ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 1, pp: (58-67), Month: January - March 2018, Available at: www.researchpublish.com

Predictive Value of Cyclic Citrullinated Peptide Reactive Antibodies in Diagnosis of Early-Undifferentiated Rheumatoid Arthritis in Families with History of Rheumatoid Arthritis

[1]Mohannad Gaiballa Mohammed Abdelgader, [2]Abu Elgasim Mohamed Ali Musa, [3]Samia Elhaj, [4#]Sayed Ibrahim Ali

[1]PhD student, immunology in tropical medicine, Sudan academy of Science
[2]MD in Internal medicine, Assistant professor, faculty of medicine, Bahri universit
[3]PhD immunology, Head department of immunology in tropical medicine Sudan academy of science
[4#]Assistant Professor of Biostatistics, College of Medicine, King Faisal University, Saudi Arabia

 # Corresponding Author: drsamas38@gmail.com

Abstract: **This prospective cross-sectional study was conducted during the period between March 2015 and October 2016.**

Objective: to investigate the reliability of cyclic citrullinated peptide reactive antibodies (Anti-CCP) as a predictive tool for detection of early-undifferentiated Rheumatoid Arthritis (RA) in first-degree relatives of patients with RA in Khartoum state, Sudan.

Methods: Four hundred serum samples were drawn from individuals in the study, aged between (17-40 years) without symptoms of RA were enrolled, Anti-CCP levels were estimated using Enzyme Linked Immune Sorbent Assay (ELISA) while Rheumatoid Factor (RF) tested in the serum from each individual using Beckman culture Au 480, and Statistical Package for Social Science (SPSS version 22) computer software was used for data analysis.

Results: of study indicates that Anti-CCP test has a predictive value in first-degree relatives of RA patients without presence of RA diagnostic criteria.

Conclusion: emphasized that Anti-CCP test has predictive value when used in investigating early presence of RA within the same family on specific populations who are considered as risk group (first degree relatives of patients) due to sharing genetic and environmental properties .The current study recommends to run Anti-CCP on first degree relative of patients with RA as an early diagnostic tool.

Keywords: Predictive Value, cyclic citrullinated peptide, Rheumatoid Arthritis (RA), Anti-CCP.

1. INTRODUCTION AND LITERATURE REVIEW

1.1 Definition of Rheumatoid Arthritis:

The name depend on the term "rheumatic fever", an illness which includes joint pain , take from the Greek word ῥεύμα-rheuma (nom.), ῥεύματος-rheumatos (gen.) ("flow, current"). The suffix -oid ("resembling") mean the translation as joint inflammation that resembles rheumatic fever. Recover description of rheumatoid arthritis was made in 1800 by Dr. Augustin Jacob Landré-Beauvais (1772–1840) of Paris (Landré-Beauvais *et al.*, 2001).Rheumatoid Arthritis (RA) is

one of the most common systemic autoimmune inflammatory illnesses, in about 0.5–1.0% of the world population. (Wener, 2002) Women are more times as likely to be affected as men (Sugiyama *et al.*, 2010).Typically it strikes people between the ages of 35 and 50, and effect any age (Lipsky, 2005). RA are describe as a chronic, symmetric, and damage arthritis of the terminal joints and affect must organs like the heart and the lungs (Von Mühlen *et al.*, 2007; Narain *et al.*, 2006).In lead to dysfunctional ability, there is a decreased life expectancy (Hill, 2008; Wolfe *et al.*, 2003). Mode of the disease different, because there may be choric or an acute active disease in some person that rapid operation to joint affected and dysfunction (Emery *et al*., 2008).The first discover of Rheumatoid Arthritis (RA) Known in remains of Indian skeletons among from 4500 BC and devolved as Tennessee. Persistence of the disease in that region could be documented in multi areas, as well as expansion into Ohio, first in a time about 1,000 years ago. As importance it start possible that RA then seem to the Old World before it was another time seen in the in 1785 (Till Uhlig, 2011). Rothschild has hypothesized how RA may have spread by distribution from the original appearance area of RA into areas with French, and other contrary incursion (Rothschild, *et al*., 1992).This hypothesis could understanding how RA, distributed geographically localized in an area protected from served , may have seem into the clumping New World and entered into the Old World in Europe (Till Uhlig, 2011).Rothschild and co-workers demonstrate that RA was discovered in personal of the New World before Columbus among 6,500 years. As in other modern times disease same with RA among to 1785, Rothschild has the hypothesis that RA lived in the Old World and spread to the New World (Rothschild *et al*., 1992).About more than 25,000 skeletons from Europe, Africa and the Middle East, document of RA supports the hypothesis of Rothschild that RA is a disease of the New World. RA has been clearly distinguished in pre-Columbian North America This has been surely by the sings multi joint pattern seen with damage arthritis which was the same and affected women with the same gender ratio as see in RA recent. And bone lesions consistent with RA had unknown in the Old World in 1785. In differentiation between osteoarthritis, gout and spondyloarthropathy had been well discovered in the Old World. The reasons of failure in RA to distribution in the Old World before the colonial time is saved by the examination of multi hundred skeletons (Till Uhlig, 2011).

1.2 Evidences of Rheumatoid Arthritis:

Before the document of Landré-Beauvais in 1800 there is no convincing appearance of RA in the past. The first document reference to arthritis among to 123 AD where an Indian note (Caraka Samhita) symptoms associated with swollen and painful joints initially appear in hands and feet, then devolving and causing loss of appetite, and gradate fever (Landré-Beauvais *et al.*, 2001).RA is not told in the Bible or in the works of Shakespeare, while may character resemble the clinical future of gout. In 1591 Guillaume de Baillou, the French physician and Dean of the University of Paris medical faculty wrote a book on arthritis, using the term 'rheumatism' to describe a condition characterized by inflammation, soreness, stiffness in the muscles and pain in and around the joints: "The whole body hurts, in part the face is flushed; pain is high severity around the joints, so that the small movement of the joint causes a cry of pain." A must number of character in the literature have before contained towards RA, including that of Thomas Sydenham (1624–1689), who give a mentally account. The illness of Constantine IX appear by Psellus in 1063 multipart of a chronic inflammatory illness. The diseases of Desideratum Erasmus (1466–1536) and Siebrandus Sixtus, a 17 th-century Dutch priest, had some same with RA. mentally, scientist in literature will have more formal training and other vision than physicians. Thus there is the challenge of validating reports on arthritis. In figurative art Flemish and Dutch paintings exhibit some document of RA, such as the practice of Rubens, who suffered from arthritis himself (Till Uhlig, 2011).When was 1859 that RA was taking its term. The nomenclature for rheumatic conditions had confused and deferens terms were used interchangeably such as rheumatic gout, chronic rheumatism, rheumalgia and scorbutic rheumatism. Mister Alfred Garrod, a London physician, is discover the increase in uric acid in the blood of people with gout, because there was different in acute forms of rheumatism. In 1859, he coined the Medical ward 'rheumatoid arthritis' and the earliest reference medical literature. Discussing the differential diagnosis he also have symptoms and sings , divided into acute, chronic and atypical form. However, Garrod'sknown of RA also included multi joint osteoarthritis (Garrod ,1859).Current Perspective In 1922, the term 'RA' finally received official consent, and it was other divided by the American Rheumatism Association in 1958 and 1987.RA is recent classified a heterogeneous disease, and ongoing practice not only main a revision of classification criteria, but also a known of early arthritis in connect between the American College of Rheumatology (ACR) and the European League to Rheumatism (EULAR) (Uhlig et al., 2008).

1.3 Etiology of Rheumatoid Arthritis:

Rheumatoid arthritis is a one of autoimmunity, the costive agent which still not completely discovered. It is a systemic (whole body) disorder principally affecting synovial tissues. There is no evidence that physical and emotional effects or stress could be a trigger for the disease. The multi findings may be that either the trigger varies, or maybe it might in fact be a chance event hortatory with the immune system (Edwards *et al.*, 1999).

1.3.1 Rationale and Objectives:

1.3.1.1 Rationale:

Auto anti bodies direct against cyclic citrullinated proteins (anti-CCP) propose as a potential marker of disease activity if the disease is early and undifferentiated. This study may help in early diagnosis of rheumatoid arthritis by measuring anti-CCP levels to detect individuals at-risk for RA in Khartoum state.

1.3.1.2 Objectives:

To investigate the reliability of cyclic citrullinated peptide reactive antibodies as a predictive tool for detection of early-undifferentiated RA in sero negative first-degree relatives of patients with RA.

1.3.1.3 Specific objective:

To assess the reliability of Anti-CCP and RF in early detection of RA, and to study the possibility of consequence of RA within the same family.

2. MATERIALS AND METHODS

2.1 Ethical consideration:

Ethical clearance for the current study was obtained from the Ethical Committee of Tropical Medicine Institute. Aims of the study were explained for all cases included in the study and an informed consent was obtained from each volunteer. The confidentiality of the individuals was established by coding of the questionnaires and the data list by a different code from their files to insure the anonymity of respondents. All investigations were carried out for individuals free of charge and they were not claimed for analysis expenses.

2.2 Study side:

This study was conducted in the Department of Immunology, Al-Riada modern medical Laboratory, Khartoum – Sudan

2.3 Study design:

This was prospective cross-sectional study conducted from 2015 and continued till the right number of participants and their controls were enrolled in Khartoum state.

2.4 Study population (targets and controls):

Sudanese individuals aged between (17-40) were enrolled. Targets were defined as healthy individuals who were first degree relative of patients with RA, while controls were defined as healthy individuals with no previous history of any type of arthritis.

2.4.1 Inclusion criteria:

- First degree relatives of RA patients without symptoms of RA were included.

-Mother or Father have Rheumatoid Factor Positive, if first-degree relative have Anti cyclic citrullinated proteins positive well do to him.

2.4.2 Exclusion criteria:

First-degree relatives of RA patients with symptoms of RA were excluded.

2.5 Sampling:

2.5.1 Sample collection and preparation:

Serum samples were obtained from 400 individuals (male and female) who were first degree relative of patients with RA.(199) ages matched healthy volunteers with no previous history of any type of arthritis were tested as controls.

Venous blood was collected in two container one EDITA container (container with anticoagulant) used to complete Blood count and Tri sodium citrate to Erythrocytes Sedimentation Rate and other plain container (container without anti-coagulant) and centrifuged in order to obtain serum. All serum samples were stored at -20˚C until use.

2.6 Anti CCP test:

Anti CCP was estimated quantitatively by indirect ELISA kits for Anti CCP from EUROIMMUN (Germany) Company, using Calibrator 1,2,3,4,5 and positive control in calculation of result following the kits instructions which include sample preparations, preparation of reagents and immunoassay procedure.

2.6.1 Sample preparations:

All individuals and controls samples were pre diluted 1:100 with sample buffer before assay. Therefore, 10 µl of sample diluted with 1.0 ml of sample buffer in plain test tube, and mixed well .controls and calibrators were ready to use.

2.6.2 Preparation of reagents:

The content of each vial of the wash buffer concentrate (10xs) were diluted with distilled to a final volume as required.

2.6.3 Immunoassay procedure:

1. A sufficient number of micro plate modules were prepared to accommodate calibrators and pre diluted patient samples.

2. 100 µl calibrators, controls and pre diluted patient samples were pipetted into the wells.

3. The content of micro wells were incubated for 60 minutes at room temperature (20- 28˚C).

4. The content of micro wells were discarded and washed 3 times with 300 µl of working wash solution.

5. 100 µl of enzyme conjugate were dispensed into each well.

6. The content of micro wells were incubated also for 30 minutes at room temperature (20- 28˚C).

7. The content of micro wells were discarded and washed 3 times with 300 µl of wash solution.

8. 100 µl of substrate were dispensed into each well.

9. The content of micro wells was incubated for 30 minutes at room temperature (20- 28˚C).

10. 100 µl of stop solution were dispensed into each well.

11. The optical density was read at primary wave length 450nm and reference wavelength 620 nm.

At the end the result were obtained by reading the O.D (optical density) of the colour using ELISA reader and compared with the O.D of known concentration standard (Calibrator).

The result were in form of ratio and the cut-off ratio is 5 RU/ml as fallow

> 7 RU/ml	positive
≤ 7 RU/ml	negative

2.7 Rheumatoid factor Test:-

2.7.1 Contents, Reagent Composition in the Test:

Final concentration of reactive ingredients:

Glycine buffer (pH 8.0) 170 mmol/L ,Latex coated with human IgG < 0.5% and Preservative 0.09%.

2.7.2 Principle of test:-

When a sample is mixed with R1 buffer and R2 IgG latex suspension, RF reacts specifically with IgG coated on the latex particles to yield insoluble aggregates. The absorbance of these aggregates is proportional to the RF concentration in the sample.

Reference Intervals:- Negative up to 15 IU/ml.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 1, pp: (58-67), Month: January - March 2018, Available at: www.researchpublish.com

2.8 Statistical analysis:

Variables were analyzed using chi-square tests. Statistical significance was accepted when P<0.05. The statistical analysis was undertaken using the SPSS (version 22) software package.

3. RESULTS

Four hundred samples were estimated for Anti cyclic citrullinated peptide antibodies (Anti-CCP) and Rheumatoid factor (RF) in healthy first degree relatives of patients with RA. In the study mean age of 30.34 years ± 5.77, Anti cyclic citrullinated proteins 3.26 ± 3.09 . Rheumatoid factor 68.72±63.4. (Table3.1).Only (28) individuals was positive for Anti-CCP and first degree relatives of patients with RA (18) from mother and (10) from father . the rest were negative for Anti-CCP. according to chi square test there was non significant difference between individuals with family history of RA and individuals without family history at Anti-CCP levels (P-value>0.05)equal ((0.537) (table 3.11).Rheumatoid Factor Correlated with Anti cyclic citrullinated proteins were significantly (P. value < 0.05) (Table 3.3).Also we found Anti cyclic citrullinated proteins significantly Correlated with Rheumatoid Factor (P. value < 0.05). (table3.5)There is no relationship between Rheumatoid Arthritis in family with sex (P. value > 0.05), (table 3.60) shows the distribution of individuals of this study by Family history (father and mother), which shows that only (28) positive were slightly below than negative that means insignificant guide line to diagnosis (table 11).Table 3.13 shows that Anti cyclic citrullinated proteins test considered as gold standard test to be compared with Rheumatoid Factor .

Specificity of Anti- CCP antibodies in Rheumatoid Factor tested determines is reliable in early detection of the disease. When negative predictive value (NPV) is calculated it was high percentage, thus the screening test is very good in confirming that all negative results were confidential.

Table 3.14 shows that Rheumatoid Factor test considered as gold standard test to be compared with Anti cyclic citrullinated proteins.

Sensitivity of Rheumatoid Factor all tested determines that the test is reliable in early detection of the disease. When negative predictive value (NPV) is calculated it was high percentage, thus the screening test is very good in confirming that all negative.

The frequencies of the studied Comparison between experimental group and control group for age, Rheumatoid Factor , Anti cyclic citrullinated proteins. were significantly greater than in the controls (P value =0.0001, respectively).(Table 3.12).

The most frequent diseases of the study participants were the infectious diseases (56%), which assumed that it may have affect on the presence of the disease (table 3.3).

Table 3.4 shows that RF test considered as gold standard test to be compared with Anti-CCP test. Specificity of Anti-CCP antibodies in first degree relatives tested determines that the test is reliable in early detection of the disease. When negative predictive value (NPV) is calculated it was 100%, thus the screening test is very good in confirming that all negative results were confidential.

Table 3.1: Descriptive statistics for study variable (quantities):-

Variable	Mean	SD
age	30.34	5.77
Anti CCP	3.26	3.09
RF	68.72	63.4

Table 3.2: Descriptive statistics for study variable (qualitative):-

Variable		Number	Percentage (%)
Sex	Male	194	48.5
	Female	206	51.5
RA in family	Mather	235	58.8
	Father	165	41.3
Anti CCP	Positive	28	7

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 1, pp: (58-67), Month: January - March 2018, Available at: www.researchpublish.com

	Negative	372	93
RF	Positive	328	82
	Negative	72	18

Table 3.3: Correlation Between Anti cyclic citrullinated proteins with Rheumatoid Factor

	R	P. value	Significant
RF	0.122	0.015	*

** Significant

Table 3.4: Correlation Between Rheumatoid Factor with Anti cyclic citrullinated proteins,

	R	P. value	Significant
Anti CCP	0.122	0.015	**

** Significant

Table 3.5: Relationship between Rheumatoid Arthritis in family with sex:-

Sex	Father	Mather	P. value	Significant
Male	83	111	0.544	
Female	82	124		

Table 3.6: Comparison of Anti cyclic citrullinated proteins and Rheumatoid Arthritis in family

Family history	Negative (Anti CCP)	Positive (Anti CCP)	Total
Father	155	10	165
Mather	217	18	235

P value > 0.05 not significant (0.537)

Table 3.7: Comparison between experimental group and control group for age, Rheumatoid Factor ,Anti-Nuclear Antibody Anti cyclic citrullinated proteins.

		Number	Mean	Std. Deviation	T. Test	P value
age	experimental group	400	30.34	5.771	7.38	0.0001
	control group	199	26.87	4.637		
Anti CCP	experimental group	400	3.261350	3.0872900	7.313	0.0001
	control group	199	1.644221	.6195096		
RF	experimental group	400	68.7157	63.39946	9.67	0.0001
	control group	199	34.7800	21.16868		

P value < 0.05 significant (0.001).

Table 3.8: Sensitivity and Specificity of Anti cyclic citrullinated proteins with Rheumatoid Factor with Anti-Nuclear Antibody , C. Reactive Protein, Human leukocyte antigen and Acetylcholine receptor antibody.

Anti CCP	Sensitivity (%)	Specificity (%)	Positive predicted value (%)	Negative predicted value (%)
95% CI	4- 30%	9 -95.6%	4.7 -33.6%	89.2-94.9%
RF	7.3%	94.4%	85.7%	18.3%
95% CI	4.8 -10.8%	85.7 -98.2%	66.4- 95.3%	14.6- 22.7%

NA= Not applicable

PPV= Positive predictive value, NPV=Negative predictive value

CI= Confidence of interval

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 1, pp: (58-67), Month: January - March 2018, Available at: www.researchpublish.com

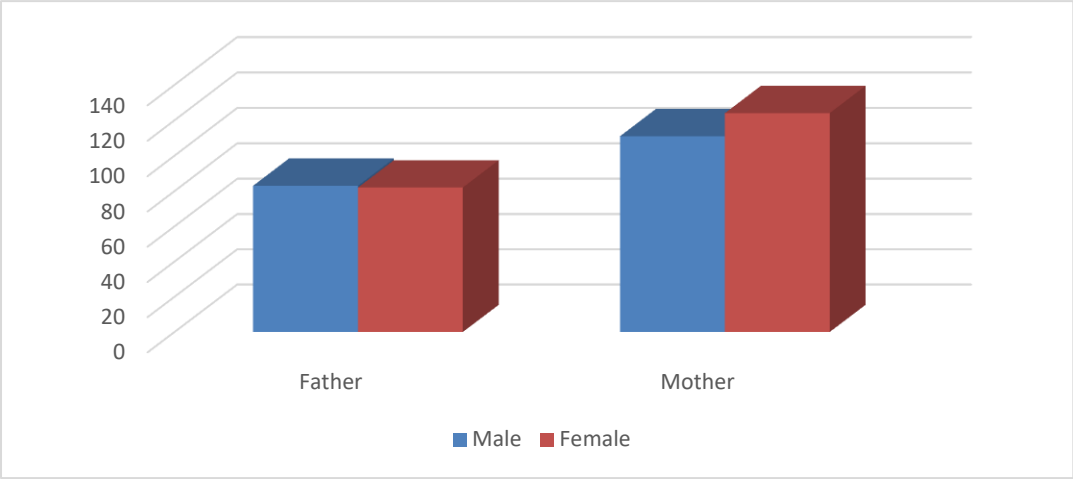
Table 3.9 Sensitivity and Specificity of Rheumatoid Factor with Anti cyclic citrullinated proteins , Anti-Nuclear Antibody , Acetylcholine receptor antibody and Human leukocyte antigen.

RF	Sensitivity (%)	Specificity (%)	Positive predicted value (%)	Negative predicted value(%)
Anti CCP	85.5%	18.3%	7.3%	94.4%
95% CI	66.4-95.3%	14.5 -22.7%	4.8 -10.8%	85.7- 98.2%

NA= Not applicable

PPV= Positive predictive value, NPV=Negative predictive value

CI= Confidence of interval



4. DISCUSSION

4.1. Discussion:

A prospective cross-sectional study was conducted through the period from march 2015 to October 2016 to investigate the reliability of cyclic citrullinated peptide reactive antibodies as a predictive tool for detection of early undifferentiated RA in sero-negative first degree relatives of patients with RA.

Relationship Between Anti cyclic citrullinated proteins with Rheumatoid Factor.

Connection Between Rheumatoid Factor and Anti cyclic citrullinated proteins.

Relationship between main parameter and symptoms and sings.

Six hundred individuals (male and female) serum and whole blood samples were drawn from each individual in the study, aged between (17-40years) without symptoms of RA were enrolled, Anti-CCP level, was estimated using enzyme linked immune sorbent assay (ELISA) while Rheumatoid factor, Uric tested in the serum in each individual by using Beckman Coulter and Statistical package for social science (SPSS version 22) computer software was used for data analysis**.**

The results of study indicate Anti-CCP test has weak predictive value in RF sero-negative and without presence of RA diagnostic criteria in first degree relatives of RA patient.There is no association between Anti-CCP and RF at prediction level. the result also showed no relation between history of the disease and the presence of the disease within the same family according to the results P-value>0.05 (not significant),

Among the Rheumatoid Factor Correlated with Anti cyclic citrullinated proteins were significantly (P. value < 0.05) . There is no association among Rheumatoid Arthritis in family with gender (P. value > 0.05). Women are the similar as possible to be affected as men.

Specificity of Anti- CCP antibodies and Rheumatoid Factor in all tested determines that the test is reliable in early detection of the disease. When negative predictive value (NPV) is calculated it was high percentage , thus the screening test is very good in confirming that all negative results were confidential.

From the results of this study it is concluded that the Anti-CCP test has predictive value more than RF test as early diagnostic tool.

According to our result there is no association between history of the disease and the presence of the disease within the same family according to the outcome P-value was not significant, although it had been established that there is a relationship between RA and its presence within the same family (Isabela *et al.,* 2010),.Although an relationship between the development of citrullinated antigens and Rheumatoid Factor (RF) has been established in early discovery of RA in primary degree relations of individual with RA (Nielen, *et al.*, 2004), but in difference the present study, shows no relationship between anti-CCP positivity and RF in the relatives evaluated .during concordance with our outcome previous study revealed that Anti-CCP antibodies have a predictive facility which confirmed by Nielen and others (Nielen, *et al.*, 2004) who studied 79 patients with RA who donated blood prior to indication beginning. They experiential that 40.5% of patients became anti-CCP-positive previous to indication beginning, and the median time starting primary anti-CCP positivity to warning sign arrival was 4.8 years, with a range from 0.1 to 13.8 years. These investigators analyzed the analytical characteristics of anti-CCP antibodies for the 5 years prior to symptom onset, and found that the 5 year positive predictive value (PPV) for anti-CCP in the RA blood donor patient population was 96.6%. They also calculated the risk of developing RA within 5 years for the universal population as well as for those at "high risk," which they defined as having 2 or more first-degree relatives with RA. They discovered that the 5 year PPV for anti-CCP in the "high risk" population was 69.4%, and in the general population it was 5.3%.Its suggested to verify the elevated predictive value of ACCP in premature diagnosis of Rheumatoid arthritis on primary degree relation of patients with rheumatoid arthritis, by monitoring these persons for adequate period of time. Rheumatoid Arthritis (RA) Is one of the most ordinary systemic autoimmune inflammatory diseases, affecting about 0.5–1.0% of the world population (Wener , 2002), Women are three times as possible to be artificial as men (Sugiyama *etal.,* 2010).Typically it strikes individuals between the ages of 35 and 50, although it may occur at any age (Lipsky, 2005). RA can be characterized as a chronic, symmetric, and erosive arthritis of the tangential joints that can in addition affect several organs such as the heart and the lungs (Von Mühlen *et al.*, 2007; Narain *et al.*, 2006).In adding together to a decline in functional capacity, there is a summary life expectation (Hill, 2008; Wolfe *et al.,* 2003). development of the disease varies, for the reason that there may be spontaneous remissions or an more and more active disease in some persons that quickly progresses to joint irregularity and disability (Emery *et al*., 2008).A negative RF does not rule out RA; rather, the arthritis is called *sero-negative*. This is the casing in about 15% of patients (Nishimura *et al*., 2007). through the primary year of illness, rheumatoid factor is more likely to be negative with some individuals converting to sero-positive status greater than time. RF is in addition seen in other illnesses, for example Sjögren's syndrome, Hepatitis C, chronic infections and in approximately 10% of the healthy population, therefore the test is not very specific .RF are antibodies directed to the constant region of immunoglobulin's of the IgG class and are establish in 70-80 % of individuals with RA. IgM RF, the iso-type the largest part characteristically detected, is seen not simply in RA but also in a range of other situation like other autoimmune diseases, infections and in up to 5-10% of strong individuals (Swedler *et al.,* 1997). The united detection of IgM and IgA RFs in a serum is a strong indicator of RA (Bas *et al.,* 2003) . However, IgA RFs are not widely available.

A cohort of 83 RA patients had blood samples available in a blood bank predating their diagnosis. Anti-CCP antibodies were positive prior to diagnosis in 33.7% of the RA patients vs. 1.8% in controls taken from the same pool of subjects ($p < 0.0001$) (Rantapaa-Dahlqvist *et al.,* 2003). Median time between blood sampling and the development of disease was 2.5 years, with a maximum interval of 9 years. Rheumatoid factor was positive in 19.3% of donors who would eventually be diagnosed with RA, compared to 6% of control donors, which was not significant in logistic regression models. A second similar study identified 79 RA patients who had donated blood to a regional blood bank prior to their diagnosis (Nielen *et al.,* 2004).Until recently, the only serological test routinely performed for the detection of RA was for the presence of igm RF. RF is found in approximately 50%–90% of these patients, but it is also found in patients with infections, other autoimmune diseases, and some healthy individuals with increasing frequency in older age groups, thus limiting its specificity for RA. In RA, IgM antibodies combine with IgG , and these immune complexes become deposited in the joints, resulting in a type III (or immune complex) hypersensitivity reaction. The complement protein C1 binds to the immune complexes, activating the classical complement cascade. During this process, C3a and C5a are generated, which act as chemotactic factors for Neutrophiles and macrophages. The continual presence of macrophages leads to the chronic inflammation usually observed which damages the synovium it self (Narain *et al*., 2006; Lee *et al*., 2006).This family of antibodies is detected using cyclic citrullinated peptides (CCP); hence the antibodies are called anti-CCP. Anti-

CCP is now the lead marker for detection of RA, because it is much more specific than RF (Wild *et al*., 2008).The present potential cross sectional study is the primary study in Sudan that using Anti-CCP as analytical tool among first degree relation of patients with rheumatoid arthritis which revealed weak prognostic value of Anti-CCP as predictive tool.

4.2. Conclusion:

- Anti-CCP test has weak prognostic value in RF sero-negative and without presence of RA analytical criteria in primary degree relations of RA patient.
- There is no association between Anti-CCP and RF at prediction level.
- Associated anti cyclic citrullinated proteins with Rheumatoid Factor .
- Anti cyclic citrullinated proteins significantly correlated with Rheumatoid Factor .
- There is no connection between Rheumatoid Arthritis in family with sex ,
- Specificity of Anti- CCP antibodies in Rheumatoid Factor determines that the test is reliable in early detection of the disease. When negative predictive value (NPV) is calculated it was high percentage, thus the screening test is very good in confirming that negative results were classified.

4.3. Recommendations:

1. This study require to verify the high prognostic value of ACCP in early judgment of Rheumatoid arthritis on primary degree relative of patients with rheumatoid arthritis, by monitoring these individuals for adequate phase of time .
2. To verify the option of outcome of RA within the same family need to create studies on genetic level.
3. In this study the majority of our patients were Sudanese for whole ethnic population in Sudan which consist of multiracial population.
4. The difference of ethnic group should be measured to represent a Sudanese population .
5. Because high specificity and positive predictive value Anti CCP antibody should be included in the future classification as adjunct to Rf a laboratory test for diagnosis of Rf.
6. To use Anti CCP as supportive diagnostic test to RF for RA especially in ambiguous cases or patients whom clinical feature resemble.

REFERENCES

[1] Bas S, Genevay S, Meyer O, Gabay C (2003). "Anti-cyclic citrullinated peptide antibodies, IgM and IgA rheumatoid factors in the diagnosis and prognosis of rheumatoid

[2] Edwards JC, Cambridge G, Abrahams VM (1999). "Do self-perpetuating B lymphocytes drive human autoimmune disease?". *Immunology* 97 (2): 188–96. doi:10.1046/j.1365-2567.00772.x. PMC 2326840. PMID 10447731.

[3] Egerer K, Bastian H, Koehler A, et al. (2008). "Marker profile in early rheumatoid arthritis reveals correlation of SE-selectin with disease activity, high sensitivity of rheumatoid factor and high specificity of ACPA". *Annals of the Rheumatic Diseases*. vol. 67, supplement 2, p. 299.

[4] Garrod AB (1859). "Treatise on nature and treatment of gout and rheumatic gout", london: *walton and maberly*.

[5] Hill, J. (2008) "The what, whys and wherefores of rheumatoid arthritis".*Nurs Res Care*. 10:123–126.

[6] Isabela Goeldner, Thelma L. Skare, Iara T. de Messias Reason, Renato M. Nisihara, Marı´lia B. Silva, Shirley R. da Rosa Utiyama (2010). " Anti-cyclic citrullinated peptide antibodies and rheumatoid factor in rheumatoid arthritis patients and relatives from Brazil ". Rheumatology. 49:1590–1593.

[7] Landré-Beauvais AJ (2001). "The first description of rheumatoid arthritis. Unabridged text of the doctoral dissertation presented in 1800", *Joint Bone Spine*. 68:130–43.

[8] Leventis P, Patel S (2008). "Clinical aspects of vitamin D in the management of rheumatoid arthritis". *Rheumatology (Oxford)*47 (11): 1617- 21. doi:10.1093/rheumatology/ken296.PMID 18682414.

[9] Lipsky, PE (2005). "Rheumatoid arthritis. In Kasper, DL, Braunwald, E, Fauci, AE, Hauser, SL, et al. (eds*)*:Harrison's Principles of Internal Medicine, ed. 16. McGraw-Hill, New York, pp. 1968–1976.

[10] Narain, S, Kosboth, M, and Hahn, P (2006)."Autoantibody testing in rheumatoid arthritis". In Detrick, B, Hamilton, RG, and Folds, JD, et al. (eds*)* Manual of Molecular and Clinical Laboratory Immunology, ed. 7. ASM Press, Washington, DC, pp. 1033–1045.

[11] Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, Habibuw MR, Vandenbroucke JP, Dijkmans BA (2004). "Specific auto antibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors". *Arthritis Rheum.* 50:380–6.

[12] Nishimura K, Sugiyama D, Kogata Y, Tsuji G, Nakazawa T, Kawano S, Saigo K, Morinobu A, Koshiba M, Kuntz KM, Kamae I, Kumagai S (2007)."Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis". *Ann. Intern. Med.* 146 (11): 797–808.PMID 17548411.

[13] Rantapää-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, Sundin U, van Venrooij WJ (2003). "Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis". *Arthritis Rheum*. 48:2741-9.

[14] Rothschild BM, Woods RJ, Rothschild C, Sebes JI (1992). "Geographic distribution of rheumatoid arthritis in ancient North America: implications for pathogenesis ".*Semin Arthritis Rheum*.22:181 7.

[15] Sugiyama D, Nishimura K, Tamaki K, Tsuji G, Nakazawa T, Morinobu A, Kumagai S (2010). "Impact of smoking as a risk factor for developing rheumatoid arthritis: a meta-analysis of observational studies". *Ann Rheum Dis.* 69 (1): 70–81.doi:10.1136/ard.2008.096487. PMID 19174392.

[16] Swedler W, Wallman J, Froelich CJ, Teodorescu M (1997). "Routine measurement of IgM, IgG, and IgA rheumatoid factors: High sensitivity, specificity, and predictive value for rheumatoid arthritis". *J Rheumatol*. 24:1037-44

[17] Till Uhlig (2011)." The History of Rheumatoid Arthritis", : *EuropeanMusculoskeletal Review*. 6(3):145-7.

[18] Uhlig T, Heiberg T, Mowinckel P, Kvien TK (2008). "Rheumatoid arthritis is a milder disease in

[19] Von Mühlen, CA, and Nakamura, RM (2007)."Clinical and laboratory evaluation of systemic rheumatic diseases. In McPherson, RA, and Pincus, MR (eds*)": Henry's Clinical Diagnosis andManagement by Laboratory Methods*, ed. 21. Saunders Elsevier, Philadelphia, pp. 916–932.

[20] Wener MH (2002)."Rheumatoid factors. In: NR Rose et al, eds". Manual of Clinical Laboratory Immunology. Washington, DC: American Society for Microbiology Press; 961-972.

[21] Wild N, Karl J, Grunert VP, Schmitt RI, Garczarek U, Krause F, Hasler F, van Riel PL, Bayer PM, Thun M, Mattey DL, Sharif M, Zolg W (2008). "Diagnosis of rheumatoid arthritis: Multivariate analysis of biomarkers". *Biomarkers.* 13:88–105.

[22] Wolfe, F, Freundlich, B, and Straus, WL. (2003) "Increase in cardiovascular and cerebrovascular disease prevalence in rheumatoid arthritis". *J Rheumatol*. 30:36–40.