"PROFILE OF AUTO-ANTIBODIES AND IT'S CORRELATION WITH CLINICAL PROFILE IN TYPE 1 DIABETES MELLITUS SUBJECTS – A CROSS SECTIONAL STUDY FROM SOUTH INDIA"

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LIST OF ABBREVATIONS

BCG	–	Bacillus Calmette-Guerin
CD	–	Cluster of Differentiation
CLIA	–	Chemiluminescence immunoassay
DKA	–	Diabetic ketoacidosis
ECL	–	Electrochemiluminescence
eGFR	–	Estimated Glomerular Filtration Rate
ELISA	–	Enzyme Linked Immunosorbent Assay
GABA	–	Gamma-aminobutyric acid
GADA-65	–	Glutamate Decarboxylase-65 Autoantibody
HLA	–	Human leucocyte antigen
IAA	–	Insulin Autoantibody
IA2A	–	Insulinoma-Associated Protein-2 Autoantibody
IAP	–	Indian Academy of Pediatrics
ICA	–	Islet cell autoantibody
ICMR	–	Indian Council of Medical Research
Ig	–	Immunoglobulin
ISPAD	–	International Society for Pediatrics and Adolescent Diabetes
O.D	–	Optical Density
RBA	–	Radio-binding Assay
SARS CoV-2	–	Severe Acute Respiratory Syndrome Coronavirus-2
T1DM	–	Type 1 diabetes mellitus
T2DM	–	Type 2 diabetes mellitus
TPO	–	Thyroid Peroxidase
TSH	–	Thyroid Stimulating Hormone

TTG	–	Tissue Transglutaminase
UACR	–	Urine Albumin Creatinine Ratio
WHO	–	World Health Organization
YRD	–	Youth Onset Diabetes in India Registry
ZnT8A	–	Zinc Transporter 8 Autoantibody
kDa	–	Kilo Dalton
mcIU/ml	–	Micro International Units per milliliter
mg/dl	–	Milligram per deciliter
mg/g	–	Milligram per gram
ng/ml	–	Nanogram per deciliter
μL	–	Microgram

ABSTRACT

OBJECTIVE: Type 1 Diabetes Mellitus (T1DM) is multifactorial in origin. Pathogenesis of T1DM involves destruction of pancreatic beta-cells by autoantibodies. The data on disease causing autoantibodies is sparse in Indian children. This study primarily aims to study the profile of autoantibodies causing T1DM in South Indian children.

METHODOLOGY: A cross-sectional study was conducted in a tertiary care hospital in Belagavi, Karnataka, involving 74 T1DM children. T1DM children from age 1 to 18 years, with less than 4 years of duration of T1DM and those who did not have any complications of T1DM were recruited for the study. Demographic details, family history, anthropometric details, details of disease onset, HbA1C and C-Peptide levels at the time of diagnosis were noted. T1DM autoantibodies – GADA-65, ZnT8A, IA-2A, IAA and ICA were estimated by ELISA method. TSH was tested via CLIA method.

RESULT: Autoantibody positivity was detected in 55.4% of T1DM children. The prevalence of individual autoantibody were: GADA-65 in 5.4%, ZnT8A in 28.4%, IA-2A in 25.7%, IAA in 33.8%. ICA was absent in all the 74 T1DM children. 59.5% of T1DM children were females. Female prevalence of 56.1% was noted in autoantibody positivity T1DM children. There was no association noted between autoantibodies and DKA, onset of disease, HbA1C level, c-peptide level, hypothyroidism and celiac disease.

CONCLUSION: The prevalence of disease related autoantibodies in T1DM children from Southern India is 55.4%. The autoantibody prevalence was noted to significantly reduce after 3 years of onset of T1DM.

KEY WORDS: Type 1 diabetes, GAD-65 Autoantibody, Zinc Transported 8 Autoantibody, Insulin Autoantibody, Insulinoma associated protein 2 Autoantibody, Islet Cell Autoantibody, HbA1C, C-Peptide, Diabetic Ketoacidosis, Hypothyroidism and Celiac Disease.

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INTRODUCTION

Diabetes mellitus is a heterogeneous group of disorder with distinct genetics, etiology and pathophysiology, which ultimately leads to impairment in glucose metabolism, either through impaired insulin production or insulin action. Type 1 Diabetes Mellitus (T1DM) is an endocrine and metabolic disorder, which has a major impact on physical and psychological development of children. Type 2 diabetes mellitus (T2DM) incidence is also seen to be increasing in adolescents due to obesity and sedentary lifestyle.

Diabetes mellitus is not a recently identified disease. The history of diabetes can be traced back to the early human civilizations. The *Ebers Papyrus* of the ancient Egyptian civilization dating back to 1550 BCE, has description of the disease which resembles diabetes. *Sushrutha Samhita* which is an Ayurvedic text from ancient India dating back to 600 BCE described diabetes as "*madhumeha*" which literally means honey urine. There is also mention of ants infesting onto the urine of the subjects who were suspected with "madhumeha". The dietary regulations as well as herbal remedies can also be found in ancient Ayurvedic, Egyptian and Greco-Roman texts. The term "Diabetes" was coined by Greek physician Aretaeus of Cappadocia, which literally means "to go through" – the excessive passing of urine, and he also gave the first accurate description of diabetes. Thomas Willis in 17th century added the term "*mellitus*" which means extremely sweet. Edward Albert Sharpey-Schafer in 1926 was the first to coin the term "*insulin*". A major achievement was made by Frederick Banting and Charles Best by discovering insulin for which they received Nobel Prize in Medicine in 1923. Further in 1936, Harold Himsworth coined the terms "insulin sensitive" and "insulin insensitive" diabetes.

The classical manifestation of T1DM is constituted by a triad of symptoms which include polydipsia, polyphagia and polyuria. They can also present with life threatening diabetic ketoacidosis or rarely may be incidentally detected with hyperglycemia when they come to a pediatrician or general physician with osmotic complaints or complaints of undue weight loss, pain abdomen, vomiting. When the hyperglycemia phase has set in, about 90% of beta-cells would have already been destroyed in T1DM [1].

Many children present with life-threatening episode of Diabetic ketoacidosis (DKA) or will encounter one or more episodes of DKA in the disease course. The SEARCH study done in United States, in 2002-2003 has reported the prevalence of presentation with DKA as the initial manifestation of T1DM as 25.5% [2]. Further study done by Dana Dabelea Et al. in USA reported prevalence of DKA to be 30.2% in 2002-2003, 29.1% in 2004-2005, and 31.1 % in 2008-2010 [3]. The prevalence of DKA at diagnosis of T1DM in the EURODIAB project in 2001, which included 11 centers of study in Europe has reported a wide varying range of prevalence from 11% to 67% [4]. A systematic review of 65 studies which included 29,000 children from 31 different countries showed the prevalence of DKA at diagnosis to range from 12.8% to 80% [5], with highest prevalence seen in United Arab Emirates with 80% and lowest prevalence in Canada and Sweden with 19% and 14% respectively [5]. There is no data available in India regarding the prevalence of DKA at diagnosis with T1DM.

T1DM children are also potentially at a higher risk of developing micro and macrovascular complications like neuropathy, nephropathy, retinopathy, ischemic heart disease and peripheral vascular disease, leading to decreased quality of life and increasing the health care burden. T1DM is also associated with other autoimmune

diseases like autoimmune thyroiditis (Hashimoto's Thyroiditis and Graves' disease), celiac disease, Addison's disease, autoimmune hypoparathyroidism, autoimmune gastritis, autoimmune skin diseases, Systemic lupus erythematosus, rheumatoid arthritis, alopecia areata, urticaria [6].

T1DM is due to defect in insulin production due to destruction of pancreatic beta-cells. It is a chronic multifactorial disease. Interaction of genetically predisposed individuals with specific environmental factors, triggers the inflammatory process causing beta-cells destruction in pancreas, leading to the disease onset. The HLA haplotypes DR3-DQ2 and DR4-DQ8 are seen to be present in individuals with T1DM (30-40%), than in the general population (2.5%) [7]. More than 60 non-HLA loci are also identified to be associated with type 1 diabetes mellitus. [8] Many infections and non-infectious agents are responsible to trigger the immune destruction of the beta-cells in pancreas. Multiple environmental factors are attributed to development of disease, progression and also preventive against pathogenesis of T1DM.

Diabetes is classified based on etiology into 4 major groups: Type 1, Type 2, other specific types (genetic defects of beta cells and insulin, neonatal diabetes, genetic syndromes, autoimmune syndromes, pancreatic exocrine diseases, endocrinopathies, infections, drug & toxin mediated) and Gestational diabetes.

There are two forms of T1DM - 1A autoimmune and 1B idiopathic. In the autoimmune type, there is antibody mediated β-cell destruction resulting in insulin deficiency and thus hyperglycemia. According to systemic review done in 2014 by Watkins RA Et al., in those individuals presenting with fasting hyperglycemia, more than 90% already had one or more autoantibodies against beta-cells [9]. The presence of either of the antibody confirms the autoimmune T1DM. These autoantibodies

against beta-cells are present months to years before the diagnosis of the disease. The number and type of autoantibody predicts the rate of progression from asymptomatic abnormal glucose tolerance phase to overt diabetes mellitus phase [10].

Patients with T1DM commonly have autoantibodies to the islet cells – Islet Cell antibodies (ICA), 65-kD form of glutamate decarboxylase antibody (GADA-65), insulinoma associated protein-2 antibody (IA2A), Insulin autoantibodies (IAA) and zinc transporter ZnT8 antibody (ZnT8A).

There is a wide heterogeneity in the pathogenesis, profile of autoantibodies and disease course of T1DM children worldwide. The incidence of T1DM also varies in different parts of the world. Many studies are in ongoing in different part of the world to understand the complex pathogenesis and disease course in different race, ethnic groups, environment and regions, so that an effective prevention strategy can be planned in high risk individuals and also may help in slowing down the disease progression.

T1DM children have considerable life-style alterations like dietary restrictions, daily insulin administration and frequent blood glucose monitoring. The modalities for management of T1DM include – lifestyle modification, dietary modifications, and exogenous insulin replacement along with blood glucose monitoring. Pancreas transplant and islet cell transplant are upcoming modalities for treatment of T1DM in children. [11]

The year 2021 marked the 100th anniversary of insulin discovery. Over years various types of insulin have been developed which include – rapidly acting insulin (Lispro, Aspart, Glulisine), short-acting insulin (Regular human insulin),

Intermediate-acting insulin (NPH), Long-acting insulin (Detemir, Glargine, Glargine-yfgn, Degludec), Insulin mixtures (NPH/Regular-70%/30%, Protamine/Lispro-50%/50%, Protamine/Lispro-75%/25%, Protamine/Aspart-70%/30%) [12]. Now even inhaled form of insulin is under trial, named Technosphere insulin – which is a rapidly acting insulin [12]. As the technology is advancing, newer modalities in management of the diabetes – especially in blood glucose monitoring and insulin delivery are being developed. Currently there are Continuous subcutaneous insulin infusion, Real-time continuous glucose monitoring system, Flash glucose monitoring system, Sensor-augmented pump, Hybrid closed-loop insulin delivery system, "Do-it-yourself" artificial pancreas systems (DIYAPS) are helping in having a better blood glucose control [13]. These devices can be even connected to smartphones and be monitored by the patient as well as the healthcare provider. Even thought so many advanced options are available and many newer technologies are upcoming, there is no equity in the accessibility of resources.

Children withT1DM have psychological stress and there is an added financial burden to the family. Overall it leads to increase burden on the healthcare system of the country and in turn on gross national development of the country.

NEED FOR THE STUDY

The presence of one or more antibodies is required to have an immunological diagnosis of type 1A diabetes. From the available data, the incidence of T1DM in India is 4.9 cases/100000/year [14]. Even though the incidence is less compared to other countries, the total number of children with T1DM is large as the under 18 population in India is huge. An estimated 2,82,832 cases are there in India out of the global 1.52 million estimated cases according to the 10th edition of IDF Atlas[15].

Even though there is a huge number of T1DM children in India, there is no registry available to maintain the exact incidence and prevalence and the outcomes of the children with T1DM. Also the data on autoantibodies profile is limited. Few hospital based studies done in different parts of the country have included only one or two autoantibodies and have shown wide varying results regarding the presence of different autoantibodies in T1DM children in India. Also the data on the type of T1DM is limited – hence the prevalence of immune mediated and non-immune mediated forms of T1DM in India is not known.

The prevalence of the different types of autoantibody in autoimmune T1DM and the prevalence of other non-immune T1DM will help in formulating the guidelines for screening of at-risk children. Evidences have shown that the children who present with DKA have poorer long-term control [16]. Hence screening at risk children is prudent to prevent life threatening episode of DKA as the initial presenting feature. It will also pave way for further research to prevent T1DM in at risk population or to slow down the progression of disease by understanding the autoantibody profile and pathogenesis of the disease and also in formulating protocols for screening.

OBJECTIVES OF THE STUDY

PRIMARY OBJECTIVE:

- Prevalence of autoantibodies in children with type 1 diabetes mellitus

SECONDARY OBJECTIVE:

- Compare the clinical characteristics in children with number of autoantibodies

REVIEW OF LITERATURE

The term diabetes mellitus means "*A group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both*" [17]. This will lead to abnormalities in carbohydrate, fat and protein metabolism and is also associated with long term damage to various organs.

ISPAD Criteria for diagnosis of diabetes in children [18]:

- Classical symptoms of diabetes with plasma glucose of >200mg/dl

  OR

- Fasting plasma glucose of more than or equal to 126mg/dl

  OR

- Plasma glucose of more than or equal to 200mg/dl after two-hour of OGTT

  OR

- HbA1c of more than or equal to 6.5%

Diabetes mellitus is broadly categorized into four types [18]:

- T1DM - Deficiency/absence of insulin secretion by β-pancreatic cells. These patients will require insulin from the beginning of the diagnosis of the disease.
- T2DM - Due to peripheral resistance of action of insulin and comparative reduced production on insulin. These patients will require insulin later on in the disease course.
- Other specific types – Monogenic diabetes, genetic defects in insulin action, diseases of exocrine pancreas, endocrinopathies, drug or chemical induced, infections, genetic syndromes associated with diabetes.
- Gestational diabetes mellitus (GDM)

Table 1. Etiological classification of diabetes [18]

I. Type 1	
Immune mediated (IAA, GAD, IA-2, ZnT8)	
Idiopathic	
II. Type 2	
Resistance to insulin with relative insulin deficiency	
III. Other specific types	
A. Common forms of monogenic diabetes	***E. Drug- or chemical-induced***
MODY	Insulin resistance and deficiency
HNF4-A MODY	Glucocorticoids
GCK-MODY	Nicotinic acid
HNF1A-MODY	Atypical antipsychotics
HNF1B-MODY	Protease inhibitors (first generation)
Neonatal diabetes	Statins
KCNJ11,INS, ABCC8,GATA6	Insulin deficiency
B. Genetic defects in insulin action	β-blockers, calcineurin inhibitors, diazoxide, phenytoin
INSR	L-asparaginase, pentamidine, thiazide diuretics
Congenital-generalized lipodystrophy	Insulin resistance
Familial partial lipodystrophy	β-adrenergic agonists, Growth hormone
PIK3R1 (Short Syndrome)	***F. Infections***
	Congenital rubella, Enterovirus, Cytomegalovirus
C. Diseases of the exocrine pancreas	***G. Uncommon forms of immune-mediated diabetes***
Pancreatitis	Anti-insulin receptor antibodies
Trauma/pancreatectomy	Polyendocrine autoimmune deficiencies APS I and II
Neoplasia	
Cystic fibrosis-related diabetes	
Hemochromatosis	
Transfusion-related iron overload	
D. Endocrinopathies	***H. Other genetic syndromes sometimes associated with diabetes***
Acromegaly	Down syndrome
Cushing's syndrome	Klinefelter syndrome
Hyperthyroidism	Turner syndrome
Phaeochromocytoma	Friedreich's ataxia
Glucagonoma	Myotonic dystrophy
Somatostatinoma	Porphyria
	Prader-Willi syndrome
IV. Gestational diabetes mellitus (GDM)	

EPIDEMIOLOGY OF TYPE 1 DIABETES MELLITUS

In 2022, according to the International Diabetes Federation, the global estimate of T1DM children below the age of 20 years was 1.52 million [15]. In this, 2, 82,832 cases (18.6%) are estimated to be from India [15]. The worldwide incidence in 2022 was 2, 01,000 [15]. The incidence differs between countries, ethnic population and the region. Highest incidence is found in Finland. The life expectancy ranges from 13 years to 65 years, for a child diagnosed with T1DM at 10 years of age, based on the income of the country [19]. The incidence is increasing worldwide due to increasing environmental risk factors and susceptible population and the prevalence is also increasing due to awareness, diagnosis and accessibility of insulin. This increase will lead to short and long term social and developmental implications and is also a burden on the healthcare system of the country.

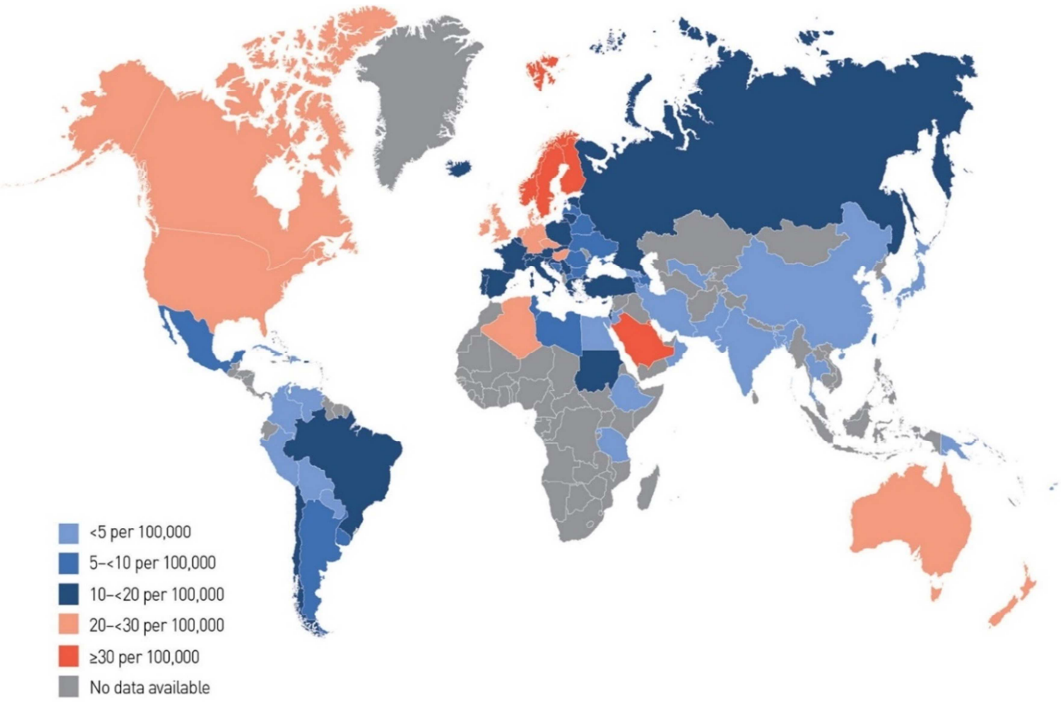


Fig. 1. Incidence rates (per 100,000) in children under 15 years in 2021. [20]

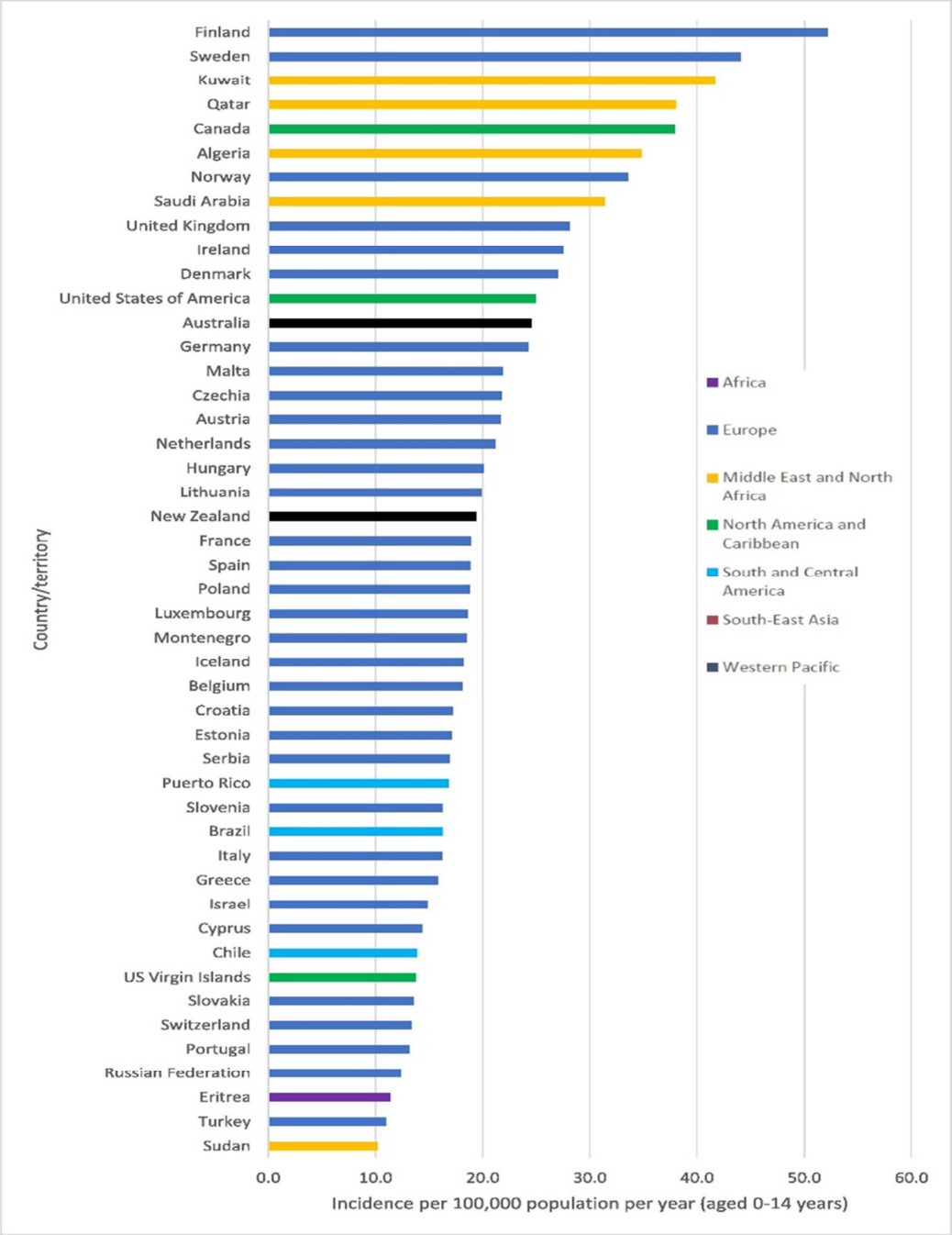


Fig. 2. Incidence of T1DM in children aged less than 14 years in different countries [21]

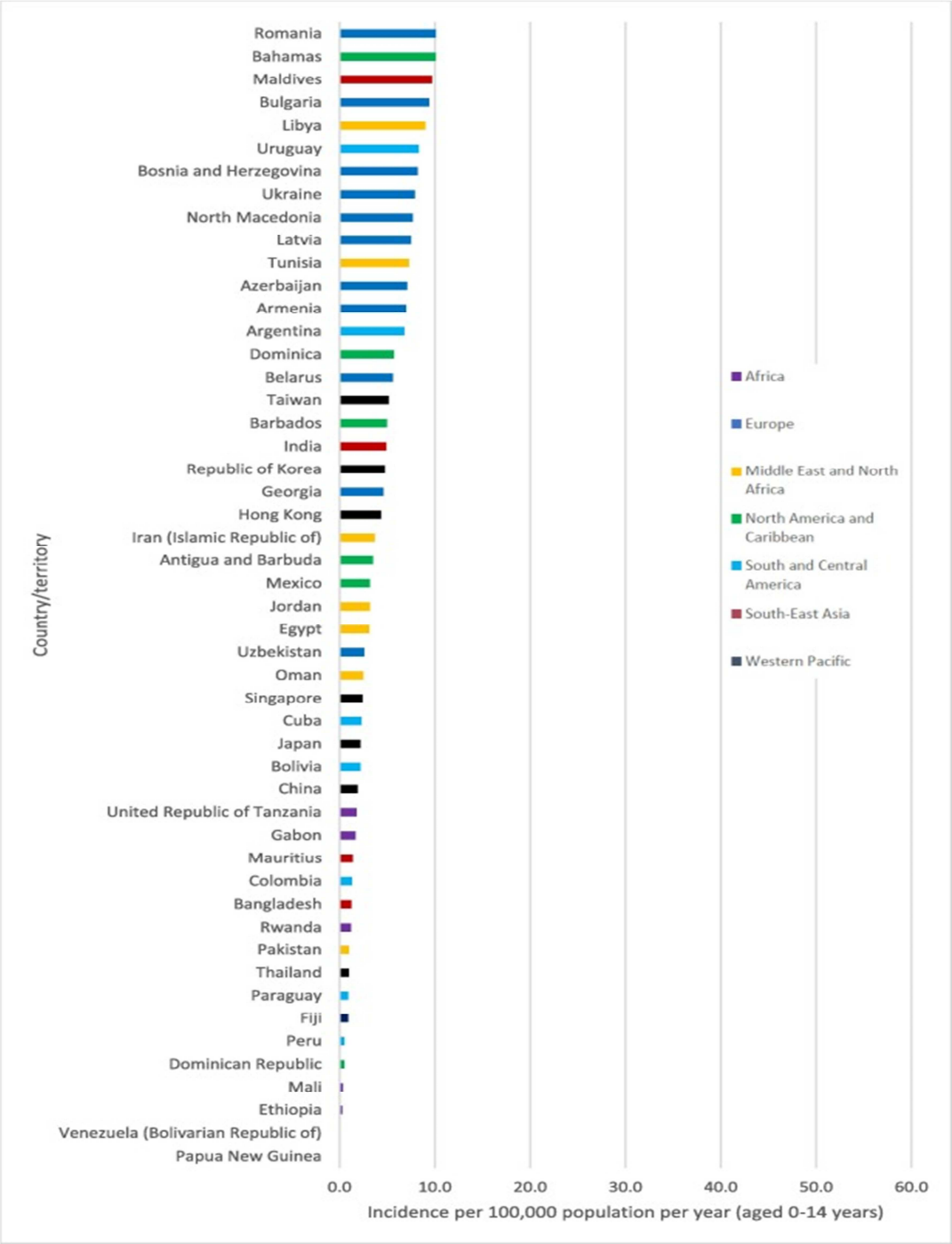


Fig. 2 (continued). Incidence of T1DM in children aged less than 14 years in different countries [21]

Table 2. Estimated incident cases. [21]

IDF Region	Number of countries with incidence rates available (%)	Incident cases per annum (1000 s)	
		0–14 years	0–19 years
AFR	6/48 (12.5)	7.7	19.7
EUR	44/59 (74.6)	24.7	31.0
MENA	12/21 (57.1)	18.1	25.0
NAC	8/23 (34.8)	18.7	24.4
SACA	12/19 (63.2)	9.5	12.3
SEA	4/7 (57.1)	20.5	25.7
WP	11/38 (29)	0.1	11.6
World	**97/215 (45.1)**	**108.3**	**149.5**

IDF - International Diabetes Federation;
AFR - Africa;
EUR - Europe; MENA - Middle East and North Africa,
NAC - North America and Caribbean;
SACA - South and Central America;
SEA - South-East Asia; WP - Western Pacific

Table 3. The ten countries/territories with the highest a) published age-standardized incidence of T1DM reported in children less than 14 years; and b) estimated number of incident cases of T1DM in children less than 14 years. [21]

Highest age-standardized incidence					Highest incident cases (2021)			
Rank	**Country/territory**	**IDF Region**	**Incidence (per 100,000 per annum)**		**Rank**	**Country/territory**	**IDF Region**	**Incident cases**
1	Finland	EUR	52.2		1	India	SEA	19,194
2	Sweden	EUR	44.1		2	United States of America	NAC	15,288
3	Kuwait	MENA	41.7		3	Brazil	SACA	7,117
4	Qatar	MENA	38.1		4	China	WP	4,900
5	Canada	NAC	37.9		5	Algeria	MENA	4,874
6	Algeria	MENA	34.8		6	Russian Federation	EUR	3,345
7	Norway	EUR	33.6		7	Germany	EUR	2,845
8	Saudi Arabia	MENA	31.4		8	United Kingdom	EUR	2,713
9	United Kingdom	EUR	28.1		9	Saudi Arabia	MENA	2,680
10	Ireland	EUR	27.5		10	Canada	NAC	2,274

Of the total cases in the world, the South-East Asian region accounts for about 23% of T1DM children, with India accounting for the majority of this proportion [21]. First population based study of prevalence of T1DM was done in South India in 1992, which documented the prevalence to be 0.26/1000 children, with peak age of diagnosis of T1DM at 12 years of age [22]. This prevalence was higher that the prevalence of T1DM reported from other Asian countries [22]. According to ICMR-YRD registry from Delhi and Chennai, the incidence of T1DM is 4.9cases/100000/year in India [23]. Whereas the SEARCH study from USA reported 21.2 cases/100,000/year [23]. However, India has a huge population of more than 1.4 billion and in that 40% are below 20 years of age. Hence, even when the incidence rate is comparatively less, the total number of cases is more.

The incidence varies largely among different regions in India. The Karnataka state T1DM registry over 13 years of study from 1995-2008, has reported the incidence of T1DM to be 3.7/100,000 in boys and 4.0/100,000 in girls [24].

In Karnal district of Haryana, the highest prevalence of 10.2/100,000 was documented with prevalence of 26.6/100,000 and 4.27/100,000 in urban and rural areas respectively in the year 2008 [25].

Under the National Programme for prevention and control of Cancer, Diabetes, Cardiovascular diseases and Stroke program of the Ministry of Health and Family Welfare, India, a study was conducted on 92,047 school children in three cities of three different states. Of this, 1,351 (1.467%) were suspected to be diabetic. An estimated 18,000 children under the age of 15 were newly diagnosed for T1DM in the year 2011 in these regions [26].

ETIOLOGY OF TYPE 1 DIABETES MELLITUS

The etiology of T1DM is an amalgam of genetic predisposition, environmental trigger and a dysregulated immune response which leads to beta-cell destruction and deficiency of insulin, thus leading to T1DM (Fig. 3) [27]. The genetic susceptibility of type 1 diabetes is attributed to HLA class II genes and other non-HLA genes like PTPN22, CTLA-4 and IL2RA [28]. Environmental factors like viral infections, early infant gluten exposure, toxins and psychological stress are hypothesized to trigger immune response.

The chronic and complex interaction between the genes, multiples environmental factors that lead to alteration in the immune mechanisms leading to development of autoantibodies which further cause immunological damage to beta-cells of pancreas.

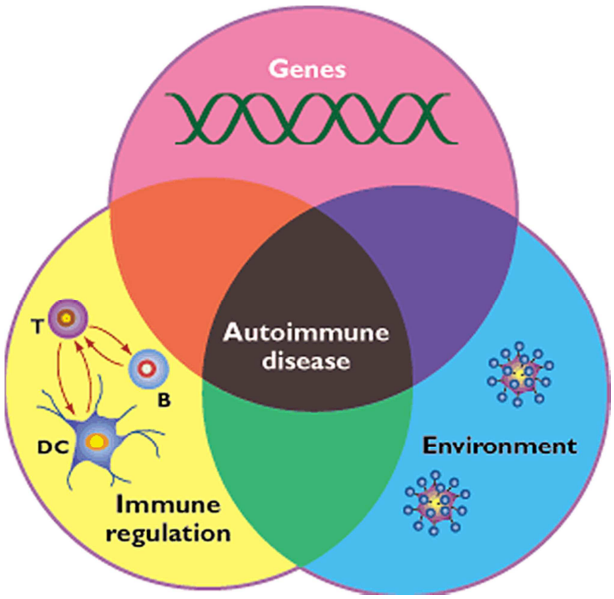


Fig. 3. Multifactorial causes in development of autoimmune disease [27]

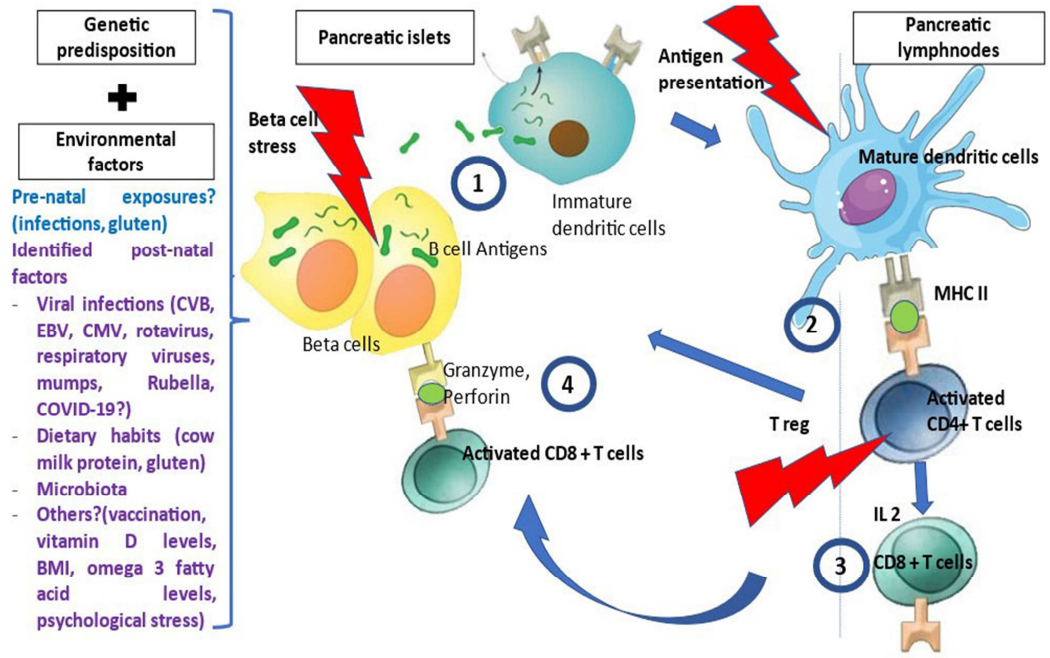


Fig. 4: Etiological triad of T1DM – Genetic predisposition, Environmental trigger and Autoimmunity [29]

GENETICS

Human Leucocyte Antigen

Individuals with HLA genotypes susceptible to T1DM are at a 30-50% risk of developing the disease. The genes of the greatest importance are: DRB1, DQA1 and DQB1. The highest risk is seen in those with *DRB1*03:01-DQA1*05:01-DQB1*02:01* and *DRB1*04-DQA1*03:01-DQB1*03:02* haplotypes [30]. Whereas the haplotypes *DRB1*15:01-DQA1*01:02-DQB1*06:02,* DRB1*14:01-DQA1*01:01-DQB*05:03 and DRB1*07:01-DQA1*02:01-DQB1*03:03 are protective against T1DM [30]. Also there is a wide variation of the HLA haplotypes seen in different regions (Fig. 5) [32].

In India, HLA association with T1DM is found commonly with DR3 haplotype. T1DM data from Orissa has shown significant increase in prevalence of DRB1*03, DQA1*501 and DQB1*0201 in T1DM [31].

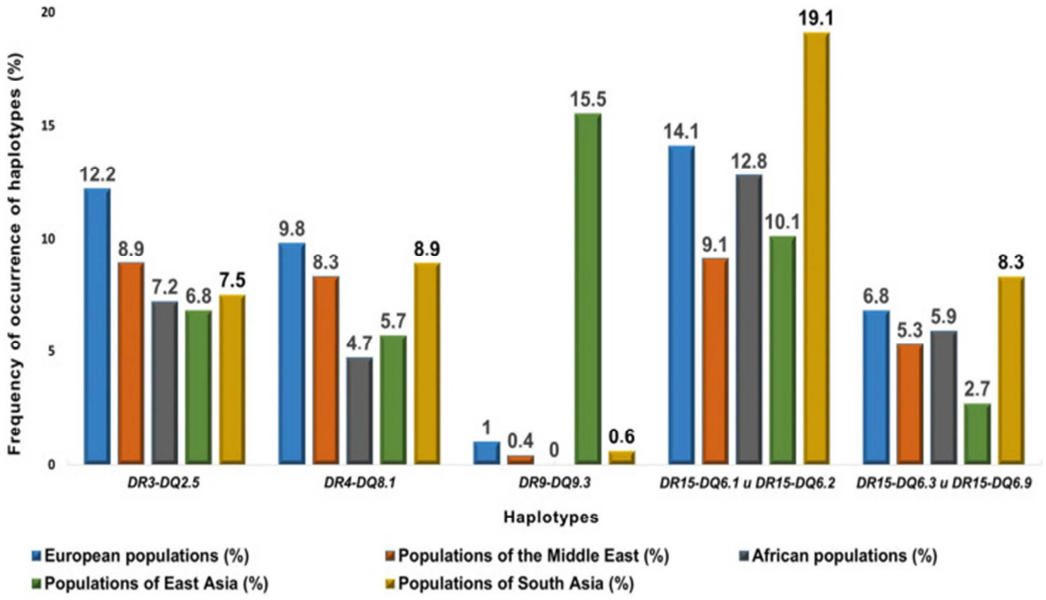


Fig. 5 Prevalence of five known *HLA-DR-DQ* haplotype groups, involved in susceptibility/resistance to T1DM, in five different regions of the world. [32]

Non- HLA Genes

There are many non-HLA genes that are identified to be associated with T1DM (Table 1) [33]. GWAS study has reported more than 50 non-HLA genetic loci which may predispose an individual to T1DM [34]. These genes are involved in progression of the disease that development of disease. Some of them are noted to be associated with beta-cell autoantibodies. Of these PTPN22, CTLA-4, IL2RA and insulin gene are of importance [28]. Fig.6 shows the various genetic loci in decreasing order associated with T1DM [28].

PTPN22 is involved in functioning of T-cell signaling pathway. In T1DM it involved in initiation of development of islet autoantibodies. CTLA-4 is involved in T-cell mediated autoimmunity in T1DM. IL2RA is involved in T-regulatory function. Shorter VNTR region near the 5 prime end of the INS gene is associated with increased risk of development of insulin autoantibodies. [35]

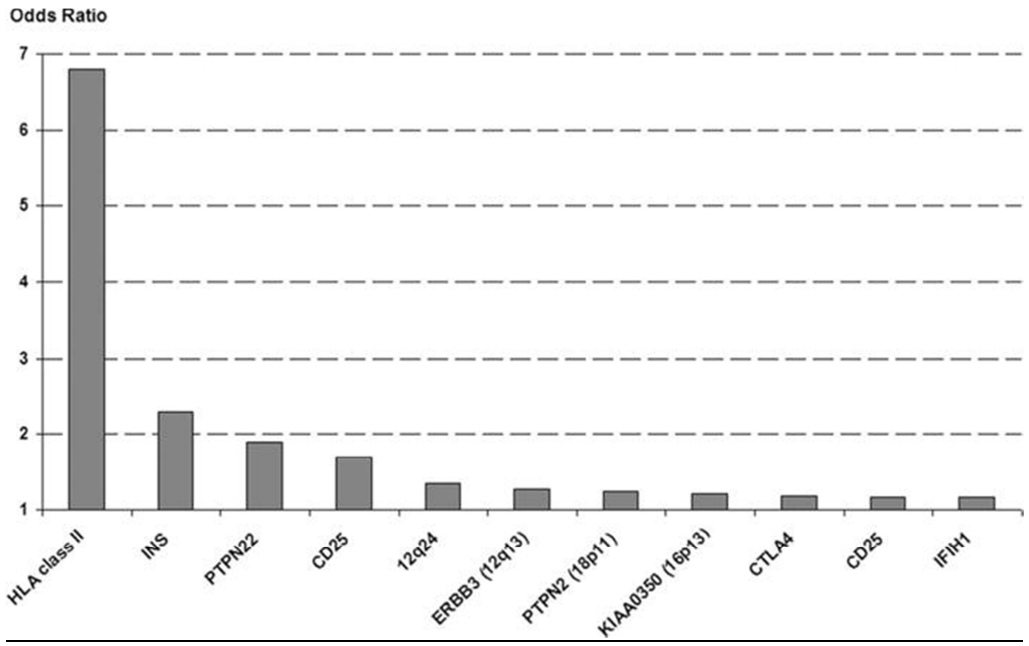


Fig. 6. Genetic loci associated with T1D [28]

Table 4: Genes connected to T1D pathology and their influence on β-cell and/or immune cell functions. [33]

Gene	Gene Function(s)
BACH2	Regulating proinflammatory cytokine-induced apoptotic pathways in pancreatic β-cells (crosstalk with PTPN2)
C1QTNF6	Participating in the BCR signalling pathway/cytotoxicity
CCR5	T_h cell development/chemokine-induced signaling
CD226	Modulating thymic T cell selection Impact on peripheral memory/effector $CD8^+$ T cell activation and function Reducing regulatory functions of $Foxp3^+$ Tregs
CD69	Participating in early lymphocyte activation, Limiting the inflammatory response Influencing the signalling of NK cells
CLEC16A	Regulating the mitophagy for mitochondrial quality control Possible involvement in β-cell fragility
COL6A6	
CTLA4	Controlling the proliferation of Tregs in the periphery Regulating pancreas autoimmunity
CTSH	Regulating cytokines inside β-cells for proapoptotic signal transduction
ERBB3	Modulating antigen presentation, Modulating cytokine-induced β-cell apoptosis
GLIS3	Implication in the generation of β-cells, insulin expression Maintaining β-cell functions and mass, Exerting antiapoptotic effects
HIP14	Regulating β-cell apoptosis and insulin secretion
IFIH1	Mediating the innate immune system's interferon response to certain viruses Participating in β-cell response to viral dsRNA
IKZF1	Regulating immune cell development
IL2/IL21	Influencing $T_{(h)}$ cell differentiation and inflammatory response
IL27	Modulating T cell subsets and regulating inflammatory response
IL2RA	Variants causing abnormalities in sensitivity to IL2, which is critical to T-regulatory cell function Potential altering of the balance between Tregs and Teffs
IL7R	Involvement in antigen binding, Ig production, and cytotoxicity
MRPS21-PRPF3	
NRIR	Negative regulator of interferon response

Gene	Gene Function(s)
PRKCQ	Influencing T cell function/apoptosis/innate immune response
PTPN2	Inducing β-cell apoptosis after interaction with increased local levels of interferon, Influencing β-cell response to viral dsRNA
PTPN22	Participating in T cell receptor signalling pathway
SH2B3	Participating in growth factor and cytokine signaling
STX4	Associated with insulin secretion Downregulating the expression of chemokine genes associated with inflammation and the apoptosis of pancreatic islets Decreasing the translocation and activation of NF-kB, thus decreasing the apoptosis
TASP1	Cleaving the MLL protein, which is required for proper *HOX* gene expression
TNFAIP3	Downregulating the intrinsic apoptotic pathway, Regulating the expression levels of ZnT8, Essential for insulin production and secretion
TYK2	Regulating the effects of cytokines inside β-cells for proapoptotic signal transduction Mediating interferon response in connection to resistance to various infections Mediating Th1- and Th17-type immune reactions
UBASH3A	Downregulating the NF-kB signalling pathway upon T cell receptor stimulation, thus reducing the IL2 expression

The susceptible HLA haplotype along with mutations in a number of other regulatory genes involved in beta-cell functioning, play an important role in pathogenesis and progression of T1DM. The gene-gene and gene–environment interactions by epigenetic modifications or virus-mediated interaction is thought to induce changes in pancreatic beta-cell functioning, predisposing the individual to T1DM.

RISK OF DEVELOPMENT OF DISEASE

The prevalence of T1DM is about 0.3% in the general population. It increases to about 5% in the family with T1DM in the first degree relatives [36] and to about 20% in extended family. The risk of developing T1DM under 20 years of age is 1:300 in families with no genetic predisposition, 1:50 those with T1DM mother, and 1:15 with T1DM father [37]. The twin with identical twin having T1DM has a 50% higher risk of developing T1DM as compared to a fraternal twin. The risk of disease development in a sibling is 4% and 9.6% at the age of 20 years and 60 years of age respectively [36]. Also there is a higher risk associated with individuals whose siblings were diagnosed at younger age, those parents who had young-onset diabetes, male sex and those born to elderly parents.

Table 5. Risk of developing T1DM for individuals who have an affected relative [38]

SIBLING	***RISK***	***OFFSPRING***	***RISK***
Overall	6%	Overall	5%
Identical twin	<50%	Father with T1DM	6%
HLA identical	15%	Mother with T1DM	2%
HLA haploidentical	6%		
HLA nonidentical	1%		

ENVIRONMENT AND T1DM

In the past decade an increase in the incidence of T1DM has been noted worldwide. This can be correlated with the changing lifestyle and surrounding environment. The environment plays an important role in either protecting or triggering the autoimmune response and also in progression of T1DM (Fig. 7) [39]. Viral infections, early exposure to cow milk protein and gluten, toxins, altered gut microbiome, psychological stress are postulated to be involved in the pathogenesis of T1DM by triggering the immune system. Whereas Vitamin D, omega-3 fatty acid, exclusive breastfeeding, zinc, nicotinamide, and vitamins C and E have shown to be protective against the development of autoimmune reaction.

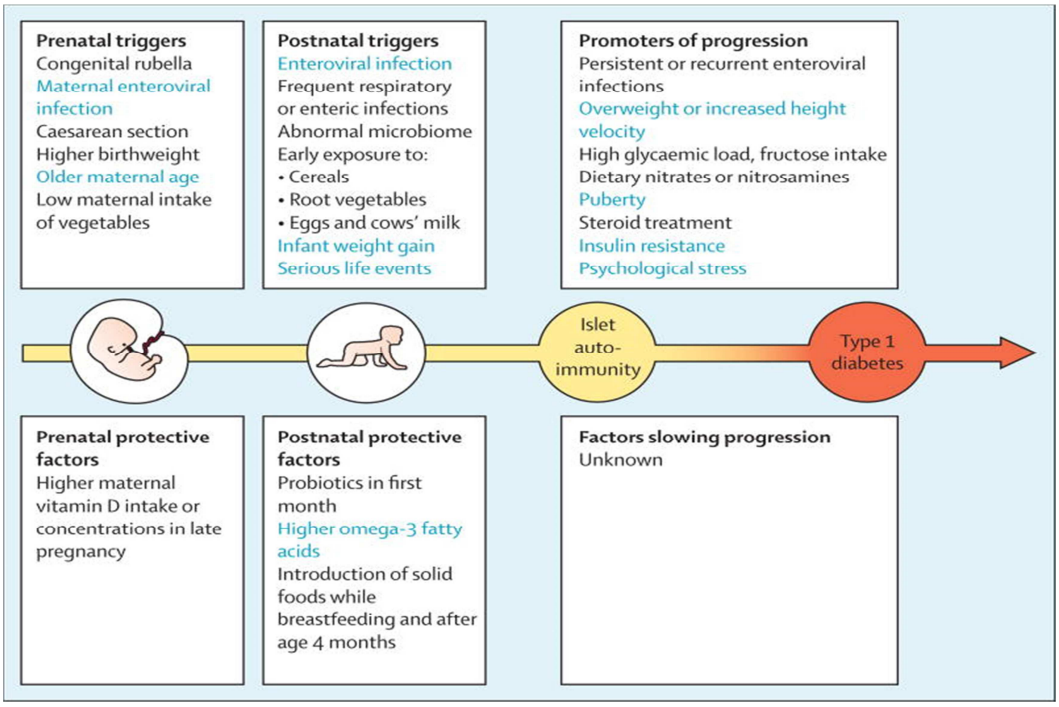


Fig 7. Environmental triggers and protective factors in development of T1DM [39]

Environmental role in initiating pathogenesis of T1DM is evidenced by the seasonal variation in incidence of T1DM. E V Moltchanova Et al. did a study on Seasonal variation of diagnosis of T1DM in children 0-14 years of age worldwide, by analyzing the data collected by the World Health Organization Diabetes Mondiale (WHO DiaMond) Project from 1990-1999 and concluded that there was a seasonality noted in occurrence of T1DM. [40]

Many studies have also shown that the region of residence has also an impact on development of T1DM. In a study done by Anita Kondrashova Et al. the incidence rate of T1DM in children less than 15 years, from the year 1990-1999 were compared between Russian and Finland population and it was seen that there is six-fold higher incidence of T1DM in Finland population as compared to Russian Karelia population who had similar HLA DQ genotypes [41]. Thus an environmental trigger might exists which makes the genetically predisposed population of Finland progress to T1DM.

Also it is seen that there is high incidence of developing T1DM in immigrants who immigrate to a region of high prevalence of T1DM. A study done in Sweden in 2006 by Ulf Söderström Et al. showed that there was an increased risk of T1DM in children born to Sweden immigrants from low incidence countries. The study concluded that the increase in incidence may be due to some environmental exposures in utero or very early in childhood. [42]

An increase in the incidence of T1DM in Polish children was noted in a study done by P. Jarosz-Chobot E. al. and it also postulated that there can be two fold higher incidence of from 2005 to 2025 [43]. This raising incidence can be linked to the environmental changes.

Infection:

There is plenty of evidence that infections triggers the development of T1DM. Although even bacteria can cause infection of pancreas and beta-cell damage, majority of the infectious etiology for development of T1DM is attributed to viral infections. Demonstration of Anti-viral antibodies [44] or viral elements in the beta cells of pancreas [45] has provided evidence for the hypothesis of viral infection triggered beta cell autoimmunity. Viral infections that predispose the beta cells to autoimmune process include congenital rubella infection, enterovirus, measles, mumps, chickenpox, coxsackievirus-B and echovirus.

Of these viruses, enteroviral infection and development of diabetes has been largely studied. Strong evidences of association are demonstrated by many studies. The Diabetes autoimmunity study done from 1993 to 2004, in Denver showed that those with serum enteroviral RNA positivity had high rate of progression from autoantibody positivity to T1DM [46]. In a critical review done on enterovirus link in T1DM, it concluded that some cross sectional studies show higher prevalence of enteroviral infection in newly detected T1DM subjects as compared to healthy population, but a strong evidence is absent and further longitudinal studies are required to be done [47]. The Finnish DIPP cohort study has also reported the appearance of islet autoantibodies following the seasonality of viral infection [48].

SARS-CoV-2 has also been associated with development of autoimmunity and progression of T1DM. This was evidenced by increase in the number of T1DM and DKA presentations post coronavirus pandemic. But there is a lacunae in the information as to whether there SARS-CoV-2 causes direct beta-cell damage or

triggers autoimmunity by molecular mimicry or contributes in progression of the disease in genetically susceptible individuals [49].

The autoantibodies formation may be due to molecular mimicry between the viral proteins and the beta-cell proteins. It is also postulated that the genetically susceptible individuals are associated with altered interferon responses and resultant cytokine storm, cellular infiltration and inflammatory response leading to pancreatic beta cell destruction. Hygiene hypothesis has also been implicated in development of T1DM [50].

Hygiene hypothesis: The increasing incidence of autoimmunity may be attributed to decreased infections in childhood due to improved hygiene. It is postulated that early exposure to pathogens enhances immune system and thus suppressing the autoimmune reaction [50]. Thus in children who have had no or less exposure to infectious organisms may have an altered immune response to infections later in life. This leads to development of various autoimmune diseases including T1DM.

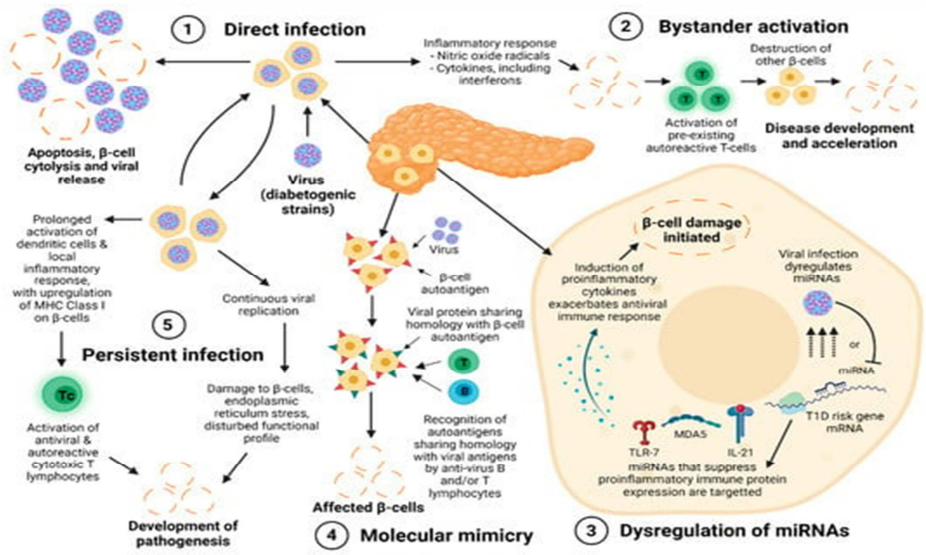


Fig 8. Mechanisms of initiation and acceleration to T1DM by viruses [51]

Dietary factors and gut microbiota:

Diet is an important factor in altering intestinal microbiome composition and it, in turn, exerts its influence on the immune system and immune response through the epigenetic regulation of immune cells. Lower or altered gut microbial environment has been seen in children with T1DM in some studies.

In the study done by Marcus C. de Goffau, et al. on fecal microbiota in children with and without islet autoimmunity, from March 2009 to February 2010 showed that low lactate-producing and butyrate-producing species in the gut was associated with beta-cell autoimmunity [52]. Metagenomic analysis on gut microbiome by Christopher T. Brown, Et al. concluded that in healthy individuals the lactate and butyrate producing bacteria are present which produce mucin and maintain the integrity of the gut. Whereas in the individuals with autoimmunity, non-butyrate-producing and lactate-utilizing bacteria are present which prevent mucin synthesis [53]. Exposure to gluten before 3 months of age, early exposure to cow milk, egg and root vegetables have shown an increased risk for development of T1DM.

FINDIA study done in three hospitals of Finland from May 2002 to November 2005, reported that weaning to bovine insulin free formula milk reduced the cumulative incidence of islet autoantibodies in children with high genetic predisposition at 3 years age [54]. TRIGR study done from May 2002 to January 2007 in 78 centers from 15 different countries showed that the children who received casein hydrolysate formula feeds had decreased risk of autoimmunity as compared to those who were given cow milk [55].

Prospective birth cohort study done by Norris JM Et al. which included 1183 subjects from 1994 to 2002, showed that the timing of introduction of gluten and cereals was associated with a higher incidence of autoantibodies against beta-cells [56]. BABYDIAB cohort study conducted in Germany from 1989 to 2003 also showed increased risk of developing islet autoimmunity with exposure to gluten [57]. A prospective birth cohort study done from 1996 to 2004 by Virtanen SM Et al. concluded that early exposure to root vegetables had higher risk of beta-cell autoantibody among genetically predisposed Finnish children [58].

Vitamin D is known to play a role in immune regulation by inhibiting INF-γ and IL-17 production and regulating T-cell function. A meta-analysis showed decreased risk of development of T1DM in the infants who were supplemented with calcitriol [59]. Whereas in the DAISY study done in Denver, USA, there was no association of Vitamin D levels in infants and children with islet autoimmunity or progression to T1DM [60].

Diet rich in omega-3-fatty acid may be protective in development of T1DM. In a done by Norris JM Et al. as a part of DAISY study, higher omega-3 fatty acid intake had negative correlation with development of T1DM [61]. Where as in India, the consumption of omega-3-fatty acid is very less, but the incidence of T1DM is comparatively lower than those countries with higher omega-3-fatty acid intake.

Chemicals and toxins are attributed to cause direct damage to pancreas and beta-cells. N-nitroso compounds present in processed food is seen to be associated with T1DM in population based study in Sweden [62].

Vaccine:

Bacillus Calmette-Guerin (BCG) vaccine was thought to cause immune modulation and prevent T1DM. But no studies have confirmed this hypothesis. A randomized controlled trial was done by Elliott JF Et al. to see if BCG vaccine can slow down the beta-cell destruction by giving BCG vaccine to T1DM children at diagnosis. But there was no positive outcome that was noted [63]. A meta-analysis of 23 observational including 16 different vaccinations done by Morgan E, Et al. showed no association between vaccines and T1DM [64].

Others:

Low birth weight and later rapid weight gain is also seen to be associated with T1DM. This is based on the accelerator hypothesis [65].

Accelerator hypothesis: Excess weight gain and obesity causes insulin resistance leading to stress on beta cells to secrete more insulin. This leads to beta-cell destruction and T1DM [65].

Beta cell stress hypothesis: Increased insulin production results in beta-cell stress and stimulation of the autoimmune process and leads to overt diabetes [66]. The increased insulin demand during rapid growth in infancy, overweight, puberty, low physical activity, trauma, infections, and glucose overload has direct impact on development of T1DM.

Stressful life events like financial crisis, divorce or death are also shown to increase the risk of T1DM. Early exposure to broad-spectrum antibiotics is positively correlated with an increased risk for developing T1DM by altering the gut microbiota and dysregulation of immune system.

AUTO-ANTIBODIES RELATED TO TYPE 1 DIABETES MELLITUS

The hallmark of autoimmune T1DM is the presence of one or more of the autoantibodies against the proteins of beta-cells. Islet cell antibody was first discovered by Bottazzo in 1974 [67]. Later in the year 1982, autoantibodies against an islet protein with a molecular weight of 64,000 (64 kDa antibody) was discovered by Baekkeskov and coworkers [68]. Further in 1983, Palmer reported insulin autoantibody in newly diagnosed T1DM individuals [69]. Fig. 9 shows the chronology of discovery of autoantibodies [70].

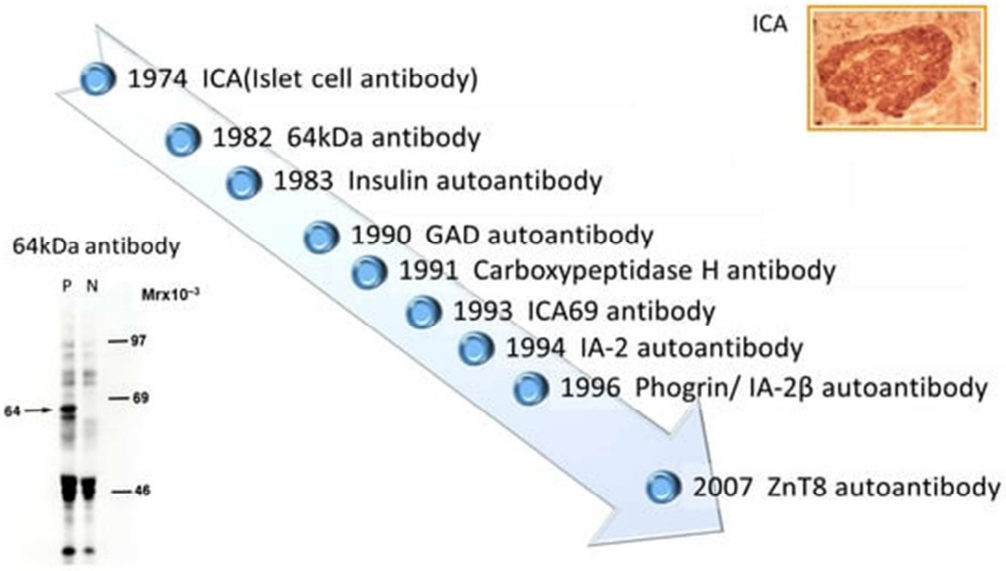


Fig. 9. Chronology of anti-islet autoantibody discovery [70].

The autoantibodies are formed as a result of molecular mimicry due to viral infection, and they attack the beta-cells leading to inflammation and destruction of beta-cells in the islets and thereby resulting in reduced production of insulin.

More than 10 autoantibodies against various proteins of the beta-cells are identified till date (Table 6) [71]. Of these the most important antibodies are formed against the islet cell antigens – GAD-65, IA-2, ZnT8. Antibody is also identified to be formed against the Insulin. The number of different antibodies present rather than the titer of the antibody level predicts the disease course. More the number autoantibodies, greater the risk of development of T1DM and faster is the progression to overt diabetes state.

TEDDY study [70] on hierarchical order of distinct autoantibody spreading and progression to T1DM, it was concluded that the first appearing antibody depends on the environmental trigger and age. The second appearing autoantibody has genetic association and leads to variation in the progression to T1DM. More the duration between first and second appearing antibody, less is the risk of progression to disease. Those who have multiple autoantibodies are at a greater risk of progression to T1DM [72]. The time of appearance of second antibody also depended on the first antibody (Fig.10) [70]. The time of progression to T1DM also depend on the second appearing antibody (Fig. 11) [70].

Table 6. Localization and function of autoantigens against anti-islet autoantibodies [71].

Antigen	Localization	Function
Insulin	Insulin secretory granules	Regulate glucose levels in the blood and induce glucose storage in the liver, muscles, and adipose tissue
GAD65	Synaptic-like vesicles in the cytoplasm of β-cells	Rate-limiting enzyme engaged in the synthesis of the neurotransmitter γ-aminobutyric acid from L-glutamate
GAD67	Cytosol of β-cells	Rate-limiting enzyme engaged in the synthesis of the neurotransmitter γ-aminobutyric acid from L-glutamate
IA-2	Insulin secretory granule membrane	Regulate insulin secretory granule content and β-cell growth
Phogrin/IA-2β	Insulin secretory granule membrane	Regulate insulin secretory granule content and β-cell growth
Carboxypeptidase H	Insulin secretory granules and granule membrane	Convert proinsulin into insulin and C-peptide by catalyzing the release of C-terminal arginine or lysine residues from polypeptides
ICA69	Insulin secretory granule membrane	Dense-core vesicles signaling and maturation
ZnT8	Insulin secretory granule membrane	Transport zinc ion from the cytosol into the insulin secretory granules
GM2-1 ganglioside	Secretory granules in β-cells and non-β-cells	Unknown
Heat shock protein 60	Insulin secretory granules	Assist correct folding of partially folded polypeptides and presentation of antigen to MHC molecules
GLUT2	β-cell surface membrane	Uptake glucose from the blood into β-cells
Tetraspanin-7	Insulin secretory granule membrane	Regulate Ca^{2+}-dependent insulin exocytosis
ICA12/SOX13	Cytoplasm and nucleus in β-cells and non-β-cells	Transcription factor (Function in the islets is unknown)

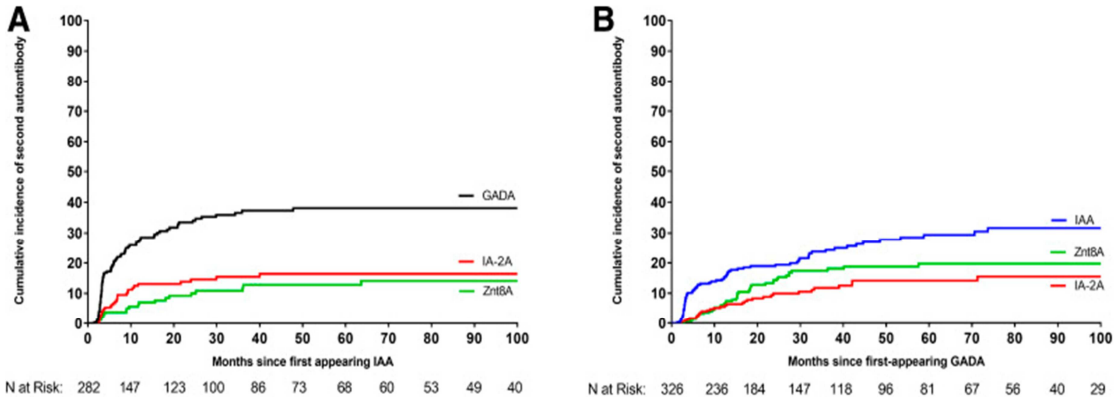


Fig. 10 Incidence of progression from single to second-appearing autoantibody positivity [70].

A: Cumulative incidence of the second-appearing autoantibody by months since first-appearing IAA.

B: Cumulative incidence of the second-appearing autoantibody by months since first-appearing GADA.

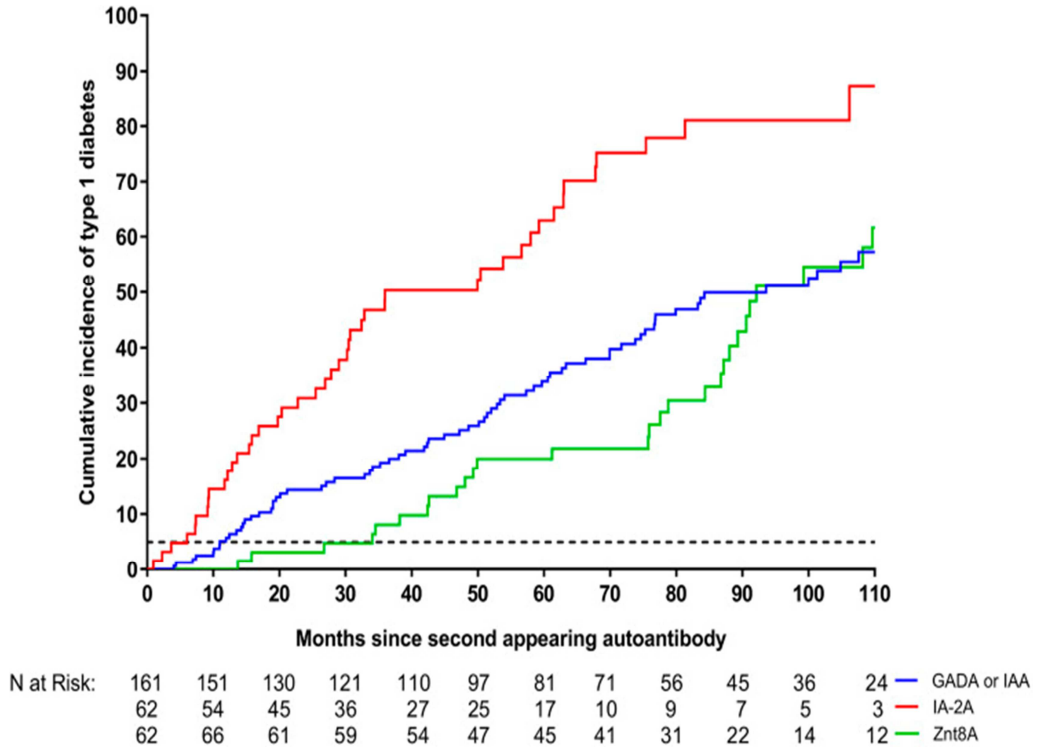


Fig. 11 Cumulative incidence with a distinct single second-appearing autoantibody for progression to T1D [70]

In a study done by Sosenko JM, et al. [74] which involved the TrialNet Natural History Study cohort showed a strong association between composite of the levels of the five autoantibodies and progression to T1DM. It concluded that multiple autoantibodies testing in at risk relatives of T1DM individuals is helpful in prediction of T1DM.

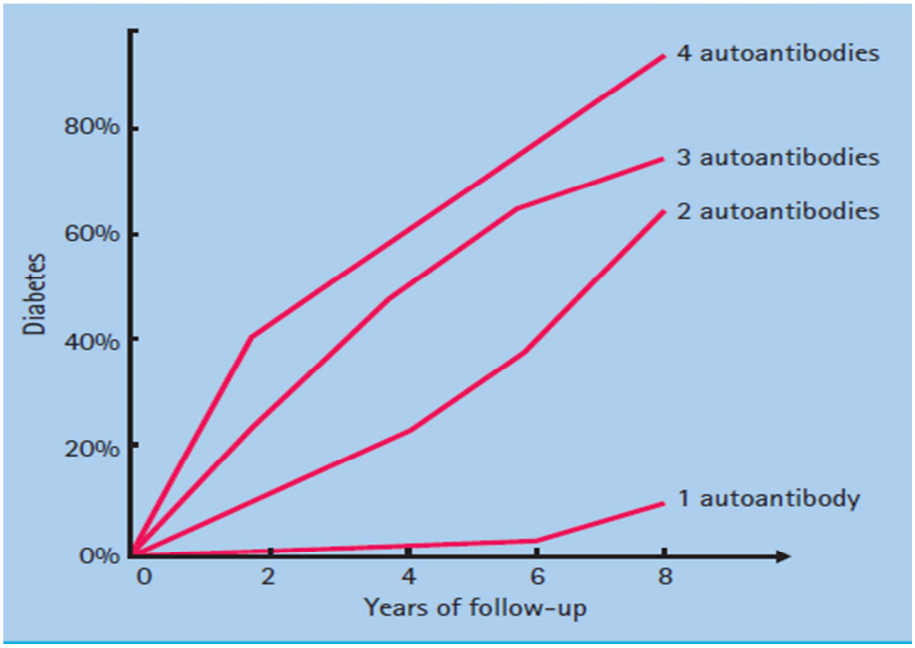
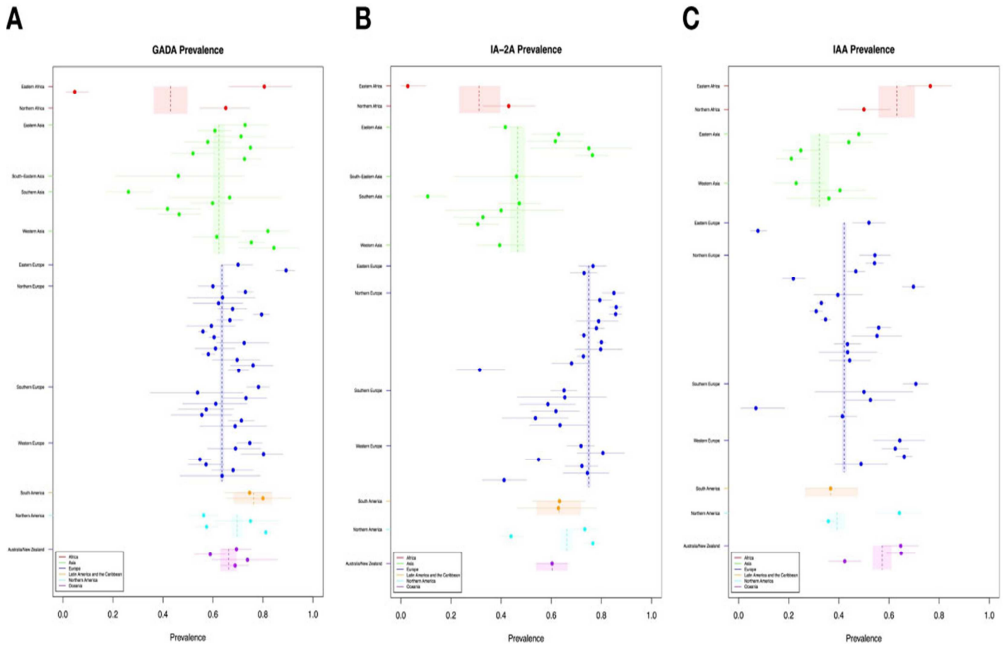


Fig. 12 Effect of multiple autoantibodies on risk of developing T1DM [73]

In a systemic review on global prevalence of autoantibodies in T1DM children and adolescents done by Carlo Ross et al. [74] analyzed the data on autoantibody prevalence which included 125 studies (from year 1990 to 2021) of 139 different population groups from 48 different countries across the world. There was wide variation in the autoantibody profile among different continents and regions. According to the global review, the most prevalent autoantibody in newly diagnosed T1DM children is IA-2A followed by ICA, ZnT8A, GADA-65 and IAA in the decreasing order of prevalence [74]. Fig. 13 shows the different antibody prevalence in

newly diagnosed T1DM children from different regions of the world [74]. However in children with established T1DM, GADA-65 was the most prevalent followed by ZnT8A, IA-2A, IAA and ICA. Fig. 14 shows the different autoantibody prevalence in established T1DM children from different regions of the world [74]. This systematic review also showed that the prevalence of autoantibodies in new onset as well as in established T1DM children was lower in Asian population as compared to that of other continents (Fig.15) [74].

A cross-sectional study done by Vipin VP et al. in India reported about 30% of T1DM children to be having no autoantibodies [75]. A study done in Barbada Davis Center, Colorado from October 1992 to October 2004also reported 19% of autoantibody negativity in newly detected T1DM children and the autoantibody negativity was more with increasing age [76].



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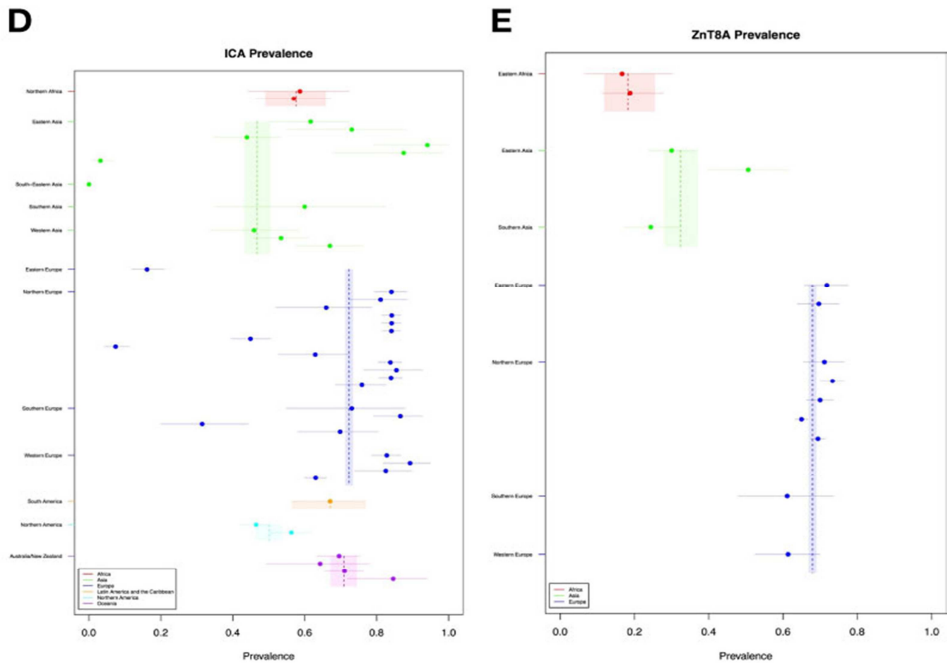
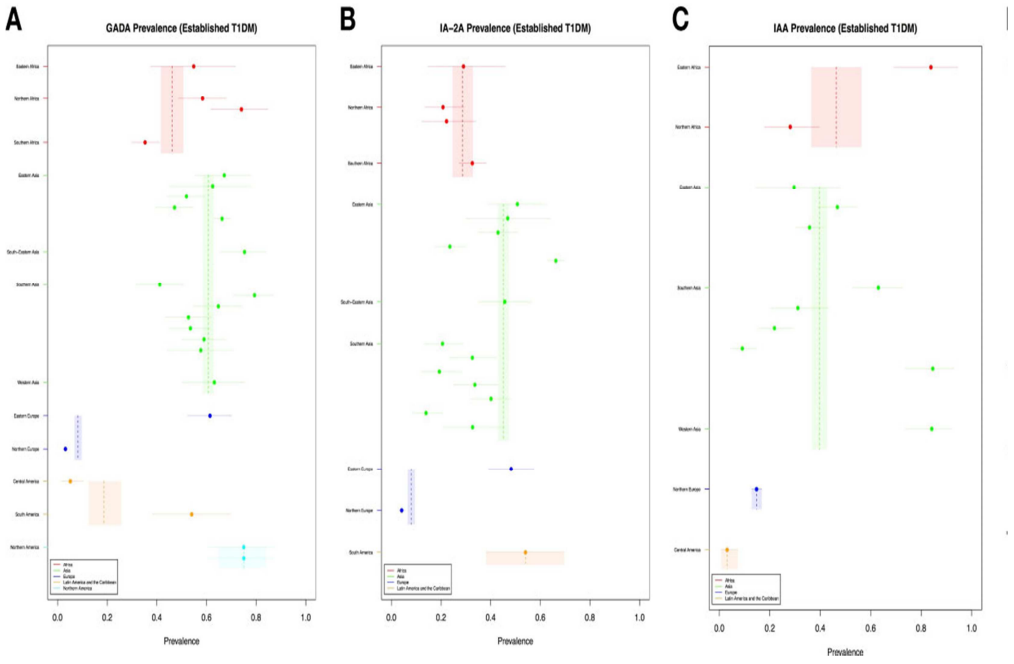


Fig. 13 Autoantibody prevalence in new onset T1DM children in different regions of the world [74]



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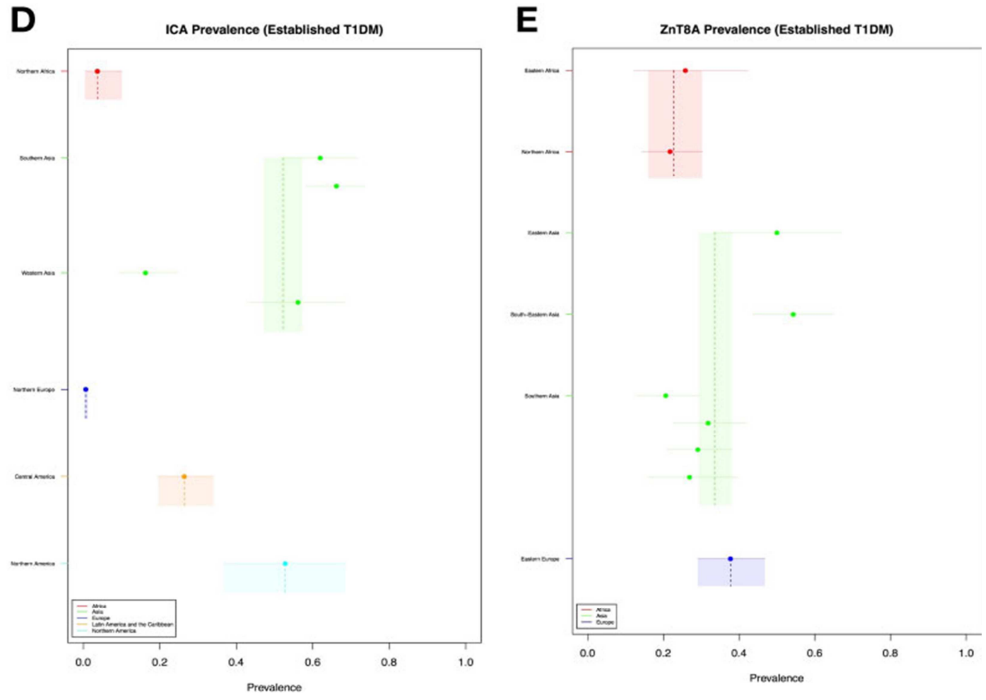


Fig. 14 Autoantibody prevalence in established T1DM children in different regions of the world [74]

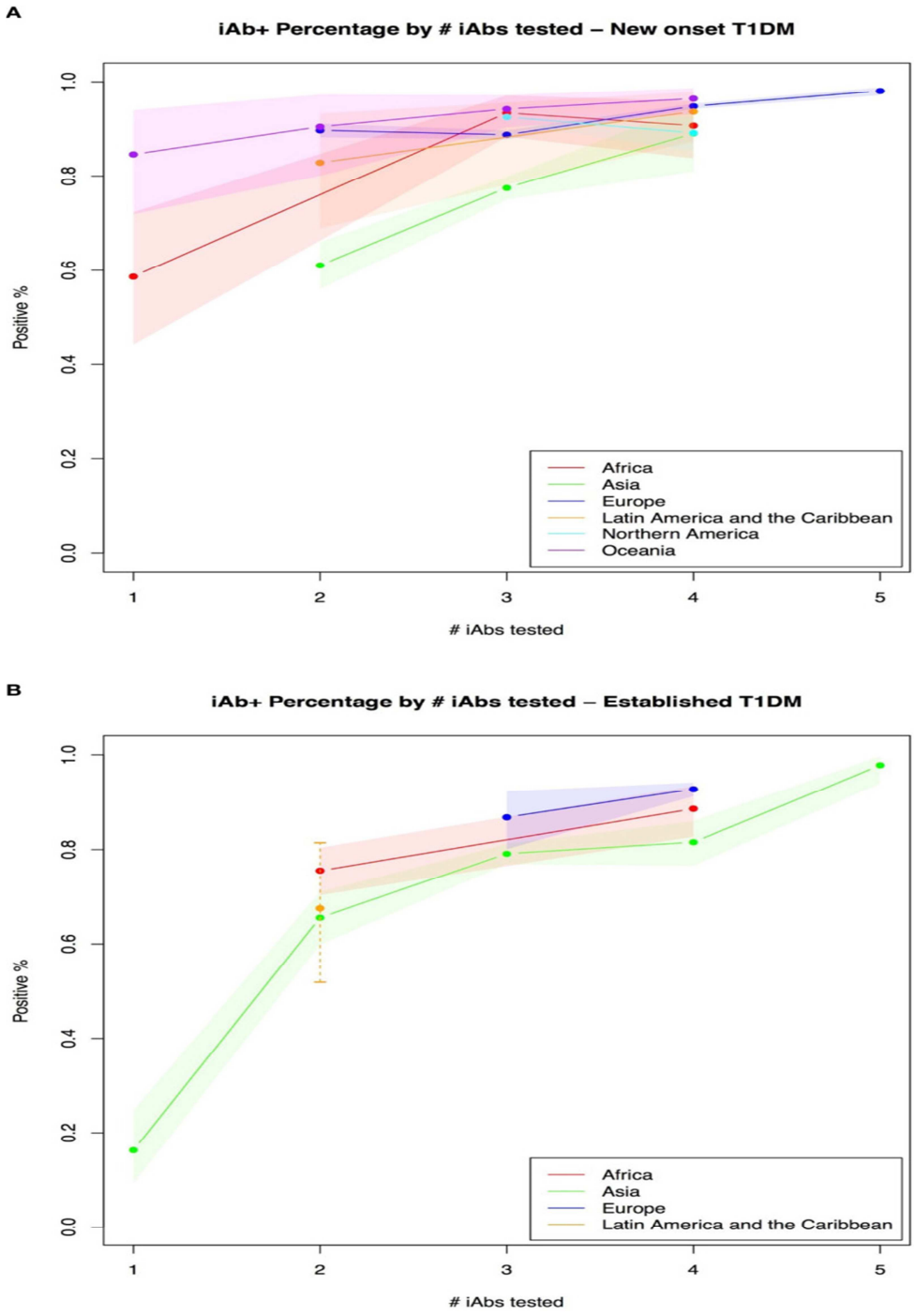


Fig. 15 A. Autoantibody positivity in new onset T1DM children in different regions.

B. Autoantibody positivity in established T1DM children in different regions. [74]

The major antigens – GAD65, ZnT8 and IA-2 are present inside the beta-cells in the insulin granule or the secretory granule as show in the Fig. 16 [77]. These antigens may get exposed to the immune system due to beta-cell damage caused by viral infections or toxins. It is also postulated that there is some molecular mimicry between the antigens of the virus and these proteins in the beat-cells which leads to cross-reactivity and beta-cell destruction.

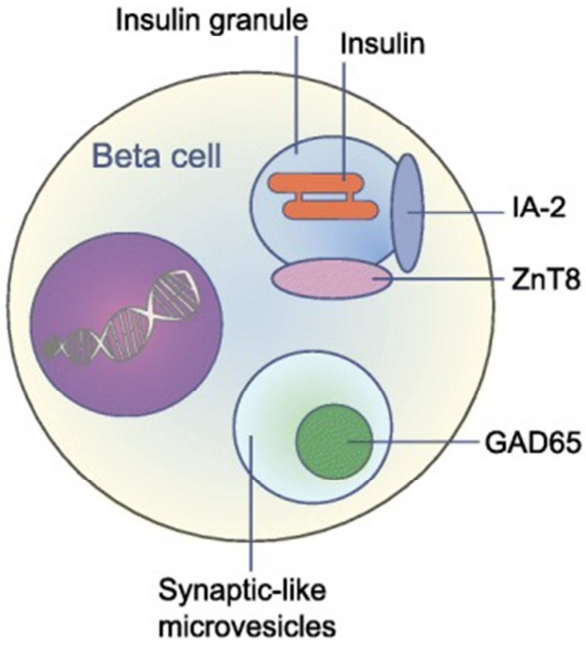


Fig. 16 Location of the major antigens against which antibodies are formed in T1DM [77].

GAD-65 AUTOANTIBODY

Glutamic acid decarboxylase (GAD)

Glutamic acid decarboxylase is present in 'GABA-ergic' nerve cells. Apart from the nervous system, GAD is also found in spermatozoa, epithelium of fallopian tube and in beta-cells of pancreas. In the beta-cells, it is present in synaptic-like vesicles. But the exact function of GAD in beta-cells is not known. Two isoforms of GAD based on their molecular weight are known – GAD65 & GAD67. 65kDa isoform is present in the beta-cells of pancreas. The protein is synthesized from GAD65 gene located on chromosome 10p11.23 [78]. 64kDa protein was first discovered in 1982. The schematic representation of GAD is shown in Fig. 12 [79]. The middle and the carboxyl regions are the sites of antibody recognition.

There is similarity found between GAD protein and Coxsackie B4 virus [79] (Fig. 17). Due to this similarity, post viral infection there is a molecular mimicry resulting in autoimmunity against the GAD-65 protein leading to beta-cell destruction.

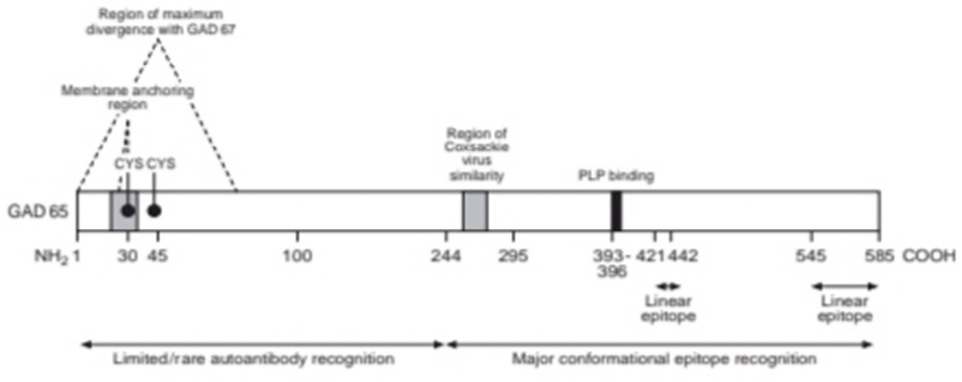


Fig. 17 Schematic representation of GAD-65 antigen showing region of Coxsackie virus similarity [79].

GAD-65 antibodies (GADA)

Antibody against 64kDa was discovered in 1984. Later it was found that 64kDa was GAD-64 by Baekkeskov in 1990 [80]. The presence and frequency of GAD-65 antibody detection in T1DM varies among different regions. Many studies have shown a wide variation from 50-80% in presence of Anti-GAD65 in T1DM. Also GAD-65 antibodies are seen in 2% of the general population. Autoantibodies to the GAD65 antigen is associated with HLA-DR3-DQ2. Studies have shown DQA1*03-DQB1*0303 HLA haplotype is commonly found in individuals recognizing central and middle epitopes of the GAD65 antigen [79].

GAD-65 autoantibodies formed in T1DM recognize the GAD65 proteins in beta-cells and initiate an immunological reaction. Also GAD-specific CD4-positive T cells and CD8-positive cytotoxic T-cells have been noted in recently diagnosed T1DM individuals. Thus GAD-65 antigen is involved in immune mediated destruction of beta-cells [81].

***Subclass*:**

During the early prediabetic phase, IgG1 subclass is the dominant type of GADA. Antibodies of subclasses IgG2 and IgG3 often appear together with IgG1 or soon after the initial IgG1 response, while IgG4 is the last subclass to appear [82].

IA-2 AUTOANTIBODY

Insulinoma associated antigen 2 (IA-2)

Insulinoma associated antigen-2 is also known as protein tyrosine phosphatase-2 [79]. It is encoded by the gene located on chromosome 7q36 [79]. IA-2 belongs to protein tyrosine phosphatase enzyme family. IA-2 is primarily found in neuroendocrine cells and is also seen to be found in the secretory granules of beta-cells of pancreas. IA-2 protein is believed to function as regulator of insulin granules, beta-cell growth and proliferation. The structure of IA-2 protein has 979 amino acids and consists of three domains – extracellular, transmembrane and intracellular (Fig. 18) [79]. The antibody reacts with the carboxyl terminal which is from 771 to 979 amino acids of intracellular part and amino terminal which is from 604 to 776 amino acids of the intracellular part of IA-2 [79].

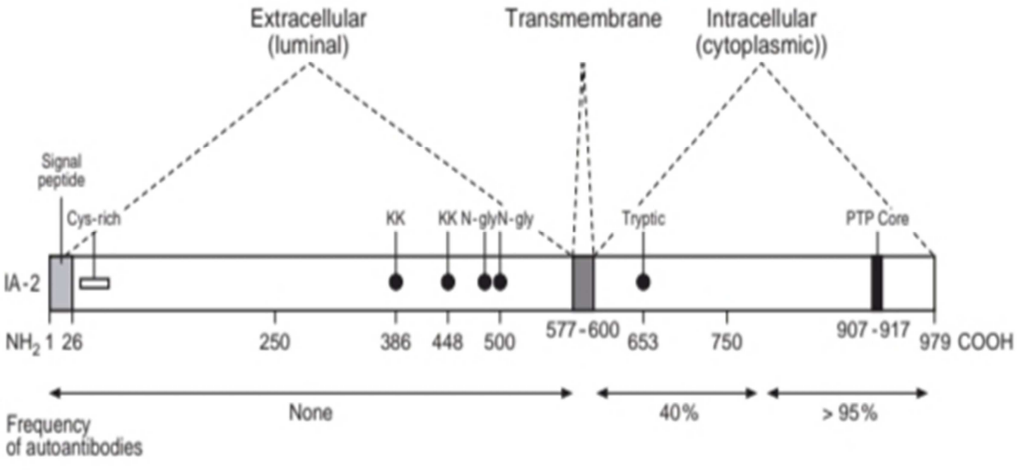


Fig. 18 Schematic representation of IA-2 protein [79].

IA-2 Antibody (IA-2A)

Autoantibodies against IA-2 protein were first detected by Payton and Hawkes in 1995 [83]. IA-2A depends on age and HLA genotype. In is seen at younger age and those with HLA DR4 and DQA1*0301-DQB1*0302 genotype [84]. IA-2A seems is be a strong predictor for development of T1DM. Those with IA-2A positivity will progress to T1DM in the near future. After IA-2 antibodies are present in the sera for only few years and tend to disappear soon after diagnosis of overt T1DM.

Subclasses:

IgG1 subclass is the predominant antibody in pre-diabetic stage and in newly diagnosed T1DM. High risk of progression to T1DM is associated with IgG2, IgG3 and/or IgG4 subclasses.

ZINC TRANSPORTER 8 AUTOANTIBODY

Zinc Transporter 8 (ZnT8)

ZnT8 is a protein highly specific to beta-cells of pancreas. ZnT8 is encoded by SLC30A8 gene present on chromosome 8q24.11 [85] and belongs to zinc transporter family. ZnT8 is a multispanning transmembrane protein present in the insulin secretory granules of beta-cells of pancreas and its function is to supply zinc ions for insulin synthesis and storage. It is a 369 amino acid protein with N-terminal and C-terminal ends in the cytoplasm of the cell (Fig. 19) [77].The antibody is against the N- and C-terminal ends of ZnT8 [86].

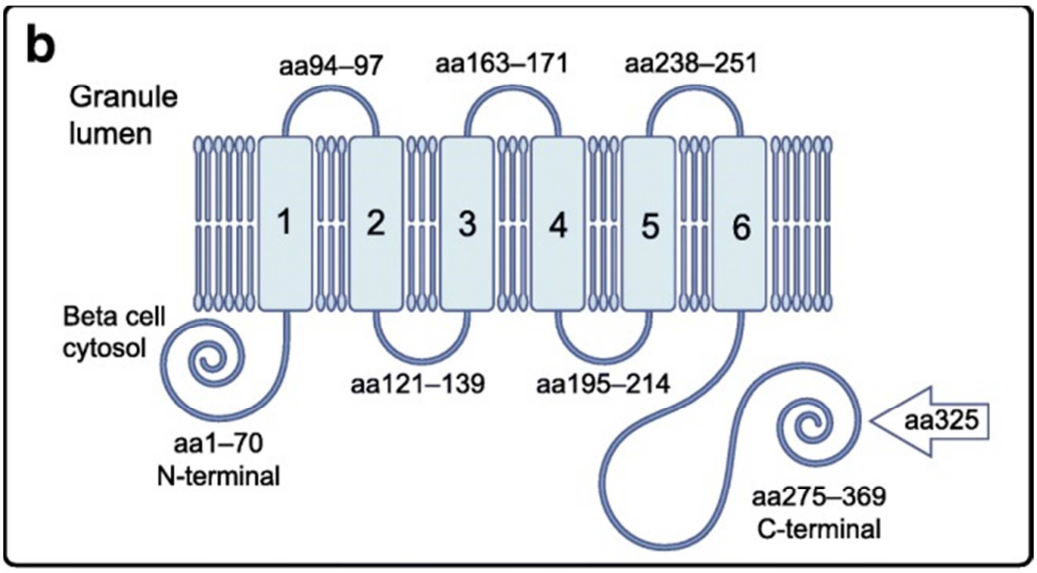


Fig. 19 Structure of ZnT8 [77]

ZnT8 antibody (ZnT8A)

It was first recognized in 2007 and a major antigen against which the autoantibodies are formed in T1DM [87]. It is one of the most recently identified autoantibody and is one of the four major autoantibody present in T1DM. The levels and the prevalence of ZnT8A increase with age, and the antibodies appear frequently in children by 3 years of age. ZnT8A usually appears after GADA and IAA in the course of disease and it precedes the disease by many years. It is also seen that the levels of ZnT8 autoantibodies start to wane after 4 years of T1DM disease course and finally disappear [87].

ZnT8 antibody is associated with HLA-DQ6.4 in individuals with T1DM. ZnT8 antibody is seen to cross react with viral proteins, Mycobacterium avium paratuberculosis. Thus molecular mimicry could be contributing factor in the initial antibody production against ZnT8 [88].

INSULIN AUTOANTIBODY

Insulin

Insulin is a peptide hormone which is initially secreted as proinsulin from the beta-cells of pancreas. The gene encoding the proinsulin is present on chromosome 11p15. Proinsulin consists of A-chain with 21 amino acids, B-chain with 30 amino acids and a C-peptide with 31 amino acids. The final insulin is formed by cleavage of the C-peptide from the proinsulin. Thus the insulin has 51 amino acids, with A and B chains cross-linked by two disulfide bridges [89]. Diagrammatic representation of insulin structure is shown in Fig. 20 [90]. Insulin is an anabolic hormone and its function is to regulate glucose metabolism and homeostasis in blood. It is a major antigen against which antibodies are formed in T1DM.

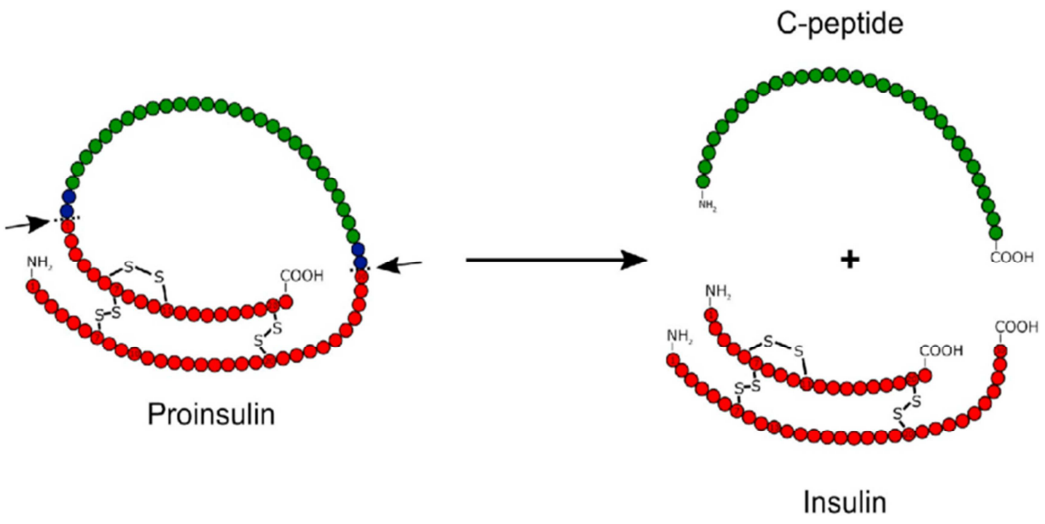


Fig. 20 Structure of proinsulin and insulin [90]

Insulin autoantibodies

Palmar in 1983 identified IAA in newly diagnosed and untreated diabetic patients [69]. The insulin antibodies react with the B-chain of the insulin. High affinity has been associated with HLA DRB1*04, young age of IAA appearance and progression to multiple autoantibodies or type 1 diabetes [91]. Also the A8-A13 region on insulin is important for binding of high-affinity IAA.

***Subclass*:**

IAA of the IgG1 subclass is the predominant type in both pre-diabetic and T1DM individuals. IgG3 subclass is also frequently found in pre-diabetic individuals. The risk of progression to T1DM is higher in individuals with IAA of subclasses IgG2, IgG3 or IgG4 as compared to those without these IgG subclasses.

AUTOANTIBODY SCREENING GUIDELINES

Many countries have developed their own guidelines for antibody estimation in T1DM. But none of them recommend antibody testing for the establishment of T1DM. Many of the guidelines just recommend screening of at risk individuals or recommend testing in research related studies.

The American Diabetes Association (ADA) supports autoantibody testing for diagnosis of T1DM but does not mention that it needs to be routinely done for establishing a diagnosis of T1DM [92]. The ADA also recommends screening can be offered to first degree relatives of a proband with T1DM [93].

Whereas International Society for Pediatrics and Adolescent Diabetes (ISPAD) guidelines suggest screening before the onset of T1DM symptoms to be conducted only during a research [94].

The United Kingdom's NICE guidelines [95] have no mention regarding the use of screening or in research setting. However it mentions that antibody testing should not be done to differentiate T1DM from T2DM.

METHODS FOR DETECTION OF AUTOANTIBODY

Many methods have been developed in the recent years to detect autoantibodies involved in pathogenesis of T1DM and also to identify antibodies of the autoimmune conditions associated with T1DM. Radio-immuno assay or radio-binding assay (RBA) is currently the gold standard for measuring the antibodies against the islet cells [96]. But the RBA cannot be used routinely for screening and in conforming the autoimmune T1DM due to limitations like need for high-tech labs, radiation exposure and radio-chemical waste produced, lack of sensitivity in early detection of antibodies and lack of identification of disease specific autoantibodies.

The immunoblotting assay and enzyme-linked immunosorbent assay (ELISA) are used widely for detection of autoantibodies [97]. Immunoblotting assay is a traditional method which uses the gel electrophoresis principle. However it is a complex procedure and needs skilled technician. Results depends on naked eye and thus, results may vary due to inter-observer variation.

ELISA has long been used as a primary tool for detection of antibodies in both research and clinical diagnostic purpose [97]. It gives reliable results for individual antibody in a shorter time period. It is based on the principle of binding of antigen to plate and detection of bound autoantibody with labeled anti-antibodies. Schematic representation of the principle of direct ELISA is shown in Fig.21 [98] and indirect ELISA in Fig.22 [98]. However the development of these antigen coated ELISA plates is difficult. Also the sensitivity is less as compared to other methods like radio binding assay and electrochemiluminescence.

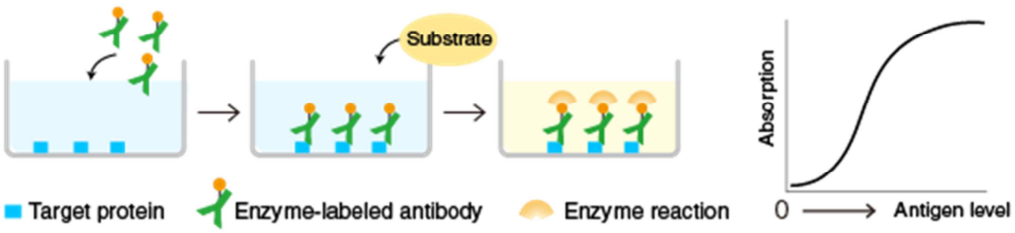


Fig. 21 Principle of Direct ELISA [98]

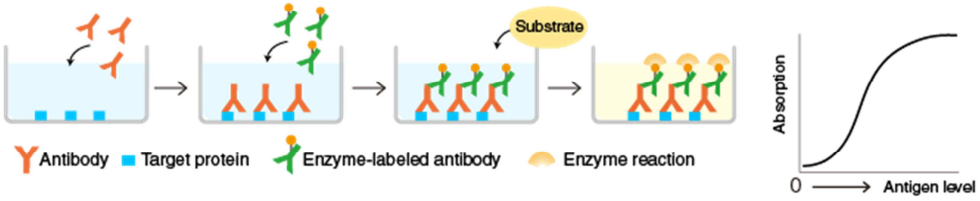


Fig. 22 Principle of Indirect ELISA [98]

Electrochemiluminescence (ECL) assay is the most recently developed method to measure antibodies against the beta-cell proteins and also other autoimmune disease autoantibodies. The validation of ECL has been done in multiple clinical trials like TEDDY, DAISY, and TrialNet [99]. The ECL assays for autoantibody detection has been demonstrated to be having both higher sensitivity and specificity compared to current RBA. The main advantage of ECL assay are that it is highly specific and sensitive as compared to gold standard RBA without involving any radiation and the hazards of radiation. Also, it can identify the antibodies early in the course of the disease process and thus can identify the at risk individuals.

THE PANCREAS – MORPHOLOGY AND PATHOLOGY

The pancreas is located in retroperitoneum of abdomen. It has both exocrine and endocrine function. The exocrine part is made up of acinar cells and the ducts which are meant for secreting and transporting the digestive enzymes like Trypsinogens, Chymotrypsinogen, Procarboxypeptidase-A, Procarboxypeptidase-B, Prophospholipase, Proelastase, and Mesotrypsin and also ions and water into the duodenum. The endocrine part consists of the Islets of Langerhans.

The islets cells constitute about 2% of the entire pancreatic cells. The islets are scattered all throughout the pancreas, but the density is more in the tail of pancreas. The islet is made of 5 different types of cells:

- Beta-cells (65-80%) – Secrete insulin in response to increase levels of blood glucose.
- Alpha-cells (15-20%) – Secrete glucagon in response to lowered blood glucose.
- Delta-cells (3-10%) –Secrete somatostatin, an inhibitor of growth.
- Epsilon-cells (<1%) – Secrete ghrelin, which increases appetite.
- Pancreatic Polypeptide-cells (3-5%) – Secrete pancreatic polypeptide, which has several putative functions, including appetite regulation.

In T1DM, the β-cells are destroyed by the auto-immunity (Fig.23) [100], which will lead to deficiency of endogenous insulin production. Histopathology of pancreas in early stages of T1DM, have shown a decreased beta-cell mass and cellular infiltration in the islets (Fig.24) [101]. CD8 lymphocytes are predominantly seen, along with CD4 lymphocytes, B lymphocytes and macrophages. These cellular infiltrates reduce significantly when all the beta-cells are destroyed.

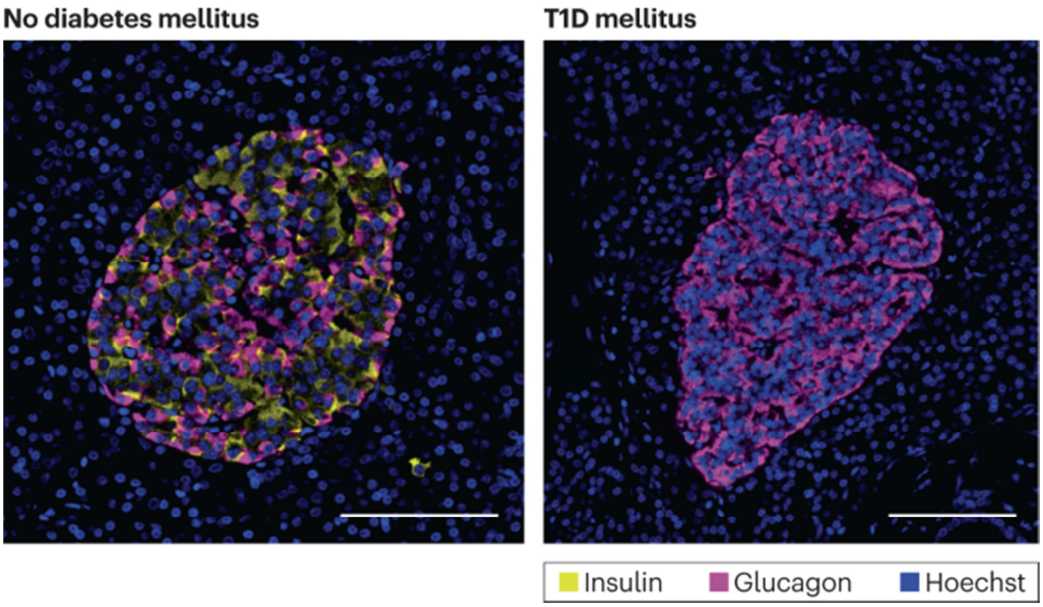
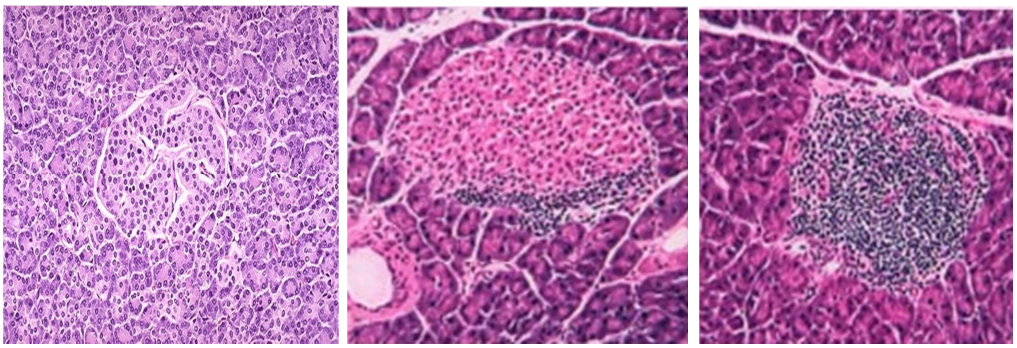


Fig. 23 Immunofluorescence imaging of islets in individuals with T1D and with normoglycaemia [100]



A. B. C.

Fig. 24 (A) Normal islet of Langerhans, stained with Hematoxylin & Eosin.

(B) Initial infiltration of islet with lymphocytes

(C) Complete infiltration of Islet with lymphocyte infiltrates [101]

PATHOGENESIS OF T1DM

STAGES OF T1DM

There are 4 stages in disease progression of T1DM (Fig. 25) [102].

Stage 1: Appearance of autoantibodies in the individuals with genetic risk and environmental predisposition marks the beginning of T1DM. The blood glucose level is normal in this stage. The islets are infiltrated with lymphocytes and there is destruction of beta-cells. The children are asymptomatic in this stage.

Stage 2: Multiple islet antibodies are present in the serum. The blood glucose levels are impaired in this stage. However, there are no symptoms of hyperglycemia. Rarely children can be identified in this stage when they present to the pediatrician for with other complaints.

Stage 3: Children usually present in this stage of T1DM. They either have symptoms of hyperglycemia like polyuria, polydipsia and polyphagia or they may present with DKA or atypical symptoms like pain abdomen, loose stool, vomiting, and loss of weight. The antibodies are still detectable in the serum in this stage. By now the beta-cells in the pancreas will be 90-95% destroyed.

Stage 4: Long standing T1DM. In this stage the antibodies gradually wane off and are no more detectable.

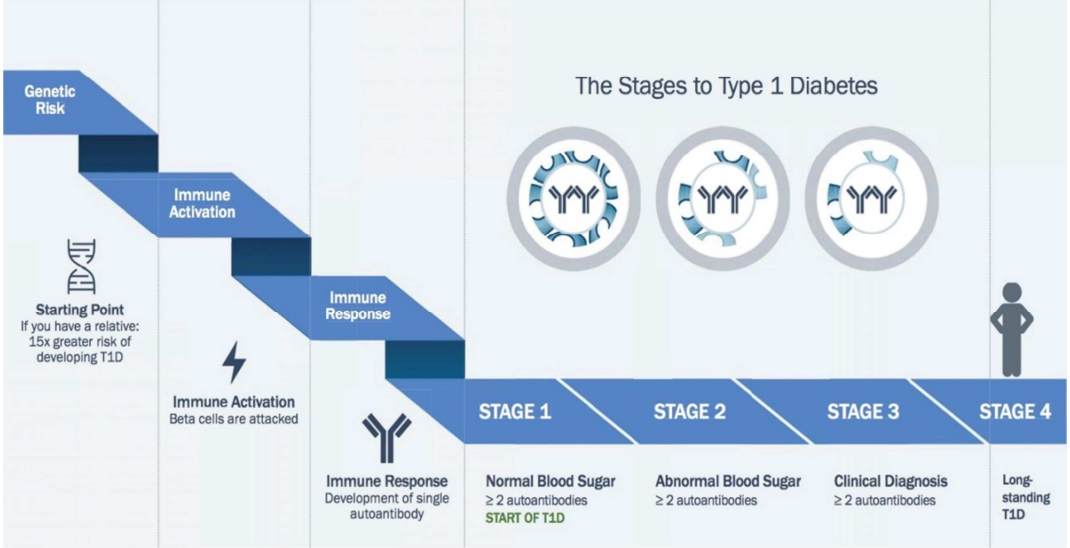


Fig. 25 Stages of T1DM progression: The appearance of two or more autoantibodies defines stage 1 disease, abnormal blood glucose levels indicates stage 2 and, finally clinical diagnosis according to American Diabetes Association criteria is stage 3 [102]

CLINICAL PROFILE OF T1DM

Majority of T1DM cases occur in children. There is a bimodal distribution in age of presentation – one peak occurring at 4 to 6 years of age and another peak occurring at onset of puberty. Also the boys are seen to be more affected than the girls. Whereas, girls are more prone for other associated autoimmune conditions.

CLINICAL PRESENTATION

Children with T1DM can initially present with classical new onset – which is the most common type of presentation followed by life threatening diabetic ketoacidosis. The least type of presentation is the silent presentation.

Classical new onset

It is the most common type of T1DM presentation. The children present with history of polyuria, polydipsia, and polyphagia and generalized weakness, fatigue, weight loss over weeks to months. In the initial phase, insulin reserve is limited and hence episodic postprandial asymptomatic hyperglycemia occurs. As the insulin producing capacity of the beta cells decline, there is persistent hyperglycemia which reaches above the renal threshold of glucose reabsorption, leading to glycosuria which in turn causes osmotic diuresis and polyuria. Excessive loss of glucose (calories) and water will cause compensatory polyphagia and polydipsia. But still there is a net loss of calories and water leading to weight loss and dehydration.

Diabetes ketoacidosis

It is the second most common presentation of T1DM. It is commonly seen in children less than 6 years where the classical symptoms go unnoticed and they end up in life-threatening DKA. There is hyperglycemia, ketonuria and acidosis along with severe dehydration, nausea, vomiting, lethargy, altered sensorium, infection, ketotic breathing. In extreme cases child can present in comatose state. DKA as a presenting feature of T1DM is because of inability of the cells to utilize glucose in the blood due to insulin deficiency leading to ketogenesis → ketonemia → ketonuria and metabolic acidosis. Ketonemia cause abdomen pain, nausea and vomiting leading to decreased oral intake of fluids, thus aggravating the pre-existing dehydration. The clinical picture of ketoacidosis include Kussmaul breathing, acetone or fruity breath, prolonged Q-T interval and impaired neurocognitive function.

A systematic review of 65 studies including 29,000 children from 31 different countries showed the incidence of DKA at diagnosis varied from 12.8% to 80% [5], with highest incidence of 80% in United Arab Emirates and lowest incidence of 14% in Sweden [5] (Fig.26). The recent data from India regarding the prevalence of DKA at diagnosis with T1DM is not available.

Silent presentation

Here the children are asymptomatic and are diagnosed at stage 1 or stage 2. Mostly these are the children who have family history of T1DM and are in close monitoring for development of T1DM.

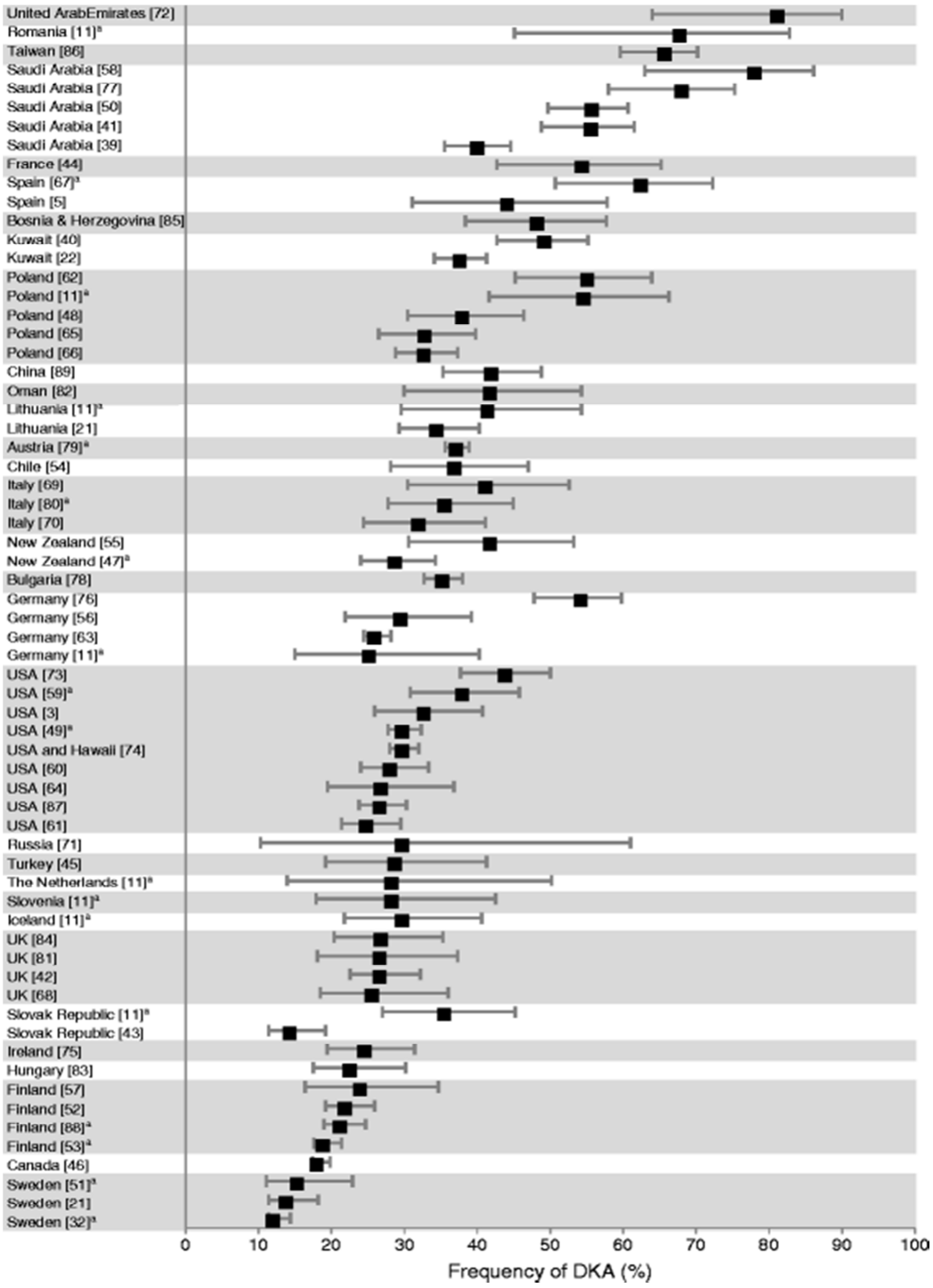


Fig. 26 Incidence of DKA at diagnosis of T1DM in different countries [5].

COMPLICATIONS OF T1DM

Uncontrolled hyperglycemia can cause acute and chronic complications. The acute complications include severe dehydration, diabetic ketoacidosis and non-ketotic hyperosmolar coma. Irregular eating habits and insulin administration can cause hypoglycemic events. Prolonged uncontrolled T1DM with persistent hyperglycemia results in development of microvascular and macrovascular complications.

Multiple mechanisms like polyol pathway, advanced glycation end products, protein kinase C activation and hexosamine pathway are involved in the pathophysiology of microvascular occlusion. Microvascular complications are a result of microvascular thrombosis, endothelial dysfunction, impaired neovascularization, altered vascular permeability leading to end organ damage. The microvascular complications include diabetic retinopathy and diabetic nephropathy. Macrovascular complications include cerebrovascular events, coronary artery disease and peripheral artery diseases. It also causes diabetic peripheral and autonomic neuropathy and diabetic osteopathy leading to increased risk of osteoporosis and fractures.

Other complications include dyslipidemia, obesity, repeated infections and lipodystrophy at insulin injection site, Mauriac syndrome. T1DM children are also at higher risk of developing psychiatric issues like depression, anxiety and eating disorders.

Diabetic Retinopathy

Diabetic retinopathy is one of the most common microvascular complication of T1DM. T1DM children are also at a higher risk of developing cataract due to glycation of tissue proteins and also at a risk of developing glaucoma. It is the leading

cause of blindness in United States in adults. About 98% of T1DM patients have the risk of developing diabetic retinopathy after 15 years of the duration of the disease. A dilated fundus examination is done by an ophthalmologist.

American Diabetes Association recommends [103] screening of diabetic retinopathy within 5 years of onset of the disease OR if the child is above 10 years of age irrespective of the duration of the disease. If there is no retinopathy then yearly screening is advised. If there is retinopathy, then depending on the severity further follow-up and treatment is planned.

Diabetic Nephropathy

About 20-30% are affected by 20 years of onset of T1DM. It is one of the leading cause of end stage renal disease (ESRD). 30-40% of T1DM patients end up having End stage renal disease. Presence of albuminuria with or without symptoms is used for diagnosing diabetic nephropathy.

Albuminuria is detected by urine albumin creatinine ratio (UACR) on a spot urine sample or on 24-hours collected urine sample. UACR below 30mg/g is normal, 30-299mg/g is microalbuminuria, and 300mg/g is termed macroalbuminuria.

According to American Diabetes Association, screening is started after 5 years of the disease onset and there after an annual screening is advised with UACR. If the UACR test is abnormal, it should be always confirmed with 2-3 repeat tests done over a period of next 6 months [103].

Diabetic Neuropathy

It includes peripheral neuropathy and autonomic neuropathy. Autonomic neuropathy is diagnosed based on symptoms and clinical tests like - breathing tests, tilt table tests, urodynamic, sudomotor axon reflex and thermoregulatory tests. An early sign of autonomic neuropathy is decreased heart rate variability in long standing T1DM with poor glycemic control.

Distal polyneuropathy is identified by comprehensive foot examination. 10g monofilament test for touch perception along with one of the following additional test – pinprick OR vibration test with 128Hz tuning fork OR temperature OR ankle reflex is for diagnosis of diabetic peripheral neuropathy.

As per American Diabetes Association, screening begins at 10 years of age or 5 years from onset of T1DM, whichever is earlier. Later annual screening is advised [103].

Diabetic Osteopathy

T1DM is associated with low bone mineral density. Prolonged hyperglycemia leads to advanced glycation end products in the bone (AGEs), chronic inflammation and abnormal bone mineralization due to urinary loss of calcium. This leads to weakening of the bone and increasing the susceptibility to fractures easily.

No guidelines or recommendations are there for routine screening of diabetic osteopathy. Bone density can be assessed by Dual-energy X-ray absorptiometry (DEXA) scan in those with fracture history. More studies needs to be done to formulate screening recommendations.

Table 7 Screening Guidelines according to American Diabetes Association recommendations – children and adolescents: standards of medical care in diabetes, 2022 [103].

	INITIAL TESTING	FREQUENCY	TEST
Thyroid disease	At diagnosis	Every 1-2 years or sooner if symptoms present	TSH, thyroid antibodies
Celiac disease	At diagnosis	Within 2 years and again at 5 years or sooner if symptoms present	IgA level and TTG antibody
Hypertension	At diagnosis	Each visit	BP recording with appropriate size cuff
Dyslipidemia	≥10 years age at diagnosis once glucose control is established	Initially every 5 years, annually if abnormal	Lipid profile
Nephropathy	At puberty or ≥10 years age, whichever is prior, if T1DM is ≥5 years	Annually	Urine albumin creatinine ratio
Retinopathy	T1DM ≥3-5years when ≥10 years or puberty, whichever comes first	Annually	Dilated eye and fundus examination
Neuropathy	At puberty or ≥10 years, whichever earlier if T1DM >5 years	Annually	Foot examination

AUTOIMMUNE DISORDERS ASSOCIATED WITH T1DM

Autoimmune thyroid disease

It is a major autoimmune disorder associated with T1DM. The children have thyroid peroxidase and/or thyroglobulin autoantibodies. They can develop either Hashimoto's Thyroiditis or Graves' disease. It is more common in females compared to males, risk increases with increasing age and duration of T1DM. Current American Diabetes Association guidelines recommend screening for thyroid disorder using Thyroid stimulating hormone (TSH) after the acute symptoms of T1DM are settled and annually thereafter [103]. Screening for Thyroid peroxidase (TPO) antibodies is not routinely done but is recommended to screen every 1-2 years. If TPO is positive and thyroid function test is normal, then 6 months thyroid function test screening is advised.

Celiac disease

The incidence and prevalence of celiac disease is rare. However in recent studies, celiac disease is present in a higher rate in T1DM as compared to normal population. Celiac disease is mostly a clinical diagnosis, augmented with autoantibody positivity against tissue transglutaminase (TTG). Small intestinal biopsy is the gold standard for confirming celiac disease. Clinical features of celiac disease include abdominal pain, bloating sensation, loss of appetite, loose stool and weight loss.

Current recommendations for screening of celiac disease is to test for TTG autoantibody in those children are having symptoms of celiac disease when T1DM is diagnosed. There after 2 yearly screening with TTG autoantibodies is recommended

[103]. If TTG autoantibody is positive, confirmatory small intestinal biopsy is to be done to confirm the diagnosis.

Adrenal disease

Autoimmune adrenal disease is found to co-exist with autoimmune thyroiditis in T1DM. The T1DM children with autoimmune adrenal disease will develop Addison disease and are positive for 21-hydroxylase autoantibodies. There is no guidelines for routine screening.

In those patients who are positive for 21-hydroxylase autoantibody, Adrenocorticotropic Hormone (ACTH) test should be done.

Other autoimmune diseases

Systemic Lupus Erythematosus, rheumatoid arthritis, autoimmune hypoparathyroidism, autoimmune gastritis, and autoimmune skin diseases like vitiligo, rheumatoid arthritis, alopecia areata, urticaria and early onset myasthenia gravis are also associated with T1DM. [6].

METHODOLOGY

SOURCE OF DATA

Children between the ages of 1 year to 18 years with confirmed type 1 diabetes mellitus, with a duration of disease for less than 4 years or newly detected type 1 diabetes mellitus, who were attending the pediatric diabetic OPD or those who were admitted in KLES Dr. Prabhakar Kore Hospital and Medical Research Centre during the study period were considered for the study.

STUDY DESIGN

Hospital based Cross- sectional observational study

STUDY PERIOD

One year study period from October 2022 to September 2023

SAMPLE SIZE

The sample size for the study was calculated based on the following formula and data.

Formula used for sample size calculation was

$$n = \frac{p\,(100-p)\,Z^2}{E^2}$$

n – Sample size required

p – The percentage of occurrence of a state or condition (proportion or prevalence)

E – Percentage maximum error required

Z – Value corresponding to the level of confidence that is required

Prevalence of autoantibody mediated type 1 diabetes was considered to be 90%. [104] With percentage of maximum error as 10% and 95% confidence level sample size was

$$n = 90 \frac{x\ 10\ x\ (1.96)^2}{9\ x\ 9}$$

$$n = 43$$

Minimum sample size required was 43.

SAMPLING TECHNIQUE

All type 1 diabetes mellitus children between 1-18 years, attending diabetic OPD or admitted in the hospital during the study period and those who fulfilled the inclusion and exclusion criteria were considered for the study.

INCLUSION CRITERIA

All children between 1 to 18 years and with type 1 diabetes mellitus for a duration of less than 4 years.

EXCLUSION CRITERIA

1. Children with evidence of chronic diabetic complications or chronic organ dysfunction.
2. Pancreatic exocrine disease
3. Other types of diabetes – Monogenic diabetes, Type 2 diabetes, Drug induced
4. Children on immunosuppressive drugs
5. Children with immunosuppressive diseases
6. Children with genetic syndromes

STUDY PROTOCOL

The child (if able to understand) and parent/guardian of child, who fulfilled the eligibility criteria were briefed about the nature of study. After their approval, written informed consent was obtained from the parents of children less than 7 years, verbal consent from children aged between 7-12 years along with written informed consent from their parents, and written informed consent from both the child and parent for those aged above 12 years. The consent forms were prepared in English as well as major regional languages used in the region – Kannada, Marathi, Hindi (ANNEXURE 1). Then the necessary details were recorder in a pre-designed proforma (ANNEXURE 2). All the data collected were the statistically analyzed.

DATA COLLECTION PROCEDURE

Demographic data, clinical history, past history, drug history, immunization history, family history, socio-economic history were recorded in a pre-designed and structured proforma. Anthropometric measurements – Height, weight, BMI, waist to hip ratio were also recorded. WHO and IAP integrated growth charts and IAP Growth Chart Application, developed by IAP Growth Chart Committee 2014 was used to assess the growth parameters. General physical and systemic examination of the child was done to look for any signs of diabetic complications. Diabetic neuropathy was assessed by examining for vibration, touch sensation (10g monofilament test), proprioception and ankle jerk. Ophthalmology examination was done to look for diabetes induced cataract and diabetic retinopathy. Diabetic nephropathy was assessed by testing for Urine albumin creatinine ratio and by calculating eGFR using Modified Swartz formula. Lipid profile was also done to look for hyperlipidemia. If any of

these above mentioned complications were present, then the child was excluded from the study.

HbA1C and C-peptide levels at the time of initial diagnosis were noted. Children were also screened for hypothyroidism by estimating early morning serum thyroid stimulating hormone (TSH) by CLIA method. Probable celiac disease was considered based on symptomatology as mentioned in European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines for diagnosis of Celiac disease [105] – unexplained chronic or intermittent diarrhea, persistent nausea or vomiting, chronic pain abdomen, chronic constipation, abdomen bloating, recurrent aphthous ulcer in mouth, failure to thrive, loss of weight, not gaining height, chronic fatigue – were the symptoms considers for labelling the child as probable celiac disease.

For T1DM autoantibody profile test, 5ml of venous blood was taken in a plain vacutainer and was allowed to clot. After clotting, the sample was centrifuged in Eppendorf Centrifuge 5702R centrifuge machine for 10 minutes at 3500 revolutions per minute (RPM) to separate the serum. The separated serum was transferred to a new plain vacutainer and was processed in Hi-Tech lab in KLES Dr. Prabhakar Kore Hospital and Medical Research Centre. The serum samples was stored in ice lined refrigerator in diabetic OPD at -20^{o}C and later were analyzed in batches in the Hi-Tech lab.

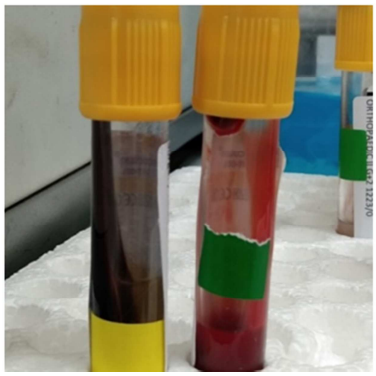
Estimation of anti-GAD-65 antibody was done using BIOMERCA ELISA kit,

IA-2 antibody with MEDIPAN ELISA kit

Insulin autoantibody with BIOMERCA ELISA kit,

Zinc transporter 8 antibodies with BIOVENDOR ELISA kit and

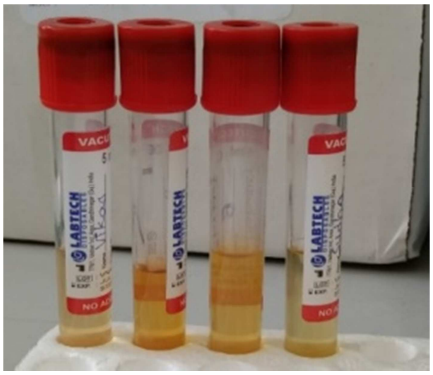
Islet Cell Antibody with BIOMERCA ELISA kit.



1. Sample is collected in yellow plain vacutainer

2. Samples are centrifuged for 10 minutes at 3500 RPM.

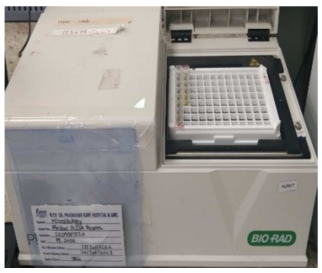
3. Serum is separated by centrifugation.



4. Serum is transferred to red plain vacutainer

5. Samples are stored in Ice Lined Refrigerator (ILR) at -20°C

6. ELISA method is used for detecting the antibodies



7. BioRad ELISA plate reader is used for reading the color

Fig. 27 Procedure of antibody testing

DATA PROCESSING AND ANALYSIS

Descriptive analysis for quantitative variables, frequency, and proportion for categorical variables was carried out by mean and standard deviation. Data was schematically represented using appropriate bar graphs, cluster bar graphs, pie charts and box plots.

The association between the explanatory variables and the categorical outcomes was evaluated by cross tabulation and comparison of percentages. Statistical significance was tested using Chi square test.

The association between the quantitative explanatory variables and the categorical outcomes for 2 groups was evaluated by independent sample T-test. For more than 2 groups ANOVA was used to assess the statistical significance. P value < 0.05 was considered statistically significant.

IBM SPSS version 22 was used for statistical analysis [106].

ELISA TEST FOR GAD-65 AUTOANTIBODY

Principle:

Purified GAD antigen are coated into the microwells. GAD specific IgG antibodies present in the serum will react with the antigen in the microwell. An enzyme (alkaline phosphatase) labeled goat-antibody, specific to human IgG is added to GAD-antibody complex. Later a substrate, p-Nitrophenyl phosphate is added and the color generated is measured spectrophotometrically. The intensity of the color is proportional to the concentration of GAD antibodies in serum sample.

Assay procedure:

1. The microwell columns were labelled and 0.1(100μL) ml each of three different calibrators, positive control and negative control are put in separate wells.
2. 0.01ml (10μL) of serum sample was pipetted and diluted with 1ml of sample diluent provided in the kit. Later 0.1ml (100μL) of each of the diluted serum sample was pipetted into appropriate microwells.
3. After all samples to be tested were put into appropriate wells, it was covered with a plastic wrap to prevent contamination and incubated for 1 hour at room temperature of 25° ± 1°C.
4. After 1 hour of incubation, the contents in the microwells were dumped and blotted dry by gently tapping into a paper towel.
5. The microwells were then filled up with wash buffer and then dumped from the microwells and blotted dry again. The process of washing was repeated for two more times.

6. 0.1ml (100µL) of enzymes conjugate reagent was added to each well and covered by plastic wrap and incubated for 1 hour in dark at room temperature of 25° ± 1°C.
7. After incubation the microwells were washed again with buffer solution for 3 times and blotted dry.
8. 0.1ml (100µL) of substrate solution was added to all the microwells and again covered and left in dark for 30 minutes at room temperature of 25° ± 1°C.
9. After 30 minutes, 0.05ml (50µL) of stopping solution was added into each microwells and the intensity of color developed was read by the automated spectrometer.

Calculation of data:

A linear dose response curve was plotted with X-axis corresponding to the 3 calibrator values which was mentioned on the calibrator solution bottles and Y-axis corresponding to their corresponding absorbance value. The 3 points are connected to get a linear curve. The GAD value of each serum sample was then obtained by using the absorbance value and extrapolating from the dose response curve on X-axis.

Interpretation:

GAD value (U/ml)	***Result***
<1.00	Negative
>1.05	Positive
1.00-1.05	Intermediate

The intermediate reports were retested with a fresh serum sample.

Quality control:

Every time the serum samples were run, positive and negative controls along with calibrators were run along with the batch of serum samples, in order to validate the results.

The negative control values were <1.00U/ml and positive control values were >1.05U/ml.

Fig. 28 GAD-65 autoantibody ELISA kit.

ELISA TEST FOR INSULIN AUTOANTIBODY

Principle:

Human insulin is coated inside the microwells. Specific IgG antibodies to insulin in the serum bind to the insulin molecules in the microwell. Alkaline phosphatase labeled goat antibody, specific to human IgG is added to the antigen-antibody complex. Then p-Nitrophenyl phosphate substrate solution is added. If the antigen antibody complex is present the color develops in the microwell. The intensity of color is directly proportional to the concentration of IAA, which is measured spectrophotometrically.

Assay procedure:

1. 0.01ml (10µL) of serum sample was pipetted into 1.0ml of sample diluent given in the kit and mixed thoroughly.
2. 0.1ml (100µL) of IAA – reference control, negative control and positive control were added to 3 different microwells. Then 0.1ml (100µL) of each diluted serum samples were added to the other microwells after labelling/numbering them properly.
3. The plate was then covered properly with plastic wrap to prevent contamination and incubated at 2-8°C for 12-16 hours.
4. The microwells were decanted and blotted dry by gently tapping on a paper towel.
5. The microwells were filled with wash solution using a squeeze bottle and then the wells were decanted and blotted dry. The washing procedure was done for two more times.

6. 0.1ml (100µL) of IAA-IgG enzyme conjugate reagent was added to all microwells and again incubated for one hour at room temperature of 25° ± 1°C. Later, the washing procedure as mentioned in previous step was repeated.
7. 0.1ml (100µL) of substrate solution was added to all microwells and left in dark for 30 minutes at a room temperature of 25° ± 1°C.
8. After 30 minutes, 0.05ml (50µL) of stopping solution was added into each well and the intensity of the color developed in each well was read by an automated spectrophotometer at an absorbance of 405nm (as per instructions of the ELISA kit manufacturer).

Calculation of data:

1. The average O.D. reading of the reference, negative and positive controls and serum samples was calculated by taking two simultaneous readings in the spectrophotometry.
2. The average O.D. of serum samples and controls was divided by the reference value. This gave a ratio value for each sample.

Interpretation:

IAA Ratio Value (U/ml)	***Result***
<0.95	Negative
>1.05	Positive
0.95 – 1.05	Intermediate

All the samples which showed intermediate results were retested with a fresh serum sample.

Quality control:

The positive and negative controls along with calibrators were run with each batch of test been done, in order to confirm the validity of the serum sample results.

Every time the positive control showed a value >1.05U/ml and negative control showed a value <0.95U/ml.

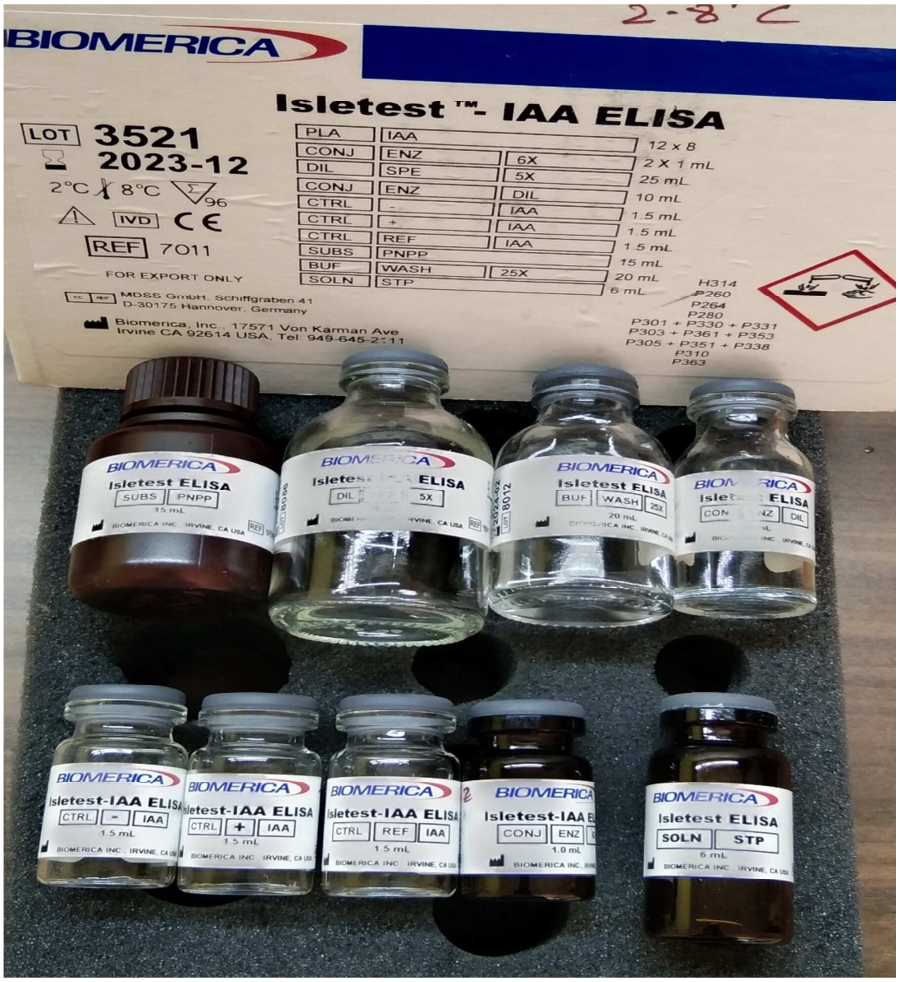


Fig. 29 Insulin Autoantibody ELISA kit

ELISA TEST FOR ZINC TRANSPORTER (ZnT8) AUTOANTIBODIES

Principle:

ZnT8 is coated onto ELISA plate wells. ZnT8 antibody present in the serum binds to the ZnT8. Biotin is then added which binds to the bivalent or polyvalent ZnT8 autoantibodies. Streptavidin Peroxidase is then added which binds to the ZnT8-Biotin complex. Addition of the peroxidase substrate 3,3',5,5' – tetramethyl-benzidine (TMB) will form a blue color product. Stop solution is added to stop the reaction. Finally a yellow color product is formed in the well. The absorbance at 405 nm and 450 nm is then read using an ELISA plate reader. The absorbance value corresponds with the level of ZnT8A.

Assay method:

1. 0.25ml of five calibrators (B1-5), two positive controls (C1, C2) a negative control (D) and serum samples were pipetted into respective wells, after identifying and labelling them properly.
2. The plate was covered and shaken for 5 seconds and incubated for 16-20 hours at 2-8°C
3. The contents were then discarded and was washed three times manually with concentrated washing solution provided in the kit and dried on blotting paper.
4. 0.1ml (100µL) of the cold reconstituted ZnT8-Biotin (2-8°C) was added into each well and covered and incubate at 2–8°C for 1 hour.
5. The contents were discarded and the plate was washed three times as described earlier.

6. 0.1ml (100µL) of diluted SA-POD was added into each well and the plate was covered and incubated on ELISA plate shaker at 20-25°C for 20 about minutes (500 shakes/min).
7. Again the plate was washed 3 times and dried and 0.1ml (100µL) of TMB was added. Then the plate was incubated in the dark at 20-25°C for 20 minutes.
8. 0.1ml of stop solution was pipetted into each well and shaken for 5 second.
9. Within 5 minutes the absorbance of each well was read using ELISA plate reader at 405nm and 450 nm.

Calculation of data:

Calibrator concentration was plotted on x-axis (log scale) against the absorbance of the calibrators on y-axis (linear scale) to obtain a calibration curve. The ZnT8 antibody levels in patient sera was interpreted by using calibration curve.

Interpretation:

Negative – <15U/ml

Positive – >15U/ml

This cut off value was according to the specification mentioned by the ELISA kit manufacturer.

Quality control:

The two positive controls (C1, C2) and a negative control (D) were always run with each batch of serum samples that were tested. The positive controls always showed a value >15U/ml and negative control always showed a value <15U/ml.

Fig. 30 Zinc Transporter 8 Autoantibody ELISA kit

ELISA TEST FOR IA-2 AUTOANTIBODY

Principle:

The wells of the microtiter plate are coated with test-specific antigens. If antibodies are present in the serum, they bind to the antigens. A second biotinylated antigen binds the bivalent immobilized fixed antibody. Streptavidin is conjugated with the enzyme peroxidase which detects the generated immune complex. A colorless substrate is converted into the colored product by the peroxidase. The signal intensity of the reaction product is proportional to the antibody levels. After stopping the reaction, the signal intensity of the reaction product is measured photometrically.

Assay method:

1. 0.05ml (50µL) each of the five calibrators (CAL0 - CAL 4), positive control (CII) and undiluted serum samples were pipetted into the microwells of the ELISA plate.
2. The plate was then covered and incubated in room temperature for 60 min along with shaking at 500rmp using a plate shaker.
3. The plate was decanted and washed with 0.3ml (30µL) of washing solution in each well for 5 seconds and was dried by tapping on a paper towel. This washing process was repeated for two more times.
4. 0.1ml (100µL) of IA2-Biotin solution was added and the plate was covered and incubated at room temperature for 60 minutes along with shaking it at 500rpm on a plate shaker.
5. The contents were again decanted and the washing process as described above was done for three times.

6. 0.1ml (100µL) of SA-POD was added to each well and plate was covered and incubated for 20 minutes in room temperature along with shaking it at 500rpm using a plate shaker.
7. The plate was decanted and washed three times as mentioned above.
8. 0.1ml (100µL) of the substrate 3,3',5,5' – tetramethyl-benzidine is added and plate shaken manually for 10-15 seconds and covered and incubated for 20 minutes at room temperature without shaking.
9. 0.1ml (100µL) of stop solution was added and plate was shaken manually for 10-15 seconds to stop the reaction occurring.
10. The optical density of the color produced in the wells was read at 420nm and 620nm within 30 minutes of the previous step.

Calculation of data:

A standard curve was generated by plotting the optical density values of the calibrators on X-axis and their antibody levels as mentioned in the kit on Y-axis, and connecting these 5 dots to obtain a linear curve.

Antibody levels of the serum samples was derived directly from their optical density by use of the generated standard curve.

Interpretation:

Antibody activity	<8U/ml	–	Negative
Antibody activity	8-10U/ml	–	Borderline
Antibody activity	>10U/ml	–	Positive

The borderline samples were retested with a fresh serum sample.

Quality control:

The optical density of calibrators was CAL0 < CAL1 < CAL2 < CAL3 < CAL4.

Also the optical density of CAL4 was >1.2.

The positive control and calibrators were always run with the batch of samples tested and was always >10IU/ml.

These criteria validated the test results.

Fig. 31 Insulinoma Associated Protein- 2 Autoantibody ELISA kit

ELISA TEST FOR ISLET CELL AUTOANTIBODIES

Principle:

A purified mixture of pancreatic antigens is coated into microwells. If antibodies are present in serum sample they bind with the antigens coated in the microwell. An enzyme alkaline phosphatase labeled goat antibody, specific to human IgG, is added to the antigen-antibody complex. Later p-Nitrophenyl phosphate substrate is added and the color generated is measured spectrophotometrically. The concentration of ICA in serum is directly proportional to the intensity of the color developed at the end of the reaction.

Assay method:

1. 0.01ml (10µL) of serum sample was pipetted into 1ml of the working sample diluent given in the ELISA kit and was mixed thoroughly.
2. 0.1ml (100µL) of negative control, 0.1ml (100µL) of positive control and 0.1ml (100µL) of reference control was dispensed into three different microwells. And 0.1ml (100µL) of diluted sample serum was added into microwells and after proper identification and labelling.
3. The plate was covered by a plastic wrap to prevent contamination and was left for 1 hour at room temperature of 25° ± 1°C.
4. After incubation the contents were decanted and blotted dry by tapping gently onto a paper towel.
5. The wells were then filled with the wash solution provided in the kit by using a squeeze bottle and then decanted and blotted dry. This process of washing was repeated for two more times.

6. 0.1ml (100µL) of ICA-IgG enzyme conjugate reagent was added to all microwells, covered and left at room temperature of 25°±1°C for one hour and then the washing step and blotting was repeated as mentioned previously.
7. 0.1 ml (100µL) of substrate solution was added to all microwells and kept in the dark for 30 minutes at room temperature of 25° ± 1°C.
8. After 30 minutes, 0.05ml (50µL) of the stopping solution was added into each well at a rapid steady pace without any interruption.
9. Automated microplate reader was used to read the absorbance at 405nm according to manufacturing instructions.

Calculation of data:

1. The average O.D. reading of the reference, negative and positive controls and serum samples was calculated by taking two simultaneous readings in the spectrophotometry. [Average OD: Reference (*R*), Negative (*N*), Positive (*P*), Samples (*S*)]
2. The average O.D. of serum samples and controls was divided by the *R* value. This gave a ratio value for each sample.

Interpretations:

ICA Ratio value	***Result***
< 0.95	Negative
> 1.05	Positive
0.95 – 1.05	Indeterminate

The samples with intermediate results were retested with a fresh serum sample.

Quality control:

Negative and positive controls along with calibrators were always run along with the samples to be tested each time for validating the results.

The negative controls value were always < 0.95 U/ml and the positive control values were always > 1.05 Units/ml.

Fig. 32 Islet Cell Autoantibody ELISA kit

RESULTS

BASIC DEMOGRAPHIC DETAILS

Parameter	Mean ± SD	Median	Minimum	Maximum
Age (Years)	10.18 ± 3.85	10.5	1.0	16.3
Age At Diagnosis	8.11 ± 3.73	9.0	1.0	15.0
Duration Of T1Dm In Years	1.97 ± 1.11	2.0	0.1	4.0
SD of Weight for Age	-0.69 ± 0.82	-0.7	-2.6	0.7
SD of Height for Age	-0.6 ± 1.04	-0.8	-3.3	1.9
BMI	15.9 ± 2.53	15.1	11.5	25.7
SD of BMI	-0.55 ± 0.82	-0.4	-3.1	1.5
Waist To Hip Ratio	0.92 ± 0.06	0.9	0.7	1.1

Table 8: Descriptive analysis of baseline parameters of the study population (N=74)

Age: The mean age of T1DM children in the study is 10.18 years, with a standard deviation of 3.85 years.

Age at Diagnosis: On average, children were diagnosed with T1DM at around 8.11 years of age, with a standard deviation of 3.73 years.

Duration of T1DM: The average duration of T1DM in the studied population is approximately 1.97 years, with a standard deviation of 1.11 years.

Anthropometric Measurements:

Weight for Age (SD): The standard deviation of weight for age is -0.69, indicating that, on average, children with T1DM have a weight below the mean for their age.

Height for Age (SD): The standard deviation of height for age is -0.6, suggesting that, on average, children with T1DM have a height below the mean for their age.

BMI (Body Mass Index): The mean BMI is 15.9, with a standard deviation of 2.53. This indicates that, on average, children with T1DM have a BMI below the normal range for their age.

BMI for Age (SD): The standard deviation of BMI for age is -0.55, indicating that, on average, BMI of children with T1DM is below the mean for their age.

Waist to Hip Ratio: The mean waist to hip ratio is 0.92, with a standard deviation of 0.06. This suggests that, on average, children with T1DM have a waist to hip ratio within the normal range.

Overall, these descriptive statistics provide insights into the demographic characteristics and anthropometric measurements of children with T1DM. The data suggests a relatively young population with early-stage of T1DM and generally lower weight, height and BMI compared to their age peers.

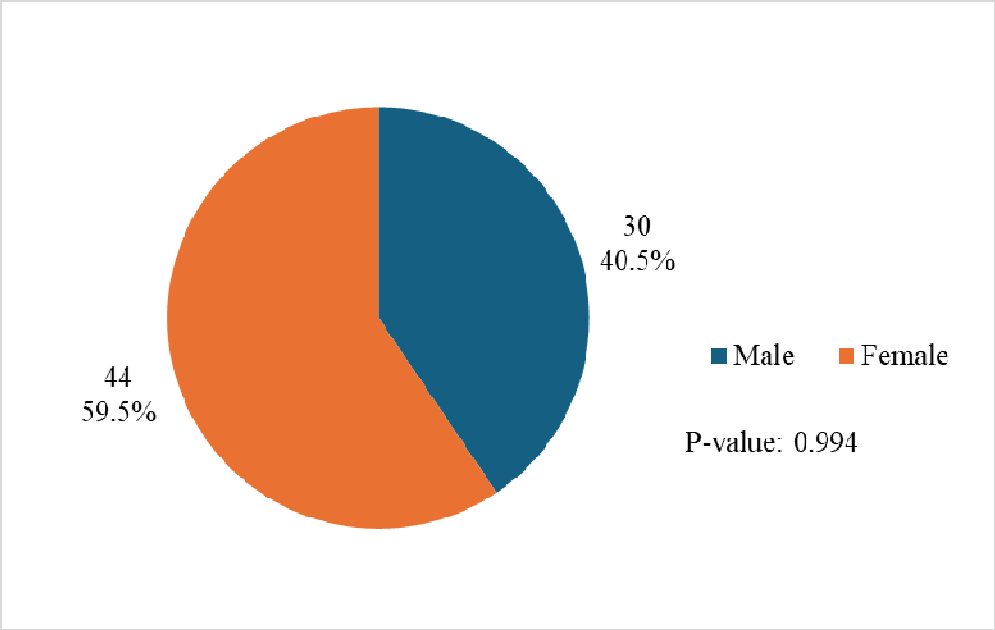


Fig. 33: Pie chart of gender distribution in the study population (N=74)

Gender Distribution: There are 30 males and 44 females in the study population of children with T1DM.

Gender Proportions: Females constitute a larger proportion (59.5%) of the dataset compared to males (40.5%).

Gender Disparity: There's a notable difference in the number of males and females, with females outnumbering males by 14 individuals.

The male: female ratio is 1:1.47.

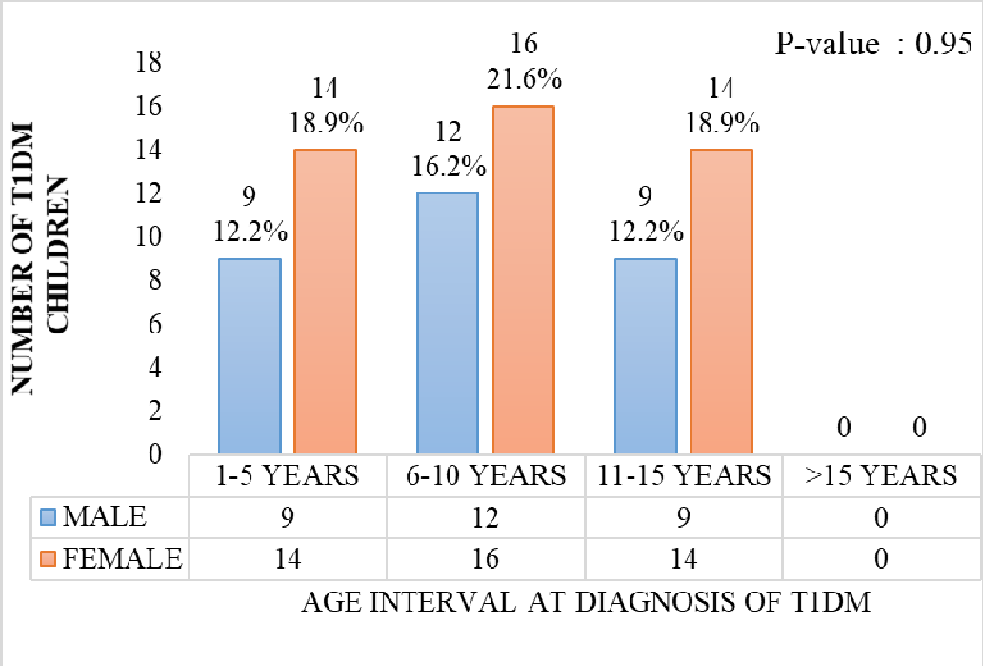


Fig. 34 Cluster bar graph of males and females in different age groups at the time of onset of T1DM

Age Interval Distribution:

1-5 Years (31.1%): There are a total of 23 children, with 9 males (12.2%) and 14 females (18.9%).

6-10 Years (37.8%): There are a total of 28 children, with 12 males (16.2%) and 16 females (21.6%).

11-15 Years (31.1%): There are 23 children, with 9 males (12.2%) and 14 females (18.9%).

>15 Years: There are no individuals in this age group.

Age and Gender Analysis:

There is slightly higher proportion of females with T1DM across all age groups as compared to males with T1DM. The highest number of children with T1DM is observed in the age group of 6-10 years for both males and females.

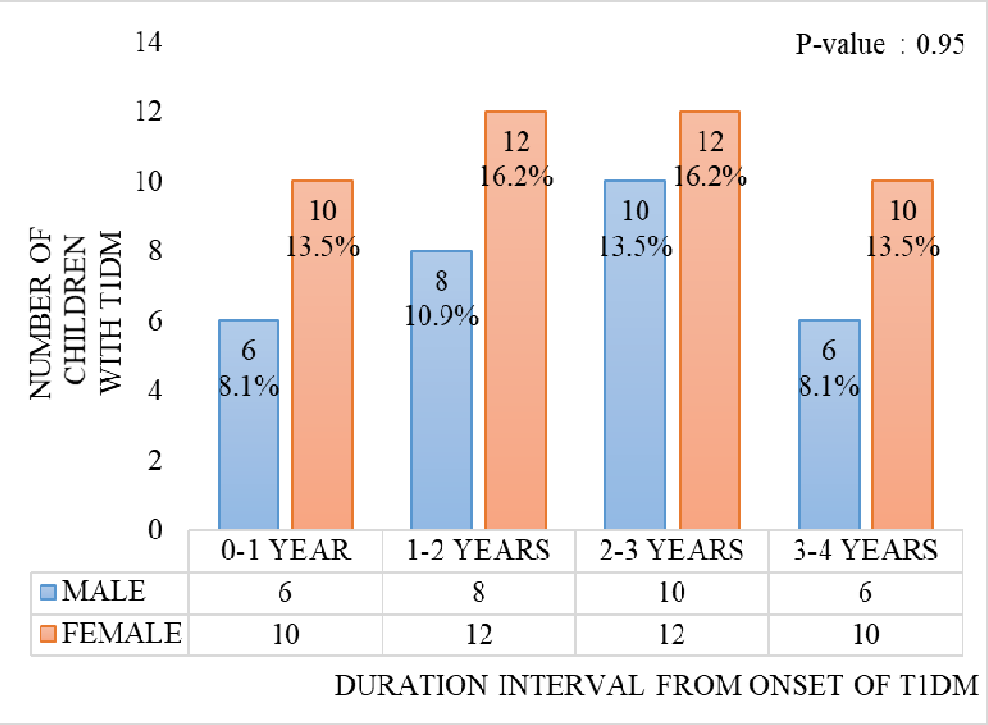


Fig. 35 Cluster bar graph of males and females in different duration intervals from onset of T1DM

The mean duration of T1DM in the study population is 1.97 ± 1.11 years. The number of children in different duration intervals of T1DM is as follows: 16 (21.6%) in 0-1 year interval of onset of T1DM, 20 (27.1%) in 2-3 years of onset of T1DM, 22 (29.7%) in 2-3 year of onset of T1DM and 16 (21.6%) in 3-4 years of onset of T1DM. The percentage of T1DM children in each duration group were almost similar. The female constituted 13.5% in 0-1 year and 3-4 years duration interval from onset of T1DM, 16.2% in 1-2 years and 2-3 years duration interval from onset of T1DM. Males constituted 8.1% in 0-1 year and 3-4 years duration interval from onset of T1DM, 10.9% in 1-2 years and 13.5% in 2-3 years duration interval from onset of T1DM.

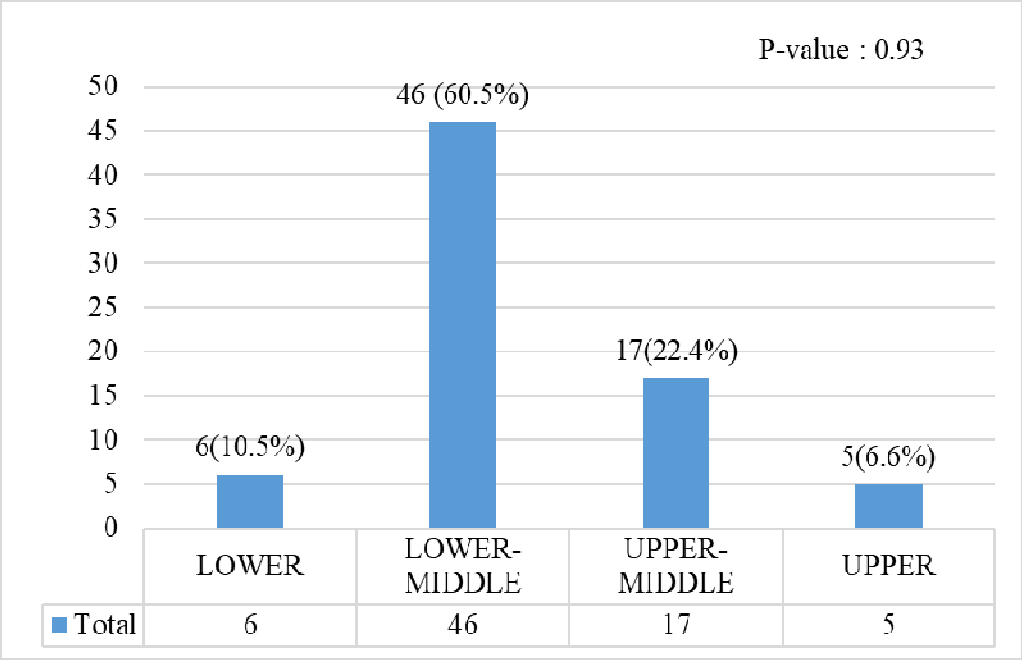


Fig. 36 Socio-economic status of the family of T1DM children

According to Modifies Kuppuswamy's classification, lower socio-economic class constituted only 10.5% of the children with type 1 diabetes. The majority of children, accounting for 60.5% of the total cases of T1DM children, belong to the lower middle socio-economic status group. A significant portion of children, comprising 22.4%, come from families classified as upper middle socio-economic status. The smallest proportion of children of 6.6% belong to upper socio-economic status.

The majority of children with T1DM come from families belonging to the lower middle and upper middle socio-economic status group. This data suggests that there may be some correlation between socio-economic status and T1DM.

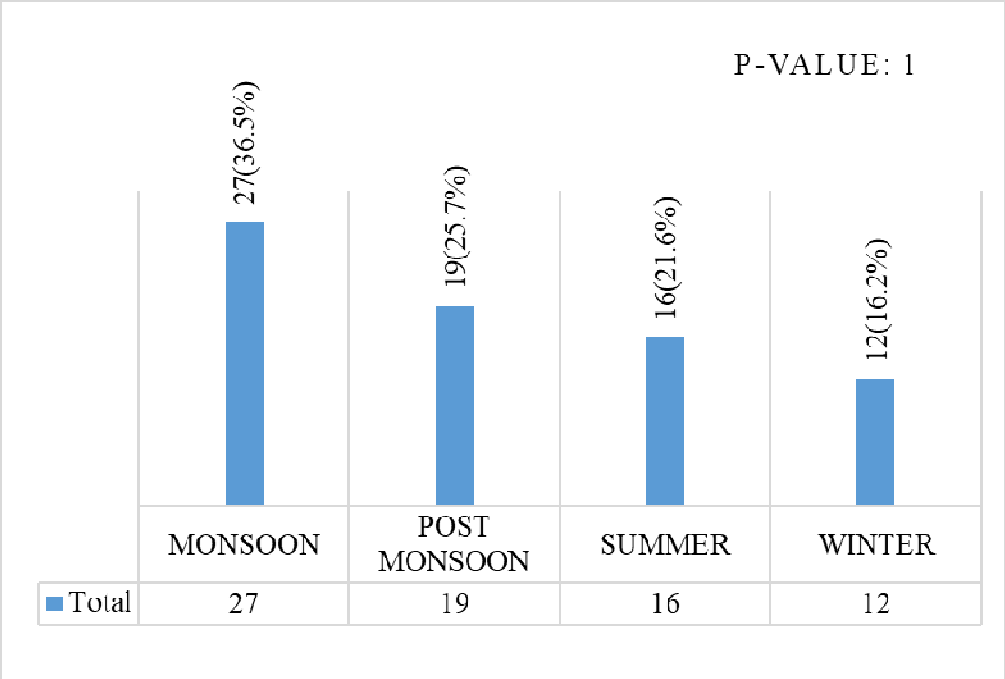


Fig. 37 Seasonal variation of onset of T1DM in children

The highest number of cases of T1DM onset in children occurred during the monsoon season, comprising 36.5% of the total cases studied. Following the monsoon season, the post-monsoon period had the second-highest number of cases, accounting for 25.7% of the total cases. The summer season had the third-highest number of onset of T1DM, with 21.6% of the total cases occurring during this period. Winter had the lowest number of cases among the seasons studied, comprising 16.2% of the total cases.

Monsoon season appears to correlate with the highest incidence of T1DM, followed by post-monsoon season.

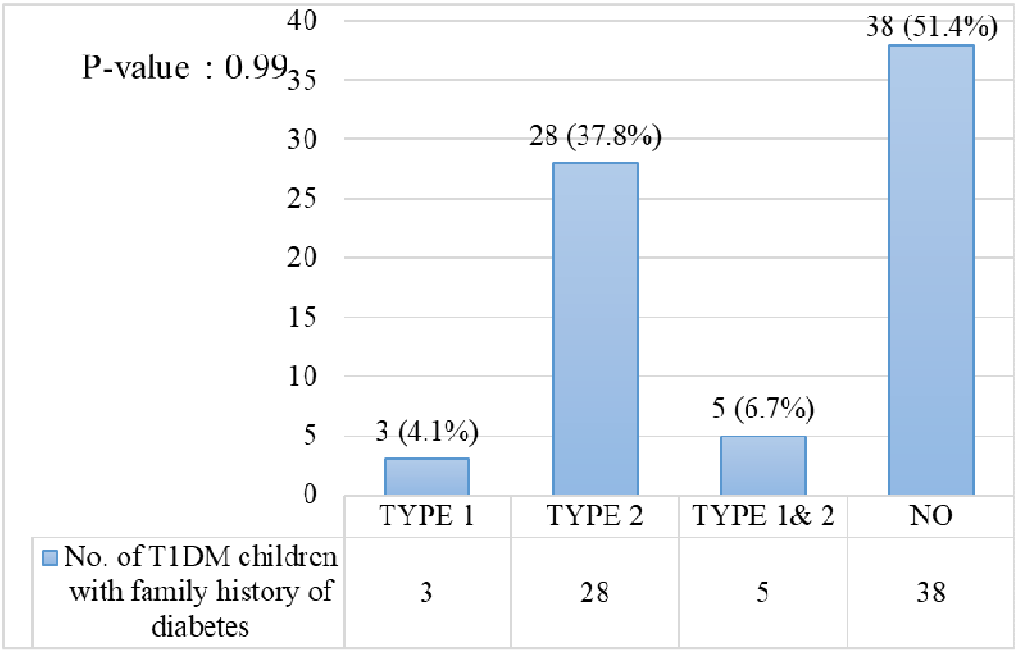


Fig. 38 T1DM children with family history of diabetes

Children with family history of diabetes:

- T1dm only): 3 children (4.1%)
- T2DM only: 28 children (37.8%)
- T1DM & T2DM: 5 children (6.7%)
- Total children with family history of diabetes: 3 + 28 + 5 = 36 children (48.6%)
- No family history of diabetes: 38 children (51.4%)

Nearly half of the T1DM children have a family history of diabetes (48.6%). This shows that there is a substantial link between family history of diabetes and development of T1DM in children. Genetic factors play an important part in the development of T1DM in children.

Over half of the T1DM children (51.4%) do not have a family history of diabetes. This highlights that factors such as environmental influences, lifestyle, or spontaneous genetic mutations, are also critical in the onset of T1DM.

PROFILE OF AUTOANTIBODIES

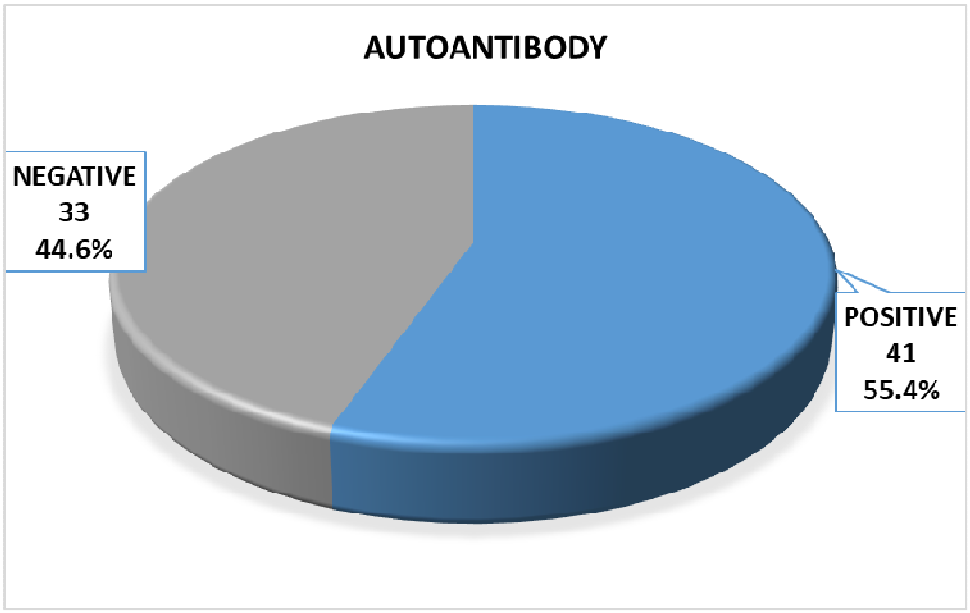


Fig.39 Proportion of autoantibody positive and negative T1DM

41 out of 74 children with T1DM tested positive for autoantibodies associated with T1DM. Conversely, 33 children with T1DM tested negative for disease associated autoantibodies. Autoantibodies associated with T1DM were detected in 55.4% of T1DM children. However, a notable portion of 44.6%, tested negative for these autoantibodies.

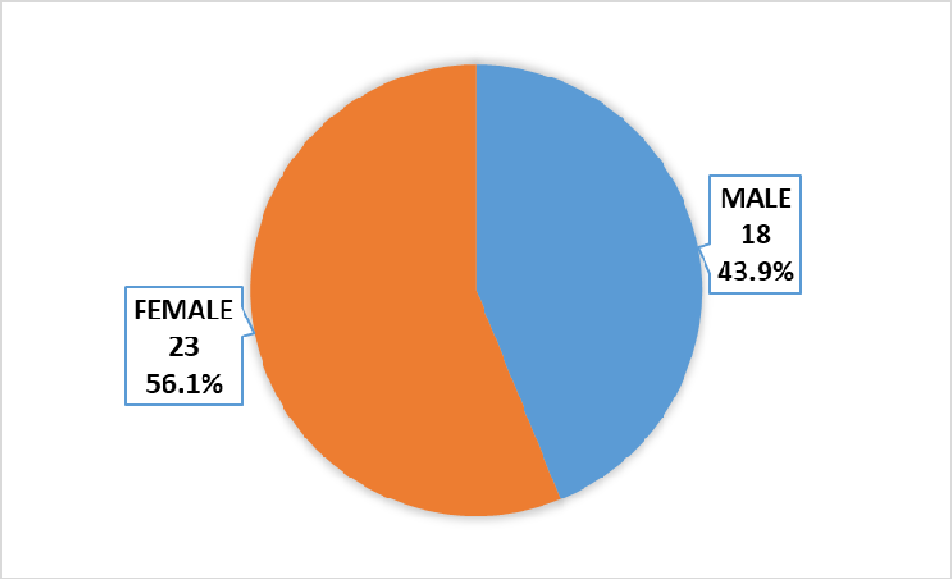


Fig. 40 Gender proportion of autoantibody positive T1DM children

Out of the 41 autoantibody positive T1DM children, 23 are female accounting for 56.1% of the total antibody positive T1DM children. 18 are males constituting for 43.9%. There are slightly more females with antibody positivity compared to males. The male: female ratio for the children with autoantibody positivity is 1:1.28.

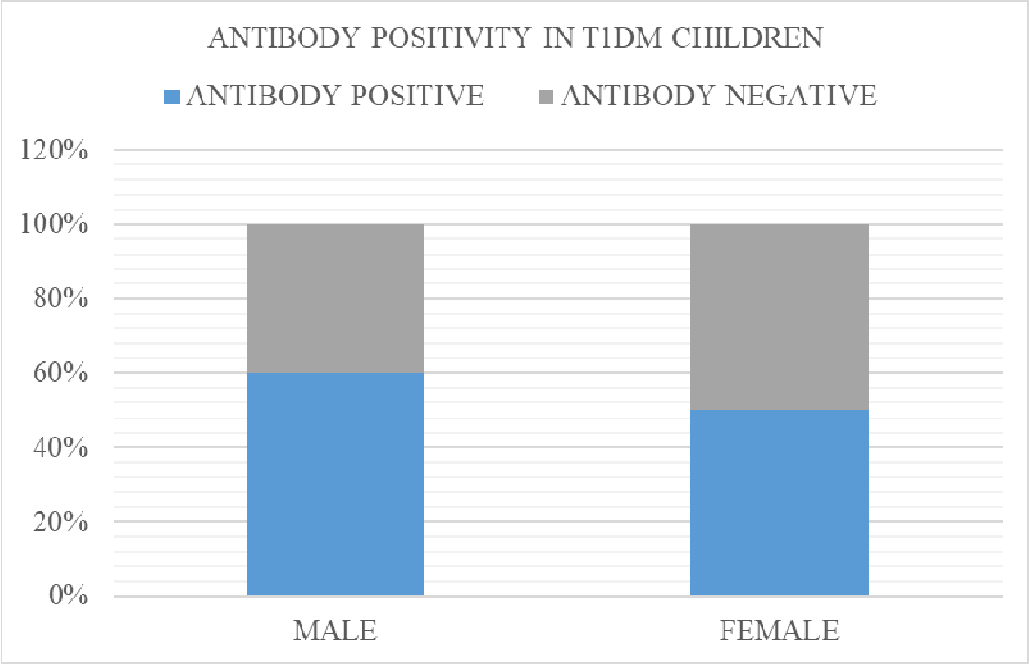


Fig. 41 T1DM Autoantibody Positivity Rate by Gender

Males: 60% of T1DM male children, 18 out of 30 have autoantibodies. This indicates a higher prevalence of autoantibodies in males.

Females: 50% of T1DM female children, 22 out of 44 have autoantibodies, showing an equal distribution of antibody-positive and antibody-negative results among females.

Comparison Between Genders: T1DM male children have a higher percentage of antibody-positive results (60%) compared to females (50%).

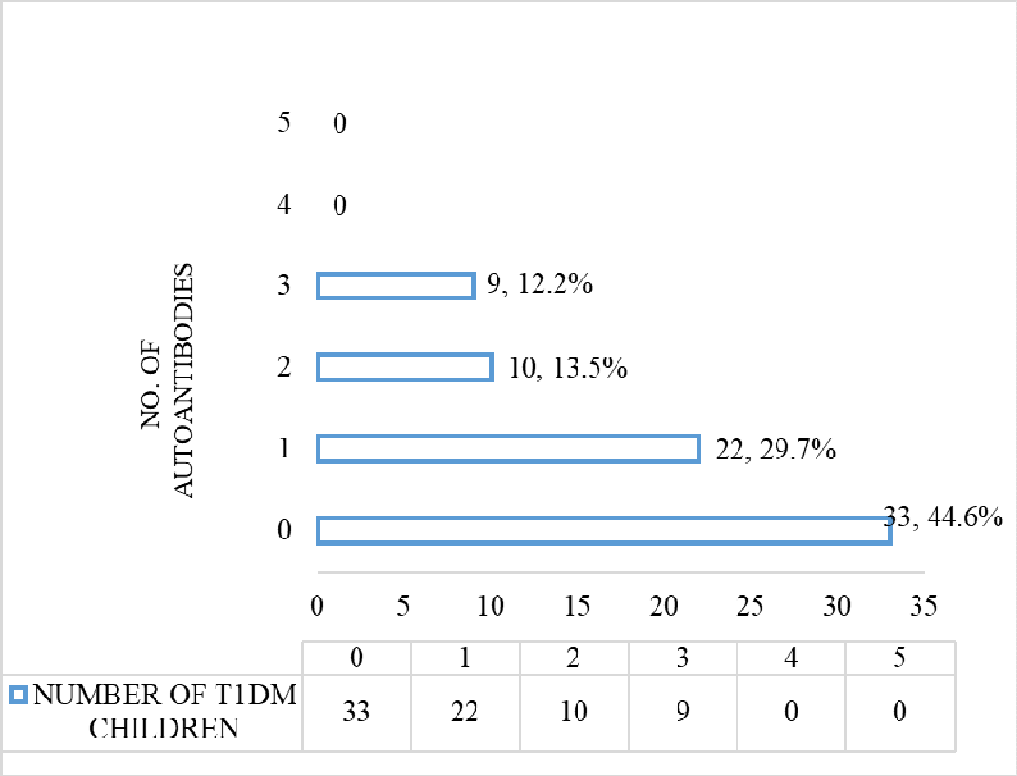


Fig. 42 Number of autoantibodies positive in each T1DM child

A significant number of T1DM children (33 of 74 children, 44.6%) have no detectable antibodies.

Of the total 74 T1DM children, 22 (29.7%) are positive for only one antibody, 10 (13.5%) are positive for any two antibodies, 9 (12.2%) are positive for any three antibodies. There are no children with 4 or 5 antibodies detected.

The 44.6% children with no antibody indicated that there is non-autoantibody mediated pathogenesis of T1DM in these children or that the autoantibodies have waned off after the onset of T1DM. This could imply that having such a high number of different antibodies is extremely rare among children with T1DM in our study population.

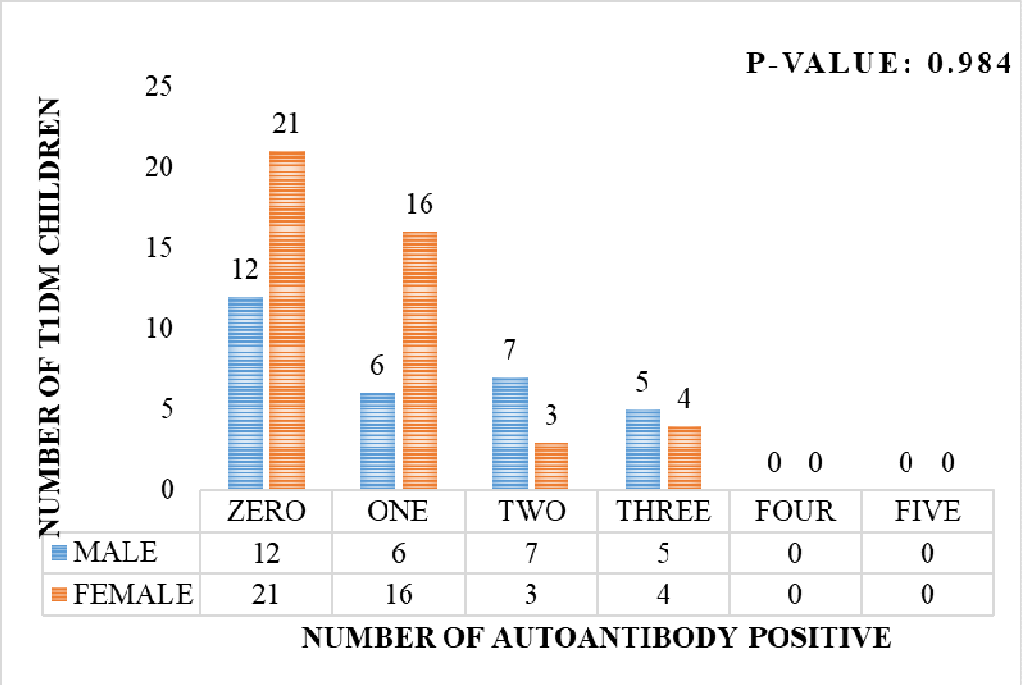


Fig. 43 Number of antibodies in male and female children with T1DM.

No antibodies are seen in 12 males and 21 females which is 16.2% and 28.4% respectively. Only one antibody is positive in 6 males (8.1%) and 16 females (21.6%). Two antibodies only is seen in 7 males (9.5%) and 3 females (4.1%). Three antibodies are present in 5 males (6.7%) and 4 females (5.4%).

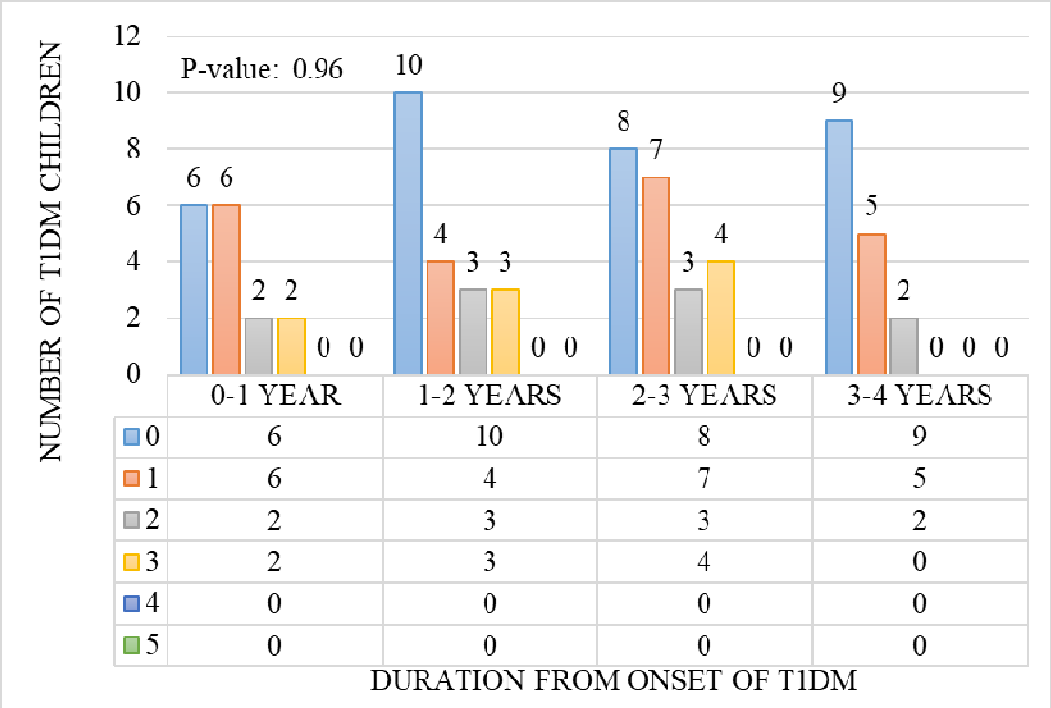


Fig. 44 Number of autoantibodies in each duration interval from the time of onset of T1DM

In the group of 0-1 year of duration of T1DM, 10 out of 16 (62.5%) T1DM children are positive for any one antibody. In group of 1-2 years 10 out of 20 (50%), 2-3 years 14 out of 22 (63.6%) and in 3-4 years 7 out of 16 (43.8%) are positive for any one antibody. The percentage of antibody positivity is highest within 2-3 years of onset of T1DM accounting for about 29.7%, followed by 1-2 of onset of T1DM with 27%. The percentage of antibody positivity is noted to decrease after 3 years of onset of T1DM.

The number of autoantibodies generally decreases as the duration since the onset of type 1 diabetes increases. This is evident from the decreasing counts as we move from left to right across the rows. There is no significant difference seen in number of children positive for one or more antibodies till three year duration of onset of T1DM. However there is a significant decrease in the number of autoantibody positivity more than 3 years of age.

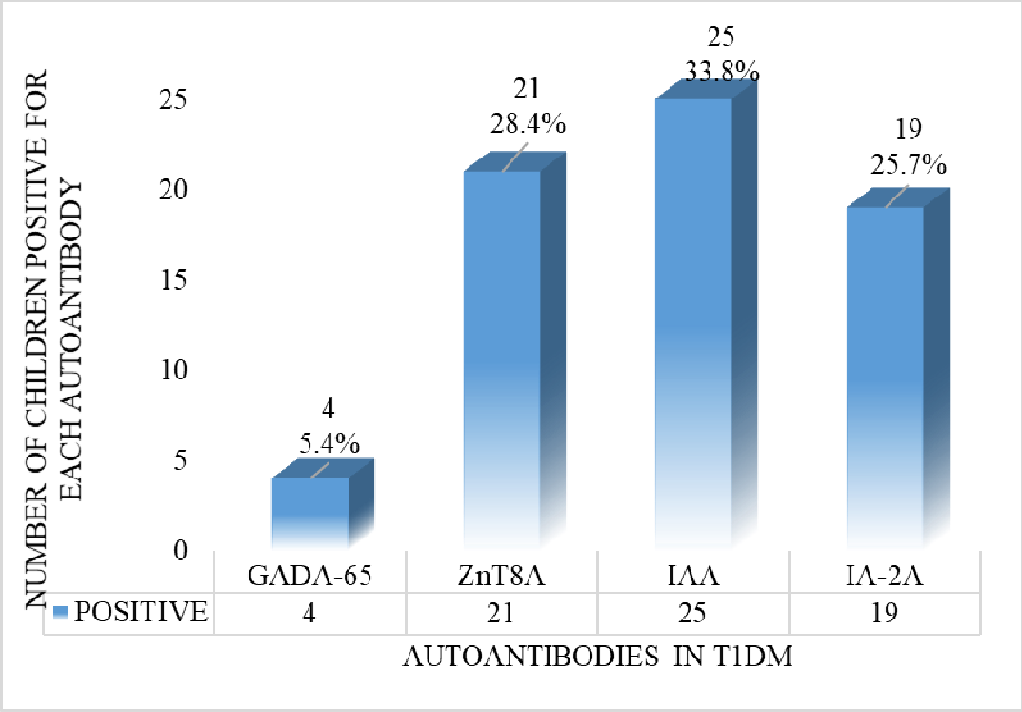


Fig. 45 Prevalence of different autoantibodies in T1DM children

The descending order of prevalence of specific autoantibody is as follows: IAA in present in 33.8% of T1DM children, ZnT8A in 28.4%, IA-2A in 25.7% and GADA-65 in 5.4%. ICA is seen to be absent in all T1DM children in our study population.

ZnT8A and **IAA** are the most prevalent autoantibodies among the T1DM children, with 21 and 25 cases, respectively. This suggests that these autoantibodies are commonly associated with T1DM in the studied population.

IA-2A is also prevalent, with 19 children with T1DM being positive for IA-2A.

GADA-65 has the lowest prevalence among the detected autoantibodies, with only 4children with T1DM.

Table 9 Profile of autoantibody in study population

AUTOANTIBODY	***TOTAL SUBJECTS (N=74)***	***PERCENTAGE***
GADA-65	1	1.35%
IA-2A	7	9.46%
ZnT8A	5	6.76%
IAA	9	12.17%
ICA	0	0
GADA-65 + IAA	1	1.35%
IA-2A + IAA	2	2.7%
ZnT8A + IAA	5	6.76%
ZnT8 + IA-2A	2	2.7%
ZnT8A + GADA-65 + IA-2A	1	1.35%
ZnT8A + GADA-65 + IAA	1	1.35%
ZnT8A + IA-2A + IAA	7	9.46%
NEGATIVE FOR ALL	33	44.59%

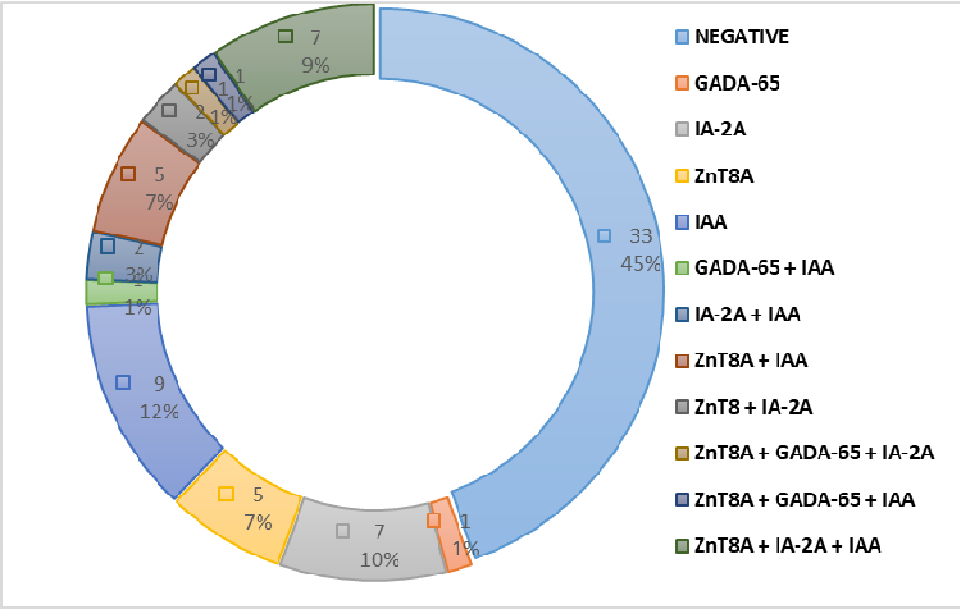


Fig. 46 Proportion of autoantibody in study population

Prevalence of Individual Autoantibodies:

IAA (Insulin Autoantibodies): With a prevalence of 12.17%, IAA is the most commonly detected autoantibody in the studied population of T1DM children. This suggests a high likelihood of insulin autoimmunity.

IA-2A (Islet Antigen 2 Autoantibodies): IA-2A follows IAA with a prevalence of 9.46%.

ZnT8A (Zinc Transporter 8 Autoantibodies): ZnT8A is detected in 6.76% of T1DM children, marking another considerable proportion exhibiting autoimmunity against zinc transporter 8.

GADA-65 (Glutamic Acid Decarboxylase 65 Autoantibodies): GADA-65 has the lowest prevalence among individual autoantibodies at 1.35%.

Notably, islet cell autoantibodies (ICA) are absent in the studied T1DM population.

Combinations of Autoantibodies: Combinations of autoantibodies are also observed, suggesting complex immune responses of children with T1DM. The combination of ZnT8A, IA-2A, and IAA is the most prevalent, accounting for 9.46%. Other combinations such as ZnT8A+ IAA and IA-2A + IAA are also notable, each representing 6.76% and 2.7% of cases, respectively.

Negative for All Autoantibodies: A significant portion of 44.6% T1DM children tested negative for all autoantibodies.

The high prevalence of IAA underscores its importance in the diagnosis and pathogenesis of autoimmune diabetes. The variety of autoantibody combinations highlights the heterogeneity of autoimmune responses in type 1 diabetes. The high percentage of autoantibody negativity indicate the need for further investigation into the diagnostic criteria and potential additional autoimmune markers in T1DM.

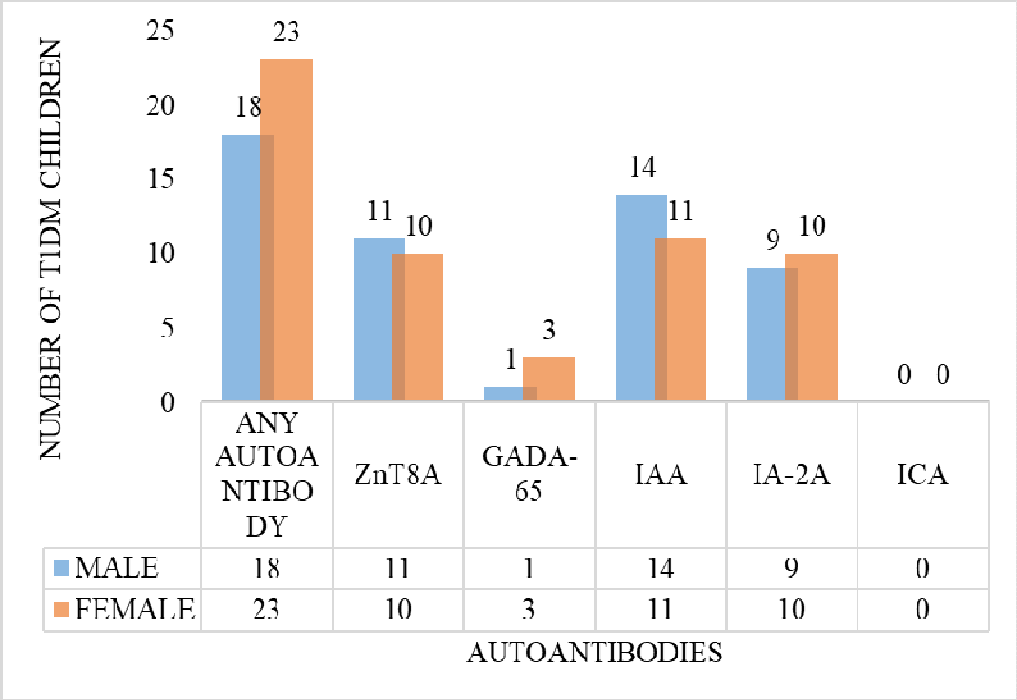


Fig. 47 Gender prevalence for each type of antibody of T1DM children

The gender prevalence of each individual autoantibody in T1DM children is as follows: ZnT8A is positive in 11 males and 10 females. 1 male and 3 females tested positive for GADA-65. 14 males and 11 females tested positive for IAA. 9 males and 10 females tested positive for IA-2A.

There were no positive tests for ICA in either males or females.

Female T1DM children have higher antibody positivity for GADA-65 and IAA. However, ZnT8A and IA-2A are higher in male children with T1DM.

The data suggests that there might be gender-specific differences in the prevalence of certain autoantibodies related to diabetes.

	Any antibody positive (n=41)	ZnT8A positive (n=21)	GADA-65 positive (n=4)	IAA positive (n=25)	IA-2A positive (n=19)	Chi Square	P value
Gender							
Male	18 (43.9%)	11 (52.4%)	1 (25%)	14 (56%)	9 (47.4%)	1.972	0.749
Female	23 (56.1%)	10 (47.6%)	3 (75%)	11 (44%)	10 (52.6%)		
Age group at onset of disease							
1-5 Years	12 (29.3%)	4 (19%)	1 (25%)	8 (32%)	2 (10.5%)	5.029	0.754
6-10 Years	17 (41.4%)	9 (42.9%)	2 (50%)	11 (44%)	8 (42.1%)		
11-15 Years	12 (29.3%)	8 (38.1%)	1 (25%)	6 (24%)	9 (47.4%)		
>15 Years	0	0	0	0	0		
Duration interval from disease onset							
0-1 Year	10 (24.4%)	5 (23.7%)	1 (25%)	6 (24%)	4 (21.1%)	6.907	0.864
1-2 Years	10 (24.4%)	6 (28.6%)	1 (25%)	9 (36%)	3 (15.8%)		
2-3 Years	14 (34.1%)	9 (42.9%)	2 (50%)	7 (28%)	7 (36.8%)		
3-4 Years	7 (17.1%)	1 (4.8%)	0	3 (12%)	5 (26.3%)		

Table 10 Demographic details of T1DM children with different autoantibody

This table gives data on the demographic details of the T1DM children in our study population with each different antibody positivity. Even though the P-value is not <0.05. It is seen that there is a female predisposition for overall antibody positivity. It is also noted that there is a greater proportion of males positive for ZnT8A and IAA. Whereas females constitute a larger proportion in those with IA-2A and GADA-65.

41.4% of children who developed T1DM between 6 to 10 years of age have antibody positivity. Whereas only 29.3% are positive when T1DM was diagnosed between 1 to 5 years of age and 29.3% when T1DM was diagnosed between 11 to 15 years of age. It is also noted that after a duration of 3 years of onset of T1DM, the number of antibody positivity significantly declines.

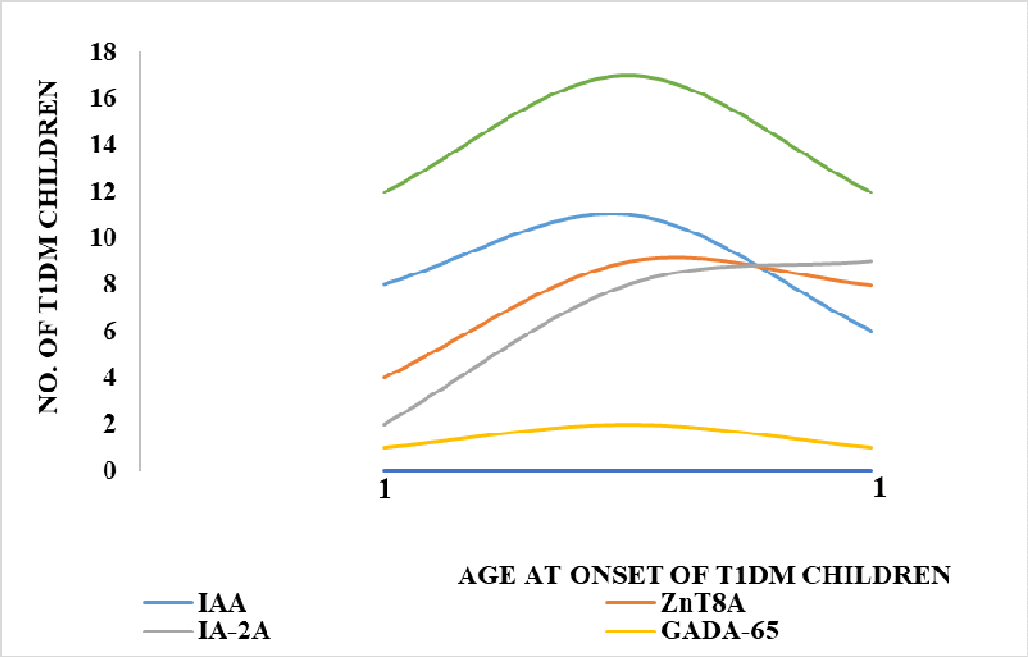


Fig. 48 Prevalence of different autoantibodies in comparison to age of onset of T1DM

Onset of T1DM at 1-5 Years: This age group has relatively low levels of ZnT8A, IA-2A, and GADA-65, with moderate levels of IAA.

Onset of T1DM at 6-10 Years: This age group has the highest levels of IAA, ZnT8A, and IA-2A, indicating a higher autoimmune activity.

Onset of T1DM at 11-15 Years: This group has high levels of IA-2A and ZnT8A but lower levels of IAA compared to the 6-10 years group.

The age group 6-10 years has the highest overall antibody count, suggesting a peak in autoimmune activity during these years. This group shows the highest levels of IAA, ZnT8A, and IA-2A, which are major autoimmune markers for T1DM.

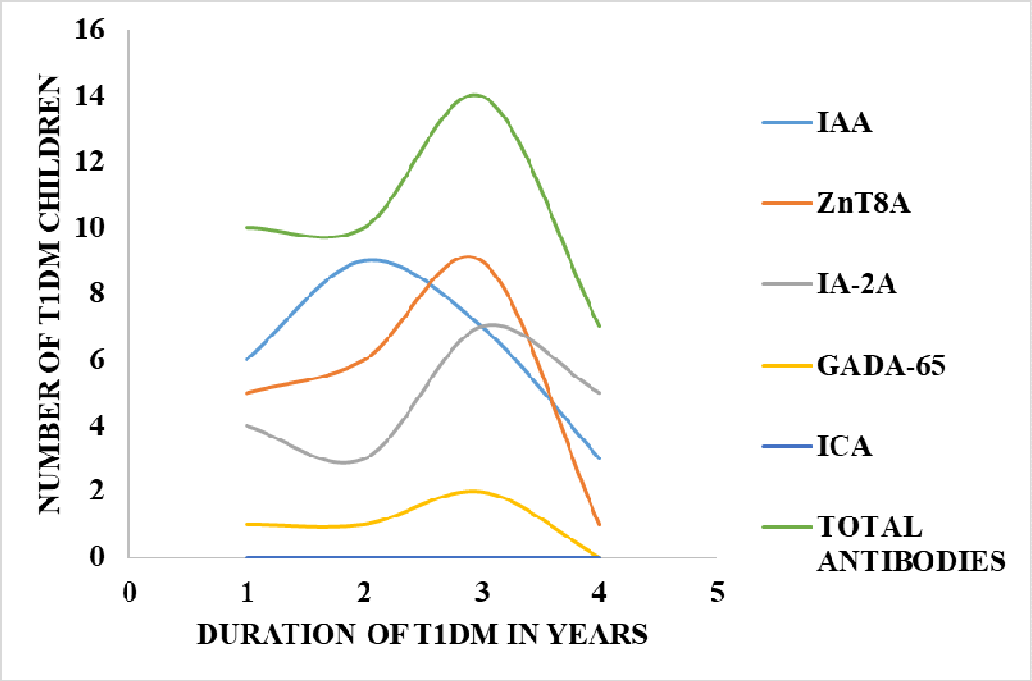


Fig 49 Prevalence of antibodies with duration of T1DM

In the first year of T1DM, the antibody levels are moderate with IAA being the highest. By 1-2 years of onset T1DM, there is an increase in IAA but a decrease in IA-2A. There is a peak in ZnT8A and IA-2A antibodies, and a slight decrease in IAA by 2-3 years of duration of onset of T1DM. After 3-4 years of T1DM, there is a significant decrease in all antibody levels except for IA-2A.

The data indicates that in children with T1DM, IAA is the most prominent in initially, but as the disease progresses, ZnT8A and IA-2A become more prevalent. The highest autoimmune activity is observed at 2-3 years post-diagnosis, with a subsequent decline.

CLINICAL PROFILE OF T1DM CHILDREN

Lab Parameter	Mean ± SD	Median	Minimum	Maximum
Hba1C at diagnosis (%)	12.23 ± 2.56	12.6	6.4	18.0
C-Peptide at diagnosis (ng/ml)	0.41 ± 0.55	0.3	0.0	3.2
TSH (mcIU/ml)	2.49 ± 1.31	2.1	0.3	6.6

Table 11 Lab parameters of T1DM children in the study

HbA1C at diagnosis (%): The mean HbA1C level at diagnosis of T1DM in the study population is 12.23%. HbA1c is a measure of blood sugar levels over the past 2-3 months. A value of 12.23% is significantly higher than the normal range (less than 5.7% is considered normal, 5.7%-6.4% indicates prediabetes, and 6.5% or higher indicates diabetes). The Standard Deviation of HbA1C is 2.56%.

C-Peptide at diagnosis (ng/ml): The mean C-Peptide level at diagnosis of T1DM in the study population is 0.41ng/ml. A value of 0.41 ng/ml is quite low, suggesting that the T1DM children, on average, have significantly reduced insulin production, which is common in T1DM. The Standard Deviation **is** (0.55 ng/ml) indicating considerable variability in C-peptide levels at the time of diagnosis of T1DM.

TSH (Thyroid-Stimulating Hormone (mcIU/ml): The mean TSH estimated is 2.49mcIU/ml. A mean of 2.49mcIU/ml falls within the normal range of TSH. Whereas the Standard Deviation of TSH is 1.31mcIU/ml indicating that there are few higher or lower values, due to some patients with subclinical or overt thyroid dysfunction.

	ANTIBODY POSITIVE T1DM CHILDREN	**ANTIBODY NEGATIVE T1DM CHILDREN**
Mean HbA1Cat diagnosis (%)	12.52	11.87
Mean C-Peptide at diagnosis (ng/ml)	0.29	0.56
Mean TSH (mcIU/ml)	2.60	2.34

Table 12 Difference in Lab parameters between autoantibody positive and negative T1DM children

The mean HbA1C levels at diagnosis is more in antibody positive group, which is a HbA1C level of 12.52% as compared to the antibody negative group with HbA1C level of 11.87%.

The mean C-peptide level at diagnosis is lesser in the antibody positivity group which is 0.29ng/dl as compared to 0.56ng/dl in the antibody negative group.

The mean TSH levels in each group is similar.

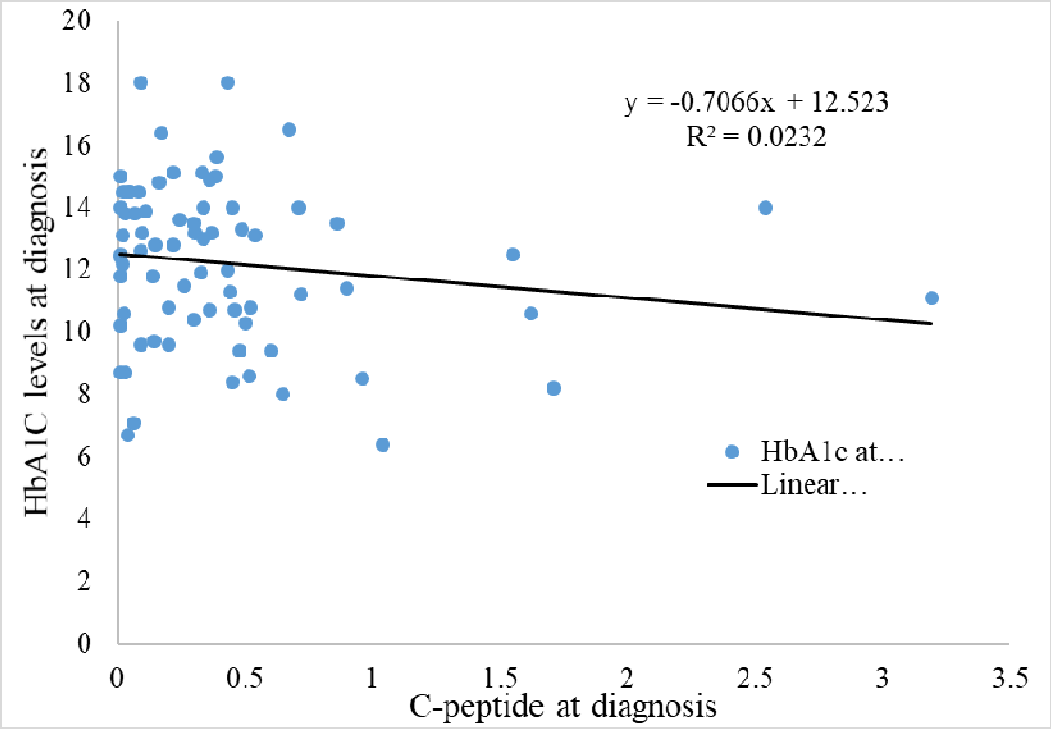


Fig. 50 Correlation between HbA1C and C-peptide at diagnosis of T1DM

The relationship between the HbA1C levels at diagnosis of T1DM and C-peptide levels at diagnosis of T1DM was evaluated using correlation graph. The coefficient of determination, R^2, was found to be 0.0232. This value indicates that approximately 2.32% of the variability in the initial HbA1C levels at diagnosis can be explained by the C-peptide levels at diagnosis. The C-peptide level has weak influence on the HbA1C levels at onset of T1DM.

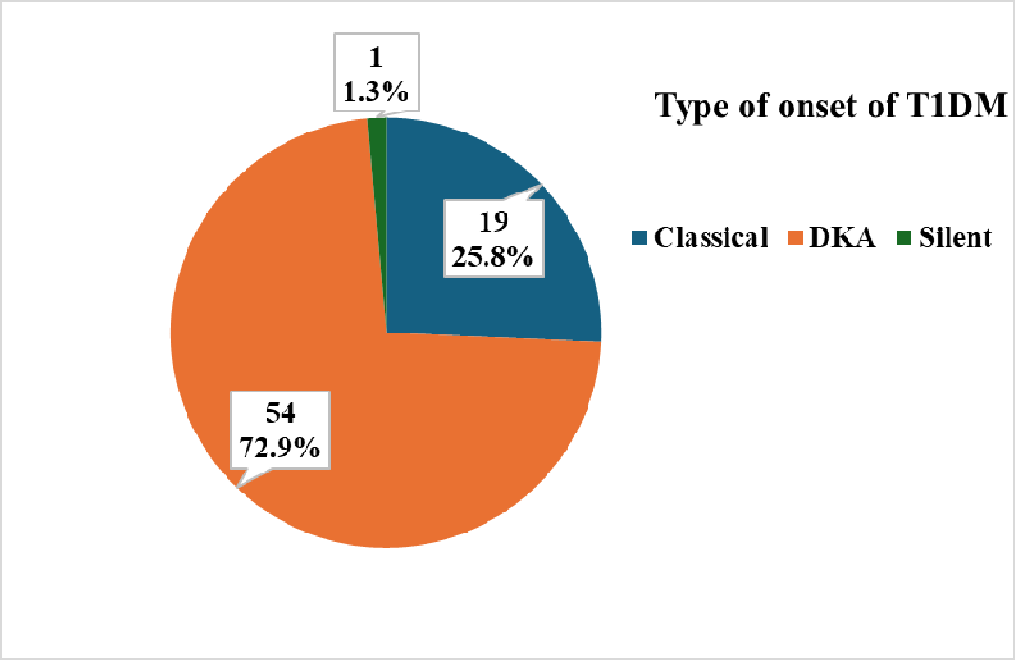


Fig. 51 Proportion of different type of presentation at onset of T1DM

The most common type of onset of T1DM in the study population is DKA, with 54 children, which constitutes a significant majority of 72.9%. Classical onset is the second most common presentation with 19 children, which is about 25.8%. Silent onset is rare, with only 1 child being diagnosed in this way.

The high prevalence of DKA as an onset type suggests that a substantial number of children are being diagnosed in a more acute, potentially severe state of T1DM. The lower incidence of classical onset might imply fewer cases being caught early and hence more awareness needs to be created in the community regarding T1DM in children. Silent onset, being very rare, indicates that more efforts in screening the at risk population to be formulated and implemented.

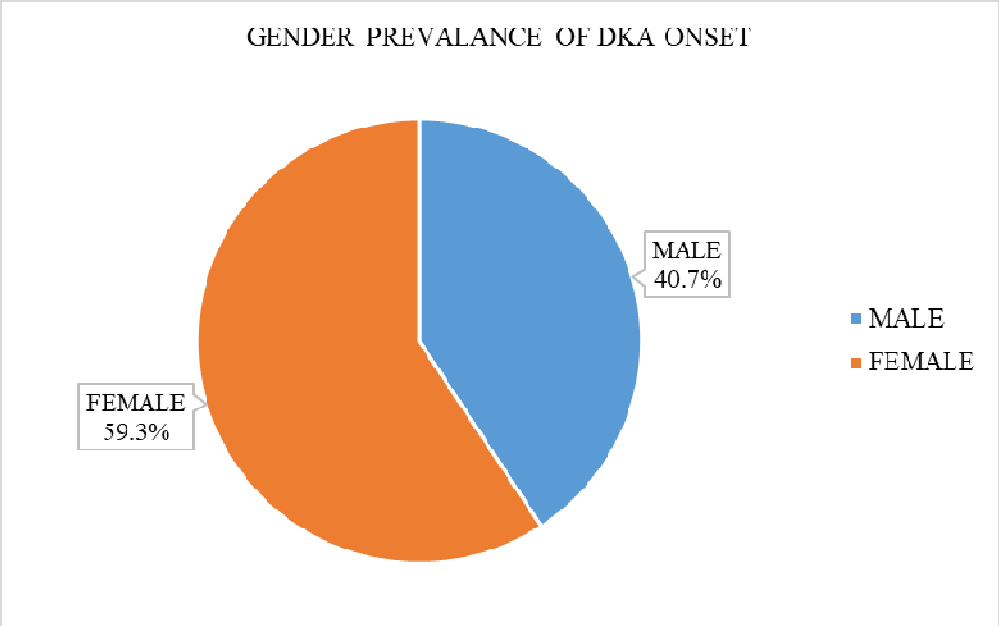


Fig. 52 Gender distribution in DKA onset of T1DM

Out of 54 DKA onset T1DM, female T1DM children are 32, which accounts for 59.3% of DKA onset cases. Male T1DM children who presented with DKA are 22 in number which is 40.7%.

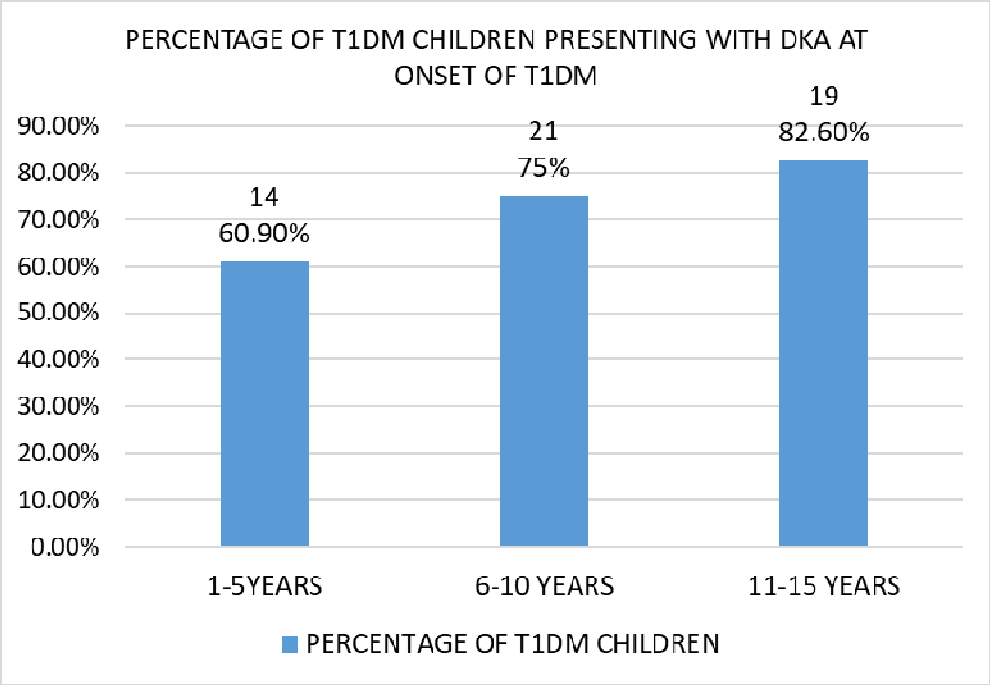


Fig.53 Percentage prevalence of DKA onset in different age group

1-5 Years: 60.90% (14 of 23) of children with T1DM are diagnosed within this age group.

6-10 Years: 75% (21 of 28) of children with T1DM are diagnosed within this age group.

11-15 Years: 82.60% (19 of 23) of children with T1DM are diagnosed within this age group.

This suggests that the likelihood of being diagnosed with T1DM increases as children grow older, with the highest percentage of diagnoses occurring in the 11-15 years age group.

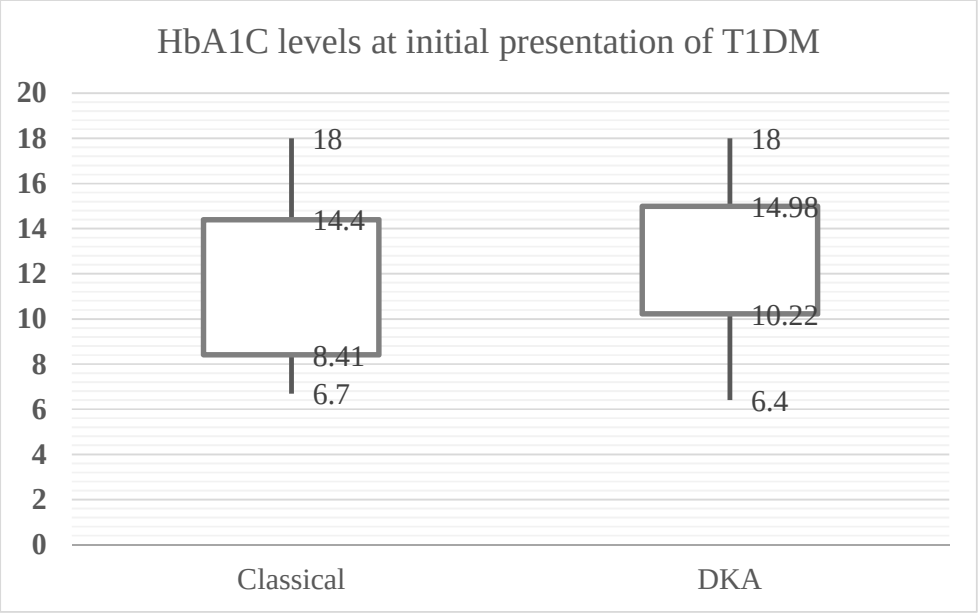


Fig. 54 Variation in HbA1C levels in T1DM children with type of presentation

The mean range of HbA1C levels at initial diagnosis of T1DM was 10.22 to 14.98 in the group which presented with DKA. Whereas, it was 8.41 to 14.4 in those who did not have DKA onset. This shows that those T1DM children with DKA onset had a higher HbA1C levels.

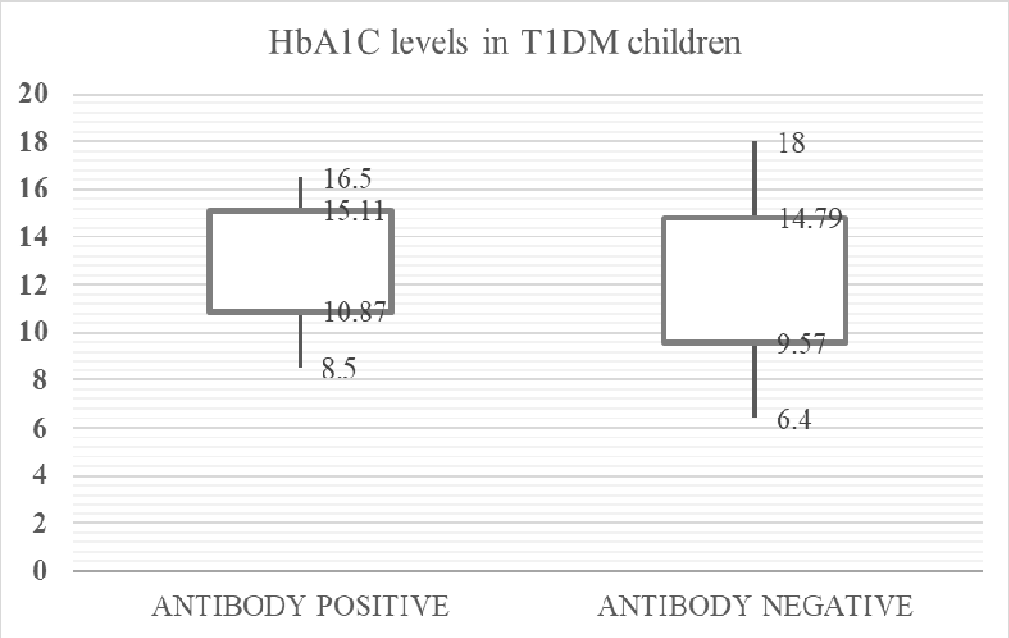


Fig. 55 Variation in HbA1C levels in T1DM children in relation to autoantibody

The mean range of HbA1C level at initial diagnosis of T1DM in those with autoantibody positivity was 10.87 to 15.11. Whereas, it was 9.57 to 14.79 in those who did not have autoantibodies.

Type of onset	Any antibody positive (n=41)	ZnT8A positive (n=21)	GADA-65 positive (n=4)	IAA positive (n=25)	IA-2A positive (n=19)	P value
DKA	28 (68.3%)	17 (81%)	4 (100%)	16 (64%)	14 (73.7%)	
Classical	12 (29.3%)	4 (19%)	0	9 (36%)	4 (21.1%)	0.657
Silent	1 (2.4%)	0	0	0	1 (5.2%)	

Table 13. Type of T1DM onset in relation to autoantibody positivity

The majority of T1DM children with antibody positivity presented with DKA, indicating a severe form of onset. A high proportion of ZnT8A positive T1DM children presented with DKA, suggesting this antibody is strongly associated with a severe onset of diabetes. All children with GADA-65 positivity presented with DKA, indicating an extremely strong association with severe diabetes onset. The majority of IAA positive patients presented with DKA, but a notable portion also had a classical onset, indicating a mixed association with diabetes onset. Most IA-2A positive patients had DKA, with some classical and silent onset, suggesting a strong but slightly less exclusive association with severe onset compared to other antibodies.

Overall, the presence of antibodies, especially ZnT8A, GADA-65, and IA-2A, is strongly associated with DKA onset. GADA-65 positivity showed a 100% association with DKA, making it a strong marker for severe diabetes onset. IAA positivity showed a higher proportion of classical onset compared to other antibodies, suggesting a slightly less severe onset pattern in some cases.

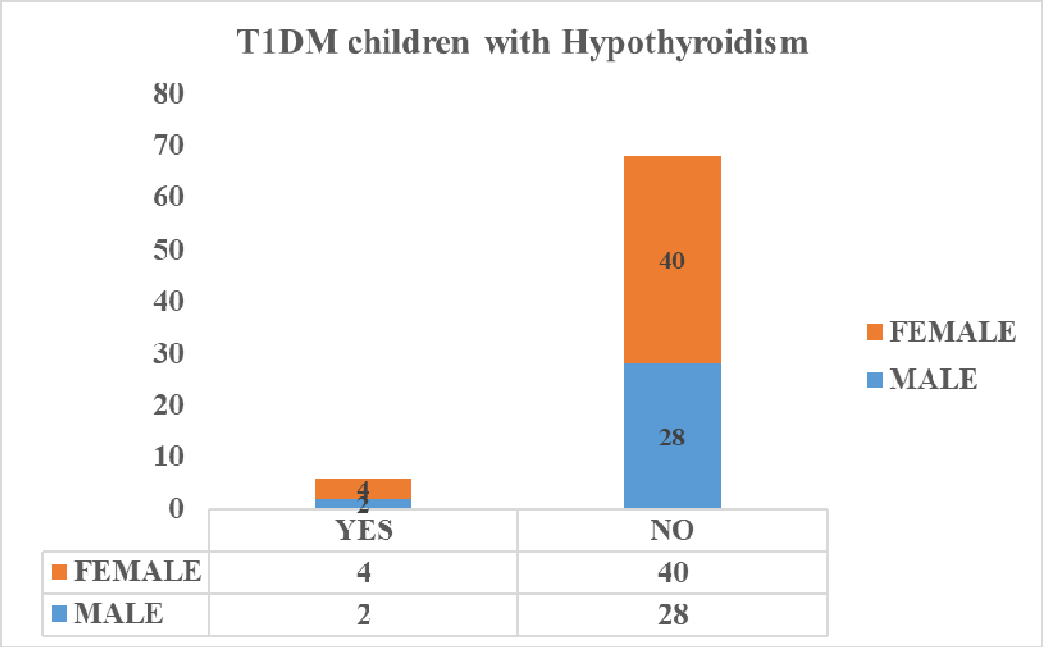


Fig. 56 Gender prevalence of hypothyroidism in T1DM children

Hypothyroidism is seen in 6 out of 74 children with T1DM in this study which accounts for prevalence of about 8.1%. Hypothyroidism is slightly more common in female children with T1DM (4 out of 44 T1DM female children, 9.09%) compared to male children with T1DM (2 out of 30 male T1DM children, 6.67%).

The number of females with hypothyroidism is double that of males. Of the total hypothyroid children with T1DM, 66.7% are female and only 33.3% are male. While hypothyroidism affects both males and females, it is more prevalent among females.

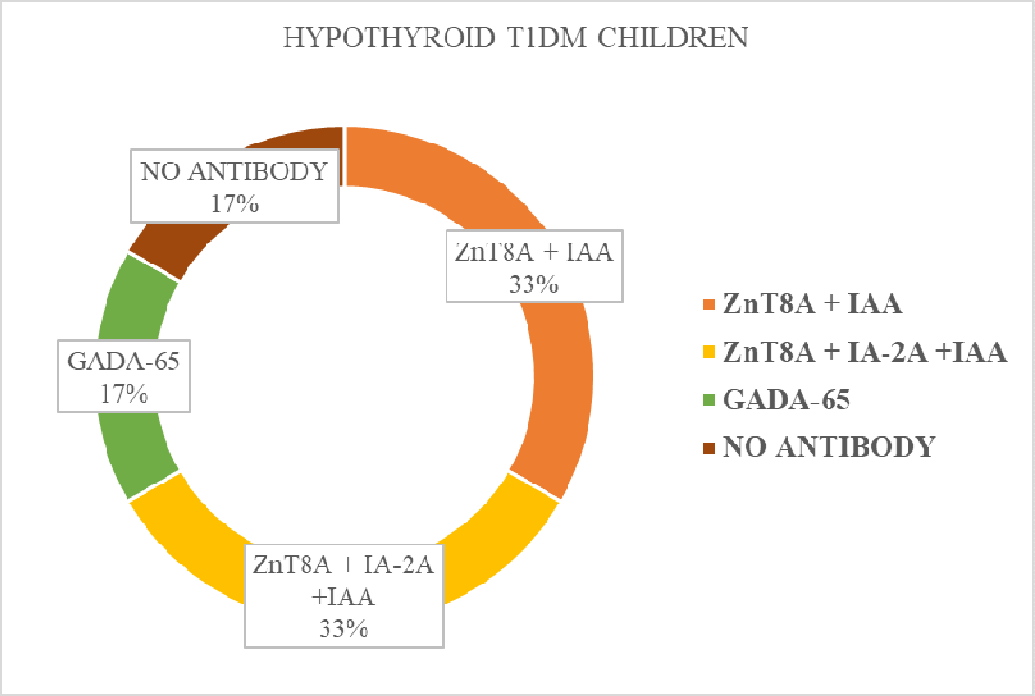


Fig. 57 Profile of T1DM autoantibody in hypothyroid children with T1DM

Prevalence of Autoantibody Profiles:

- ZnT8A + IAA: Found in 2 out of 6 children – 33.3%
- ZnT8A + IA-2A + IAA: Found in 2 out of 6 children – 33.3%
- GADA-65: Found in 1 out of 6 children – 16.7%
- No Antibody: Found in 1 out of 6 children – 16.7%

Among hypothyroid T1DM children, the ZnT8A + IAA and ZnT8A + IA-2A + IAA autoantibody profiles are equally prevalent and more common. This suggests that these two combined autoantibody profiles might be significant markers in this group of children.

Autoantibody	Hypothyroidism (N=6)		P-value
	Yes	No	
ZnT8A	4 (66.7%)	2 (33.3%)	**0.124**
GADA-65	1 (16.7%)	5 (83.3%)	
IAA	4 (66.7%)	2 (33.3%)	
IA-2A	2 (33.3%)	4 (66.7%)	
Any antibody	5 (83.3%)	1 (16.7%)	

Table 14 Hypothyroidism in relation to different autoantibodies in T1DM

4 out of 6 T1DM children with hypothyroidism tested positive for ZnT8A, accounting for 66.7%. This suggests that ZnT8A autoantibodies are present in a significant portion of T1DM children with hypothyroidism in this study. Only 1 out of 6 T1DM child with hypothyroidism tested positive for GADA-65, which is 16.7%. GADA-65 autoantibodies appear to be less prevalent with hypothyroidism compared to other autoantibodies tested. Similar to ZnT8A, 4 out of 6, 66.7% of T1DM children with hypothyroidism tested positive for IAA. This indicates a notable presence of IAA autoantibodies with hypothyroidism in this study. 2 out of 6 individuals with hypothyroidism tested positive for IA-2A, accounting for 33.3%. IA-2A autoantibodies appear to be less prevalent compared to ZnT8A and IAA but more prevalent than GADA-65.

The majority (5 out of 6 children, 83.3%) with hypothyroidism tested positive for at least one of the autoantibodies listed. Only 1child (16.7%) tested negative for all autoantibodies tested. This suggests that the presence of autoantibodies is common among T1DM children with hypothyroidism in this study.

Autoantibody	Celiac disease symptoms (N=5)		P-value
	Yes	**No**	
ZnT8A	1 (20%)	4 (80%)	
GADA-65	1 (20%)	4 (80%)	
IAA	1 (20%)	4 (80%)	**0.924**
IA-2A	1 (20%)	4 (80%)	
Any antibody	2 (40%)	3 (60%)	

Table 15 Celiac disease in relation to different autoantibodies in T1DM

The data indicates that among T1DM children with celiac disease symptoms, the prevalence of autoantibodies associated with T1DM (ZnT8A, GADA-65, IAA, IA-2A) is low. Only a small proportion of T1DM children with celiac disease symptoms tested positive for any of these autoantibodies, suggesting a weaker association between these autoantibodies and celiac disease symptoms in this cohort.

Autoantibody	Cow milk exposure <1year age (N=35)		P-value
	Yes	No	0.561
Any antibody	19 (54.3%)	16 (45.7%)	

Table 16 Autoantibodies in T1DM in relation to exposure to cow milk in infancy

35 out of 74 T1DM children were started with cow milk in their infancy. Of these 35 T1DM children, only 19 had autoantibody positivity. In those who were fed with cow milk at age less than 1 year, 7 were positive for ZnT8A, 2 for GADA-65, 11 for IAA and 22 for IA-2A.

Overall, the exposure to cow milk does not have an impact on development of autoantibody against beta-cells.

DISCUSSION

This study is a hospital based, single centered, cross-sectional study done in a cohort of 74 T1DM children from Southern India, aged 1 year to 18 years, with a total duration of disease onset < 4 years.

The mean age of T1DM cohort in this study is 10.18±3.85years. Whereas, in a study done by Basu M, et al., in Kolkata, the mean age of the T1DM children during their study period was 14±3.99 years [107].

The mean age at diagnosis in this study is 8.11 ± 3.73 years with a minimum age of 1 year and maximum age of 15 years. This finding is similar to study done by Verma H, et al., where T1DM was diagnosed among children around 8.8±4.09 years [108]. Whereas the United States SEARCH registry has reported a mean age of diagnosis to be 10±4.5 years [109] and Sharma B, et al. study has also reported the age of diagnosis to be 10±3.63 years [110]. Compared to these two reports the mean age at diagnosis of T1DM in our study group was earlier.

The duration from onset of T1DM in this study is 1.97±1.11 years. Contrastingly, it was 6.3±4 years in the study done by Basu M, et al [107]. This difference is because our study included only those T1DM children with 4 years of duration from onset of T1DM.

The anthropometric values of the study cohort are below the average for given age and sex. Mean SD of height for age is -0.6, with 3 of 74 (4.1%) having short stature. The mean SD of weight for age is -0.69, with 4 of 74 (5.4%) having thinning. The mean BMI is 15.9 and mean SD of BMI for age is -0.55, with 69 (93.2%)

children having normal BMI, 3 (4.1%) undernourished and 2 (2.7%) being overweight. In a study done by Naaraayana SA, Et al. in Chennai, reported 14.4% short stature, 16% had thinning, 3.2% overweight and 2.2% were obese [111]. Compared to this study result, our study population has lesser short stature and thinning, indicating a healthy population. The mean waist to hip ratio is 0.92 with a variation of 0.06, which shows that there is no associated central obesity in the study population of T1DM children.

Our study shows female predisposition to T1DM with 59.5% of the study population of T1DM children being females and the remaining 40.5% being males, with male: female ratio being 0.68. Our result is in concordance with studies by Narayan SA, et al. which also showed a female preponderance with male-female ratio of 0.67 [111] and Basu M, et al. which reported 64.1% females with male: female ratio of 0.56 [107]. Whereas the findings from Indian YRD registry (Youth Onset Diabetes in India) and SEARCH registry of United States show less female proportion of 47.1& and 46.7% [109]. T1DM is common in boys as compared to girls in European countries like Finland, UK, Italy, Norway and Sweden [5].

In this study, 37.8% were diagnosed with T1DM around 6-10 years of age. From 6-15 years of age, 68.9% were diagnosed with T1DM. Our findings were similar to a study done by Al-Fifi SH in Saudi Arabia where only 27.6% of T1DM children presented before 5 years and the rest 72.4% presented after 5 years of age [112]. Whereas in a multicentric study done by DIAMOND Project Group [2], the incidence of T1DM was reported to increase with increase in age. Where as in our study the proportion of children in all the three age groups were almost similar with

31.1% in 1-5years age group, 37.8% in 6-10years age group and 31.1% in 11-15years age group.

Our study is comprised of 60.5% of cohort belonging to lower-middle and 22.4% belonging to upper-middle socio-economic class, indicating that environmental factors are triggering the development of T1DM in this socio-economic group. Finding of our study is similar to that in the study done in a tertiary care center near Foothills of Himalayas by Verma H, et al., where 56.2% belonged to lower-middle class and 34.4% belonged to upper-middle class [108]. However our study varies from the SEARCH registry of United States and Indian YDR registry which have reported 53.6% and 60.8% of T1DM children belonging to high socioeconomic class [109].

Seasonal variation was also noted in our study population with maximum percentage of children with onset of disease during monsoon and post-monsoon. The viral infections which are common during the cold weather, triggers the autoimmunity resulting in beta-cell destruction. Whereas in a study done by Moltchanova EV, et al, analyzing the findings of DIAMOND study (A project of World Health Organization) in UK, reported T1DM incidence to be more in winter and autumn months [7]. This difference in study result can be due to difference in the temperate zones of the place of study.

In this study, 48.6% of the T1DM children have a family history of diabetes with 4.1% of them having history of only T1DM in the family, 37.8% have family history of T2DM only and 6.7% having family history of both T1DM and T2DM. In those with T1DM history in the family, 9.5% of them had sibling with T1DM and 1.4% had father with T1DM. The percentage of history of T2DM in family members in our study was similar to that reported in the study done by Naaraayan SA, et al.,

where 37.5% had family history of T2DM. However the percentage of siblings with T1DM was reported to be only 1.6%, which is less as compared to our study which shows 9.5% [111].

Our study has 55.4% autoantibody positivity in T1DM children. The remaining 44.6% are autoantibody negative. Lower rates of antibody positivity in T1DM children has been noted in Asian population in a global review done by Ross C, et al., as compared to Western and European population [74]. The exact reasons for this low prevalence of autoantibodies in Asian population and in India population is not known. In the study done by Basu M, Et al, on autoantibody profile in T1DM, 97.8% positive prevalence of autoantibodies was reported [107]. A cross-sectional study done by Vipin VP et al. in India reported about 30% of T1DM children to be having no autoantibodies [75]. A study done in Barbada Davis Center, Colorado from October 1992 to October 2004 also reported 19% of autoantibody negativity in newly detected T1DM children and the autoantibody negativity was more with increasing age [76]. Our study reports a higher percentage of autoantibody negativity as compared to other studies.

In our study, 29.7% have any one antibody positivity, 13.5% have any two antibody positivity, 12.2% have any three antibody positivity and none are positive for four or more antibodies. Whereas an Indian study by Basu M, Et al. study showed any single antibody in 97.8%, two antibodies in 67.3%, three antibodies in 21.7%, four antibodies in 3.2% and all five antibodies in 3.2% [107]. This may be due to difference in the genetic predisposition in developing certain antibodies in different ethnic groups.

The descending order of prevalence of specific autoantibody in our study is IAA, ZnT8A, IA-2A, GADA-65. ICA is absent in all the individuals of our study population. According to the global review by Ross C, et al., , the most prevalent autoantibody in newly diagnosed T1DM children is IA-2A followed by ICA, ZnT8A, GADA-65 and IAA in the decreasing order of prevalence [74]. In children with established T1DM, GADA-65 was the most prevalent followed by ZnT8A, IA-2A, IAA and ICA [74]. Our study findings doesn't correlate with the global review report.

The prevalence of GADA-65 autoantibody in our study is 5.4%, ZnT8A is 28.4%, IAA is 33.8% and IA-2A is 25.7%. ICA is absent is all the T1DM subjects in our study. An Indian study done by Basu M, et al. have reported GADA-65 positivity to be 79.3%, ZnT8A to be 20.65%, IAA to be 63%, IA-2A to be 32.6% and ICA to be 61.9% [107]. ZnT8A and IA-2A prevalence are almost correlating, whereas the IAA and GADA-65 prevalence is low in our study. ICA prevalence is zero maybe because of difference in genetic makeup and the pathogenesis of development of T1DM and beta-cell destruction.

Also in our study, in those with autoantibody positivity, again the gender predisposition is more towards females who constitute about 56.1%, whereas male account for only 43.9%. Whereas when we took individual gender and looked for antibody positivity, 60% of T1DM males had antibody positivity and only 52.3% females with T1DM had autoantibodies. The gender prevalence of GADA-65 and IA-2A is again towards female with female percentage being 75% and 52.6% respectively for GADA-65 and IA-2A. Higher GADA-65 positivity finding in our study was similar to the study done by Naaraayan SA, et al., where GADA-65 positivity in females was 61.6% [111]. Whereas, number of males are to be more in

those with ZnT8A and IAA accounting for 52.3% and 56% respectively. Females were found to be more in the group with those having only one antibody positivity. However, males are more in number as compared to females in those group with two or more antibody positivity.

Our study showed peaking of autoantibody in children who presented with T1DM between the age of 6 to 10 years. Where as in a study done in Qatar by Haris B, et al., the autoantibody positivity increased from 1 year to 15 years of age and then showed decline [113]. In our study it was noted that the percentage of T1DM children with antibody positivity declined after 3 years of duration of onset of the disease. The study done by Rodacki M, et al., in Brazil mentions that the ICA and IA-2A decline after T1DM is established [114]. Study on ZnT8A kinetics by Wenzlau JM, et al., reported that the levels of ZnT8A decline after a duration of 4 years of onset of T1DM [87]. The early fading away of antibodies can be the cause of low antibody prevalence in our study.

The DKA onset in our study is 72.9%. Whereas the studies from Saudi Arabia have reported prevalence of DKA onset to range from 55 to 77 % [112]. Indian study by Naaraayan, et al. has reported DKA onset to be 57.6% [111]. The higher prevalence in our study is due to lack of awareness about T1DM in the community.

Our study reports prevalence of hypothyroidism in T1DM children to be 8.1%. Sanyal, et al. study on thyroid profile in T1DM have reported 6% of hypothyroidism and 32-68% of subclinical hypothyroidism [115].

Our study also reports prevalence of suspected celiac disease to be 6.8%. In a longitudinal study done by Lindgren M, et al., the prevalence of celiac disease in T1DM children was 9.8% and 95.9% were diagnosed within 5 years of duration of onset of T1DM [116].

Our study shows no statistical significance between exposure to cow milk and development of different autoantibodies related to T1DM. FINDIA study done in Finland, has reported that weaning to bovine insulin free formula milk reduced the cumulative incidence of islet autoantibodies in children with high genetic predisposition at 3 years age [54]. TRIGR study which was done in 15 different countries showed that the children who received casein hydrolyzed formula feeds had decreased risk of development of autoantibodies as compared to those who were given cow milk [55].

LIMITATION AND SCOPE OF THE STUDY

- This is a cross sectional study, measuring disease related autoantibodies in T1DM. Our study is one of the first to evaluate for all the major autoantibodies related to T1DM in children in India within four years of onset of the disease. We also tried to correlate the number of autoantibodies present with DKA onset, hypothyroidism and celiac disease.
- However, all T1DM children were not evaluated at the time of initial presentation of the disease for autoantibodies. The duration of disease ranged from 1 month to 4 years which may be the reason for only 55.4% of T1DM children having autoantibodies in our study.
- The sample size is 74. If more number of children were evaluated there would have been a better statistical significance.
- As T1DM children with duration of disease less than 4 years were included in the study, exact time of onset of hypothyroidism and celiac disease in T1DM and the prevalence of these conditions in long standing T1DM could not be estimated.
- So, we recommend to conduct more studies on prevalence of different disease related autoantibodies in T1DM at the time of initial presentation of the disease, so that exact prevalence of autoimmune T1DM is established in India.

CONCLUSION

Our study shows 55.4% prevalence of autoantibodies in T1DM children. This could be because of early fading away of autoantibodies after onset of T1DM. Also the minor autoantibodies may be present in our study population which were not estimated in our study. The correlation between autoantibodies and T1DM associated autoimmune diseases like hypothyroidism and celiac disease could not be established statistically, as it was a cross-sectional study and the cohort included only those T1DM children with duration of onset of disease of 4 years. Further well designed studies and estimation of major autoantibodies related to T1DM and also estimation of minor autoantibodies at initial presentation of the T1DM is required to understand the exact pathogenesis of the disease in Indian population.

SUMMARY

Type 1 Diabetes Mellitus (T1DM) is an endocrine and metabolic disorder, which has a major impact on physical and psychological development of children. Patients with T1DM commonly have autoantibodies to the islet cells – Islet Cell antibodies (ICA), 65-kD form of glutamate decarboxylase antibody (GADA-65), insulinoma associated protein-2 antibody (IA2A), Insulin autoantibodies (IAA) and zinc transporter ZnT8 antibody (ZnT8A). There is a wide heterogeneity in the pathogenesis, profile of autoantibodies and disease course of T1DM children worldwide.

The prevalence of the different types of autoantibody in autoimmune T1DM and the prevalence of other non-immune T1DM will help in formulating the guidelines for screening of at-risk children. The data on the prevalence of autoantibodies present in T1DM children in India is limited.

A one year cross sectional study was done from October 2022 to September 2023 in KLE'S Dr. Prabhakar Kore hospital and medical research center, Belagavi, Karnataka, India. The study enrolled 74 Type 1 diabetes mellitus children with duration of onset of diabetes of less than four years. The salient findings of the study are as follows:

- The mean age of T1DM children in the study population was 10.18±3.85 years.
- The mean age at which T1DM was diagnosed was 8.11±3.73 years.
- The mean duration of T1DM from onset of disease was 1.97±1.11 years.
- Female constituted 59.5% and males 40.5%.
- 46 children, 60.5% belonged to lower-middle socio-economic class and 17 children, 22.4% belonged to upper-middle socio-economic class.

- 62.2% children had T1DM onset during monsoon (36.5%) and post-monsoon (25.7%).
- 48.6% T1DM had family history of diabetes, and 10.8% had history of T1DM in the family.
- Autoantibodies were positive in 55.4% of the children.
- IAA was present in 33.8% of T1DM children, ZnT8A in 28.4%, IA-2A in 25.7% and GADA-65 in 5.4%. Whereas ICA was noted to be absent in all T1DM children.
- The autoantibody positive T1DM was prevalent in those diagnosed between 6 to 10 years of age as compared to other groups.
- Autoantibody prevalence was seen to decline after 3 years of duration of onset of T1DM.
- DKA was the most common type of presentation which accounted for about 72.9%. The HbA1C levels at diagnosis was found to be comparatively higher in those who presented with DKA. C-peptide levels at diagnosis was found to be comparatively lower in those who presented with DKA.
- The prevalence of hypothyroidism in T1DM children in our study is 8.1%.
- The prevalence of probable celiac disease in T1DM children in our study is 6.8%.
- There was no association between autoantibody positivity and DKA onset, hypothyroidism and celiac disease in T1DM.
- Our study at the end recommends the need for further studies to be done on prevalence of autoantibodies in different population groups and also to evaluate for presence of other minor autoantibodies.

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ANNEXURE I

INFORMED CONSENT FORM

"PROFILE OF AUTO-ANTIBODIES AND ITS CORRELATION WITH CLINICAL PROFILE IN TYPE 1 DIABETIES MELLITUS SUBJECTS – A CROSS SECTIONAL STUDY FROM SOUTHERN INDIA"

Name of Student/Principal Investigator: _____

Name of Guide/Co Investigators: _____

Objectives:

PRIMARY OBJECTIVE: Prevalence of autoantibodies in type 1 diabetes mellitus

SECONDARY OBJECTIVE: Compare the clinical characteristics in children with number of autoantibodies.

Introduction: Type 1 diabetes is the most common form of diabetes in children. Studies have shown that one or more autoantibodies are present in more than 90% of individuals. These autoantibodies are present months to years before the child comes with symptoms to a doctor. But there is very less information as to which autoantibody is more common in India children. By doing this study we will be able to gather information regarding the most common autoantibody so that later we can use it as a screening test to all children at risk of developing diabetes and prevent life-threatening complications like diabetic ketoacidosis. This may further help in developing some preventive strategies and as may help for further research related to type 1 diabetes meatus in children.

Explanation of procedure: Participants and parents will be asked questions related to their symptoms and clinical presentation, drug history, social and family history and details will be entered in a proforma, clinical examination of the child will be performed, anthropometric measurements will be recorded and two 3ml each of plain blood samples will be taken for antibodies estimation.

Withdrawal from participation in the study: Participation in this study is voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: You will/will not have nor get any benefits by participating in this study. The data gathered will help the population at large.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person from identifying you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purposes and or presented to scientific groups. However, your identity will never be revealed.

Legal rights: By signing this consent form, we are not waiving any of your legal rights.

Questions:

If you have any question or complaints with regard to your right as study participant you may contact Dr. Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waiving any of your legal rights.

CONSENT STATEMENT

I am making a voluntary decision to participate in the study **"PROFILE OF AUTO-ANTIBODIES AND ITS CORRELATION WITH CLINICAL PROFILE IN TYPE 1 DIABETIES MELLITUS SUBJECTS – A CROSS SECTIONAL STUDY FROM SOUTHERN INDIA"** My signature below indicates that I have decided to participate/ let my child participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the child:

Age of the child:

Signature or left thumb impression of the child:

(If more than 12years)

Verbal consent from child:

(7 to 12 years)

Name of the parent/ guardian:

Signature or left thumb impression of the parent/guardian:

Name of the investigator:

Signature of investigator:

ANNEXURE II

PROFORMA

ID NO.:

Name: **OP No.:**

DOB: **Age:** **Sex:** **Education:**

Address:

Ph. No.:

Mother's age: **Education:** **Occupation:**

Father's age: **Education:** **Occupation:**

Family income:

Socio-economic status:

HISTORY:

Date of diagnosis -

Age at diagnosis -

Duration of diabetes –

Season at presentation -

Type of presentation – Classical new onset

Silent presentation

DKA

Any complaints at present -

Compliance to intake of insulin- Regular / Irregular

Increase / Decrease in insulin dosage since time of diagnosis -

Number of DKA episodes since diagnosis –

Episodes of hypoglycemia – Frequent / Rare

History of weight loss –

History of loose stools, pain abdomen, loss of appetite -

History of any associated chronic complications –

History of any other comorbidities –

PAST HISTORY:

Infection – Measles, Mumps, Rubella, CMV, EBV, Rota, Coxsackie B, SARS CoV2, HIV

Autoimmune disorders – Hypothyroidism, Hyperthyroidism, Celiac disease, Addison disease, Vitiligo, Alopecia, SLE, Rheumatoid arthritis, Vasculitis

Other endocrine disorders – Yes/No, if yes, name the condition

History of any toxic drug intake in past – Yes/No

FAMILY HISTORY:

Consanguinity: Yes/ NO

H/O Diabetes in family: Yes / No. If yes: Type1/Type2/Others

H/O any autoimmune disorders in family: Yes / No

If yes, mention the disease

H/O any endocrine disorders in family: Yes / No

If yes, mention the disease

Siblings with diabetes: Yes / No

If yes, Age of presentation:

Type of presentation:

Duration:

History of any other endocrine or autoimmune disorder:

Any other comorbidity:

BIRTH HISTORY:

Antenatal*:* H/o GDM in mother Yes/No

H/o fever with rash in mother Yes/ No

TORCH infections Yes/No

Intake of any drugs

Natal*:* Birth weight

Gestation

Type of delivery with indication

Post natal*:* Type of feeding- DBF/Top feeds

Duration of exclusive breast feeding

Pre lacteal feeds

Weaning started at

H/o NICU admission

H/o any infection

Any deformities

H/o cow milk exposure before 1 year of age

IMMUNIZATION HISTORY:

Immunization completely taken as per age- Yes / No

Are below vaccines taken –

Measles Yes/No; Mumps Yes/No; Rubella Yes/No; Influenza Yes/No

Rotavirus Yes/No; Covid-19 Yes/No

Any other viral vaccine if taken –

CLINICAL EXAMINATION:

Vitals: HR- BP- RR-

Anthropometry:

PARAMETERS	MEASURED	EXPECTED	PERCENTILE	INFERENCE
Weight [in kg]				
Height [in cm]				
BMI				
Waist to hip ratio				

Head to toe examination:

Thyroid examination:

Hyperpigmentation:

Lipodystrophy/lipoatrophy:

Any other features of diabetic and complications of diabetes:

SMR staging (if applicable):

Systemic examination:

RS- Normal/Any positive findings

PA- Normal/Any positive findings

CVS- Normal/Any positive findings

CNS-

Vibration sensation – Normal/ Reduced sensation

Touch (10g monofilament test) – Normal/Reduced sensation

Proprioception – Normal/Abnormal

Ankle jerk – Normal/Diminished reflex

Foot examination: Normal/Any unhealed wound/ Multiple healed scars present

Ophthalmic examination: Right eye Left eye

Visual acuity

Lens

Fundus (Diabetic retinopathy)

INVESTIGATIONS:

HbA1C (at the time of diagnosis)

C-peptide level (at the time of diagnosis)

Lipid profile: Cholesterol

HDL

LDL

Triglycerides

RFT: Urea Creatinine eGFR

LFT: SGOT SGPT

Thyroid: TSH

Urine examination: Urine albumin creatinine ratio

Autoantibodies:

GAD-65

IAA

IA-2

ZnT8

ICA

Other tests if any:

ANNEXURE III – MASTER CHART

ID NO.	AGE	GENDER	AGE AT DIAGNOSIS	DIAGNOSIS AGE INTERVAL	DURATION OF T1DM	DURATION INTERVAL	SOCIO-ECONOMIC ST	SEASON AT PRESENTATION	DIABETES IN FAMILY	T1DM IN FAMILY MEMBER	TYPE OF ONSET	PROBABLE CD	HYPOTHYROIDISM	COW MILK EXPOSURE	SD OF WEIGHT FOR AGE	SD OF HEIGHT FOR AGE	BMI	SD OF BMI	WAIST:HIP	HbA1c at diagnosis	C-Peptide at diagnosis	TSH	Ab POSITIVE	NO. OF POSITIVE Ab	GADA-65	IAA	ICA	IA-2A	ZnT8A
1	15	M	11	11-15 YEARS	3.58	3-4 YEARS	LOWER-MIDDLE	POST MONSOON	TYPE 2	NO	CLASSICAL	NO	NO	YES	-0.45	0.31	17.21	-0.66	0.98	14	0.45	3.68	YES	2	N	P	N	P	N
2	9.75	F	7	6-10 YEARS	2.75	2-3 YEARS	LOWER-MIDDLE	MONSOON	TYPE 1	SIBBLING	CLASSICAL	NO	NO	YES	-2.07	-1.98	13.38	-1.46	0.94	13.2	0.304	1.32	YES	1	N	N	N	P	N
3	3.5	F	2	1-5 YEARS	1.66	1-2 YEARS	LOWER-MIDDLE	POST MONSOON	TYPE 1	SIBBLING	DKA	NO	NO	YES	-0.04	0.23	14.9	-0.31	0.99	6.4	1.04	1.38	NO	0	N	N	N	N	N
4	6.5	F	5	1-5 YEARS	0.75	0-1 YEARS	LOWER-MIDDLE	MONSOON	TYPE 1	SIBBLING	SILENT	NO	NO	YES	-0.91	-1.1	14.05	-0.38	0.94	7.1	0.064	1.69	YES	1	N	N	N	P	N
5	15.08	F	13	11-15 YEARS	1.5	1-2 YEARS	UPPER-MIDDLE	POST MONSOON	NO	NO	DKA	NO	YES	NO	-0.08	-3.19	25.7	1.52	0.82	16.5	0.67	4.26	YES	2	N	P	N	N	P
6	11.16	M	9	6-10 YEARS	2.16	2-3 YEARS	LOWER	SUMMER	NO	NO	DKA	NO	NO	YES	-1.65	-1.06	13.28	-1.77	0.82	12.2	0.02	1.84	NO	0	N	N	N	N	N
7	10.33	F	7	6-10 YEARS	2.91	2-3 YEARS	LOWER-MIDDLE	MONSOON	NO	NO	CLASSICAL	YES	NO	YES	-0.24	0.23	15.6	-0.48	0.8	10.2	0.01	3.61	NO	0	N	N	N	N	N
8	15.33	M	12	11-15 YEARS	3.5	3-4 YEARS	LOWER-MIDDLE	POST MONSOON	NO	NO	DKA	NO	NO	YES	-1.64	-0.81	15.23	-1.5	0.8	14	0.01	2.82	YES	2	N	N	N	P	P
9	5.41	F	4	1-5 YEARS	1.58	1-2 YEARS	LOWER-MIDDLE	POST MONSOON	NO	NO	DKA	NO	NO	YES	0.02	-0.79	15.8	0.64	1.1	10.8	0.2	2.17	NO	0	N	N	N	N	N
10	6	F	5	1-5 YEARS	1.16	1-2 YEARS	UPPER-MIDDLE	MONSOON	NO	NO	CLASSICAL	NO	NO	NO	0.48	1.1	14.24	-0.18	0.92	11.9	0.325	0.28	NO	0	N	N	N	N	N
11	6	M	5	1-5 YEARS	0.41	0-1 YEARS	LOWER-MIDDLE	WINTER	TYPE 2	NO	CLASSICAL	NO	NO	YES	-0.08	-0.52	15.15	0.15	0.91	13.5	0.298	2.81	YES	2	N	P	N	P	N
12	6.25	F	3	1-5 YEARS	3.08	3-4 YEARS	LOWER-MIDDLE	SUMMER	TYPE 2	NO	DKA	NO	NO	NO	-0.7	-0.76	14.03	-0.39	0.94	12.4	0.01	2.54	NO	0	N	N	N	N	N
13	11	M	10	6-10 YEARS	0.91	0-1 YEARS	UPPER-MIDDLE	MONSOON	TYPE 2	NO	DKA	NO	NO	NO	-1.71	-0.78	12.73	-2.12	0.93	8.5	0.96	2.3	YES	3	N	P	N	P	P
14	10.66	F	7	6-10 YEARS	3.41	3-4 YEARS	UPPER-MIDDLE	WINTER	TYPE 2	NO	DKA	NO	NO	YES	-0.14	0.23	18.65	-0.34	0.94	14.5	0.08	1.85	YES	1	N	N	N	P	N
15	11.41	M	7	6-10 YEARS	3.75	3-4 YEARS	LOWER-MIDDLE	MONSOON	TYPE 2	NO	DKA	NO	NO	NO	-2.49	-1.28	11.62	-2.97	0.9	11.4	0.9	3.67	NO	0	N	N	N	N	N
16	13.08	M	10	6-10 YEARS	2.83	2-3 YEARS	UPPER-MIDDLE	MONSOON	NO	NO	DKA	NO	NO	YES	0.23	0.47	18.27	0.01	0.95	14.5	0.034	0.992	YES	3	N	P	N	P	P
17	6	F	3	1-5 YEARS	3.08	3-4 YEARS	UPPER-MIDDLE	SUMMER	NO	NO	DKA	NO	NO	NO	0.19	0.43	14.42	-0.08	0.95	8.7	0.03	2.01	NO	0	N	N	N	N	N
18	9.5	M	6	6-10 YEARS	3.41	3-4 YEARS	LOWER-MIDDLE	WINTER	NO	NO	CLASSICAL	NO	NO	YES	-1.38	-1.43	14.21	-0.95	0.96	8	0.649	1.25	NO	0	N	N	N	N	N
19	14.33	F	13	11-15 YEARS	0.83	0-1 YEARS	LOWER-MIDDLE	MONSOON	TYPE 2	NO	DKA	NO	NO	YES	0.36	-1.3	23.69	1.07	0.9	14.8	0.16	2.4	YES	1	N	N	N	P	N
20	10.5	F	7	6-10 YEARS	3.25	3-4 YEARS	UPPER-MIDDLE	SUMMER	TYPE 2	NO	DKA	YES	NO	NO	0.71	0.71	18.45	0.56	0.92	8.6	0.515	1.99	YES	1	N	N	N	P	N
21	6.41	F	12	11-15 YEARS	2.08	2-3 YEARS	LOWER-MIDDLE	SUMMER	NO	NO	DKA	NO	NO	YES	-1.07	-1.3	17.59	-0.59	0.97	12.6	0.09	3.22	YES	1	N	N	N	P	N
22	6.58	M	5	1-5 YEARS	1.33	1-2 YEARS	LOWER-MIDDLE	WINTER	NO	NO	DKA	NO	NO	NO	-0.74	-1.22	14.85	-0.07	0.92	11.8	0.138	1.45	YES	1	N	P	N	N	N
23	10	M	7	6-10 YEARS	2.41	2-3 YEARS	LOWER-MIDDLE	WINTER	TYPE 2	NO	DKA	NO	YES	YES	-0.72	-0.46	14.76	-0.76	0.92	10.7	0.46	5.18	YES	3	N	P	N	P	P
24	15.75	M	13	11-15 YEARS	2.5	2-3 YEARS	LOWER-MIDDLE	WINTER	TYPE 1 & 2	FATHER	DKA	NO	NO	YES	-0.73	0.12	17.05	-0.9	0.94	16.4	0.17	1.22	YES	2	N	N	N	P	P
25	13.33	F	11	11-15 YEARS	1.66	1-2 YEARS	UPPER-MIDDLE	POST MONSOON	TYPE 2	NO	DKA	NO	NO	YES	-1.92	-1.41	14.71	-1.61	0.95	14	0.335	1.07	YES	3	P	N	N	P	P
26	14.16	F	12	11-15 YEARS	2	2-3 YEARS	LOWER-MIDDLE	MONSOON	NO	NO	DKA	NO	YES	NO	-1.59	-0.85	15.32	-1.45	0.95	14	0.708	3.44	YES	3	N	P	N	P	P
27	3	F	1	1-5 YEARS	0.83	0-1 YEARS	UPPER	MONSOON	TYPE 1 & 2	SIBBLING	CLASSICAL	NO	NO	NO	-0.74	-1.06	15.22	-0.14	0.9	10.3	0.5	1.05	YES	1	N	P	N	N	N
28	6.5	F	5	1-5 YEARS	0.75	0-1 YEARS	LOWER-MIDDLE	POST MONSOON	NO	NO	DKA	NO	NO	NO	-0.95	-0.93	13.72	-0.59	0.92	14.5	0.05	3.98	YES	2	N	P	N	N	P
29	15.08	F	12	11-15 YEARS	2.66	2-3 YEARS	UPPER	POST MONSOON	NO	NO	DKA	NO	NO	NO	-1.28	-1.42	17.54	-0.76	0.93	18	0.43	1.36	NO	0	N	N	N	N	N

30	16.25	F	13	11-15 YEARS	2.58	2-3 YEARS	LOWER-MIDDLE	POST MONSOON	NO	NO	DKA	NO	NO	NO	0.57	1.82	19.57	-0.27	0.93	13.5	0.86	5.13	NO	0	N	N	N	N	N
31	13.25	M	11	11-15 YEARS	2.16	2-3 YEARS	LOWER	SUMMER	TYPE 2	NO	CLASSICAL	NO	NO	NO	-2.15	-1.99	14.08	-1.68	0.96	18	0.09	1.45	YES	3	N	P	N	P	P
32	10.41	F	8	6-10 YEARS	2.25	2-3 YEARS	UPPER-MIDDLE	SUMMER	NO	NO	DKA	NO	NO	YES	-0.04	1.56	14.43	-1.02	0.93	9.4	0.48	1.17	NO	0	N	N	N	N	N
33	5.58	M	4	1-5 YEARS	1.08	1-2 YEARS	LOWER-MIDDLE	MONSOON	NO	NO	DKA	NO	NO	NO	-0.91	-1.13	14.15	-0.36	0.94	13.8	0.07	1.37	NO	0	N	N	N	N	N
34	15.66	F	14	11-15 YEARS	1	1-2 YEARS	UPPER-MIDDLE	MONSOON	NO	NO	DKA	NO	NO	YES	-0.17	0.22	19.03	-0.33	0.91	15.6	0.39	1.53	YES	1	N	P	N	N	N
35	12.66	F	10	6-10 YEARS	2.33	2-3 YEARS	LOWER	SUMMER	TYPE 2	NO	CLASSICAL	NO	NO	NO	0.55	0.66	19.56	0.36	0.81	10.6	1.62	1.4	YES	1	N	N	N	N	P
36	9.83	F	6	6-10 YEARS	3.58	3-4 YEARS	LOWER-MIDDLE	WINTER	NO	NO	CLASSICAL	NO	NO	NO	-1.41	-1.76	14.85	-0.7	0.79	15	0.011	2.95	YES	1	N	P	N	N	N
37	15.33	F	11	11-15 YEARS	3.91	3-4 YEARS	LOWER-MIDDLE	POST MONSOON	NO	NO	DKA	NO	NO	YES	-1.57	-0.56	15.68	-1.54	0.73	13.2	0.094	2.06	NO	0	N	N	N	N	N
38	8.25	M	7	6-10 YEARS	1	0-1 YEARS	LOWER-MIDDLE	MONSOON	NO	NO	DKA	NO	NO	NO	-0.67	-0.81	14.96	-0.35	0.92	15.1	0.332	1.74	NO	0	N	N	N	N	N
39	11.66	F	10	6-10 YEARS	1.5	1-2 YEARS	LOWER-MIDDLE	SUMMER	TYPE 2	NO	CLASSICAL	NO	NO	YES	0.06	1.46	15.66	-0.74	0.8	12	0.43	2.37	NO	0	N	N	N	N	N
40	6.41	M	4	1-5 YEARS	1.91	1-2 YEARS	LOWER-MIDDLE	POST MONSOON	TYPE 2	NO	CLASSICAL	NO	NO	NO	-0.24	-0.14	14.46	-0.29	0.86	6.7	0.038	2.84	YES	1	N	P	N	N	N
41	13	M	12	11-15 YEARS	0.91	0-1 YEARS	LOWER-MIDDLE	POST MONSOON	NO	NO	DKA	NO	NO	YES	0.58	1.89	17.37	-0.28	0.92	12.5	1.55	4.79	NO	0	N	N	N	N	N
42	6.16	M	2	1-5 YEARS	4	3-4 YEARS	UPPER-MIDDLE	MONSOON	NO	NO	DKA	NO	NO	NO	0.09	-0.15	15.01	0.08	0.94	11.8	0.01	2.79	NO	0	N	N	N	N	N
43	7.5	F	5	1-5 YEARS	2.58	2-3 YEARS	LOWER-MIDDLE	WINTER	NO	NO	DKA	NO	YES	NO	-0.57	-0.38	14.03	-0.58	0.9	13.8	0.028	6.32	YES	1	P	N	N	N	N
44	8.33	F	7	6-10 YEARS	0.75	0-1 YEARS	LOWER	POST MONSOON	NO	NO	DKA	YES	NO	NO	0.25	0.25	15.92	0.16	0.89	12.8	0.22	2.87	YES	3	P	P	N	N	P
45	13.83	M	11	11-15 YEARS	2.08	2-3 YEARS	UPPER	MONSOON	TYPE 2	NO	DKA	NO	NO	YES	-0.08	0.02	15.63	-1.07	0.88	9.4	0.6	3.04	NO	0	N	N	N	N	N
46	12.08	M	10	6-10 YEARS	2	2-3 YEARS	LOWER-MIDDLE	MONSOON	TYPE 2	NO	DKA	NO	NO	YES	-1.14	-0.98	15.04	-1.01	0.82	10.8	0.519	1.76	YES	2	P	P	N	N	N
47	3.41	F	3	1-5 YEARS	0.08	0-1 YEARS	UPPER	MONSOON	TYPE 2	NO	CLASSICAL	NO	NO	NO	-0.04	-0.35	15.62	0.22	0.94	10.7	0.36	1.85	NO	0	N	N	N	N	N
48	15.25	F	11	11-15 YEARS	3.75	3-4 YEARS	UPPER-MIDDLE	POST MONSOON	TYPE 2	NO	DKA	NO	NO	NO	0.1	0.83	19.05	-0.32	0.85	13.2	0.37	3.22	NO	0	N	N	N	N	N
49	5.25	F	5	1-5 YEARS	0.08	0-1 YEARS	LOWER	MONSOON	NO	NO	DKA	NO	NO	NO	-1.46	-1.38	13.2	-0.8	0.96	13.6	0.24	1.78	NO	0	N	N	N	N	N
50	10.5	F	9	6-10 YEARS	1.16	1-2 YEARS	LOWER-MIDDLE	MONSOON	NO	NO	DKA	NO	NO	YES	-1.55	-2.41	15.86	-0.38	0.97	13.3	0.49	1.68	YES	3	N	P	N	P	P
51	12.41	F	9	6-10 YEARS	3.58	3-4 YEARS	LOWER-MIDDLE	SUMMER	TYPE 2	NO	DKA	NO	NO	NO	-1.53	-1.06	14.8	-1.32	0.97	13.9	0.11	1.5	YES	1	N	N	N	P	N
52	15.91	M	14	11-15 YEARS	1.41	1-2 YEARS	LOWER-MIDDLE	SUMMER	TYPE 2	NO	DKA	NO	NO	NO	-0.46	-1.51	21.16	0.34	0.96	11.1	3.19	2.49	NO	0	N	N	N	N	N
53	5.33	F	2	1-5 YEARS	3.25	3-4 YEARS	UPPER-MIDDLE	MONSOON	TYPE 2	NO	DKA	NO	NO	YES	0.38	0.61	14.47	0.04	0.93	11.3	0.44	1.03	NO	0	N	N	N	N	N
54	12.91	M	10	6-10 YEARS	2.5	2-3 YEARS	UPPER-MIDDLE	WINTER	NO	NO	DKA	NO	YES	NO	-0.21	-0.29	17.75	-0.15	0.93	10.4	0.3	6.03	YES	2	N	P	N	N	P
55	13.08	M	12	11-15 YEARS	1.16	1-2 YEARS	LOWER-MIDDLE	MONSOON	TYPE 2	NO	DKA	NO	NO	NO	0.31	-0.92	21.47	0.87	0.95	13.1	0.54	0.96	YES	3	N	P	N	P	P
56	5.58	M	3	1-5 YEARS	2.66	2-3 YEARS	UPPER	SUMMER	TYPE 1 & 2	SIBBLING	CLASSICAL	NO	NO	NO	-0.91	0.21	12.45	-1.62	0.96	9.7	0.144	1.48	YES	1	N	N	N	N	P
57	11.08	M	9	6-10 YEARS	1.58	1-2 YEARS	LOWER-MIDDLE	SUMMER	NO	NO	DKA	NO	NO	YES	-2.62	-1.48	11.48	-3.05	0.83	11.5	0.26	1.72	YES	1	N	P	N	N	N
58	1	F	1	1-5 YEARS	0.08	0-1 YEARS	LOWER-MIDDLE	MONSOON	NO	NO	DKA	NO	NO	NO	-1.59	-3.26	17.52	0.52	0.95	12.8	0.15	2.68	YES	1	N	N	N	N	P
59	16.16	M	15	11-15 YEARS	1.16	1-2 YEARS	LOWER-MIDDLE	MONSOON	NO	NO	CLASSICAL	YES	NO	YES	-1.57	-0.14	14.7	-1.84	0.96	8.2	1.71	2.7	NO	0	N	N	N	N	N
60	7.08	F	4	1-5 YEARS	3.33	3-4 YEARS	LOWER-MIDDLE	MONSOON	TYPE 2	NO	CLASSICAL	NO	NO	YES	0.56	0.74	15.41	0.26	0.96	9.6	0.2	6.6	YES	1	N	P	N	N	N
61	14.16	F	12	11-15 YEARS	1.66	1-2 YEARS	LOWER-MIDDLE	POST MONSOON	NO	NO	DKA	NO	YES	YES	-0.89	-1	17.71	-0.55	0.94	8.4	0.45	3.86	NO	0	N	N	N	N	N
62	12.08	F	9	6-10 YEARS	2.83	2-3 YEARS	UPPER-MIDDLE	WINTER	TYPE 2	NO	DKA	NO	NO	NO	-1.98	-1.73	14.22	-1.49	0.94	13.1	0.022	4.12	YES	1	N	P	N	N	N
63	5	M	5	1-5 YEARS	0.08	0-1 YEARS	LOWER-MIDDLE	MONSOON	TYPE 2	NO	CLASSICAL	NO	NO	YES	-0.32	-0.58	14.51	-0.09	0.96	11.2	0.72	3.6	YES	1	N	P	N	N	N
64	5.58	M	4	1-5 YEARS	1.58	1-2 YEARS	UPPER-MIDDLE	SUMMER	TYPE 2	NO	DKA	NO	NO	YES	0.31	0.59	14.37	-0.23	0.96	12.5	0.01	1.96	YES	2	N	P	N	N	P
65	10.25	F	9	6-10 YEARS	1.66	1-2 YEARS	LOWER-MIDDLE	WINTER	TYPE 1 & 2	SIBBLING	DKA	NO	NO	YES	-1.01	-0.77	14.71	-0.89	0.95	15	0.386	2.84	NO	0	N	N	N	N	N
66	11.5	F	10	6-10 YEARS	1.58	1-2 YEARS	LOWER-MIDDLE	SUMMER	TYPE 1 & 2	SIBBLING	DKA	NO	NO	YES	-0.61	-0.84	16.84	-0.28	0.97	13	0.339	2.11	NO	0	N	N	N	N	N
67	10.25	F	9	6-10 YEARS	1	1-2 YEARS	LOWER-MIDDLE	POST MONSOON	NO	NO	CLASSICAL	NO	NO	NO	-1.47	-1.46	14.44	-1.02	0.94	10.6	0.026	1.48	YES	2	N	P	N	N	P
68	12.58	F	12	11-15 YEARS	0.08	0-1 YEARS	LOWER	POST MONSOON	TYPE 2	NO	DKA	NO	NO	NO	-0.33	-0.78	18.57	0.05	0.93	15.1	0.22	3.54	YES	1	N	N	N	N	P
69	13.75	F	13	11-15 YEARS	0.33	0-1 YEARS	LOWER-MIDDLE	MONSOON	TYPE 2	NO	CLASSICAL	YES	NO	YES	-0.89	-1.89	19.24	-0.05	0.94	14	2.54	2.81	NO	0	N	N	N	N	N
70	7.41	M	4	1-5 YEARS	3.5	3-4 YEARS	UPPER-MIDDLE	SUMMER	NO	NO	DKA	NO	NO	NO	-1.1	-1.8	15.04	-0.13	0.92	8.7	0.01	2.92	NO	0	N	N	N	N	N
71	8.9	F	6	6-10 YEARS	2.75	2-3 YEARS	LOWER-MIDDLE	WINTER	NO	NO	DKA	NO	NO	NO	-1.3	-1.39	14.11	-0.83	0.93	14.5	0.02	1.16	NO	0	N	N	N	N	N
72	6.58	M	6	6-10 YEARS	0.08	0-1 YEARS	LOWER-MIDDLE	POST MONSOON	NO	NO	DKA	NO	NO	NO	-0.43	-0.67	14.77	-0.11	0.96	14.5	0.05	2.02	NO	0	N	N	N	N	N
73	12.75	F	15	11-15 YEARS	2.08	2-3 YEARS	LOWER-MIDDLE	MONSOON	NO	NO	DKA	NO	NO	NO	-0.66	-0.61	17.35	-0.47	0.95	14.9	0.36	1.97	NO	0	N	N	N	N	N
74	13.58	M	10	6-10 YEARS	2.5	2-3 YEARS	LOWER-MIDDLE	POST MONSOON	NO	NO	DKA	NO	NO	NO	-1.45	-1.26	15.13	-1.2	0.94	9.6	0.09	1.96	YES	1	N	N	N	N	P