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Evaluate the prevalence of CCP and RF antibodies as a marker for diagnosis and progression of Rheumatoid Arthritis disease and assess the prevalence of HCV in RA patients

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by the presence of autoantibodies like rheumatoid factor (RF). In the last few years, several other autoantibodies have been described of which anti-cyclic citrullinated peptide (CCP) antibody is the most specific. Chronic hepatitis C virus (HCV) has extrahepatic autoimmune properties and a variety of autoantibodies were found in patients with HCV. This study is conducted to evaluate the sensitivity and specificity of Anti-CCP in compare with RF in RA patients and assess their association with severity of the disease and evaluate if HCV have been one of the infectious agent for rheumatoid arthritis. Sixty blood samples were collected from RA patients and twenty from both apparently healthy group and HCV patients. The serum from each subject was tested for anti- CCP, RF and anti HCV by enzyme- linked immunosorbent assay (ELISA). Estimation of erythrocyte sedimentation rate (ESR) by Westergreen' method was included in this study. The result indicated that anti-CCP postivity for RA patients sera (78.3%), (20%) for HCV patients and (0.0%) for healthy group, it showed highly significant differences in RA group in compare with control group ($P<0.005$).While the percentage of RF positive was (70%) for patients with rheumatoid arthritis with significant differences and 20% for patients with viral hepatitis type (c) with no significant differences in compare with control group (5%). Results also showed that (1.7%) of patients with rheumatoid arthritis showed positive result to HCV.

Introduction

Rheumatoid factor (RF) is classically used as a serologic indicator of RA. Its presence is included as a criterion in the American college of rheumatology classification criteria for RA [1]. However, this autoantibody lacks specificity. It may be found in patients with other autoimmune diseases, with chronic infections or in healthy elderly individuals [2, 3].

Anti-CCP antibodies have a high specificity, mostly above 95%, for RA combined with sensitivity comparable to the traditional RF. In addition, anti-CCP antibodies appear early in the disease, often even preceding the symptoms of RA [4].

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Finally, anti-CCP is the most reliable predictor of a progressive and erosive course of RA [5]. Citrullinated proteins have been discovered in the joints of patients with rheumatoid arthritis but not in other forms of joint disease. The citrullinated proteins in the joints correspond to the presence of the citrulline antibodies in the blood and suggest a possible role for these antibodies in the development of rheumatoid arthritis [6].

Citrulline antibody directed against a circular peptide (a ring of amino acids) called Citrulline is a nonstandard amino acid, as it is not incorporated into proteins during protein synthesis. It can, however, be generated via post-translational modification of arginine residues by peptidylarginine deiminase

(PAD) enzymes [7, 8]. Conversion of arginine into citrulline involves the replacement of an amine group by an oxygen atom in the side chain of this amino acid, and is associated with the loss of a positive charge (at neutral pH). Although this conversion results in a relatively small chemical alteration of the protein involved, the reactivity of autoantibodies reactive with citrulline-containing epitopes seems to be critically dependent on the presence of a citrulline residue [9].

Erythrocyte Sedimentation Rate is a measure of the rate at which red blood cells settle through a column of liquid. ESR is sometimes helpful in distinguishing between inflammatory and non-inflammatory conditions. This test may be useful for monitoring patients with rheumatoid arthritis, polymyalgia rheumatica, etc. [10].

Various viruses have been implicated in the cause and pathogenesis of rheumatoid arthritis (RA). Hepatitis C virus (HCV) infection, which has been recognized as a cause of some autoimmune diseases, and which has been described as sometimes presenting with rheumatic manifestations indistinguishable from RA, might be a candidate [11]. Patients with HCV infection may have rheumatic signs and symptoms, and 50–70% of the cases may contain rheumatoid factor (RF) [12]. The increased prevalence of RF in patients with HCV infection diminishes the diagnostic specificity of serum RF for rheumatoid arthritis (RA) in patients with HCV [13].

Material and Methods

Sample collection

Sixty blood samples were collected from RA patients and twenty samples from HCV patients and twenty samples from apparently healthy control in the period between December/ 2011 and June / 2012. Those patients had RA as defined by the American Rheumatism Association 1987 criteria. The diagnosis of those patients has been performed under the supervision of rheumatic disease Consultant Rheumatologist, and the patients were having four or more of the 1987 ACR criteria.

Serology

Serum samples from patients and control groups were kept at -20˚C.Rheumatoid factor RF isotypes (IgG , IgM, IgA) were detected using ELISA kit (Euroimmun, Germany), Anti-CCP antibodies was detected by ELISA (Euroimmun, Germany) and Antibodies against Hepatitis C Virus were detected by ELISA (DIALAB, Austria).

Hematology

Erythrocyte Sedimentation Rate(ESR) was measured by Westergren's method in which the blood was diluted with Trisodium citrate 3.8% as anti-clotting and then the value of ESR was measured, expressed as mm/1hr.

Statistical Methods

The mean ± SD were given, difference between means of patients and healthy control group were assess by least significant differences (LSD**).** Diagnostic characteristics were determined by means of sensitivity and specificity. All the statistical analysis were done by using Pentium-4 computer through the SPSS program (version-10) and Excel application.

Note:

The comparison of significant (P-value) in any test were significant difference if (P<0.005).

Result:

A rheumatoid factor positivity in the current study showed (70%) for RA cases with significant difference (P<0.05) in comparing with the positivity of RF which was (20%) for HCV patients and (0.00 %) for control. (table:1)

High frequency of anti-CCP positivity for RA patient's sera (78.3%) in compare with (20.0%) and (0.0%) for HCV and healthy cases respectively. (Table: 2)

In addition, among these patients with RA, (63.33%) were positive for both tests (RF and CCP) as shown in table:3.

The current study showed that the sensitivity and specificity of Anti-CCP as recorded in table (4) which presents in the validity test that appear the highest (78.3%) in comparison with low (70%) sensitivity for RF. Moreover, the specificity of Anti-CCP is very high (100%), while the specificity of RF is high (95%).

There is significant alteration occurs on the mean of ESR level of RA patients (48.98 ± 20.027) in comparison with control group (9.95 ± 5.083) (P < 0.005).(Fig.1)

By comparing the mean values between RF and CCP with ESR, the result shows that the increase in RF and CCP means associate with the increase of ESR. (Fig.2) By testing the presence of anti-HCV in RA patients serum, the result showed that one of the patients (1.7%) had positive result.(Table:5).

Table1: Frequency table for RF in all study groups (RA patients, HCV patients and healthy control)

Group			Frequency	Percent %	Valid Percent %	Cumulative Percent %
RA	Valid	positive	42	70	70	70
		negative	18	30	30	100.0
		Total	60	100.0	100.0	
Control	Valid	Positive	1	5	5	5
		negative	19	95	95	100.0
		Total	20	100.0	100.0	
HCV	Valid	positive	4	20.0	20.0	20.0
		negative	16	80.0	80.0	100.0
		Total	20	100.0	100.0	

Table 2: Frequency table for Anti-CCP in all study groups (RA patients, HCV patients and healthy control)

Group			Frequency	Percent %	Valid Percent %	Cumulative Percent %
RA	Valid	positive	47	78.3	78.3	78.3
		negative	13	21.7	21.7	100.0
		Total	60	100.0	100.0	
Control	Valid	negative	20	100.0	100.0	100.0
HCV	Valid	positive	4	20.0	20.0	20.0
		negative	16	80.0	80.0	100.0
		Total	20	100.0	100.0	

Table 3: Distribution of rheumatoid arthritis patients into serologic subgroups according to RF and anti-CCP antibody status

variables	RF ve+	RF –ve	Total
Anti – CCP +ve	38 (63.33 %)	9 (15 %)	47 (78.33 %)
Anti – CCP –ve	4 (6.67 %)	9 (15 %)	13 (21.67 %)
Total	42 (70 %)	18 (30 %)	60 (100 %)

Table 4: Validity test (%) of immunological parameters (Anti-CCP and RF).

Tests	Sensitivity %	Specificity %	PPV %	NPV %
Anti-CCP	78.3 %	100 %	100 %	39.3 %
RF	70 %	95 %	97.67 %	48.6 %

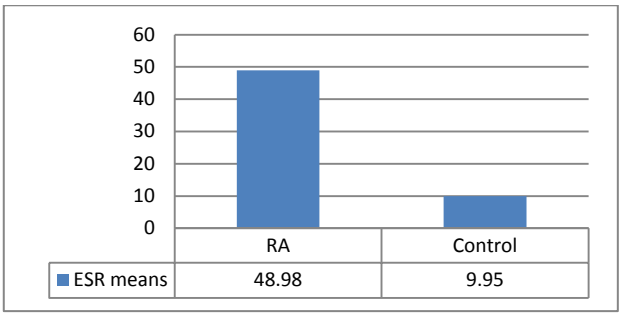

Fig 1: The mean values of ESR in RA patients and Control groups

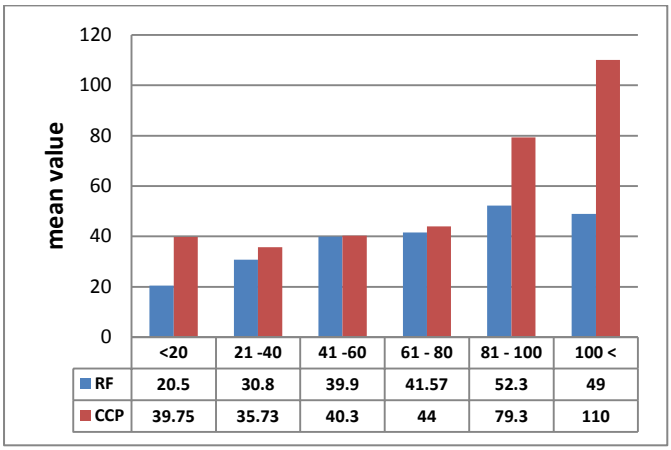

Fig 2: The comparison between RF and CCP mean values with ESR

Table 5: Frequency table for Anti-HCV in RA patients.

		Frequency	Percent %	valid Percent %	Cumulative Percent %
Valid	positive	1	1.7	1.7	1.7
	negative	59	98.3	98.3	100.0
	Total	60	100.0	100.0	

Discussion

Rheumatoid factor (RF) has been used as a marker of rheumatoid arthritis included into RA classification criteria for more than half a century. RF presents in nearly all patients with extra-articular manifestations and almost in variably in patients with nodules. The diagnostic value of tests is increased when titer is higher than 20 IU/ ml in Latex fixation test. But alone it is not sufficient for diagnosis. High titer of RF is associated with more severe clinical picture and poor prognosis [14].

A rheumatoid factor positivity in the current study showed (70%) from RA cases with significant difference (P<0.005). While other studies showed lower than that percentage (53%) which is mentioned by [14] and (47 %) which was mentioned by [15].

Chronic liver disease is characterized by a variety of serum autoantibodies. In current study HCV patients had (20 %) positivity of RF, it's approximately similar to that of [16] (14.6%). The presence of RF is generally not helpful in establishing a diagnosis of RA in patients with concurrent HCV infection [12].

In the last few years, several other autoantibodies have been described of which anti-cyclic citrullinated peptide (ACCP) antibody is the most specific. In current study high frequency of anti-CCP positivity for RA patient's sera (78.3%) in compare with (20%) and (0.0%) for HCV and healthy cases respectively, this percentage is higher than that of [15] (69%).

The percentage (20 %) of CCP positive in HCV patients and it's higher than that of [16](4.9 %). The presence of anti-CCP antibodies in HCV patients proof of the presence of RA . Anti-cyclic citrullinated peptide (CCP), a highly specific biomarker for RA in the general population, may be useful for the diagnosis of RA in the HCV population [16].

By testing the presence of anti-HCV antibodies in RA patients serum, the result showed that 1.7% of patients with rheumatoid arthritis appeared they are infected with the hepatitis C virus (Table 5). That could lead to suggest that the hepatitis c virus was one of the causative agents for injury of rheumatoid arthritis.

From analyzing the result in table 3, there was 15 % of RA patients had CCP positive and RF negative. A negative RF does not rule out RA; rather, the arthritis is called seronegative. This is the case in about 15% of patients. During the first year of illness, rheumatoid factor is more likely to be negative with some individuals converting to seropositive status over time [17].

There was 6.67 % of RA patients had CCP negative and RF positive. Some reports describe a decrease in titer of anti-CCP antibodies following successful treatment of RA [18], as well as in case of negative for both tests.

Anti-CCP antibodies have been clearly associated with the development of RA, although a distinct pathogenic role in the development of disease remains to be established. In this study, the relative roles of the two common autoantibodies in RA patients have assessed and evaluate their sensitivity and specificity to the disease.

From analyze the result by validity test, the sensitivity of CCP (78.3%) and specificity (100%) were higher than that of RF (70 %) and (95 %) respectively. From this outcome it can conclude that Anti-CCP antibody as a significant diagnostic marker with high specificity

(100%). Also, high specificity of Anti-CCP antibodies had been investigated by other researchers. Most of them reported specificity of (90-99%), and sensitivity (64-74%)[19]. It was noticed that the result of different studies were heterogeneous. This may be caused by the manufacturing companies with different serum dilutions and different CCPs used in the assays. These all may alter the result and necessitate the need for international standardization. Since the difference was not significant between the specificity for both tests, this result confirms previous studies with combination of RF plus Anti-CCP.

The erythrocyte sedimentation rate (ESR) is a nonspecific, most commonly used inflammatory biomarker in clinical practice. In current study the result showed that there is significant differences occurs on the mean of ESR level of RA patients (48.98 ± 20.027) in comparison with control group (9.95 ± 5.083) ($P < 0.005$).

Anti-CCP antibodies were positively correlated with higher erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), swollen joint count, and worse physician global assessment ratings. Presence of rheumatoid factor was positively correlated with increased ESR and CRP, but there was no association with other disease activity markers. From compare the mean values between RF and CCP with ESR [20]. In current study the result shows that the increase in RF and CCP means associate with the increase of ESR. (Fig.2) That result confirms that which mentioned by [21], Anti-CCP is a prognostic indicator for RA progression, although generally not more useful than RF.

Conclusions:

1. Frequency of rheumatoid factor among RA cases was (70%) while anti-cyclic citrullinated peptide was (78.33 %). The sensitivity of CCP and specificity were higher than that of RF which made this test (anti-CCP) considered as a good parameter for diagnosis of rheumatoid arthritis.
2. The increase in RF and CCP means associate with the increase of ESR lead to suggest that anti-CCP is a prognostic indicator for RA progression, although generally not more useful than RF.
3. There were 20% of HCV patients who had anti-CCP positive and there were 1.7% of RA patients were infected with HCV, which may lead to the suggestion that HCV may be one of the causative agents of RA or it may trigger the progression of RA.

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تقييم انتشار الأجسام المضادة CCP وRF كعلامة لتشخيص وتطور مرض التهاب المفاصل الروماتويدي وتقييم مدى انتشار فيروس التهاب الكبد (سي) في مرضى التهاب المفاصل الروماتويدي

قبس راغب علي محمد قيس العاني هناء ناجي عبد الله سليم عبيد المولى

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يعد التهاب المفاصل الرثوي مرض مزمن من أمراض المناعة الذاتية التي تتميز بوجود الأجسام المضادة للعامل الروماتويد (RF). وقد وصفت في السنوات القليلة الماضية العديد من الأجسام المضادة الاخرى منها الاجسام المضادة لضد الببتيد الستروليني الحلقي التي تعتبر الأكثر خصوصية للمرض. كما وجد ان التهاب الكبد الفايروسي المزمن نوع (ج) له خصائص المناعة الذاتية خارج الكبد وتم العثورعلى مجموعة متنوعة من الأجسام المضادة في المرضى الذين يعانون من فيروس (ج). الهدف من الدراسة الحالية تقييم حساسية وخصوصية الاجسام المضادة لضد الببتيد الستروليني الحلقي بالمقارنة مع العامل الروماتويدي في مصل مرضى التهاب المفاصل الروماتويدي مع تقييم مدى ارتباطهما مع شدة المرض وتقييم ما إذا كان فايروس الكبد نوع (ج) من العوامل المساعدة للاصابة بالتهاب المفاصل الرثوي. تم قياس المؤشرات المناعية الثلاثة (عامل الروماتويد، الاجسام المضادة للببتيد الستوليني الحلقي و الاجسام المضادة لفايروس الكبد نوع (ج)) بطريقة الاليزا في مجموعات الدراسة الثلاثة، كما تم تقدير معدل ترسيب كريات الدم الحمراء في مصل مرضى التهاب المفاصل الرثوي ومجموعة السيطرة. تم جمع ٦٠ عينة دم من مرضى التهاب المفاصل الرثوي وعشرين من كل من مجموعة الاصحاء ومرضى التهاب الكبد الفايروسي نوع (ج) و تم اختبار الأمصال لكل العينات. واظهرت النتائج ان نسبة الايجابية لضد البتيد الستروليني الحلقي ٨٧.٣% في مصل مرضى التهاب المفاصل الرثوي و ٢٠% في مصل مرضى التهاب الكبد الفايروسي نوع (ج) والتي اظهرت فروقات معنوية عالية لكلاهما مقارنة بمجموعة الاصحاء ٠ %، بينما كانت نسبة الايجابية للعامل الرثوي ٧٠% لمرضى التهاب المفاصل الرثوي مع فروقات معنوية و ٢٠% لمرضى التهاب الكبد الفايروسي نوع (ج) مع عدم وجود فروقات معنوية مقارنة مع مجموعة السيطرة.كما اظهرت النتائج ان ١.٧ % من مرضى التهاب المفاصل الرثوي اظهر نتيجة موجبة لضد فايروس الكبد نوع (ج) .