4 ± 5.9 ng/mL. It was compared using unpaired students' t-test. The difference in mean Sr. prolactin between the cases and controls were statistically significant with the p value < 0.001.

In female RA patients, 33.88 ± 18.75 ng/mL, was the mean prolactin concentration and 14.11 ± 7.9 ng/mL was the mean prolactin concentration in female controls. The difference in mean prolactin concentration between the two groups was statistically highly significant with the p value < 0.0001. 31.16 ± 7.9 ng/mL was the mean prolactin concentration in male RA patients. 16.1 ± 3.2 ng/mL was the mean prolactin concentration among male controls. The difference in mean between the groups was statistically significant with the p value being 0.0059.

22 cases with moderate disease activity had a mean DAS score of 4.4 ± 0.4 with mean serum PRL concentration 25.03 ± 10.3 ng/mL. 33 cases had high disease activity score, with a mean DAS score 6 ± 0.7 with a mean serum PRL concentration of 39.2 ± 19.4 ng/mL.

The difference in mean among patients with moderate and high disease activity scores was statistically highly significant with a p value - 0.0028. 36.58 ± 21.6 ng/mL was the mean prolactin concentration in patients with RF positivity.

31.13 ± 14.35 ng/mL was the mean prolactin concentration in RF negative cases. The difference in mean prolactin levels among RF positive and negative patients by applying unpaired students t- test was statistically not significant, p value - 0.266.

Group	Number	Mean Anti CCP Levels in U/L
Cases	55	47.5 ± 64.8
Control	27	5.3 ± 3.9

Table 1. Comparison of Mean Anti CCP between Cases and Controls

The mean anti CCP levels between cases and controls was analysed by applying unpaired student t- test. The difference in mean between the two groups was statistically significant with a p value of 0.0012.

Group	Number	Mean PRLng/mL	Mean Anti CCP U/mL
Anti CCP weak positive (20 - 39.9 U/L)	27 %	36.67 ± 10.24	25.8 ± 4.75
Anti CCP positive (40 - 59.9 U/L)	5 %	41.8 ± 3.7	49.7 ± 7.4
Anti CCP strong positive (_60 U/L)	22 %	34 ± 2.1	158.86 ± 51.87
Anti CCP negative (< 20 U/L)	46 %	30.45 ± 19.9	6.75 ± 5.2

Table 2. Mean Serum Prolactin Concentration in Anti CCP Positive and Anti CCP Negative Individuals among Cases

The mean prolactin concentration is high among Anti CCP positive patients.

Variable	Groups	N	Mean	Std. Dev	P-Value
PRL ng/mL	Both RF & anti CCP positive	13	38.654	19.6836	0.448
	Only RF positive, anti CCP negative	10	34.540	24.9064	
	Only anti CCP positive, RF negative	17	34.141	11.7759	
	Both RF & anti CCP negative	15	27.687	16.8622	
	Total	55	33.520	17.8998	

Table 3. One Way ANOVA to Compare Mean Values between Groups

Variable	Sum of Squares		df	Mean Square	F-Value	P-Value
PRL	Between groups	870.013	3	290.004	0.900	0.48 NS
	Within groups	16431.675	51	322.190		
	Total	17301.688	54			

Table 4. ANOVA Table

Sl. No.	Variables		Prolactin
1	Anti CCP	Correlation	0.042
		p-value	0.760
		N	55
2	ESR	Correlation	0.236
		p-value	0.082
		N	55
3	RF values in RF positive patients	Correlation	0.089
		-value	0.681
		N	24

Table 5. Pearson Correlations between Prolactin and Variables among Cases

Table 5 shows the correlation of serum PRL concentration with variables like Anti CCP, ESR, RF. The correlation was found using Pearson's correlation coefficient. NO correlation exists between anti CCP with serum prolactin concentration. A fair correlation exists

between ESR with serum prolactin concentration but they were not statistically significant.

DISCUSSION

In the present research, 55 patients diagnosed with rheumatoid arthritis recently, who were not on any treatment were included, from the rheumatology outpatient department of Rajiv Gandhi Govt. General Hospital Chennai. The diagnosis of RA was based on 2010 American College of Rheumatology criteria.[5] 27 age and sex matched apparently healthy volunteers without any evidence of rheumatoid arthritis clinically were selected. In both cases and controls, serum TSH was measured to rule out hypothyroidism. Serum urea and serum creatinine was measured to assess the renal function. ESR in blood and C-reactive protein (CRP) in serum was measured to assess the level of inflammation. Serum rheumatoid factor, prolactin and anti CCP were determined in all the study participants. The cases and control group had been matched with respect to age, sex, serum urea, creatinine, TSH as shown by p value less than 0.05. Among the cases, 87.3 % (48 out of 55) were females which matches with the literature that auto immune diseases are common in women.[6] The disease onset was in the age group of 30 to 40 years that matches with the existing literature; about 80 % of patients develop RA between 35 and 50 years of age. The criteria for inclusion as recently diagnosed RA patients and not under any treatment was devised, the major contribution in the study is by patients in 31 to 40 years of age. In this research, based on the menstrual cycle, premenopausal women were more that matches with the existing literature.[7] About 18 % (n = 10) of cases presented within 3 months of symptoms onset, 65 % of patients (n =3 6) presented after 3 to 6 months of symptoms onset. RF positivity among patients were 42 % (n = 23). Rheumatoid factor was negative in the serum of 58 % (n = 32) patients. Though rheumatoid factor presence in serum is not specific for rheumatoid arthritis, it is one of the cardinal feature of RA. The mechanism of rheumatoid factor formation is by mutations in germ line cells that is of somatic in nature and by gene rearrangements. The formed rheumatoid factor activates the classical complement pathway by binding with IgG Fc fragment. In diagnosis of rheumatoid arthritis, rheumatoid factor present in serum has a poor predictive value (PPV) because 2/3rd of RF positive individuals did not develop the disease. Strong predictor of RA is combined detection of IgM and IgA RF.[7]

When taking into account, Anti CCP antibody and RA fator, anti CCP antibody alone was positive for 31 % of patients. 23.6 % of cases had both RF and anti–CCP antibodies. RF alone was positive in 18.2 % of cases. Both the antibodies were negative in 27.3 % of cases. According to 2010 ACR/EULAR criteria, "In a patient with classical features of rheumatoid arthritis namely morning stiffness for more than 1 hour for at least 6 weeks, involvement of two or more small joints, RF and anti – CCP antibodies does not exclude the diagnosis of rheumatoid arthritis".[7] Hence, absence of antibodies in serum does not rule out disease in that individual. Citrullinated proteins are synthesized in RA patients by post translational modification of arginine by the action of enzyme PADI. Proteins like vimentin, fibrinogen, fibronectin undergo citrullination. Glycine in the primary sequence of these proteins is converted to arginine by PADI. ACPA are produced against these citrullinated proteins. These peptides bind better in the HLADR 4 Ag binding groove when compared with other peptides. Detected in healthy individuals years before disease onset. Anti CCP is not useful to predict the future development of disease since 1.5 % of normal individuals possessing this antibody did not develop the disease.[8]

In this research, 33.53 ± 17.93 ng/mL was the mean PRL concentration in cases (n = 55) that was significantly higher when compared with control group (n = 27) 14.4 ± 5.9 ng/mL; p value of < 0.0001. Even among males the mean serum PRL concentration in male patients 31.16 ± 7.9 ng/mL was higher than male controls 16.1 ± 3.2 ng/mL; which is statistically highly significant p value 0.0059. All these findings match with existing literature.[8-17] Balance between Th1 and Th2 maintains the homeostasis of immune system. Th1 and Th2 cells are activated by prolactin. Th1 is activated more by PRL than Th2. In autoimmune diseases with either Th1 or Th2 dominance, increased prolactin level is seen. Many animal models have shown that Th1 cytokines like TNF-α, INF-γ, IL-2 are involved in organ specific autoimmune diseases like rheumatoid arthritis, multiple sclerosis and insulin dependent diabetes mellitus. Th2 is associated with systemic lupus erythematosus (SLE), Hashimotos' throiditis and allergy.[18] Immuno-stimulatory effect is shown by prolactin. PRL activates autoimmunity by making immune cells anti-apoptotic, in the process of maturation of self reactive B- lymphocytes impaired negative selection these cells occur and immunogenic response to antigens is increased by prolactin. Hence, by increasing immunoglobulin production, cytokines favours autoimmunity. Association between disease progression with increasing prolactin levels has been noted in murine models. Moderate hyperprolactinemia has been seen in systemic lupus erythematosus, Rheumatoid arthritis, Sjogren's syndrome, Hashimoto's thyroiditis, and multiple sclerosis.[19]

The difference in mean between serum PRL concentration in both RF & anti CCP negative (30.45 ± 19.9 ng/mL, n = 25) and positive (36.1 ± 15.3, n = 35). Individuals were not significant statistically (p value - 0.2394).

The comparison of mean PRL concentration among the 4 different groups: RF & anti CCP positive, only RF positive, only anti CCP positive, both RF & anti CCP negative using ANOVA did not show any significant statistical difference in serum concentration of PRL. On comparing RA patients with erosive and non-erosive joint involvement, the levels of ESR and anti-CCP antibodies were significantly higher in RA patients with erosion. HLA-class II alleles is one of the cardinal genetic marker, whose presence increases the risk of RA. HLA- DRB1 molecule contains the shared epitope

(SE) allele. The serum prolactin concentration was highest in patients who visited the hospital between 3 and 6 months of symptoms onset (35.86 ± 19.9 ng/mL) than who presented earlier i.e. within 3 months of joint symptoms (27.45 ± 9.7 ng/mL) as well as individuals who visited OPD later than 6 months of symptoms onset (31 ± 13.2 ng/mL). Elevated ESR was observed in 89.1 % patients and 6 patients had normal range of ESR at the end of one hour. The above discussion tells us that concentration of serum PRL in RA patient is high. But markers of RA namely RF & anti CCP antibodies positivity had no significant correlation with PRL concentration. Hyperprolactinemia due to macroprolactin (a complex of PRL with IgG) is common in patients with slow renal clearance. The chance of macroprolactin causing hyper prolactenemia in RA patients is least likely because IgM type is the predominant antibody formed in RA that, may not bind with PRL. 70.9 % of patients had hyperprolactinemia, this elevation is not because of macroprolactin. Certain studies favour this.[20,21]

CONCLUSIONS

This study shows the role of prolactin in rheumatoid arthritis. In RA patients, the serum prolactin concentration was higher when compared with controls. This study tell us that,

1. Prolactin has a role in rheumatoid arthritis.
2. During treatment, monitoring prolactin concentration in serum will be useful, because there was a significant difference in serum PRL levels in patients with moderate and high disease activity.
3. Dopamine agonist can be included as an adjuvant in RA treatment for remission in individuals with elevated serum prolactin.

No correlation exists between serum prolactin levels and anti CCP levels in this study group; which needs to be further investigated.

Limitations of the Study

Male patients in this study were low and it skewed towards female population. There was lack of juvenile rheumatoid arthritis patients in this study. Serum was not screened for macro prolactin in both the study groups. The pulsatile secretion of prolactin was not considered in sample collection.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
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