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Original article

Independence of carbohydrate-deficient isoforms of transferrin and cyclic citrullinated peptides in rheumatoid arthritis

Monika Gudowska[a], *Ewa Gindzienska-Sieskiewicz*[b], *Ewa Gruszewska*[a], *Bogdan Cylwik*[c], *Stanislaw Sierakowski*[b], *Maciej Szmitkowski*[a], *Lech Chrostek*[a,*]

[a] *Medical University of Białystok, Department of Biochemical Diagnostics, Białystok, Poland*
[b] *Medical University of Białystok, Department of Rheumatology and Internal Diseases, Białystok, Poland*
[c] *Medical University of Białystok, Department of Pediatric Laboratory Diagnostics, Białystok, Poland*

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ABSTRACT

Objective: The aim of this study was to assess the relationship between the two types of posttranslational modifications of proteins in RA: glycosylation on the example of carbohydrate-deficient transferrin and citrullination by means of autoantibodies to cyclic citrullinated peptides.

Methods: The study was carried out in 50 RA patients. CDT was measured using N Latex CDT immunonephelometric test, the results were presented in absolute and relative units. Anti-CCP were measured using the chemiluminescent method and rheumatoid factor by immunoturbidimetric method.

Results: 80% of RA patients were positive for anti-CCP, 70% for RF and 62% for both, anti-CCP and RF. The level of %CDT was significantly elevated, but absolute CDT level was not changed. The mean absolute CDT concentration was higher in anti-CCP positive patients than that in anti-CCP negative. CDT (absolute and relative concentration) did not correlate with anti-CCP and RF. However, serum RF significantly correlated with anti-CCP. %CDT did not correlate with anti-CCP, but absolute level correlated with anti-CCP only in anti-CCP negative and RF negative patients. CDT did not correlate with RF, but solely with anti-CCP in anti-CCP negative patients. Anti-CCP correlated with DAS 28 only in anti-CCP negative RA, but CDT (absolute and relative units) correlated with DAS 28 in all patients and in anti-CCP positive RA.

Conclusions: These results suggest that the changes in CDT and anti-CCP concentrations are not associated with oneself and indicate on the independence of these posttranslational modifications in rheumatoid arthritis. Only the alterations in transferrin glycosylation reflected the activity of RA.

* *Corresponding author.*
E-mail: chrostek@umb.edu.pl (L. Chrostek).

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Independência de isoformas de transferrina deficiente em carboidrato e peptídeos citrulinados cíclicos na artrite reumatoide

Palavras-chave:
Anticorpos antipeptídeo citrulinado cíclico
Transferrina deficiente em carboidrato
Fator reumatoide
Artrite reumatoide

RESUMO

Objetivo: O objetivo deste estudo foi avaliar a relação entre os dois tipos de modificações pós-translacionais de proteínas na AR: glicosilação no caso da transferrina deficiente em carboidrato (TDC) e citrulinação por meio dos anticorpos no caso do antipeptídeo citrulinado cíclico (anti-CCP).

Métodos: O estudo foi realizado em 50 pacientes com AR. A TDC foi medida utilizando o teste imunonefelométrico N Latex CDT, e os resultados foram apresentados em unidades absolutas e relativas. O anti-CCP foi mensurado usando o método quimioluminescente e o fator reumatoide (FR) pelo método imunoturbidimétrico.

Resultados: 80% dos pacientes com AR foram positivos para anti-CCP, 70% para FR e 62% para ambos (anti-CCP e FR). A percentagem de transferrina total (%TDC) esteve significativamente elevada, mas o nível absoluto de TDC não esteve alterado. A concentração média de TDC absoluta foi maior nos pacientes anti-CCP positivos do que naqueles anti-CCP negativos. A TDC (concentração absoluta e relativa) não se correlacionou com o anti-CCP e o FR. No entanto, o FR sérico se correlacionou significativamente com o anti-CCP. O percentual de TDC não se correlacionou com o anti-CCP, mas seu nível absoluto se correlacionou com o anti-CCP apenas em pacientes FR negativos e anti-CCP negativos. A TDC não se correlacionou com o FR, somente com o anti-CCP em pacientes anti-CCP negativos. O anti-CCP se correlacionou com o DAS 28 apenas nos pacientes com AR anti-CCP negativos, mas a TDC (unidades absolutas e relativas) se correlacionou com o DAS 28 quando considerados todos os pacientes com AR e em pacientes com AR anti-CCP positivos.

Conclusões: Estes resultados sugerem que as alterações na TDC e as concentrações de anti-CCP não estão associadas entre si e indicam a independência destas modificações pós-translacionais na artrite reumatoide. Apenas as alterações na glicosilação da transferrina refletem a atividade da AR.

Introduction

Rheumatoid arthritis (RA) is a chronic, autoimmune disease of connective tissues, with the appearance of several types of autoantibodies.[1] The specific autoantibodies generated in RA have been associated with the posttranslational modifications of proteins and peptides.[2] These modification involve glycosylation, citrullination, methylation, acetylation and ubiquitination occur in physiological conditions and have an important role in the normal function of the immune system.[3] The first type of autoantibodies founded in RA patients was rheumatoid factor (RF), which is an autoantibody to Fc domain of IgG. The changes in glycosylation (galactosylation/sialylation) IgG have been found to be associated with a pathogenesis of RA and can be diagnostically and therapeutically useful.[4] The several studies were conducted to recognize the alterations in glycosylation of others glycoproteins such as IgA, alpha 1-acid glycoprotein, fibronectin, haptoglobin and transferrin. Human transferrin exists as an heterogeneous population glycosylated variants differing in carbohydrate composition.[5] In healthy people mostly occur tetrasialylated glycoforms and glycoforms which lack one or both of complete N-glycans are called carbohydrate-deficient transferrin (CDT).[5] It has been assumed that CDT is the sum of three isoforms: asialo-, monosialo- and disialotransferrin. The glycosylation of plasma transferrin changes in RA and can lead to increase the CDT level.[6] The newly known posttranslational modification in RA is citrullination.[7] It is an enzymatic conversion of arginine to citrulline catalysed by peptidylarginine deiminases (PAD). In patients with RA, PAD may leak out of the dying cells in the synovial joints which can citrullinate of arginine and generate an cyclic citrullinated peptides (CCP).[8,9] Autoantibodies against CCP are considered as early diagnostic and prognostic biomarker of RA.[10] Their presence in RA-negative patients testifies for the early RA with worse prognosis.[8] In RA patients many citrullinated proteins have been detected, as fibrinogen, vimentin, enolase and type II collagen.[3] The aim of this study was to assess the relationship between different types of posttranslational modifications – glycosylation and citrullination – in RA by means of the concentration of CDT and anti-CCP.

Material and methods

The study was carried out in 50 RA patients (41 females, 9 males; mean age 53) recruited from the Department of Rheumatology and Internal Diseases (Medical University in Bialystok). The diagnosis of RA was confirmed according to criteria which were set by the American College of Rheumatology (ACR). All patients were interviewed regarding their use of alcohol. The patients drank alcohol only occasionally.

Table 1 – The results of laboratory tests in rheumatoid arthritis (RA) patients and controls.

	CRP (mg/L)	RF (IU/mL)	IgG (g/L)	CDT (mg/L)	%CDT	Anti-CCP (U/L)
RA ($n=50$)	22.96 ± 25.54	172.23 ± 229.37	11.79 ± 3.06	42.70 ± 69.40	2.03 ± 0.24	185.42 ± 325.02
	$p=0.000$[a]	$p<0.01$[a]	$p=0.904$	$p=0.564$	$p<0.01$[a]	$p=0.000$[a]
Controls ($n=33$)	1.04 ± 0.78	21.58 ± 1.23	11.43 ± 1.42	42.52 ± 9.23	1.77 ± 0.22	0.57 ± 0.21

Data are mean and standard deviation.

[a] $p<0.05$ the differences between RA patients and control group (Mann–Whitney U test).

Control group consisted of 33 healthy subjects (mean age 41) recruited from hospital workers. Blood fasting samples were taken by vein puncture after 12 h of fasting. All (healthy and sick) subjects gave their informed consent to participate in the studies. This study was in accordance with the Bioethical Committee at the Medical University in Bialystok and with the Helsinki Declaration.

RA activity was determined by disease activity score (DAS 28) according to the formula:

$$\text{DAS}\,28 = 0.56 * \text{sqrt(t28)} + 0.28 * \text{sqrt(s28)} + 0.7 * \ln(\text{ESR}) + 0.014 * \text{VAS}$$

The number of tender joints (t28), swollen joints (s28), erythrocyte sedimentation rate (ESR) and visual analogue scale (VAS) were used in that formula. Low activity of RA (DAS 28 between 2.6 and 3.2) had none of patients, moderate activity (DAS 28 in the range of 3.2–5.1) had 27.08% patients and high activity (DAS 28 above 5.1) had 72.92% of patients. CDT was measured using N Latex CDT immunonephelometric test on BN II analyser (Siemens Healthcare Diagnostics, Somerville, USA). The results were given as absolute CDT units (mg/L) and percentage of total transferrin (%CDT). The antibodies against cyclic citrullinated peptide (anti-CCP) were measured on the Architect i2000 analyser. The results above 5 U/L are considered negative and ≥5.0 U/L positive. RF (normal values < 30 IU/mL), C-reactive protein (CRP; normal range: 0.8–1.2 mg/L) and immunoglobulin G (IgG; normal range: 5.52–16.31 g/L) were determined using immunoturbidimetric methods on the Architect c8000 (Abbott Laboratories, Abbott Park, IL, USA). ESR (mm/h) was measured by Westergren method on the Sediplus S 2000 (Sarstedt, Germany).

Statistical analysis

The differences between tested and control group were evaluated by Mann–Whitney U test. We considered p-values <0.05 as statistically significant. The correlation between variables was assessed using Spearman's rank correlation coefficient.

Results

The results of laboratory tests in RA patients and controls are presented in Table 1. The mean levels of CRP, RF, %CDT and anti-CCP were significantly higher in RA patients in comparison to the control group. 80% of RA patients were positive for anti-CCP, 70% for RF and 62% for both, anti-CCP and RF.

Table 2 – Correlation of CDT with anti-CCP.

	Test	R	p
All RA	CDT	0.088	0.547
	%CDT	0.033	0.821
Anti-CCP positive	CDT	−0.212	0.195
	%CDT	−0.069	0.677
Anti-CCP negative	CDT	0.696	0.025[a]
	%CDT	0.364	0.301
RF positive	CDT	−0.063	0.741
	%CDT	0.223	0.235
RF negative	CDT	0.629	0.012[a]
	%CDT	−0.016	0.954

[a] Significant correlation.

CDT concentration did not correlate with anti-CCP in all RA patients (Table 2), but only in anti-CCP negative ($r=0.696$) and RF negative ($r=0.629$) subjects. The comparison of CDT levels (absolute units) in relation to anti-CCP and RF concentrations exhibited the significant difference only considering the anti-CCP levels. In the anti-CCP positive patients, CDT concentration (43.1 ± 7.4 mg/L) was significantly higher than in anti-CCP negative subjects (38.9 ± 2.4 mg/L) ($p=0.022$) (Fig. 1).

CDT concentration (absolute and relative units) correlated with RA activity, it means with DAS 28 in all patients (Table 3). The high RA activity is associated by the low absolute CDT and high relative CDT concentration. CDT (expressed in both units) correlated with DAS 28 regardless of anti-CCP levels but % CDT in RF positive patients. Anti-CCP concentration did not

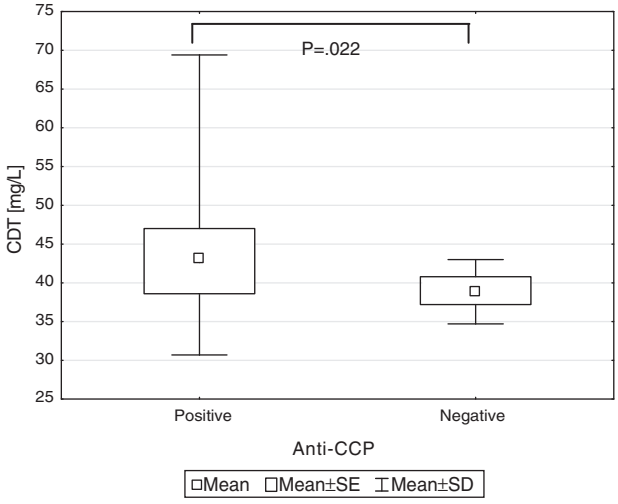

Fig. 1 – Carbohydrate-deficient transferrin (CDT) concentration in anti-cyclic citrullinated peptides (CCP) positive and anti-CCP negative patients.

Table 3 – Correlation of disease activity score (DAS) 28 with anti-CCP and CDT.

	Test	R	p
Correlation between DAS 28 and CDT			
All RA patients	CDT	−0.350	0.014[a]
	%CDT	0.321	0.026[a]
Anti-CCP positive	CDT	−0.446	0.003[a]
	%CDT	0.324	0.047[a]
Anti-CCP negative	CDT	0.567	0.112
	%CDT	0.627	0.070
RF positive	CDT	−0.306	0.094
	%CDT	0.399	0.026[a]
RF negative	CDT	−0.428	0.144
	%CDT	0.319	0.288
Correlation between DAS 28 and anti-CCP			
All RA patients		0.050	0.739
Anti-CCP positive		0.193	0.246
Anti-CCP negative		0.748	0.020[a]
RF positive		0.113	0.550
RF negative		−0.198	0.517

[a] Significant correlation.

correlate with DAS 28 in all RA patients, but only in anti-CCP negative subjects.

CDT did not correlate with RF in all RA patients but considering the anti-CCP and RF values (positive or negative) ($p > 0.05$ for each comparisons). In contrary, anti-CCP correlated with RF in all RA subjects ($r = 0.348$; $p = 0.019$), and in the anti-CCP negative subjects ($r = 0.750$; $p = 0.032$).

DAS 28 did not differ between anti-CCP positive (5.63 ± 1.37 U/L) and anti-CCP negative patients (6.14 ± 1.20 U/L) ($p = 0.337$) and between RF positive (5.84 ± 1.22 U/L) and RF negative subjects (5.06 ± 1.41 U/L) ($p = 0.113$). RF value was significantly higher in anti-CCP positive RA (176 ± 216 IU/mL) than that in anti-CCP negative (142 ± 307 IU/mL) ($p = 0.047$), but anti-CCP did not differ between RF positive (222 ± 378 U/L) in comparison to RF negative RA (117 ± 224 U/L) ($p = 0.126$).

Discussion

The present study demonstrated the lack of association between concentration of carbohydrate-deficient isoforms of transferrin (CDT) and the level of citrullinated proteins and peptides measured by autoantibodies (anti-CCP) in RA patients. Taking into consideration the relationship of anti-CCP or CDT with DAS 28, there were only the association with CDT concentration. Our results are in accordance with the study of Serdaroğlu et al.[11] and Aridoğan et al.[12] who proved no significant correlation between anti-CCP and DAS 28. Likewise, they did not found a significant difference in DAS 28 between anti-CCP negative and anti-CCP positive patients and between RF positive and RF negative subjects. Similarly, they found the significant differences between anti-CCP positive and anti-CCP negative RA patients for RF. The only one study, Önder and co-workers, revealed that anti-CCP were positively associated with higher scores of DAS-28, while these were not associated with ESR and CRP.[13] In the other study, a weak correlation between anti-CCP and DAS 28 was observed, but all RA patients were positive for anti-CCP.[14] Although, there was no correlation between CDT and anti-CCP in RA, there was association between two serological markers of RA, it means RF and anti-CCP. CDT correlated with anti-CCP only in anti-CCP negative and RF negative patients, thus in seronegative RA. This phenomena may be responsible for significantly higher CDT concentration in anti-CCP-positive than in anti-CCP-negative RA patients. Although, as demonstrated Aridoğan et al.,[12] antibodies against CCP were more specific for RA than RF (100% vs. 96.8%), anti-CCP did not correlate with disease activity (DAS 28) but with RF. The diagnostic accuracy (area under the ROC curves) of anti-CCP was also higher than that of RF.[15] The lack of correlation of disease activity with anti-CCP and its existence with CDT may be indicated on the time-dependent difference in the activity of these two different posttranslational modifications of the proteins. DAS 28 indicates the current disease activity, but the activation of PAD and citrullination of various proteins overtakes the clinical symptoms of disease, but the alterations in transferrin glycosylation undergo parallel with the development of disease. Hamad et al. also found that the presence of high anti-CCP level is not associated with higher disease activity.[16] However, anti-CCP positivity and titre correlated with disease duration, what can enforce our hypothesis about time-dependent shift in citrullination towards glycosylation in RA. Anti-CCP can be found early in the course of RA, even years before the onset of clinical symptoms.[17] It is very interesting that the anti-CCP status may change (from negative to positive and vice versa) after 3 years from the onset of RA and the mean serum anti-CCP level declines during the years.[18]

A major conclusion of this study is that, in RA patients the changes in transferrin glycosylation measured by means of carbohydrate-deficient isoforms (CDT) are not related with citrullination, other type of posttranslational modification in RA.

Conflicts of interest

The authors declare no conflicts of interest.

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