ORIGINAL ARTICLE

Anti-mutated citrullinated vimentin antibodies in rheumatoid arthritis patients: Relation to disease activity and manifestations

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KEYWORDS
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Abstract Aim of the work: To evaluate the frequency of anti-mutated citrullinated vimentin antibodies (MCV) in rheumatoid arthritis (RA) patients and to correlate it with disease activity and various disease manifestations.

Patients and methods: Fifty RA patients were recruited from the rheumatology and rehabilitation outpatient clinic, Kasr Al-Aini. Thirty healthy subjects served as controls. All patients were subjected to full history taking and clinical examination including general and joint assessment. Disease activity was assessed by the disease activity score (DAS-28) and functional ability was evaluated by the Modified Health Assessment Questionnaire (MHAQ). Anti-MCV and anti-cyclic citrullinated peptide (anti-CCP) were assayed by ELISA in patients and controls. Plain X-ray was performed on the hands and wrists and Sharp score was used to assess the erosions and joint space narrowing.

Results: A highly significant elevation of serum anti-MCV in RA patients (135.82 ± 126.81 U/ml) compared to controls (13.63 ± 8.48 U/ml) (p < 0.0001) was found. Anti-MCV showed a sensitivity of 84% and specificity of 80%. There was a significant difference between anti-MCV positive and anti-MCV negative patients as regards MHAQ (1.07 ± 0.74 vs. 0.52 ± 0.37, p = 0.005) and Sharp erosion score (12.93 ± 23.55 vs. 4 ± 2.2, p = 0.02). Anti-CCP showed a
1. Introduction

Rheumatoid arthritis (RA) is a common systemic autoimmune disease. Its prevalence is between 0.5% and 1% worldwide. It is mainly characterised by persistent joint inflammation that results in loss of joint function and morbidity [1]. The American College of Rheumatology (ACR) 1987 revised criteria could still be used in the classification of RA and is primarily based on clinical parameters. The only serological criteria being IgM rheumatoid factor (IgM RF). However, the criteria may be insufficient for the diagnosis of early RA, as they are based upon measurements of disease classification predominately featuring manifestations typical of later-stage disease [2].

Numerous serological markers of RA have been described over the past 50 years. Among all these, Anti-Cyclic Citrullinated Peptide (CCP) antibodies have been proven to be highly specific, diagnostic and prognostic markers in RA [3]. In July 2010, the 2010 ACR/European League against Rheumatism (EULAR) rheumatoid arthritis classification criteria was introduced. These new classification criteria included anti-CCP testing [4], overruled the “old” ACR criteria 1987 [5] and are adapted for early RA diagnosis. The newest member of this autoantibody family is anti-mutated citrullinated vimentin (MCV) [3].

Vimentin is a protein found in mesenchymal and endothelial cells, monocytes and activated macrophages, citrullinated by peptidyl arginine deaminase enzyme [6]. In RA this citrullinated peptide activates T-lymphocytes by binding on HLA-DR4 on the surface of antigen presenting cells and may contribute to certain pathways in the pathogenesis of RA [7]. Studies on the diagnostic accuracy of anti-CCP antibodies report a higher sensitivity (up to 78%) and specificity (up to 95%) of anti-MCV. Besides the higher sensitivity it has been shown that anti-MCV is an even better prognostic marker for the outcome of RA, it correlates well with the disease activity score [7].

The aim of this work is to evaluate the frequency of anti-MCV antibodies in RA patients and to correlate its relation to disease activity and manifestations.

2. Patients and methods

Fifty RA patients attending the Kasr Al Aini Rheumatology and Rehabilitation Outpatient Clinic, and 30 healthy controls were involved in this study. All patients were previously diagnosed according to the 2010 ACR/EULAR RA classification criteria [4].

All patients gave informed consent to participate in the study, which was approved by the Kasr Al Aini medical ethics committee. Patients were subjected to detailed history, general and musculoskeletal examination and measurement of the 28-joint count of tender and swollen joint with calculation of the disease activity score (DAS-28) for each RA patient by DAS-28 score calculator [8].

Modified Health Assessment Questionnaire (MHAQ) was used for assessing the functional ability of the patients [9,10].

2.1. Laboratory tests

All patients were subjected to routine laboratory investigations.

Rheumatoid factor was assayed with a quantitative immunonephelometry test (Behring, Marburg, Germany).

Anti-CCP was measured using the Immunoscan CCPPlus® test kit using enzyme-linked immunosorbent assay (ELISA) for qualitative and semiquantitative detection of IgG antibodies to Cyclic Citrullinated Peptides (CCP) in human sera. Normal reference level is up to 25 U/ml.

Anti-MCV was measured using an indirect solid phase ELISA for the quantitative measurement of IgG class autoantibodies against Mutated Citrullinated Vimentin (MCV) in human serum (using kit from ORGENTEC Diagnostika GmbH). Normal reference level is up to 20 U/ml.

2.2. Conventional radiography

Sharp score was calculated to evaluate radiographic changes in RA patients and included the Sharp erosion score and the joint space narrowing (JSN) score [11].

Statistical methods: Comparison was done using Student’s t test for independent samples comparing 2 groups and the Mann Whitney U test. Comparison of numerical variables between more than two groups was done using the Kruskal Wallis test. For comparing categorical data, Chi square ($\chi^2$) test was performed. Correlation between various variables was done using Spearman rank correlation equation for non-normal variables. A linear multiple regression analysis for anti-MCV levels (dependent variable) was performed to assess the effect of possible independent variables. p-values less than 0.05 were considered statistically significant. All statistical calculations were done using computer programs SPSS version 15 for Microsoft Windows.

3. Results

The study included fifty RA patients, 47 females (94%) and 3 males (6%). The demographic, clinical and laboratory characteristics of the RA patients are demonstrated in Tables 1 and 2.

Serum Anti-MCV levels in the patients were detected with a no-nephelometry test (Behring, Marburg, Germany).

Comparison was done using Student’s t test. Comparison of numerical variables between more than two groups was done using the Kruskal Wallis test. For comparing categorical data, Chi square ($\chi^2$) test was performed. Correlation between various variables was done using Spearman rank correlation equation for non-normal variables. A linear multiple regression analysis for anti-MCV levels (dependent variable) was performed to assess the effect of possible independent variables. p-values less than 0.05 were considered statistically significant. All statistical calculations were done using computer programs SPSS version 15 for Microsoft Windows.

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higher than the corresponding values of the control group which had a mean of 13.63 ± 8.48 U/ml (p < 0.0001) (Fig. 1). The mean level of MCV in the 3 male patients was higher (165.3 ± 165.97 U/ml) compared to the level in females (133.94 ± 125.98 U/ml).

Methotrexate was received by 42 patients (84%) with a dose ranging from 12.5 to 25 mg/week (17 ± 7.94 mg/week). Eighteen patients (36%) received Hydroxychloroquine and corticosteroids (prednisone) were received by 22 (44%) with a dose ranging from 2.5 to 10 mg/day (3.25 ± 3.75 mg/d). Leflunomide (20 mg/day) was received by 12 patients (24%). Non steroidal anti-inflammatory drugs (NSAIDs) were taken regularly by 7 patients (14%) and at need by 36 (72%).

The sensitivity of anti-MCV was 84% with 80% specificity. The serum anti-CCP levels were detected with a mean of 252.63 ± 284.51. These values were significantly higher than the corresponding values of the control group which had a mean of 1.72 ± 1.104 (p < 0.0001). The sensitivity of anti-CCP was 70% with 100% specificity. There was no significant difference between the sensitivities of both anti-MVC and anti-CCP (p = 0.602), however, there was a statistically high significance between the specificities of both markers (p = 0.0314).

The patients were divided into 2 groups; anti-MCV positive and anti-MCV negative. Both groups were compared as regards various disease parameters (Table 3). There was no significant difference between anti-MCV positive and negative patients as regards to age, age of onset and disease duration. Anti-MCV positive patients had higher ESR and DAS28 scores, however, they were statistically insignificant (p = 0.27 and 0.17, respectively). The MHAQ score, Sharp erosion score, and anti-CCP titres were significantly higher in the RA patients with a positive anti-MCV compared to those with a negative anti-MCV (p = 0.005, p = 0.02, and p = 0.01, respectively) (Fig. 2). There was no significant difference between anti-MCV positive and negative patients as regards to ESR and DAS28.

There was no significant difference between anti-MCV and anti-CCP regarding the demographic, clinical or laboratory features of the RA patients. The rheumatoid factor (RF) positivity was more frequent in those with a positive anti-CCP (31/35 patients = 88.57%) compared
to those with a positive anti-MCV (34/42 patients = 80.95%).

No significant correlation could be elicited between serum anti-MCV and DAS28, MHAQ, ESR, Sharp score and frequency of extra-articular manifestations. The relation was negative with the age, age at disease onset, disease duration, presence of Sjögren syndrome and pulmonary manifestations, yet insignificant. Only the correlation of the anti-MCV with the anti-CCP antibodies was highly significant ($r = 0.49, p < 0.0001$) (Fig. 3). Furthermore, on performing a regression analysis, on the predictors of an increased anti-MCV level, only the anti-CCP was significant ($B = 0.18, p = 0.006$) among other independent factors including the age, disease duration, ESR, DAS28, erosion score and methotrexate dose.

### Table 3

Comparison between anti-MCV positive and negative rheumatoid arthritis (RA) patients as regards some of the demographic, clinical, laboratory and radiological characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Anti-MCV Positive ($N = 42$)</th>
<th>Anti-MCV Negative ($N = 8$)</th>
<th>$p$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-MCV (U/ml)</td>
<td>160.28 ± 124.02</td>
<td>7.39 ± 6.32</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.21 ± 10.79</td>
<td>44.50 ± 11.14</td>
<td>0.8 (NS)</td>
</tr>
<tr>
<td>Onset (years)</td>
<td>36.02 ± 10.13</td>
<td>40.25 ± 10.47</td>
<td>0.33 (NS)</td>
</tr>
<tr>
<td>Duration (years)</td>
<td>9.14 ± 7.54</td>
<td>4.03 ± 2.73</td>
<td>0.06 (NS)</td>
</tr>
<tr>
<td>ESR (mm/1st hour)</td>
<td>56.05 ± 30.63</td>
<td>42.75 ± 22.8</td>
<td>0.28 (NS)</td>
</tr>
<tr>
<td>DAS28</td>
<td>5.29 ± 1.516</td>
<td>4.58 ± 1.24</td>
<td>0.18 (NS)</td>
</tr>
<tr>
<td>MHAQ</td>
<td>1.07 ± 0.74</td>
<td>0.52 ± 0.37</td>
<td>0.005 (S)</td>
</tr>
<tr>
<td>Anti-CCP (U/ml)</td>
<td>291.56 ± 287.13</td>
<td>71.1 ± 171.7</td>
<td>0.01 (S)</td>
</tr>
<tr>
<td>Sharp Score</td>
<td>Erosion</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.93 ± 23.55</td>
<td>4 ± 2.2</td>
<td>0.02 (S)</td>
</tr>
<tr>
<td></td>
<td>JSN</td>
<td>68 ± 16.63</td>
<td>57.5 ± 11.92</td>
</tr>
</tbody>
</table>

ESR: Erythrocyte Sedimentation Rate; MCV: Mutated Citrullinated Vimentin; MHAQ: Modified Health Assessment Questionnaire; DAS28: Disease Activity Score 28; Anti-CCP: Anti-Cyclic Citrullinated Peptide; JSN: Joint space narrowing; NS: Non significant; S: Significant.

4. Discussion

The Rheumatoid arthritis disease course can follow two distinct clinical phenotypes characterised by mild disease activity with less joint destruction and fewer co-morbidities or high disease activity, more joint destruction and higher co-morbidities. Therefore, it is important to select patients who would benefit most from aggressive treatment in the early phase of the disease [12].

Anti-citrullinated protein antibodies (ACPA) have been reported as more specific serological markers of RA. They provide a superior alternative to the RF test in laboratory diagnostics of RA. This autoantibody family is an overlapping group of antibodies dependent on the citrullination of arginine residue [13]. Only Anti-Cyclic Citrullinated Peptide (CCP) antibody is used in clinical practice. Due to the high clinical potential of ACPAs, this biomarker was deservedly included in the new RA classification criteria released based on collaborative efforts between the ACR and EULAR [4]. A new member of this autoantibody family is anti-MCV [3]. With this as a background, we were stimulated to study the role of anti-MCV as a marker in RA and compare it to anti-CCP.

In the present study, we found that the sensitivity of anti-MCV is 84% and the specificity is 80%. This agrees with the studies of Poulson and Charles [2] and Bang et al. [14] who reported a sensitivity of 84% and 82% and specificity of 87% and 88%, respectively. However Liu et al. [3] reported a lower sensitivity and higher specificity (78.2% and 93.4%, respectively). Sizova [15] reported a lower sensitivity (53.3%) and comparable specificity (83.3%).

In this study there was no significant difference between RA patients and healthy controls from one side and between patients who were anti-MCV positive and those who were anti-MCV negative with respect to age, age of onset, disease duration, sex and extra-articular manifestations. This was in agreement with the results found in the studies of Wagner et al. [7] and Engelmann et al. [16].
No significant difference was found between anti-MCV positive and anti-MCV normal groups as regards parameters of disease activity represented in the Disease Activity Score 28 (DAS28) and ESR, although anti-MCV positive patients had higher ESR and DAS28 scores than the anti-MCV negative group. Furthermore, no significant correlation was present between anti-MCV level and these parameters. This finding was in agreement with the findings of other studies [3,16,17] who showed that the DAS28 was higher in the anti-MCV-positive group than in the negative group, however no significant statistical correlation was found between circulating levels of anti-MCV with DAS28 and ESR. They all concluded that it is not useful to monitor disease activity with anti-MCV levels.

The present study did not find any correlation between anti-MCV and MHAQ, however, there was a statistically significant difference between anti-MCV positive and negative groups as regards MHAQ. This finding suggests that anti-MCV could be useful for the assessment of functional disability in RA.

A significant difference between anti-MCV positive and negative groups as regarding the radiographic damage of joints was present with a higher mean Sharp score in the positive group. This finding points to the important role anti-MCV positivity in disease severity as assessed by the radiological damage of joints. This was in agreement with the results of other studies [3,17,18] that proved aggressive peripheral joint disease in anti-MCV positive groups. In addition, Liu et al. [3] remarkably proved that anti-MCV-positive patients with early RA already had more severe radiographic damage than those with a negative anti-MCV. However, other coworkers [7,19,20] did not find a significant correlation between anti-MCV level and radiographic scores.

In the current work, anti-MCV was strongly correlated with anti-CCP but weakly correlated with RF. This finding is consistent with the literature in the studies done by Mutlu et al. [21] and Mathsson et al. [19]. In the present study, the anti-CCP was positive in 35/50 (70%) of the RA patients while none of the controls had a positive anti-CCP.

In our study, the reported sensitivity for anti-CCP was 70% and specificity was 100%. The anti-MCV sensitivity for RA was found to be higher than that of the anti-CCP (84% vs. 70%). Comparable results have been found in the studies of Wagner et al. [6] (71.5 vs. 69%), Bang et al. [14] (sensitivity 82 vs. 72%), Coenen et al. [22] (74.5 vs. 66.4%), Soós et al. [23] (75.6 vs. 66.4%) and Mathsson et al. [24] (70.7 vs. 57.9%). This concludes that the sensitivity of anti-MCV is superior to anti-CCP.

As for the specificity, anti-CCP was reported to be higher when compared to anti-MCV in our study (100 vs. 80% respectively). This was in agreement with the results of Wagner et al. [7] (97.6 vs. 81.3%), Dejaco et al. [24] (98.7 vs. 90.8%), Coenen et al. [22] (96% vs. 90.3%) and Soós et al. [23] (98.3% vs. 91.5%). Only one study done by Bang et al. [14] found a higher specificity of anti-MCV compared to the anti-CCP (98% vs. 96%).

In conclusion, despite the great expectations, the anti-MCV assay did not provide significant additional diagnostic value over the anti-CCP assay. Although credited with higher sensitivity, the anti-MCV assay obtained lower specificity in comparison to the anti-CCP assay which is clinically the most...
relevant. However, it could be of diagnostic importance in anti-CCP negative patients due to its high sensitivity. Further studies on a larger number of cases and with a longitudinal study design are required to prove the relation of anti-MCV with disease activity and different manifestations. For now, it seems that the only obvious advantage of anti-MCV over anti-CCP antibodies is a closer association with radiologic progression, thus making the anti-MCV titre useful in detecting a more aggressive and erosive disease. Testing for these antibodies early in the disease can guide the choice of initial therapy, reserving aggressive regimens for those with positive anti-MCV titres and high radiographic scores who are suspected to have an aggressive early erosive disease course.

Conflict of interest

The authors declare no conflict of interest.

References


