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Original Research Article

Inflammatory Markers in Insulin-Dependent Children with Diabetes

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Abstract:

Aim: To determine inflammatory markers in insulin dependent diabetes mellitus (T1DM) children.

Materials & Methods: Following clearance from the Institutional Ethics Committee, the researchers started the investigation. This study included 30children with T1DMwho were previously diagnosed and 30 children who were newly diagnosed with T1DM (controls).As the age of the child was less than 18 years, the present study provided consent from the parents of the children. Exclusion criteria were children without diabetes, co morbidities other than diabetes, apparently healthy children, and parents who did not consent to participate in the study.

Statistical Analysis: Regressions were employed, and the percentages were calculated. Significance was set at the level of 0.05.In summary, this finding is of utmost importance.

Results: Children with a new diagnosis of T1DM had decreased levels of IL-1 and TNF, while those with a prior diagnosis with T1DM showed elevated levels. The two groups had significantly different serum IL-1 and TNF levels. Children with newly diagnosed T1DM had higher IL-2 and IL-15 blood levels than those with previously diagnosed T1DM. Significant differences were observed in IL-1, IL-2, IL-4, TNF-α, and IL-15 blood levels between the two groups. No notable disparities were observed in IL-1 and IFN levels between the two groups of the present study. The levels of IL-15 and TNF-α were negatively correlated in children with previously diagnosed T1DM.

Conclusion: T1DM secondary disorders are influenced by both pro-inflammatory and anti-inflammatory cytokines, according to this new study's findings.

Keywords: Type 1 diabetes mellitus, hyperglycemia, lipid profile, proinflammatory cytokines, anti-inflammatory cytokines.

Introduction

Type 1 diabetes [T1DM] is an autoimmune condition that leads to the destruction of pancreatic beta cells which in turn causes insufficient insulin production, resulting in hyperglycemia [1]. Type 1 diabetes is a chronic disease requiring insulin replacement and intensive effort by the patient. The common symptoms of T1DM in children are increased thirst, frequent urination, possibly bedwetting in a toilet-trained child, extreme hunger, unintentional weight loss, fatigue, irritability or behavior changes, and fruity smelling breath [2,3].

Research has indicated that cytokines play a role in the development of T1DM. Two inflammatory cytokines, namely tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), have been associated with T1DM. The presence of inflammation in pancreatic beta cells is a defining feature ofT1DM, and it is believed that these cytokines contribute to their progression [3,4]. Furthermore, adipose tissue, namely fat cells, have the ability to produce inflammatory cytokines such as TNF-α and IL-6, which can stimulate lower-level inflammation throughout the body [6-10]. Consequently, chronic inflammation may exacerbate and increase the likelihood of developing T1DM. Hence, the suppression of cytokines and the mitigation of inflammation could serve as a valuable therapeutic approach for the management of T1DM [7,9].

This category encompasses medications that specifically target cytokines and inflammation, as well as lifestyle modifications such as engaging in physical exercise and adopting a healthier diet. The successful identification of risk factors for T1DM will facilitate the development of effective approaches for early disease detection and prevention [8,9]. This is one of our objectives that we aim to accomplish. The unique aspect of this study is in its central objective of investigating the potential differentiation

in inflammatory markers between those diagnosed with T1DM and those newly diagnosed the condition in children.

Materials & Methods

The investigation was initiated by the researchers after obtaining approval from the Institutional Ethics Committee. The sample for this study consisted of 30 children who had previously been diagnosed with T1DM and 30 children who had recently been diagnosed with T1DM (control group). Given that the child's age was below 18 years, the current study obtained parental consent. The study's exclusion criteria were children who did not have diabetes, comorbidities unrelated to diabetes, children who appeared to be in good health, and parents who declined to provide consent for their participation.T1DM was done by the clinicians basing upon the diagnostic criteria laid by American Diabetes Association [10]. The children age included in the study was between 10 to 18 years of age.

The quantification of cytokines in the serum was performed using a multi-analyte Elisarray kit provided by Qiagen laboratories. After undergoing incubation, the capture antibodies exhibit enhanced binding affinity towards their respective target proteins. Following the removal of unbound protein, the gathered analyte can be attached to the wells using biotinylated detection antibodies. Following the completion of a final washing step, an avidin-horseradish peroxidase conjugate is employed to eliminate any residual unbound particles.

Following an additional washing step, a colorimetric substrate solution is introduced into the wells, resulting in the transformation of the sample into a specific shade of blue. This change in color is directly correlated with the concentration of the protein analyte present in the initial sample. Following the addition of a stop solution, it is possible to assess the absorbance of the samples at a wavelength of 450 nm, enabling the establishment of significant comparisons among them. A 5.2% disparity was observed among the duplicates, while a 7.9% disparity was observed among the tests. The researchers found a sensitivity of 0.8 pg/mL.

Statistical Analysis: For this statistical analysis, IBM SPSS version 20 was utilized. We compared the two groups' means on the variables using an unpaired "t" test. The percentages were also calculated. Significant at the 0.05 level. Regressions were employed to ascertain the nature of the association between the two variables. The percentages were also calculated. Significant at the 0.05 level.

Results

Table 1 shows the baseline characteristics of the study population. Newly diagnosed T1DM group consists of thirty patients of which 18 were male and 12 were female. On the other hand, previously diagnosed T1DM group consists of 30 patients of which 16 were male and 14 were female. No notable differences were observed in the parameters of glucose, Glycosylated hemoglobin (HbA1c), microalbuminuria, creatinine, and urea levels when compared between the two groups of the present study.

For both groups, Table 2& 3 shows that the mean levels of IL-1, IL-2, IL-4, IL-5, IL-10, IL-15, IFN and TNF-α were elevated. A prior diagnosis of T1DM in children was associated with elevated IL-1 and TNF levels, whereas a recent diagnosis of T1DM in children was associated with decreased levels. The two groups also differed significantly in terms of their serum IL-1 and TNF levels. Blood levels of IL-2, IL-4, and IL-15 were higher in children with newly diagnosed T1DM than in those with previously diagnosed T1DM.

We observed large variations in these metrics between the two patient sets. On average, cytokine levels were lower in children with T1DM who had already been diagnosed with the disease than in those who had just been diagnosed, as shown in Table 3.The results showed that the IL-1, IL-2, IL-4, TNF-α, and IL-15 blood levels were significantly different between the two groups.

Nevertheless, no notable disparities were observed when comparing the levels of IL-10, and IFN among the groups (Table 3).The levels of IL-15 and TNF-α were negatively correlated in children with previously diagnosed T1DM ($y = -0.129x + 3.7$).

Table 1: Baseline Characteristics of the study population

Parameters	Newly diagnosed T1DM	Previously Diagnosed T1DM	P Value
Number of Patients	30 Male: 18 Female: 12	30 Male: 16 Female: 14	NA
Glucose (mg/dL)	172.6 ±22.4	168.4±31.9	> 0.05
HbA1c (%)	7.8 ± 1.4	8.1 ± 2.1	> 0.05
Microalbuminuria (mg/L)	9.8 ± 3.1	8.2 ± 2.8	< 0.05
Creatinine (mg/dL)	0.8 ± 0.2	0.7 ± 0.3	> 0.05
Urea (mg/dL)	37.5 ± 9.9	36.1 ± 8.2	> 0.05

Table 2: Increased Inflammatory markers in the present study population of newly diagnosed T1DM

Parameters	Newly diagnosed T1DM (n=30)	Previously Diagnosed T1DM (n=30)	P Value
IL-2	54.6 ±11.4	41.2 ± 11.3	**< 0.05**
IL-4	46.2 ±13.2	32.9 ± 8.2	**< 0.05**

Table 3: Decreased Inflammatory markers in the present study population of newly diagnosed T1DM

Parameters	Newly diagnosed T1DM (n=30)	Previously Diagnosed T1DM (n=30)	P Value
IL-1	13.5 ± 2.6	28.9± 4.5	**< 0.05**
TNF-α	4.4 ± 2.06	7.8 ± 2.3	**< 0.05**
IL-5	19.2 ± 1.8	20.2 ± 3.4	> 0.05
IL-10	22.1 ± 2.8	23.9 ± 4.8	> 0.05
IL-15	4.8 ± 1.9	8.1 ± 3.3	**< 0.05**
IFN	56.7 ±6.9	58.2 ±7.5	> 0.05

Discussion

The differences in the concentrations of blood serum levels of IL-2, IL-4, and IL-15 were observed to be increased among children with T1DM diagnosed previously and those newly diagnosed. The concentrations of all three cytokines were found to be different among the children with T1DM diagnosed previously. The serum concentrations of IL-4 were found to be lower in children with T1DM newly diagnosed than in those diagnosed previously. This finding was supported by a scholarly publication and the findings of the research conducted by the researcher and his colleagues [11,12]. The results of the research revealed a significant decrease in IL-4 expression among children with T1DM diagnosed previously. Additionally, the research also found elevated levels of IL-4 gene expression in children with T1DM diagnosed previously compared to those newly diagnosed. A separate investigation revealed that there is no association between the existence of IL-4 and the incidence of children with T1DM diagnosed previously [13]. The present investigation provides definitive data supporting the association between children with T1DM diagnosed previously and a decrease in IL-4 production. These findings are statistically significant when compared to children with T1DM newly diagnosed who have normal levels of IL-4 production. Conversely, children with T1DM diagnosed previously exhibited elevated levels of IL-5 and IL-13 compared to children with T1DM newly diagnosed [14]. However, the blood levels of IL-10 and IL-12 did not show any notable differences between the two groups.

The likelihood of this event occurring is increased by the aging process, even in individuals who generally enjoy good health. It is not uncommon for children who have previously been diagnosed with T1DM to exhibit elevated levels of IL-5, a factor that has been linked to increased levels of oxidized low-density lipoprotein (oxLDL) [4,13]. Our understanding of the relationship between IL-5 and children with T1DM diagnosed previously is primarily based on a limited number of global studies that have been conducted. In addition, the study groups were compared with respect to serum levels of IL-2, IL-15, IL-1, interferon- (IFN-), and tumor necrosis factor- (TNF-). Statistically significant differences were observed when comparing the levels of IL-2, IL-15, IL-1, and TNF- in the blood of children with T1DM diagnosed previously to those of children with T1DM newly diagnosed. Notably, significant changes in the concentrations of IL-2, IL-15, and TNF- were observed.

This study demonstrates a consistent and increasing relationship between weight and IL-2 levels in children with T1DM newly diagnosed. Furthermore, the efficacy of this correlation appears to be increasing gradually. IL-2 is a cytokine with anti-inflammatory properties that regulates white blood cells, particularly T-lymphocytes [5,16]. A recent investigation into the role of IL-2 revealed that children with T1DM diagnosed previously exhibited notably reduced levels of IL-2 in comparison to children with T1DM newly diagnosed. [16] The IL-2 study [10] demonstrated that children with T1DM diagnosed previously exhibited elevated levels of IL-2 compared to children with T1DM newly diagnosed. The study's outcome differed significantly when compared to children with T1DM newly diagnosed. This study only included children with T1DM diagnosed previously for a period of less than five years. Another study [11-13] found that children with T1DM diagnosed previously exhibited reduced levels of anti-inflammatory cytokines.

Children with T1DM diagnosed previously had a negative correlation between IL-15 levels and TNF-a levels. This was our third finding throughout the duration of our inquiry. The connection seen Children with T1DM diagnosed previously is subject to various alternative interpretations due to the intricate nature of pathophysiology. To comprehend the biology of the inflammatory response, it is vital to possess knowledge regarding this particular cytokine. As

indicated in [13,14,17-18], IL-15 is clearly implicated in both the initiation and progression of inflammation associated with cardiovascular diseases. This represents the culmination of their investigation. Based on the results of several studies [17,18], it was observed that there were elevated levels of IL-15 in atherosclerotic lesions in both human and animal subjects [19]. Diabetes and hyperglycemia are two additional risk factors associated with the development of atherosclerotic plaques.

Several investigations [16,17] have found a correlation between high TNF- levels and children with previously diagnosed T1DM, obesity, and nephropathy. The findings of this study support the observations reported by the researcher [15,16]. Based on the results of two studies [17-19], it was demonstrated that individuals with peripheral neuropathy exhibited significantly increased levels of serum TNF-. This discovery was also linked to the observed nerve conduction velocity in children with previously diagnosed T1DM.

Our research reveals an unusual finding: there is no significant correlation between IL-15 and TNF- in the healthy control group. A notable association was observed between the two variables among the cohort exhibiting children with previously diagnosed T1DM.

The therapy of secondary disorders, such as cardiovascular and atherosclerotic lesions, is significantly influenced by the pro-inflammatory cytokines IL-15 and TNF-. These cytokines induce systemic inflammation and play a substantial role in the development of subsequent complications. The aforementioned view is substantiated by the observation that children with previously diagnosed T1DM who were involved in our research also exhibited heightened concentrations of IL-15 and TNF-. The finding enhances the reliability of this analysis of the data. Children with previously diagnosed T1DM is a commonly observed metabolic condition characterized by a gradual and escalating inflammatory response, leading to an upregulation in the synthesis of cytokines, including IL-15 and TNF- [20,21].

Conclusion

This study's findings establish a connection between the history of T1DM and the inflammatory cytokines IL-4, IL-5, and IL-13 in children. It is impossible to exaggerate the importance of this finding. Both pro-inflammatory and anti-inflammatory cytokines play a role in the development of type 1 diabetes in children according to the current study's findings. The examined cytokines may also have biomarker potential for the early identification of type 1 diabetes in children.

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