LYMPHOCYTES VALUES IN TYPE 1 DIABETES MELLITUS NEWLY DIAGNOSED IN CHILDREN

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Type 1 diabetes mellitus (T1DM) is an autoimmune disease, caused by the destruction of the pancreatic β cells. T lymphocytes are activated as a response to the dominant islet antigens, the result being the development of T1DM, characterized by insulin deficiency. Due to the fact that T lymphocytes decrease mainly in poorly controlled diabetes mellitus patients, in this study there were observed the values of lymphocytes in children with newly diagnosed diabetes mellitus. Also, we checked the C reactive protein in these patients, due to the islet inflammatory reaction. There were taken into study 26 subjects distributed as it follows: 13 children with T1DM newly diagnosed and 13 children clinically healthy, who represented the control cohort. In all cases there was analyzed: the blood count and there were considered the absolute number of lymphocytes, blood glucose level, glycated hemoglobin (HbA1c) and C reactive protein (CRP), determined from peripheral blood and blood serum, respectively. The lymphocytes values in children with T1DM was significantly lower as compared to the control cohort ($p < 0.0001$). The glucose level in the blood and the HbA1c were also significantly increased ($p < 0.001$, $p = 0.00006$, respectively). Checking the correlation between the lymphocytes number and CRP, it shown to be positive, but with no statistic significance ($r = 0.60$; $p = 0.5$). The results suggest a lower number of lymphocytes in the peripheral blood in children with T1DM newly diagnosed, which would represent their increase in the pancreatic β-cells, as inflammatory response.

Keywords: type 1 diabetes mellitus, lymphocytes, autoimmune disease, C reactive protein.

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is an autoimmune disease associated with destruction of pancreatic beta cells in the islets of Langerhans, being the most common type of diabetes in children and adolescents. The destruction of beta cells is characterized by two factors autoantibodies and lymphocyte infiltration of the pancreas[1]. The disease is characterized by progressive infiltration of the islets of Langerhans with the cells of the immune system, particularly of CD4+ and CD8+, as well as with macrophages. This infiltration leads to the production of insulitis and decreased insulin production. In some persons, this infiltration occurs under control, without obvious manifestation of the disease, while to others it may progress through a destructive immune response, selective beta cells being destroyed.[2]

Studies on the mechanisms of progression of the disease have focused on identifying the types of cells involved in T1DM. The evidence from animal model studies have shown the involvement of T cells in the CD4 + and CD8 + T1DM development[3,4]. However, it seems that diabetes does not develop in the absence of macrophages, which are mediators of inflammation due to their ability to secrete cytokines, especially interleukin-1β and tumor necrosis factor (TNF-α)[5]. Other cells may be also involved in the development of diabetes, such as B cells, natural killer cells (NK) and natural killer T-cells (NKT)[6].

In our study we followed the absolute number of lymphocytes in children with newly diagnosed

type 1 diabetes, as well as their relationship with C reactive protein (CRP).

MATERIAL AND METHODS

We studied a total of 26 subjects distributed in two groups: 13 children with newly diagnosed diabetes (group I) and 13 clinically healthy children as a control group, aged between 6–13 years. For diagnostic criteria there were used the criteria from the expert committee on the diagnosis and classification of diabetes mellitus[7].

In all subjects, venous blood was collected after an overnight fast and there were determined: cell blood count (CBC), blood glucose, glycosylated hemoglobin (HbA1c), C-reactive protein (CRP) and lipid metabolism parameters (total cholesterol, serum triglyceride, HDL and LDL cholesterol). For CBC, venous blood was collected using tubes containing EDTA, the analyses being performed on Sismex XT analyzer and for the biochemical parameters, venous blood was collected in tubes with coagulation activator the samples being tested on the Cobas 6000 automatic analyzer modul 501c, using reagents from Roche.

Statistical analysis: data was analyzed statistically, using Microsoft Excel 2003. Normally distributed data are expressed as means and standard deviation (SD). To determine the correlation, we used the Pearson coefficient. Statistically significant were considered the differences in the cases where the bilateral value, $p < 0,05$.

The parental consent for data use was obtained.

RESULTS AND DISCUSSIONS

The results are shown in Table 1. Among patients with T1DM taken into the study, three had diabetic ketoacidosis (DKA) as the first manifestation of the disease. The rest presented with abdominal pain and investigations have shown elevated blood sugar and glycosylated hemoglobin.

The absolute number of lymphocytes presented values significantly lower in children with diabetes than the control group (2.79 ± 0.63 *vs.* 3.87 ± 0.18, $p < 0.0001$). Most studies show alterations of CD4+ and CD8+ in the blood of patients diagnosed with T1DM[8]. More elevated activated T lymphocytes were observed in the blood of patients with prediabetes[9]. A more recent article reported low percentages in the peripheral blood of the CD3, CD4+, CD8+ and HLA-DR, and decline in the CD4+/CD8+, both in patients with newly diagnosed T1DM as well as in the development of this type of diabetes[10].

Table 1

Parameter tested

Parameters	Group I Type 1 diabetes mellitus	Group II Control	p value
Lymphocytes absolut number ($x10^3$)	2.79±0.65	3.87±0.18	p<0.0001
Glycemia (mg/dl)	281.2±247.4	82.82±4.50	p<0.0001
HbA1c (%)	8.52±2.08	5.04±0.23	p<0.0001
Total cholesterol (mg/dl)	192.58±22.39	162.23±4.11	p<0.001
Triglyceride (mg/dl)	172.70±101.69	123.38±7.21	p<0.05
HDLc (mg/dl)	47.16±16.7	48.22±2.19	NS
LDLc (mg/dl)	114.06±26.11	89.33±4.70	p<0.05
CRP (mg/l)	3.13±2.46	0.53±0.33	p<0.0001

Mean blood glucose and HbA1c levels were statistically significantly higher in patients with newly diagnosed T1DM compared to the control (p < 0.0001, respectively p < 0.0001).

C-reactive protein showed values significantly increased statistically in patients with newly diagnosed diabetes than the control group (3.13 ± ± 2.46 *versus* 0.53 ± 0.33, p < 0.0001), but it not exceeded the normal average values. Four patients had CRP values greater than 5 mg/L, and the same patients having normal levels in terms of absolute number of lymphocytes in children.

Following the relationship between CRP and absolute number of lymphocytes it was observed a statistically significant positive correlation in patients with newly diagnosed T1DM (r = 0.60; p < 0.01) (Fig. 1).

Mean total cholesterol, triglycerides, respectively LDLc values showed values significantly higher statistically in patients with newly diagnosed diabetes than the control, but the average level of HDLc showed no significant decreases between the two groups (Table 1).
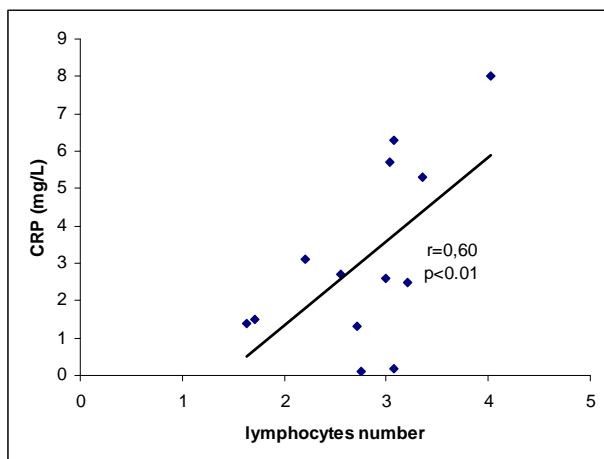

Figure 1. The relationship CRP-lymphocytes absolute number in newly diagnosed T1DM.

Schalkwijk and colab. reported elevated CRP levels in type 1 diabetes comparative with control subjects.[11] The same thing was proved by Gomes *et al.* in 2003, showing the involvement of the inflammatory markers in the pathogenesis of diabetes[12]. In the studys mentioned above, CRP levels were correlated with lipid metabolism parameters showing the involvement of inflammation in atherogenesis in these patients[11,12]. In our study we did not obtain the CRP correlation with lipid metabolism parameters. Correlation was observed

only related to the increasing number of lymphocytes, elements involved in particularly in the viral inflammatory process.
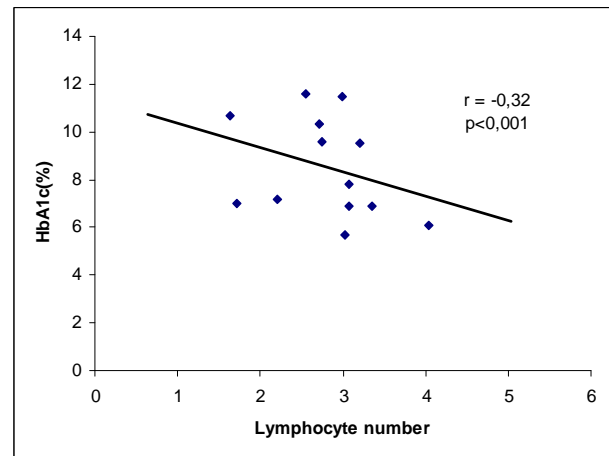

Figure 2. The relation between HbA1c and the lymphocytes absolute number in newly diagnosed T1DM.

Six patients presented on admission, values of glycosylated hemoglobin (HbA1c) greater than 8.5%; the remaining seven patients had HbA1c values below 8%.

Also in our study, we obtained a significant correlation between the number of peripheral lymphocytes and HbA1c (p < 0.001) (Fig. 2). Sherfi *et al.* showed, in a study, correlations between HbA1c and CD95 expression[13].

The low number of lymphocytes in peripheral blood in children with newly diagnosed diabetes was put on the fact that they could represent lymphocytes number increase in the pancreatic beta cells. To prove this hypothesis would require biopsy of the pancreas. But for safety reasons biopsy is difficult to achieve and autopsy tissue from patients recently diagnosed with T1DM is very rare.

LIMITATIONS

Our study is limited in that we only determined the total number of lymphocytes without differentiating the subsets.

CONCLUSIONS

The results suggest a lower number of lymphocytes in the peripheral blood in children with T1DM newly diagnosed, which would

represent their increase in the pancreatic β-cells, as inflammatory response.

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