ANTI-CYCLIC CITRULLINATED PEPTIDE (ANTI-CCP) AUTOANTIBODY AS A USEFUL DIAGNOSTIC TEST FOR THE DIAGNOSIS OF RHEUMATOID ARTHRITIS

by

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ABBREVIATIONS

Anti-CCP antibodies	Anti-cyclic citrullinated peptide antibodies
DIP	Distal interphalengeal joints
DMARDs	Disease modifying anti-rheumatic drugs
ELISA	Enzyme linked immunosorbent assay
HUSM	Hospital Universiti Sains Malaysia
IgA RF	Immunoglobulin A rheumatoid arthritis
IgG RF	Immunoglobulin G rheumatoid factor
IgM RF	Immunoglobulin M rheumatoid factor
KPP	Klinik Pakar Perubatan
MCP	Metacarpophalengeal joints
MTP	Metatarsophalengeal joints
PIP	Proximal interphalengeal joints
RA	Rheumatoid arthritis
RF	Rheumatoid factor

ABSTRAK

Pengenalan : Faktor reumatoid adalah salah satu dari kriteria yang digunakan oleh 'American College of Rheumatology' (ACR) untuk diagnosa penyakit radang sendi (rheumatoid arthritis). Walau bagaimanapun faktor reumatoid mempunyai spesifisiti yang rendah untuk diagnosa penyakit radang sendi kerana ianya juga boleh didapati di kalangan individu yang mempunyai penyakit autoimun yang lain dan penyakit infeksi. Ianya juga boleh didapati di kalangan individu yang sihat. Antibodi kepada cyclic citrullinated peptide telah ditemui dan dikatakan lebih spesifik di dalam pendiagnosaan penyakit radang sendi.

Objektif : Untuk menentukan sensitiviti dan spesifisiti antibodi kepada cyclic citrullinated peptide di kalangan pesakit radang sendi yang mengunjungi Hospital Universiti Sains Malaysia menggunakan kriteria 'American College of Rheumatology' sebagai piawai dan untuk membezakan sensitiviti dan spesifisiti di antara antibodi kepada cyclic citrullinated peptide dan faktor reumatoid.

Metodologi : Ini adalah kajian rentas yang dijalankan dari bulan Januari sehingga Disember 2008 ke atas 261 pesakit yang terdiri dari 96 orang pesakit radang sendi (kes) dan 165 orang pasakit yang mengalami sakit sendi (kontrol). Serum untuk ujian antibodi kepada cyclic citrullinated peptide dan faktor reumatoid dari setiap subjek dilakukan dengan menggunakan teknik 'enzyme linked immunosorbent assay' (ELISA). Walau bagaimanapun ujian antibody ini tidak dapat dilakukan ke atas 12 sampel darah

daripada kumpulan control kerana masalah sel darah yang tidak normal. Sensitiviti dan spesifisiti setiap ujian ditentukan dengan menggunakan diagnosa klinikal sebagai piawai.

Keputusan : Ujian antibodi kepada cyclic citrullinated peptide menunjukkan sensitiviti 69.8% dan spesifisiti 94.8% sementara faktor reumatoid menunjukkan sensitiviti 84.4% dan spesifisiti 74.5%. Nilai prediktif positif untuk antibodi kepada cyclic citrullinated peptide adalah 89.3% berbanding faktor reumatoid 67.5%. Ujian sensitiviti dan spesifisiti untuk antibodi kepada cyclic citrullinated peptide dan faktor rheumatoid menunjukkan perbezaan yang signifikan dengan p-value < 0.001.

Kesimpulan : Antibodi kepada cyclic citrullinated peptide mempunyai diagnosis spesifisiti dan nilai positif prediktif yang lebih tinggi daripada faktor reumatoid tetapi nilai sensitiviti yang kurang berbanding faktor reumatoid.

ABSTRACT

Introduction: Rheumatoid factors are currently used in the diagnosis of rheumatoid arthritis and constitute one of the classification criteria proposed by the American College of Rheumatology. However, rheumatoid factor positivity shows low diagnostic specificity because it is also present in patients with other autoimmune and infectious disease and even in a proportion of normal healthy individuals. Recently, another test of interest in the diagnosis of rheumatoid arthritis is the assay for anti-cyclic citrullinated peptide (anti-CCP) antibodies.

Objectives : To determine the sensitivity and specificity of anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis patients attending HUSM using American College of rheumatology (ACR) criteria as a gold standard and to compare the sensitivity and specificity of anti-CCP and rheumatoid factor.

Methodology: This was a cross sectional study which conducted from January 2008 to December 2008. The study consisted of 261 patients, 96 patients with rheumatoid arthritis (cases) and 165 patients with arthritis or arthralgia but not fulfilled ACR criteria for rheumatoid arthritis (controls). Serum from each subject was tested for anti-CCP antibodies and IgG RF by enzyme linked immunosorbent assay (ELISA). However, antibodies testing unable to do to 12 blood sample from the control group due to blood lysis. Sensitivity and specificity of the test were evaluated using the clinical diagnosis as the gold standard.

Results: The sensitivity of anti-CCP was 69.8% with 94.8% of specificity. For rheumatoid factor the sensitivity was 84.5% and specificity was 74.5%. The positive predictive value for anti-CCP was 89.3% whereas for rheumatoid factor was 67.5%. The sensitivity and specificity of anti-CCP antibodies and rheumatoid factor was significantly different with p-value of < 0.001.

Conclusions: Anti-CCP antibody has a higher diagnostic specificity and positive predictive value than rheumatoid factor, however sensitivity was lower than rheumatoid factor.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Rheumatoid Arthritis (RA) is a systemic inflammatory autoimmune disease and the most common inflammatory arthritis, affecting from 0.5 to 1% of the general population worldwide (Gabriel, S.E., 1999). It is more common in women with female to male ratio of 2 to 4:1 (Gabriel, S.E., 1999). In Malaysia, it affects about five in 1000 people (Arthritis Foundation Malaysia, 2009). The incidence increased with age, peaking between the fourth and sixth decades (Lee D.M. & Weinblatt, M.E., 2001).

Despite many years of intensive research, the exact aetiology of RA is still unknown. However, general risk factors have identified, such as infectious agents, smoking and oral contraceptives (Merete, P. *et al.*, 2006). Genetic factors are believed to be responsible for approximately 60% of the risk factor for the developing RA (Mac Gregor A.J *et al.*, 2000). In addition, gender is also a risk factor for the development of RA (Cutolo M *et al.*, 2002).

RA is an autoimmune disorder of unknown aetiology characterized by symmetric, erosive synovitis and in some cases with extra-articular involvement. Primary target organ of the disease is the synovium of diarthrosis. The disease begins in the small joints of the hands and feet and progresses in the centripetal and symmetrical fashion. Early indications of RA are swelling and pain of the proximal interphalengeal and

metacarpophalengeal joints. Later the larger joints become affected, especially those of the knee, elbow and ankle. Since RA is a systemic autoimmune disease, other part of the body may become affected at the later stage (Rindfleish, J.A. & Muller, D. 2005).

1.2 Clinical Manifestations of Rheumatoid arthritis

Rheumatoid arthritis is a chronic inflammatory characterized by progressive damage of synovial-lined joints and variable extra-articular manifestations. Generally, signs and symptoms of RA begin insidiously within weeks to months. However about 15% of patients, onset occurs more rapidly over days or weeks. Its clinical manifestations are divided into articular and non articular involvement (Haris, E.D. 2005).

1.2.1 Articular Manifestations

RA usually presents as a polyarticular (an arthritis affecting more than 3 joints) with the predilection for the wrists, the metacarpophalengeal joints and the proximal interphalengeal joints (Tehlirian C.V., *et al.*, 2008). Being symmetrical it tends to affect the same joints on the hands and because of inflammation process, the joints are red, swollen, painful and tender. One of the major components of the inflammation seen in RA is morning stiffness that lasts at least an hour. Although joints that commonly affected by RA are small proximal joints of the upper limbs, other joints of the upper and lower limbs might be affected also. However for unknown reason the distal interphalengeal joints and sacroiliac joints tend not to be affected (Grassi W, *et al.*, 1998).

The damage done to the joints and periarticular supporting structures can lead to characteristic permanent deformities. The damage is caused by cellular and humoral inflammatory process that erodes cartilage, bones and digests the joint capsule and also the periarticular soft tissues.

1.2.2 Non articular manifestations

Extra-articular manifestations tend to be more frequent in patients with severe disease (Gordon D. A. *et al.,* 1973). Study of a community based sample reported that among RA patients, 15% that extra-articular manifestations of RA occur in about 40% of all patients and 15% developed severe extra-articular RA at certain time (Turesson C. *et al.*, 2002).

Rheumatoid nodules are the most common cutaneous manifestation of the disease. It occurs in up to 50% of patients with RA and more usually seen in patients with positive rheumatoid factor (Anderson R. 2001). It has been shown that rheumatoid nodules predict severe extra-articular disease (Voskuyl A.E *et al.* 1996). Based on this, it would be expected that some extra-articular manifestations tend to occur together. Rheumatoid nodules can grow anywhere in the body, but are commonly found along the pressure points, such as extensor surfaces of the joints. Typical site for rheumatoid nodules include the dorsum of the arm near the elbow, along the archilles tendon and along the dorsal tendons of the hands and feet. It also can be found in other tissues throughout the body including the eyes, lungs, heart and central nervous system (Haris E.D., 2005).

Pulmonary involvement may include development of perenchymal pulmonary nodules as well as interstitial lung disease. Pleural effusions are common finding in RA and may present in up to 70% of patients at some point during the course of their disease. Parenchymal pulmonary disease, pleural effusion and pericardial effusion are more common in patients with a positive rheumatoid factor or other extra articular disease (Haris E.D., 2005).

Ophthalmic complications of RA include episcleritis and scleritis. Vasculitis is a rare complication of RA. Involvement of vessels that provide the blood supply to peripheral valves can lead to mononeuritis multiplex, in which patients may present with functional deficit in individual nerves.

Anaemia of chronic disease is the most typical haematologic abnormality in RA. It correlates with ESR and activity of the disease (Rosenstein E.D. & Kramer N., 1991).

1.3 Diagnosis of Rheumatoid arthritis

RA is difficult to diagnose in its early stages especially in primary care for several reason. There is no single and specific test for the disease. The symptoms differ from person to person and can be more severe in some patients than in others. It might be similar to other types of arthritis and joints conditions and it may take some times for other conditions to be ruled out. Finally the full range of symptoms develops over times, and only a few symptoms may be present in the early stages (Janet M.K &Michael H.W., 2000).

For decades, the diagnosis of RA has been primarily based on clinical manifestations supported by radiological features and laboratory investigation that primarily only based on presence of RF.

Early stage RA characteristically shows no overt clinical evidence of joint damage and no signs of cartilage or bone loss on plain radiographs. At this stage, it is difficult to determine if the disease course will be mild or more severe. The diagnosis of RA at this stage can be challenging, onset may be acute or may take place over several months (Weinblatt M.E & Kuritzky L., 2007). The most common presentation of RA is insidious pain, stiffness and swelling of the small peripheral joint (MCP, MTP and PIP) and wrist, in some cases the large joints can be affected first. The symmetrical involvement of the hands and wrists is the most characteristic and early finding of RA. Symmetrical swelling and tenderness are usually noted first at the MCP and PIP joints. The DIP joints are usually not affected. If the DIP joints affected, it must be distinguished from coincident appearance of osteoarthritis which can also occur (Jacob J. *et al.,* 1986). Although some patients have painless swelling, symmetrical involvement of the wrists usually is painful and limits function. Chronic inflammation may produce synovial expansion and proliferation that can cause compression of the nerves. This may lead to erosion or rupture of tendons in the wrists and finger that can cause deformity and loss of function (Gordon G.A & Hastings D.E., 2003). The accepted symptoms for diagnosis of RA include the presence at least three swollen or more arthritic joints, but it is not occur in the early course of the disease (Arnett F.C *et al.*, 1988). Therefore, the diagnosis of RA is difficult at the early stages.

Involvement of the knees is common and is often a primary occurrence in the early course of RA. This is usually characterized by swelling and synovial effusion and thickening. The muscle around the knee can atrophy and result in weakness that can be detected in the early stages of the disease. Chronic and persistent synovitis can limit movement due to cartilage destruction, ligament laxity, joint instability and contractures (Gordon G.A & Hastings D.E., 2003).

Inflammation of the small joints of the feet is another common manifestation, with ankle joint involvement less common. As the disease progress it causes deformity and difficulty in walking. The hip is affected later than most other joints, but involvement of the hips is rare (Gordon G.A & Hastings D.E., 2003).

Rheumatoid arthritis is an erosive arthritis that destroys bone. The use of radiograph may help to identify erosive changes or eliminate competing diagnosis of RA. Characteristic radiograph finding of RA include periarticular osteopenia, loss of joint space and marginal joint erosion. These changes are often not seen in early disease. There was only 15 to 30% of people with RA will have changes on x-rays in the first year of disease (Dixey J., 2004). However, after the first year of rheumatoid arthritis, more than 90% of the people have changes on x-rays. X-rays may also help to measure bone mineral density, which was often decreased in the later stages of the disease. Therefore x-rays are useful for monitoring the status of RA rather than for the diagnosis (O'dell J.R., 2004)

Magnetic resonance imaging (MRI) scanning is more sensitive than plain radiograph for detecting the bone damage caused by RA. MRI is not only more sensitive in detecting erosions but in addition it is capable of identifying bone marrow oedema and synovial hyperthrophy which are the early changes and strong predictor of bone destruction, hence useful for early diagnosis and prognostic decision for RA (Hoving J.L. *et al.*, 2004). However, the cost for MRI is greater than plain x-rays, so MRI is not widely used to diagnose or monitoring the course of the disease.

Ultrasonography is used infrequently in establishing a diagnosis of RA. It is more sensitive in the detection of synovial and tendon inflammation than clinical examination alone (Kane D. *et al.*, 2003). Ultrasonography may also be useful in guided joint aspiration and injection.

For laboratory investigation, rheumatoid factor (RF) was regarded as a key element of the serological diagnosis of RA. Although it is not very specific to RA, RF testing was performed routinely because it was an easy method with a reasonable sensitivity (60 to 85%). (Shmerling R.H *et al.*, 1991)

In 1988, the classification of RA was developed which relies mainly on the criteria described by the American College of Rheumatology (ACR) (Arnett, F.C., *et al.*, 1988). However, especially during the first few months of the disease, the 1987 revised criteria of the American College of Rheumatology (ACR) are rarely met. About one-third of the patients with persistent arthritis do not fulfil the classification criteria, so it is often difficult to diagnose RA (Vallbracth I *et al.*, 2005).

ACR 1987 revised criteria for the classification of RA

No	Criteria	Definition
1.	Morning stiffness	Morning stiffness in and around joints, lasting at least 1 hour before maximal improvement
2.	Arthritis of three or more joints areas	At least three joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible areas are right or left PIP, MCP, wrist, knee, ankle and MTP loints
3.	Arthritis of hand joints	At least one area swollen (as define in 2) in a wrist, or in an MCP or PIP joint
4.	Symmetrical arthritis	Simultaneous involvement of the same joints areas (as defined in 2) on both sides of the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry)
5.	Rheumatoid nodules	Subcutaneous nodules over bony prominences, or extensor surfaces or in juxta-articular regions observed by a physician
6.	Serum rheumatoid factor	Demonstration of abnormal amount of serum rheumatoid factor by any method
7.	Radiographic changes	Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which include erosions or

unequivocal bony decalcification localized in, or most marked adjacent to the involved joints (osteoarthritis alone do not qualify)

Rheumatoid arthritis is defined by the presence of four or more criteria and criteria 1 till 4 must be present for at least 6 weeks (Arnett FC *et al.*, 1988)

1.4 Why need a good diagnostic test for RA?

The majority of RA patients have progressive disease that result from joint damage, a decline in physical function over time, depression, disability and other co-morbidities. If left untreated, 20 to 30% of patients with RA become permanently work disable within three years of diagnosis (Sokka T., 2003). After 20 years with RA, the physical functions of more than 60% of affected patients were significantly impaired (Lee, D.M. *et al.*, 2001). According to the World Health Organization (WHO), more than 50% of patients with RA completely stop working within 10 years of disease onset (World Health Organization, 2009). Therefore disability from RA causes major economic loss and can have a profound impact on families. Studies showed risk of mortality ratio of 2.26 among people with RA compared to general population. They are two times more likely to die compared to people in the same age in the general population (Wolfe F. *et al.*, 1994).

However, study showed irreversible joint destruction can be prevented by intervention during the first months of disease. Therefore early diagnosis of rheumatoid arthritis is

important in managing the disease (ACR Subcommittee on Rheumatoid Arthritis, 2002).

1.5 Management of Rheumatoid arthritis

Early treatment of RA decreases the rate of disease progression (Emery P *et al.*, 2002). Therefore, it is important to diagnose the disease and initiating treatment as soon as possible as these can decreases the rate of disease progression. The American College of Rheumatology Subcommittee on Rheumatoid Arthritis (ACRSRA) recommends that patient with suspected rheumatoid arthritis is referred within three months of presentation for confirmatory of diagnosis and initiation of treatment (ACR Subcommittee on Rheumatoid Arthritis Guidelines, 2002).

Primary care physician play an important role in the successful management of RA through early recognition of symptoms, provisional diagnosis, referral to a rheumatologist, continue monitoring of patient during the course of the disease and its treatment (Combe B *et al.*, 2007).

Pharmacotherapy for RA generally involves non steroidal anti-inflammatory drugs (NSAIDs) for control of pain with selective use of low dose oral or intra-articular glucocorticoid and initiation of disease modifying anti rheumatic drugs (DMARDs). DMARDs are the mainstay of therapy in RA (ACR Subcommittee on Rheumatoid Arthritis Guidelines, 2002). The goal of pharmacotherapy includes preservation of function and quality of life, minimization of pain and inflammation, joint protection and control of systemic complications without causing permanent and unacceptable side

effects (ACR Subcommittee on Rheumatoid Arthritis, 2002). However the risk of side effects from treatment must be weighed against the benefit.

The DMARDs that are commonly used include methotrexate, hydroxychloroquine, sulphasalazine, leflunamide, etanercept and infliximab (ACR Subcommittee on Rheumatoid Arthritis, 2002). Those are less frequently used azathioprin, D-penicillamine, gold salt, minocycline and cyclosporine. DMARDs generally have slow onset of action and the benefit usually are seen after 4 to 6 months (ACR Subcommittee on Rheumatoid Arthritis, 2002).

In the past decades, the pharmacologic treatment of RA was managed using a pyramid approach. Symptoms alleviating treatment was started at the diagnosis and only with progression of symptoms, dosage were changed or additional medication was added. However, a 'reverse pyramid' approach now is in favour, in which DMARDs are initiated earlier to slow down the disease progression (Boers M, 2001). This change of approach is a result of several research findings; such as (1) joint damage begins early in the disease (Emery P, 2002), (2) DMARDs have significant benefits when used early, (3) the benefits of DMARDs may be enhanced when the drugs are used in combination (Pincus, T. *et al.,* 1999), (4) a number of new DMARDs are available with a good evidence of benefit effects (Oslen NJ, Stein CM, 2004).

Other newer DMARDs include tumour necrosis factor (TNF) antagonists, anakinra and rituximab. It has been recommended as a second line therapy after trying the traditional DMARDs (Combe B *et al.,* 2007). This is due to the cost, serious side effects and also

difficulty in administration of medication for example, TNF need to be given by intravenous infusion or subcutaneous injection.

1.6 Rheumatoid factor

RF is an autoantibody which binds to other antibodies. It was first developed by Dr. Eric Waaler and Dr. H.M. Rose in 1940. Therefore it is occasionally referred to as Rose-Waaler or Waaler-Rose test (Waaler E, 2007). It is an antibody that react with the crystalizable fragment (Fc fragment) of immunoglobulin G (IgG). RF and IgG join to form an immune complex which contributes to the disease process. In RA, this immune complexes deposited in the synovium of the joint or other tissues. RF has three subclasses which include IgM, IgA and IgG antibodies. Each subtype is associated with a different symptoms or disease process and simultaneous presence of all three subtypes is usually only seen in RA (Del Puente A *et al.*, 1988).

RF is detected in 70 to 80% of RA patients (Shmerling R.H. *et al.*, 1991). In seropositive RA, the incidence of RA increases with the duration of disease. At three months the incidence is 33%, while at one year it is 75%. Up to 20% of RA patients remain negative for RF (seronegative RA) throughout the course of their disease (Shmerling R.H. et al., 1991). The concentration of RF tends to be highest when the disease peaks and tends to decrease during prolonged remission (6). However these antibodies are not very specific to RA. They can be detected in relatively high percentage in other autoimmune diseases in which early presentation is similar to RA such as, systemic lupus erythematosus (30%), systemic sclerosis to (20 to 30%),

Sjogren syndrome (75 to 95%) and up to 5% in healthy individual (Shmerling R.H *et al.*, 1991).

There was few method of determination of RF. Firstly, agglutination test detecting IgMRF. It is the earliest test and the most common methods used in the laboratory diagnosis of RA (Renaudieau Y. *et al.*, 2005). There were two methods in detecting RF by agglutination test. One method was mixed the patient's blood with tiny beads covered with human antibodies (IgG). The latex beads clumps or agglutinate if RF (IgM RF) is present. Another method mixed the patient's blood with sheep red blood cells clump if rheumatoid factor is present (US Food and Drug Administration, 1997). This method relatively easy to perform but it is difficult to quantitative without experience and is prone to observer variability. This is also has a large variation in titres for the same sera between different laboratories. In addition it is not possible to quantify RF isotypes (Bampton J.L., 1985).

The second method is nephelometry. In this method latex particle are coated with human IgG that captures RF. Complex IgG and RF are detected by light scattering. The degree the light scatter is dependent upon the concentration of immune complexes formed, making this a quantitative test (US Food and Drug Administration, 1997).

The third method of RF detection is by enzyme linked immunosorbent assay (ELISA). These assays represent a quick and cost effective method if processing large numbers of samples. It is also the most sensitive assay compared to other methods. Unlike agglutination test, ELISA can individually quantify RF isotypes.

1.7 Anti-CCP antibodies

Before 1998, the only serological laboratory test that could contribute to the diagnosis of RA was rheumatoid factor. However, major breakthrough came with the important series of investigations that described the test which was remarkable diagnostic specificity for RA with sensitivity comparable with RF (Nijenhuis S, *et al.*, 2004).

1.7.1 What is an anti-cyclic citrullinated peptide (anti-CCP) antibody?

Anti-citrullinated protein or peptide antibodies are auto antibodies that frequently detected in RA patients. Cyclic citrullinated peptide is also known as CCP. It is a cyclic peptide incorporating the amino acid citrulline. Citrulline is a 'non standard' amino acid. Amino acids are the building blocks of proteins in the body. About 20 standard amino acids used by the body to make proteins. Citrulline is made by the modification of the standard amino acid arginine by the action of peptidylarginine deiminase (PAD), and several proteins in the body are known to contain citrulline. Many patients with RA develop an immune response against protein containing citrulline (Vossenar E.R. & van Venrooij, W.J. 2004)

The first citrullinated binding autoantibodies 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). (Nienhuis R.L.F *et al.*, 1964). Few years after that another group of RA specific antibodies which called anti-keratin antibodies (AKA) was found (Young B.J *et al.*, 1979).

Subsequent studies demonstrated that AKA and APF recognized a similar epitope and were perhaps the same antibodies (Sebbage M *et al.*, 1995). It was also discovered that conversion of arginine to citrulline on peptide was essential for anti-keratin antibody and perinuclear factor binding (Schellekens G.A *et al.*, 1998). Therefore, anti-nuclear factor and anti-keratin antibodies was broadly categorized as anti-citrullinated peptide antibodies. The formation of antibodies to citrullinated peptides seem to be specific for RA patient. Assay for the detection of anti-citrullinated peptide antibodies using linear stretches of citrullinated peptides proved difficult to standardize and relatively low sensitivity (Schellekens G.A *et al.*, 1998). To optimize the exposure of the citrulline residue and or to introduce structural constrains that might improve their antigenicity, cyclic variants of the peptide were developed, resulting in increased in sensitivity without loss of specificity. These cyclic citrullinated peptides (CCP) were subsequently used as antigens in the first generation of CCP test (Schellekens *et al.*, 2000). 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-CCP 1 antibodies (Van Gaalen F.A *et al.*, 2005). Anti -CCP 2 antibodies is currently the most widely use anti-citrullinated peptide assay.

1.7.2 Advantages

Measurement of anti-CCP antibodies for this purpose has few advantages:

1. Anti-CCP as a diagnostic potential

Over the last few years many independent studies have confirmed the diagnostic performance of anti-CCP antibodies in diagnosing RA. A meta-analysis compared the sensitivities and specificities from 37 studies on anti-CCP antibodies and 50 studies of RF (Nishimura K. *et al.*, 2007). The result showed that anti-CCP antibodies had comparable sensitivity to RF (67% versus 69%) but with higher specificity (95% versus 85%). High specificity of anti-CCP antibodies may be valuable in distinguishing RA from other disease which is clinically very similar to RA in its early stages and in which RF is negative. From the reported studies it helps clinician to be more effective in the diagnosis of RA and will lead to proper management of the patients.

2. Anti-CCP antibodies as a predictive marker for disease

Study found that anti-CCP antibodies could be detected in some patients 10 years before appearance of clinical symptoms (Rantapaa-Dahlquist S. *et al.*, 2003).

3. Anti-CCP antibodies are also predictive for the outcome of early arthritis patients.

A recent study showed that 90% of the patients with unclassified arthritis were positive anti-CCP at baseline were classified as established RA patients within 12 months of follow up (Vittecoq O. *et al.*, 2004). It can be concluding that anti-CCP antibodies are present early in disease, and their presence enables accurate prediction of the development of RA.

4. Prognostic ability of anti-CCP antibodies

Few recent studies showed that anti-CCP antibodies can predict the severity of either the clinical or radiological outcome in RA patients (Kastbom A *et al.*, 2004 & Forslind K *et al.*, 2004). It prognostic potential may aid rheumatologist in reaching decisions on the most optimal treatment strategies.

1.7.3 Disadvantages

Anti-CCP antibodies test is not available in the government hospital in Malaysia; however it is available in certain private hospital and private laboratory. Furthermore the cost of the test is higher when compared to RF test. Therefore it make the test is not widely used in this country.

1.7.4 Anti-CCP antibodies versus rheumatoid factor

Anti-CCP antibodies have been studied in various populations and it showed that it has become a key serologic marker in RA. Because it presence early in the course of the disease, it is useful as a test for early diagnosis of RA. Anti-CCP antibodies also has higher specificity but comparable sensitivity to RF, therefore it can be used to distinguishing RA from other rheumatic diseases. Anti-CCP also used for prediction of prognosis of RA (Chou C.T *et al.*, 2007).

Many studies showed that anti-CCP antibodies test enables clinicians to effectively distinguished RA from other RA resembling diseases in its early stages and in cases where the RF is not discriminative. Mediwake, R. *et al.*, (2001) showed that anti-CCP antibodies are used to distinguish RA from SLE patients who present with erosive

polyarthritis which is often accompanied by RF seropositivity. Another example, a significant proportion of patients with chronic hepatitis C (HCV) infection suffers from a symmetric inflammatory polyarthritis that closely resembles the symptoms of RA. Since the majority of patients are RF positive, RF cannot be used to discriminate HCV associated arthritis from RA (Nijenhuis S *et al.*, 2004). In a study by Bombardieri *et al.*, (2004), showed that anti-CCP antibodies were detected in 77% of RA patients but none in patients with chronic hepatitis C, however 15% of patients with chronic hepatitis C was RF positive.

The presence of anti-CCP antibodies also has been associated with a less favourable prognosis. Presence of anti-CCP antibodies in RF negative patients was associated with more severe joint damage than in patients with positive RF and negative for anti-CCP antibodies (Meyer O *et al.*, 2003). Although anti-CCP antibodies are associated with severity of RA and erosion but study has shown that there was no positive correlation between anti-CCP antibodies and extra-articular manifestations. However presence of RF was associated with extra-articular manifestations (De Rycke L *et al.*, 2004).

Although studies showed that anti-CCP antibodies is a better diagnostic test for the diagnosis of RA but there is not available in government hospital in Malaysia and due to the cost of the test, it is not widely used in this country.

At present the gold standard for the diagnosis of RA is based on ACR revised criteria, which include RF, clinical and radiological criteria (Arnett F.C. *et al.*, 1988). However, RF positivity is nonspecific for RA. RF is also found in 5% of healthy person and its

prevalence in the population increases with age. RF will be positive in 10 to 20% of individuals over the age of 65 years old. In addition RF also present in other conditions other than RA, such as connective tissue diseases and infections. RF may appear transiently in normal individuals following vaccination or transfusion and may also be found in relatives of individuals with RA (van Boekel M.A.M. *et al*, 2002). Therefore, RF remains suboptimal as a diagnostic test. However, it has been established that high titres of RF indicate aggressive disease (ACR subcommittee on Rheumatoid Arthritis, 2002).

RF was widely available in most of the hospital in Malaysia and it is incorporated in the ACR criteria for the diagnosis of RA, therefore it is commonly used in a screening for RA. The cost of RF also was lower when compared to anti-CCP antibodies.

1.7.5 Sensitivity and specificity of anti-CCP antibodies

Evidence has demonstrated that anti-CCP antibodies are highly specific for RA (Schellekens GA *et al.*, 2000). A recent systemic literature review concerning the diagnostic value of anti-CCP antibodies in RA has been shown that the specificity for the second generation of anti-CCP ELISA test was 96% and the sensitivity was 68% (Avouac J *et al.*, 2006). A study by van Venrooij (2002) showed anti-CCP antibodies positive in 82% of chronic RA patient, 1% in healthy controls and 2% in disease controls. In the above study, IgM RF test was equally sensitive but less specific (varying from 62% to 88%) (van Venrooij WJ *et al.*, 2002).

A study done in Netherland, in comparison with IgM RF ELISA, the anti CCP ELISA had a significantly higher specificity (96% versus 91%) but sensitivity of both tests was comparable (Schellekens *et al.*,2000). Apart from that, a study by Lee D.M and Schur P.H (2003) showed that among a group of patients with diversity of rheumatic diseases, sensitivity and specificity of anti-CCP antibodies for RA was 66% and 90% respectively. This compared with the sensitivity and specificity of RF for RA at 71% and 80% respectively. Thirty four percent of patients with RA who were RF negative showed reactivity to anti-CCP antibodies. (Lee D.M & Schur PH, 2003).

Study in Tokyo Japan, using a cohort of 549 RA patients and 208 patients with other rheumatic diseases showed that the specificities of anti-CCP was 88.9% and sensitivity of 87.6% whereas RF has sensitivity of 69.8% and specificity of 81.7%. It clearly showed higher discriminative ability for the CCP test than RF (Suzuki K. *et al.*, 2003).

In the study to evaluate the diagnostic performance of anti-CCP antibodies among RA patients in Korean population, authors found that the sensitivity of anti-CCP antibodies was 72.8% and specificity of 92.0% (Choi S.W *et al.*, 2005)

Another study done in Singapore to evaluate the sensitivity and specificity of anti-CCP antibodies in rheumatoid arthritis patients showed that the sensitivity and specificity of anti-CCP antibodies were 62.3% and 92.1% respectively whereas the sensitivity and specificity of RF were 82.0% and 65.0% respectively. Anti-CCP antibodies was more specific but less sensitive than RF, therefore it may be used to distinguish RA from other rheumatic diseases (Koh E.T *et al.*, 2004).

1.8 Justification

For many years rheumatoid factor has been widely used in the diagnosis of RA apart from clinical manifestations. However RF is also present in other rheumatic diseases and in a proportion of healthy individual, makes it is not very specific for RA. The new test is anti-CCP antibodies has been shown highly specific compared to RF. Many studies have been done on sensitivity and specificity for anti-CCP antibodies. However there is no similar study done in Malaysia to assess the sensitivity and specificity of anti-CCP antibodies among Malaysian population.

In this study we would like to know the sensitivity and specificity of anti-CCP antibodies among our patients who fulfilled the ACR criteria for rheumatoid arthritis at HUSM. It might be the initial step to determine the sensitivity and specificity of anti-CCP antibodies before it involved through out Malaysian population.

Result from this study might be give a new hope to our patient in detecting early RA and subsequently starting early treatment especially DMARDs. In long term it will minimize the associated complications and its damage.

CHAPTER 2

OBJECTIVES

2.1 General Objective

To determine the sensitivity and specificity of anti-CCP antibodies and to compare with rheumatoid factor among rheumatoid arthritis patients in HUSM

2.2 Specific Objectives:

1. To describe the sociodemographic and clinical characteristics of rheumatoid arthritis patients
2. To determine the sensitivity and specificity of anti-CCP antibodies in rheumatoid arthritis patients attending HUSM using ACR criteria as the gold standard.
3. To compare the sensitivity and specificity of anti CCP antibodies with rheumatoid factor in rheumatoid arthritis patients attending HUSM using ACR criteria as the gold standard.

CHAPTER 3

METHODOLOGY

3.1 Study Design

This is a cross sectional study which was conducted from June 2008 to December 2008.

3.2 Study Location

This study was conducted at Klinik Rawatan Keluarga (KRK), Rheumatology Clinic at Klinik Pakar Perubatan (KPP) and Immonology laboratory, HUSM, Kubang Kerian, Kelantan. HUSM is one of the teaching hospitals in Malaysia, situated at Kubang Kerian, Kelantan. HUSM is also one of the tertiary referral centres for the state of Kelantan, other than Hospital Raja Perempuan Zainab II in Kota Bharu.

3.3 Population and sample

Patients with arthritis and/or who arthralgia attended Klinik Rawatan Keluarga and Rheumatology Clinic, Klinik Pakar Perubatan between June 2008 and December 2008.

The study population comprised of adult RA patients who fulfilled the ACR criteria as cases and patients with symptoms of arthralgia or arthritis as controls.

Criteria of cases

Inclusion criteria:

1. RA patients who fulfilled the ACR criteria
2. Age of 18 years old and above

Exclusion criteria:

1. Patients who were already been diagnosed or treated for sarcoidosis, systemic lupus erythematosus and Sjogren's syndrome
2. Pregnant women

Criteria for controls

Inclusion criteria:

1. Patients with symptoms of arthritis or arthralgia
2. Age of 18 years old and above.

Exclusion criteria:

3. Patients who were already been diagnosed or treated for sarcoidosis, systemic lupus erythematosus and Sjogren's syndrome
4. Pregnant women