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USING SERUM BIOMARKERS FOR THE EARLY DIAGNOSIS AND PREDICTION OF TYPE 1 DIABETES

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Abstract:

Diabetes mellitus type 1 (T1D) is an autoimune disease where the pancreas fails to insulin production. This is a cronic disorder observed mainly in young ages and is caused by an autoimune response against pancreatic β cells, leading to reduced levels or lack of insulin in circulation. There are different factors, such as genetic, epigenetic, immunologic and environmental factors, leading to the development of type 1 diabetes.

Clinical similarity to type 2 diabetes (T2D) and the lack of clinical symptoms at the beginning of the disease, makes its diagnosis more difficult and this is one of the main reasons leading to late diagnosis of the disease. Monitoring specific parameters like C-peptide, HbA1c, fasting glucose and autoantibodies is of great importance in prediction and evaluation of individuals predisposed to develop diabetes mellitus type1.

Based on the importance of these autoimune markers for type 1 diabetes, the aim of our work, was 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 diagnosed with type 1 diabetes. After blood tests and data analysis, the results showed that the increase of positive autoantibodies affects the reduction of C-peptide levels, which means that pancreatic β cells have begun to lose their functionality, as a result of cell death which comes after the autoimmune attack. Based on these results we can say that low C-peptide levels and high blood glucose are an indicator of type 1 diabetes. A significant correlation between C-peptide level and age, as well as between high glicemic level and high triglicerides is present. High levels of urea and electrolite disbalance is observed. Taking into consideration these facts, clinical testing for the presence of specific serum biomarkers, helps in the early detection of the disease.

Keywords: diabetes mellitus 1, serum biomarkers, C-peptide, autoantibody

Introduction

Type 1 diabetes mellitus (T1DM) is known as a condition in which our pancreas does not produce the hormone insulin, which is crucial for maintaining proper blood glucose levels. When the beta cells of pancreas detect an increase in blood glucose level, they release insulin

hormone in order to mediate the glucose uptake by the cell and decrease the circulating glucose level back to normal. Diabetes mellitus type 1 is the most common chronic metabolic disease diagnosed in children and adolescents. The age of symptomatic onset is usually during childhood or adolescent with a peak incidence rate at 12–14 years of age, but the symptoms can also develop at much later ages (Katsarou A, et al. 2017). Although it is not contagious, the disease is the first and only condition regarded by the United Nations as an epidemic of the 21st century (P.Zhang et.al 2009). The incidence of the disease in children increases for unknown reasons at a rate from 3 to 5 % every year worldwide (Krzewska A, Ben-Skowronek I, 2016).

Type 1 diabetes, is a chronic autoimmune disorder, in which the immune system, attacks and destroys the insulin-producing pancreatic β-cells. Serum biomarkers, including a combination of glucose, glycated molecules, C-peptide and autoantibodies have been well established for the diagnosis of T1D. There is a great need for prognostic biomarkers to predict T1D development or progression. This would allow us to identify individuals at high risk for early prevention and intervention (Lian Yi et al. 2018). Some of the main autoantibody biomarkers tested more often (in prevention and intervetion of T1D) are: Islet cell cytoplasmic autoantibodies (ICA), Glutamic acid decarboxylase autoantibodies (GADA), Insulinoma-associated-2 autoantibodies (IA-2A), Insulin autoantibodies (IAA) and zinc transporter 8 AAb (ZNT8A).

Islet autoantibodies are markers of an autoimmune (self-reactive) response to the islets, and are the result of this response and not the cause of type 1 diabetes. Serum islet autoantibodies help in the early prediction of T1D, during the asymptomatic phase that precedes T1D. The presence of these specific autoantibodies and C-peptide rate can be used also in the differentiation of T1D and T2D, which is of great importance especially during the preoperative evaluation for bariatric surgery (Pilla, S. J. et al. 2018). For patients with an atypical clinical phenotype, these measures may be valuable in predicting clinical course (Catherine P. Et al. 2005).

Other biomarkers can be used in the prediction of diabetes in general, but do not differentiate between T1D and T2D. Glucose for example, is a simple and useful metabolic biomarker used for the diagnosis of diabetes, but it is not specific to T1D (Lian Yi et al. 2018). C-peptide and insulin are important parameters also to be measured as they are released in equimolar amounts and with the ability of c-peptide to have a longer half-live time, we can preddict the initial amounts of insuline. Stimulated serum C-peptide level (as a surrogate for insulin) has been considered a consistent and sensitive measure of β-cell function and can be used to help differentiate autoimmune-diabetes from other diabetes subtypes (Leighton E. et al. 2017). Form other studies is shown that the elevated ratio of serum proinsulin to C-peptide, as an indication of β-cell ER dysfunction, precedes T1D onset especially in younger children (McLaughlin KA, et al. 2016). Althought, C-peptide measurement may not be as reliable with the increasing obesity epidemic (Lian Yi et al. 2018), future studies measuring proinsulin to C-peptide ratios, combined with other biomarkers, are needed to demonstrate their utility in predicting the onset of T1D in the pre- symptomatic phase (McLaughlin KA, et al. 2016; Truyen I.et al. 2005). According to other studies (Shivani AP. Et al. 2016) BMI and waist circumference were both reasonable predictors of prevalent diabetes and hypertension.

Recently, other biomarkers, such as metabolomics biomarkers are of great interest to study. A few amino acids and lipid metabolites were found to be associated with T1D. Methionine is one

typical amino acid that is involved in DNA methylation and could be relevant to the timing of appearance of autoantibodies (Pflueger M.et al. 2011; Lian Yi et al. 2018).

The application of biomarkers should provide benefits greater than its cost. Benefits range from learning about the disease process to preventing complications such as diabetic ketoacidosis at the diagnosis of diabetes, or even the prevention of diabetes entirely. The long-term cost/benefit ratio of estimating the risk of type 1 diabetes risk is still under investigation (Ezio B. 2015).

Method

This study is focused on the assessment of the importance of serum biomarkers in the early diagnosis and prediction of type 1 diabetes. Our sample includes a small group of thirty individuals from Fier city, a place located near the capital of Albania. Most of the individuals included in this study, after having some unknown and unexpected symptoms (increased appetite and rapid unexpected weight loss, some of them were feeling lethargic continuously, etc.), were addressed to the Regional Hospital of Fier without a doctor's recommendation. All clinical data and biochemical parameters are recorded carefully in each case. According to their declaration to the physician they had no health problems and were not suffering from some other disease.

Glycosylated hemoglobin, c-peptide, anti-IAC, anti IA-2, anti-GAD, fasting glucose level, cholesterol, Na/Ca/Cl, urea levels, ALP/AST/CK/LDH, total protein/Albumin and hematological profile was evaluated to these individuals. The average age of patients is 13.5 years old, where the youngest patient is 6 years old and the oldest is 21 years old. Ten ml of fasting blood (overnight fasting or at least eight hours fasting) was collected from each individual in tubes containing anticoagulants such as EDTA and sodium heparin. Blood samples are placed on ice or centrifuged directly at room temperature and separate plasma from cells. Then after the plasma is separated from the mass cell, it is centrifuged again to eliminate residual cellular elements.

For the measurement of these parameters are used different methods according to the parameter that is going to be measured. For the measurement of glycosylated hemoglobin different methods can be used, such as immunologic assays, enzymatic methods, electrophoresis, chromatographic methods, but the most used method is HPLC. Regarding the measurement of c-peptide and autoantibodies are used elechtrochemiluminiscence method (ECL), radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA). Colorimetric methods were used to measure the level of cholesterol, plasma proteins and urea, while for ALP/LDH/AST/CK specific appropriate tests were used.

Results and Discussion

Clinical type 1 diabetes is preceded by an asymptomatic phase that can be identified by serum islet autoantibodies which can be used as biomarkers to diagnose type 1 diabetes that is already at this asymptomatic stage, so that attempts to prevent clinical hyperglycemia become a feature of disease management (Ezio B. 2015). The presence of these autoantibodies helps in the prediction of T1DM.

In the 1970s, using frozen parts of the human pancreas, the first autoantibody (ICA) was identified. Later autoantibodies were discovered and others that were now widely offered for the onset and diagnosis of diabetes. The Possible Population Market Survey (DAISY), the

German Study (BabyDIAB) and the Diabetes Prevention Completion Study (DIPP) have established that autoantibodies appear several years before diabetes activity (Li Zhang, George S E. et al. 2011).

In this study were included patients of different age groups, and it turned out that the highest frequency of occurrence of type 1 diabetes belongs to the age group 12-16 years old (figure 1.1). As we know from other studies too, T1D affects more the young and adolescents (Goitom M. et al. 2021).

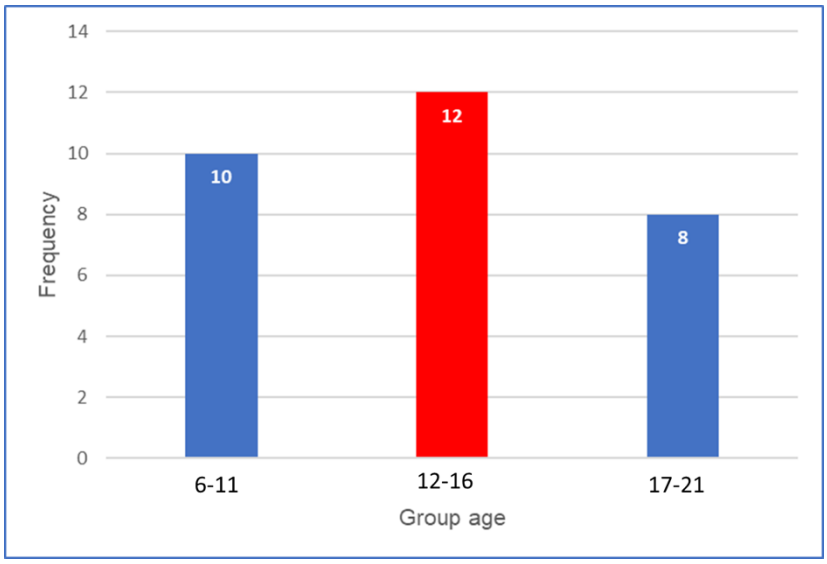

Figure 1. Frequency of case distribution by age groups

From this study, resulted that the frequency of incidence of T1D is higher in man (57 %) when compared to women (43%). Most of the patients (67 %) were oriented to the hospital after facing the first symptoms of the disease, without suffering from any other disease before. In 70 % of the cases there was no previous family history for T1D. According to many studies except genetics there are other environmental factors (Rewers, M., & Ludvigsson, J. 2016) and epigenetic factors leading and affecting T1D (Stankov K. et al.2013; Wang Z. et al.2017). Other immunologic factors, life style, dietary habits, etc. combined with other factors can lead to T1D. in most of the cases with T1D there is more than one factor leading to the disease.

T1DM is a metabolic disorder, and in recent decades it has been shown that metabolites other than glucose play an important role in insulin deficiency and the development of diabetes (Lanza IR. Et al. 2010; Bain JR. 2013; Bervoets, L.et al. 2017). In this study, we investigated other parameters linked with the metabolic profile and variations in T1DM children and adolescents using diferent tests and methods in order to understand better the biochemical pathways that are altered in early stages of T1DM. Besides the expected higher concentration of glucose, there are other parameters that are higher than normal, such as HbA1c, autoantibodies concentration, and when analyzing the lipidic profile of these patients, we notice also high levels of triglycerides. There are observed changes in the level of some electrolytes and specific enzymes like ALP (Table 1.).

Table 1. Parameters measured at T1D individuals

Parameter	T1DM (mean)	Reference	Range
Glycaemia (mg/dl)	288.33	70-110	136
HbA1c (%)	11.41	4.2-6.3	7
Anti -IA-2 (U/ml)	674.46	< 7.5	420.04
Anti-ICA (U/ml)	122.46	< 28	69.258
Anti-GAD (U/ml)	147.79	< 5	86.19
C-Peptide (mmol/L)	0.53	0.3-3.7	0.41
Triglicerydes (mg/dl)	229.53	20-150	128
Cholesterol	149.41	50-200	25
HDL-Chol (mg/dl)	33.31	40-60	19.9
LDL-Chol (mg/dl)	64.66	< 130	20.32
Na (mmol/L)	135.61	135-145	16
K (mmol/L)	3.9	3.5-5.2	0.8
Cl (mmol/L)	94.12	90-100	17.61
Urea (mg/dl)	23.6	7-20	7.9
ALP (U/L)	165.93	45-115	71.63
AST (U/L)	13.89	8-48	13.58
CK (U/L)	48.28	30-200	25.77
LDH (U/L)	130.31	122-222	59.88
Total Protein (g/dl)	7.8	< 8	0.55
Albuminemia (g/dl)	4.34	3.5-5.0	0.61
RBC (mm^3)	5251666.6	$4.5\text{-}6.1x10^6$	430000
WBC (mm^3)	7195.3	$4\text{-}11x10^3$	300

Recently, it has been shown that metabolites other than glucose play an important role in insulin deficiency and the development of diabetes (Bervoets, L. Et al. 2017).

Fasting glycemia gives a general information about the basic levels and ability of insulin to metabolise glucose. When there is insufficient concentration of insulin (low basic level), hyperglycemia will appear. Glycemic levels from 70-110 mg/dl are considered normal, whereas glycemic values from 110-126 mg/dl can be considered as broken glucose tolerance and glycemic level above 126 mg/dl indicates diabetes. In this group of untreated patients for T1D, mean glycemic levels are 288.33 mg/dl, much higher than normal glycemic index. According to other studies, glycemic level, if untreated, can go much higher than normal reaching till 500 mg/dl (Informed Health.org 2020).

We found also very hight HbA1c level 11.41 % compared to reference (4.2-6.3 %). HbA1c test is very important and helpful in the diagnose and diferentiation of T1D as it indicates the

average blood sugar for the last two to three months (about the past 12 weeks). The higher levels of glucose in the blood, the more hemoglobin with sugar attached to this molecule and the higher the percentage of HbA1c.

The chemistry of glycation predicts a straightforward relationship between mean glucose concentrations and HbA_{1c} values over the average lifespan of a patient's red cells (Cohen RM. Et al. 2004). There is substantial individual variability between the measured versus calculated mean glucose concentrations. Consequently, estimated average glucose concentrations calculated from measured HbA_{1c} values should be used with caution (Wilson, D. M. Et al. 2011).

In addition to the HbA1c test, other blood and urine analysis are needed. According to the doctors recommendation, a periodical control of cholesterol levels, thyroid function, liver and kidney function has to be done. This, because T1D is often complicated with other autoimmune diseases, and anti-islet autoantibodies precede the clinical onset of disease. The most common coexisting organ-specific autoimmune disease in patients with T1D is autoimmune thyroid disease, and its frequency is estimated at > 90% among patients with T1D and autoimmune diseases (Kawasaki E. 2014).

We used bidirectiona t-test to see if there was a possible correlation between HbA1c level and age of the patients, but not significant correlation was found ($ts=1.1694<t\alpha_{0.05(28)}=[-2.0484:2.0484]$), which means that there is not a direct correlation between these two parameters (although there are young and adolescents only included in our study).

Autoantibodies on the other hand have a major impact on the accuracy of predicting type 1 diabetes but not all island autoantibodies are the same. The IAA are the first to show up. GADA and IAA are the most common islet autoantibodies in childhood. From other studies we know that GADA is the hallmark of type 1 diabetes in adults and IA-2 antigens are very specific for the development of diabetes (Bonifacio E. et al. 2015). A similar situation was observed in some individuals involved in our study who were positive for autoantibodies.

These patients were also tested for C-peptide levels. Low C-peptide and high blood glucose levels may be an indicator of type 1 diabetes as shown in Table 1. In order to understand and evaluate the prevalence of C-peptide over age the linear regression test was performed. This test was performed in three different age groups: 6-11 years old, 12-16 years old and 17-21 years old. In the first two age groups the test was not significant while in the third age group the test resulted to be significant ($p = 0.027$), which indicates that C-peptide levels are age-related.

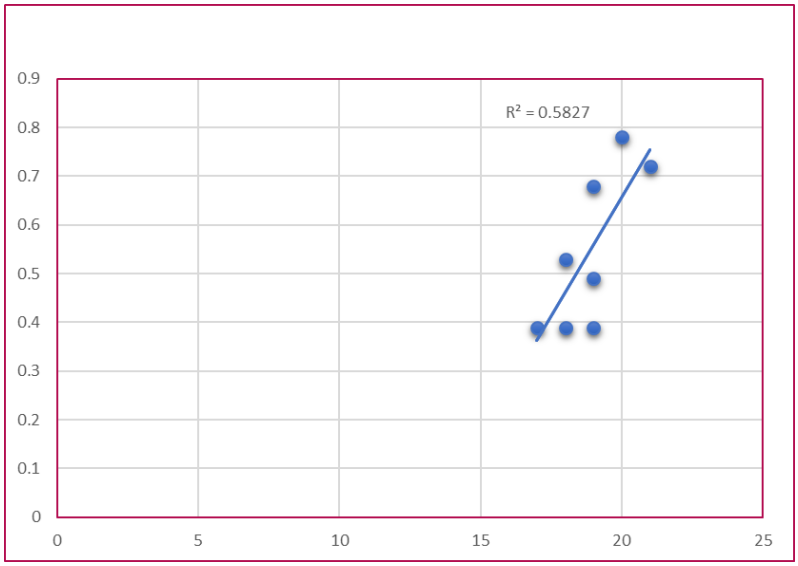

Figure 2. Linear regression test to evaluate the relationship between C-peptide level and age of the patients

Other studies by the American Diabetes Association showed that at all durations of disease, diagnosis in adulthood is associated with greater frequency and higher values of C-peptide (Asa K Davis et al.2015). C-peptide level may also correlate with microvascular and macrovascular complications and future use of insulin therapy, as well as likely response to other individual therapies. Many studies explore the potential uses of c-peptide measurement in clinical practice (Leighton, E. Et al.2017).

As patients with T1D often have problems with dyslipidemias we tested the possible correlation between glycemic level and triglycerides, because as we can see from table 1 there are present high levels of triglycerides in this patients. Abnormal lipid levels in T1D have been shown to affect the cardiovascular system (Soedamah-Muthu et al. 2004; Verges B. et al. 2011).

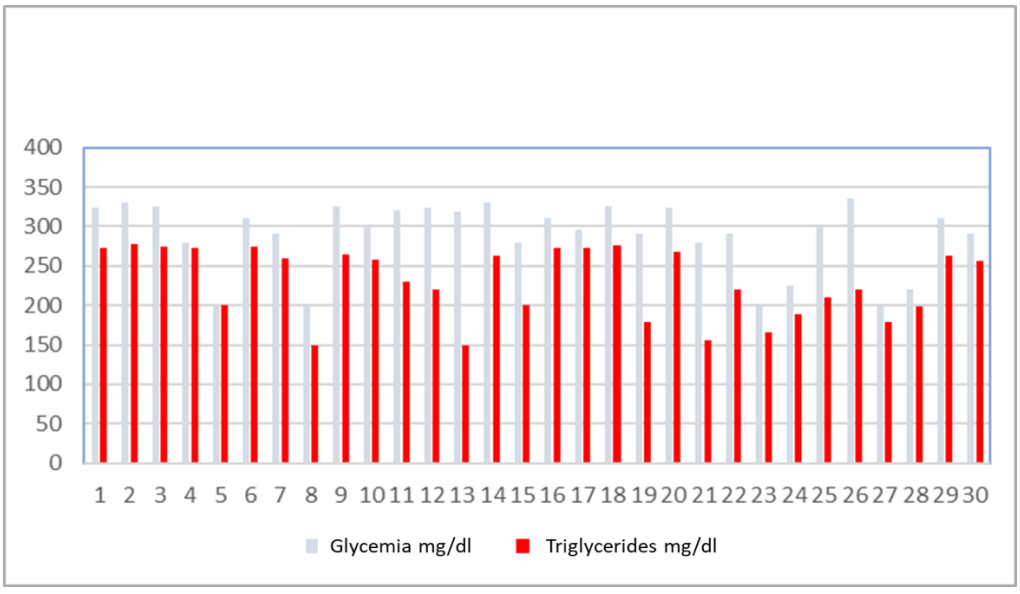

Figure 3. Correlation analysis between glycaemia and triglycerides

A correlative analysis was performed in order to see and determine if high glycemic levels affected triglyceride levels in these patients. Two-way t test was used to perform this correlative analysis. T test resulted to be significant ($t_s=4.3436>t\alpha_{0.05(28)}=[-2.0484: 2.0484]$), which means that high glycemic levels affect triglyceride levels.

According to many studies there is a strong connection between hyperglicemia and hyperlipidemia, mainly reflected in high levels of tryglicerides in the blood. Hyperglycemia and hyperlipidemia, which are usually diagnosed by analysis of blood glucose (GLU) and lipid levels, are two of the most common diseases in modern society (Hao, G. Et al. 2017). Glucose and lipid metabolism are linked to each other in many ways. The most important clinical manifestation of this interaction is diabetic dyslipidemia, characterized by elevated triglycerides, low high density lipoprotein cholesterol (HDL-C), and predominance of small-dense LDL particles (Parhofer K. G. 2015).

Diabetic patients often show electrolyte disorders. Diabetes mellitus is associated with both hypo and hypernatremia/potassium that reflect the mechanisms associated with hyperglycemia (Liamis G. et al. 2014). There was observed high electrolyte levels in our group of patients too. These elevated values may be an indication of the problems affecting the renal system, which is known to happen in these conditions.

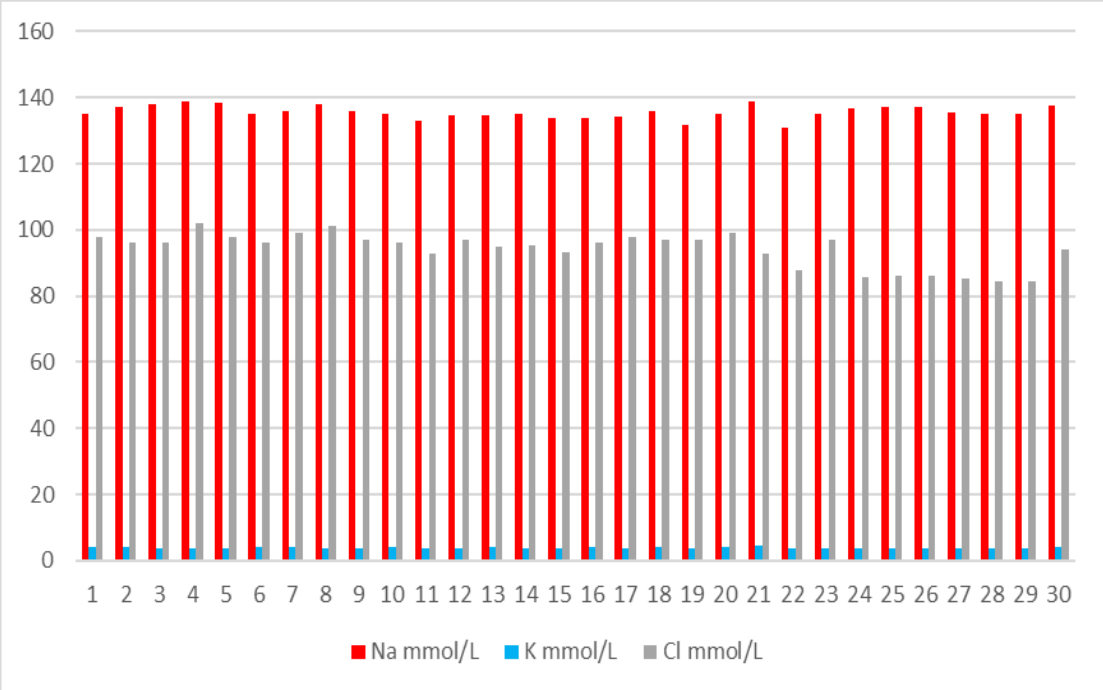

Figure 4. Electrolytes distribution

Approximately 20% to 30% of all diabetics develop nephropathy, and a high percentage of patients with type 1 diabetes develop end-stage renal disease. Diabetic nephropathy is characterized by macroalbuminuria and abnormal renal function represented by a decrease in glomerular filtration and an increase in serum urea and creatinine concentration. Clinically diabetic nephropathy is evidenced by proteinuria and decreased rate of glomerular function (Banamikar SA. et. Al. 2016). In our case it can be confirmed from the high levels of urea at a mean value of 23.6 mg/dl, which can be the result of a decrease of the renal function in these individuals.

Conclusion

The study highlights the importance of measuring autoantibodies for early diagnosis of type 1 diabetes. Since T1DM is preceded by an asymptomatic stage, autoantibodies play a crucial role as a biomarker and their presence indicates the predisposition that in the future the individual may develop diabetes.

Low C-peptide levels and high blood glucose levels are an indicator of type 1 diabetes. C-peptide results to be linked with the age of the patients, especially in adulthood.

We found out a correlation between glycemic and triglyceride levels, where high glycemic levels affect triglyceride levels also.

High urea, changes and disbalance in electrolyte levels indicates that these individuals are predisposed to develop abnormalities in the renal system.

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