Antibodies to Cyclic Citrullinated Peptides (Anti-CCP) as a Marker of Association between Type 1 Diabetes Mellitus (T1DM) and Rheumatoid Arthritis (RA) in Children and Adolescent

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Abstract: Aims/Introduction: The association between Type 1 diabetes mellitus (T1DM) and rheumatoid arthritis (RA) remains controversial. The aim of this work was to find out the prevalence of anti-CCP in children and adolescences with T 1 DM and to determine whether anti-CCP could serve as marker for the development of RA in these patients. **Material and Methods:** We studied 42 children and adolescents with T1DM, 20 patients with RA, and 40 healthy age and sex matched controls. The studied groups were investigated for anti-CCP and RF and the result were statistically analyzed. **Results:** The number of anti-CCP positive patients in the diabetic group was not statistically different when compared with the healthy control group (1 vs. 0, $P = 1$) but was statistically lower than in the RA group (1 vs. 9, $P < 0.0001$). Family history of RA was detected in only two of the diabetic patients with no significant difference than the control group. Family history of first degree relative with type 1 DM was not statistically significant different between the RA group and the control population ($p = 0.4$). **Conclusions:** No significant difference in anti-CCP antibodies expression in patients with T1DM than the healthy control. Screening of anti-CCP antibodies may not appear to be useful in the follow-up of patients with type 1DM unless there are joint abnormalities.

[Doaa Shahin, Rawia A. Swelam, Abeer Fathy, Dina A. Shahin and Mohamed Attiya. **Antibodies to Cyclic Citrullinated Peptides (Anti-CCP) as a Marker of Association between Type 1 Diabetes Mellitus (T1DM) and Rheumatoid Arthritis (RA) in Children and Adolescent.** *Life Sci J* 2013;10(1):3061-3065]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 378

Key words: anti-CCP, Type 1 diabetes mellitus**,** rheumatoid arthritis.

1. Introduction:

The co-occurrence of autoimmune diseases such as rheumatoid arthritis (RA) and type 1 diabetes mellitus (Type 1 DM) has been reported in individuals and families. [(1)]. Although the exact etiologies of RA and T 1 DM are unknown, they are likely attributable to a combination of genetic susceptibility and interactions between environmental risk factors and genes. RA and T1DM are associated with the same human leukocyte antigen (HLA) specificity; HLA-DR4. Thus, T1DM and the Auto immune connective tissue diseases are considered to be in the same immunologic cluster. [(2)] In addition, both diseases were associated with the 620W allele of the protein tyrosine phosphatase N22 (PTPN22) gene, prompting the researchers to suggest that this gene variant might "represent a common pathway for the pathogenesis of these two diseases." [(3)].

Few studies addressed the question of an association between prevalent RA and T1 DM. A systematic review examining the prevalence of organ-specific autoimmune diseases in patients with RA and their relatives found an excess of such disease in comparison with control subjects, but specific excess of T 1 DM remains to be confirmed [(4)]. Moreover, in the last years, several studies showed that anti-CCP represent a sensitive and specific serologic marker for RA. Moreover, a large body of evidence has shown that anti-CCP may also serve as an early and prognostic marker in RA [(5)]. Liao *et al.* [(1)]. had shown that the odd ratios of co-existing T1DM in RA patients was only significantly increased in individuals with antibodies against anti-CCP.

The anti-CCP antibodies had found to exhibit better specificity than IgM rheumatoid factor (IgM-RF) [(6)] in diagnosing RA. However, high titres of anti-CCP can occasionally be found in patients with inflammatory myopathy [(7)], and psoriasis [(8)]

Furthermore, Anti-CCP antibodies are found in the sera of RA patients a median of 4.5 years before the overt disease .The importance of such a notion lies not only in the ability to prevent life-threatening manifestations but also in the ability to treat and even prevent overt autoimmune diseases. [(9)].

So, the aim of this work was to find out the prevalence of anti-CCP in children and adolescences

with type 1 DM and to determine whether anti-CCP could serve as marker for the development of RA in these patients.

2. Patients and methods

I. Study design and study population:

A case control study was conducted and approved by the local ethical committee. The studied population was divided into three categories:

1. Forty two children and adolescents who fulfilled the American Diabetes association diagnostic criteria [10]. These patients were randomly selected independently to their RA status.
2. Twenty patients fulfilled the International League of Associations for Rheumatology (ILAR) criteria for diagnosis of juvenile idiopathic arthritis (JIA) [11]. All patients were under eighteen and the diagnosis of JIA had been confirmed for at least six months.
3. Both the DM and JIA patients groups were studied in comparison with 40 healthy age and sex matched controls.

All the guardians of study participants gave their informed consent. The clinical data were assessed at the time of blood donation, and all the demographic, clinical and serological characteristics of the studied subjects were evaluated and recorded by a pediatrician and a rheumatologist.

II. Exclusion criteria:

Individuals with a personal history of malignancy, systemic lupus erythematosus or autoimmune connective tissue disease (other than RA), viral hepatitis or treatment with interferon-α were not eligible for the present study.

III. Laboratorial analysis:

Anti-CCP: was measured by electrochemiluminescence immunoassay (ECLIA) using COBAS INTEGTRA 400 machine (Roche Diagnostics, Indianapolis, Ind) with a titre of >17 U/ml considered as positive. [12]

RF: RF was measured using immunoturbidimetric technique on the Cobas Integra 400 analyser (Roche Diagnostics, Indianapolis, IN, USA), using reagents and calibrators from Roche [13]

Statistical analysis

Data were analyzed using SPSS (version 17). Data were expressed as mean reanalyz frequencies. Differences between continuous variables were analyzed by t-test, Dichotomous variables were analyzed by Fishermous variables, were analyzed by t-test mean <0.05 were considered significant.

3. Results:

Overall population and TIDM:

Table 1 shows the clinical data and the laboratory parameters of the studied groups. Anti CCP antibodies were found to be positive in only one diabetic patient and in 9 of the RA group. The number of anti-CCP+ patients in the diabetic group was not statistically different when compared with the healthy control group (1 vs. 0, ***P*** = 1) but was statistically lower than in the RA group (1 vs. 9, ***P***< 0.0001). The only Anti CCP positive diabetic patient was 12 years old male with no joint abnormalities. He had negative rheumatoid factor and no family history of RA. Family history of RA was detected in only two of the diabetic patients with no significant difference than the control group. Family history of first degree relative with type 1 DM was not statistically significant different between the RA group and the control population (*p* 0.4).

JIA patients:

Positive **anti-CCP** was found in 9/20(45%) of JIA cases while 11/20(55%) were negative for **anti-CCP** . Out of 9 JIA cases with positive **anti-CCP** ,9 patients had positive RF, versus only one out the 11 JIA cases with negative **anti-CCP** with statistically significant difference between them (*P*< 0.001). of note most the patient with polyarticular disease (72.7%) had positive anti-CCP (*P*= 0.01) as shown in table (2).

Table 1. Demographic, laboratorial and serological characteristics of the studied groups.

	Diabetic group (n = 42)	***Rheumatoid arthritis group* (n = 20)**	***Control group (n =40)***	***P****
Age (years) (mean ± SD)	8.6± 2.9	7.9± 3.4	7.4± 3.1	ns
Gender(male /female)	14/6	8/7	23/19	ns
Duration of diabetic state in years (mean ± SD)	4.2 ± 2.1	-	-	
Family history of type 1 DM	6(14.4%)	1(5%)	2 (5%)	ns
Associated other autoimmune diseases**	3 (7.2%)	0	0	
Family history of rheumatoid arthritis	2 (4.8%)	4 (20%)	3 (7.5%)	ns
+ve rheumatoid factor	0	7 (35%)	0	<0.0001
+ve anti-CCP	1 (2.4%)	9 (45%)	0	<0.0001

* *P* for fisher's exact test for 2X3 contingency table.

** Two patients had associated autoimmune thyroiditis, one patient had vitiligo

Table (2): Demographic and clinical and laboratorial characteristics of anti-CCP positive and anti-CCP negative RA patients

	Anti-CCP-ve (n = 11)	Anti-CCP +ve (n = 9)	*P* value
Age (years)	7.4± 3.1	10.4± 3.1	ns#
Gender female/male	4/7	7/2	ns*
Tender joint count	2.6± 3.0	5.2 ± 5.7	(ns)#
ESR mm/hr	33.6 ± 21.3	37.9 ± 26.8	ns#
CRP mg/L	29.16± 15.6	30.51± 25.6	ns#
+ve rheumatoid factor	1	9	0.0001*
Polyarticular disease	3(27.3%)	8(72.7%)	0.01**
Oligoarticular disease	7(87.5%)	1(12.5%)	
Systemic onset disease	1	0	

*p<0.05, NS: Nonsignificant
*= Fisher's exact test
**= Fisher's exact test for a 2x3 contingency table.
#= t test.

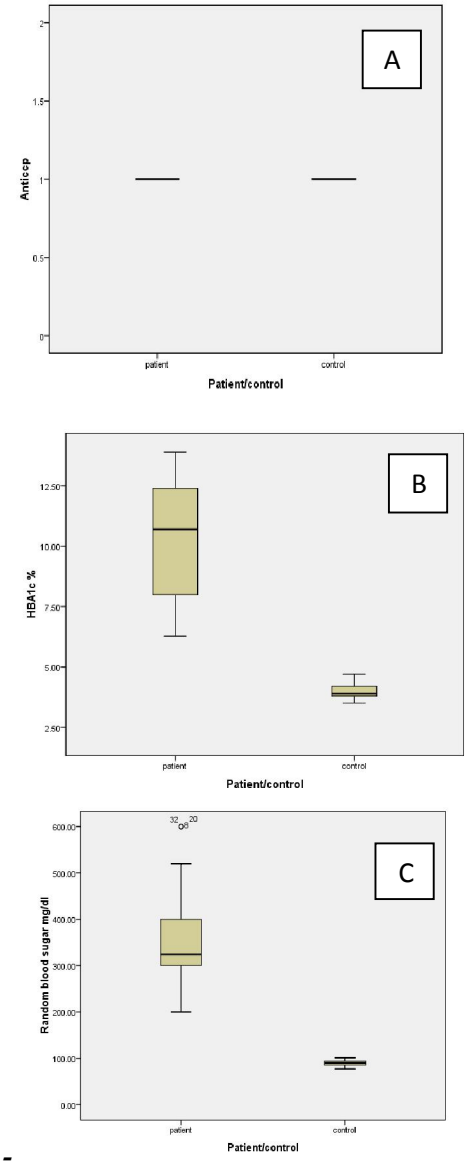

Figure 1: Interaction box plot representing the Anti-CCP, RBS, and HBA1c in DM patients and control groups (A,B,C respectively)

4. Discussion:

There are numerous genetic factors associated with increased risk for autoimmunity including HLA alleles containing the shared epitope (SE), PTPN22 polymorphism, and others [3, 14- 16]. The association between Type 1 diabetes mellitus and rheumatoid arthritis remains controversial.

In the current study, no significant difference in anti - CCP antibodies expression was found in patients with T 1 DM compared with the healthy control. This comes in agreement with the finding of Desplat-Je´go *et al.* [17] who reported rare prevalence of anti- CCP antibodies in patients with type 1 DM. They suggested weak association between type 1 DM and immune processes leading to RA. Furthermore, Simards and coworkers [18] suggested a non-significant association between diabetes and RA (despite differences in their study design, race and age of the population studied compared with our study) . On the other hand, Liao, *et al.* [1] have found that T1DM was associated with an increased risk of anti-CCP positive RA (OR 7.3). This controversy in results might be attributed to different age of the population studied. In our study, the age is under 18 however Liao *et al.* [1] Studied cases aged 18- 70. Therefore, our cases might develop RA later in life.

In the present study, non of the RA patients had type 1 DM and the family history did not differ than that of the controls. In accordance with this, the findings of Hakala *et al.*[19] that the prevalence of type 1 DM in the RA cohort was similar to that in the general population.

On the other hand, Thomas *et al.* [20] ,in their study conducted on 295 RA patients found that 13% of the studied patients had a first-degree relative with type 1 diabetes. Epidemiologic studies have identified several potential risk factors for the disease, but it is not known at what point during disease evolution these factors are important [21,22]. However, elevated autoantibodies, cytokines, and inflammatory markers occur years prior to clinical onset of joint symptoms,

suggesting that epidemiologic factors may play an early role in the development of RA [23-25]. In our study anti-CCP measurement identified only one diabetic patient with high titre without clinical signs suggestive of RA. this patient must be followed up for several years since anti- CCP might predict RA.

In the current study, anti-CCP antibodies were detected in 45% of patients with JIA most of them have polyarticular disease. This was consistent with the findings of Habib *et al.* [26] and Van Rossum and colleagues [27] however, much less figures(2%) were reported by Avcin and coworkers [28] and Sandra and associates. [29] It seems that Anti- CCP can be detected in children and adolescents with JIA but are less frequently present than in adults with RA. However it still remains to be determined whether anti-CCP could identify a subset of JIA with the potential to progress to adult RA.

This study was limited by the cross-sectional design which does not enable follow up of longitudinal changes that evolve over time for further interpretation of the significant association between TIDM and anti-CCP in predicting those individuals who evolve into the full clinical RA.

Furthermore, the relatively small sample size of the studied groups does not enable adequate power to pick up a moderate association between these two conditions and these findings need to be confirmed in a larger cohort.

Conclusion:

The association between RA and DM could not yet been ruled out, despite the lack of a significant association noted in our study. Screening of anti-CCP antibodies may not appear to be useful in the follow-up of patients with type 1DM unless there are joint abnormalities.

Author's contributions

All the authors shared the study design, data collection and literature research. DS was responsible for performing the biochemical analyses and helped in writing the initial version of the manuscript and in the statistical analysis. RAS and AF assembled the patients' cohorts, helped in writing the initial version of the manuscript. DAS coordinated clinical data collection, critically revised the statistical analysis of the clinical results and wrote the final version of the manuscript. MA helped in data collection and literature research.

Disclosure

All the authors of this paper report no conflicts of interest.

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