The clinical importance of anti-CCP in early diagnosis of Sudanese patients with rheumatoid arthritis

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Abstract

Background:

Rheumatoid Arthritis (RA) is a systemic inflammatory autoimmune disorder; it is the most common inflammatory arthritis in the world. The early diagnosis and effective treatment of rheumatoid arthritis (RA) can improve the clinical outcome of the disease. Recently, anti-Cyclic-Citrullinated protein antibodies (Anti-CCP) are found to be more specific to RA arthritis although in the early stages when the arthritis is undifferentiated. The objective of this study was to determine the clinical importance and the prevalence of anti-CCP in early diagnosis of Sudanese patients with RA.

Methodology

This work was a cross-sectional study conducted at Al Ribat teaching hospital (from June 2012 to ? 2014). The study sample included 56 Sudanese patients, known patients of RA who fulfilled the ACR criteria. Demographic and clinical data were collected by questionnaires. All patients and controls sera were investigated for anti-CPP by enzyme linked immunosorbent assay (ELISA).

Results

The mean age of RA patients in this study was 46.0 (±11.7) years. The control group consisted of 56 Sudanese apparently healthy subjects, 27 (48%) of them were female with mean age of 37 ±17.2years. The anti-CCP antibodies were detected in 60.7% of cases, and in two (3.6%) of the control group. The sensitivity of anti-CCP was 60.7% and specificity of 96.4%. Anti-CCP test has a maximum proportionate reduction in uncertainty (PRU) of 94% for a positive result and 59% for a negative result.

Conclusions

Anti-CCP antibody has a higher diagnostic specificity and positive predictive value than rheumatoid factor; however its sensitivity was low.

The consent of each patient was obtained prior to commencement of data collection.

Keywords: Anti-CCP antibodies, Rheumatoid arthritis.

Introduction:

Rheumatoid Arthritis (RA) is a systemic inflammatory autoimmune disorder characterized by widespread joint inflammation with synovial hyperplasia disease and the most common inflammatory arthritis affecting from 0.5 to 1% of the general population worldwide (1). It is more common in women with female to male ratio of 2 to 4.1 (2). The peak incidence between 30 and 50 years (3). The etiology of RA has been suggested to be combination of both genetic and environmental factors (4). Recently, studies have found a link between antibodies produced in response to Porphyromonas gingivalis (P. gingivalis) and development of RA. Associated with

periodontitis, P. gingivalis antibodies in RA patients have been linked to the production of anti-citrullinated protein antibodies (ACPAs) known to enhance the autoimmune response seen in RA **(5).**

A higher prevalence of RA has been reported in certain Native Americans, and a very low frequency of RA in some areas of rural Africa (6). Only a few number of studies reporting the prevalence of RA were identified in Africa. The majority of studies were concentrated in a few countries (South Africa, Nigeria and Uganda), while for vast areas of Africa no data was available (7). RA, like all forms of inflammatory arthritis, has no single specific pathognomonic sign, symptom or laboratory feature (8). The American College of Rheumatology (ACR) presented a core set of classification criteria for RA in 1987 (9), the it revised and new criteria was developed in collaboration with the European League Against Rheumatism (EULAR) in 2010 (10).

Before 1998, the only serological laboratory test that could contribute to the diagnosis of RA was rheumatoid factor (RF) (11), which present in the circulation of patients with RA for months to years prior to the onset of clinical disease Of either IgM or IgA isotype(12). The first citrullinated binding auto antibodies in RA sera were discovered in 1964, as autoantibodies which were able to bind to perinuclear granules in normal buccal mucosa cells and were named anti-perinuclear factor (APF) (13). The formation of antibodies to citrullinated peptides seem to be specific for RA patient. These cyclic citrullinated peptides (CCP) were subsequently used as antigens in the first generation of CCP test (14). A second generation assay was revised by screening a large number of citullinated-containing peptides with RA sera and this resulted in the identification of a number of highly reactive peptides that are currently used in the second generation CCP test (CCP 2). This CCP 2 test has slightly better performance in term of characteristic than anti-CCP1 antibodies Anti-CCP 2 antibodies is currently the most widely use anti-citrullinated peptide assay (15). High specificity of anti-CCP antibodies (95% versus 85% to RF) may be valuable in distinguishing RA from other disease which is clinically very similar to RA in its early stages (16). Anti-CCP antibodies could be detected in some patients 10 years before appearance of clinical symptoms (12). The presence of anti-CCP antibodies can predict the severity of either the clinical or radiological outcome in RA patients (17,18). To our knowledge, few studies conducted in Sudan addressed the importance anti-CPP among Sudanese

patients with RA(19,20). The objective of this study was to determine the Clinical importance and the prevalence of anti-CCP in early diagnosis of Sudanese patients with RA.

Materials and methods:

It is a cross-sectional descriptive hospital based study conducted at Al Ribat teaching hospital (from June 2012 to 2014). The study sample included fifty six Sudanese patients with known RA who fulfilled the American College of Rheumatology (ACR) criteria. The mean age of RA patients in this study was 46.0 (±11.7) years, range from 19 to 65 years old. Questionnaires were used to collect demographic and clinical data . The control group consisted of 56 Sudanese apparently healthy subjects.

After appropriate ethical approval and written consent form participants prior the commencement of the study . The blood was collected and then centrifuged, the serum was collected and stored in aliquots at -80°C immediately until assayed. All patients and controls sera were investigated for anti-CCP antibodies by enzyme linked immunosorbent assay (ELISA). Statistical analyses were performed using the biomedical Stats Direct Statistical Software v2.7.9 (7/9/2012). The conventional 5% level of significance was used for all statistical tests. The confidence interval (CI) shown for any estimate is the 95% CI.

Results:

A total of fifty six cases and fifty six controls were enrolled. Out of the 56 patients, 46 were females (82%), while only 10 were males (18%).Females and males mean age was46 ±11.7 years, 52 ±14.7 years respectively. Table 1 shows the age and sex distribution. The control group consisted of 56 healthy subjects, 29 were males (52%), 27 were females (48%).

Table 1. Age and sex distribution of the studied patients and controls

Group	No. of subjects	No. of Females (%)	Age in years	
			Female	Male
			Mean ± SD	Mean ± SD
Controls	56	27 (48%)	37 ±17.2	46 ±13.7
RA Cases	56	46 (82%)	46 ±11.7	52 ±14.7

The anti-CCP antibodies were detected in 60.7% of RA patients and the result was negative in 39.3%. in contrast, the antibodies were detected in only two subjects (3.6%) of healthy apparently control (Table.2).

Table 2. Characteristics of the two groups of subjects tested for anti-CCP

Group	Number of subjects	Anti CCP Test			
		Positive		**Negative**	
		n	**%**	**N**	**%**
Controls	56	2	3.6	54	96.4
RA Cases	56	34	60.7	22	39.3

Anti-CCP test has a maximum Proportionate Reduction in Uncertainty (PRU) of 94% for a positive result and 59% for a negative result. A positive ACCP result can clarify more than 60% of diagnostic uncertainty when the prior probability of RA ≥ 0.1. In fact, it can clarify more than 90% of diagnostic uncertainty when the prior probability of RA > 0.5. A negative result, however, can only clarify 50-59% of diagnostic uncertainty when the prior probability of RA is 0.3 or less.

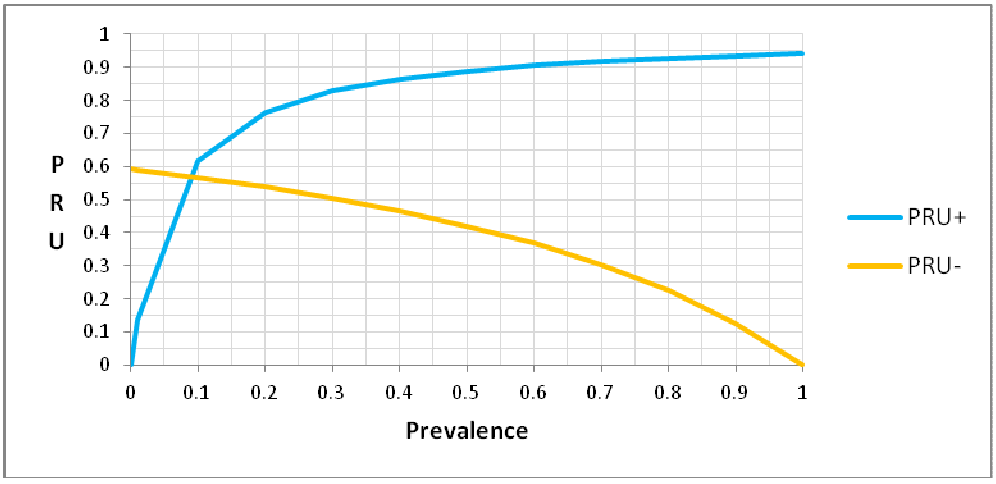

Figure 1. PRU for anti-CCP test

In Figure 2. The area under ROC curve of 0.6732 (0.5612 to 0.7851) is moderate. The optimum cut-off point selected is 20.45. The ROC curve shows a peculiar pattern in that it reverses the diagnosis at lower thresholds.

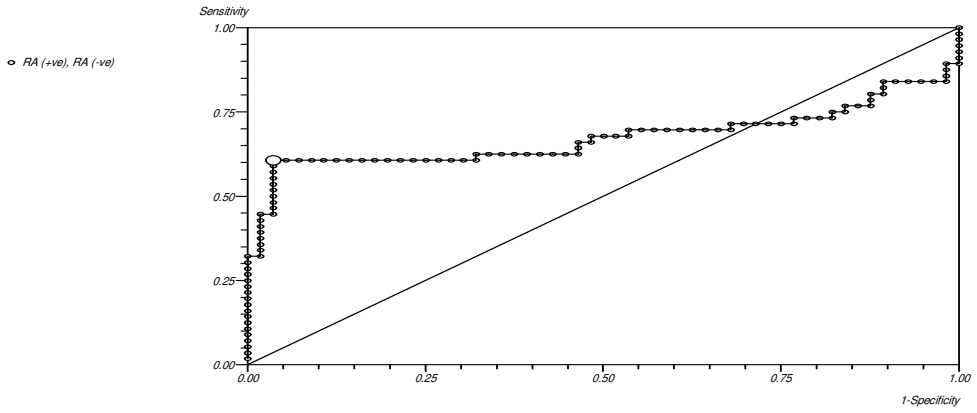

Figure 2. Receiver operating characteristic (ROC) curve for anti-CCP test

Discussion:

In the present study, 82% of patients with RA were females. The predominance of females in the current study was consistent with that detected by Teh and Wong, they observed 84.4% of their RA patients were females (21). Also it was supported by the findings of Lawrence *et al* in which female patients were four times more common than male (22). Since most of the cases in this study were female aged above forty five, the high percentage of unemployed (78.3%) was expected. Even though RA related disability can result in unemployment because of the pain, impaired physical function and transportation difficulties**.**

The mean age of RA patients in the current study (46.0) was slightly low when compared to other studies conducted in Turkey and United State (52.1 and 51.2 years) respectively (23,24).

Heliovaara *et al* suggested the smoking was one of the risk factors of RA (25). Their finding is inconsistent with the result of the current study, where 89.3% of our RA patients were non smokers.

In this study the specificity obtained for anti-CCP test was 96.4% and the sensitivity was 60.7%. In other study conducted in Sudan, Ahmed Bolad *et al* (19) found that anti-CCP specificity and

sensitivity was 86.7% and 74.3%, respectively. The high specificity in the current study is comparable to those obtained by other studies (26, 28) ranging from 96 to 100%. Our results also in accordance with another study conducted in Singapore showed that the specificity and sensitivity of anti-CCP antibodies were 92.1% and 62.3% respectively (29).

Anti-CCP assay is a very valuable tool for the diagnosis of RA, Moderate sensitivity of Anti-CCP test did not allow using as a screening test, but because of its high specificity, especially when high Antibodies titers are present, it may become one of the most useful serologic tests for the diagnosis of RA in Sudan.

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