Original Article

Indian Journal of Pathology: Research and Practice
Volume 4 Number 2, July - December 2015
DOI : https://dx.doi.org/10.21088/ijprp.2278.148X.4215.3

Emergence of Anti-Citrullinated Protein in the Early Diagnosis of Rheumatoid Arthritis: A Study of more than 1000 Cases in a Standlone Laboratory

Bhuyan M. P.*, Sarmah S.**

*Consultant Microbiologist **Consultant Pathologist, SRL Skylab, Guwahati.

Abstract

Introduction: Rheumatoid arthritis (RA) is one of the most common systemic autoimmune diseases. The aetiology of the disease, which affects up to 1-2 % of the world population, is unknown. This poor prognosis has led to an emphasis on early diagnosis and aggressive treatment. The routinely used diagnostic aid prescribed for Rheumatoid Arthritis is the serological test for the determination of rheumatoid factors (RF) in the serum. *Methods and Materials:* The anti- CCP antibody kit used is based on an ELISA method. All the anti-CCP positive cases were tested for Rheumatoid factor (RF) with turbidmetric immunoassay method. A total of 1024 cases were tested for Anti citrullinated peptide (Anti CCP). Of these 216 cases has been reported positive. *Result:* Positive Anti-CCP was found to be most prevalent in the age group: 41 – 50 years. In females the positive percent is 77. 3 %. Of the positive Anti –CCP cases only 68 were positive for Rheumatoid Factor (direct test with undiluted serum). *Discussion & Conclusion:* This study emphasised the value of anti-CCP antibodies as an early diagnostic marker of RA. The results are consistent with other relevant reviews. Anti-CCP antibodies precede the onset of RA and their presence is indicative of the future development of RA, making early therapeutic intervention possible.

Keywords: Rheumatoid Arthritis (RA); Anti-CCP Antibodies; Rheumatoid Factors (RF); ELISA; Immunoturibimetry; Early Diagnosis.

Introduction

Rheumatoid arthritis (RA) is one of the most common systemic autoimmune diseases. The aetiology of the disease, which affects up to 1-2 % of the world population, is unknown. The diagnosis of RA depends primarily on clinical manifestation of the disease. It is characterised by chronic inflammation of the synovial joints, which leads to progressive joint erosions and eventually to disability and loss of quality of life. This poor prognosis has led to an emphasis on early diagnosis and aggressive treatment.

Till now, the routinely used diagnostic aid prescribed for Rheumatoid Arthritis is the serological test for the determination of rheumatoid factors (RF) in the serum. RF is antibodies of the IgG class.

However these antibodies are also present in relatively high percentage in other autoimmune disease, infections and in up to 15 % of healthy individuals.

But over the past few years, several new antibodies have been described in patients with RA (antiperinuclear factor antibodies, antikeratin antibodies, anti RA33), but not all have been successfully incorporated into routine clinical practice [4]. A new group of autoantibodies that have generated particular interest are the anti cyclic citrullinated peptide (anti CCP) antibodies, which appear to be of value for the diagnosis of RA [5].

This study is to assess the early detection ability of anti cyclic citrullinated peptide (anti CCP) antibodies in the development of RA- years before the

Corresponding Author: Spandan Sarmah, SRL Skylab, Guwahati-Shillong Rd, Near Ulubari Flyover, Ulubari, Guwahati, Assam 781007.

E-mail: dr_spandan@rediffmail.com

development of clinical symptoms.

Methods and Materials

The anti-CCP antibody kit used is based on an ELISA (Enzyme linked immunosorbent) method (IMMCO Diagnostics, Inc.). The test utilizes microtitre plate wells coated with citrullinated synthetic peptides (antigen). Diluted patient serum is applied to the wells and incubated. If specific antibodies are present, they will bind to the antigen in the wells. Unbound material is washed away and any bound antibody is detected by adding horse radish peroxidise (HRP) labelled anti-human IgG, followed by a second washing step and an incubation with substrate. The presence of reacting antibodies will result in the development of colour, which is proportional to the quantity of bound antibody, and this is determined photometrically.

Microplate reader with filter 450 nm and calibrated micropipettes has been used in the procedure. Precautions of not touching the under surface of the wells and preventing damage and dirt and also careful pipetting and washing throughout the procedure are taken for optimal results.

Manufacturer recommended protocol for semi-quantitative manual assay has been strictly followed. Samples with results < 25 U / mL are negative, and >= 25 U / mL are defined positive.

The positive percent agreement of the kit used is 99.3 %. All the anti-CCP positive cases were tested for Rheumatoid factor (RF) with turbidmetric immunoassay method based on the principle of agglutination reaction.

Undiluted or serially diluted samples were used.

Study Population

During the period of January 2014 to July 2015, a total of 1024 case were tested for Anti citrullinated peptide (Anti CCP) using the ELISA method. Patients' age ranged from 6 years to 80 years with varied socio economic status mostly from sub urban areas. Most patients had complains of joint pain.

Total Male patients: 246

Total Female patients: 778

Total: 1024

Of these 216 cases has been reported positive, a percentage of 21.1 %.

Results

Table 1:

Patient Age group	Female	Male	Total
1-10			
11-20	1	1	2
21-30	17	2	19
31-40	38	8	46
41-50	47	13	60
51-60	33	15	48
61-70	24	7	31
71-80	7	3	10
Grand Total	**167**	**49**	**216**
	Female	**Male**	
Percentage:	77.3	22.7	

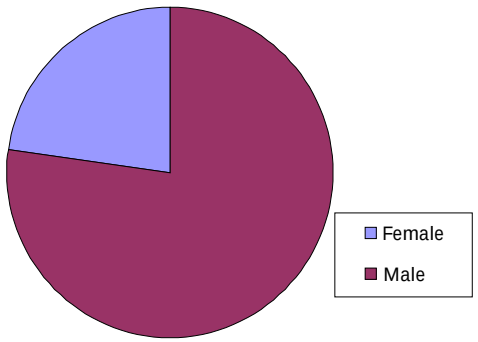

Fig. 1:

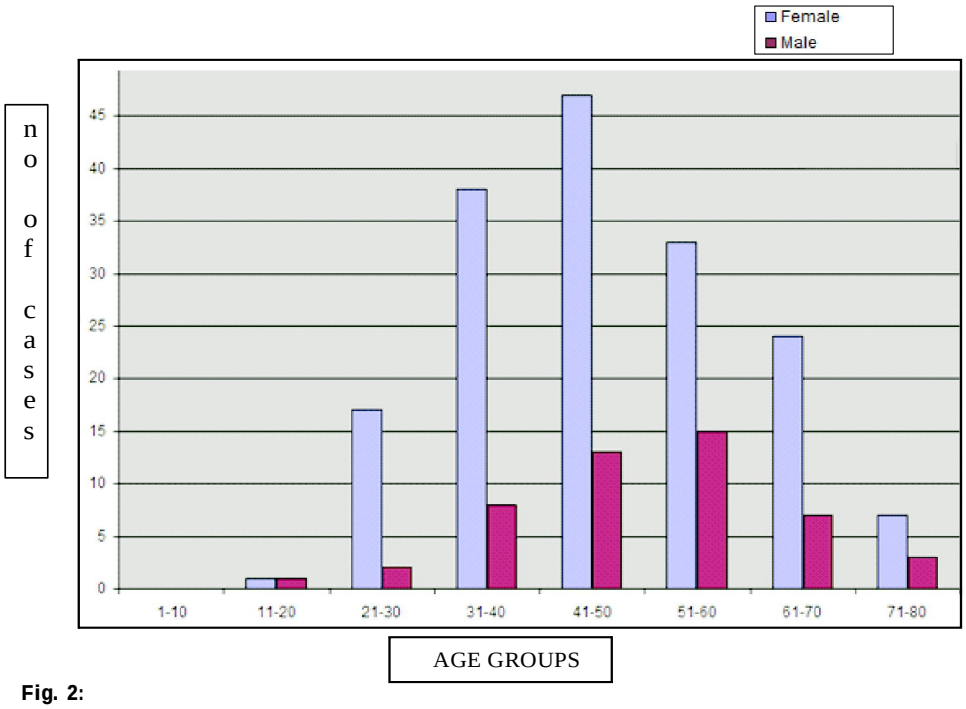

Fig. 2:

Positive Anti-CCP was found to be most prevalent in the age group: 41 – 50 years. In females the positive percent is 77. 3 %.

Rheumatoid factor (RF) testing with standard quantitative immunoturbidimetric assay was done for all positive cases. Of the positive Anti –CCP cases only 68 were positive for Rheumatoid Factor (direct test with undiluted serum). But on serial dilution of the –ve RF test, positive result was obtained in higher dilution which meant that the false -ve RF were due to prozone phenomenon (Antibody access).

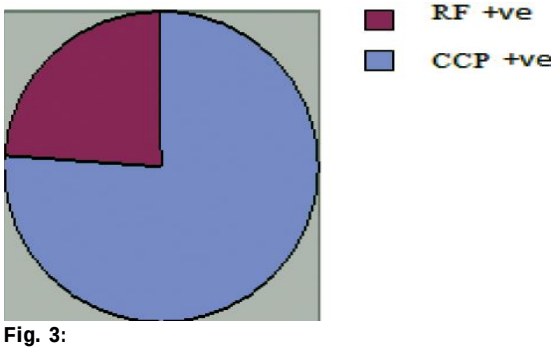

Fig. 3:

Discussion

Autoimmune diseases may be defined using Witebsky's postulates. These postulates require that 1) an autoimmune reaction is identified in the form of autoantibody or cell-mediated immune reaction, (2) the corresponding Ag is known, and (3) an analogous response causes a similar disease in experimental animals [2,3].

Recent data indicate that the immune response against citrullinated Ags is an attractive candidate for the fulfilment of the three Witebsky postulates.

There has been considerable interest in recent years in the observation that a very high proportion of patients with RA have IgG Abs to citrulline-containing proteins.

This study emphasised the value of anti-CCP antibodies as an early diagnostic marker of RA. The results are consistent with other relevant reviews [6]. This analysis highlighted the fact that the anti-CCP test had a higher sensitivity and specificity than the RF test for the diagnosis of RA demarcating its advantage over the immunoturbidimetric Rheumatoid Factor test.

Most of our clinicians are prescribing RF test only, for diagnosing Rheumatoid Arthritis (RA) and not anti-CCP whereas this study shows that the anti-CCP test appears to be better than rheumatoid factor tests in predicting which patients develop joint erosions and physical deformities. The detection of these auto-antibodies very early in the disease shall be useful to the clinician / rheumatologist in decisions on optimal treatment strategies.

Conclusion

To conclude, the anti-CCP antibodies precede the onset of RA and their presence is indicative of the

future development of RA, suggesting that the initial trigger for the development of RA may occur before the appearance of symptoms. Also, monitoring anti-CCP in people who may have an increased risk for the development of RA (shared epitope or other genetic factors) and in whom the antibody titres are increasing [7], makes possible the early therapeutic intervention.

Acknowledgement

The authors like to thank SRL Skylab, Guwahati, for support in publishing this article.

Emergence of Anti-citrullinated protein in the early diagnosis of Rheumatoid arthritis - A study of more than 1000 cases in a standalone laboratory.

References

1. Van Gaalen F, Ioan-Facsinay A, Huizinga TW, Toes RE. "The devil in the details: the emerging role of anticitrulline autoimmunity in rheumatoid arthritis". J. Immunol. 1 November 2005; 175 (9): 5575–80. http://www.jimmunol.org/content/175/9/5575.full
2. Rose, N. R., C. Bona. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). Immunol. Today 1993; 14:426.-430.
3. Witebsky, E., N. R. Rose, K. Terplan, J. R. Paine, R. W. Egan. Chronic thyroiditis and autoimmunization. J. Am. Med. Assoc. 1957; 164: 1439-1447.
4. van Boekel M A, Vossenaar E R, van den Hoogen F H, van Venrooij W J. Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. Arthritis Res 2002; 487–93.93 [PMC free article] [PubMed]
5. Schellekens G A, de Jong B A, van den Hoogen F H, van de Putte L B, van Venrooij W J. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis specific autoantibodies. J Clin Invest 1998; 101273–281.281 [PMC free article] [PubMed]
6. Riedemann J P, Munoz S, Kavanaugh A. The use of second generation anti-CCP antibody (anti-CCP2) testing in rheumatoid arthritis—a systematic review. Clin Exp Rheumatol 2005; 23S69–S76.S76 [PubMed]
7. Pruijn G J, Vossenaar E R, Drijfhout J W, van Venrooij W J, Zendman J W. Anti-CCP antibody detection facilitates early diagnosis and prognosis of rheumatoid arthritis. Curr Rheumatol Rev 2005; 11–7.7.