Sensitivity and specificity of Rheumatoid factor and anti-cyclic citrullinated protein antibody positivity in patients with rheumatoid arthritis in karbala city.

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ABSTRACT:

Rheumatoid arthritis (RA) is systemic autoimmune disease of unknown etiology which characterize by joint and systemic involvement can lead to sever disability. The rheumatoid factor is one of the main serological test for diagnosis but is of low specificity since it is found in healthy people and other diseases . Anti- citrullinated protein antibody (anti-CCP) which is more specific and less sensitive is considered as another serological marker for diagnosis of RA. So that this was done to measure the sensitivity and specificity of RF and   anti-CCP in patients with rheumatoid arthritis. The study included 260 patient with RA and 130 healthy subjects their sera were used for measuring RF by latex agglutination technique and measuring anti-CCP by ELISA technique.   This study was found the sensitivity ,specificity , positive predictive value and negative predictive value   for RF were (86.9%) , (96%) ,(97%)and (78%) respectively , while those   for AntiCCP were 73% ,100% , (100%)and (65%) respectively. The p value for RF anti-CCP antibodies test was < 0.01 which indicate significant difference between patients and control group .So anti-CCP more specific and less sensitive than RF ,although it can be depended on RF for initial diagnosis ,   treatment and prevention of joint disabling .Seronegative patients did not exclude RA so must seek for another investigation such as anti-CCP. Measuring RFand anti-CCP at the same time increase sensitivity for diagnosis.

حساسية و خصوصية rheumatoid factor و anti cyclic citrullinated بروتين الجسم المضاد الإيجابية في المرضى الذين يعانون من التهاب المفاصل الروماتويدي في مدينة كربلاء

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الخلاصة:

التهاب المفاصل الروماتويدي (RA) هو مرض المناعة الذاتية النظامية مجهول السبب الذي يتميز بالتهاب المفاصل و⬜هرة مختلفة من الجسم يمكن أن تؤدي إلى الإعاقة. وكان عامل الروماتويد الاختبار المصلي الرئيسي للتشخيص ولكن ذو خصوصية منخفضة لكونه مو⬜ود لدى الأشخاص الأصحاء وامراض اخرى . مكافحة citrullinated بروتين الأ⬜سام المضادة (المضادة للCCP) وهو أكثر خصوصية، ولكن أقل حساسية كعلامة المصلية آخر لتشخيص التهاب المفاصل الروماتويدي. لذلك تم ⬜راء هذه الدراسة لمعرفة حساسية

وخصوصية RF وanti-CCP في المرضى الذين يعانون من التهاب المفاصل الروماتويدي. وشملت الدراسة 260 مريض بالتهاب المفاصل الروماتيدي و 130 من الأصحاء .امصال المرضى والاصحاء خضعت لقياسRF بواسطة تقنية ال latex وقياسanti-CCP بواسطة تقنية ELISA. تم العثور على هذه الدراسة الحساسية والخصوصية والقيمة التنبؤية الإيجابية والقيمة التنبؤية السلبية لـRF كانت (86.9٪)، (96٪)، (97٪) و (78٪) على التوالي. اما بالنسبة لـanti-CCP كانت القيم (73) ٪ ،(100)٪، (100٪)، و (65٪) على التوالي . وكانت قيمة احتمالية لـ anti-CCP وRF اقل من واحد بالمئة والتي تشير إلى اختلاف كبير بين المرضى ومجموعة الاصحاء. لذا مضاد CCP أكثر خصوصية وأقل حساسية من RF، وعلى الرغم من أنه يمكن الاعتماد على فحص RF للتشخيص الأولي والعلاج والوقاية من الاعاقة .المرضى ذوي الامصال الخلية من RF لايستبعد التهاب المفاصل لذلك يجب أن يخضع المرضى الى فحص اخرمثل anti-CCP . قياسanti-CCP وRF في الوقت نفسه زيادة حساسية لتشخيص المرض.

Introduction :

Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology and it is the most common of the inflammatory joint diseases, affecting 0.5-1% of the world population which is more common in women than in men about 75% of RA patient are women [1,2].The disease is characterized by chronic inflammation of the synovial membrane which spreads symmetrically from small to large joints leading to destruction of joints in the late phase accompanied by a systemic involvement of the soft tissue that can lead to severe disability[1,3] .There is important associations between specific HLA alleles (HLA-DR4 and HLA-DR1) and susceptibility to RA [4-8]. Although the diagnosis of RA depends primarily on clinical manifestations of the disease but the serological test routinely used is to determine the presence of rheumatoid factors (RF) in the serum. RF are antibodies directed to the constant region (FC) of immunoglobulins of the IgG subclass. RF can be detected in up to 70–80% of RA patients, but is also detected in relatively high percentages in other autoimmune and infectious diseases, and in up to 15% of healthy individuals [9]. Anti-CCP is antibody directed against amino acid citrulline , which present in filagrin, is a substantial component of the antigenic epitope [10]. The presence of RF and anti-CCP that detect in patient with RA have been associated with poor outcomes, such as increased disease activity, radio graphic progression and disability [11-15].

Patients and method :

The total 260 patient with RA and 130 healthy subjects were enrolled in this study from January 2015 to February 2016. All patient were selected from rheumatology out patient clinic from Al-Hussein teaching hospital in Kerbela city . All   serum samples were tested by agglutination technique for RF (RF-Latex Kit) and ELISA technique for ACPA (anti-CCP ELISA IgG EUROIMMUNE EA 1505-9601 G).

Results:

In the current study 260 patient with RA about 226 (87%) are seropositive for RF, 34 (13%) who are seronegative for RF while healthy control patients with RF positive are 5(4%) and those who are RF negative 125 (96%). RF has sensitivity 86.9% specificity 96% ,positive predictive value(PPV)(97%)and negative predictive value NPV(78%).The p value which is significant for RF antibodies test <0.01 as show in table 1. The 260 patients with RA about 190 (73%) seropositive for anti-CCP while those who are negative for anti-CCP 70 (27%) and 130 healthy control whose are

100% negative for anti-CCP . The anti-CCP has Sensitivity 73% specificity 100% , ppv(100%)and NPV(65%). The p value for anti-CCP antibodies test which are significant < 0.01 as shown in table 2. 186 (82%) of RF positive have anti-CCP positive and 40 (18%) of RF positive are negative for anti-CCP while those are RF negative about 14 (41%) positive for anti-CCP and 20 (59%) are negative for both RF and anti-CCP , PPV (82%), NPV (58%) and the p value <0.01 as shown in table 3.

Table 1:The number and percentage of patients for RF seropositivity .

total	Seropositvity for RF		Seronegative for RF	
	No.	Percent%	No.	Percent%
RA patients No=260	226	87%	34	13%
Control No=130	5	4%	125	96%

p value <0.01

Table 2.: The number and percentage of patients for anti-CCP seropositivity .

	Anti-CCP seropositive		Anti-CCP seronegative	
	No.	Percent%	No.	Percent%
RA patient No=260	190	73%	70	27%
Control No=130	0	0%	130	100%

p value <0.01

Table 3: RF and anti-CCP result in study patients.

	Anticcp +ve		Anti-CCP-ve	
	No.	Percent%	No.	Percent%
RF+ve No=226	186	82%	40	18%
RF-ve No=34	14	41%	20	59%

p value <0.01

Table 4: Performance characteristics of RF and anti-CCP

	RF	Anti-CCP	Combind RF and anti-CCP
Sensitivity%	86.6	73	93
Specificity%	96	100	33
Positive predictive value(PPV)%	97	100	82
Negative predictive value(NPV)%	78	65	58

Discussion :

The study included 260 patients diagnosed with rheumatoid arthritis according to ACR/EULAR Classification Criteria for Rheumatoid Arthritis [16]. The serological markers play an important role in diagnosis and prognosis of patients with rheumatoid arthritis. RF has been utilized for RA diagnosis for several years, and forms part of ACR diagnostic criteria. Anti-CCP test is reported to have superior performance characteristic and is part of ACR criteria [17]. In the study the sensitivities of RF and anti-CCP were 86.9% and 73% respectively, which had been shown that RF higher sensitivity than anti-CCP which may be due to the technique that had been used which ELISA for anti-CCP which is more accurate than latex technique for diagnosis of RF which is less accurate but it cheep method that used in most laboratories when compare the result with other studies the obtained result is had been found the sensitivity of RF and anti-CCP   higher than the result had been down by Amayo AA 2014 in which   the sensitivity of RF and anti-CCP 50% and 60% respectively [18] .When we compared the study   with the study of RA in black south African, the RF sensitivity in the study (86.9%) was higher than the sensitivity of RF (81.7%)   which had been studies in black American south African   [19] while sensitivity of the anti-CCP (73%) lower than sensitivity of anti-CCP in the study of RA in black south African (82.5 %) [19] may be need more patients in further study to explain this .In the current study the specifity of RF was lower than anti-CCP (96%

and 100%) respectively .The specificity of RF (96%) was higher than specificity of RF in the study had been done by Amayo AA 2014 was (90.3%) [18] and also higher than that the study RA in black south African had been ranged from (77-90.7%) [19] . The specifity of anti-CCP (100%) in the study was higher than the specificity of ant-CCP in the study had been down by Amayo AA 2014 was (83.9%) [18] and the study RA in black south African in which the specificity of anti-CCP ranged from (84.9-98%) [19] this may be due to the study included only healthy control and RA patients did   not include other diseases for example other autoimmune diseases or other connective tissue diseases .The predictive values of diagnostic tests depict the likelihood that a patient has the disease if the test is positive (positive predictive value –PPV), and the likelihood that a patient does not have the disease if the test is negative (negative predictive value- NPV) .They are important in clinical utilization of diagnostic tests as they inform the clinician whether additional confirmatory tests are required or treatment can be initiated base on the results. In this study the PPVs for RF and anti-CCP were (97%) and (100%) respectively while negative predictive value for RF and anti-CCP were (78%) and (65 %) respectively. The PPVs found in this study are similar to those reported in a study was had been done by Amayo AA 2014 were (91.5%) and (89%) [18] ,this was similar to those had been done among

black south African were (92.5%) and (87.6%) [19] and indicate the likelihood of RF being present was very high if RF or anti-CCP was positive. The NPVs found in this study   for RF and anti-CCP were (78%) and (65%) respectively were higher than NPV found in Amayo AA 2014 study were (46%) and (52%) for RF and anti-CCP respectively [18] and similar to those had been done among black south African were (68%) and (79%) for RF and anti-CCP respectively [19] . less than one fourth of patient classify as RA in our study tested negative for anti-CCP while (59%) of them were negative for RF   as shown in table 3. Better diagnostic performance characteristics have been reported if RF and anti-CCP are combined and some have advocated for both marker to be used [20] . The sensitivity of RF (93%)in our study was increase when both RF and anti-CCP positive from (86.6% to 93%) than use of RF alone while the specificity was decrease when both RF and anti-CCP positive this might be due to FR measured by latex agglutination and anti-CCP measured by ELISA technique .

Conclusion:

The current study has measured sensitivity and specificity of RF and anti-CCP in patient with RA which showed that RF more sensitivity and less specific than anti-CCP . Although anti-CCP more specific than RF but can depend on RF initial diagnosis and treatment for prevention of joint disabling. Although RF useful for initial diagnosis but negative RF does not exclude RA so must   seek for other investigation such as anti-CCP. Measuring RF and anti-CCP at the same time increase sensitivity for diagnosis   .

References:

[1] Klareskog L, Catrina AI, Paget S. Rheumatoid arthiritis. Lancet 2009; 373: 659-72.

[2]Weyand CM, Schmidt D, Wagner U, et al. The influence of sex on the phenotype of rheumatoid arthritis. Arthritis Rheum 1998;41(5):817-822.

[3]Gabriel SE. The epidemiology of rheumatoid arthritis .Rheum Dis Clin North Am 27 (2001) 269-281.

[4]Stastny P. Association of the B-cell alloantigen DRw4 with rheumatoid arthritis. N Engl J Med 1978;298:869-871.
[5] Gregerson P, Silver J, Winchester R. The shared epitope hypothesis: an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 1987;30:1205-1213.
[6] Nepom GT, Hansen JA, Nepom BS. The molecular basis for HLA class II associations with rheumatoid arthritis.J Clin Immunol 1987;7:1-7.

[7] Willkens RF, Nepom GT, Marks CR, et al. Association of HLA-Dw16 with rheumatoid arthritis in Yakima Indians. Further evidence for the shared epitope hypothesis. Arthritis Rheum 1991;34:43-47.
[8] Del Rincon I, Escalante A. HLA-DRB1 alleles associated with susceptibility or resistance to rheumatoid arthritis, articular deformities, and disability in Mexican Americans. Arthritis Rheum1999;42:1329-1338.

[9] Smolen, J.S. 1996. Autoantibodies in rheumatoid arthritis. In Manual of Biological Markers of Disease. W.J. van Venrooij and R.N. Maini, editors. Klu-wer Academic Publishers, Dordrecht, The Netherlands. C1.1/1–C1.1/18.

[10]Schellekens GA, Visser H,de jong BA,etal.the diagnostic properties of rheumatoid arthritis antibodies recognizing acyclic citrullinated peptide. Arthritis Rheum43(2000)155-163.

[11]Quinn MA, Gough AK, Green MJ, Devlin J, Hensor EM, Greenstein A, Fraser A, Emery P: Anti-CCP antibodies measured at disease onset help identify . seronegative rheumatoid arthritis and predict radiological and functional outcome. Rheumatology 2006, 45:478–480

[12] De Rycke L, Peene I, Hoffman IE, Kruithof E, Union A, Meheus L, Lebeer K, Wyns B, Vincent C, Mielants H, Boullart L, Serre G, Veys EM, De Keyser F: Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestations. Ann Rheum Dis 2004, 63:1587–1593.

[13] Ronnelid J, Wick MC, Lampa J, Lindblad S, Nordmark B, Klareskog L, van Vollenhoven RF: Longitudinal analysis of citrullinated protein/ peptide antibodies (anti-CP) during 5 year follow up in early rheumatoid arthritis: anti-CP status predicts worse disease activity and greater radiological progression. Ann Rheum Dis 2005, 64:1744–1749.

[14]Kudo-Tanaka E, Ohshima S, Ishii M, Mima T, Matsushita M, Azuma N, Harada Y, Katada Y, Ikeue H, Umeshita-Sasai M, Miyatake K, Saeki Y: Autoantibodies to cyclic citrullinated peptide 2 (CCP2) are superior to other potential Abbreviations diagnostic biomarkers for predicting rheumatoid arthritis in early undifferentiated arthritis. Clin Rheumatol 2007, 26:1627–1633.

[15]Bos WH, Bartelds GM, Vis M, van der Horst AR, Wolbink GJ, Van De Stadt RJ, Van Schaardenburg D, Dijkmans BA, Lems WF, Nurmohamed MT, Aarden L, Hamann D: Preferential decrease in IgG4 anti-citrullinated protein antibodies during treatment with tumour necrosis factor blocking agents in patients with rheumatoid arthritis. Ann Rheum Dis 2009, 68:558–563.

[16] www.american college of rheumatology

[17] Saag KG,Teng GG, Patkar NM, *et al* .american college of rheumatology 2008recommendations for the use of non biologic and biologic disease modifying antirheumatic drugs in rheumatoid arthritis.Arthritis rheum.2008; 59 (6) :762-784.

[18] Amayo AA , Ayunga AO and Oyoo GO. Performance characteristics of anti-cyclic citrullinated peptide and rheumatoid factor test in rheumatoid arthritis and undifferentiated arthritis at Kenyatta National Hospital 2014, vol1:23-27.

[19]Hodkinson B , Meyer PW , Musenge E , Ally MM, *et al.* The diagnostic utility of anti-ccp antibody test in no better than rheumatoid factor in south Africans with early rheumatoid arthritis .Clin Rheumatol 2010;29(6) :615- 618.

[20]Mikuls TR, Holers VM, parrish L and Kuhn KA.Anti-cyclic citrullinated peptide antibody and rheumatoid factor isotopes in African Americans with early rheumatoid arthritis. Arthritis rheum.2006; 54:3057-3059.