

## Correlation between Insulin antibody and HbA1c in Diabetes Mellitus

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**Abstract-** Insulin antibodies (INAs) are found in both types of diabetic patients and have low binding capacity and high affinity leading to hypoglycemia or hyperglycemia in type II patients taking insulin, which differs from the insulin antibodies found in type I that contain a high capacity of insulin binding and low affinity. This work aims to detect the levels of insulin antibodies and HbA1c in diabetic patients, and find the correlation between them and its potential role in glucose control. The study involved 60 patients with diabetes, divided into two groups type I and type II, each group containing 30 patients, in addition to 30 healthy individuals as a control group. High-performance liquid chromatography in a D-10 analyzer was used to measure HbA1c while ELISA was used to measure INAs in control and patients. Results: The current finding showed significant an elevated mean levels of HbA1c and INAs in both of patients groups compare to healthy, and there was non-significant weak positive correlation between them ( $R= 0.107$ ,  $P= 0.4$ ). Together, elevated levels of HbA1c and INA, and the positive correlation between their levels, provide evidence of the effect of insulin antibodies on glycemic control and the effects of HbA1c levels in patients with diabetes.

**Keywords:** Insulin antibody, HbA1c, poor glycemic control

### I. INTRODUCTION

Insulin is an anabolic pathway hormone that has cardinal roles in glucose homeostasis, the growth of cells, and metabolism process. It was isolated and purified in Toronto from 1921 to 1922 by Dr. Frederick Banting [1-2]. Autoantibodies against islet beta cell antigens "ex: antibodies against glutamic acid decarboxylase (GADA), insulin antibodies (INAs), islet antigen-2 (IA-2A)", are markers that in turn serve as significant predictors of the onset of Type 1 diabetes so far. The presence of one or more of them is a hallmark of an ongoing autoimmune process that leads to type 1 diabetes [3].

Insulin antibodies (INAs) are proteins found in 50% of patients with type 2 in response to taking insulin to control diabetes [4-5]. Published papers have demonstrated a significant relationship between insulin antibodies and control on glucose, and high IgG antibody titers may limit the action of insulin, leading to potential clinical effects such as insulin resistance because they bind or release insulin in unexpected ways, recurrent diabetic ketoacidosis, and low blood sugar [6-7]. Furthermore, INAs can compete with insulin for sites of binding, leading to hyperinsulinemia

and hypoglycemia, which represent risk factors for a variety of complications, including cardiovascular disease [8].

Glycated hemoglobin A1c (HbA1c) is an accurate measure of chronic blood sugar and is linked to the risk of prolong diabetes complications, so it is the best choice for chronic diabetes monitoring and management in an individual's blood with diabetes [9].

Detecting the role of insulin antibodies in diabetes management and their relationship to HbA1c is a subject of ongoing research and clinical interest. Therefore, this study is designed to determine the effect of insulin antibodies and HbA1c levels in diabetic patients, and to find the relationship between high insulin antibodies and high HbA1c in diabetic patients of both types.

### II. PATIENTS AND METHODS

#### A. Design of study

This case-control study was conducted the Department of Pathological Analysis at the University of Thi-Qar, College of Science.

Sixty diabetic patients who visited the Diabetes and Endocrinology Center of the Health Directorate in Thi-Qar province were randomly selected after taking informed consent and 30 subjects of healthy people as a control group. Samples were collected during the period of six months August (2023) to February (2024) An information form was approved for the two groups of patients and healthy control. The patients group was divided into 30 patients diagnosed with T1DM aged between 4–33 years and 30 patients with T2DM aged between 33-80 years.

#### B. Method

Five milliliters of participants' venous blood were drawn using a disposable syringe and divided into two parts. Two milliliters were placed in an EDTA tube and used to analyze glycosylated hemoglobin. Three milliliters of blood were transferred into a gel tube and allowed to clot at room temperature to get serum after separate it by centrifuging to 4000 xg for 10 min. The serum of the gel tube was put into an Eppendorf tube and stored at (-20°C) until used to estimate the insulin antibody (INA). The Bio-Rad D-10 instrument is used for determining glycohemoglobin HbA1c



that uses ion exchange high performance liquid chromatography (HPLC) to separate analyses based on their ionic interactions with the cartridge material. The D-10 software reduces raw data, and two-level calibration is used for quantitation, and the INAs Serum levels in DM patients and controls were evaluated by using the Sandwich-ELISA Kit pre-coated with an antigen specific to INS-Ab with catalog NO: SL2124Hu of the provided company (Sun Long - China).

### C. Statistics Analysis of the Data

The data was analyzed using SPSS version 26. Percentages were used with categorical variables such as age and gender. One way ANOVA test was used to compare the difference of means between groups. The difference was significant when the probability (P) value was  $\leq 0.05$ . Pearson's correlation coefficient was used as an indicator to express the correlation between INA and HbA1c and if one variable depends on the other.

### III. RESULTS

Demographic information of the study individuals was listed in Table 1. The mean age of population is 37.26 years. Patients with DM were about 60/90 (66.7%) of the investigated people. The majority of patients with T1DM belonged to the age group  $\geq 20$  (86.7%) while most of the patients with T2DM were in elderly ages groups Table 1. Also, the distribution of sex among studied groups listed in Table 1.

Table 1: Demographic details of the study population

	groups	T2DM	%	T1DM	%	Con.	%
Age	$\leq 20$	0	0.0	26	86.7	9	30
	21-41	2	6.6	4	13.3	9	30
	42-62	14	46.7	0	0.0	9	30
	>62	14	46.7	0	0.0	3	10
	<b>Total</b>	<b>30</b>	<b>100</b>	<b>30</b>	<b>100</b>	<b>30</b>	<b>100</b>
Sex	Male	17	56.7	15	50	15	50
	Female	13	43.3	15	50	15	50
	<b>Total</b>	<b>30</b>	<b>100</b>	<b>30</b>	<b>100</b>	<b>30</b>	<b>100</b>

T1DM : Type 1 diabetic mellitus , T2DM : Type 2 diabetic mellitus

Table 2 indicates some clinical manifestations of patients with diabetes regarding the type of treatment that used and glycemic control. More than half of the patients used insulin as a glucose control medication. HbA1c was used to determine glycemic control. The findings reported that 78.3% of diabetic patients had good glycemic control, while only 21.7% of patients showed poor glycemic control.

Table 2: Types of medication and Glycemic control in DM patients

Variable	Category	T2DM	T1DM	Total	Percent
Type of medication	Insulin	3	30	33	55.5
	Oral hypoglycemic	27	0	27	45.5
	Total	30	30	60	
Glycemic control based on HbA1c	Bad (>7.5%)	4	9	13	21.7
	Good (<7.5%)	26	21	47	78.3
	Total	30	30		

Furthermore, the results indicated high significant different in the concentration of INAs and HbA1c among study groups. Where patients with type I DM had the highest mean levels of insulin antibodies and Glycated hemoglobin HbA1c comparison to type II DM and healthy as listed in Table 3.

Table 3: INAs and HbA1c Means in DM Patients and Healthy

Parameters	T1DM <sup>3</sup>	T2 DM <sup>4</sup>	Healthy	F test	P value
INAs <sup>1</sup> Mean± SD	38.629 ± 7.269	36.182± 9.374	20.275 ± 9.771	36.33	0.01**
HbA1c <sup>2</sup> Mean± SD	9.88±1.82	8.63±2.03	4.89±0.43	79.62	0.0**

<sup>1</sup>INAs: Insulin antibodies , <sup>2</sup>HbA1C : Glycated hemoglobin ,

Also, Table 4 demonstrated the comparison among patients groups and healthy individuals according to insulin antibody titers.

Table (4): Comparison of serum levels of insulin antibodies between diabetic patients and healthy people

Groups	Mean± SD of INS- Abspg/ml	Sig. between groups	P value
T1DM	38.629±7.269	DM I X DM II	0.287 <sup>NS</sup>
T 2 DM	36.182±9.374	DM I X Healthy	0.00**
Healthy	20.275 ± 9.771	DMI II X Healthy	0.00**

#### A. Correlation between INAs & HbA1c

The present data found that there was a weak and non-significant positive Pearson correlation between HbA1c and INAs at (R = 0.107, P = 0.4) as illustrated in Figure 1.

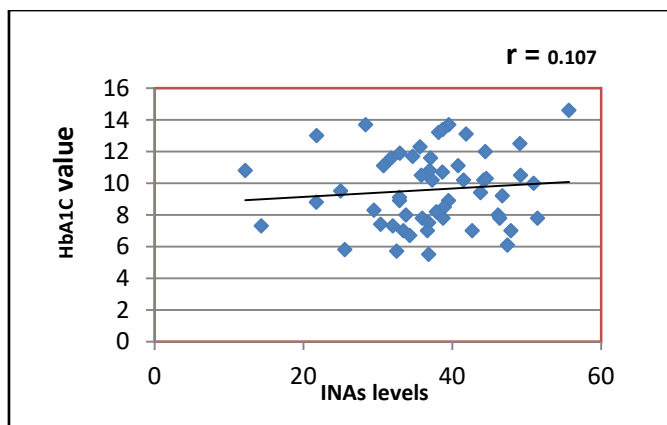


Fig. 1 : Correlation between INAs & HbA1c

Also, the results found that most patients with poor glycemic control suffered from type I DM and had higher levels of INAs.

#### IV. DISCUSSION

Insulin antibodies may impact a patient's glycemic control because of differences in both binding and releasing of insulin [10]. The hemoglobin A1c "HbA1c" test represents an excellent measure of glycemic control in the most of patients with diabetes [11]. In one Iraqi study, HbA1c level was used as a monitoring indicator to control blood sugar levels in patients in the city of Babylon [12].

Good glycemic control was found in 78.3% of diabetic patients, while only 21.7% of patients had poor glycemic control. This result is consistent with Kurai and his colleagues who found that only 27% of both type I and type II diabetic patients had raised levels of HbA1c, which represents poor blood sugar control [13].

The high significant difference was demonstrated in the concentration of INAs and HbA1c among patients categories and healthy, Where patients with type I DM had the highest means levels of insulin antibodies and Glycated hemoglobin comparison to type II DM and healthy as listed in Table 2. The data of Al-Fayyadhetal showed that people with diabetes had increased levels of HbA1c compared to control samples at  $P \leq 0.05$  [14].

Thijail and Mousa found a significant increase in the level of anti-Gad autoantibodies in both groups of DM patients compared with the healthy. The results also recorded a highly significant increase in anti-Gad serum levels in type 1 patients compared to type 2 patients [15].

Previous studies indicated that high titers of insulin antibodies can lead to insulin un-impactand hyperglycemia in number of diabete patients [16-18].

Takeuchi *et al.* suggested that diabetic patients with insulin antibodies, especially type I DM patients have high capacity of binding with low affinity, may exhibit great fluctuations in plasma glucose, as evidenced by an elevated levels of glycated albumin and the ratio of GA/HbA1c [19]. Kurai *et al.* referred that 54% of type 1 diabetes patients had poor control while 24% were in type 2 diabetes patients with statistically significant differences [13].

In figure 1, non-significantly weak positive correlation between HbA1c and INAs ( $r = 0.107$ ,  $P=0.4$ ) was indicated, this result is relatively in line with Bistritzeret *et al.* regarding the existence of a similar positive relationship

between the means of HbA1c concentration and the concentration of insulin antibodies, but with significant difference at ( $P$  less than 0.01), which was not found in the current study [20].

#### V. CONCLUSION

This study concluded that the direct relationship between insulin antibody levels and HbA1c provides evidence of the effect of insulin antibodies on blood sugar control, and the effect of HbA1c levels in diabetic patients.

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#### CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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