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Original article

Type 1 diabetes mellitus and its complications in children aged ≤20 years from Punjab, Pakistan

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Abstract: *Background* — Type 1 diabetes mellitus causes serious disease complication in children. *Objectives*- The main objective of this study was to assess the frequency and severity of diabetic complication in children ≤ 20 years of age from Punjab, Pakistan.
Methods — The data of diabetic patients and their blood samples were collected from the diabetic registries at three districts of Punjab, Pakistan. Fasting plasma glucose, random plasma glucose, HbA1c, and GAD-65 autoantibodies were measured in sampled blood serum. The data on other clinical symptoms at the onset of disease were recorded as well.
Results — Out of 310 patients, 54.2% were male, and their mean age at the onset of disease was 13.22 years. Among all patients, according to clinical indicators, high severity of the disease and serious complications were revealed.
Conclusions — The diabetic complications were severe in all patients from three districts of Punjab, Pakistan, at and below the age of 20 years.

Keywords: Pediatric DM, acute and chronic complications, mode of presentation of T1DM, BMI, HbA1c, GAD 65 antibodies.

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Introduction

Type 1 diabetes mellitus (T1DM) is one of the most common immune-mediated childhood diseases that can lead to early morbidities and mortalities [1]. It can occur at any age, but the peak of its incidence is noted below 18 and over 60 years of age [2-4]. The results of the SEARCH for Diabetes in Youth Study 2009 (USA) suggested that environmental or behavioral factors (or both) play larger parts in the higher incidence of type 1 diabetes mellitus [5] than previously suspected. In Canada [6], compared with decades ago in Europe [7], a similar trend was observed. Beyond incidence, the reports on clinical symptoms and complications of T1DM in children from Pakistan are limited, while from Punjab these are scarce. There are published reports on type 1 diabetes in children from Karachi, Pakistan [1], entire Pakistan [8], China [9], Canada [10], Egypt [11], Japan [12], Saudi Arabia [13], and other parts of the world [4, 14]. These publications described the prevalence, clinical features and diabetes complications in diabetic children. Apart from these studies, data on the symptoms of clinical complications (neuropathy, nephropathy, retinopathy and DKA) in children suffering from T1DM were reported worldwide; while such reports from Punjab, Pakistan, on chronic complications due to T1DM in children under the age of 20 years are a missing link. This could be due to a very limited access to health facilities, poor awareness about the ailment and/or non-acceptance of pediatric diabetes as a serious health issue.

Later studies focused on life-threatening condition such as diabetic ketoacidosis (DKA) as an acute complication and medical emergency in children and adolescents with diabetes and other demographic and clinical symptoms [14-18], as well as polyuria, polyphagia, polydipsia, HbA1c, neuropathy, nephropathy, and retinopathy [3, 12, 13, 19-21].

The peak of T1DM was reported at the ages of 10-14 years throughout the world [22]. Registries in Europe suggested that the incident rates of T1DM were the highest in the youngest age-group (0-4 years) [23]. However, after puberty, incidence rates declined and appeared stabilized at the age of 15-29 years, but in the later study, the highest incidence was recorded at the age of 10-13 years [24].

Poor metabolic control may result in the acute complications of hyperglycemia and ketoacidosis, chronic microvascular and macrovascular complications [11, 18, 25, 26]. If the patients are left untreated, diabetes may cause many complications. Such complications include DKA and hyperosmolar hyperglycemic state [27]. Majority of diabetic children worldwide exhibit conventional symptoms of polyuria, polydipsia and weight loss [1, 13, 25, 28]. The frequency of occurrence of the life-threatening condition of DKA reported in the literature is variable, ranging from 10% to 80% [14, 16, 18].

Furthermore, there is a noteworthy variation in the T1DM complications in different countries worldwide, particularly the incidence of neuropathy, nephropathy and retinopathy and DKA. The latter carries a significant risk of mortality and morbidity. Such broad variation in the clinical symptoms of T1DM may reflect a heterogeneity in the pathogenesis of the disease [29]. The rate of b-cell destruction is mirrored clinically by different preclinical duration, age of onset, and severity of the symptoms. Moreover, it

is also known that among children at the age of 20 and less, boys and girls are equally affected [14, 16]. There are reports that the incidence has increased due to COVID-19 pandemic in Germany [30], UK [31], Romania [32] and Italy [33].

The current study was conducted at the Pediatric Diabetes Clinics (PDCs), following standard WHO criteria [34], and at the laboratory of Molecular Endocrinology, Institute of Zoology, University of the Punjab, Lahore, Pakistan. A sample of 213 new and 97 previously diagnosed patients of T1DM patients at the age of ≤ 20 years was included in our study to examine their demographic, clinical, biochemical and serological parameters in order to describe the onset of diabetes and its complications.

Material and methods

Study site

The study was carried out during four years (from 2019-2022) based on registries of Pediatric Diabetes Clinics (PDCs) satisfying standard WHO criteria [34] for the diagnosis of T1DM and its complications. The medical experts employed at PDCs from three districts of Punjab participated in the study. These districts were Dera Ghazi Khan (DGK) representing southern part, Sahiwal (SWL) representing central part, and Gujranwala (GLA) representing northern part of Punjab Province of Pakistan.

Study subjects

The subjects at the age of 20 years and less were screened for T1DM at their first visits at PDCs for medical attention/treatment and those already under treatment were reassessed. The conduct, diagnosis, blood collection and patient handlings were done strictly by paramedics working under the supervision of medical doctors. Peripheral blood was aspirated using venipuncture into vacutainer tubes after an overnight fast. Fresh blood samples were assessed for fasting glucose and spun down immediately for collection of serum, which was stored at -20° C in a freezer for further screening via biochemical and serological analyses. All procedures performed in this study were approved by the Advanced Studies and Research Board, University of the Punjab, Lahore, Pakistan) and were in accordance with the 1964 Declaration of Helsinki and its later amendments, or comparable ethical standards.

Demographic data

The collected demographic characteristics included first name, family name, age, sex, height and weight through interviews of parents and children of the patients. Height and weight were measured with subjects with light clothing on and without shoes. Height was recorded to the nearest centimeter and weight to the nearest 0.1 kg. BMI was calculated by dividing a person's weight in kilograms by the squared height in meters. BMI scores were calculated using the WHO standards for those <5 years of age [35] and for 5-19 years old [36]. For those 20 years of age, BMI was calculated using an age of 19.0 years.

Biochemical and serological parameters

Determination of the type of diabetes was made at PDCs by the medical experts according to available clinical characteristics and anamnesis. Clinical features such as polyuria, polydipsia, polyphagia, sudden weight loss, fatigue, blurred vision, nausea and diabetic ketoacidosis (DKA) were recorded at the time of registration at respective PDCs. A total of 1,035 subjects were screened for T1DM through the repeated measurements of fasting plasma glucose (FPG) and random plasma glucose (RPG) by the blood glucose monitor kit (ACCU-CHEK Instant S; Roch Diabetes Care Gmbh, Germany). Glycosylated hemoglobin A1c (HbA1c) was assessed using a BioRad D-10 analyzer (BioRad Laboratories Inc., Hercules, USA), and anti-GAD 65 autoantibodies were measured by commercially available ELISA kits (IBL, Hamburg, Germany, in Pakistan) from frozen serum samples at the laboratory of Molecular Endocrinology, Institute of Zoology, University of the Punjab, Lahore, Pakistan.

Diabetic complications

Peripheral neuropathy was defined as diminished or absent touch or vibratory sensations of the feet. Nephropathy was defined as the level of protein ≥ 1^+ on dipstick (Combur 10; Roche Diagnostics, Mannheim, Germany), with no other abnormal findings on urinary examination. Retinopathy was classified as normal, background (presence of microdots and hard exudates), preproliferative, proliferative (presence of exudates and new vessels), maculopathy, or prior to laser photocoagulation for diabetic retinopathy according to Shera [1].

All patients with confirmed T1DM were divided into two groups: group I included newly diagnosed patients and group II encompassed individuals already diagnosed with T1DM 1 or more years before.

Statistical analysis

The data were subjected to Statistix version 8.1, data analysis software for researchers (Analytical Software, 2105 Miller Landing Rd., Tallahassee, FL 32312, USA). Chi-squared test was applied to examine the relationship between variables. An overall α value of 0.05 was used to assess significant differences between the variables. Data are presented as mean ±SD.

Results

During the study period of four years, we have screened 1,035 subjects aged ≤ 20 years for T1DM. Of all screened subjects, only 310 were confirmed through repeated clinical assessments of FPG, RPG, HbA1c and GAD 65 autoantibodies. Our data revealed that 336 subjects (32.46%) were confirmed as T1DM patients, of which 26 refused to provide disease information and therefore were excluded from the study. The remaining 310 were taken as study subjects. Of them, 213 (68.7%) were newly diagnosed (group I), while 97 (31.3%) were previously diagnosed with T1DM (group II) for 1 or more years and were undergoing the insulin treatment.

The mean age of the subjects diagnosed with T1DM was 13.22±3.1 years (range 10-15 years) with a peak range at 11-15 years. The median age in group I was 12.1±3.1 years, while in group II, it was 15.75±1.9 years with a peak of disease ranging from 6-15 years and 10-20 years, respectively.

The overall data has revealed that T1DM was more prevalent in male (54.2%) than female (45.8%) patients. Based on the district-based data for DGK (male: 55.3% and female: 44.7%) and SWL (male: 55.4% and female: 44.6%), we observed unequal percentage of disease; while in GLA district, the T1DM was almost equally prevalent among male and female subjects (male: 51.0% and female: 49.0%). Similar results were obtained for group I and

II, except in group II in GLA, where the share of females (63.2%) was significantly higher (P<0.05) than in group I, as well as other in districts (*Table* 1).

Among all T1DM patients, 93 (30.0%) were underweight, followed by 147 (47.4%) with a normal BMI, 68 (21.93%) were overweight and only 2 (0.65%) were categorized as obese (level I). In group I, 88 patients (41.3%) were underweight, 114 (53.5%) were normal, 11 (5.16%) were overweight and no one was obese. In group II, very few 5 (5.15%) were underweight, 33 (34.0%) were normal, 57 (58.7%) were overweight and only 2 (2.06%) were obese (level 1) (*Table* 1). We also observed some statistically significant trends: in GLA, 60 patients (58.8%, P<0.05) had normal BMI; 34 individuals (33.0%, P<0.05) were overweight in SWL. In DGK PDCs registries, there was non-significantly high number of 39 underweight patients (37.2%). The mean value of FPG in all patients was 175.2±10.4 mg/dl, while random plasma glucose was measured at 218.5±18.4 mg/dl in all patients. The levels of FPG and RPG between groups I and II were statistically different (p<0.05). The mean value of glycosylated hemoglobin (HbA1c) was 11.89±2.02%, and GAD-65 autoantibodies were measured as 617.03±209.12 nmol/L in 275 out of 310 patients (because 35 patients refused to follow the diagnosing procedures). The mean GAD value for 275 patients was 617.03±209.12 nmol/L. In group I, all 213 patients were diagnosed as positive, and autoantibodies were measured as 550.1±184.5 nmol/L; while in group II, out of 97 patients, 62 were assessed for GAD autoantibodies, and their mean value was 846.98±186.79 nmol/L, which was significantly higher (P<0.05) than in group I.

It was noticed that majority of the patients had poor glycemic control leading to a higher percentage of diabetic complications such as DKA 50.27%, neuropathy 37.84%, nephropathy 47.56% and retinopathy 49.23%. In group I, patients had significantly lower (P<0.05) values of DKA incidence (32.5%), neuropathy (26.6%), nephropathy (32.3%) and retinopathy (29.0%). The percentage of DKA in patients of group II was recorded at 82.5%, of neuropathy at 55.7%, of nephropathy at 64.3±%, and of retinopathy at 88.0%, which was significantly higher than in group I (P<0.01) as differed from overall cumulative data (P<0.05). There were slight variations in these values for males and females in different age classes in groups I and II (*Table* 2).

It was obvious that majority of patients with T1DM (306) exhibited classical clinical symptoms of polyuria and polydipsia (98.7%); 246 (79.4%) had polyphagia; 275 (88.7%) suffered from sudden weight loss; 220 (71.0%) had fatigue; 132 (42.6%) experienced blurred vision, and 88 (28.4%) were experiencing nausea. Similar results were obtained among newly and previously diagnosed patients (groups I & II).

Table 1. Baseline data of T1DM patients from PDCs from three districts of Punjab, Pakistan

Parameters		*Group I*		*Group II*		*All*	
n		213		97		310	
Age (years)	Peak of infection	Mean 12.1±3.1	Range 6-15	Mean 15.75±1.9	Range 10-20	Mean 13.22±3.1	Range 5-20
Gender	Male	n=118 (55.4%)		n=50 (51.6%)		n=168 (54.2%)	
	Female	n=95 (44.6%)		n=95 (44.6%)		n=142 (45.8%)	
BMI	Under wt.	n=88 (41.3%)		n=5 (5.15%)		n=93 (30.0%)	
	Normal	n=114 (53.5%)		n=33 (34.0%)		n=147 (47.4%)	
	Over wt.	n=11 (5.16%)		n=57 (58.76%)		n=68 (21.9%)	
FPG (mg/dl)	Male	n=118 (191.5±9.6)		n=50 (162.8±21.9)		n=168 (180.9±12.2)	
	Female	n=95 (188.1±7.3)		n=47 (159.2±8.3)		n=142 (169.6±5.8)	
RPG (mg/dl)	Male	n=118 (255.6±19.6)[a]		n=50 (185.7±13.6)[b1]		n=168 (219.5±17.8)	
	Female	n=95 (249.0±23.5)[a]		n=47 (188.6±17.3)[b1]		n=142 (216.8±18.9)	
DKA	Male	n=28 (30.77%)		n=41 (82.0%)		n=69 (48.94%)	
	Female	n=2 (34.21%)[a]		n=39 (83.0%)[b2]		n=64 (51.61%)	
Neuropathy	Male	n=27 (29.67%)		n=28 (56.0%)		n=55 (39.01%)	
	Female	n=17 (23.61%)		n=26 (55.3%)		n=44 (36.67%)	
Nephropathy	Male	n=31 (36.47%)		n=27 (62.8%)		n=58 (45.37%)	
	Female	n=20 (28.17%)		n=25 (65.8%)		n=46 (41.82%)	
Retinopathy	Male	n=24 (30.38%)[a]		n=36 (90.0%)[b3]		n=60 (50.42%)	
	Female	n=18 (27.69%)[a]		n=31 (86.11%)[b4]		n=49 (48.04%)	
District wise data							
DGK		Group I (80)		Group II (25)		All (105)	
	Male	n=42 (52.5%)		n=16 (64.0%)		n=58 (55.24%)	
	Female	n=38 (47.5%)		n=9 (36.0%)		n=47 (44.76%)	
SWL		Group I (65)		Group II (38)		All (103)	
	Male	n=37 (56.9%)		n=20 (52.6%)		n=57 (55.4%)	
	Female	n=28 (43.1%)		n=18 (47.4%)		n=46 (44.6%)	
GLA		Group I (64)		Group II (38)		All (102)	
	Male	n=38 (59.4%)		n=14 (36.8%)		n=52 (51.0%)	
	Female	n=26 (40.6%)		n=24 (63.2%)		n=50 (49.0%)	

Data are presented as mean±SD, b is statistically different from a in the same row as b1 (P=0.046), b2 (P=0.038), b3 (P=0.029) and b4 (P=0.036).

Table 2. Physical, physiological and biochemical characteristics of all subjects, newly and previously diagnosed with Type 1 diabetes mellitus

Parameters	*Group I (n=213)*				*Group II (97)*	
Age groups (yrs)	<5	6-10	11-15	16-20	11-15	16-20
n	2	84	81	46	46	51
Weight (kg)	24.0±0.0	31.82±6.33	47.2±5.8	55.77±4.42	50.46±6.71	64.53±5.28
Height (cm)	127.5±3.5	136.19±8.36	151.74±5.56	157.22±4.82	156.59±4.46	158.16±4.60
BMI (kg/m^2)	14.79±0.81	16.96±2.09	20.46±2.0	22.55±2.33	24.55±2.83	25.83±1.88
HbA1c (%)	11.90±1.0	11.04±1.08	12.34±2.62	11.42±1.53	11.46±1.15	13.39±3.95
GAD 65 (nmol/L)	442.0±86.0^{a1}	423.8±130.5	585.2±158.7	723.76±149.3	812.54±155.7	897.96±228.8^{b1}
Polyuria (%)	100.0	100.0	96.3	100.0	100.0	98.04
Polydipsia (%)	100.0	98.81	98.77	95.65	100.0	100.0
Polyphagia (%)	100.0^{a2}	88.10^{a2}	91.36^{a2}	73.91	52.17^{b2}	74.51
Sudden wt. loss (%)	100.0	92.86	88.89	86.96	86.96	84.31
Fatigue (%)	0.0	66.67	71.6	71.74	71.74	78.43
Blurred vision (%)	0.0	25.0	25.93	43.48	78.26	66.67
Nausea (%)	0.0	21.43	27.16	30.43	36.96	33.33
D Ketoacidosis (%)	0.0	9.09 ^{b3}	30.88	61.36^{a3}	78.26^{a3}	86.27^{a3}
Neuropathy (%)	0.0	16.67	31.82	34.88	47.83	62.75
Nephropathy (%)	0.0	15.79	44.44	41.67	54.76	74.36
Retinopathy (%)	0.0	20.0^{a}	25.42^{a}	48.57^{a}	86.84^{b}	89.47^{b}

n, number of patients; data are presented as mean with standard deviation (M±SD); b is statistically different from a in the same row as b1 (P=0.035), b2 (P=0.041), b3 (P=0.026) and b4 (P=0.033).

Discussion

The study investigated the clinical, biochemical, and serological characteristics of newly and previously diagnosed type 1 diabetic children ≤ 20 years of age in selected areas of Punjab, Pakistan. The data has revealed that males prevailed (54.2%) over female subjects (45.8%) diagnosed with T1DM, which agreed with previous findings from Karachi, Pakistan [1, 3]. The pattern of the starting age of the disease has suggested a peak of disease at 13.22±3.1 years (range:10-15 years) among all patients. The age of onset of the disease in group I was 12.1±3.1 (range: 6-10 years), which was lower than in group II, 15.75±1.9 (range: 10-20 years), but the difference was not statistically significant. Similarly, the data from three districts (DGK, SWL and GLA) separately, also exhibited unequal shares of the disease among male and female patients (*Table 1*). The results of current study have revealed that the onset age of diabetes in district-based data demonstrated similar age range as documented in some previous studies [1-3, 37]. The age of the disease onset in our cohort peaked from 10 to15 years old, which was similar to the results shown in a study conducted in Saudi cohort (10-20 years of age) among T1DM patients [38], as well as in children of Kuwait [39] and in other studies [18, 40, 41].

The clinical features identified in T1DM patients demonstrated that incidence values of polyuria, polydipsia, polyphagia, sudden weight loss, fatigue, blurred vision and nausea were very high but similar to the results presented in other studies [1, 14, 25, 27, 28]. The frequency of symptoms of the life-threatening condition (DKA) reported in the literature varies from 10% to 80% [35]. In our study, 50.27% among all children (48.95% male and 51.6% female) had symptoms of DKA, but in group I, DKA (32.5%) was observed at a significantly lower level than in group II (82.0%). The percentage of DKA in group I patients was similar to the values documented in [39] in children studied in 2011-2017. The older patients (group II) had onset of diabetes for some years; thus, they had significantly higher percentage of DKA. These findings implied that as the disease progressed, along with poor glycemic control (RPG=218.2±18.4 mg/dl), the diabetic complications were more frequent and more severe as documented in [4, 42, 43].

The GAD65 autoantibody titers were high in our study; the mean value based on 275 patients was 617.03±219.12 nmol/L. The group I patients had a significantly lower value of GAD 65 autoantibodies as compared to group II patients, because in group I, all patients were newly diagnosed, while in group II, all patients were previously diagnosed with T1DM, and the mean duration of the onset of diabetes was 11.72±4.7 years (range: 5-25 years). This means that a later onset of diabetes would have higher GAD 65 autoantibodies value in the patient's blood. Higher titers of GAD 65 autoantibodies in diabetic patients were associated with more severe clinical symptoms of the disease [38]. Moreover, the autoantibodies for GAD 65 were r higher in DGK patients (707.17nmol/L), which depicts significant beta cell destruction in a patients' pancreas. Simultaneously, the GAD 65 autoantibody values in patients from GLA (584.04 nmol/L) and SWL (555.94 nmol/L) were lower, which revealed a relatively better glycemic control, follow up and disease management. It was noted through interviews that 42.31% of diabetic children were either not properly diagnosed during their first visit at the nearby health centers, or unable to obtain timely medical help because of being from rural areas far from their respective PDCs. The delay in proper treatment caused continued hyperglycemia, which resulted in a significant beta cell destruction measured in those patients as a high titer of GAD 65 autoantibodies.

The frequencies of neuropathy, nephropathy and retinopathy among T1DM patients in the current study were 37.9%, 43.7% and 49.3% respectively; these values were slightly higher from those reported by Najem [26] but not significantly different. Possibilities of these complications are not uncommon among pediatric diabetic patients [28] and also depend on quality of disease control and management. In Pakistan, particularly in underprivileged areas of Punjab, health care system does not exist; thus, all patients suffering from any disease have to undergo a private medical treatment at private clinics on the self-pay basis. Unaffordability of this expenditure incurred during diagnosis and treatment results in the mismanagement of the disease.

The current study was conducted during 2019-2022. It was a peak time of COVID-19 pandemic, which offered great difficulties

in consulting and taking patient's records of the disease, and also negatively affected the overall situation at PDCs. The studies showed that COVID-19 pandemic have affected the diabetic patients more severely in different parts of the world [30-33]. Furthermore, we investigated T1DM issue among children in unprivileged areas of Punjab, Pakistan, where medical facilities of acceptable quality are not readily available for general public. There is indeed a dire need of the better health facility access to people of such underprivileged areas. We thereby strongly recommend a complete and thorough examination of pediatric diabetes and its complications in other cities of Punjab in order to portray a complete picture of T1DM and its complications among children with a purpose of a proper control, care and management of this disease. The presented research is the only study of such detail in three districts of Punjab, Pakistan, and unfortunately, it demonstrates very alarming results regarding the T1DM.

Conclusion

The study has revealed that the pediatric diabetes patients experienced severe and life-threatening complications of the disease, probably, because of their unawareness of the ailment, thus experiencing complications due to the poor glycemic control and disease management. The study period fell into the peak time of COVID-19, thereby presenting great difficulties to attend medical/health centers for proper diagnosis and timely medical treatment. The delay in the onset of treatment caused hyperglycemia for longer time, which resulted in more severe diabetes complications in patients.

Competing interests

The authors declared no competing interests.

Author contributions

The first author (KSA) conducted the research under the supervision of the research supervisor (NB) and prepared the draft manuscript. The latter was corrected by MSA, while MSR provided technical assistance in its preparation. Statistical analyses were performed by NH, and the manuscript was completed and finalized by MSA.

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Advanced Studies and Research Board, University of the Punjab, Lahore, Pakistan; and in compliance with the 1964 Declaration of Helsinki and its later amendments, or comparable ethical standards. Our study was retrospective in its nature; hence, the formal consent was not required.

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