

Autoantibodies as important biomarkers in early diagnosis of type-1 diabetes

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Abstract

Type 1 diabetes (T1D) is an autoimmune disorder characterized by the inability of beta cells in pancreas to produce insulin. Lack of the clinical symptoms until the disease has advanced, makes it difficult to diagnose in an early stage. The use of some specific serum biomarkers will make it easier to diagnose or assess the risk for diabetes earlier and most importantly in the cases that have a familiar history. Based on that, in this study, we aim to study some potential biomarkers related to the early diagnosis and prediction of type 1 diabetes, and the relationship between these biomarkers in a group of individuals suspected of type 1 diabetes. Fifty individuals, from Tirana region, suspected for diabetes type I, were enrolled in this study. Their mean age was 22.5 (± 19.7), with a range from 2 months old to 47 years old. Fasting blood was collected in vacuum tubes and tested for C-peptide, anti IA2, anti ICA and anti-GAD levels. The results showed that the increase of positive autoantibodies affects the decrease of C-peptide levels, which means that the pancreatic β -cells have started to lose their functionality, as a result of cell death which is caused by the autoimmune attack. There can be many reasons that lead to this autoimmune attack, starting from genetic and epigenetic factors, immunologic and other factors based on the lifestyle of these individuals as well. After blood tests and data analysis, it was observed that individuals who have more than two positive autoantibodies have a higher prevalence of developing type 1 diabetes, even though they have no clinical signs at the time of diagnosis. It was noticed also that there is no statistically significant difference between the presence of positive autoantibodies and gender. This indicates that any individual can develop positive autoantibodies regardless of gender.

Key words: Diabetes type-1, biomarkers, β -cells, anti-GAD, anti-ICA, anti-IA2, C-peptide

1. Introduction

Based on the clinical observations and recent studies there is noticed a fast increase in the incidence of diabetes mellitus type 1. The number of blood examinations for diabetes in the biomedical centers is increasing fast in the recent years. There are many factors that help diabetes mellitus type 1, but till now it is not possible to stop or prevent it. Based on these recent observations, this paper aims to study the laboratory parameters related to the early

diagnosis of type 1 diabetes as well as to evaluate the relationship between these parameters in a group of individuals suspected of type 1 diabetes.

Type 1 diabetes is known as an autoimmune disorder that leads to the failure of pancreatic β -cells function, which with time becomes totally insulin dependent. This autoimmunity issue needs time to develop until the problem appears clearly with clinical symptoms. Based to different studies, the vast majority (~90%) of the T1D patients develop autoantibodies against pancreatic β -cells before the clinical onset (Purohit et al., 1997; Harrison, 2001). There are different autoantibodies, used as serum biomarkers that can help in early detection and risk stratification of at-risk populations. These serum biomarkers can also be used for therapeutic monitoring during the treatment of the disease. Diabetes can be classified using genetics and occurrence of autoantibodies which are present in 85-90% of the cases of classical T1D in the western world (Tuomilehto, 2013) and to some extent, also via C-peptide (Ludvigsson et al., 2012).

The techniques of autoantibodies measurement for diabetes type 1 are relatively new in our laboratories and quite expensive, but based on their importance in the early diagnosis of diabetes (T1DM), it is necessary to test the efficacy and emphasize their role in the early diagnosis process. Type 1 diabetes mellitus (T1DM) is an autoimmune disorder (with evidence of autoantibodies) and decreased beta-cell function (measured using C-peptide levels) (Siraj et al., 2018). Goals of biomarkers use in Type 1 diabetes clinical trials is linked to early detection of insulinitis, prediction of development of overt diabetes in at risk subjects, identification of responders to treatment and direct measurement of β cell mass and death (Tooley & Herold, 2014).

Regarding the statistics on type 1 diabetes mellitus developments, the results of meta-analysis on “Prevalence and incidence of type 1 diabetes in the world” from Mobasseri et al. (2020), showed that the incidence of type 1 diabetes was 15 per 100,000 people and the prevalence was 9.5% (95% CI: 0.07 to 0.12) in the world, which was statistically significant. The worldwide incidence of type 1 diabetes mellitus (T1DM) has increased in recent decades (Ogrotis et al., 2023). An annually increase of the prevalence of T1DM by 0.34% is observed (Liu et al., 2020 & Ogrotis et al., 2023). According to a modeling study from Gregory et al. (2022), on Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040, they predict an increase in prevalent cases to 13.5-17.4 million (60-107% higher than in 2021) with the largest relative increase versus 2021 in low-income and lower-middle-income countries. The incidence of type 1 diabetes in Europe was 15 per 100 000 population, which was statistically significant (Incidence = 0.015, 95% CI = 0.013 to 0.018, $P < 0.001$ (Mobasseri et al., 2020).

The situation in Albania is also problematic. According to type 1 diabetes index in Albania, there is a growing 4.7% of T1D compared with 3.1% for type 2 diabetes (Kollçaku, 2022).

Based on these data and the fast increase in the number of cases of individuals affected by type 1 diabetes, there is an urgent need to take measures to diagnose these cases in time and prevent the progression of the disease. Serum biomarkers could be of great importance in that case, based also on the etymology of beta cells autoimmunity, which is still not clear. There are observed different situations of diabetes type 1 progression, such as asymptomatic beta cells autoimmunity with normoglycemia, asymptomatic beta cells autoimmunity with dysglycemia and symptomatic type 1 diabetes (Insel et al., 2015). The sequence of events that occurs from autoimmunity to dysglycemia and then diabetes is predictable, but the duration of each stage varies greatly between individuals.

2. Materials and methods

Fifty individuals, with mean age of 22.5 (± 19.7) years old from Tirana region, suspected for diabetes type 1, were enrolled in this study. These individuals came to “Genius” laboratory, in Tirana, 2019-2020, for the measurement of parameters that diagnose type 1 diabetes, as requested by their family doctor. We ensured that each participant was fully informed about our intention to study their case and monitor their progress while adhering to all privacy terms. All subjects willingly agreed and provided their consent to participate in this study.

Fasting blood was collected and tested for C-peptide, anti IA2 (autoantibodies, islet antigen 2; or tyrosine phosphatase-related islet antigen 2), anti ICA (Pancreatic islet-cell antibodies) and anti-GAD (anti-Glutamic acid decarboxylase) levels. For the measurement of autoantibodies, anti IA2, anti ICA and anti-GAD, ELISA method is used and the results are obtained after the exposure of ELISA plate on microplate reader rt-2100c (Figure 1).



Figure 1. Elisa microplate reader (type RT-2100c)

For the measurement of C-peptide, we used an immunological assay based on Electrochemiluminescence method (CLIA), using MAGLUMI 4000 PLUS apparatus (Figure 2).



Figure 2. MAGLUMI 4000 PLUS apparatus

Statistical methods of analysis: Data collected after blood sample analysis, were organized in Microsoft Office Excel 2017 and then they were converted to the statistical program IBM SPSS Statistics 20 (Statistical Package for the Social Sciences).

We used descriptive analysis for the data collected from this sample (target group) observations in the laboratory. χ^2 tests were used to test for independence of the categorical variables taken in the study. In addition, we used histograms and barplots according to the case, to present the data for the parameters mentioned above. We used also the regression analysis, for the relationship between two continuous variables. Scatterplot graph was used as well to present and evaluate this relationship.

3. Results

A total of 50 individuals, suspected with type 1 diabetes, 29 male and 21 female were included in this study. Patients of different age groups have participated in this study; 51.7% of the patients belong to the 26-50 years old age group, 42% belong in the 0-13 years old age group and 6.3% belong to the 14-25 years old age group as shown in the Figure 3.

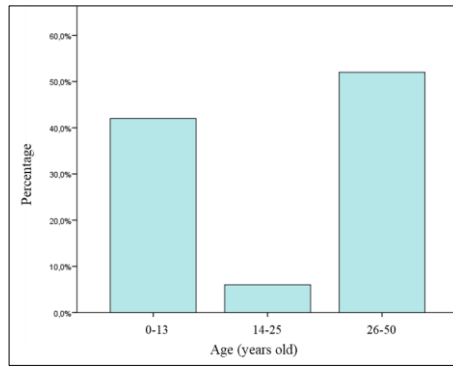


Figure 3. Distribution in percentage of different age groups included in the study

From the results of our sample study about the presence of autoantibodies, there are individuals diagnosed with T1DM as well as individuals with normoglycemia. It is noticed that 25% of individuals in the group diagnosed with type 1 diabetes, have three (3) positive autoantibodies (anti-ICA; anti-GAD; anti-IA2) present (Table 1), 20% of them have two (2) positive autoantibodies (anti-GAD and anti-IA2) present, 17.5% have only one (1) autoantibody present (mainly anti-IA2) and 37.5% of the individuals, do not have positive autoantibodies.

Table 1. Presence of autoantibodies as indicator of development of type 1 diabetes.

No positive autoantibodies	1 positive autoantibodies	2 positive autoantibodies	3 positive autoantibodies
37.5%	17.5%	20%	25%

The measurement and quantification of positive autoantibodies is of great importance in predicting the progress of the disease, since autoimmune diabetes does not show symptoms since the beginning of the disease. Because of the presence of more positive autoantibodies, individuals are more likely to develop type 1 diabetes. From our observations and other

studies as mentioned above, the presence of more autoantibodies increases the risk for the development of autoimmune diabetes mellitus type 1.

In order to better understand the relationship of autoantibodies, gender and age and the importance of autoantibodies in the prediction and diagnosis of diabetes mellitus type 1, we run different statistical tests, by testing one parameter at a time. Autoantibodies associated with pancreatic β -cell autoimmunity tend to appear early in an individual's life. For this reason, testing these autoantibodies in suspected individuals is important in the early diagnosis of type 1 diabetes.

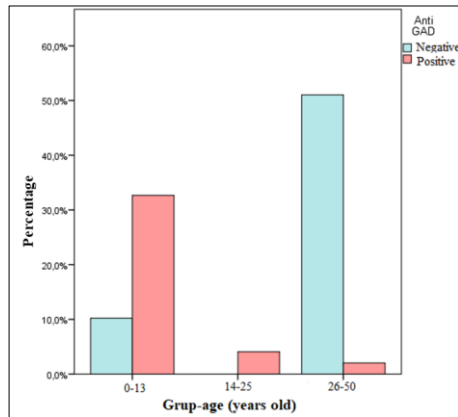


Figure 4. Presence of GAD-autoantibodies according to age

In graph showing the presence of GAD-autoantibodies according to age (Figure 4), we can see the association of age groups with the presence of GAD autoantibodies. Anti-GAD and other autoantibodies against pancreatic cells can be detected many years before clinical symptoms of diabetes appear (Sørgjerd et al., 2012). The results show that positive GAD autoantibodies are more present in individuals aged 0 to 13 years old. This indicates the existence of these autoantibodies from an early age. As it has been cited in other studies as well, the appearance of autoantibodies is closely related to age ($p < 0.001$) (Bravis et al., 2018).

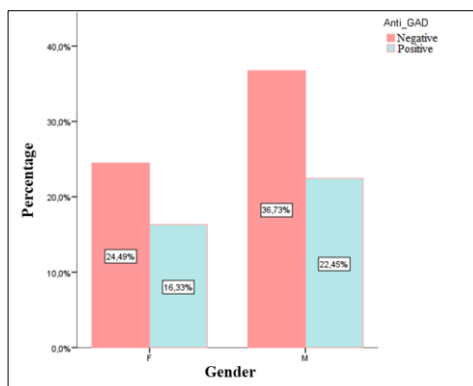


Figure 5. Relationship between individuals' gender and the presence of GAD autoantibodies (F-female, M-male)

Chi-square test was used for the two categorical variables, gender and anti-GAD (categorized as positive or negative). It turns out that from our data there is no statistically significant relationship between the gender of the individual and the positive result for GAD autoantibodies (Figure 5), ($\chi^2(1)=0.884$, $p=0.559$), which means that both sexes are equally predisposed to develop this pathology. Other studies report the same results as ours, showing that gender does not have a significant effect on the frequency of GAD autoantibody positivity (Rodacki et al., 2004).

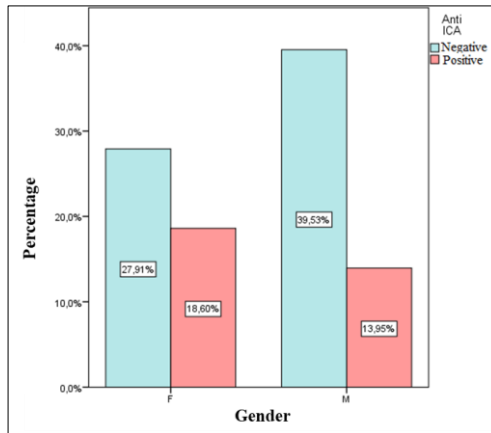


Figure 6. Relationship between individuals' gender and the presence of ICA autoantibodies (F-female, M-male)

Regarding the prevalence of ICA autoantibodies, according to gender, we applied chi-square test for the independence of these two categorical parameters (gender and anti-ICA), Figure 6. The results show that the presence of autoantibodies is not affected by the gender of the patient, ($\chi^2(1)=0.331$, $p=0.251$), although the graph shows that positive autoantibodies are more present in women.

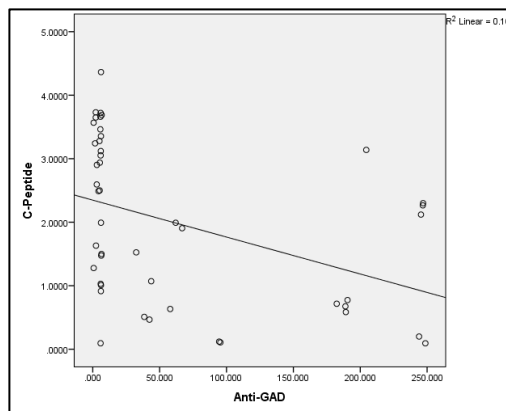


Figure 7. Relationship between C-peptide and anti-GAD autoantibodies presence

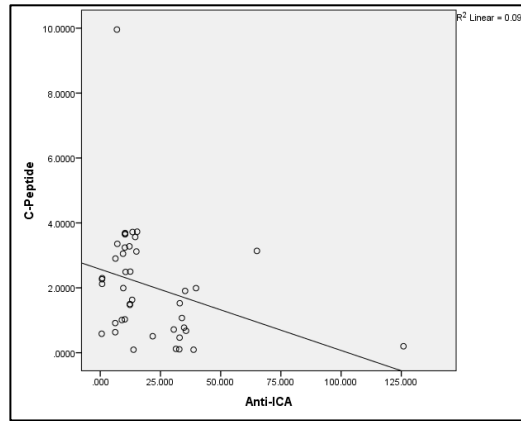


Figure 8. Relationship between C-peptide and anti-ICA autoantibodies presence

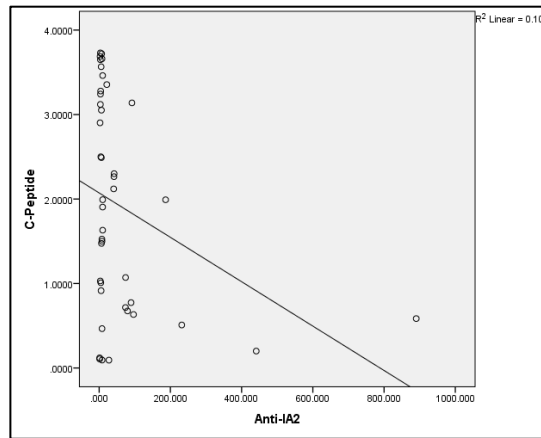


Figure 9. Relationship between C-peptide and anti-IA2 autoantibodies presence

C-peptide is another very important biomarker, which can help in the early detection and diagnosis of type 1 diabetes mellitus. Most of the proinsulin is cleaved into equimolar amounts of insulin and connecting (or C)-peptide in the secretory granules (Yau et al., 2021). The appearance of autoantibodies is related to the amount of C-peptide found in the patients' serum. Based on the generated boxplots, it is observed that C-peptide levels decrease when individuals have positive autoantibodies. In Figure 7, boxplots show that C-peptide levels are lower in individuals with positive GAD autoantibodies. The same result is observed in the other graphs on Figure 8 and 9, where the level of C-peptide follows the same logic with anti-ICA and anti-IA2 positive autoantibodies. Measurement of C-peptide levels is one of the main tests to assess the development of type 1 diabetes in suspected individuals. As can be seen from the presentation of the boxplots, a low level of C-peptide indicates that the pancreatic β -cells have begun to lose their function and cannot produce enough insulin to meet the body's needs.

One of the main causes of the autoimmune disease type 1 diabetes has to do with the total destruction of β cells, so they cannot secrete enough insulin. Another important test,

used as a clinical indicator of the stage of the disease, is the measurement of C-peptide level. Other studies conducted by the American Diabetes Association confirm the importance of measuring C-peptide levels as an important clinical indicator to determine the stage of autoimmune disease progression and the need or not, for medical treatment (Davis et al., 2015).

4. Discussion

This study points out and emphasizes the importance of clinical testing for some autoantibodies responsible in the autoimmune mechanism against pancreatic β cells, as a key indicator in the early diagnosis of type 1 diabetes. It has been estimated that over 90% of newly diagnosed T1DM will have a measurable amount of antibodies, with the most common autoantibodies being glutamic acid decarboxylase (GAD)-65 antibody (GADA), islet antibody (IA)-2 antibody (IA-2A), or insulin autoantibody (IAA) (Fenner et al., 2022; DiMeglio et al., 2018).

Based on other studies as well, on the importance of positive autoantibodies and the number of autoantibodies present, among those with two (2) autoantibodies, autoantibody positive pairs at baseline screening can predict risk of progression, providing a more personalized approach to clinical trial recruitment and facilitating recruitment of higher risk participants in prevention clinical trials. Similar risk for type 1 diabetes between those with two (2) and those with more than two (>2) autoantibodies was notable (Jacobsen et al., 2020). It is noticed also that, single GADA and single IAA autoantibodies that are positive by electrochemiluminescence are indicative of much higher risk of type 1 diabetes than those that are negative (Jacobsen et al., 2020; Fouts et al., 2026; Steck et al., 2016; Sosenko et al., 2017). Autoantibody titer also appears to affect the risk of type 1 diabetes (Jacobsen et al., 2020; Steck et al., 2016; Giannopoulou et al., 2015; Achenbach et al., 2004; Sosenko et al., 2013) and would be valuable data to add in future studies (Jacobsen et al., 2020). As noticed in these studies, in our study also, was observed that individuals who have more than two positive autoantibodies have a higher prevalence of developing type 1 diabetes, even though at the time of diagnosis they have no clinical signs.

Regarding C-peptide levels and its relationship with the presence of positive autoantibodies, we noticed that C-peptide levels are lower in individuals with increased autoantibodies and this is linked to the stage of the development of the disease. Based on a study from American Diabetes Association in 2018, until recently, it was believed that insulin and C-peptide production stopped within years of the diagnosis of T1DM, but from later studies, new research has shown that C-peptide production can persist for decades following T1DM diagnosis (Fenner et al., 2022; Nunes, 2015). This is a very important finding as; higher C-peptide levels are associated with lower rates of complications such as nephropathy, neuropathy, foot ulcers, and retinopathy (Fenner et al., 2022). According to research evidence collected over the past 20 years, C-peptide is also a biologically active peptide that may influence diabetic complications (Luppi et al., 2009; Chen et al., 2023).

5. Conclusions

The measurement of autoantibodies plays a pivotal role in both the diagnosis and prevention of type 1 diabetes. By identifying individuals at risk early on and monitoring their progression, healthcare professionals can intervene promptly, potentially delaying or even preventing the onset of the disease. This underscores the importance of including autoantibody testing into

routine screening protocols to improve outcomes and enhance the management of type 1 diabetes.

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Conflict of interests

The authors declare that they have no competing interests.

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