The Evaluation of Some Biomarkers According to Rheumatoid Factor in Early Diagnosis of Rheumatoid Arthritis Patients in Baghdad City

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Abstract

Rheumatoid arthritis (RA) a chronic inflammatory autoimmune disease that primarily affects small joints and leads to chronic inflammation in the synovial fluid. The aim of this study was to identify the relationships between early diagnosis of RA with some serological markers, namely the antibodies to citrullinated protein/peptide antigens (ACPAs), anti-mutated citrullinated vimentin (anti-MCV), anti-carbamylated protein (Anti-Carp), anti- heterogeneous nuclear ribonucleoproteins (anti-hnRNP) and Glucose-6-phosphate isomerase (GPI) .This study involved 60 newly diagnosis RA patients (mean age 46.80±11.96) who were divided into two subgroups (30 RF positive and 30 RF negative) and 30 subjects as healthy control (mean age 43.6±14.15). The serological data of serum concentrations of ACPAs, Anti-MCV, Anti-Carp, Anti-hnRNP, and G6PI were estimated by ELISA method, whereas RF was estimated by latex agglutination method. The results revealed that ACPAs, Anti-MCV, Anti-Carp, Anti-hnRNP, and G6PI have significantly higher mean±SD titers among the refractory RA patients in comparison with the control group. Also, the effective distinguishing of RA patients as RF+ve and RF-ve showed different sensitivity and specificity values of ACPAs (90.8% , 94.1%, and 88.2%, 86.6%); anti-MCV (66.7% , 33.0% and 70.0%, 70.2%); anti-CarP (76.7% , 90.0% and 93.3%, 78.5%); anti-hnRNP (74.9% , 61.9% and 71.4%, 70.9%) and GPI (77.3% , 76.7% and 84.4%, 80.1%), respectively. This study confirms the importance of measuring multiple serum biomarkers and their combinations as having a high diagnostic value for RA which provides support for the early diagnosis the disease.

Keywords: autoimmune disease, RA, ACPAs, Anti-MCV, Anti-Carp, Anti-hnRNP, GPI, RF.

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تقدير بعض المؤشرات الحيوية وفق العامل الرثوي في التشخيص المبكر لالتهاب المفاصل الرثوي لدى المرضى العراقيين

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الخلاصة

التهاب المفاصل الرئوي هو من امراض المناعة الذاتية المزمنة يكون تاثيره بصوره اولية على المفاصل الصغيره ويقود الى التهاب اغشية المفاصل المزمن. وهذه الدراسة شملت 60 من المرضى بمتوسط اعمار (80, 46 ±96, 11) المشخصين حديثا بالتهاب المفاصل الرئوي , مقسمين الى مجموعتين فرعيتين (30 لديهم العامل الرئوي RF موجب و30 ليهم العامل الرئوي RF السالب) و30 من الاصحاء ثمثل مجموعة السيطره بمتوسط اعمار (43,6 ±14,15) . المعلومات المصلية اخذت من تراكيز المعلمات(ACPAs وAnti-MCV وAnti-CarP وAnti-hnRNP و GPI) المقدرة بطريقة الاليزا والعامل الرئويRF مقدر بطريقة التكتل التلازني latex agglutination. ظهرت النتائج بان المعلمات لديها فروق معنوية لتراكيز (المتوسطات ± الانحراف المعياري) لكل من مرضى مجموعتين العامل الرئوي عند مقارنتها بمجموعة السيطرة وكذلك التمييز بفعالية بين مرضى التهاب المفاصل الرئوي لكل من العامل الرئوي الموجب و العامل الرئوي السالب اللتي تظهر حساسية وخصوصية لكل من ACPAs (8و90% و1و94% مع 2و88%و6و86%) و-Anti MCV (7و66% و33% مع 70% و2و70%) و Anti-CarP (7و76% و 90% مع 3و 93% و 5و 78%) و Anti-hnRNP (9و74% و 9و61% مع 4و71% و 9و70%) وGPI (3و77% و7و76% مع 4و84% و 1و80%) بالتعاقب. تهدف الدراسة الى معرفة العلاقة بين بعض المعلمات المصلية (ACPAs وAnti-MCV وAnti-CarP وAnti-hnRNP و GPI) والتشخيص الاولي لالتهاب المفاصل الرئوي. هذه الدراسة تؤكد اهمية قياس عدة معلمات حيوية وارتباطها مع تشخيص عالي المستوى لالتهاب المفاصل الرئوي وتدعم التشخيص الاولي لالتهاب المفاصل الرئوي.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that primarily affects small joints and leads to chronic inflammation in the synovial fluid, resulting in the destruction of small joints, deformity, and disability [1]. RA prevalence is reported to be 0.5–1% in the general population [1]. RA etiology is not fully understood, and it was attributed to the incorporation of genetic and environmental factors [2, 3]. The American College of Rheumatology (ACR) and The European League Against Rheumatism (EULAR) classified RA according to new criteria. Overall clinical symptoms involve the two immunological markers of rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs). Furthermore, RA patients are typically classified into two sub-groups, designated as seropositive and seronegative, with seropositivity referring to the existence in serum of high concentrations of the autoantibodies RF and ACPA [4]. The serological status is a crucial factor in the current diagnosis and prognosis of RA disease. The seropositive RA patients demonstrate specific genetic and environmental risk factors and were observed to have a more severe course of disease [5, 6]. Correlations were reported between the biomarker genetic variation and various single nucleotide polymorphisms (SNPs) in RA [7]. The genetic correlation for negative ACPAs in RA was also demonstrated [8]. The clinical symptoms and status of seronegative RA was always recorded as less acute than those of seropositive RA [9]. In several studies, it was demonstrated that the anti-mutated citrullinated vimentin (anti-MCV) antibodies might represent a precious factor for RA diagnose in anti- CCP-negative patients. Moreover, anti-MCV antibodies could be practical in monitoring the effects of infliximab therapy as well as in RF and anti-CCP negative juvenile idiopathic arthritis patients [10]. New immunologic markers associated with RA include anti-CarP and anti-hnRNP A2/B1, RA33. A recent study observed that the presence of anti-CarP antibodies was significantly associated with the development of RA in ACPA and RF-negative patients [11]. Glucose-6-phosphate isomerase (GPI) could be a practical biomarker for the newly diagnosed clinical RA, specifically their correlation with RA acute states. GPI has the same function of autocrine motility factor (AMF), a cytokine with various functions that is able to organize cell immigration, penetration, survival and proliferation [12]. The aim of this study was to identify the relationships between early diagnosis of RA with some serological markers, namely the antibodies to citrullinated protein/peptide antigens (ACPAs), anti-mutated citrullinated vimentin (anti-MCV), anti-carbamylated protein (Anti-Carp), anti- heterogeneous nuclear ribonucleoproteins (anti-hnRNP) and Glucose-6-phosphate isomerase (GPI)

Materials and Methods

Data were collected from the register of patients attending the Rheumatology Consultation Clinic /Baghdad Teaching Hospital and Al-Imamein AL-Kadhimein medical city in Baghdad during the period of July 2018–May 2019. This study included a group of 60 patients of newly diagnosis RA with less than 1 year of disease duration. They were classified according to ACR criteria into 30 newly diagnosed RF + and 30 newly diagnosed RF- patients, in addition to 30 healthy controls. Venous blood specimens (5 ml) were collected from patients and healthy controls and used for serum separation. Study protocols involved quantitative measurement of ACPA, Anti-MCV, Anti-CarP, Anti-hnRNP and GPI by Enzyme Linked Immuno-Sorbent Assay (ELISA), according to manufactures protocol (Sunlong System- China).

Results

Table-1 shows the results of different concentrations of markers along RA as well as control groups. These results showed highly significant differences between studied groups at $p<0.01$.

Table 1- Serum concentrations of biomarkers among study groups

Concentration	Case	Groups	No.	Mean ± SD	SE	P-value
ACPAs Ng/L	Patients	RF+ve	30	144.76± 5.20	28.52	0.001** (HS)
		RF-ve	30	115.40± 6.04	46.82	
	Control		30	80.70± 4.59	25.19	
Anti- MCV U/ML	Patients	RF+ve	30	119.57± 17.14	93.90	0.025* (S)
		RF-ve	30	60.58 ± 4.45	34.53	
	Control		30	45.46± 2.42	13.25	
Anti- CarP U/ML	Patients	RF+ve	30	13.25± 1.72	9.44	0.001** (HS)
		RF-ve	30	11.99 ± 0.95	7.39	
	Control		30	7.04± 0.48	2.64	
Anti- hnRNP Pg/ML	Patients	RF+ve	30	1962.22 ± 199.78	1094.27	0.002** (HS)
		RF-ve	30	1277.49± 96.82	749.96	
	Control		30	840.20± 47.51	260.22	
GPI Pg/ML	Patients	RF+ve	30	622.45 ± 23.42	128.28	0.000** (HS)
		RF-ve	30	539.51± 18.46	143.00	
	Control		30	465.90± 14.82	81.17	

(**) HS: Highly Sig. at P<0.01; S: Sig. at P<0.05; NS: No Sig. at P>0.05.

Receiver Operative Characteristic Curve (ROC) for the Biomarkers

Table-2 shows the results of the analysis of in depth sensitivity and specificity along with the positive and negative predictive values, which are the most basic and understandable parameters assessing diagnostic performance. The groups were divided according to + and - RF antibodies as a consequence of the detection of the elevated cut-off titters reflecting the most optimal or balanced sensitivity and specificity. In addition, significant differences among the parameters was considered less than fifty precent, with 95% confidence interval of area parameter in each status (RF+/-) of newly diagnosed RA patient groups.

Table 2- ROC values of study groups and biomarkers.

Study	Con.	Cut off Point	Area under curve	Asymp. Sig. (*)	Asymptotic 95% C.I.		Sensitivity	Specificity	Predicted Values	
					L.b.	U.b.			PP V	NP V
RF+ve	ACPA	125.06	0.714	0.001**	0.61	0.81	90.8	94.1	52.2	82.0
	Anti-MCV	68.00	0.685	0.004**	0.55	0.81	66.7	33.0	52.6	80.0
	Anti-CarP	11.05	0.521	0.001**	0.39	0.64	76.7	90.0	37.2	29.8
	Anti-hnRNP	1289.85	0.717	0.000**	0.60	0.82	74.9	61.9	52.5	82.0
	GpI	542.75	0.667	0.010**	0.54	0.78	77.3	76.7	50.0	89.5
RF-ve	ACPA	125.06	0.286	0.001**	0.18	0.39	88.2	86.6	91.9	50.9
	Anti-MCV	68.00	0.219	0.004**	0.12	0.31	70.0	70.2	80.4	53.2
	Anti-CarP	11.05	0.137	0.001**	0.06	0.21	93.3	78.5	76.7	38.3
	Anti-hnRNP	1289.85	0.081	0.000**	0.02	0.13	71.4	70.9	74.5	45.7
	GpI	542.75	0.148	0.010**	0.06	0.23	84.4	80.1	87.5	49.0

(**) HS: Highly Sig. at P<0.01; S: Sig. at P<0.05; NS: Non Sig. at P>0.05; L.b.: lower bound; U.b.: upper bound; " PPV: positive predictive value; NPV: negative predictive value".

The results showed higher sensitivity and specificity of ACPAs in RF+ than RF- patients, while anti-MCV and GPI showed higher sensitivity and specificity in RF- than RF+ patients. However, anti-CarP showed higher sensitivity and lower specificity in RF- than RF+ patients, whereas hnRNP showed lower sensitivity and higher specificity in RF- than RF+. It could be concluded that these markers can be used as good indicators for diagnosing RA.

Discussion

The present study is consistent with that of Mohamed and his co-workers who reported that RF and ACPA titres (mean± SD 186.84±258.05) in RA patients were significantly higher (P<0.0001) as compared to that of the control (3.59±6.31) [2]. Also, many studies revealed statistically significant differences between ACPA + and ACPA - RA patients for RF among patients and control groups [13]. This study is also in agreement with Sulaiman and his co-workers who reported a significant association of RF and ACPA antibody co-occurrence (P=0.002) was observed for both RF + or- when compare with ACPAs [14]. A previous functional study observed that the responses of the immune system in ACPAs + RA patients take place in a citrulline-specific manner and these antibodies to citrulline are able to enhance arthritis. Moreover, effectiveness of basophils from ACPAs +RA patients, contrary to that from of ACPAs - patients, was shown to occur upon exposure to citrullinated antigens. These findings indicated markedly different responses of immune cells to citrulline antigen [15].

Similarly, another study reported that the mean anti-MCV antibody serum level in RF+ RA patients (162.34 ± 128.33 U/ml) showed a highly statistically significant increase (*P* < 0.001) compared with RF- patients (86.3 ± 72.03 U/ml) [16]. Highly significant different levels of ACPAs and anti-MCV in RF + patients than those in RF - patients (P=0.005 vs. 0.011) were also reported [17]. This could be explained by the notion that vimentin might exert the primary response of the immune system in RA. That effectives T lymphocytes by joining with HLA-DR allele encoded the "shared epitope" has

determination. Furthermore, an existing of citrullination- vimentin in immune complexes of RA patients in synovium fluid for ACPAs and the specificity was high of anti-MCV additional emphasize the role of pathogenesis in RA [18]. Although anti-MCV antibodies used the panel for the diagnosis of RA (RF and ACPAs) could improve the early diagnosis of patients, mainly in patients that were seronegative for RF and ACPAs [19].

Serum anti-Carp level in the present study reported high significant at both groups, similar to study investigation whether anti-Carp levels was correlated with RF a statistical high significant in RA patients than healthy control (p=0.019) [20]. This agreement with the search results of an earlier studies [21, 22].The high significantly levels of anti-Carp in RA patients were determination than the control group (p < 0.001) [23].Thus, think out the first prove of correlation among the anti-Carp immune response and the incitement of RF like reactionary. That favour a level was high of defined or interaction among peptides that T- helper cells presentation, which consequence activate the B cells polyclonal to production two anti-carp and responses to RF. These events, can be serious to explain, either the region of Fc in IgG synovial fluid contains citrullination arginine or carbamylation lysine residues or what peptides were able to stimulate an antibodies response in patients of RA [24].

Similar to these finding of Lashkari and his co-workers, who demonstrated the anti-hnRNP/ RA33 concentration was (28.34±16.21) in patients with RA in compare with control (21.66±7.31) with high significant different at p=0.016 [25]. Which were in agreement with other studies that recorded significant different [26, 27].

Similar to the results of the current study, serum concentrations of G6PI Ag in RA patients and a healthy control group showed significant a difference ((5.0±1.7, 3.7±1.8 ng/ml, respectively, *P* < 0.001). Therefore, it seems to be a reliable marker for the diagnosis of RA [28]. Several hypotheses can explain the elevated GPI in sera and synovial fluids of RA patients. Hypoxic microenvironment can be the main factor participating to overexpression of GPI. Further, hypoxia can stimulate angiogenesis and elevate the expression of GPI in combination with human dermal microvascular endothelial cells (HDMECs) and rheumatoid arthritis synovial fibroblasts (RASFs). Hypoxia stimulation of angiogenesis is based on the expression of GPI in HDMECs and vascular endothelial growth factor (VEGF) Production by RASFsthe latter being organized by GPI [29]. The hypoxia modulation cellular bioenergetics by inducing dysfunction of mitochondrial and promoting a switch to glycolysis, that leading to abnormal angiogenesis [30]. The current study agrees with a previous report which recorded significantly elevated serum levels of biomarkers in RA patients than those in the control group (p≤0.001) [31].

Amongst the parameters used, ACPAs was the best prognosis and diagnosis tool for evaluation. The sensitivity of ACPAs assay in RA was 60–80% with a very high specificity was 95–99% the assay has a high significant predictive value and autoantibodies can be found early, even in the preclinical phase of RA [32].

In the present study, higher sensitivity and specificity of ACPAs were recorded in RF+ than those in RF- patients, with highly significant differences. This is in agreement with a study on Iraqi patients that observed the significantly higher sensitivity and specificity (40% and 100%, respectively, p=0.01) in early RA. Therefore, ACPAs are considered as a good indicator for RA disease because of their high level in both RF+ and RF- groups of RA patients [33].

In the present study, we found higher sensitivity and specificity in anti-MCV RF- than RF+ patients. Previous research suggested that the anti-MCV assay may be + in patients with RF and ACPAs – RA, allowing for improved classification of such patients as having RA, especially in the newly diagnosed disease [34]. Anti-MCV The study [35] was incorporated to evaluate the serial tests for anti-MCV and RF, with the combined sensitivity (90.7%–98.0%). All the calculation regarding the diagnostic values of combinations of the markers suggested p<0.0001 [36]. On the contrary, the results demonstrated a higher sensitivity of anti-MCV (68.6%) over the ACPAs (61.7%) in RA diagnosis. However the specificity of anti-MCV (94.2%) remained lower than that of ACPAs (97.1%) [37]. The elevation of anti-MCV levels is considered predictive of quick erosion in newly diagnosed RA disease and linked to disease activity and worse consequence [38, 39].

Also, in the current study, anti-carP had high specificity (90%) in RF+ and high sensitivity (93.3%) in RF- patients. Moreover, the positive predictive value (PPV) more than negative predictive value(NPP) these results agree with the low sensitivity of anti-CarP (32.2%) and the lower diagnostic potential as compared to ACPAs. As related to the specificity (96.7%), ACPAs had a higher potential

which was comparable to that of anti-CarP antibodies. However, ACPAs had higher AUC than that of anti-CarP. Also, in concordance with the current study, a previous work demonstrated anti-hnRNP sensitivity, specificity, PPV and NPV values of 79.4%, 76.47%, 77.14% and 78.79%, respectively [40]. On the contrary, our results are in disagreement with a study that found that anti-hnRNP occurred in a low sensitivity for diagnosing RA (7.32%), and considered as inefficient marker for in the diagnosis of early RA [41]. Other studies showed that the sensitivity of anti-hnRNP antibody was between 14.5% and 36%.9 [42, 43]. The present study reported that anti-hnRNP sensitivity was higher of among rheumatoid arthritis patients than those previously reported.

The result of this study confirmed the importance of GPI higher sensitivity and specificity as well as the positive predictive value which was higher in RF- than RF+ newly diagnosed RA patients. In an earlier study [44], the sensitivity of GPI ranged from 33% to 81.6% and the specificity ranged from 55.7% to 91.5% in patients with RA. There was also a positive correlation between RF and GPI (P < 0.01), as well as markedly higher PPV of ACPAs than that of GPI, while the NPV of GPI was markedly higher than that of anti-hnRNP antibody [45]. Also, there was a significant difference in serum G6PI concentration (p<0.001), which proposes it as a reliable marker for the diagnosis of RA [30].

The current study assessed sensitivity, specificity, PPV, and NPV of five serum markers and their various combinations and identified their high diagnostic value for RA and the support they provide for the early diagnosis of this disease.

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