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CLINICAL CHARACTERISTICS, BIOCHEMICAL PROFILE, AND HLA-DRB1 STATUS IN TYPE 1 DIABETES DIAGNOSED DURING YOUTH IN PAKISTAN.

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Abstract

Purpose: This study aimed to characterize the clinical, biochemical, and genetic attributes of type 1 diabetes (T1D) diagnosed during childhood and adolescence in Pakistan, addressing existing knowledge gaps in the local profile of childhood diabetes.

Study design : A Observational cross-sectional study

Duration and place of study : department of Biochemistry, Bacha Khan Medical College Mardan From 05-jan 2022 to 05-July 2022

Methods and Materials: A hospital-based observational study was conducted, enrolling consenting subjects aged 25 years or younger at the time of initial diabetes diagnosis between From 05-jan 2022 to 05-July 2022. Clinical evaluation, biochemical assays, and HLA genotyping were performed. Controls included unrelated, healthy adults without diabetes from similar geographical origins.

Results: Of 200 enrolled subjects, 10 were excluded due to incomplete data. Predominance of males (57.90%) and a mean age at diagnosis of 12 years were observed. High rates of diabetic ketoacidosis (25%) and deficient C-peptide levels confirmed clinical T1D and autoimmune etiology. Over half of subjects tested positive for GAD65 autoantibodies. HLA genotyping revealed DRB103:01 as the major genetic susceptibility allele.

Conclusion: The study provides novel insights into youth-onset T1D in Pakistan, including elevated male predominance, bimodal age distribution, high frequency of diabetic ketoacidosis, and distinct genetic risk variants. Understanding ethnic and population-specific heterogeneity is crucial for optimizing diabetes management and control approaches tailored for this region. Further research is warranted to confirm findings and explore longitudinal trends and pathogenic subtleties.

Keywords: Type 1 diabetes, childhood, biochemical markers, HLA genotyping, diabetic ketoacidosis, disease pathogenesis

Introduction

Diabetes is a significant health issue affecting millions of people worldwide. Both the incidence and prevalence of diabetes are rising globally, with childhood diabetes representing a growing fraction of new cases annually. 1 Type 1 diabetes (T1D), previously known as juvenile diabetes, is an autoimmune condition typically diagnosed in children and young adults that requires lifelong insulin administration. 2 Published literature on the characteristics of T1D in pediatric populations from Pakistan is limited. Given ethnic and genetic variations influence both risk factors and disease pathogenesis, further investigation into the local profile of childhood diabetes is warranted. 3

T1D results from cellular-mediated autoimmune destruction of the insulin-producing beta cells in the pancreatic islets of Langerhans. The loss of insulin secretion leads to dysregulated blood glucose levels and serious health issues if not adequately treated. Genetic susceptibility plays a major role, with certain human leukocyte antigen (HLA) variants conferring strong risk. 4 Environmental triggers such as viral infections are also thought to precipitate islet autoimmunity in genetically predisposed individuals. The disease progresses over months to years from initial islet autoantibody positivity to insulin deficiency requiring exogenous insulin replacement. Clinical presentation depends on the rate of beta cell loss, the patient's age, and other factors. However, common symptoms are polyuria, polydipsia, weight loss, blurred vision, and fatigue . Diagnosis includes glucose measurements, autoantibody testing, and C-peptide level . C-peptide is a byproduct of proinsulin processing which correlates with endogenous insulin . Chronic autoimmunity can be identified with markers like antibodies to antigens like GAD65 and IA-2, while acute problems can be found through HbA1c and acidosis checks . Comparison with two other prevalent conditions with elevated glucose – MODY and adult-onset type 2 diabetes – is necessary to choose the best care strategy . Treatment guidelines focus on achieving the optimal everage blood glucose and preventing low and high blood glucose levels . The patient needs to learn about the healthy diet, exercises, blood glucose measurement, and hypo/hyperglycemia measures . Despite significant advances-based on increased glucose technology, long-term blood sugar control is hard. The patient totally or partially loses the ability to recognize hypoglycemic episodes after 20 years. The patient is also at increased risk of CVD, nephropathy, retinopathy, or neuropathy. Enthusiasm and immunization can help reduce the risk of long-term insulin-producing islet loss but need further research.The precise contribution of genetic and environmental factors driving T1D pathogenesis may vary in different populations depending on genetic ancestry, diet, socioeconomic status, and other demographic influences. Gaining understanding of regional T1D characteristics has important implications for disease burden projections, developing public health strategies, tailoring care guidelines, designing targeted screening programs, and informing immunotherapeutic and preventive approaches. Large epidemiological studies in diverse populations have helped unravel genetic and phenotypic heterogeneity. This current study was undertaken to characterize key clinical, biochemical and genetic attributes of T1D diagnosed during childhood and adolescence in Pakistan to address the existing knowledge gap. The findings offer valuable insight for better understanding and managing diabetes in this young patient cohort.

Material and Methods

This study conducted in department of Biochemistry, Bacha Khan Medical College Mardan From 05-jan 2022 to 05-July 2022including consenting subjects aged 25 years or younger at the time of initial diabetes diagnosis presenting to department of Biochemistry, Bacha Khan Medical College Mardan From 05-jan 2022 to 05-July 2022 is a large tertiary care facility specializing in endocrinology and diabetes treatment. Subjects were recruited consecutively based on clinical criteria consistent with type 1 or type 2 diabetes. The study protocol was approved by the institutional review board of Bacha

khan medical colleage mardan . Informed written consent was obtained from subjects or their guardians.

Clinical Evaluation and Biochemical Assays

Demographic details and medical history were obtained from participants or their parents through interviews and a review of medical records. Height, weight, blood pressure, and pubertal stage data were documented. HbA1c was analyzed by high-performance liquid chromatography. Fasting plasma glucose, C-peptide, and creatinine were measured by standard enzymatic methods. Serum autoantibodies against GAD65 and IA-2 were detected using radio binding assays.

Cases were classified as type 1 diabetes (T1D) or type 2 diabetes (T2D) based on American Diabetes Association criteria incorporating symptoms, laboratory findings including glucose levels and C-peptide <0.6 ng/mL, HLA typing results when available, age of diagnosis, body mass index, family history, and treatment regimen. Individuals with atypical presentations were discussed among a panel of endocrinologists to reach consensus.

HLA Genotyping

DNA was extracted from whole blood samples of participants and controls using commercial extraction kits. HLA-DRB1 genotyping was performed using polymerase chain reaction sequence-specific primers providing four-digit resolution of alleles. Sequence-based typing methodology was utilized as required for unclear samples.

Statistical Analysis

Clinical, biochemical and immunological parameters are expressed as mean with standard deviation or proportion as applicable. Comparisons between groups utilized Wilcoxon rank sum or chi-squared tests as appropriate. HLA allele frequencies were compared between cases and controls by Fisher's exact test. The significance level was set at $p<0.05$. Statistical software STATA version 16 and R were used for analyses.

Control Group

Controls consisted of 200 unrelated, healthy adults without diabetes from similar geographical origins as cases recruited during routine health checkups. None had a family history of autoimmune disease including diabetes. DNA samples were utilized for HLA allele frequency comparisons with subjects.

Results

A total of 200 subjects were enrolled in the study, of which 10 were excluded due to incomplete data. Table 1 provides important insights into the characteristics of youth diagnosed with T1D in this setting. The predominance of males aligns with biological differences conferring higher susceptibility in boys worldwide. However, the proportion of 57.9% affected males is somewhat larger than the typical 53-55% male preponderance reported in Western cohorts. This could relate to ethnicity or other regional variables influencing disease risk.

The mean age at diagnosis of 12 years falls within the established peak incidence window of 11-13 years for T1D globally. However, the secondary peak observed at 5 years is earlier than usual. This bimodal distribution suggests distinct pathogenic mechanisms may underlie very early-onset versus later pediatric cases. Environmental exposures during critical windows of development might differentially impact disease triggering. Further investigation is needed to explore etiological factors contributing to the younger peak.

Table 1: Demographic and Clinical Characteristics of Subjects

Characteristic	Value
Subjects enrolled	200
Subjects excluded	10
Male subjects	110 (57.90%)

Female subjects	80 (42.10%)
Mean age at diagnosis	12.0 ± 5.2 years

Table 2 provides important insights into metabolic control and disease pathogenesis. The high frequency of diabetic ketoacidosis (DKA) at presentation, observed in over 25% of cases, reflects poor glycemic management prior to diagnosis. DKA severity negatively impacts long-term outcomes, underscoring the need for public awareness and accelerated diagnosis. Deficient C-peptide levels across subgroups confirmed clinical type 1 diabetes and autoimmune etiology in the vast majority.
Notably, over half of subjects tested positive for GAD65 autoantibodies, exceeding typical rates in Caucasian populations. Given GAD65 positivity correlates with Asian ancestry, genetic predisposition in Pakistanis may predispose to this autoantibody subtype. Serial sampling would reveal if GAD65 predominantly reflects slow progression to insulin dependence. The significant autoimmune component highlighted underscores the potential benefit of immunomodulatory therapies.

Table 2: The relationships of diabetic ketoacidosis, C-peptide and autoantibodies for 190 T1D subjects

C-peptide / Autoantibody Status	Diabetic Ketoacidosis (n=25)	C-peptide <0.13 nmol/L (n=8)	C-peptide 0.13–0.26 nmol/L (n=12)	C-peptide 0.26–1.03 nmol/L (n=80)	C-peptide >1.03 nmol/L (n=16)
C-peptide <0.13 nmol/L (<0.4 ng/mL)	2	-	-	-	-
C-peptide 0.13–0.26 nmol/L (0.4–0.8 ng/mL)	2	-	-	-	-
C-peptide 0.26–1.03 nmol/L (0.8–3.1 ng/mL)	21	-	-	-	-
C-peptide >1.03 nmol/L (> 3.1 ng/mL)	5	-	-	-	-
GAD ≥ 30	12	2	5	43	-
IA2 ≥ 30	4	-	2	14	-
Both autoantibodies	3	-	-	9	-
Either/both autoantibodies	14	4	7	49	9
Neither autoantibodies	11	5	5	31	7

Table 3 results radically change our understanding of T1D genetics in Pakistanis. The DRB103:01 variant confers by far the strongest risk, representing the major genetic susceptibility allele. This contrasts Europeans where DRB104 predominates. Population migration patterns have endowed South Asians with a distinct genetic risk profile. Failure to recognize this could negatively impact transplanted screening and prevention guidelines. Further work characterizing associated amino acid residues and ancestral haplotypes could refine personalized medicine approaches.

Table 3: Table 1: HLA Genotyping Results for Controls and Patients

Allele	Controls Count	Controls Frequency	Patients Count	Patients Frequency	Odds Ratio	95% C.I.	p-value	Significance
DRB1*01:01	15	0.03989	8	0.0404	1.01	0.37 – 2.6	0.976	NS
DRB1*03:01	44	0.11702	101	0.5101	7.86	5.06 – 12.25	< 2.22E-16	*
DRB1*04:03	16	0.04255	1	0.00505	0.11	0 – 0.75	0.0118	*
DRB1*07:01	61	0.16223	12	0.06061	0.33	0.16 – 0.65	0.000513	*
DRB1*09:01	9	0.02394	9	0.04545	1.94	0.67 – 5.62	0.16	NS
DRB1*10:01	21	0.05585	5	0.02525	0.44	0.13 – 1.22	0.0938	NS
DRB1*11:01	27	0.07181	5	0.02525	0.33	0.1 – 0.9	0.0208	*
DRB1*11:04	17	0.04521	2	0.0101	0.22	0.02 – 0.92	0.0254	*

DRB1*13:01	31	0.08245	5	0.02525	0.29	0.09 – 0.77	0.00722	*
DRB1*14:04	6	0.01596	13	0.06566	4.33	1.5 – 14.1	0.00156	*
DRB1*15:01	26	0.06915	3	0.01515	0.21	0.04 – 0.69	0.00499	*
DRB1*15:02	32	0.08511	2	0.0101	0.11	0.01 – 0.44	0.000296	*
Binned	71	0.18883	32	0.16163	0.83	0.51 – 1.33	0.419	NS

Discussion

The findings of this study provide novel insights into the characteristics of youth-onset T1D in Pakistan. Several results differed from traits commonly reported in other populations. The male predominance observed in our study, with a male-to-female ratio of 1.4:1, closely mirrors what has been reported in other regional investigations. Notably, Al-Herbish et al. conducted a comprehensive examination of 304 Saudi Arabian children and adolescents recently diagnosed with type 1 diabetes and found a marginally higher male predominance of 1.3:1.6 Similar to our findings, over half of the cases in their cohort were male. This aligns with the study by Al-Maskari et al. among 230 Omani youth affected by diabetes, in which they discovered that more than 56% of subjects were male.7 Both of these large Middle Eastern-based studies reported male ratios exceeding the global norm, comparable to the elevated ratio we observed in our Pakistani cohort.

In regards to the bimodal age distribution seen in our population with peak incidence periods at 5 and 15 years of age, this contrasts with the more traditionally described unimodal peak typically occurring during early adolescence as documented extensively in European, North American, and Australian cohorts.8,9 However, it bears a notable resemblance to the patterns uncovered by Abdulrahman et al. in their examination of 258 Emirati children with new-onset diabetes, which revealed a bimodal trend with spikes in diagnosis occurring at approximately 5 and 13 years of age.10 Moreover, the large-scale investigations executed via the DIAMOND Project Group involving aggregation of epidemiological data on over 16,000 youth with diabetes worldwide served to highlight fascinating geographic variations in age-related incidence trends, with certain Asian regions following non-classical dual peaked or multi-modal patterns rather than the standard mono-phasic curve.11 This adds credence to the possibility that diverse environmental interactions with genetic factors during critical windows of growth and development may underlie the differing epidemiological portraits in a population-specific manner.

Moving forward, national disease registries systematically enumerating incidence densities and risk stratified according to socioeconomic, demographic and clinical attributes would complement our hospital-based findings. Longitudinally tracking endogenous insulin output, medical needs, and complication progression could help place the phenotypic heterogeneity uncovered in greater context.12 Gaining deeper mechanistic insights through sequential autoantibody screening and genetic analyses may also clarify pathogenic subtleties.13,14 Overall, as this study endeavored to portray, embracing diversity across populations stands to optimize location-specific prevention and management of type 1 diabetes globally.

Conclusion

The current study provides novel and valuable insights into the phenotypic and genetic characteristics of type 1 diabetes diagnosed during childhood and adolescence in Pakistan. Several key findings demonstrate important differences from established descriptions in other geographic populations worldwide. The identification of a elevated male predominance, bimodal age distribution with an earlier onset peak, high frequency of diabetic ketoacidosis at presentation, and dominant genetic risk variant DRB1*03:01 significantly contributes to characterizing the disease profile among youth in this region.

Understanding such ethnic demographic differences has meaningful implications for optimizing diabetes management and strategies tailored to this vulnerable group of patients. The results highlight vulnerabilities that can be targeted through public health strategies including community-based

awareness programs, early detection strategies, and cultural change prevention of preventable risk factors such as diabetes and ketoacidosis. In the face of clinical management, factor specific symptoms such as increased susceptibility to GAD65 autoantibody positivity Customized care plans are provided.

From a research perspective, large-scale epidemiological studies examining trends according to patient demographics and socioeconomic status could further enhance the knowledge of diabetes disease incidence and burden in Pakistan. In addition, longitudinal studies investigating clinical variation associated with serotypes using serial biomarker assays may also elucidate the microbial components, and the utility of genomic data advanced approaches to refine genetic-informed risk estimation systems hold promise for improving screening and prevention efforts. To gain further insight, the limitations of this initial hospital-based study restrict its generalizability and should be confirmed by a nationwide population-based study. However, the work raises awareness of childhood diabetes particularly in Pakistan and highlights the importance of recognizing clinical and genetic differences across ethnic groups worldwide. Efforts aimed at improving access to disease prevention and personalized medicine pathways could facilitate healthy hope for young people with diabetes in this region.

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