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PREVALENCE OF ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES IN PATIENTS CLASSIFIED AS RHEUMATOID AND UNDIFFERENTIATED ARTHRITIS AT KENYATTA NATIONAL HOSPITAL.
A. O. Ayunga, MBChB, MMed, Garissa Provincial General Hospital, G. O. Oyoo, MBChB, MMed, (Int. Med) FACR, FRCP (Edin) Clin. Rheum. (LSU, USA), Senior Lecturer, E. O. Amayo, MBChB, MMed, Professor, Department of Clinical Medicine and Therapeutics and A. A. Angela, MBChB, MMed, Lecturer, Department of Clinical Pathology, School of Health Sciences, University of Nairobi, P. O. Box 19626-00202, Nairobi, Kenya

PREVALENCE OF ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES IN PATIENTS CLASSIFIED AS RHEUMATOID AND UNDIFFERENTIATED ARTHRITIS AT KENYATTA NATIONAL HOSPITAL

A. O. AYUNGA, G. O. OYOO, E. O. AMAYO and A. A. ANGELA

ABSTRACT

***Background*: Rheumatoid arthritis(RA) is a debilitating condition.Early diagnosis of RA can be difficult as the disease may initially be indistinguishable from Undifferentiated arthritis(UA). American College of Rheumatology criteria(ACR)is not suitable for early diagnosis as its characteristics are fulfilled when bone damage has already taken place.Anti-cyclic citrullinated antibodies(Anti-CCP) are highly specific for RA and have been used to confirm early diagnosis.**

***Objective*:To determine the prevalence and clinical utility of Anti-CCP antibodies in patients with rheumatoid and undifferentiated arthritis at presentation to KNH medical clinics.**

***Design*: A cross-sectional descriptive study.**

***Setting*: Kenyatta National Hospital Medical Outpatient Clinics (MOPCs) between the month of October 2008 to February 2009.**

***Results* :A total of 95 patients were recruited.The mean age of the patients studied in the RA and UA was 44.7 and 41.2(p=0.356) respectively.Sixty four patients(64) satisfied ACR criteria.The overall prevalence of Anti-ccp antibodies in the population studied was 47.4%.The prevalence of Anti-ccp antibodies in patients who satisfied the ACR criteria was 62.5% .The prevalence of Rheumatoid Factor (RF) in patients who satisfied the ACR criteria was 50% compared to 9.7% for those who did not(p=0.000).The male to female ratio of subjects studied was 1:11**

***Conclusion*:Anti-ccp antibodies are more prevalent in this cohort of patients with rheumatoid and undifferentiated arthritis than RF. It was also concluded that ACR characteristics correlated well with Anti-ccp and RF. A greater percentage of patients who were RF negative were Anti-ccp positive.**

INTRODUCTION

The emphasis in the management of Rheumatoid Arthritis is early diagnosis and intervention. The hypothesis on which this approach is based is that a window of opportunity exists where therapy has a disproportionate impact on outcome (1). It would appear logical to introduce therapy prior to irreversible damage (2).

An inherent problem with earlier assessment is accurate disease classification. Pathognomonic features of RA such as deformity and nodules are related to chronicity and are absent at presentation. The American College of Rheumatology (ACR) classification criteria for RA (3) include such parameters, making them insensitive when applied early (4). Also routine laboratory investigations such as acute phase markers and X-rays may be normal in up to 60 and 70% of patients respectively (5). Current best practice for early diagnosis of RA is reliant on the history and examination findings, with supplementary additional investigations.

Rheumatoid Factor (RF) has been widely used as a screening test for patients with arthritis. Although RF is prognostically useful,as it correlates with functional (6) and radiographic (7) outcomes in both RA and early inflammatory arthritis (8),as a diagnostic test it performs poorly, with low sensitivity and moderate specificity (9). Used in isolation, RF has little diagnostic utility, but has retained its place in practice because of its prognostic ability and the lack of an alternative test.

More recently a highly specific autoantibody system has been described for RA, in which patients develop antibodies to modified(citrullinated) arginine residues, and this has resulted in the development of the Anti-cyclic citrullinated peptide(Anti-ccp) antibody test, which has a sensitivity of 68% and a specificity of 97%(10). Further development yielded novel peptides and a second generation test. The Anti-ccp2 test has improved sensitivity (80%comparable to Immunoglobulin M-RF(IgM-RF)(70-75%) and equivalent specificity(>98%)(11).Moreover,35 to 40% of RF-negative patients are Anti-ccp antibody positive. Anti-ccp antibodies have also demonstrated prognostic utility with regard to radiographic outcomes (12).

An ideal strategy for RA diagnosis would involve sensitive and specific laboratory markers that detect RA early in its course, differentiate RA from other rheumatic diseases and provide prognostic information regarding an individual's disease progression. Treatment could therefore be started early and might help to diminish progression to severe erosive arthritis.

Of patients with RA,65-85% have evidence of circulating RF, IgM or Immunoglobulin G(IgG) antibodies directed against the Fc portion of IgG(3). Immunoglobulin A(IgA) and Immunoglobulin E(IgE) can sometimes be detected.

Unfortunately, 3 to 5% of general population also have low levels of circulating RF and this level increases to 10 to 20% by age 65(13).The majority of these patients however will not develop RA. As RF can be detected in numerous other rheumatologic and inflammatory disorders, it is somewhat limited in its usefulness as a sole laboratory test for the diagnosis of RA. While RF has proven useful in providing some prognostic information regarding disease severity, the need for an additional and prognostic marker for RA is evident (14).

Anti-ccp antibodies are often found in patients with erosive or polyarticular symptoms.Anti-ccp antibody formation precedes the development of clinical symptoms in some patients with RA and multiple studies have now demonstrated that the presence of anti-ccp antibodies can provide valuable prognostic information regarding the aggressiveness of disease progression (15).

A combined analysis of publications concerning more than 2000 patients with early undifferentiated arthritis found a prevalence of 23% of Anti-ccp antibodies at baseline. Prevalence increased to 51% in more than 1000 patients who fulfilled the ACR criteria on follow up for 18 months. Anti-ccp positive patients had more severe radiological destruction during the disease course (16).

MATERIALS AND METHODS

Study population: The study was conducted on patients attending the medical outpatient clinics at Kenyatta National Hospital.

Patient recruitment: Patients 18 years and above were consecutively recruited. They were referred to medical clinics with arthritis and gave signed informed consent. We excluded patients with acute febrile illnesses, those who had autoimmune disease and those with gouty and septic arthritis. All patients who were classified as inflammatory arthritis using elevated Erythrocyte Sedimentation Rate(ESR) had their ages, sexes and other socio-demographic characteristics recorded by the principal investigator and assistants on an already prepared questionnaire.

Physical examination was carried out and the musculosketal system was screened using the Gait, Arms, Legs and Spine (GALS) screening system.ACR criteria was then applied to classify patients into RA and UA. The number of joints swollen and deformed were recorded. Patients were also examined to find out whether they had rheumatoid nodules, deformity of the upper and lower limb joints.

Case definition: Arthritis-patient with inflammation of the joint, swelling, pain, stiffness and tenderness. Rheumatoid arthritis-patient with signs and symptoms that satisfy the ACR criteria for RA. At least four elements of the criteria to be fulfilled.

Undifferentiated arthritis: patient with non specific signs and symptoms of arthritis not satisfying the classification criteria for RA.
Gout – patient known to have had characteristic crystals in the joint fluid and or more than one attack of acute arthritis.
Septic arthritis - patient known to have an infection (bacterial) in the joint cavity.

Laboratory methods: Consenting patients had about five millilitres of venous blood drawn from the forearm using a non heparinised needle and syringe and this blood was allowed to stand at room temperature to get serum for Anti-ccp and RF antibodies. Two millilitres of blood was collected in an Ethylene Diamine Tetra Acetate(EDTA) bottle for ESR estimation which was done immediately. RF was also done immediately. Serum for Anti-ccp test was frozen and done as a batch at the end of the study period.RF was measured by use of agglutination method.Anti-ccp antibodies were measured by using Axysm Machine that uses automated ELISA for the detection of IgG and IgA anti-CCP3 and results were interpreted thus:Negative<5.0IU, Positive>5.1IU.ESR was measured using the Winthrop method.Elevated ESR was interpreted as follows:>15mm/hr and >20mm/h

for men and women under 50yr of age respectively and >20mm/hr and >30mm/h for men and women older than 50yrs respectively.

Data management and analysis: All data were collected on the study proforma and entered into a computer database. The data were then cleaned and verified. Statistical analysis was done using statistical package for social scientists (SPSS) version 15.0 software. Data were summarised into means, ranges, ratios and then presented in form of pie charts and tables. A significant association was deemed present at a P-value of <0.05

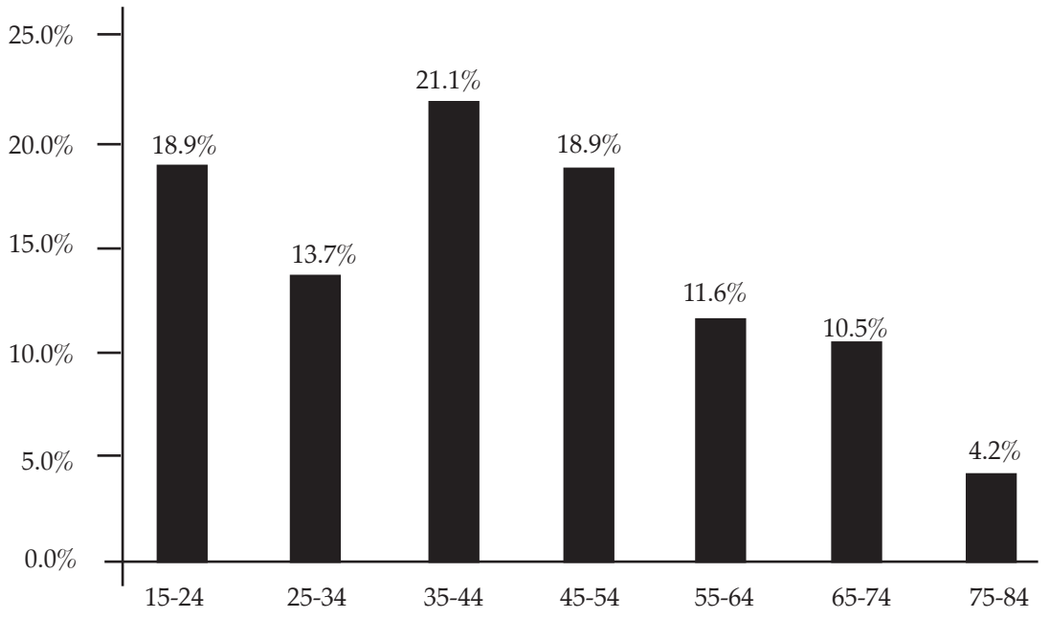
Ethical considerations: The study was carried out after approval from the department of medicine (University of Nairobi) and Kenyatta National Hospital ethics committee. Patients were included in the study after being explained to the purpose of the study and giving a signed informed consent. Participation in the study was voluntary and patients were at liberty to withdraw from the study without any prejudice. The patients right to privacy was respected. Study results were given to the primary care physicians to allow for provision of better healthcare and confidentiality was maintained.

RESULTS

One hundred and thirty one patients with arthritis were screened at the Medical Outpatient Clinics. One declined informed consent. History and Physical Examination and the screening tool GALS was used to screen patients.ESR was carried out on these patients and was found elevated in 95 patients and these were presumed to have either rheumatoid or undifferentiated arthritis. The ACR characteristics were applied and 64 patients satisfied the ACR criteria and 31 had Undifferentiated Arthritis (UA) as they did not satisfy the ACR criteria. Both RF and Anti-ccp were carried out on these patients. Fourty patients were positive for Anti-ccp in the RA group as compared to five patients in the UA group.Thirty patients were RF positive in the RA group as compared to three patients in the UA group.

Socio-demographic characteristics of the patients: The study population was 95 patients selected from a group of patients referred with arthritis to MOPC. The patients age ranged from 18 to 82 years and had a mean age of 43.5 years. The peak age of the patients studied was between 35 to 44 years (Figure 1).

Figure 1
Age distribution for the study group



The sex distribution of the study population was 91.6% female and 8.4% male giving a M:F ratio of 1:10 in the study population.

Rheumatoid arthritis (RA) and Undifferentiated Arthritis (UA): Using the ACR criteria, rheumatoid arthritis was identified in 67.4% of the patients.There was no difference between male and female groups of patients in terms of prevalence of RA and UA (P=0.759). In addition, mean age was not statistically significant between the RA (44.7 years) and UA patients (41.2 years), P=0.356.The prevalence of Anti-ccp (47.4%)

was higher than that of RF (36.8%), P=0.05.62.5% of RA patients were Anti-ccp positive compared to 16.1% of the UA patients (P=0.000).The titres of Anti-ccp were markedly higher for patients who satisfied the ACR criteria than those who did not. However there were few patients who had higher titres of Anti-ccp who were in the UA.Half of RA patients were RF positive. In UA patients,9.7% were RF positive(p=0.000).

In the total study population, the total number of patients who were RF was 60. Twelve (20%) of these patients were Anti-ccp positive 32 of these patients who were RF negative satisfied the ACR criteria for RA. Of the 32 who satisfied the ACR criteria for RA, ten (31%) were Anti-ccp positive. Hence a greater percentage of patients would miss treatment for RA if RF will solely be relied on.

Five patients who were classified as UA using ACR criteria had their sera test positive for Anti-ccp.Three of those patients were RF positive.Three of them had negative family history for RA.The mean duration of illness was 126 weeks which was higher than the mean (61.1weeks) duration for the total study population.The mean Anti-ccp titre was 127.64iu compared to the mean for the whole study population that was 59.68iu.The mean joint count was 10.4,similar to the mean found for patients who were classified as RA.The mean age of this group of patients was 45.4 years and hence comparable with the mean for the study population.

Family history of RA was present in 25.3% of all patients. Family history of RA was reported among 25 and 25.8% of the RA and UA patients respectively hence was not significantly associated to presence or absence of RA (P=0.932).

The range of duration of illness was 2-260 weeks with a mean duration of illness of 62.8 weeks ± 54.8SD. The mean duration of illness was higher by about ten weeks among the RA patients than the UA patients. However, the difference between the two means was not significant (P=0.4).

The range of joints was 2-20 joints with a mean number of joints involved among the 95 patients studied being 9.6±3.2SD. The number of joints was significantly different between the RA and the UA patients (P=0.011). The average number of joints involved among the RA patients was higher (10.2 joints) than the UA patients (8.4 joints).

DISCUSSION

From the results, it can be seen that the majority of the study population were female (91.6%)(M:F=1:10). This female preponderance is keeping with other studies done previously (17).The mean age of patients classified as RA as per the ACR criteria was 44.7 years as compared with that of UA which was 41.2 years but this was not statistically significant between the two groups. This figure is closely similar to one found by Oyoo (17) who found this age to be 44.5years. From our study we can deduce that inflammatory arthritis afflicts the relatively young and productive members of our society hence the need to control this condition early enough so as to prevent morbidity and mortality.

Sixty four patients (67.4%) satisfied the ACR criteria and were thus classified as RA.These results are similar with those found at baseline in an early arthritis clinic by Harrison, B. J., *et al* (18). Among 323 patients studied,67% satisfied the ACR criteria for RA.Higher number of patients may have satisfied the ACR criteria due to recall bias of the study subjects. This is because questions regarding the ACR criteria may be misinterpreted by the patient and hence explain the high number of patients who satisfied the ACR criteria.Furthermore the sensitivity and speficity of ACR is quite low for the diagnosis of early RA.

The overall prevalence of Anti-ccp was 47.4% compared to 36.8% for RF. Thus the population with inflammatory arthritis who were Anti-ccp positive was significantly higher than that of RF (p=0.05) and hence the confirmation from this study that Anti-ccp is usually picked early in the disease process as compared to RF which is usually picked late in the disease process.Vittecoq and coworkers (38) in a similar study found the prevalence of Anti-ccp to be 41% and that of RF to be 28% at presentation to the clinician and these results are comparable to those of our study.They concluded that Anti-ccp detected more positive subjects compared to RF.

The greater majority of the patients classified as RA as per the ACR criteria had their blood test positive for Anti-ccp(62.5%) as compared to UA(16.1%) which was statistically significant (p=0.000).Forty(88.9%) of the patients who tested positive for Anti-ccp were classified as RA Lee D.M *et al* (19) found almost a similar figure of 83% in these subset of patients.Hence Anti-ccp is significantly correlated with the ACR criteria in classifying patients into RA and UA.

RF was positive in 50% of the patients classified as RA as compared to 9.7% classified as UA(p=0.000). The prevalence of RF in this cohort of patients classified as RA is lower than earlier studies (21) that had a prevalence of 70 to 80%. This prevalence might have been lower given the fact that most of the patients recruited into our study had a shorter duration of disease (62.8 weeks) than earlier studies (64.97 months).This may also have been due to the fact that the researchers in these studies may not have excluded patients who had co-mobidities that can cause RF to be positive. Such patients were excluded from our study. Furthermore,RF tends to become positive as the disease progresses.

The joints involved in the two groups were statistically significant (10.2 in RA as compared to 8.4 in UA p=0.011). Since the majority of the patients who had RA as per the ACR criteria had positive Anti-ccp,

we can deduce that joint count positively correlated with Anti-ccp which as we know is associated with more erosive and severe forms of RA. Anti-ccp has been shown to be an independent predictor of radiological damage and progression (20).

In the total study population,60 patients were RF negative,20% of whom had positive Anti-ccp .This produces similar results obtained in other studies that found a prevalence of 20-43%(22). Lee D. M. *et al* (19) found 34% of patients who were RF negative testing positive for Anti-ccp.These values suggest important diagnostic utility where previously serology had been unhelpful. Hence from this prevalence of Anti-ccp in seronegative individuals, using Anti-ccp antibody would appear to select seronegative RA patients and so may have important implications for patient management.Of these patients who were RF negative, 32 satisfied the ACR criteria for RA. This again shows that RF was highly seronegative in patients who satisfied the ACR criteria.This in clinical practice may cause diagnostic and management difficulties. Of the 32 who satisfied the ACR criteria and were RF negative, ten were Anti-ccp positive.This also strongly suggests that a greater number of patients with early arthritis will test positive for Anti-ccp earlier than they do for RF.

Five patients classified as UA tested positive for Anti-ccp which was 5.26% of the total population studied.These group of patients had higher titres for Anti-ccp than the average for the whole study population hence a conclusion can be drawn that despite being negative for ACR,these patients can still be presumed to have RA.Furthermore,three out of five of these patients had their sera test positive for RF.Their mean joint count and the age never differed significantly from the total population.

Their mean Anti-ccp titres (127.64iu) were away above the average for the whole study group (59.8iu). This information is important as it tells us that Anti-ccp can be used to diagnose patients who have RA despite the fact that they have not satisfied the ACR criteria for RA.

These five patients diagnosis of RA may have been missed because of their non-specific signs and symptoms.These are the patients who benefit most from Anti-ccp when the diagnosis of RA is in doubt and hence its clinical utility.

It can therefore be concluded that there is a high prevalence of Anti-ccp in patients with inflammatory arthritis and that the prevalence was higher in those who satisfied ACR criteria.The prevalence of RF in this same group of patients was lower than that of Anti-ccp.The prevalence of RF was also lower than that of Anti-ccp in those who satisfied the ACR criteria.

A high percentage of patients with inflammatory arthritis satisfied the ACR criteria at 67.4%. Anti-ccp was found to be more sensitive than RF. Many patients (20%) who were RF negative were Anti-ccp positive. Anti-ccp should be done in a patient presenting with inflammatory arthritis where the diagnosis of RA may be in doubt.

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