**Dijlah Journal of Medical Sciences (DJMS)** Vol. 3, No. 1, January, 2026, pp. 163-169
P-ISSN: 3078-3178, E-ISSN: 3078-8625, paper ID: 18

ORIGINAL ARTICLE

# Study of V-Domain Immunoglobulin Inhibitor of T-Cell Activation Marker in Iraqi Patients with Diabetes Mellitus Type-1

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**Article Info**

***Article history:***

Received October, 09, 2025
Revised November, 03, 2025
Accepted December, 01, 2025

***Keywords:***

Diabetes Mellitus Type1,
VISTA, Immune Checkpoint,
C-Peptide,
HbA1c

**ABSTRACT**

Type 1 diabetes mellitus (T1DM) is an autoimmune ailment where the immune system destroys the β-cells in the pancreas, ensuing in absolute insulin insufficiency. Understanding immune regulatory markers may cause new techniques of diagnosis and therapy in addition to a deeper know-how of the mechanisms underlying disorder. The checkpoint protein VISTA which regulates Tcell activity and immunological tolerance is one example of such a hallmark. To evaluate serum VISTA levels in Iraqi patients with T1DM compared to healthy individuals, and to explore its potential as an immunological biomarker for early detection, risk stratification, and monitoring of the disease. A case-control study including 150 participants 100 T1DM patients and 50 healthy controls was conducted in Baghdad. The levels of C-peptide, VISTA, fasting blood sugar (FBS), and HbA1c in blood samples were measured using ELISA. T1DM patients showed significantly higher HbA1c and FBS levels compared to healthy controls. C-peptide levels were drastically reduced in patients, indicating near-total loss of β-cell function. VISTA levels were markedly elevated in patients (339.78 ng/mL) compared to controls (134.46 ng/mL). Positive correlations were observed between HbA1c and VISTA, as well as FBS and VISTA, suggesting that chronic hyperglycemia may influence immune checkpoint activity. In conclusion, T1DM, VISTA seems to be a promising biomarker that may be used for risk assessment, disease monitoring, and early identification. Also, VISTA pathway targeting could be a new therapeutic strategy to control immune responses and maintain β-cell function.

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## 1- INTRODUCTION

Type 1 diabetes mellitus (T1DM), also known as autoimmune diabetes, is a chronic disease characterized by insulin deficiency due to pancreatic β-cell loss and leads to hyperglycaemia [1, 2]. Many studies declare that VISTA has special structural characteristics, expression patterns, and features. In addition to monocytes, dendritic cells, macrophages, and basophils under constant-nation conditions, VISTA has been shown to be notably expressed in neutrophils and microglia. VISTA isn't always expressed on B cells, even though it is widely expressed on plasma cells. It is usually expressed on FoxP3T regulatory cells (Tregs) in lymphocytes and naïve CD4+T cells, although it is less abundant in CD8+T cells, NK cell subsets, and thymocytes [3].

In several studies, VISTA capabilities as a regulatory gatekeeper for T cells at rest, blocking the primary phases of T mobile activation and the trade from quiescence to priming. VISTA is wonderful from other immunological checkpoint molecules because of this [4, 5]. Furthermore, in a number of animal fashions of immune pathology, which includes GVHD, RA, EAE, and asthma, VISTA became shown to be an immunological checkpoint [2]. Although VISTA has a 25% protein series with PD-L1, it differs from PD-1, CTLA-4, Tcellular Immunoglobulin and Mucin-area containing-3 (Tim-3), Lag-three, and B and T BTLA in each structure and characteristic. Like other immunological checkpoints, it transmits coinhibitory indicators to prevent T mobile activation [6, 7].

The T1D reasons general insulin insufficiency while the immune system kills the pancreatic βcells that produce insulin [8]. Because T1D has wonderful metabolic, genetic, and immunogenetic traits, each patient's remedy should be tailored to their age-associated adjustments. There is underlying genetic chance for many people with the infection. Accordingly, T1D autoantibody trying out and screening are encouraged for first- and 2nddiploma relatives of individuals with T1D through the ADA [9]. Clinical signs sooner or later seem in those with a high number of autoantibodies related to T1D. Insulin secretion may drop both quick or step by step. Although the scientific presentation varies from man or woman to character, the same old first signs and symptoms are polyuria, polydipsia, and inadvertent weight loss. Even although their signs and symptoms may additionally seem more slowly, adults with new-onset T1D frequently showcase symptoms which might be corresponding to those of kids [10]. A comprehensive approach to patient care is vital for the efficient remedy of T1D. Maximizing T1D outcomes calls for insulin alternative remedy, diabetes self-control training, nutritional assistance, and particular identity and treatment of underlying psychological issues [11]. The aim of this study is measure serum VISTA levels in Iraqi patients with type 1 diabetes mellitus (T1DM) versus healthy controls. Test correlations between VISTA and glycemic control/β-cell function markers (HbA1c, FBS, C-peptide). Evaluate VISTA's potential as an immunological biomarker for early detection, risk stratification, and disease monitoring.

## 2- MATERIALS AND METHODS

### 2.1 Sample Characteristics

In this case-control research, the Iraqi populace in Baghdad was the subject. Research ethics at the Ministry of Health and Environment approved the project. The goals of the study were explained to all participants, and all patients gave their written and verbal permission. One hundred and fifty individuals had their blood drawn between May to August of 2025.

- Group I- One hundred T1DM individuals, ages ranging from three to nineteen, were recorded, and blood samples were obtained from forty-six male and fifty-four female patients. All T1DM patients were diagnosed by skilled endocrinologists.

- Group II- 50 patients (24 men and 26 women) who were not diabetics (as a control group) and appeared healthy were volunteers who had neither a family history nor symptoms of diabetes.

### 2.2 Collection of blood samples

To determine the glycosylated hemoglobin level (HbA1c), two milliliters of venous blood were put in ethylene diamine tetraacetic acid (EDTA) tubes.

After centrifuging 3 milliliters of venous blood for five mins at 3000 rpm in dry, easy gel tubes, the blood was allowed to coagulate for five minutes at room temperature (25 °C). The separated serum was put into Eppendorf tubes and uncovered to ELISA era that allows you to degree the fasting blood glucose (FBS) and analyze the tiers of VISTA and C-peptide.

### 2.3 Statistical Analysis

The Statistical Packages of Social Sciences-SPSS (2019) software was used to determine how various groups affected the study's parameters. Means were significantly compared using the t-test. The Chi-Square test was used to compare percentages in a meaningful way. Find the correlation coefficient between the factors that differed across the patient groups in the research.

## 3- RESULTS

The research aimed to compare beta-cell functional indicator (C-peptide) and immune-related biomarkers among patient groups. In comparison to controls (134.46 ± 3.31), VISTA was significantly higher in patients (339.78 ± 4.94). as shown in Figure and Table1.

**Table (1): Examination of metabolic and immunological indicators in a patient with type 1 diabetes**

| **Group** | **Means ±SE** | |
|---|---|---|
| | **VISTA** | **C-Peptide** |
| **Patients** | 339.78 ±4.94 | 0.0045 ±0.0003 |
| **Control** | 134.46 ±3.31 | 1.152 ±0.065 |
| **T-test** | 14.581 ** | 0.0907 ** |
| **P-value** | 0.0001 | 0.0001 |
| **** (P≤0.01).** | | |

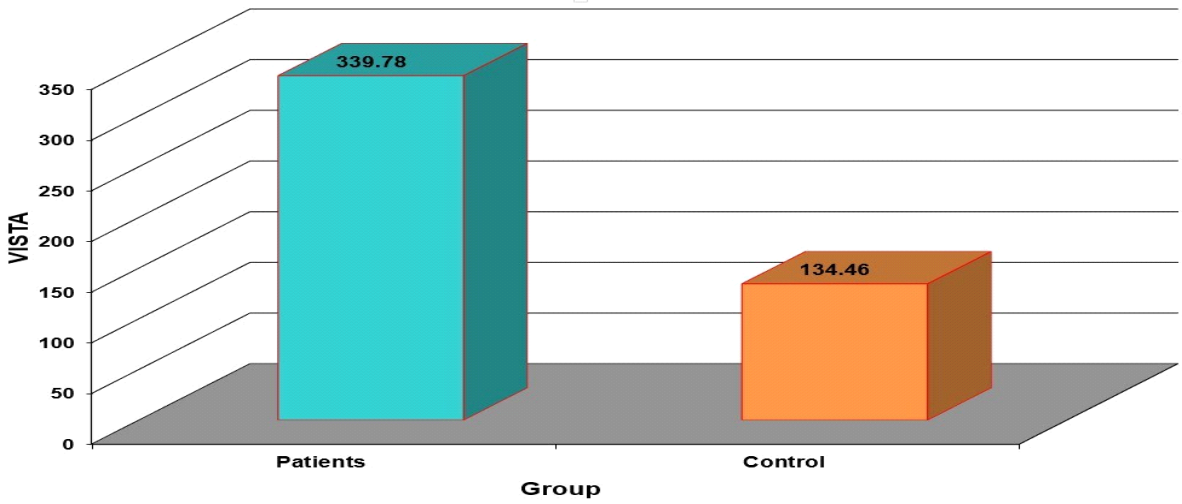


**Figure (1): The comparison of VISTA (ng/ml) between patients and controls**

Corresponding analysis of important clinical and immunological markers in patients with T1DM can provide important insights of the relationship between immune modulation and glycemic management. There was a strong and statistically significant positive correlation between HBA 1C and fasting blood sugar (FBS) (FBS) (FBS) (R = 0.54, P = 0.00001), showing that the long -term glycimic load is increased in insufficient short -term glycimic control. HBA also shown a slight but significant positive correlation at the level of 1C and Vista (R = 0.33, P = 0.0007), indicating that the degree of chronic hyperglycemia can affect immunological checkpoint activation.

However, neither FBS K HBA 1CA C-Paptide (R = 0.03, P = 0.7949) or C-Peptide (R = 0.06, P = 0.5986), which shows that residual β-cell activity is not directly reflected in this group's current glycimic state. Similarly, Vista is not related to expression and C-Peptide (R = 0.03, P = 0.7602), which shows that the Cell-cell secretary capacity operates independently of immune checkpoint control (Table 2).

**Table (2): Estimate of the Correlation coefficient between different parameters in patient groups**

| **Parameters** | | **Correlation coefficient-r** | **P-value** |
|---|---|---|---|
| **HbA1c** | **F.B.S.** | 0.54 ** | 0.0001 |
| | **VISTA** | 0.33 ** | 0.0007 |
| | **C-Peptide** | 0.06 NS | 0.5926 |
| **F.B.S.** | **VISTA** | 0.23 * | 0.0225 |
| | **C-Peptide** | 0.03 NS | 0.7949 |
| **VISTA** | **C-Peptide** | 0.03 NS | 0.7602 |
| *** (P≤0.05)**<br>**** (P≤0.01), NS: Non-significant.** | | | |

Figure 2 displays the ROC curves for five diagnostic biomarkers: VISTA, FBS, GAD, TIGIT, and HbA1c, with regards to a reference diagonal, also referred to as a risk line. The ROC curve assesses the diagnostic performance of each marker by using plotting sensitivity (proper tremendous charge) in opposition to 1-specificity (false high - quality fee). A curve closer to the higher-left nook suggests higher diagnostic accuracy. The ROC evaluation provides a sincere visible and quantitative evaluation of every biomarker's capability to differentiate between people with diabetes and healthful people.

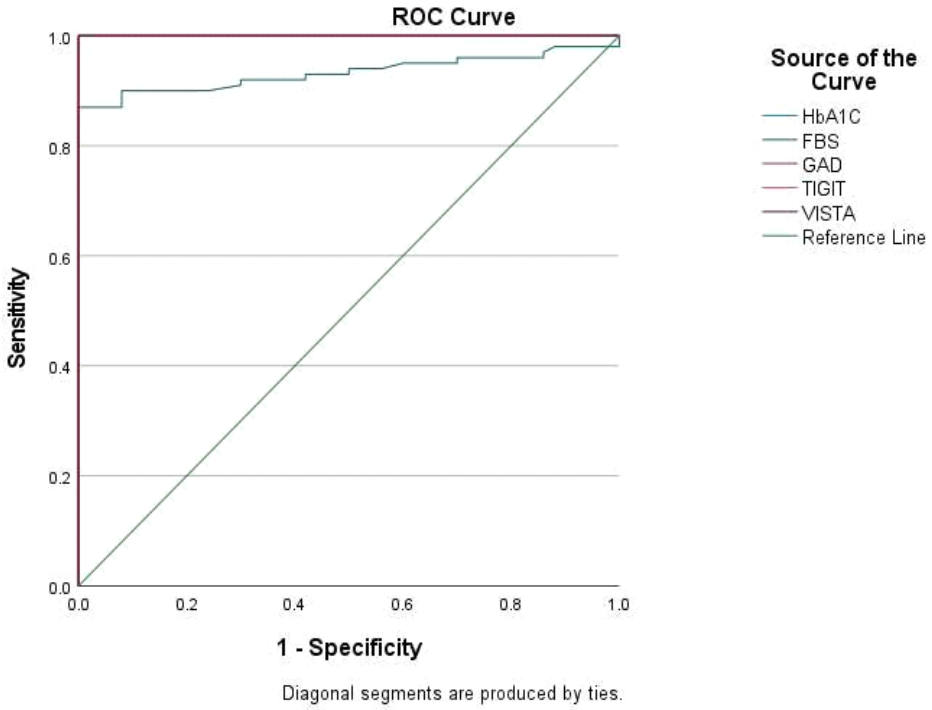


**Figure (2): Analysis of Diagnostic Biomarkers in Patients and Controls Using ROC Curves**

## 4- DISCUSSION

Immunological marker results show that the patient group's higher VISTA levels (Table 1) suggest immune hyperregulation or compensatory suppression, most likely as a result of long-term autoimmune activation. When pancreatic β-cells are gradually destroyed by autoreactive T cells in T1D, the immune system may try to suppress inflammation and stop more damage by upregulating checkpoint proteins like VISTA. This suggests that those with the illness have much higher levels of VISTA expression. According to Roy *et al.,* (2024),[12] the almost threefold rise in patients points to an immunological milieu that is actively undergoing regulatory modification. The current investigation supported research that found elevated VISTA levels, indicating that it plays a role in immune control in inflammatory or autoimmune diseases. According to a previous study, VISTA suppresses T-cell activation and inflammation. In lupus-prone Sle1.Sle3 animals missing VISTA, pro-inflammatory cytokines including IFN-γ and TNF-α increased 2-3 times more than in wild-type mice, resulting in severe glomerulonephritis [13]. Similarly, Zheng *et al.,* (2023) found that patients with systemic lupus erythematosus (SLE) had much higher levels of VISTA expression on $CD4^+$ T cells than healthy controls, with flow cytometry demonstrating a mean fluorescence intensity increase of almost 2.8 times. Studies from other autoimmune models, including EAE and lupus, have demonstrated that while VISTA blockade accelerates inflammation, enhanced VISTA signaling can lessen the severity of the disease, despite the fact that its role in T1DM has not been as thoroughly investigated as that of PD-1 or GAD. The patients' increased VISTA may be due to a protective feedback mechanism, even if it is inadequate to stop the course of the illness [14].

Additionally, VISTA has been identified as a possible biomarker representing immunological dysregulation in autoimmune disorders by recent clinical research. In one research, for instance, VISTA expression on $CD4^+$ T cells were 2.6 times higher in SLE patients than in healthy controls (mean fluorescence intensity [MFI]: 1,243 ± 115 vs. 475 ± 98, $p < 0.01$). This significant increase confirms that VISTA has a role in regulating peripheral immunological tolerance. Therefore, the present patient group's higher VISTA levels could be a compensatory immunoregulatory response to ongoing inflammation, which might be connected to chronicity or disease activity [15].

Table 1 show that the level of the C-peptide marker of endogenous insulin secretion varied statistically significantly ($P < 0.01$). This huge disparity (almost 250-fold difference) is statistically significant ($P < 0.01$), showing that whereas control participants continue to produce insulin normally, sick have substantially decreased or almost nonexistent β-cell activity. When proinsulin cleaves, a brief peptide called C-peptide is produced in equimolar levels to insulin. C-peptide testing is the most accurate indicator of endogenous insulin production and β-cell functional reserve since it is absent from exogenous insulin formulations. C-peptide levels rapidly and steadily decrease in people with T1D due to the autoimmune destruction of pancreatic β-cells. Perhaps the most clinically significant results were the C-peptide results, which revealed much lower levels (0.0045 ng/mL) in the patients, effectively suggesting near-total βcell failure. Controls, on the other hand, continued to produce insulin endogenously, as shown by their normal C-peptide levels (1.152 ng/mL). A hallmark of chronic T1D is low or undetectable C-peptide, which is highly predictive of insulin reliance. Crucially, longitudinal studies like the DCCT/EDIC trials and Joslin Diabetes Center cohorts have shown that Cpeptide levels drop significantly within the first five years after a diagnosis of T1D, frequently reaching undetectable levels in young patients and patients with poor control [16]. These findings are also used to differentiate T1DM from T2DM and MODY in ambiguous cases.

Additionally, there was a small but significant positive connection between VISTA and fasting blood sugar ($r = 0.23$, $P = 0.0225$). The possible link between glycemic status and immunological regulation is further supported by a similar trend seen between FBS and VISTA ($r = 0.23$). These moderate associations, as seen in Table 2, demonstrate how immunoregulatory mechanisms may react dynamically to metabolic dysregulation, which is in line with new findings on immune-metabolic interaction in T1DM (17).

## 5- CONCLUSION

Incorporate TIGIT and VISTA into diagnostic panels for early detection or risk stratification of T1DM in high-risk populations, particularly in settings where conventional markers are inconclusive. Utilize multi-marker approaches—combining HbA1c, C-peptide, and checkpoint markers to improve diagnostic accuracy and monitor immune activity in established patients. Clinical trials should be considered to evaluate the immunomodulatory potential of therapies aimed at checkpoint pathways (e.g., VISTA-based regulation) in slowing β-cell destruction.

Routine monitoring of immune checkpoint levels may offer a non-invasive method for assessing immunological status in T1DM patients and could inform personalized treatment strategies.

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