ISSN 1948-9358 (online)

World Journal of *Diabetes*

World J Diabetes 2024 July 15; 15(7): 1384-1653

Published by Baishideng Publishing Group Inc

World Journal of Diabetes

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ABOUT COVER

Peer Review of *World Journal of Diabetes*, Erkan Gokce, MD, Professor, Department of Radiology, Tokat Gaziosmanpasa University, School of Medicine, Tokat 60100, Türkiye. drerkangokce@gmail.com

AIMS AND SCOPE

The primary aim of *World Journal of Diabetes* (*WJD*, *World J Diabetes*) is to provide scholars and readers from various fields of diabetes with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJD mainly publishes articles reporting research results and findings obtained in the field of diabetes and covering a wide range of topics including risk factors for diabetes, diabetes complications, experimental diabetes mellitus, type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes, diabetic angiopathies, diabetic cardiomyopathies, diabetic coma, diabetic ketoacidosis, diabetic nephropathies, diabetic neuropathies, Donohue syndrome, fetal macrosomia, and prediabetic state.

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The *WJD* is now abstracted and indexed in Science Citation Index Expanded (SCIE, also known as SciSearch®), Current Contents/Clinical Medicine, Journal Citation Reports/Science Edition, PubMed, PubMed Central, Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database. The 2024 Edition of Journal Citation Reports® cites the 2023 journal impact factor (JIF) for *WJD* as 4.2; JIF without journal self cites: 4.1; 5-year JIF: 4.2; JIF Rank: 40/186 in endocrinology and metabolism; JIF Quartile: Q1; and 5-year JIF Quartile: Q2.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Lu Cai, Md. Shahidul Islam, Michael Horowitz

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-9358/editorialboard.htm

PUBLICATION DATE
July 15, 2024

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World J Diabetes 2024 July 15; 15(7): 1489-1498

DOI: 10.4239/wjd.v15.i7.1489

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Retrospective Study

Association between glucose-lowering drugs and circulating insulin antibodies induced by insulin therapy in patients with type 2 diabetes

Peng Zhang, Qing Jiang, Bo Ding, Reng-Na Yan, Yun Hu, Jian-Hua Ma

Specialty type: Endocrinology and metabolism

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade C, Grade C, Grade C, Grade C, Grade C,

Novelty: Grade B, Grade B

Creativity or Innovation: Grade B, Grade B

Scientific Significance: Grade B, Grade C

P-Reviewer: Horowitz M, Australia; Jethwani P, India; Pappachan JM, United Kingdom; Tung TH, Taiwan

Received: December 19, 2023

Revised: April 8, 2024

Accepted: May 27, 2024

Published online: July 15, 2024

Processing time: 202 Days and 1.8 Hours

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Abstract

BACKGROUND

Insulin antibodies (IAs) affect blood glucose control in patients receiving insulin therapy.

AIM

To investigate the relationship between different hypoglycemic treatments and IAs in patients with type 2 diabetes mellitus (T2DM).

METHODS

This cross-sectional, retrospective study included 1863 patients with T2DM who were receiving exogenous insulin therapy. All patients received stable antidiabetic therapy in the last 3 months and IA levels were measured using an iodine-125 array.

RESULTS

A total of 1863 patients were enrolled. There were 902 (48.4%) patients who had positive IAs (IA level > 5%), with a mean IA level of 11.06% (10.39%-11.72%). IA levels were positively correlated with high fasting blood glucose (odds ratio = 1.069, $P < 0.001$). The proportion of positive IAs was lowest in patients using glargine only (31.9%) and highest in patients using human insulin only (70.3%), $P < 0.001$. The IA levels in patients using sulfonylureas/glinides (8.3%), metformin

(9.6%), and dipeptidyl peptidase-4 inhibitors (8.2%) were all lower than in patients without these drugs (all $P < 0.05$).

CONCLUSION
Nearly half of patients on insulin therapy have positive IA antibodies, and IA antibody levels are associated with blood glucose control. Insulin glargine and a combination of oral glucose-lowering drugs were correlated with lower IA levels.

Key Words: Insulin antibodies; Insulin therapy; Glucose-lowering drugs; Glargine; Type 2 diabetes

Core Tip: In this study, we found that the proportion of positive insulin antibodies (IAs) was high in type 2 diabetes patients receiving exogenous insulin therapy. Positive IAs was correlated with high fasting blood glucose, insulin glargine was associated with the lowest IA levels among the insulin regimens, and the use of insulin secretagogues, metformin, and dipeptidyl peptidase-4 inhibitors was correlated with decreased IA levels.

Citation: Zhang P, Jiang Q, Ding B, Yan RN, Hu Y, Ma JH. Association between glucose-lowering drugs and circulating insulin antibodies induced by insulin therapy in patients with type 2 diabetes. *World J Diabetes* 2024; 15(7): 1489-1498
URL: https://www.wjgnet.com/1948-9358/full/v15/i7/1489.htm
DOI: https://dx.doi.org/10.4239/wjd.v15.i7.1489

INTRODUCTION

Many patients with type 2 diabetes mellitus (T2DM) eventually require insulin therapy due to disease progression with the reduction of effects of oral antidiabetic agents[1]. Insulin antibodies (IAs), which are found in patients treated with exogenous insulin, may affect glycemic control owing to their tendency to bind and/or release insulin in an unpredictable fashion[2-4]. Recently, Zhu *et al*[5] showed that a higher circulating IA titer was associated with increased glycemic variability and the risk of hypoglycemia[5], which are considered potential risk factors for mortality[6] and diabetes complications in patients with T2DM[7,8]. Therefore, identifying the factors that influence IA levels may help us further understand blood glucose fluctuations and improve blood glucose control in patients with diabetes.

Along with the development of recombinant human insulin and insulin analogues, several studies have found that circulating IA levels are closely related to the mode of insulin injection and type of insulin[9-11]. IA levels were usually increased during the first 12 weeks following initiation of exogenous insulin treatment and then stabilized or gradually declined[10,12]. However, previous studies usually compared the IA levels between patients using two types of insulin. Previous studies have also compared IA levels after the action of two similarly acting insulins, and comprehensive comparisons of different types of insulin are lacking. In addition, the effect of the combination of oral hypoglycemic agents (OHAs) on IAs after insulin administration has rarely been reported.

Therefore, in this study, we retrospectively analyzed the circulating IA levels in patients with T2DM using different insulin therapies and oral glucose-lowering drugs.

MATERIALS AND METHODS

Study design

This retrospective, cross-sectional study was approved by the Institutional Ethical Committee of Nanjing First Hospital, Nanjing Medical University (KY20220124-01). The study was conducted in accordance with the Declaration of Helsinki guidelines. The need for informed consent was waived as the research involved no more than minimal risk to the subjects, and the waiver did not adversely affect the rights and welfare of the subjects. All data were retrospectively collected from a medical review database without any identifiable private information with the consent of the corresponding department.

Two researchers extracted data from the consecutive medical records of patients admitted to Nanjing First Hospital. The data analysis covered the period from January 2017 to January 2019. The inclusion criteria were as follows: (1) Patients who were diagnosed with T2DM; (2) patients who were using insulin therapy, and there were no changes in glucose-lowering drugs in the last 3 months before the end of the index date; and (3) patients whose IA levels were tested. Patients were excluded if they: (1) Had infectious diseases which may promote antibody responses[13]; (2) had acute complications of diabetes on admission, such as diabetic ketoacidosis and lactic acidosis, which affect serum IA concentration through dehydration; (3) had severe systemic diseases (*e.g.*, heart failure, myocardial infarction, acute pancreatitis, severe liver and kidney dysfunction) or any other conditions that may influence IA levels (*e.g.*, heart failure, myocardial

infarction, acute pancreatitis, severe liver and kidney dysfunction); (4) were pregnant; and (5) received systemic glucocorticoids in the last 3 months.

Clinical and laboratory assessments

Patient data regarding height, weight, age, duration of diabetes, type of hypoglycemic treatment, and concomitant use of other medications were collected from the hospital admission records. Body mass index was calculated as weight divided by the square of height (kg/m^2). Blood samples were collected from all patients after fasting overnight (> 10 h). Hemoglobin (Hb)A1c was measured using high-performance liquid chromatography (Bio-Rad Laboratories, Inc., CA, United States). C-peptide levels were measured using a chemiluminescent immunometric assay which employs the Modular Analytics E170 (Roche Diagnostics GmbH, Mannheim, Germany).

The IA titers were determined using the iodine-125 insulin antibody array kit (Beijing North Institute of Biological Technology, China) in accordance with the manufacturer's instructions, and the procedure was similar to that in previous studies[5,14]. The method of IA measurement was routinely and clinically carried out in Nanjing Clinical Nuclear Medicine Center (ISO/IEC15189/17020) before February, 2019. The results were expressed in terms of bound radioactivity in the precipitate as a percentage of total counts (%B/T) in the assay. Blank values obtained by measurement of specific IA-zero serum were subtracted from sample values. IA ≤ 5% was identified as a negative result, and IA > 5% as positive[5]. The nonspecific binding rate should be ≤ 15%, the binding rate with standard IA positive serum should be > 25%, and the binding rate with standard negative serum should be < 5%. The intra- and inter-assay coefficients of variation were < 10% and < 15%, respectively. In order to compensate for the lack of specificity and sensitivity of radioimmunoassay compared with the chemiluminescence assay, we also analyzed the characteristics of patients with IA > 10% and IA < 1%.

Statistical analysis

Statistical analyses were performed using the IBM SPSS software 27.0 (SPSS, Inc., Chicago, IL, United States). All variables were tested for normal distribution. Data are presented as mean (95%CI). Insulin resistance and β-cell function were evaluated using Homeostasis Model Assessment 2 (HOMA2-IR and HOMA2-β), which were calculated using fasting C-peptide and blood glucose by the HOMA2 calculator[15]. Differences between the positive and negative IA groups were examined using the Student's unpaired *t*-test for parametric data or the Mann-Whitney *U*-test for non-parametric data, respectively. Logistic regression analysis was used to identify the factors which may affect IA levels. The differences in IA levels among the different blood glucose-lowering drugs were analyzed using the analysis of covariance (ANCOVA) to adjust for influencing factors. Categorical data were examined using the χ^2 test. All comparisons were 2-sided with a 5% significance level. A P value < 0.05 was considered statistically significant.

RESULTS

Study populations

Among 7426 patients with T2DM, a total of 2496 (33.61%) patients were receiving insulin therapy. Of these patients, two pregnant patients, two patients using systemic glucocorticoids, another 22 patients with changes in their glucose-lowering drug treatment in the last 3 months, and 134 patients with infection were excluded. Moreover, a hundred patients with positive glutamate decarboxylase antibody (GAD-Ab) were excluded from the study. A total of 1863 patients completed the IA measurement and were finally enrolled in the analysis. There were 1015 (54.5%) males and the mean age was 63.7 (63.2-64.3) years, the mean duration of insulin use was 7.35 (6.81-7.89), and the mean HbA1c was 8.9% (8.8%-9.0%). There were 902 (48.4%) patients with IA > 5% (Figure 1), and the mean IA level was 11.06% (10.39%-11.72%).

Clinical characteristics of the patients with positive IAs

Compared to patients with negative IAs (IA ≤ 5%), patients with positive IAs (IA > 5%) were associated with higher age, longer diabetic duration, higher total cholesterol, low density lipoprotein-cholesterol (LDL-c), high density lipoprotein-cholesterol, fasting blood glucose (FBG), insulin levels, GAD-Ab levels, and the proportion of cancer. Alanine transferase (ALT), triglycerides, duration of insulin use, and the proportion of smokers were lower in patients with IA > 5% than in the others (Table 1). To increase the specificity of IAs, and reduce the false positives and false negatives, we also analyzed the characteristics of patients with IA > 10% or < 1%. Further analysis showed that the patients with IA > 10% and ≤ 1% age, ALT, total cholesterol, triglyceride, LDL-c, FBG, insulin levels, duration of insulin use, and the proportion of smoking and the complication of cancer between patients with IA > 10% and ≤ 1% were greater. Patients with higher IA levels > 10% had higher HOMA-IR as compared to those with IA < 1%. HbA1c also seemed higher (but not statistically significant) in patients with IA > 10%. The prevalence of diabetic complications was similar between the patients with positive and negative IAs (Table 1).

Logistics regression analysis for risk factors of IAs

To identify the factors which were associated with IA levels, we performed a logistics regression analysis with positive IA levels or not as the dependent variable, including factors which were different between the IA-positive and IA-negative groups, such as age, diabetes duration, duration of insulin use, ALT, total cholesterol, triglyceride, LDL-c, FBG, GAD-Ab, HOMA2-IR, accompanied by cancer or smoking, and the types of OHAs as independent variables. As a result, diabetes duration, FBG, and GAD-Ab were risk factors for positive IAs (all P < 0.05; Table 2). ALT, triglyceride, types of OHAs,

Table 1 Clinical characteristics, *n* (%)/95%CI

	IA ≤ 5% (*n* = 961)	IA > 5% (*n* = 902)	*P* value	IA ≤ 1% (*n* = 278)	IA > 10% (*n* = 581)	*P* value
Age (yr)	63.1 (62.3-63.9)	64.5 (63.7-65.2)	0.029	62.6 (61.1-64.2)	65.4 (64.5-66.4)	0.004
Gender (male)	532 (55.4)	472 (52.3)	0.193	155 (55.8)	293 (50.4)	0.175
Smoking	176 (18.3)	120 (13.3)	0.003	69 (24.8)	68 (11.7)	< 0.001
Family history	219 (22.8)	187 (20.7)	0.287	71 (25.5)	121 (20.8)	0.145
BMI (kg/m^2)	24.8 (24.6-25)	24.8 (24.6-25)	0.998	24.6 (24.2-25.1)	24.7 (24.4-25.0)	0.854
Duration of insulin use (yr)	8.05 (7.23-8.84)	6.71 (5.99-7.43)	0.011	9.11 (7.42-10.79)	6.80 (5.81-7.78)	0.005
ALT (U/L)	26.7 (24.5-28.9)	23.5 (21.9-25.1)	0.049	30.4 (26.0-34.7)	23.8 (21.6-25.9)	< 0.001
AST (U/L)	20.7 (19.3-22.2)	18.9 (18-19.7)	0.691	21.7 (19.0-24.4)	19.1 (18.1-20.2)	0.513
Creatinine (μmol/L)	77.3 (74.7-80)	80.1 (76.9-83.3)	0.475	79.3 (73.7-84.8)	78.9 (75.4-82.3)	0.820
Uric acid (μmol/L)	308.5 (302.2-314.8)	307.6 (300.1-315)	0.361	310.8 (299.4-322.2)	302.3 (293.1-311.5)	0.083
Total cholesterol (mmol/L)	4.7 (4.4-5.1)	4.8 (4.4-5.1)	0.032	4.3 (4.1-4.4)	4.6 (4.4-4.8)	0.004
Triglyceride (mmol/L)	2 (1.9-2.2)	1.8 (1.7-1.9)	0.058	2.3 (1.8-2.7)	1.7 (1.5-1.8)	0.015
LDL-c (mmol/L)	2 (2-2.1)	2.1 (2.1-2.2)	0.003	1.8 (1.7-1.9)	2.2 (2.1-2.2)	< 0.001
HDL-c (mmol/L)	1.2 (1.2-1.2)	1.2 (1.2-1.3)	0.053	1.2 (1.2-1.3)	1.3 (1.2-1.3)	0.675
White blood cell (× 10^9)	6 (5.9-6.1)	6 (5.9-6.1)	0.984	5.9 (5.8-6.1)	6.0 (5.9-6.1)	0.922
Neutrophil ratio	59.4 (58.6-60.1)	59.6 (58.9-60.4)	0.829	58.7 (57.3-60.1)	59.6 (58.7-60.6)	0.339
Hemoglobin (g/L)	130.5 (129-132)	129.2 (127.9-130.5)	0.315	129.7 (127.6-131.8)	128 (126.5-129.5)	0.248
FBG (mmol/L)	8.4 (8.2-8.7)	9.1 (8.8-9.4)	< 0.001	7.9 (7.5-8.3)	9.0 (8.7-9.4)	< 0.001
Fasting insulin (mU/L)	22.2 (18.9-25.6)	41.4 (34.2-48.6)	< 0.001	27.4 (21.5-33.2)	54.0 (43.8-64.3)	< 0.001
Insulin-120 min (mU/L)	54.4 (49-59.7)	93 (79-107)	< 0.001	60.7 (52.7-68.7)	115.6 (96.2-134.9)	< 0.001
Fasting CP (ng/mL)	1.5 (1.3-1.6)	1.4 (1.3-1.5)	0.451	1.4 (1-1.8)	1.4 (1.3-1.5)	0.049
CP-120 min (ng/mL)	3.1 (3-3.3)	3 (2.9-3.2)	0.361	3.0 (2.7-3.3)	3.0 (2.8-3.1)	0.569
HOMA2-IR	1.5 (1.4-1.6)	1.7 (1.5,2)	0.191	1.3 (1.2-1.4)	1.7 (1.5-2)	0.025
HOMA2-β	49.1 (46.3-51.8)	46.6 (43.6-49.6)	0.082	48.6 (43.9-53.2)	46.5 (42.7-50.3)	0.107
HbA1c	9 (8.8-9.1)	8.8 (8.7-9)	0.177	8.6 (8.4-8.8)	8.8 (8.7-9.0)	0.102
GAD-Ab (IU/mL)	4.5 (4.2-4.9)	5.6 (5.2-6)	< 0.001	3.3 (2.7-3.9)	5.5 (5.0-6.0)	< 0.001
IA	2.2 (2.1-2.3)	20.4 (19.3-21.5)	< 0.001	0.3 (0.3-0.3)	27.8 (26.7-29.3)	< 0.001
Hypertension	566 (58.9)	547 (60.6)	0.450	176 (63.3)	346 (59.6)	0.297
Fatty liver	291 (30.3)	246 (27.3)	0.167	86 (30.9)	152 (26.2)	0.166
Cancer	36 (3.7)	63 (7)	0.002	5 (1.8)	44 (7.6)	< 0.001
DKD	191 (19.9)	207 (22.9)	0.113	55 (19.8)	124 (21.3)	0.654
Neuropathy	257 (26.7)	273 (30.3)	0.100	72 (25.9)	168 (28.9)	0.372
Retinopathy	263 (27.4)	258 (28.6)	0.570	79 (28.4)	178 (30.6)	0.525
Atherosclerosis	662 (68.9)	638 (70.7)	0.391	188 (67.6)	416 (71.6)	0.233

IA: Insulin antibody; BMI: Body mass index; ALT: Alanine transferase; AST: Aspartate transferase; LDL-c: Low density lipoprotein cholesterol; HDL-c: High density lipoprotein cholesterol; FBG: Fasting blood glucose; CP: C peptide; HOMA2-IR: Homeostasis model assessment 2 of insulin resistance; HOMA2-β: Homeostasis model assessment 2 of β cell function; HbA1c: Hemoglobin A1c; GAD-Ab: Glutamic acid decarboxylase antibody; DKD: Diabetic kidney disease.

Table 2 Logistics regression analysis for risk factors of insulin antibodies

	OR	95%CI	*P* value
Diabetes duration	1.019	1.001-1.037	0.042
Types of OHAs	0.875	0.771-0.991	0.036
ALT	0.996	0.992-1.000	0.053
Triglyceride	0.920	0.867-0.976	0.005
FBG	1.069	1.029-1.111	0.001
GAD-Ab	1.025	1.004-1.047	0.021
With cancer	0.533	0.310-0.917	0.023

OR: Odds ratio; OHAs: Oral hypoglycemic agents; ALT: Alanine transferase; FBG: Fasting blood glucose; LDL-c: Low density lipoprotein cholesterol; GAD-Ab: Glutamic acid decarboxylase antibody.

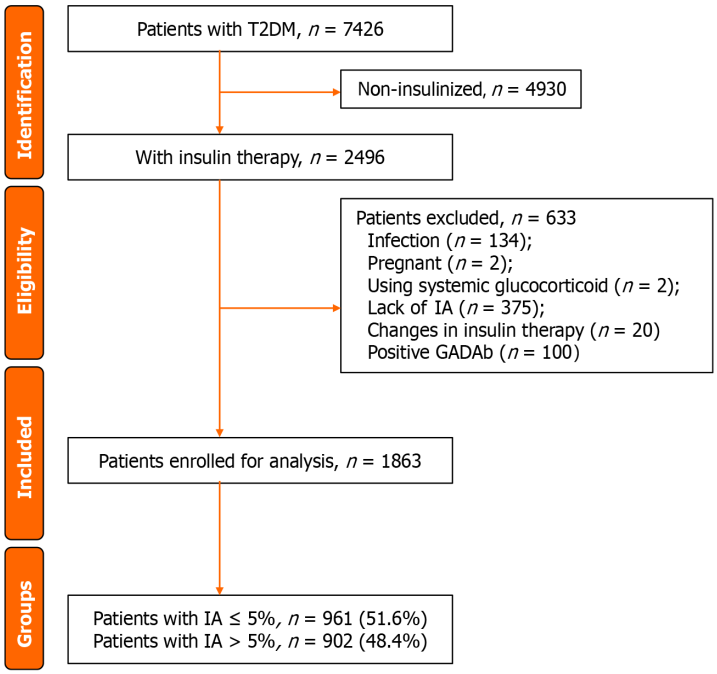

Figure 1 Flow diagram of patient screening. T2DM: Type 2 diabetes; IA: Insulin antibody; GADAb: Glutamic acid decarboxylase antibody.

and history of cancer were protective factors for positive IAs (all $P < 0.05$; Table 2).

Distribution and IA levels in patients treated with different insulin regimens

The proportions of different insulin therapies among the patients with T2DM receiving insulin therapy are shown in Figure 2A. Among the insulin therapy regimens in the population with more than 1%, glargine with OHAs was the most used (28.8%), and the patients using biphasic insulin aspart 30/50 constituted the largest proportion of patients with positive IAs (13.7%). The proportion of positive IAs was lowest in patients using glargine only (31.9%) and highest in patients using human insulin only (70.3%), $P < 0.001$ (Figure 2A). After adjusting for diabetes duration, ALT, triglyceride, FBG, types of OHAs, and cancer, which were risk factors in the logistics regression, the ANCOVA test showed that among the different insulin therapy regimens, patients on glargine therapy had the lowest IA levels [6.7% (5.8%-7.5%), and patients receiving insulin aspart had the highest IA levels 17.7% (13.6%-21.8%)], $P < 0.001$, Figure 2B.

Effects of OHAs on IA levels

Among the OHAs, α-glucosidase was the most used drug combined with insulin therapy (33.0%), and the proportion of positive IAs was highest in patients using thiazolidinediones (TZDs) (51.4%, $P = 0.121$; Figure 3A). The IA levels in patients using sulfonylureas/glinides, metformin, and dipeptidyl peptidase-4 (DPP-4) inhibitors were all significantly lower than those in patients without these drugs [the controls were the patients using all the other OHAs and patients without any OHAs or glucagon-like peptide 1 receptor agonists (GLP-1RAs)] (all $P < 0.01$; Figure 3B), and patients using DPP-4 inhibitors had the lowest IA level [8.2% (6.5%-10.0%); Figure 3B]. After adjusting for diabetes duration, ALT,

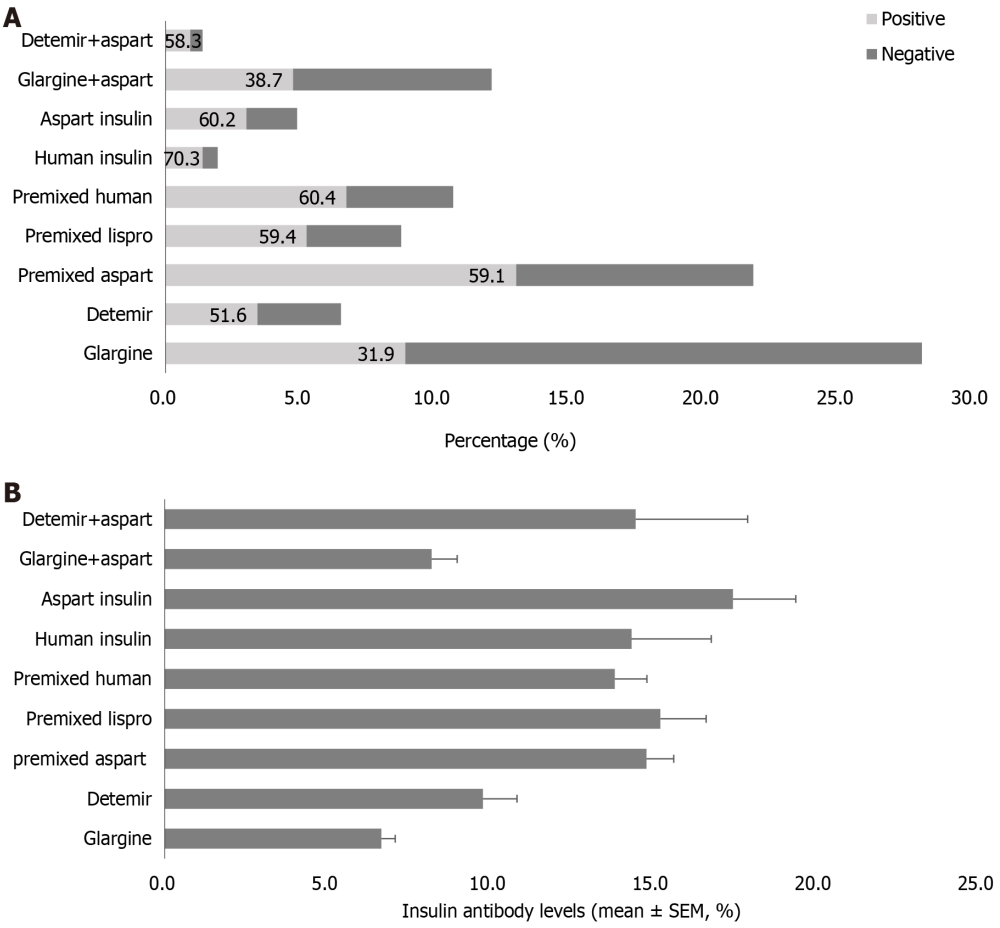

Figure 2 Insulin antibody levels in patients using different insulin regimens. A: Percentage of patients using different insulin regimens, and the proportion of positive insulin antibodies (IAs) in patients using different insulin regimens; B: IA levels in patients using different insulin regimens. SEM: Standard error of the mean.

triglyceride, FBG, and cancer, the ANCOVA test showed that the differences between patients taking or not taking these drugs remained significant (DPP-4 inhibitors, $P = 0.026$; metformin, $P = 0.002$; sulfonylureas/glinides, $P < 0.001$). The proportion of patients treated with OHAs was 83.0% in those taking insulin glargine, which was only 51.4% among patients using mixed insulin (biphasic aspart, biphasic lispro and biphasic human insulin) in the present study ($P < 0.001$). On the other hand, the proportion of patients who were using glargine and deremir was similar in patients using different OHAs (42.0%-55.1%) except sulfonylureas/glinides (75.0 %) ($P < 0.001$; Supplementary Table 1).

DISCUSSION

The present study found that the number of positive IAs were significantly lower in patients using insulin glargine than in those using other insulin therapy regimens, while the use of DPP-4 inhibitors, metformin, and insulin secretagogues may also be associated with reducing IA levels. To the best of our knowledge, there have been no previous reports of such findings.

Although circulating IAs rarely interfere with HbA1c levels, FBG levels and insulin resistance were higher in patients with positive IAs than in the others in our study, and this result was in accordance with a previous study in patients with type 1 diabetes[16]. In the present study, increased HOMA2-IR in patients with positive IAs may have contributed to a higher FBG level rather than fasting C-peptide level. However, previous studies demonstrated that insulin resistance may result from increased IAs[17,18]. The problem of high FBG and insulin resistance is more pronounced and needs to be addressed in patients with T2DM, which may lead to an increased insulin dose in these patients[17], and result in an increase in the cost of insulin and increased weight[19]. Moreover, nearly half of the patients taking insulin had positive IAs, consistent with the results of previous studies[20,21]. Therefore, attention should be paid to the detection of circulating IAs in patients with T2DM who are receiving insulin therapy, especially in elderly non-smokers with high LDL-c levels according to our study, and modification of the therapeutic regimen in patients with positive IAs should be considered.

In previous studies, the proportions of positive IAs were 27.6%-29% in patients with glargine only[22,23], and 24.5%-49.2% in patients using glargine plus aspart or lispro[10], which was lower than in patients with biphasic insulin aspart (68%), biphasic human insulin (66%)[14], and insulin detemir plus aspart (40%-50%)[24]. Yki-Järvinen *et al*[25] found that

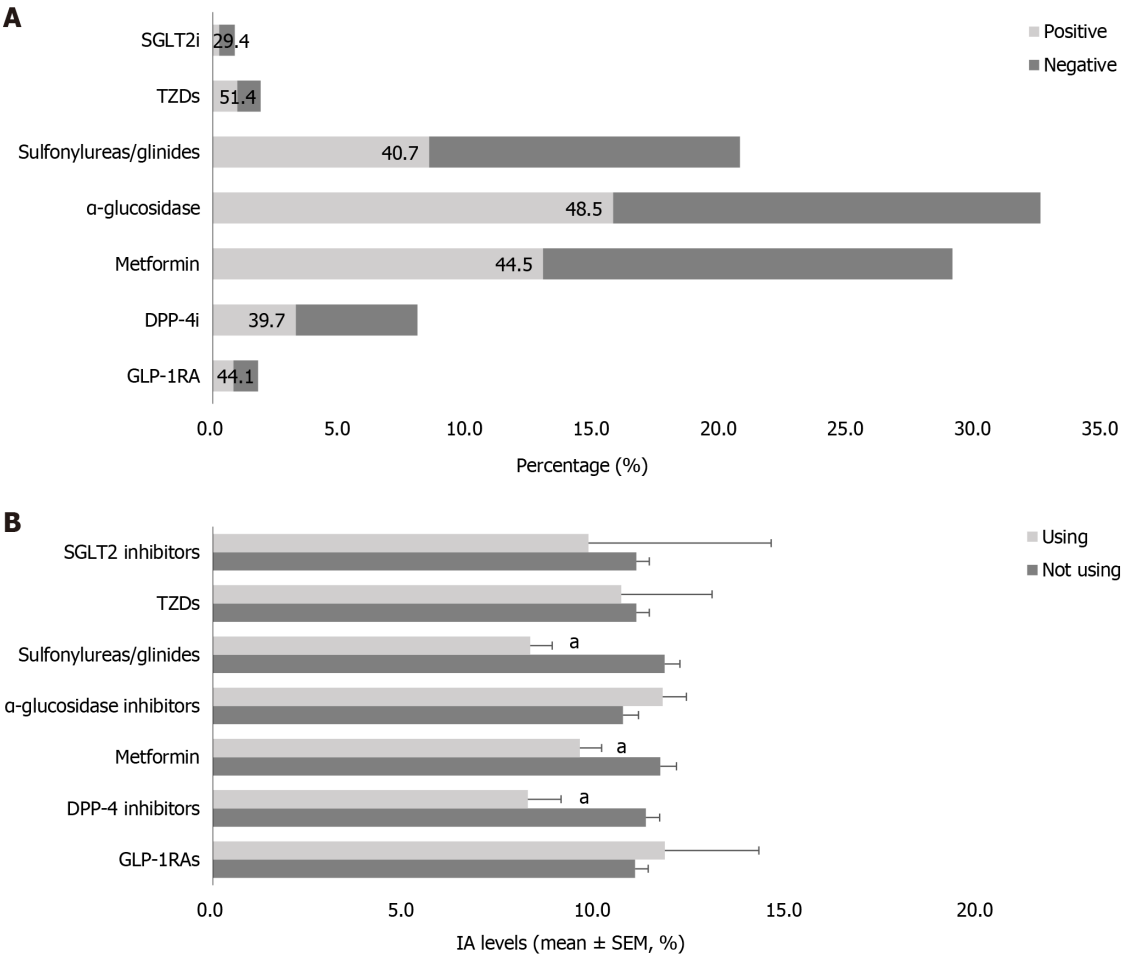

Figure 3 Insulin antibody levels in patients using different oral hypoglycemic agents and glucagon-like peptide 1 receptor agonists. A: Proportion of patients using different oral hypoglycemic agents and the rate of positive insulin antibodies (IAs) in patients using different drugs; B: IA levels in patients using different oral hypoglycemic agents and glucagon-like peptide 1 receptor agonists. $^{a}P < 0.01$ *vs* patients not using the drug. SGLT2i: Sodium-dependent glucose transporters 2 inhibitors; TZDs: Thiazolidinediones; DPP-4i: Dipeptidyl peptidase-4 inhibitors; GLP-1RAs: Glucagon-like peptide 1 receptor agonists.

antibodies against insulin glargine and human insulin were both significantly lower in patients treated with insulin glargine than in those treated with neutral protamine hagedorn (NPH)[25], while the difference in IA levels between these two types of insulin was not statistically significant in patients with type 1 diabetes[26]. Moreover, Hattori *et al*[15] found that glargine and aspart induced IAs more frequently than the others based on a small sample of 381 patients with T2DM, and only 17 patients used glargine in their study[15]. We confirmed that patients on insulin glargine had lower IA levels than other insulin regimens for the first time in such a large population. The decreased IA levels in patients using insulin glargine may contribute to its pharmacokinetic and pharmacodynamic characteristics. Following injection, insulin glargine forms a depot in the subcutaneous tissue, from which it is slowly absorbed. This provides a relatively uniform, peakless concentration over approximately 24 h after administration[27]. Therefore, insulin glargine treatment results in a lower daily basal insulin dose and fewer injection site reactions than those with insulin detemir[28] and NPH[29]. Insulin degludec, another basal insulin with an ultra-long duration of action (> 42 h), induces fewer immunogenic responses than insulin glargine[12]. However, there were only 7 patients (< 1% of the population) using insulin degludec in the present study, and we did not include them in the statistical analysis of IA levels. On the other hand, the proportion of patients treated with OHAs was much higher among those taking insulin glargine than among those treated with mixed insulin in the present study. These patients may have better endogenous insulin reserve and lower levels of extrinsic insulin use.

We unexpectedly found that IA levels in patients using α-glucosidase and TZDs were similar to the patients who were not using these drugs, although they had similar effects on the reduction of insulin resistance and insulin dose compared with metformin, DPP-4 inhibitors, and sodium-dependent glucose transporters 2 (SGLT2) inhibitors[30-32]. Therefore, the association between OHAs and IA levels may not be attributed to their effects on insulin dose reduction only. Elevation of endogenous insulin may play an important role in the prevention of IA positivity. Our study showed that a combination of insulin secretion promoters was associated with decreased IA levels in patients with T2DM. Moreover, Bae *et al*[33] found that DPP-4 inhibitor users secreted more insulin than TZD users[33]. Their findings partially explained the decrease in IA levels in patients using insulin combined with DPP-4 inhibitors but not with TZDs in our study. Ursini *et al*[34] suggested that metformin could inhibit B cell activation and antibody production in autoimmune diseases by increasing the activation of adenosine monophosphate-activated protein kinase, with a subsequent decrease in the phosphorylation of mTOR[34]. In the present study, low IA levels in patients using sulfonylureas/glinides may be

correlated with the high proportion of long-acting insulin analogues in these patients. A case report showed that Liraglutide might also have an effect on decreasing IA levels in patient with T2DM[35]. However, we did not observe IA reduction in patients treated with GLP-1RA due to the small number of patients taking a combination of GLP-1RA and insulin. Moreover, the number of patients using SGLT2 inhibitors was small, and the relationship between SGLT2 inhibitors and IA levels requires further confirmation in a study with a large sample size. Therefore, the mechanisms underlying the effects of different OHAs on IA levels are complex and require further investigation.

In the present study, old age was another relevant factor for IA levels, with the exception of OHAs types. The incidence and prevalence of many common autoimmune diseases are increasing in the elderly, although autoimmune diabetes is more common in young people and children. Ray and Yung[36] suggested that aging is associated with chronic inflammation and increased likelihood of developing autoimmune diseases[36]. Aging negatively affects the immune system, known as immunosenescence, which increases the susceptibility of elderly people to infection, autoimmune disease, and cancer[37]. We also found a higher proportion of cancer in patients with positive IAs, which may be attributed to the older age of these patients. However, the relationship between IA levels and cancers deserves further study.

One limitation of our study was that the cross-sectional design could not explain the causality between the use of blood glucose lowering drugs and changes in IA levels. Other factors, such as the duration of insulin use, age, and blood lipid levels, may simultaneously affect drug prescription and IA levels. Moreover, we observed that most hospitalized patients with type 2 diabetes in the present study had poor glycemic control. The association between IA levels and glucose-lowering drugs in insulin-treated patients with good glycemic control requires further investigation.

CONCLUSION

In conclusion, nearly half of patients on insulin therapy have positive insulin antibodies (IAs), and IA levels are associated with blood glucose control. The use of insulin glargine, DPP-4 inhibitors, metformin, insulin secretagogues, and SGLT2 inhibitors was associated with lower IA levels. The causal relationships between the changes in IA levels and these drugs require further investigation.

ACKNOWLEDGEMENTS

We thank the members of the Endocrinology Department of Nanjing First Hospital for their support.

FOOTNOTES

Author contributions: Zhang P and Jiang Q are co-authors and contributed equally to this article. Jiang Q and Hu Y wrote and reviewed the manuscript; Zhang P, Ding B and Yan RN contributed to the data collection; Hu Y and Ma JH are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have read and agreed to the published version of the manuscript.

Supported by The National Key R and D Program of China, No. 2018YFC1314103; and The National Natural Science Foundation of China, No. 81870563 and No. 82270838.

Institutional review board statement: The study was approved by the institutional research committee (No: KY20190926-01).

Informed consent statement: This is a retrospective study performed by review of participants' electronic clinical records only and therefore, patient consent was not necessary and was not obtained.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: Authors are happy to share the data on request.

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S-Editor: Liu H
L-Editor: Webster JR
P-Editor: Zhao S

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