RELATIONSHIP BETWEEN ISLET CELL ANTIBODY AND B CELL FUNCTION AMONG CHILDREN WITH DIABETES MELLITUS

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ABSTRACT

The relationship between islet cell antibody (ICA) and β cell function among patients with diabetes mellitus (DM) was investigated. Insulin-dependent diabetes mellitus (IDDM) patients admitted to the endocrinology department of XX hospital were included and divided into DM group (40 cases) and control group (24 cases). ICA and glucose tolerance tests (GTT) were performed. According to the comparison of ICA positive rate between two groups, ICA positive rate of DM group was 40.00% (16/40) and that of control group was 8.33% (2/24). The difference was remarkable (P<0.01). ICA positive fasting and postprandial blood glucose (1, 2, and 3 hours) of DM group were 15.41±4.33, 20.36±5.82, 25.15±6.73, and 23.16±5.54, respectively. Insulin levels among ICA positive patients 1 and 2 hours after meal were all significantly lower than those among ICA negative patients (P<0.05). C-peptide levels among ICA positive patients 1 and 2 hours after meal were both obviously lower than those among ICA negative patients (P<0.05). Besides, glucose areas of ICA positive and negative patients in DM group and patients in the control group were 65.12±17.45, 58.11±16.07, and 19.57±28.33, respectively. Insulin areas of ICA positive and negative patients in DM group and patients in the control group reached 29.12±7.32, 34.51±8.24, and 80.67±24.38, respectively. C-peptide areas of ICA positive and negative patients in DM group and patients in the control group were 0.57±0.44, 0.89±0.52, and 5.71±1.33, respectively. β cell function among ICA positive children with DM was inferior to that among ICA negative children.

Keywords: *Pediatric diabetes, islet cell antibody, β cell, function.*

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Introduction

The pathogenesis and autoimmunity of pediatric insulin-dependent diabetes mellitus (IDDM) result in the impairment of islet β cell function[(1)]. To investigate the relationship between autoantibody among children with DM and β cell function, islet cell antibody (ICA) among children with DM was measured and the relationship between ICA and glucose tolerance, insulin, and C-peptide release was observed. According to the classic clinical classification and different basic pathophysiology, type 1 and type 2 DM are the two main types of human DM[(2, 3)]. Islet autoantibody (IAA) is the fundamental characteristic of type I DM as an autoimmune disease. The pathogenesis of type 2 DM includes different levels of insulin resistance and relative insulin deficiency. However, considerable evidence suggests that autoimmunity of islet cells occur among some patients with type 2 DM[(4, 5)]. Islet autoimmunity among patients with type 2 DM was initially determined by IAA in different subgroups among patients with type 2 DM. The autoimmune mechanism in hyperglycemia-induced glucose toxicity is featured with the rise of islet β level. The apoptosis of autoantibody β accelerates

the expression of cell antigen[6]. IAA is the marker of islet autoimmune inflammation. About 4% to 29% of patients with type 2 DM have islet autoantibodies[7]. Short-term active insulin treatment for new patients with type 2 DM can correct metabolic disorders quickly and effectively and reverse the harmful effects of glucose toxicity and fat toxicity on cell function and insulin function β[8]. Intensive insulin treatment inevitably leads to hypoglycemia and weight gain, which are the disadvantages of intensive insulin treatment. However, few doctors pay attention to insulin immunogenicity[9]. Insulin is not only islet β. Hormone produced by cells is also the key target antigen for autoimmune islet destruction. More and more evidence demonstrate that exogenous insulin with different purity and sources still have immunogenicity in human body[10].

DM among children is an endocrine metabolic disease caused by insufficient secretion of insulin. In most cases, the metabolic disorders of carbohydrate, protein, and fat lead to fasting and postprandial hyperglycemia and glucosuria[11]. The clinical manifestations include polydipsia, polyuria, polyphagia, and weight loss. Children usually suffer from ketoacidosis and vascular diseases later, which result in eye and kidney involvement. DM occurs in each stage of childhood and its incidence becomes higher with gender difference during adolescence[12]. According to different causes of DM, DM among children is divided into primary DM and secondary DM. Primary DM is divided into IDDM (also called type 1 DM) and non-IDDM (also called type 2 DM). The main symptoms of secondary DM include pancreas diseases, abnormality in hormone receptors, and the impairment of glucose tolerance[13].

IDDM is very common among children with DM. At present, the incidence of type 2 DM increases, insulin becomes less sensitive, and the level of insulin secretion is higher than normal among children with type 2 DM. IAA can be detected 9 to 13 days after insulin treatment[14, 15]. IAA is present in patients with DM and it is very likely that IAA positivity is related to an underlying autoimmune process or the outcome of exogenous insulin treatment. According to relevant studies, IAA is produced among 13% to 61% of patients receiving insulin treatment[16]. It is well known that IAA is associated with the onset of type 1 DM and islet β is positively correlated with rapid cell injury. However, the relationship between IAA and islet β cell function among patients with type 2 DM is inconsistent. Some studies show that IAA doesn't result in pancreatic carcinoma among patients with type 2 DM and islet β cell injury[17, 18]. According to a British prospective research report on DM, islet function of patients with IAA positive β cell function is weak and they are more likely to undergo insulin treatment. The patients with positive IAA may receive insulin treatment earlier than those with negative IAA[19]. A 37-month experimental study demonstrates that islet autoimmunity among patients with type 2 DM is related to their islet β, which is associated with the significant and fast decline in cell function[20].

Besides, a 18-year clinical follow-up study reveals that islet β cell function of patients with positive IAA decreases remarkably, while islet β cell function is unknown among patients with negative IAA[21]. In this research, the relationship between ICA and β cell function was investigated. In addition, the relationship between ICA among children with DM and β cell function was explored.

Materials and methods

Research objects

IDDM patients admitted to endocrinology department of XX hospital between January 2021 and March 2022 were included and divided into DM group and control group. 40 IDDM patients were enrolled in the DM group, including 17 males and 23 females aged between 3 and 12. Their average age was 8.7±4.5 and the duration of disease course ranged from 1 to 4 months. 37 patients ever suffered from ketosis, including 23 suffering from ketoacidosis and 8 with a family history of previous DM. All included patients didn't suffer from other autoimmune diseases with normal functions of the heart, liver, kidney, and other organs. 24 patients were enrolled into the control group, including 10 males and 14 females aged between 2 and 12. Their average age was 8.6±4.3.

The inclusion criteria were as follows:

• Children treated in endocrinology department of Children' hospital with disease course over 1 month;

• Children performed with daily continuous or multiple subcutaneous injection of insulin and blood glucose monitoring with peripheral blood glucose or fast scan blood glucose monitoring system;

• Children who maintained their sustained DM management plans during the entire research.The blood glucose monitoring range was 2.3-21.3mmoL/L. During the monitoring, insulin dose could be adjusted according to the change in blood glucose.

The exclusion criteria were as follows:

• Children with other endocrine diseases and severe heart, kidney, brain, and other internal medicine diseases;

• Children suffering from serious hypoglycemia and other complications during the research;

• Children treated with glucocorticoid and blood transfusion during the research;

• Children who were uncooperative or had no complete final data;

• Children allergic to medical adhesive.

The implementation of this research had been approved by Hospital Ethics Committee. All included patients had signed informed consent forms.

ICA measurement

Paraffin sections were routinely dewaxed and then digested with 0.04% trypsin solution for 58 minutes. Then, they were rinsed with TBS for 6 to 11 minutes. After that, they were incubated with 5.2% normal goat serum at room temperature for half an hour. Next, they were rinsed with TBS and each of them was added with 23-39μL detection serum (1:31 and 1:63 diluent). After that, they were put into a humid incubator and then refrigerated at 3°C for 17 hours. Next, sections were rinsed with TBS and added with 39μL biotin-labeled goat anti-human G Ig. Then, they were incubated at room temperature for 1 hour. After that, they were rinsed with TBS and colored with diaminobenzidine (DAB) base solution for 6 to 9 minutes.

Next, they were washed with running water for 2 minutes and then re-stained with hematoxylin (HE). After that, they were sealed with neural adhesive and observed with a normal optical microscope. To determine the amount of serum, two unknown sources were observed. The cytoplasms of islets were brownish yellow positive granules. Besides, sections were stained with HE and anti-insulin serum enzyme markers. ICA positive serum was diluted with 1:16, 1:7, 1:3, 1:2 and 1:126, 1:258, 1:521 to determine the positive degree of two endpoints.

Glucose tolerance test (GTT)

The included children were instructed to orally take glucose. Children aged under 3 took 2.1g/kg and children over 3 took 1.76g/kg. The maximum dose was 76g. A blood sample was collected and blood glucose, insulin, and C-peptide levels were measured after 1.2 hours and 3 hours. On the morning of the test, children were ordered to stop taking insulin. Blood glucose was automatically measured by Weihai Baihe Biotechnology Co., Ltd.. Radioimmunoassay was adopted to measure the levels of insulin and C-peptide. The kit was purchased from Beijing Yamei Biotechnology Co., Ltd.. The calculation method of glucose, insulin, and C-peptide areas was expressed below.

$$\frac{(0\text{ h} - \text{value} + 1\text{ h} - \text{value})}{2} + \frac{(1\text{ h} - \text{value} + 2\text{ h} - \text{value})}{2} + \frac{2\text{ h} - \text{value} + 3\text{ h} - \text{value})}{2}$$

Statistical analysis

SPSS21.0 software was used for the analysis of overall data. $\bar{x}\pm s$ was employed to denote continuous variables. Percentage was adopted to represent enumeration data. The comparison between groups was carried out by Fisher's precision probability test. $P<0.05$ suggested that the difference was statistically significant.

Results

Basic information about patients

In DM group, there were 40 IDDM patients, including 17 males and 23 females aged between 3 and 12. Their average age was 8.7±4.5 and the duration of disease course ranged from 1 to 4 months. 37 patients ever suffered from ketosis, including 23 suffering from ketoacidosis and 8 with a family history of previous DM. All included patients didn't suffer from other autoimmune diseases with normal functions of the heart, liver, kidney, and other organs.

In the control group, there were 24 patients, including 10 males and 14 females aged between 2 and 12. Their average age was 8.6±4.3. The specific information about the patients in the two groups is displayed in Table 1 below.

Variables	DM Group	Control group
Gender (M/F)	17/23	10/14
Age (years old)	8.7±4.5	8.6±4.3
History of ketosis (case)	37	/
History of ketoacidosis (case)	23	/
Family history of previous DM	8	/

Table 1: Basic information about patients.

ICA positive rate of the two groups

ICA positive rate of DM group was 40.00% (16/40) while that of the control group was 8.33% (2/24). The difference was remarkable ($P<0.01$). The result was shown in Figure 1 below.

Results of GTT in the two groups

The levels of fasting and postprandial blood glucose (1, 2, and 3 hours) among the patients with

positive ICA in DM group reached 15.41±4.33, 20.36±5.82, 25.15±6.73, and 23.16±5.54, respectively, while those among the patients with negative ICA in DM group amounted to 13.34±4.01, 18.74±5.13, 22.17±5.77, and 20.81±5.61, respectively. In contrast, the levels of fasting and postprandial blood glucose (1, 2, and 3 hours) among the patients in control group were 4.43±0.38, 7.72±1.21, 7.15±1.15, and 5.17±0.61, respectively. Besides, the levels of fasting and postprandial blood glucose (1, 2, and 3 hours) among DM children with positive ICA were all higher than those among DM children negative ICA (P>0.05). The results of GTT were illustrated in Figure 2 below.

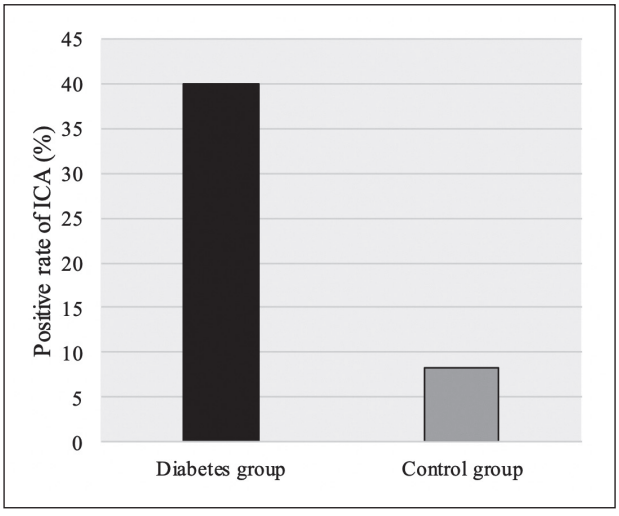

Figure 1: Comparison of ICA positive rate between the two groups (%).

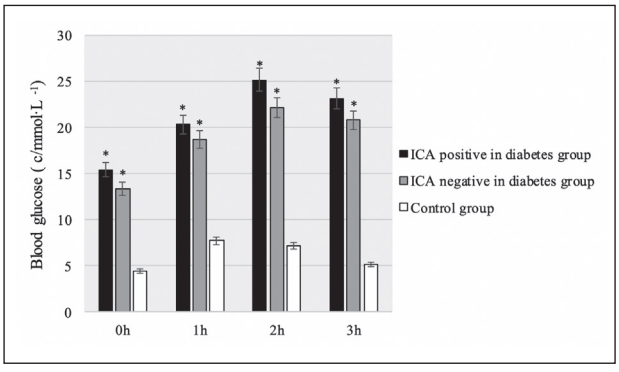

Figure 2: Glucose Results of GTT in the two groups (* indicated that the comparison with the levels of fasting and postprandial blood glucose (1, 2, and 3 hours) in the control group showed P<0.05).

Insulin results of GTT in the two groups

The levels of fasting and postprandial insulin (1, 2, and 3 hours) among the patients with positive ICA in DM group amounted to 4.31±1.85, 8.75±2.34, 12.29±2.73, and 11.63±3.44, respectively, while those among the patients with negative ICA in DM group reached 5.12±1.81, 10.54±2.13, 14.31±3.07, and 13.57±3.61, respectively. In contrast, the levels of fasting and postprandial insulin (1, 2, and 3 hours) in control group were 7.16±2.38, 42.25±12.21, 29.53±9.75, and 10.17±3.41, respectively.

It was found that the levels of postprandial insulin (1 and 2 hours) among the patients with positive ICA were both significantly lower than those among the patients with negative ICA (all P values were lower than 0.05).

Besides, fasting and postprandial insulin (3 hours) among the patients with positive ICA were also lower than those of the patients with negative ICA (P>0.05). The insulin results of GTT were shown in Figure 3 below.

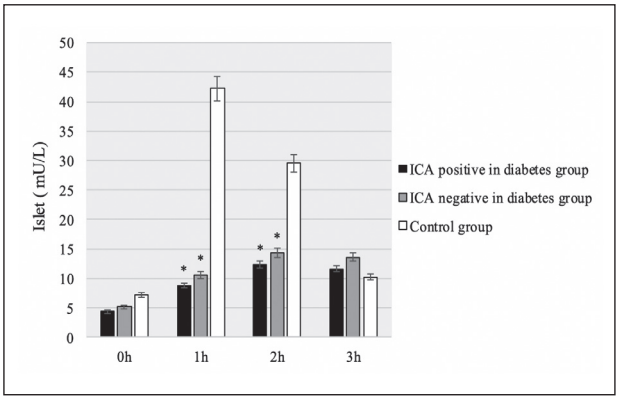

Figure 3: Insulin results of GTT in the two groups (mU/L) (* suggested that the comparison with the levels of fasting and postprandial insulin (1, 2, and 3 hours) of control group showed P<0.05).

C-peptide results of GTT in the two groups

The levels of fasting and postprandial C-peptide (1, 2, and 3 hours) among the patients with positive ICA in DM group amounted to 0.13±0.12, 0.17±0.14, 0.22±0.15, and 0.23±0.16, respectively, while those among the patients with negative ICA in DM group were 0.2±0.13, 0.28±0.15, 0.32±0.17, and 0.31±0.19, respectively. In contrast, the levels of fasting and postprandial C-peptide (1, 2, and 3 hours) in control group reached 0.61±0.15, 2.83±0.67, 2.02±0.54, and 1.01±0.22, respectively.

It was found that the levels of postprandial C-peptide (1 and 2 hours) among the patients with positive ICA were both apparently lower than those among the patients with negative ICA (all P values were lower than 0.05). In addition, the levels of fasting and postprandial C-peptide (3 hours) among the patients with positive ICA were also lower than those among the patients with negative ICA (P>0.05). The C-peptide results of GTT were displayed in Figure 4 below.

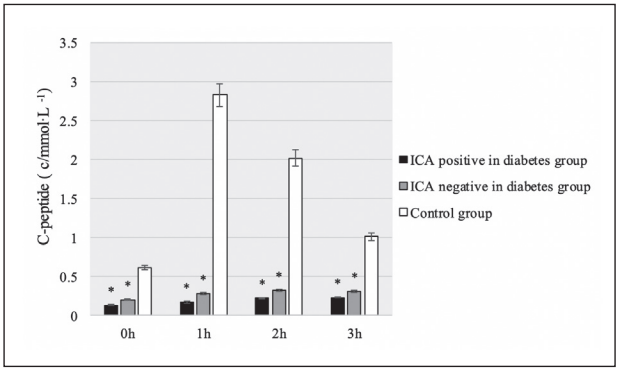

Figure 4: C-peptide results of GTT in the two groups (c/mmol·L-1) (* suggested that the comparison with the levels of fasting and postprandial C-peptide (1, 2, and 3 hours) of control group showed P<0.05).

Comparison of glucose areas in the two groups

The glucose areas of patients with positive and negative ICA in DM group and patients in the control group were 65.12±17.45, 58.11±16.07, and 19.57±28.33, respectively. It was found that the glucose areas of patients with negative and positive ICA were both larger than that of patients in the control group (all P values were lower than 0.05), as illustrated in Figure 5 below.

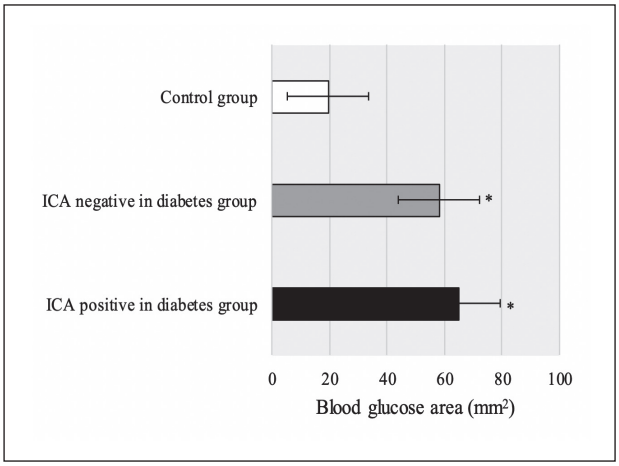

Figure 5: Comparison of glucose areas in the two groups (* indicated that the comparison with glucose area in control group revealed P<0.05).

Comparison of insulin areas in the two groups

The insulin areas of patients with positive and negative ICA in DM group and patients in control group amounted to 29.12±7.32, 34.51±8.24, and 80.67±24.38, respectively.

It was found that the insulin areas of patients with negative and positive ICA were both notably smaller than that of patients in the control group (all P values were lower than 0.05), as shown in Figure 6 below.

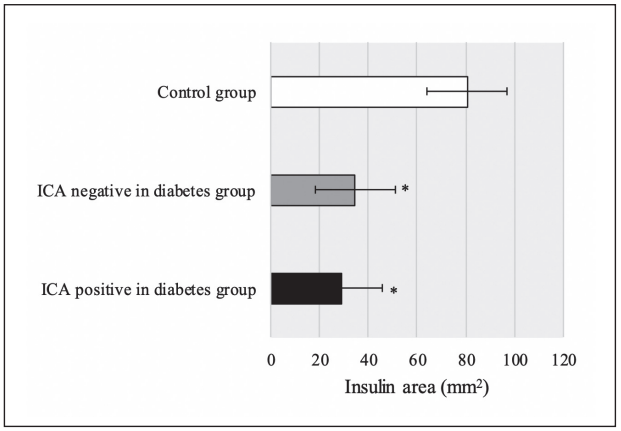

Figure 6: Comparison of insulin areas in the two groups (* indicated that the comparison with insulin area in control group revealed P<0.05).

Comparison of C-peptide areas in the two groups

The C-peptide areas of patients with positive and negative ICA in DM group and patients in control group amounted to 0.57±0.44, 0.89±0.52, and 5.71±1.33, respectively. It was found that the C-peptide areas of patients with negative and positive ICA were both remarkably smaller than that of patients in the control group (all P values were lower than 0.05), as displayed in Figure 7 below.

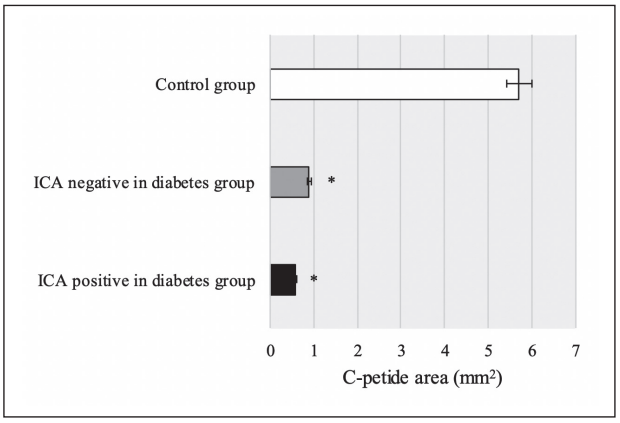

Figure 7: Comparison of C-peptide areas in the two groups (* indicated that the comparison with C-peptide area in control group revealed P<0.05).

Discussion

According to different pathogenesis, DM is mainly divided into two types. One is type 1 DM caused by absolute deficiency in insulin secretion. The majority of type 1 DM results from the destruction of islet β cell autoimmunity. Another is type 2 DM that is mainly caused by insulin resistance and the impairment of islet β cell secretion[22]. However, it is currently unknown which type of DM

is the initiating factor of the pathogenesis of DM. Among the "sequence" studies on insulin resistance and the impairment of insulin secretion function of islet β cells, the most important theory is "two-stage model" occurring among races with high incidence of DM (such as Pima Indians). In the first stage, insulin resistance transforms normal glucose tolerance (NGT) into impaired glucose tolerance (IGT). In the second stage, the deficiency of insulin resistance and β cell function results in the transformation of IGT into DM[23]. The pathogenesis of latent autoimmune diabetes in adults (LADA) is T lymphocyte-mediated autoimmune disease featured with the deficiency in insulin secretion. Insulin-independent diabetes mellitus treatment can be achieved during a long period of time. LADA is the transitional type of type 1 and 2 DM. Hence, it is also called type 1.5 DM. The early clinical manifestations of LADA are similar to those of type 2 DM.

According to relevant studies, some patients with type 2 DM had IAA no matter if they ever received insulin treatment before hospitalization. Compared with that in non-insulin treatment group, the positive rate of IAA in insulin treatment group was higher. It was found that the function of islet β cells in positive IAA subgroup was poorer than that in negative IAA subgroup of insulin treatment group. Besides, there was no apparent difference in insulin resistance between the two groups[24]. Unfortunately, the differences in islet β cell function and insulin resistance between the two subgroups of non-insulin treatment group were not detected before hospitalization. It was reported that 5% to 30% of patients with type 2 DM had IAA[25]. Since the production, storage, and administration of exogenous insulin can't completely mimic human physiology, the existing human insulin and insulin analogues still had immunogenicity to the human body. Insulin is an ideal candidate for main autoantigen because it is the specific expression of islet β cells. IAA is the commonest positive antibody among children, which is related to insulin autoimmunity during the destruction of islet β cells. Some scholars described 3 patients with type 2 DM who had hyperinsulin antibodies of insulin resistance or insulin allergy shortly after insulin treatment[26].

During the histological examination of pancreatic tissues, the invasion of islet tissues by monocyte was observed. C-peptide is the by-product of insulin production, which helps repair arterial wall and prevents vascular problems and nerve injury. β cells produce equal amounts of insulin and C-peptide. In most cases, doctors carry out C-peptide test to monitor DM patients and perform blood test to measure C-peptide level for the measurement of the amount of insulin produced in the body. As far as we know, this is the first research into IAA and islet β cell function based on grouping and regrouping[27]. β cell is in pancreas and produces and releases insulin according to glucose level. Among DM patients, β cells must work harder to produce adequate insulin to control hyperglycemia level. As a result, they can't work as normal to regulate glucose. Besides, β cells must work more diligently to produce sufficient insulin to reduce glucose levels, which results in loss of β cells or the inability to function effectively. Because of the cycle of lost β cell function, it is possible that not enough insulin can be produced in the body to control glucose level.

At present, it is still unclear about the pathogenesis of IDDM among children. It is generally believed that it is related to autoimmune response caused by virus infection and other external factors. According to some foreign reports, positive ICA rate among new patients diagnosed with IDDM reached as high as 60% to 80% and gradually decreased with the extension of disease course[28]. In terms of antibodies, many studies have been performed at the cellular and molecular levels[29-39], and research in this area is ongoing. In the future, it may be possible to link the results of cellular and molecular studies with Diabetes Mellitus.

Besides, it was reported that positive ICA rate among IDDM patients in Japan was 32%, which was close to that in the group (39.47%). The above two values were both lower than that positive ICA rate among new patients diagnosed with IDDM mentioned above. The difference in positive ICA rate among various populations in different countries might be caused by the disease course of the two groups and national differences. According to relevant reports, positive ICA rate among IDDM patients in Asian countries was apparently lower than that among white-skinned patients in European and American countries[29]. The research results demonstrated that the levels of fasting and postprandial glucose among DM children with positive ICA were higher than those among DM children with negative ICA. In addition, the levels of serum insulin and C-peptide among patients with positive ICA were remarkably lower than those among patients with negative ICA 1 and 2 hours after meal. What's more, insulin and C-peptide areas among patients with positive ICA were smaller than those among patients with

negative ICA, suggesting that β cell function of DM children with positive ICA was weaker than that of DM patients with negative ICA.

Conclusion

To sum up, β cell function of DM children with positive ICA was weaker than that of DM patients with negative ICA. Because the included sample size was small, it would be further enlarged in follow-up studies to improve the scientificity of studies.

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