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GAVIN PUBLISHERS

Journal of Diabetes and Treatment

He Y, et al. J Diabetes Treat: JDBT-155.

Research Article

DOI: 10.29011/ 2574-7568. 000055

Serum Insulin-to-C-Peptide Ratio and Prediction of the Presence of Insulin Autoantibodies in Type 2 Diabetes with Exogenous Insulin Therapy

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Citation: He Y, Gu J, Fu Q, Zhang W, Yang T (2018) Serum Insulin-to-C-Peptide Ratio and Prediction of the Presence of Insulin Autoantibodies in Type 2 Diabetes with Exogenous Insulin Therapy. J Diabetes Treat: JDBT-155. DOI: 10.29011/ 2574-7568. 000055

Received Date: 05 July, 2018; **Accepted Date:** 19 July, 2018; **Published Date:** 27 July, 2018

Abstract

Objective: The aim of the study is to develop a model for predicting the presence of Insulin Autoantibodies (IAA) in type 2 diabetes patients.

Materials and Methods: IAA, plasma insulin and C-peptide have been measured in type 2 diabetes with or without exogenous insulin therapy. Performances of the predicting models were estimated by using Receiver Operating Characteristic (ROC) curves.

Results: Compared to the group without insulin treatment, patients with insulin therapy had higher prevalence of IAA+(19.45% vs 5.5%, p<0.001). Two models for predicting the presence of IAA in type 2 diabetes patients with insulin therapy before hospitalization were developed. The ROC curves indicated the fasting insulin-to-C-peptide ratio (insulin/C-peptide) and the AUC insulin -to-AUCC-peptide ratio（AUC insulin/AUCC-peptide） have the good predicting property for the positive of IAA with the AUC [0.834 (95%CI 0.784-0.884), p<0.001, 0.824 (95%CI 0.771-0.877, p<0.001), respectively]. Serum fasting insulin/C-peptide＞0.089 had 82.83% sensitivity for predicting the presence of IAA and with 78.05% specificity. With a cutoff of 0.0236, the AUCinsulin/AUCC-peptide had considerable sensitivity (72.37%) and specificity (84.63%) for predicting the presence of IAA.

Conclusions: Fasting serum insulin/C-peptide and AUCinsulin/AUCC-peptide can be used as suitable index to predict the presence of IAA in type 2 diabetes with exogenous insulin therapy.

Keywords: Autoantibodies; Diabetes Mellitus Type 2; Insulin Antibodies.

Introduction

With the increasing prevalence of diabetes, diabetes mellitus has been a major public health problem in both developed and developing countries. More than 425 million adults in the word are living with diabetes now, and the number of people with diabetes may rise to 693 million in 2045 [1]. The overall estimated prevalence of diabetes was 11.6%, accounting for 113.9 million adults with diabetes in China [2]. Type 2 diabetes is the most common type of diabetes, accounting for around 90% of all cases of diabetes [3]. The number of adults with type 2 diabetes has doubled over the past three decades.

The pathogenesis of type 2 diabetes involves a combination of varying degrees of insulin resistance and relative insulin deficiency. But there is increasing evidence that Diabetes-Associated Autoantibodies (DAA) is present in some individuals with clinical type 2 diabetes [4-6]. To improve glucose control, type 2 diabetes patients who do not have the metabolic syndrome will have a lower cardiovascular risk but are candidates for earlier insulin therapy [7]. Temporary and aggressive insulin therapy at disease onset in type 2 diabetes can rapidly and effectively correct their metabolic imbalance and reverse the deleterious effects of

glucotoxicity and lipotoxicity exposure on β-cell function and insulin action [8,9]. Insulin Autoantibodies (IAA) can develop as early as 10-14 days after initiation of insulin therapy. Studies have shown that 14-60% of patients on unremitting insulin therapy may develop IAA [10,11].

DAA test is a regular evaluation for type 1 diabetes. Taking the cost and complexity of DAA test into account, DAA test is not a regular evaluation for every type 2 diabetes. Usually, doctors turn to testing for DAA in type 2 diabetes when they have a challenge in diabetes classification. In developed countries, DAA determination is done in a standard and regional laboratory, while DAA determination is done in the hospital laboratory in China. Due to the different protocol and kits, the significantly different prevalence of DAA was reported [12,13]. Only several hospital laboratories have participated in Islet Antibody Standardization Program in China. The GADA, IA2A assays of our laboratory achieved a laboratory-defined sensitivity of 64%, 64%, with 97.8%, 100% specificity, respectively in Islet Antibody Standardization Program of 2013 [14].

IAA affects integrated incidence of hypoglycemia, insulin dose requirements, and glucose control or contributes to β-cell failure. Maybe doctors make a false judgment about the β-cell function in type 2 diabetes due to the presence of IAA, which would influence the formulation of the patient's hypoglycemic program. To identify individuals with IAA, DAA determination is needed. However, in routine clinical practice, DAA determination is costly and inconvenient. Many hospitals do not carry out DAA examination. So an index based on anthropometric measurements or simple laboratory measurement is needed to predict the presence of IAA in type 2 diabetes. In clinical work, we have observed that abnormal Serum insulin-to-C-peptide ratio（insulin/C-peptide） indicates the presence of IAA in type 2 diabetes. Therefore, the aim of the present investigation was to test the hypothesis that serum insulin/C-peptide can predict the presence of IAA in type 2 diabetes. Our objectives were 1) to compare the prevalence of IAA among type 2 diabetes patients with or without insulin therapy before hospitalization 2) to assess the relationship between serum insulin/C-peptide and the positivity of IAA in type 2 diabetes.

Research Design and Methods

A total of 1018 Chinese Han patients diagnosed of type 2 diabetes who received treatment in the First Affiliated Hospital of Nanjing Medical University between December 2014 and April 2016 were enrolled in this study. Inclusion criteria was that study patients had a diagnosis of type 2 diabetes according to WHO criteria [15]. Exclusion criteria were pregnant or lactating females, renal failure and severe liver dysfunction. All subjects gave informed consent, and the protocol was approved by the Institutional Review Board of the First Affiliated Hospital of Nanjing Medical University. The insulin therapy group was defined as type 2 patients who were treated with subcutaneous insulin injection for more than 2 weeks. Otherwise they were grouped into the other group. Personal, historical and clinical data was obtained. After 10-12 hours overnight fasting, blood sample were taken for glycated Hemoglobin (HbA1c) measurement, autoantibody measurement and biochemical measurement. HbA1c concentrations were measured by HPLC in our hospital laboratory.

Mixed-Meal Tolerance Test

All patients underwent mixed-meal tolerance test to assess β-cell function. A mixed-meal tolerance test was performed after a time interval of treatment with insulin until a fasting blood glucose lower than 10.0 mmol/L. Plasma glucose, insulin and C-peptide were measured at 0, 30, 60, 120 and 180 min during the mixed-meal tolerance test. Plasma glucose was measured by using a hexokinase method (Beckman Coulter, USA), while plasma insulin and C-peptide concentrations were measured by chemiluminescence (Roche Diagnostics, Switzerland). The unit of plasma insulin and C-peptide concentrations is pmol/L. The 3-h insulin and C-peptide area under the curve (AUC) was calculated by using the trapezoidal rule over the 3-h period.

Autoantibody Determination

IAA was determined by ELISA (Biometric, USA). ELISA kits anti IAA allow quantitative in vitro tests for human autoantibodies against IAA in serum plasma. Patients' samples were in cubated with islet antigens coated into a micro-plate well. If they are positive, specific antibodies bind to the islet antigens. Bound antibodies form a bridge between islet antigens on micro-plate well and biotin-labeled islet antigens reagent, which is added in a second incubation step. To detect the bound biotin, a third incubation is carried out using enzyme-labeled avidin, which is capable of promoting a color reaction. The intensity of the color formed is proportional to the antibody against IAA. Our operating procedures were strictly in accordance with the protocol. According to the Biometric protocol, the suggested results' values of IAA are:<0.95 IU/ml are negative values of achieved results, and >1.05 IU/ml are positive values of achieved results.

Data Analysis and Statistics

The presence of IAA was defined as positive for IAA determination. The normally-distributed continuous variables were reported as Mean±SD and compared using an independent two-sample t test. For categorical variables, results were presented as N (%) and $\chi 2$ were used. Non-normally distributed variable(insulin/C-peptide) was log-transformed. To evaluate the index as a predictor of the presence of IAA, receiver operating characteristic curve analysis was performed. AUC was calculated to evaluate discriminating accuracy of each model. All statistical comparisons were two-tailed, and a p value<0.05 was considered significant. All analysis was performed with spss22.0.

Results

The demographic and clinical characteristics are described in Table 1. There were significant differences in HbA1c, age and diabetes duration between the group with insulin therapy and the group without insulin therapy. No statistical difference was observed in gender and BMI between the two groups. The prevalence of IAA in the groups of patients is also shown in Table 1. In the group without insulin therapy before hospitalization, 28 of the 509 patients (5.5%) were IAA+. Compared to the group without insulin treatment, the patients with insulin therapy had higher prevalence of IAA+(19.45% vs 5.5%, p<0.001).

	without insulin therapy	with insulin therapy	P value
Number	509	509	
Gender (male/female)	311/198	301/208	0.522
Age (years)	57.62±12.84	59.33±12.48	0.032
Diabetes duration (years)	6.24±6.28	11.50±7.35	<0.001
BMI (kg/m^2)	25.48±3.70	25.18±3.45	0.171
HbA1c (%)	9.15±2.40	8.76±1.87	0.004
IAA+ (%)	28 (5.5%)	99 (19.45%)	<0.001
For categorical variables n (%) is presented. For continuous variables mean (SD)/n is presented. Statistically significant, P < 0.05. BMI: body mass index; HbA1c: hemoglobin A1c; IAA+, positive insulin autoantibodies			

Table 1: Demographic and clinical characteristics of patients and prevalence of positive IAA.

Figure1 showed the ROC curves based on the fasting insulin/C-peptide and the positivity of IAA. In type 2 diabetes without insulin therapy before hospitalization, the area under the ROC curve was 0.637 (95%CI 0.510-0.764). It was seen in Figure 1A. While the ROC curves indicated that the fasting insulin/C-peptide has the good predicting property for the positivity of IAA with the AUC [0.834 (95%CI 0.784-0.884), p<0.001] in type 2 diabetes with insulin therapy before hospitalization (Figure1B).

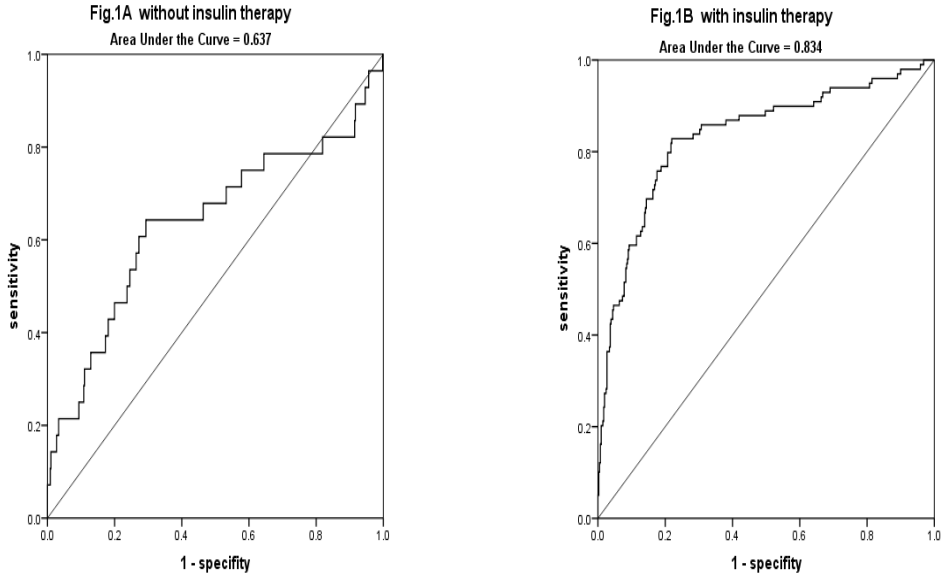

Figure 1: ROC curve of fasting insulin/C-peptide.

The ROC curves of the AUC insulin-to-AUCC-peptide ratio（AUC insulin/AUCC-peptide） and the positivity of IAA was depicted in Figure 2. In type 2 diabetes without insulin therapy before hospitalization, the area under the ROC curve was 0.640(95%CI 0.529-0.751). It was shown in Figure 2A. While the area under the ROC curve was 0.824 (95%CI 0.771-0.877, p<0.001), which indicated AUC insulin/AUCC-peptide is a good predictor for the positivity of IAA in type 2 diabetes with insulin therapy before hospitalization (Figure 2B).

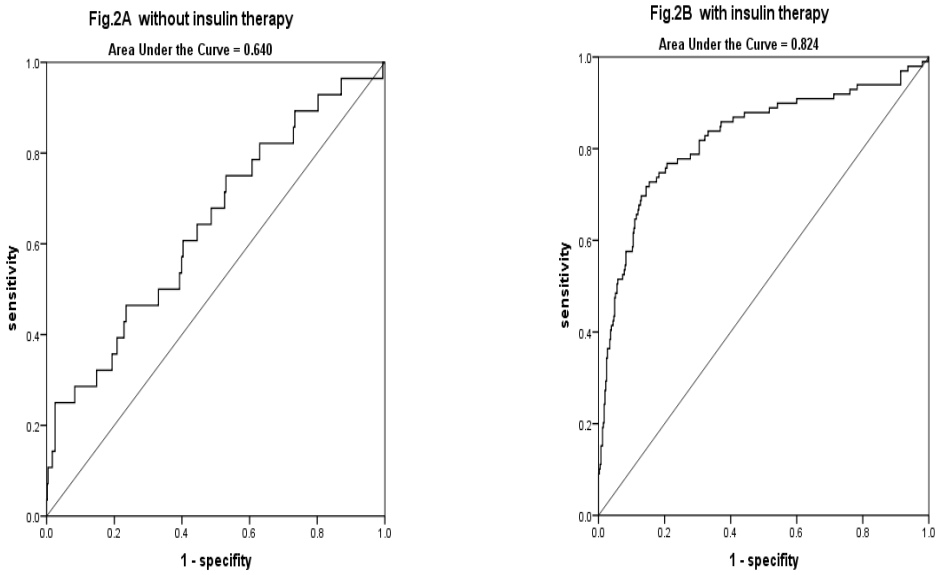

Figure 2: ROC curve of $AUC_{insulin}/AUC_{C\text{-}peptide}$.

The biggest Youden's index was explored to retrieve the cut-off value of the index. In the group with insulin therapy, serum fasting insulin/C-peptide ratio>0.089 had 82.83% sensitivity for predicting the presence of IAA and with 78.05% specificity. With a cutoff of 0.0236, the AUC insulin/AUCC-peptide ratio had considerable sensitivity (72.37% and specificity (84.63%) for predicting the presence of IAA (Table 2).

	Cutoff	Sensitivity	Specifity
Fasting insulin/C-peptide	0.089	82.83%	78.05%
$AUC_{insulin}/AUC_{C\text{-}peptide}$	0.0236	72.37%	84.63%

Table 2: Cutoff of fasting insulin/C-peptide and $AUC_{insulin}/AUC_{C\text{-}peptide}$.

Discussion

Our study is retrospective, focusing on the association of the insulin-to-C-peptide ratio with the presence of IAA in type 2 diabetes. We demonstrated that IAA also occur in clinical type 2 diabetes patients with or without insulin treatment before hospitalization and patients with insulin administration had higher prevalence of IAA than their peers without insulin administration before hospitalization. In this study, the novel finding was the establishment of two new indices to predict the presence of IAA. Serum fasting serum insulin/C-peptide and AUC insulin/AUCC-peptide showed reasonably good ability to predict the presence of IAA in type 2 diabetes with exogenous insulin therapy, with the area under ROC of 0.834 and 0.824 respectively. The two indices displayed good predictive efficiency. Whereas, they could not predict the presence of IAA in type 2 diabetes without exogenous insulin therapy.

We have shown that the prevalence of autoantibodies directed against insulin antigens was 5.5% in type 2 diabetes patients without insulin therapy before hospitalization. Exogenous insulin can induce the production of IAA. In type 2 diabetes patients with insulin therapy before hospitalization, the prevalence of IAA was 19.45%, which was higher than those of the peers without insulin therapy before hospitalization. This is in line with recently reported study. In Ethiopian patients, DAA was present in 22% of clinically diagnosed type 2 diabetes patients and the prevalence of IAA was 16% [16]. Because early insulin preparations were impure, immunological complications such as lipoatrophy, insulin allergy or insulin antibody production were frequently observed after the introduction of insulin therapy in the 1920s. Since highly purified recombinant human insulin and insulin analogs are currently in use, these complications have markedly decreased, but still occur. Due to nonphysiological means of manufacture, store and delivery, today's human insulin and insulin analogs are immunogenic in humans [17-19].

In our study, there were no significant differences in gender and BMI between the insulin therapy group and the non-insulin therapy group. Whereas there were significant differences in HbA1c, age and diabetes duration between the two groups. Type 2 diabetes patients without insulin therapy before hospitalization had higher HbA1C, younger age and shorter diabetes duration than the peers with insulin therapy before hospitalization. More young and newly diagnosed type 2 diabetes patients were assigned to the non-insulin therapy group, which may explain the significant differences in HbA1c between the two groups. The ROC curve

analysis was based on the two parameters of each group. And the ROC curve was not compared between the two groups. Therefore, the differences of HbA1c, age and diabetes duration did not influence the results of this study.

Serum fasting serum insulin/C-peptide and AUC insulin/AUCC-peptide showed reasonably good ability to predict the presence of IAA in type 2 diabetes with exogenous insulin therapy, with the area under ROC of 0.834 and 0.824, and the cutoff of 0.089 and 0.0236 respectively. Unfortunately, their predictive value was not seen in type 2 diabetes without exogenous insulin therapy. The phenomenon may be mainly explained by follow reasons. Firstly, there was differences in the titer of IAA between the two groups. Type 2 diabetes with insulin therapy before hospitalization might have higher IAA titer when compared to the peers without insulin therapy before hospitalization [17]. In this study, the titer of IAA was not measured. So we cannot see the difference between the two groups. The measured insulin concentration may include the concentration of plasma free insulin and IAA-insulin complexes. Secondly, the presence of IAA can lead to spurious results in radio immunoassays, immuno-enzymometric and immuno-fluorimetic methods [20,21]. In chemiluminescence assays, IAA can cause overestimation of plasma free insulin. The degree of overestimation depends on the comparative affinity of the autoantibodies and the antibodies in the assay [22]. Fasting serum insulin/C-peptide had a larger area under ROC than AUC insulin/AUCC-peptide. In practice, fasting serum insulin/C-peptide is easily to be obtained, while AUC insulin/AUCC-peptide is hard to be obtained and be calculated. Therefore, fasting serum insulin/C-peptide is recommended to the prior index to predict the presence of IAA in type 2 diabetes with exogenous insulin therapy.

According to our knowledge, this is the first specially established two indices for predicting the presence of IAA in type 2 diabetes with exogenous insulin therapy. They are easy to be carried out, especially the fasting serum insulin/C-peptide. However, this study has two potential limitations. A limitation of this study is that the preparations exogenous insulin or insulin analogs were not classified in details. Another limitation is that the prediction model has not been verified outside the subjects of this study. Further study is thus needed to determine the predictive value of the model in type 2 diabetes with exogenous insulin therapy.

Conclusion

In conclusion, our study has confirmed that positivity for DAA can be found in a significant proportion of patients with type 2 diabetes and the IAA prevalence of patients with insulin therapy is higher when compared to the patients without insulin therapy before hospitalization. Our data's provide substantial evidence that fasting serum insulin/C-peptide and AUC insulin/AUCC-peptide can be used as suitable index to predict the presence of IAA in type 2 diabetes with exogenous insulin therapy. Whereas, the two indices cannot predict the presence of IAA in type 2 diabetes without exogenous insulin administration. Further studies are needed to confirm these findings. Fasting serum insulin/C-peptide is recommended to the prior index to predict the presence of IAA in type 2 diabetes with exogenous insulin therapy.

Duality of Interest: The authors declare that there is no duality of interest associated with this manuscript.

Contribution Statement: Yinhui He and Jingyu Gu contributed equally to this work. All authors were responsible for drafting the article and revising it critically for important intellectual content. All authors approved the version to be published.

Acknowledgements: This work was supported by the National Natural Science Foundation of China (81270897, 81530026).

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