Original Article

# Comparison of demographic variables and clinical findings with immune biomarkers in type1 diabetes

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## ABSTRACT

**Objective**: To explore how demographic variables and clinical findings relate to immune biomarkers, assess their impact on glycemic control, and identify the most relevant immune biomarker for the Pakistani population with Type 1 Diabetes.

**Material and Methods:** This cross-sectional analytical study was conducted at Chughtai Institute of Pathology, from April 2021 to March 2022. We enrolled 130 male and female diagnosed cases of Type 1 Diabetes of age below18 years in this study. A total of 100 cases were included in the study as per defined criteria and 30 were excluded. Relevant details of demographic variables & clinical findings were noted on a predesigned proforma. 5ml whole blood was taken from each subject. All samples were analyzed for Plasma Glucose, HbA1c%, C-peptide, Anti GAD65, Anti IA2 and Anti IAA. SPSS 25.0 was used for statistical analysis.

**Results:** Mean age of the Demographic details of study participants was 14.2±3.6years. Majority of the study participants were male (57%). Mean height was 4.89±0.69feet, mean weight of the participants was 57.8±18.0 Kilograms, mean BMI was 27.0±7.7kg/m2and mean Fasting blood glucose level was 213.3 ±128.2 mg/dL. Majority of the participants (57%) belonged to middle socioeconomic class, had normal BMI with a poor glycemic control. When means were compared, it was found that there was a significant difference in the mean anti-GAD level, where group with poor glycemic control having higher values.

**Conclusion:** Anti-GAD65 is the most prevalent immune biomarker in the Pakistani population, with elevated levels linked to poor glycemic control. While low socioeconomic status correlates with worse glycemic outcomes. A targeted approach for high-risk populations may enhance clinical outcomes and alleviate financial and mental burdens for patients.

**Keywords:** Type 1 diabetes mellitus, GAD65, IA-2, IAA, Biomarker

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## INTRODUCTION

Globally, the incidence of diabetes mellitus is rapidly on the rise. People having diabetes are expected to increase from 425 million in 2017 to 629 million in 2045, and in low- and middle-income countries like Sudan, Pakistan, India, Sri Lanka, Azerbaijan and Armenia, 79% rise is estimated [1]. After China and India, Pakistan is ranked third in the prevalence of diabetes where around 33 million people are enduring with diabetes [2]. With an enhancing incidence of 2-5% annually worldwide, Type 1 diabetes is affecting a large number of individuals mostly targeting people of under 19 years of age. A wide variety is seen worldwide as some portions of the world have a very high incidence than others. One description of it can be a correlation between genetic and environmental factors [3, 4]. Type 1 diabetes is observed as one of the most frequent dreadful childhood diseases. It develops mostly in children and adolescent populations, although any age group can present with type 1 diabetes [2].

Interactions between multiple genes, environmental factors &the immune system of

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body results in autoimmunity which pave the way for the destruction of the insulin producing β cells of the pancreas that cause Type 1 diabetes. Child becomes symptomatic after going through two asymptomatic stages. Up to 70-80% of the pancreatic cells have already been damaged at this specific stage. Hypoglycemia and diabetic ketoacidosis are the most frequent acute complications and long term micro- and macro-vascular complications results from bad control which remarkably affects quality of life and public health care costs. Future results can be predicted and prevented by intervening in lengthy latent (asymptomatic) phase [2,4,5,6].

To mark & differentiate between diverse types of diabetes sometimes numerous tests may be required. To diagnose Type 1 Diabetes specially in the asymptomatic phase the role of autoantibodies has been well accepted [3,7]. Most of the time these autoantibodies are not able to cope with the diverseness inherent to type 1 diabetes progression [8]. For bulk of the people with type 1 diabetes, access to superior treatment options is difficult in the developing countries like Pakistan [9]. Therefore, these persons are sensitive to acute and chronic complications of T1D influencing their quality of life [10].

No study has been conducted in our population to look for the differences in immune biomarkers among various groups based on factors like age and gender. This study is designed to explore whether there are variations in immune biomarker levels among these groups and if categorizing into groups can help pinpoint those at a higher risk.

Inspite of the fact that we are dealing a huge number of patients with Type 1 Diabetes (T1D) in Pakistan, but sadly the documentation of local data about the role of immune biomarkers in early diagnosis of Type 1 diabetes with the impact of demographic variables on disease is not yet available. Categorizing individuals by their age, gender, and other traits and examining how these factors affect the lab tests can aid in early diagnosis. Early management can prevent the later severe complications. Moreover, tailoring treatment plans for various age groups and socio-economic classes can lead to more effective disease management. This study focused to find association between different demographic variables and immune biomarkers in T1D so that disease can be detected at an early stage if persons are classified properly.

## MATERIAL AND METHODS

This study was conducted at the Chughtai Institute of Pathology in a duration of one year (from 1st April 2021 to 31st March 2022). The research work was initiated after approval from the Institutional Ethical Review Board (IRB # CIP / IRB / 1064 B, approval date: 24-02-2021). It was a cross-sectional analytical study in which both male and female subjects < 18 years of age were included. Participants were included after explaining the nature of the study to their parents/guardians and informed consent was taken. We enrolled 130 male and female diagnosed cases of T1D of age below18 years in this study. A total of 100 cases were included in the study as per defined criteria and 30 were excluded [11]. The sample size was calculated by using Cochran formula that allowed us to calculate the ideal minimum sample size from unknown population with a desired confidence level (Z), level of precision (e) and estimated proportion of the disease in the population. In this study by reviewing the data and findings of other authors, the proportion of type-1 diabetes in the population is 10 % with e = 5% and 90% confidence level. By using this value in formula, the minimum sample size is 98 was obtained. Therefore, in this study 100 patients easily fulfilled our criteria of sample size. Individuals taking anti hyperlipidemic treatment, individuals with acute infections, undergoing surgery or admitted in intensive care units/high dependency units were excluded from the study. Also, the individuals whose parents/guardians did not give enough information about the disease history and demographic variables also excluded from the study. Relevant details of all cases i.e., age, gender, BMI, socio-economic status and medical history were noted on a predesigned proforma. After noting down the

required information, 5ml whole blood was taken from each subject and centrifuged at 1500 g for 5 minutes.

All samples were analyzed for following parameters: GAD 65 Ab: (Sandwich Chemilumine-scence Immunoassay), IAA: (Sandwich Chemiluminescence Immunoassay) and IA-2 Ab:(Sandwich Chemiluminescence Immunoassay) All above mentioned tests were performed on fully automated immunoassay analyzer (Maglumi- Snibe). Data was analyzed via SPSS version 23.0. Frequencies and percentages were calculated for demographic variables such as gender, socioeconomic status, BMI categories, glycemic control, blood pressure, symptoms, and family history. Mean and standard deviation were calculated for continuous variables including age, height, weight, BMI, and fasting blood glucose level. Normality of data was assessed using the Shapiro-Wilk test. One-way ANOVA was used to compare means of immune biomarkers (Anti GAD, AntiIA2, IAA) across different groups (gender, socioeconomic status, BMI categories, glycemic control, blood pressure, symptoms, family history). Independent sample t-test used to compare means of immune biomarkers between two groups (e.g., gender). The Mann-Whitney U test was used for comparing means between two groups when data did not follow a normal distribution, while the Kruskal-Wallis test was used as a non-parametric alternative to ANOVA for more than two groups. Both tests were employed when data did not meet the assumptions for parametric testing, with a p-value of less than 0.05 considered statistically significant.

## RESULTS

Mean age of the Demographic details of study participants was 14.2 ±3.6 years. Majority of the study participants were male (57%). Mean height was 4.89±0.69 feet, mean weight of the participants was 57.8±18.0 Kilograms, mean BMI was 27.0±7.7kg/m2and mean Fating blood glucose level was 213.3 ±128.2 mg/dL. Mean Values of immune biomarkers in the study is given in Table-I.

Distribution of cases in different study groups is given in table-II. This distribution shows that majority of the participants (57%) belonged to middle socioeconomic class, had normal BMI with a poor glycemic control. The details are given below.

Mean values of all the chemical biomarkers were analyzed in each group separately to the means were compared to see if any significant difference was present among the groups or not. When means of biomarker levels were compared on the basis of glycemic control, it was found that there was a significant difference in the mean anti-GAD level, where group with poor glycemic control having higher values. There was no significant difference in the immune biomarker levels when values among the groups were compared using ANOVA and independent sample T-test. Non-parametric tests like Mann-Whitney and Kruskal-Wallis were used for data that was not normal. Mean and IQR were used to depict non-parametric data. Variable wise distribution on immune biomarkers along with the p values are given in Table-III.

**Table-I: Mean values of immune biomarkers.**

| Immune Biomarker | Mean | Interquartile range |
|---|---|---|
| Anti GAD | 64.19 | 73 |
| AntiIA2 | 23.05 | 9 |
| IAA | 12.14 | 10 |

**Table-II: Distribution of participants in different study groups.**

| Group | Frequency (Percentage) |
|---|---|
| **Gender** | |
| Male | 57% |
| Female | 43% |
| **Socioeconomic Status** | |
| Lower | 11% |
| Middle | 57% |
| Upper | 32% |
| **BMI** | |
| Underweight | 8% |
| Normal weight | 41% |
| Over weight | 22% |
| Obese | 29% |
| **Glycemic Control** | |
| Poor control | 84% |
| Average control | 12% |
| Good control | 4% |
| **Blood Pressure** | |
| Low BP | 64% |
| Normal BP | 36% |

**Table-III: Comparison of mean values of Chemical Biomarkers analyzed in the study among all groups.**

| Variable | Anti GAD | AntiIA2 | IAA |
|---|---|---|---|
| **Gender** | | | |
| Male | 75.8 | 18.9 | 11.2 |
| Female | 48.8 | 28.4 | 13.2 |
| p-value | 0.129 | 0.38 | 0.383 |
| **Socioeconomic Status** | | | |
| Lower | 68.2 | 9.5 | 10.1 |
| Middle | 48.8 | 32.03 | 11.7 |
| Upper | 89.7 | 11.5 | 13.4 |
| p-value | 0.106 | 0.15 | 0.665 |
| **BMI** | | | |
| Underweight | 32.8 | 42.3 | 16.8 |
| Normal weight | 83.8 | 17.2 | 11.01 |
| Overweight | 58.6 | 19.9 | 10.3 |
| Obese | 49.6 | 28.4 | 13.7 |
| p-value | 0.27 | 0.594 | 0.416 |
| **Glycemic control** | | | |
| Good control | 7.6 | 11.5 | 12.7 |
| Average control | 16.7 | 32.29 | 8.7 |
| Poor control | 73.8 | 22.3 | 12.5 |
| p-value | 0.04 | 0.752 | 0.569 |
| **Blood Pressure** | | | |
| Low | 64.1 | 19.9 | 12.8 |
| Normal | 64.3 | 28.4 | 10.9 |
| p-value | 0.99 | 0.45 | 0.437 |
| **Symptoms** | | | |
| No | 68.5 | 20.7 | 11.5 |
| Yes | 50.7 | 30 | 14 |
| p-value | 0.116 | 0.173 | 0.203 |
| **Family History** | | | |
| Negative | 64.49 | 20.8 | 12.39 |
| Positive | 63.8 | 25.2 | 11.8 |
| p-value | 0.05 | 0.412 | 0.316 |

**Interpretation**:

- Significant differences in Anti GAD levels were found across groups categorized by glycemic control ($p = 0.04$), indicating higher levels in those with poor control.
- No significant differences were observed in AntiIA2 and IAA levels across any of the groups analyzed (all $p > 0.05$).

## DISCUSSION

In type 1 diabetes, there is a loss of beta cells due to autoimmune processes. Destruction of these cells results in loss of endogenous insulin production which is reversible, demanding the daily administration of insulin from outside of the body. Infection or environmental factors stimulate the immune system of those people who already have genetic susceptibility. The role of both humoral and cellular is let autoimmunity has been established [1].

Autoantibodies in type 1 diabetes have been broadly accepted as the hallmark of the disease by the scientific society. The combination of all of these autoantibodies would be a stronger and confident diagnostic measure for the patients having type 1 diabetes. Their role has been established as biomarkers of the pre-symptomatic stage of the disease [1,2].

This can be seen in our study that autoantibodies like Anti-GAD65 antibodies, Anti- IA2 antibodies, and Insulin autoantibodies are positive in almost all of the cases of type 1diabetes. This shows a strong relationship between these immune biomarkers and type1diabetes.One can confidently go for these kinds of immune biomarkers as a screening tool for the initial workup of suspected cases. In fact, it is highly encouraging practice to ask for these tests for the early intervention to minimize the disease progression and related complications. So far, the Pakistani data was not available for such type of relationship between the immune biomarkers and the type 1 diabetes. Our study has been managed to fill the gap in this particular area and successfully established the role of these immune biomarkers in diseased cases as we commonly see in other races across the globe.

The age of autoantibody development can also be used to stratify individuals with regard to the likelihood of quick progression to clinical diabetes, with more rapid disease progression being observed in children who develop islet autoantibodies early. Children who develop autoimmunity in the second decade of life or later mostly present with GAD auto antibodies earlier than any other immune biomarker [2].

In our study, the male population had a predominance over female cases. Although this is not a prevalence study, it has been noted that diabetes prevalence differs by gender depending on the study setting and the study population. There is no confident data available on gender difference in type 1 diabetes mellitus. This disease affects males more frequently than females but this may be change in different communities [3]. So, in our study no specific correlation has been found between gender difference and status of immune biomarkers. Patients of type 1 diabetes from low socioeconomic class, specifically those with low wages and less education, were more expectedly to suffer from type 1 diabetes related complications and comorbidities [4]. In our study no such type of correlation has been found with immune biomarkers. Autoantibodies are positive in every socioeconomic class of patients. Although persons of the upper class could manage their disease effectively to avoid future complications.

In the context of type 1 diabetes, several research have looked at the association between auto-antibodies and BMI. According to several studies, having a higher BMI may increase your likelihood of getting auto-antibodies and ultimately type 1 diabetes. To demonstrate a clear and consistent association between these parameters, more research is required as the results so far have been contradictory [5]. In our study there is no such kind of a relationship found and the patients even with normal BMI shows positive results for autoantibodies.

The presence of diabetes auto antibodies affects the HbA1c level and the total number of insulin units used per day by the patients; the more diabetes autoantibodies are present, the higher the HbA1c level, the more insulin units that patients need to control their blood glucose levels [6]. In our study, it is found that almost all of the patients with positive GAD65 autoantibodies have poor glycemic control meaning by that they have high values of HbA1c and Fasting Blood Sugar levels. HbA1c in our study was a marker of glycemic control whereas Fasting Blood Sugar is strongly correlated with HbA1c.

A statistically convincing relationship has been found between positive immune biomarkers and osmotic symptoms of type 1 diabetes. But this relationship is weak in childhood and more pronounced in adulthood. We studied the demographic and clinical features in terms of biomarkers in patients with T1DM.In our study, which includes patients of less than 18 year of age, no significant clinical findings had been located in type 1 diabetes cases. Even most of the cases with positive autoantibodies had no clinical presentation at that time [7].

International literature supports the fact that children who were autoantibody positive and progressed to type 1 diabetes had at least one relative with type 1 diabetes [8]. In our study population, no such relation established and results are evenly distributed among patients with positive family history and those with no such history. Overall anti- GAD65 positivity was more than any other type of biomarker.

## CONCLUSION

Anti-GAD65 is identified as the most prevalent immune biomarker in the Pakistani population. Elevated levels of immune biomarkers are associated with poor glycemic control. Although low socioeconomic status correlates with worse glycemic control, no significant differences are found in Anti-IA2 and IAA levels across groups. Implementing a targeted approach for high-risk populations may improve

clinical outcomes and reduce both financial and mental burdens for patients.

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## CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Declared none

## AUTHORS CONTRIBUTION

**Hafiz Muhammad Bilal:** Conceptualization, manuscript writing, study design, data collection, accountable for all aspects of the work

**Muhammad Dilawar Khan:** Overall supervision, final approval of the work, accountable for all aspects of the work

**Hijab Batool:** Data collection, accountable for all aspects of the work

**Akhtar Sohail Chughtai:** Critical review, accountable for all aspects of the work

**Omer Rashid Chughtai:** Revisions, proofread, accountable for all aspects of the work

**Shakeel Ashraf:** Data analysis, accountable for all aspects of the work

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