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ORIGINAL ARTICLE

# Insulin antibody as a biomarker to monitor the development of type 2 diabetes in county hospitals in China

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## Abstract

**Background** The significance of insulin antibody (IA) detection in type 2 diabetes mellitus (T2DM) has received scant attention from county hospitals in China. We aimed to introduce exogenous IA positive rate and its relative factors in T2DM treated with insulin in Xiangshan County of Ningbo City, analyzing the immunogenicity of different kinds of insulin.

**Methods** Patients who were residents from the Danxi community and six towns (Dongchen, Xizhou, Maoyang, Sizhoutou, Juexi, and Qiangtou) in Xiangshan County and diagnosed with T2DM and treated with insulins at Xiangshan Hospital of TCM Medical and Health Group between August 2019 and June 2020 were identified. Those who met the eligibility criteria were included and assigned to the IA-positive or IA-negative group. The immunogenicity of different insulins was compared between the two groups.

**Results** Among 992 patients, 781 were eligible for IA detection, and 40.2% of them were IA positive. Blood IA was closely associated with fasting and 2-h glucose, insulin, and C peptide levels and higher insulin dosage. Patients receiving basal insulin treatment showed significantly lower blood IA than those treated with mixed human insulin, premixed human insulins, rapid-acting analogs, or a combination of basal and rapid-acting analogs.

**Keywords** Insulin antibody · Type 2 diabetes mellitus · Primary hospital · Insulin

## Introduction

In 2011, 370 million people suffered from diabetes worldwide and 80% of them were from developing countries, according to the estimates released by the International Diabetes Federation. The prevalence has reached 11.6% in China, according to the 2010 report from Chinese Center for Disease Control and Prevention. Patients with type 2 diabetes mellitus (T2DM) roughly account for 90% of all diabetic cases. Insulin is an important means of diabetes treatment. Insulin antibodies (IA) can be developed upon initiation of insulin treatment [1]. Their binding with insulins, forming insulin-antibody complexes which compete with insulins for the binding site of insulin receptors or serve as an insulin pool to reduce insulin activity and irregularly release insulin, ultimately results in hyperinsulinemia and hypoglycemia. Unfortunately, hyperinsulinemia has been proven to be a risk factor of wide glycemic fluctuation [2], increased blood pressure [3], and the occurrence of several tumors [4], Alzheimer's disease [5] and microvascular dysfunction that leads to cardiovascular diseases [6, 7]. Both insulin-antibody complexes and exogenous insulin antibodies (IAs) have been shown to involve in the development of lipoatrophy and microangiopathy, except for glycemic control, via altered insulin pharmacokinetics, insulin resistance, and other mechanisms [8].

As for diabetic complications, patients detected exogenous IA positive due to intake of sulfhydryl-containing agents often suffer from insulin autoimmune syndrome (IAS), which is characterized by spontaneous hypoglycemia and endogenous hyperinsulinemia with a high titer of anti-insulin antibodies. For T2DM patients with insulin therapy, IAs may cause high glucose fluctuation and

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exogenous hyperinsulinemia, which is called exogenous insulin-related insulin autoimmune syndrome (EIAS). IAs preclude the possibility of glucose control and act as a barrier to avoid related complications.

Exogenous IAs have a non-negligible impact on T2DM in China. Dong et al. reported a relatively high ratio of 220/742 exogenous IA-positive patients in Shandong Province [9]. However, the only two studies of exogenous IAs in China were both conducted in urban tertiary 3A hospitals. Any study that provides data for IA-associated measurements of rural patients is urgently needed, who have known risks of treatment incompliance. Therefore, the goal of this study is twofolded: to detect blood IA levels in rural T2DM patients, to explore relationships of exogenous IAs with clinical characteristics of patients and insulin usage in this patient group.

## Methods

### Subjects and designs

Between August 2019 and June 2020, patients who were residents from the Danxi community and six towns (Dongchen, Xizhou, Maoyang, Sizhoutou, Juexi, and Qiangtou) in Xiangshan County Zhejiang Province and diagnosed with T2DM and treated with insulins at Xiangshan Hospital of TCM Medical and Health Group were selected. Patients diagnosed with T2DM and receiving insulin therapy for at least 2 weeks were included. But they were excluded if they had (1) IAS at or before diagnosis; (2) a history of treatment with sulfhydryl-containing agents in the past 6 months; (3) moderate-to-severe liver or renal dysfunction with alanine aminotransferase (ALT) or glutamic oxalacetic transaminase (AST) > 100 U/L, or estimated glomerular filtration rate (eGFR)< 45mL/(min*1.73m$^2$); (4) myocardial infarction, cerebral infarction, trauma, operation, and stress state occurred in the past 6 months; (5) acute complications; and (6) types of insulin changed in recent 2 years. The study protocol was approved by the ethical committee of Xiangshan Hospital of TCM Medical and Health Group (p2019-[k]-10).

All subjects were asked to undergo the 75-g oral glucose tolerance test (OGTT) after 10–12 h of fasting. No insulin injection or oral antidiabetic agents (OADs) were administered the night before and on the morning of the test and after the test, with a continuation of insulins. Blood samples were collected during the fasting glucose test and OGTT. We assessed fasting and 2-h post-OGTT glucose, insulin, and C-peptide levels. Fasting blood IA, glutamic acid decarboxylase antibody (GADA), islet cell antibody (ICA), and glycated hemoglobin (HbA1c) levels were quantitated.

### Biochemical assays

Serum insulin and C-peptide were determined by immunoelectrochemiluminescence (Roche Diagnostics GmbH, Mannheim, Roche E601, Germany), and hexokinase method was performed to quantitate fasting and 2-h blood glucose levels (Meikang biology, Beckman AU5800, China). We employed high-performance liquid chromatography (Huizhong, Medicine, MQ-2000PT, China) to detect HbA1c. Serum IA was tested with radioimmunoassay (North Institute, Gamma counter sn-6105, China), while ICA and GADA were detected by indirect immunofluorescence assay (Euroimmun, fluorescence microscope EURostarIII, China).

### Statistical analysis

Continuous variables were expressed as mean ± standard error of the mean (SEM). Differences between IA-positive and IA-negative groups were compared with the Student *t*-test if data were normally distributed. The chi-square test was applied for categorical variables. All statistical analyses were performed using SPSS24.0 (SPSS, Palo Alto, CA, USA) and Graphpad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). The significance level was set at $p < 0.05$.

## Results

### IA expression was associated with clinicopathological characteristics

Initially, 992 residents from Xiangshan diagnosed with T2DM were identified. Among others, 211 not fulfilling the eligibility criteria were ruled out, and 781 subjects (78.73%; mean age 61.69 ±11.27 years, range 25–88 years, male-to-female ratio 1.07:1) were enrolled in this study. Among the 781 cases, 40.2% (314 of 781cases) were detected of IA positive. All patients were categorized into the IA-positive (IA+) or IA-negative (IA−) group; IA+ patients showed significant increases in fasting and 2-h blood glucose (FBG, 2hBG; both $p$=0.000), C peptide ($p$=0.041 and 0.032), insulin (both $p$=0.000), and insulin/C peptide ratio (both $p$=0.000), daily insulin dosage ($p$=0.000), homeostatic model assessment of insulin resistance (HOMA-IR, $p$=0.000), and body mass index (BMI, $p$=0.000) compared to IA− patients (Table 1). There were no pronounced correlations of IA with age, gender, duration of diabetes, duration of insulin treatment, and blood

**Table 1** Correlation between IA expression and clinicopathological characteristics of T2DM patients ($n$=781)

| Characteristics | IA+ | IA− | *T/Z* | *p* |
|---|---|---|---|---|
| Number | 314 | 467 | - | - |
| Percentage (%) | 40.2 | 59.8 | - | - |
| Gender (M/F) | 174/140 | 230/237 | - | 0.091 |
| Age (year) | 61.02±10.85 | 62.14±11.54 | −1.364 | 0.173 |
| Duration of diabetes (year) | 9.68±6.75 | 9.97±7.17 | −0.572 | 0.568 |
| Duration of insulin treatment (year) | 4.22±4.26 | 4.65±4.72 | −1.320 | 0.187 |
| HbA1c (%) | 8.29±1.92 | 8.89±5.85 | −1.765 | 0.078 |
| FBG (mmol/L) | 8.38±3.11 | 9.98±3.58 | −6.465 | 0.000 |
| 2HBG (mmol/L) | 16.07±5.69 | 17.93±5.61 | −4.514 | 0.000 |
| C peptide 0 min (nmol/L) | 0.69±0.60 | 0.61±0.40 | 2.050 | 0.041 |
| C peptide 120 min (nmol/L) | 1.73±1.44 | 1.53±1.00 | 2.303 | 0.032 |
| Insulin 0 min (μIU/ml) | 66.52±94.47 | 10.89±7.33 | 10.413 | 0.000 |
| Insulin 120 min (μIU/ml) | 129.63±136.99 | 35.74±31.11 | 11.938 | 0.000 |
| insulin/C peptide 0 min | 117.18±140.08 | 23.94±38.59 | 11.505 | 0.000 |
| insulin/C peptide (120min) | 99.57±156.62 | 25.89±22.54 | 8.279 | 0.000 |
| BMI (kg/m$^2$) | 25.27±3.34 | 24.38±3.42 | 3.634 | 0.000 |
| Daily dosage of insulin (U) | 36.57±17.70 | 30.69±13.70 | 5.216 | 0.000 |
| HOMA-IR | 25.37±43.81 | 4.75±3.78 | 8.318 | 0.000 |

The normally distributed date was tested by *t*-test, and measurement data were expressed as mean ± standard deviation. The enumeration data were analyzed by chi-square

HbA1c (all $p > 0.05$, Table 1). Therefore, IA was associated with daily insulin dosage instead of treatment sessions, suggesting that higher insulin dosage may be associated with IA in T2DM.

### Serum IA decreases with age

We performed the Nonlin fit of Saturation Binding Data analysis to assess the relationship of serum IA with age in T2DM patients. The results showed that the proportion of IA+ patients decreased by 0.3344 per year of age (Fig. 1).

### Insulin regimens associated with IA elevation

Subsequently, proportions of IA+ patients were compared between those treated with different insulin analogs to identify regimens associated with IA elevation. Intriguingly, only 18.1% (19/105) of patients treated with long-acting basal insulin analogs (detemir and glargine) were IA+, significantly lower than the percentages of patients receiving premixed human insulins (novolin and humulin), of 43.1% (197/458), and those receiving premixed rapid-acting analogs (aspart and lispro) and a combination of basal and rapid-acting analogs, of 45.86% (72/157) and 42.62% (26/61), respectively ($p$<0.05, Fig. 2). No significant differences in the proportion of IA+ patients were found among premixed human insulin group, premixed insulin analogue group, and a combination of basal and rapid-acting analog group ($p$>0.05). All these indicate that the long-acting basal insulins are less likely to develop IAs.

## Discussion

IAs are immunoglobulin, antibodies against insulin, of which IgG-class IAs are most common, followed by IgM-, IgA-, and IgD-class IAs. Insulin often binds to plasma proteins to form multimers. Insulins are primarily targeted by specific antibodies, with binding capacity reaching up to 10,000μU/ml. Once supersaturated insulin is formed, one molecule of IA will antibody can bind to two molecules of insulin, forming an Ab1Ag2 complex (molecular weight 162,000). Specifically, IA-insulin binding can form Ab1Ag2 complexes when a low amount of blood IAs are present or form Ab2Ag1 complexes (molecular weight 306,000) when there are excessive IAs, or constitutes (AbAg)n immune macromolecular complexes when the amounts of insulin and IAs are comparable. However, Ab1Ag2 complexes are unstable, cannot be cleared by the reticuloendothelial system, completely contrary to (AbAg)n complexes [10]. Insulin-receiving individuals with high IA levels and recurrent hypoglycemia had a higher dissociation constant for insulin; furthermore, this study reported that IA characteristics were among the causative factors in hypoglycemic episodes [11]. Therefore, monitoring IA

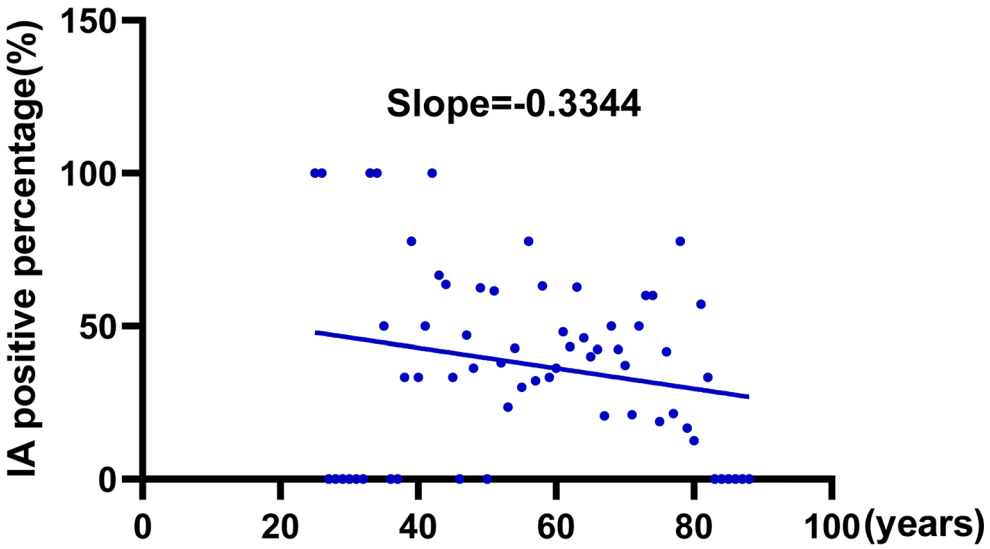


**Fig. 1** Serum IA decreases with age

expression monitoring and follow-up treatments of potential complications are particularly important.

In 1960, Harwood first reported the presence of IAs in patients receiving exogenous insulin therapy [12]. As we mentioned above, IA+ patients are more likely to develop hyperinsulinemia more often than IA− patients without IA expression. Due to the mitotic effect of insulin, hyperinsulinemia can promote cell proliferation in vascular wall cells, resulting in thickening of the vascular wall and local deposition of cholesterol. Therefore, hyperinsulinemia has a strong correlation with cardio-cerebrovascular diseases. In addition, IA+ immune macromolecular complexes can cause vascular damage and contribute to diabetic complications.

In this study, blood IAs were detectable in 40.2% (314/781) of all patients, very close to 44% reported by Fineberg et al. [13] in an Indiana population, but much higher than the percentage achieved in an urban Chinese population [9]. This difference can be explained by several reasons. Physicians in county hospitals lack knowledge of the clinical significance of blood IA in T2DM. Glucose control among IA+ patients is unsatisfactory due to glucose fluctuation without regular glucose tests. Furthermore, patients in rural areas often have higher risks of poor compliance with tests or loss to follow-up.

We found that FBG and 2hBG levels significantly decreased and 0' and 120' serum insulin and insulin/C peptide ratio markedly increased in the IA+ versus IA− groups. It can be explained by the biological characteristics of IA [1, 14]. IA accumulation or IA+ complexes in blood have been shown to have a robust ability to bind to a vast array of endogenous and exogenous insulin. Insulin can only be slowly dissociated from the complexes slowly in the absence of exogenous insulins and perform pharmacological actions later. This phenomenon is called a reservoir-like effect [14], which contributes to lower concentrations of FBG and 2HBG in T2DM patients

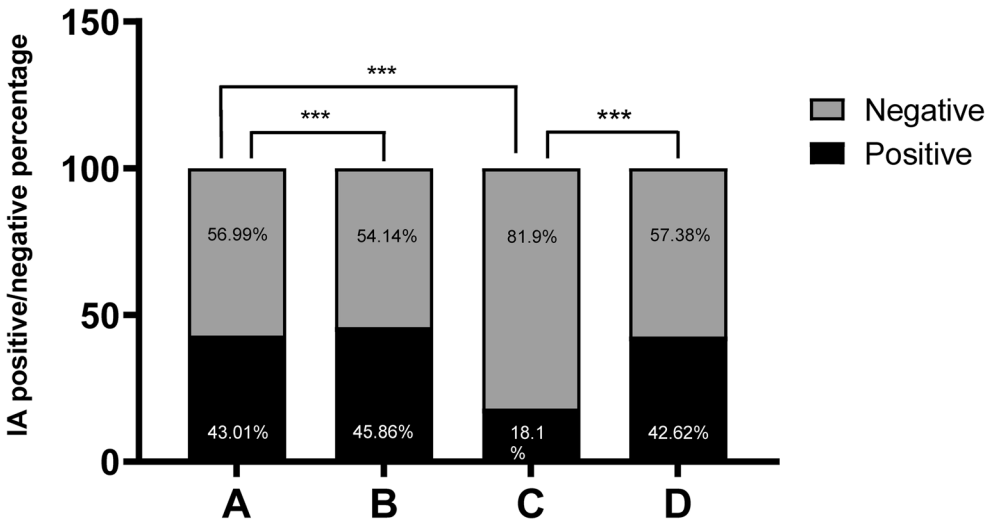


**Fig. 2** Proportions of IA+ patients compared between those treated with different insulin analogs

(Table 1). Although IA+ patients exhibited lower FPG and 2hPG levels and greater glucose fluctuation than IA− patients, both of which could not be discriminated by HbA1c.

Previous studies have ascertained that chronic hyperinsulinemia can lead to the decrease of insulin sensitivity [15]. It is reasonable to speculate that hyperinsulinemia in T2DM patients with IA will lead to an inadequate response to insulins, stimulating endogenous insulin production, as manifested by elevated serum C-peptide.

Overweight or obesity may trigger specific and nonspecific autoimmunity [16]. Therefore, BMI may be positively correlated with IA levels. Consistently, we found that BMI in the IA+ group was indeed than that in the IA− group.

Rajan et al. reported that chronic hyperinsulinemia could reduce insulin sensitivity in T2DM patients [16], which required increased insulin dosage. Furthermore, the binding of IA binds to free insulin may reduce the amount of functional insulin in blood, resulting in increased insulin requirement. When insulin combines with IAs with different ratios, insulin-IA complexes of different sizes and molecular weights may exert different biological effects. Their macromolecular complexes can stimulate downstream signaling cascades upon extensive insulin-IA binding. Therefore, blood IAs are more associated with insulin dosage than insulin treatment duration.

In this study, the proportion of IA+ patients among all T2DM patients was comparable between those receiving premixed human insulins and rapid-acting analogs, consistent with the findings reported by Home et al. [17] that there was similar immunogenicity between the two patient groups. As insulin stimulates IA production, patients treated with premixed human insulins group and rapid-acting analogs exhibited significantly higher proportions of IA+ patients than those receiving basal insulin support. The underlying mechanisms can be persistent low serum insulin levels induced by long-acting insulin analogs (insulin multimers) under the skin and weak antigenicity induced by the modified allergenic amino acid B30 in long-acting insulin analogs. Patients receiving a combination of basal and rapid-acting insulin analogs also revealed a higher proportion of IA+ cases than those treated with basal insulin therapy. This may be due to various immune responses triggered by types of insulin analogs [18] and increased risk of IA development by repeated insulin injections [19]. However, mechanisms responsible for a high percentage of IA+ patients among the three insulin regimens have not yet been fully explored.

As for physicians in county hospitals, personalized therapeutic decision-making in T2DM patients to reduce the rate of IA+ patients is feasible to improve blood glucose management and the use of insulin analogs. Thus, diabetic complications can be prevented or delayed to circumvent irreversible organ damage to ameliorate the prognosis and life quality in T2DM patients.

## Limitations

The limitations are apparent due to the nature of a single center sampling survey in some villages and towns of county hospitals in China. This cannot represent the specific situation of rural medical care in China, let alone the overall situation in China. These problems can be resolved in multicenter studies in China in the future.

## Conclusion

Blood IA is associated with hyperinsulinemia and daily insulin dosage in T2DM. IA+ T2DM patients are more likely to have significant blood glucose fluctuations and hypoglycemia after insulin injections, which can be resolved by adjusting the treatment plan. Special attention must be placed on rural residents as the proportion of IA+ patients from Xiangshan County is much higher than that reported in previous studies, conducted in urban populations.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s13410-022-01110-2.

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## Declarations

We the undersigned declare that this manuscript entitled "Analysis of the status of insulin antibody positive rate in T2DM patients in county hospitals in China" is original, has not been published before, and is not currently being considered for publication elsewhere.

We would like to draw the attention of the Editor to the following publications of one or more of us that refer to aspects of the manuscript presently being submitted. Where relevant copies of such publications are attached.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We understand that the corresponding author is the sole contact for the Editorial process. He is responsible for communicating with the other authors about progress, submissions of revisions, and final approval of proofs.

**Ethical Approval** The study protocol was approved by the ethical committee of Xiangshan Hospital of TCM Medical and Health Group (p2019-[k]-10).

**Conflict of Interest** The authors declare no competing interests.

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