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Purified protein derivative response in type 1 diabetics

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Abstract

Aim: The aims of our study were to identify the role of suppressor T1 helper cells in the immunopathogenesis of type 1 diabetes mellitus (T1D) and to investigate the purified protein derivative (PPD) response, which is a delayed type of skin sensitivity response that develops secondarily to the effect of the T1 helper cell response.

Materials and Methods: Twenty-four newly diagnosed patients with T1D were included in the study (12 girls, 12 boys), and 30 (18/12) age/sex matched healthy children were included as controls. All patients underwent the PPD skin test (TST) in the first diagnostic 24–48 h (study group-1) and again 1 month after diagnosis (study group-2). The PPD response was compared with the response of healthy children. The lower limit of PPD positivity was accepted as $\geq$ 10 mm.

Results: The mean PPD induration value taken in the study group-2 was statistically greater than both the study group-1 and the control group PPD (p=0,014, p=0.001). Regarding the increase in duration, the PPD positivity rate was statistically higher after the first month than in the control group (p= 0.005) and the initial PPD positivity rate (p=0.023).

Conclusion: Interesting results emerged for the PPD response of the study group-2, because the PPD positivity rate increased over time.

Introduction

Type 1 diabetes (T1D) develops as a result of the destruction of pancreatic beta cells, but its pathogenesis has not yet been fully defined as an autoimmune disease. Pancreatic beta cell destruction is thought to be caused by autoantibodies that develop against pancreatic beta cell proteins [1, 2, 3]. The T helper cells (Th), a subgroup of T lymphocytes, play an important role in the immune pathogenesis of the disease. T helper cells 1 and 2 (Th1 and Th2) are two other subgroups of CD4+ T-cells. Based on their cytokine responses these two groups of T cells have different functions, and the TH1/TH2 response is kept in balance in the organism.

These two cell types have the ability to suppress each other's immune response. Th1 cells mediate the response to intracellular bacterial and parasitic infections via a delayed-type hypersensitivity reaction such as the purified protein derivative (PPD) response and by acute allograft rejection. When Th1 cell functions are dominant, autoimmune inflammatory diseases (e.g., juvenile rheumatoid arthritis, T1D, etc.) emerge. When the Th2 cell response is dominant, humoral immune-mediated atopic diseases develop. The Th2 functions may be effective in preventing the development of organ-specific autoimmune diseases, such as multiple sclerosis, thyroiditis, Crohn's disease and T1D [4]. In T1D, pancreatic beta cells are destroyed locally because of a Th1 dominant response. The most important feature of T1D is the development of a complete lack of insulin as a result of the destruction of pancreatic beta cells [1,2].

T1D results from destruction of pancreatic beta cells by T cells of the immune system. Th1 cell functions are dominant in T1D. Another effect of Th1 dominance is seen with the PPD skin test, a simple and inexpensive test that has been widely used for years to screen for tuberculosis (TB). The PPD response, which materializes as a delayed-type skin hypersensitivity response, is also a Th1-mediated immune response; however, the response differs in diseases that develop with predominant Th1 versus Th2 cell responses. While a significantly reduced PPD response is reported in children with juvenile idiopathic arthritis, which is thought to result from a Th1-dominant immune response [5], some studies have reported significant increases, decreases or no changes at all in the PPD response in children with allergic asthma caused by a Th2

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immune response compared with control children [6, 7, 8, 9]. One report has indicated that in vitro PPD response does not change in patients with T1D; however, no study has yet examined the effects on PPD skin testing [10].

In this respect, newly diagnosed children with T1D are an interesting cohort, as they could reveal how the immune system is affected by evaluating the PPD skin test (TST) responses in the initial stage and during the course of the disease. Therefore, the aim of this study was to determine whether the PPD response changes between the beginning of T1D disease and one month after the metabolic disorders are resolved in patients with T1D, a disease in which the CD4+ Th1 immune response is reported to be dominant.

Materials and Methods

This study was conducted in the Istanbul University Cerrahpaşa Medical Faculty Child Emergency Polyclinic from April 2012 to November 2013. A total of 24 children (12 girls, 12 boys) diagnosed with T1D were included in our study. These children were diagnosed based on T1D and diabetic ketoacidosis criteria determined by the World Health Organization and the American Diabetes Association [11, 12].

The children in the study group drank large quantities of water and had complaints of very frequent urination, weight loss and high blood glucose. The levels of consciousness among the children varied between normal and significant drowsiness and coma. None of these patients had recently used antibiotics or any drugs. Plasma glucose, venous blood gas, HBA1C and full urine tests were analyzed to confirm the diagnosis of T1D in the children in the study group.

Children were excluded from the study if they met any of the following exclusion criteria: receiving immune suppressing drugs or recent steroid treatment, having active TBC diagnosed or suspected, and having a family health history of a chronic disease, such as active TB or immune insufficiency [13, 14].

In our clinic, the control group included 30 healthy children (18 girls, 12 boys) who attended the General Children's Polyclinic. The controls were age- and gender-matched with the children in the study group. Exclusion criteria for the control group included a family member with T1D history, a history of chronic disease or any infectious disease, immune-suppressing drug use and a TBC history in the family. In our study, information was collected regarding age (year), weight (kg), height (cm) and body mass index, which was calculated and recorded [15]. For all participants, the gender, date of arrival in the emergency department and BCG vaccine information were retrieved from the participants' own vaccination card.

Parents were provided with sufficient information about the research aims and its importance to the children. The study strictly adhered to protocols to protect volunteering and confidentiality, and the parents provided written consent. The Clinical Research Ethics Committee of Cerrahpaşa Faculty of Medicine approved the study (Ethics Committee Decision No: 83045809/11265).

PPD testing: TST was applied to children in the study group immediately after diagnosis (24–48 hours) (Study group-1) and again 1 month after the diagnosis (study group-2). TST was applied to the control group only once. TST was performed by placing an intradermal injection of 0.1 mL of PPD (Statens Serum Insitute, Copenhagen, Denmark) containing 5 tuberculin units (TU) into the volar surface of the forearm. The test was applied by the same expert throughout the study, using a 27-gauge needle and an insulin syringe for all children [14]. The induration size was measured across the long axis of the forearm with a pencil boot method after 48 h. Erythema or redness was ignored. TST was considered positive if the induration was $\geq$ 10 mm in size [13, 14]. One patient in the study group was evaluated for TBC disease because the PPD induration size was 17 mm one month after inclusion in the study.

Statistical analysis

Kıray et al. reported a significantly reduced PPD response in children with juvenile idiopathic arthritis, which is thought to result in a Th1-dominant immune response, as in Type 1 diabetes [5]. In this study, the response to PPD, one of the Th1 cell type responses, was found to be significantly lower in BCG vaccinated children with JIA than in healthy children. We planned to collect a similar number of patients using this study as an example. However, since patients diagnosed with diabetic ketoacidosis are rare in our center, we conducted an interim evaluation when we reached 24 patients and terminated the study because a significant difference was detected between the two groups. Data were analyzed using the Statistical Package for the Social Sciences program (SPSS 22.0; IBM). The Kolmogorov–Smirnov normality test was used to test whether quantitative variables showed a normal distribution. Quantitative data are presented as mean or SD and categorical data are shown as numbers or percentages. Induration sizes were compared between the groups using a One Way ANOVA test with post hoc analyses (Bonferroni correction, the mean difference is significant at the 0.017 level). Categorical variables were summarized using frequencies and percentages, and were compared between the groups using a Pearson Chi-square analysis. The McNemar test was used with dependent categorical variables. The Pearson correlation test was applied in cases in which an association was evaluated. Results were evaluated at a 95% confidence interval at a significance level of $p < 0.05$.

Results

Age, gender, and body mass index distributions of children in both groups were similar (p= 0.110, p= 0.462 and p = 0.723, respectively) (Table 1). Overall, 50% of the children in the study group and 17% of the children in the control group had been vaccinated twice with the BCG vaccine, but the injection status was not significantly different between the two groups (p= 0.09) (Table 1).

Based on the age and gender of the children in the study group, 75% were normal weight and 25% were overweight. No patients in the study group were obese. In the control group, 83.3% of the children were normal weight, 6.7% were overweight and 10% were obese. No significant difference was observed between the two groups in terms of being overweight and obese (p= 0.063).

Table 1. Demographic features of the study and the control groups.

Variables	Study group (n=24)	Control group (n=30)	p value
Age, year, mean±SD	8.79 ± 4.60	6.95 ± 3.73	0.110*
Gender, n (%)			
Girl	12 (50%)	18 (60%)	0.462**
Boy	12 (50%)	12 (40%)	
BMI, kg/m^2, mean±SD	17.25 ± 3.78	17.82 ± 3.92	0.723*
BCG vaccinations, n (%)			
One	12 (50%)	25 (83.3%)	0.09**
Two	12 (50%)	5 (16.7%)	

BCG: Bacillus Calmette-Guérin vaccination, BMI: Body mass index, n: Number of patients, SD: Standard Daviation. Qualitative variables were performed by Pearson Chi-square analysis. Quantitative data were presented as mean, and standard deviation. p-value of less than 0.05 was considered significant. * t test, ** Pearson Chi-Square test.

The average plasma glucose level first analyzed in the hospital for children in the study group was 442 ± 121.52 mg/dL, average venous blood gas pH level was 7.23 ± 0.16, and average bicarbonate level was 12.21 ± 6.55 meg/L. The HBA1C level was first analyzed in the hospital for children in the study group (11.695 ± 0.48%) was quite high, but after 3 months (7.43 ± 2.23%), it decreased significantly according to the initial value (p=0.00). This result shows the 3-month condition of glycemic control in patients in the study group. The first urine analyzed in the hospital for children in the study group had glycosuria and except for one, all of them had ketonuria.

Among the patients in the study group, 29% (n=7) were admitted to hospital in autumn, 33% (n=8) were admitted in winter, 8% (n=2) admitted in spring, and 25% (n=6) were admitted in summer.

PPD evaluation

The initial average PPD induration size did not differ between the study group (study group-1 =5.04 ± 3.08 mm) and the control group (control group =3.5 ± 3.54 mm; p = 0,344). The average PPD induration size at 1 month after the diagnosis for the study group (Study group-2=8.045 ± 4.07 mm) was significantly larger than both the control (3.5 ± 3.54 mm) and the study group-1 PPD (5.04 ± 3.08 mm) responses (p = 0.001; p=0.014).

Whereas the initial PPD positivity ratio did not differ between the study group-1 and the control group (p=0.02), the PPD positivity ratio was significantly higher for the study group-2 than for both the control group (p = 0.005) and the study group-1 (p=0.023) (Table 2).

No significant correlation was found between the HBA1C level, venous blood gas pH or bicarbonate levels, which are assumed to affect the PPD induration size and the initial average PPD induration size. The patients' initial PPD induration size was 12 mm; however, one patient in the study group had an initial PPD induration size of 17 mm. That patient's computed lung tomography was evaluated and a gastric juice test was performed three times for that patient, and TBC was excluded.

Discussion

The TH1 response is known to play an important role in T1D immunopathogenesis [4]. The aim of this study was to investigate how the dominant TH1 response, a Th1-mediated delayed-type skin hypersensitivity immune response, affects the PPD response in children with T1D and previous BCG vaccination.

In our study, the initial PPD induration size did not differ between the study group-1 and the healthy controls of similar age and gender. The number of BCG vaccinations also did not differ between the two groups. However, interesting results emerged for the PPD response of the study group-2, as the induration size was significantly larger than the size in either the controls and or the study group-1 (p = 0.001; p=0.014).

Another interesting and important result of our study is related to the PPD positivity rate. Evaluation of our results according to the PPD positivity limit (PPD induration size ≥ 10 mm) accepted by the World Health Organization in vaccinated children revealed that the initial PPD positivity rate did not differ between the study group-1 and the control group (p=0.02). However, a statistically significant difference was evident between the PPD positivity rate of the study group-2 and either the study group-1 or the control group (p=0.023, p=0.005) [13, 14].

One interesting finding in this study is that the rate of PPD induration and PPD positivity did not differ from the healthy controls in the patients in the initial period of their T1D disease. A change in the PPD response was expected in patients with T1D, as the Th1 dominant response plays an important role in the immune pathogenesis of this disease. Hyperglycemia and/or ketoacidosis can suppress the immune system in patients with type 2 diabetes [16]. Initially, this suppression is a result of the metabolic changes that suppress the immune system. However, in our study, we could not determine any statistical relationship between PPD positivity, induration size, and metabolic (blood sugar, acidosis, HBA1C) changes. This might reflect the small number of cases included in our study.

Conversely, the increase in the PPD induration size and in PPD positivity in the study group-2 leads us to suspect that the Th1 dominant response gradually increases with disease progression. However, the repetition of the TST may also be responsible for this increase [17].

The available literature indicates that the in vitro PPD proliferative response does not differ in T cells from patients with T1D compared to T cells from healthy controls [10]. A longitudinal study conducted in children with T1D reported that the CD4+Th1 response became increasingly transformed into a Th2-dominant response [18]. No study has yet been published in the literature regarding TST in patients with T1D; therefore, we cannot compare our results to other studies. Nevertheless, our findings lead us to think that the Th1 cell response is becoming increasingly dominant in patients with T1D as their disease develops.

Table 2. Evaluation of PPD response of the study and the control groups.

PPD response, n (%)	Study group – 1 (n=24)	Study group – 2 (n=22)	Control group (n=30)	p value
PPD induration size, mm				<0.001
mean±SD	5.04 ± 3.08	8.00 ± 3.90	3.50 ± 3.54	0.344*
(min-max)	(0-12)	(1-17)	(0-13)	0.014**
				<0.001***
PPD positivity ratio,				0.020*
n (%), ≥ 10 mm (%)	3/24 (12.5%)	7/22 (32%)	1/30 (3%)	0.005**
				0.023***

PPD: Purified protein derivative, n: Number, SD: Standard deviation, Study group-1: PPD skin test (TST) was applied to children in the study group immediately after diagnosis (24–48 hours), Study group-2: TST was applied to children in the study group again 1 month after the diagnosis. PPD induration sizes were presented as mean±SD (min-max) and compared between the groups using a One Way ANOVA test with post hoc analyses (Bonferroni correction, the mean difference is significant at the 0.017 level). One Way ANOVA test showed that there was a significance between the groups. Then post hoc analyses with Bonferroni correction showed in pairwise comparisons. PPD positivity were performed by Pearson Chi-square analysis. * between Study group-1 and Control group, ** between Study group-1 and Study group-2 and *** between Study group-2 and Control group.

Longitudinal studies are needed to determine how long the predominant Th1 cell response lasts.

TST is widely used as a diagnostic aid in TBC screening. While PPD positivity detects that the person has encountered mycobacteria, it does not provide sufficient information to establish the presence or absence of TB disease. Vaccination with the BCG vaccine also causes PPD positivity and the PPD positivity we detected in our study is due to vaccination with BCG. In our country, if the PPD induration size is greater than 15 mm, the recommendation is to conduct TBC research. [13]. In our study, one patient had a PPD induration size greater than 15 mm, but subsequent research and tests indicated that the patient had no TB disease. TB and infectious diseases in general are common in adult and childhood patients with diabetes [19], and the risk of TB disease increases by 2- to 8-fold in people with type 2 diabetes [14]. A study conducted in our country revealed that 7% of TBC patients have type 2 diabetes. In children with T1D, the frequency of TBC is reported to be lower than 3.6% [20, 21]. This low frequency of TB in patients with T1D in our country is interesting, as the frequency of TB is reported to be high (26/100,000). Investigating whether the response of dominant TH1 is effective in T1D cases would be an interesting line of research.

Limitations

In our study, because our number of cases was small, the short-term monitoring of the cases was a shortcoming of our study. An investigation into the PPD response at an older age in patients with T1D could provide interesting new information.

Conclusion

In conclusion, our data suggest that the PPD response may give misleading results in the diagnosis of TB in patients with T1D due to the predominant Th1 response in patients with this disease. In addition, the Th1 active response continues to increase 1 month after the diagnosis in these patients, leading us to recommend investigating the effectiveness of immune suppressive treatments after diagnosis in patients with T1D.

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Statement of human rights

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Conflict of interest

The authors declare that they have no conflict of interest.

Informed consent

Written informed consent was obtained from patients' parents who participated in this study.

Ethical approval

The Clinical Research Ethics Committee of Cerrahpaşa Faculty of Medicine approved the study (Ethics Committee Decision No: 83045809/11265).

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Author contributions

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