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ORIGINAL ARTICLE

ANTI-GLUTAMIC ACID DECARBOXYLASE-65 AND INSULIN AUTOANTIBODIES IN PATIENTS WITH TYPE 1 DIABETES MELLITUS OF EASTERN NEPAL

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ABSTRACT

Type 1 Diabetes Mellitus (T1DM) has been a rising trend in the younger population globally. There are limited data available on T1DM in the Nepalese population with negligible reports on the antibody status in the T1DM of the Nepalese population. This study aimed to estimate the autoantibodies namely glutamic acid decarboxylase-65 (GADA) and Insulin autoantibody (IAA) positivity and assess the serum vitamin D levels in Type 1 DM patients of eastern Nepal. A hospital-based cross-sectional study was conducted among 54 diagnosed cases of T1DM attending the B.P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal for one year (15th January- 15th December 2020). Convenient sampling was used to recruit the patients. Serum GADA and IAA were estimated using a chemiluminescence immunoassay (CLIA). Quantitative variables were expressed as a mean and standard deviation. The level of significance was established as p< 0.05. We recruited fifty-four patients (36 female and 18 male). The mean age of the patient was 22.44 ± 9.69 years. GADA positivity was present in 18.5% (n=10) of the patients and IAA was positive in 24.07% (n=13). Our study unveiled a lower prevalence of GADA and IAA in the Nepalese population compared to previous studies. In addition, we report female preponderance in patients with both GADA and IAA positivity.

Keywords: *Autoantibodies, Autoimmunity, GADA, IAA, Type 1 diabetes*

Abbreviations:
ADA: American Diabetes Association
GADA: Antibodies to Glutamate acid Decarboxylase-65
IAA: Insulin autoantibody
T1DM: Type 1 Diabetes Mellitus

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease resulting from selective destruction of the ß-cells of the pancreas. (Alshiekh S et al., 2017; American Diabetes Association.,2017; Expert Committee., 2003). Predominantly, the destruction of ß- cells is cell-mediated but ß-cell-specific autoantibodies have been detected in the serum of patients suggesting a probable role for the humoral immune system too. (Baker II et al., 2009). Commonly detected autoantibodies in T1DM are antibodies to glutamic acid decarboxylase 65kDa (GADA), insulin autoantibodies (IAA), tyrosine phosphatase-associated islet antigen-related antibody (islet antigen 2 or IA-2), islet cell autoantibodies (ICA), and zinc transporter (ZnT8) autoantibodies. (Alshiekh S et al., 2017; Amutha A et al., 2013). Some studies have reported the prevalence of GADA to be 70%–80 in children with new-onset T1DM and insulin autoantibodies (IAA) in 90% of children who progress to T1DM before the age of 5 years with only 40%–50% of those older than 15 years. (Alshiekh S et al., 2017; American Diabetes Association.,2017). There is variability in prevalence values concerning the prevalence of GADA antibodies in India (25%- 79.4%) (Basu M et al., 2020; Sanyal D et al., 2019) and China (66.3%) (Cheng B et al., 2018). Numerous factors can be ascribed to the low prevalence of autoantibodies in T1DM in southeast Asia. The common factor is etiologic heterogeneity with idiopathic variety of T1DM in Southeast Asian countries like India in contrast to the Western countries. (Balasubramanian K et al., 2003).

Also, there is no explicit delineation of the time and process of screening for autoimmune diseases in Type 1DM patients. (Goncalves C et al., 2013; WHO.,2023). There has been very little research on T1DM patients in Nepal (Shakya B et al., 2020; Paudel U et al., 2021) with negligible reports on autoantibody status and genetic characteristics of T1DM patients in Nepal. Thus, this study aimed to determine the autoantibodies (GADA and IAA) levels in T1DM patients attending B.P. Koirala Institute of Health Sciences one of the largest tertiary care centers in eastern Nepal.

METHODS:

Study Design

A hospital-based cross-sectional study was conducted as a collaborative work between the Department of Biochemistry, the Department of Pediatrics and Adolescent Medicine, and the Department of Internal Medicine, BPKIHS.

The patient population included in this study was diabetic patients aged ≤ 30 years of age visiting the Pediatrics and Adolescent Medicine and Internal Medicine (Endocrinology OPD), BPKIHS, patients who had been admitted to the ward or ICU under Internal Medicine of BPKIHS due to diabetic ketoacidosis (DKA) during the study period. The diagnosis of DKA was made in the emergency department by the presence of 3 laboratory findings: a plasma glucose level of 250 mg/dL or higher; a serum bicarbonate level of 15 mEq/L or lower, an arterial blood pH of 7.30 or lower, or a venous blood pH of 7.25 or lower; and moderate or large urinary ketones. C-Peptide is

not commonly used in our setting due to its unavailability in most of the laboratories, hence it was not included due to resource constraints.

Convenient sampling was used to recruit the patients. Patients were conscripted from the Department of Pediatrics and Adolescent Medicine and the Department of Internal Medicine (Endocrinology OPD), BPKIHS. Patients were included only after receiving informed consent from the patients themselves (age ≥ 16 years) and their parents in the case of minors.

Inclusion Criteria

Diagnosis of T1DM was done following the American Diabetes Association (ADA) criteria (ADA., 2020). The patients diagnosed with T1DM were included and sent for autoantibodies (GADA and IAA) investigations. Patients with a short history of osmotic symptoms, initial ketonuria, and hyperglycemia were included.
Since our hospital did not perform autoantibody investigations before the commencement of this study, thus the diagnosis of T1DM was made based on age, characteristic features of T1DM, and responsiveness to insulin therapy. We included diagnosed patients of T1DM under insulin treatment.

Exclusion Criteria

Patients diagnosed with Type 2 Diabetes Mellitus (T2DM), and monogenic and secondary diabetes were excluded. The study was conducted for 1 year from 15th January- to 15th December 2020. A self-formed structured proforma was used to collect all the information related to the patients. Blood was collected in a serum vial (gold top with clot activator and serum separating gel) from the study population for GADA, and IAA estimation.

Details of the subject enrollment with the patients excluded is depicted in **Figure 1**.

Sample Collection and Preparation

3 ml blood was collected in a gold color top/ vial (Becton Dickinson BD Vacutainer® tubes. The serum was separated in a cooling centrifuge at 4000 rpm and stored at − 20 °C until the test was performed. The serum sample was used for the analysis of autoantibodies.

Laboratory Analysis

Fasting (FBG) and post-prandial blood glucose (PBG) were estimated by the Hexokinase method in Cobas c311 autoanalyzer (Roche Diagnostics). HbA1c was done by turbidimetric inhibition immunoassay (TINIA) method in Cobas c311 Autoanalyser [Tina Quant., 2022]. HbA1c is expressed in percentage (%). The normal range is 4.5–6.3%. The HbA1c determination is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood. Glycohemoglobin (HbA1c) in the sample reacts with anti-HbA1c antibodies to form soluble antigen-antibody complexes. Only one specific HbA1c antibody site is present on the HbA1c molecule, complex formation does not take place. Addition of R2 (Polyhapten reagent) and the start of reaction: The polyhaptens react with excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex measured turbidimetrically. Autoantibodies to glutamic acid decarboxylase 65 (GADA) and Insulin autoantibodies (IAA) were measured by chemiluminescence immunoassay (CLIA) in Maglumi 2000 autoanalyzer (Snibe Diagnostics) with intra-assay % CV less than 5% and interassay % CV less than 10% and according to the procedure recommended by the reagent manufacturer. Values for GADA ≥ 30IU/ml and IAA ≥ 20IU/ml were considered to be positive.

This study has been approved and obtained ethical clearance from the Institutional Review Committee (IRC, BPKIHS). The ethical clearance number for the study is IRC/1522/019. All the study participants were enrolled only after obtaining written informed consent. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Statistical Analysis

Sample Size

We enrolled a total of 54 patients during the study period of one year. Data were collected and entered using Microsoft Excel™ and analyzed using Statistical Package of Social Science (SPSS) version 22.0. Data were expressed in terms of figures, percentages, mean, standard deviation (For parametric variables), and median (25^{th}, 75^{th} Percentile) for non-parametric variables. Quantitative variables were expressed as a mean and standard deviation. The level of significance was established as $p < 0.05$.

RESULTS

The clinico-demographic profile of the T1DM patients is illustrated in **Table 1**. The mean age of the patients was 22.94 ± 9.69 with the majority of the patients being females (66.6%). Most of the patients in the study population had a history of diabetes for <1 month (30%) followed by 3- 6 months (28%) with a family history of Diabetes Mellitus present in 16% of the total patients respectively. Comparing GADA and IAA in the study population, we found that IAA was positive (n=13) in a greater number of patients compared to GADA (n=10) as depicted in **Figure 2**.

We found female preponderance in autoantibody positivity as well as double antibody positivity (GADA +IAA positive) in females as shown in **Tables 2 & 3** respectively.

We also assessed the GADA positivity in different age groups and duration of T1DM and found that maximum GADA positivity was seen in the age group of 10-20 years with the disease duration being < 5 years as represented in **Tables 4** and **5** respectively.

Similarly, IAA positivity as per the age group and disease duration depicted that maximum patients with IAA positivity belong to the age group of 10-20 years and disease duration < 5 years as shown in **Tables 6 and 7** respectively.

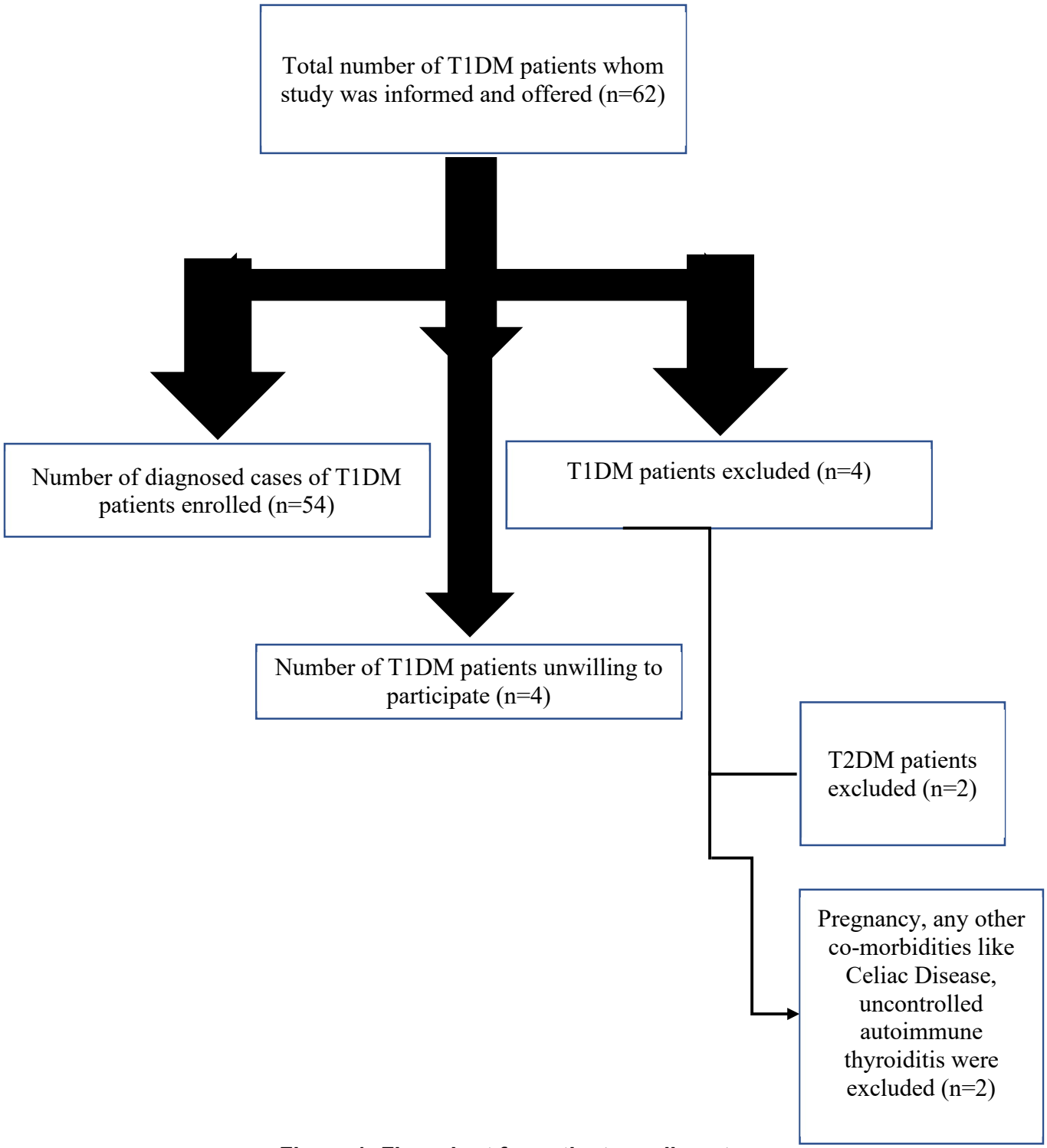

Figure 1: Flow chart for patient enrollment.

Table 1: Demographic and Clinical Profile of the study population

Variables	**Values (n=54)**
Age (years)	22.94 ± 9.69
Gender (M/F)	17/37
BMI (kg/m^2)	20.98 ± 7.39
Duration of DM (Months)	24 (2.75, 72.00)
Family history of DM (Present)	8
FBG (mg/dl)	231.94 ± 96.27
PPG (mg/dl)	367.10 ± 176.48
HbA1c (%)	9.83 ± 2.94
GADA (IU/ml)	7.12 (5.92, 11.10)
IAA (IU/ml)	6.46 (4.63, 25.06)

Table 2: Autoantibody status in male and female T1DM patients

Parameter	Males	Females	Odd's Ratio (CI)	p value
GAD-A (>30 IU/ml)	1	9	5.14 (0.59-44.38)	0.10[a]
IAA (>20 IU/ml)	2	11	3.17 (0.61-16.27)	0.15 [a]

a=Chi Square test

Table 3: GADA +IAA positivity in male and female patients

GADA +IAA	Gender		p value
	Male	**Female**	
Positive	0	4	0.15[a]
Negative	17	33	

a=Chi-Square test

Table 4: GADA positivity as per the age group

Age	GADA positive (n=10)	p value
< 10 years	1	0.34[a]
10-20 years	5	
20-30 years	3	
30-40 years	0	
>40 years	1	

a=Chi-Square test

Table 5: GADA positivity and titer as per the duration of DM

Duration of DM	GADA positive (n=10)	GADA titre	p value
<1 year	2	11.48 ± 12.75	0.33[a]
1-5 years	5	15.36 ± 16.21	
5-10 years	3	13.02 ± 15.56	
> 10 years	0	8.41± 4.46	

a=Chi-Square test

Table 6: IAA positivity as per the age group

Age	IAA positive (n=13)	p value
<10 years	1	0.01[a*]
10-20 years	9	
20-30 years	3	
30-40 years	0	
>40 years	0	

a=Chi-Square test;*p value <0.05 is considered to be statistically significant

Table 7: IAA positivity and titer as per the duration of DM

Duration of DM	IAA positive (n=13)	IAA titre	p value
< 1 year	4	16.27 ± 23.93	0.59[a]
1-5 years	6	23.22 ±28.37	
5-10 years	2	18.55 ± 29.37	
> 10 years	1	22.36 ± 21.89	

a=Chi-Square test

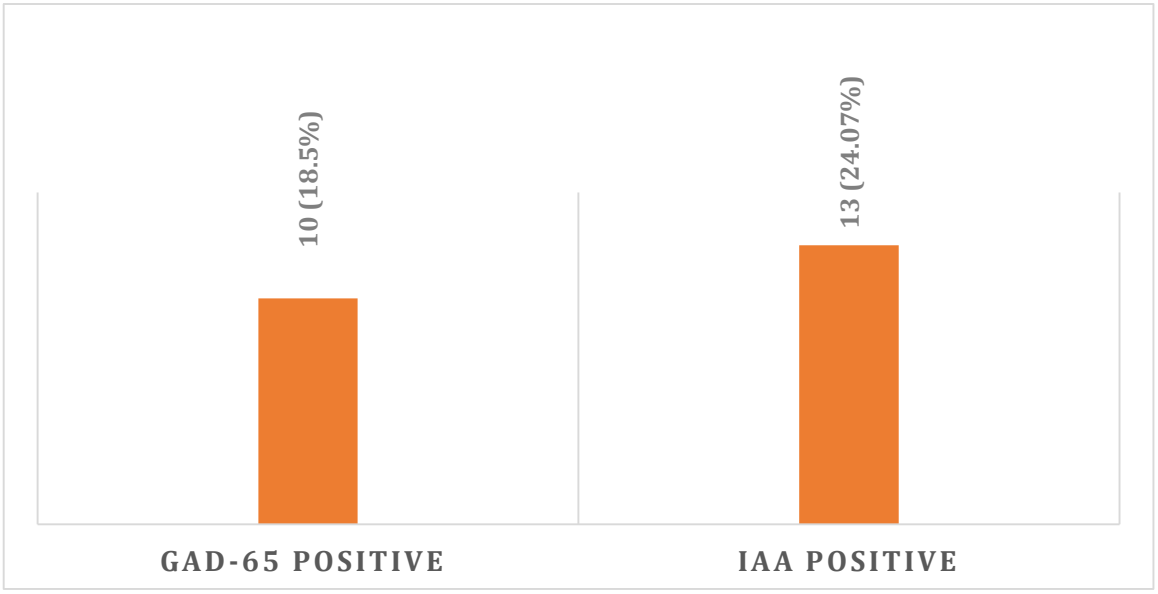

Figure 2: Comparison of GAD-65 and IAA in the T1DM patients

DISCUSSION

The present study intended to determine the autoantibody (GADA and IAA) positivity in the diagnosed cases of T1DM at a tertiary care center in eastern Nepal. Autoantibodies are not commonly done in routine practice in most of the laboratories in Nepal, despite the repeated clinician's requests. We estimated the autoantibody levels in T1DM patients. The major findings of our study are the outlining of autoantibody positivity in T1DM patients of eastern Nepal. Antibodies against GAD were positive in 18.05% of the patients and IAA in 24.07%. These findings were comparable to the studies done in northern India (Dayal D et al., 2015) but lower than those reported from the eastern part of India (Sanyal D et al,2017) and other countries (Mayer-Davis EJ et al., 2013; Wenzlau JM et al., 2010; Wenzlau JM et al., 2015; William Cl et al., 2022; Zamanfar D et al., 2020; Zhang M et al., 2022). The variable frequency in autoantibody positivity across the globe is not known. Autoantibodies detection is directly linked with the onset of the disease. Hence, the difference in the time of estimation of autoantibodies since the time of disease onset, variations in the detection methods among medical laboratories, kit-based range, etc. can be a few of the reasons for the fluctuations in values. (Marita, A. R et al., 2004). The mean age of the patient in the present study was 22.94 ± 9.69 years with the majority of the patients being females (66.6%). Among the study population, the majority of the patients belong to the age group 20-30 years (35.2%) followed by 10-20 years (31.5%) and 30-40 years (20.4%) respectively. The finding is similar to that reported by Sanyal D et al., 2017) from eastern India, (Bronner et al.,2020) from the Netherlands, and the United Kingdom respectively (Thomas NJ et al., 2018). A known fact accounts that T1DM is typically considered a disease of childhood and adolescence, but can occur at any age. Correct diagnosis of type 1 diabetes in young people (<20 years) is forthright as most (≥85%) cases of diabetes are due to T1DM in the respective population. (Thomas NJ et al., 2018; Dayal D et al., 2015; Scottish Diabetes Survey Monitoring Group; 2016).

It is commonly observed that organ-specific autoimmune disorders are preponderant in females. Contradictorily, T1DM does not show a female prevalence with studies reporting a prevalence roughly of 45% in females (Thomas NJ et al., 2018; Maahs, D. M., 2010). Our study reports a higher proportion of T1DM in females, similar to the studies reported from India (Naaraayan, S. A., 2021; Varadarajan P et al., 2011; Tandon N et al., 2002; Wenzlau, J. M et al., 2007). The reason might be attributed to ethnic or geographical variations. Our study reports GADA positivity in 18.5% and IAA in 24.07% of T1DM patients. The values are lower than those reported from Western countries and India. (Sanyal D et al., 2017; Tandon N et al., 2002; Wenzlau, J. M et al., 2007). Low antibody seropositivity is a consistent feature of T1DM in the Asian population (Sanyal D et al., 2017). We had only 3 patients with both antibodies positive which are contradictory to the study reported from India (Sanyal D et al., 2017) and also by the pioneering studies such as the SEARCH for Diabetes in the Young Study and the Finnish DIPP Study (Dabelea D et al., 2011; Kimpimaki T et al., 2002) which reported that the majority of their participants were positive for one or more autoantibodies (Sanyal D et al., 2017; Dabelea D et al., 2011; Kimpimaki T et al., 2002). Researchers on the comparison of clinical characteristics between GADA-positive and negative patients illustrated a high GADA positivity in younger females (Naaraayan, S. A. et al., 2021). We found similar findings in our study, where nine of the ten GADA-positive T1DM patients were females.

The trend of increasing cases of T1DM is being noted in southeast Asia more commonly in the Indian population (Dhanwal, D. K et al., 2014; Sanyal D., 2020). The cause has been ascribed to global warming/environmental change, lifestyle changes, and microbial factors (Kumar K. M. P., 2015). It has been postulated that changes in enterobiome might have a role in the surge in autoimmunity leading to T1DM (Gale E.,2002). We mainly took the reference from the Indian population due to geographical and cultural similarities and found few studies that reported antibody positivity in the T1DM population (Sanyal D., 2020; Kumar KMP., 2015; Balasubramanian K et al., 2003). A study by Balasubramanian et al reported that 45% of recent onset T1DM were negative for GADA and IA2A (Balasubramanian K et al., 2003). Another study done among children with T1DM found 97.8% antibody positivity, including GADA, IAA, IA2A, and ZnT8A (Sanyal D., 2020). Among the antibodies, GADA was the most common, two antibodies'

positivity was present in 67.3% of T1DM patients, (Sanyal D., 2020), and the number of antibodies well correlated with the duration of the disease (Gale E., 2002; Balasubramanian K et al., 2003). Studies have shown that levels of IAA positivity are common among younger children. (Sanyal D., 2020; Endesfelder D.,2016) There is a probable association between IAA positivity and a fulminant course as a consequence of the destruction of β cells and complete insulin deficiency with rapid onset of T1DM (Sanyal D., 2020; Endesfelder D.,2016). Our study supports this finding with IAA being significantly positive in recently diagnosed T1DM within the duration of <2 years and age group of 10-20 years.

The most commonly done and important autoantibodies in T1DM is GADA. Levels of GADA summits later, more common in older children and adolescents with T1DM and LADA. This typical characteristic of GADA suggests a persistent but less intense autoimmune process. (Sørgjerd EP., 2019). We report the majority of GADA positivity in the age group of 10-20 years with the duration of DM being less than 5 years respectively.

No published record of the susceptibility of HLA alleles and T1D-susceptibility variants and their distribution in our population is found. Few studies have been reported highlighting the common HLA alleles in variable populations like renal transplant recipients and donors (Tuladhar A. et al., 2013) and unrelated healthy populations (Shrestha S et al., 2021) but no data has been reported for HLA alleles and susceptibility of T1DM in the Nepalese population. The study findings suggest that the proportion of autoantibodies positivity (i.e., GADA and IAA) is slightly lower than that reported from India and other parts of the world. Majority of th-antibody-negative subjects could be due to the presence of other T1DM autoantibodies not analyzed in this study eg. Islet Cell Cytoplasmic (ICA), Insulinoma-Associated-2/Tyrosine Phosphatase (IA-2A), and, Zinc Transporter-8 Autoantibodies (ZnT8A). We could not assess these patients' organ-specific autoantibodies, particularly thyroid and adrenal gland positivity. Thus, there can be the possibility of other organ-specific autoantibodies in T1DM patients.

Limitations

We do not have any previously published data on autoantibodies status in T1DM patients in the Nepalese population, therefore, it is difficult to compare and conclude in terms of the proportion of positivity of autoantibodies in our population. In addition, we do not have any published record for the susceptibility of HLA alleles and T1D-susceptibility variants and their distribution in our population. This was a single-centered and cross-sectional study; hence the findings could not be generalized.

CONCLUSIONS

Our study outlines the prevalence of GADA and IAA positivity levels in T1DM patients attending a tertiary care center in eastern Nepal. To the best of our knowledge, this is the first-ever report from Nepal depicting the prevalence of autoantibodies in T1DM patients. We recommend screening the possible autoimmune disease in T1DM by measuring GADA, IA2A, and IAA autoantibodies for early diagnosis, better patient care, and prevention of complications in Nepalese patients.

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FUNDING

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AUTHORS' CONTRIBUTIONS

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST DECLARATION

We affirm that there is no Conflict of Interest among the author(s) concerning the subject matter or materials discussed in this manuscript. We further certify that the article represents the original work of the Authors and Co-Authors. The manuscript has not been previously published and is not currently under consideration for publication elsewhere. This research/manuscript has not been submitted for publication nor published in whole or in part elsewhere. We attest that all authors have made significant contributions to the work, ensuring the validity and legitimacy of the data and its interpretation, thereby warranting its submission to the Malaysian Journal of Clinical Biochemistry.

DECLARATIONS

Ethical approval and consent to participate:

This study has been approved and obtained ethical clearance from the Institutional Review Committee (IRC, BPKIHS). The ethical clearance number for the study is IRC/1522/019. All the study participants were enrolled only after obtaining written informed consent. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication

Not applicable.

Patient and public involvement

Patients and/or the public were not involved in the design, conduct, reporting, or dissemination plans of this research.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analyzed during the current study are not publicly available due to the unavailability of disclosure of patient's identification but are available from the corresponding author upon reasonable request.

PRESENTATION

The abstract of this paper was presented at the EuroMedLab Munich 2021 – Munich, held at April 10–14, 2022 as a poster presentation with interim findings. The poster's abstract was published in "Poster Abstracts" in Clinical Chemistry and Laboratory Medicine (CCLM). URL: https://www.degruyter.com/document/doi/10.1515/cclm-2021-5008/html.
The principal author also presented the paper as an oral presentation at the 10th National Conference of the Association of Pathologists of Nepal (ACPN) on April 2023. The principal author received the Young Scientist Award for this research at the 34th Malaysian Association of Clinical Biochemists (MACB) held in Kuala Lumpur, Malaysia from 22-23 July 2024.

REFERENCES

1. Alshiekh, S., Larsson, H. E., Ivarsson, S. A., & Lernmark, Å. (2017). Autoimmune type 1 diabetes. In R. I. G. Holt, C. S. Cockram, A. Flyvbjerg, & B. J. Goldstein (Eds.), *Textbook of diabetes.* Wiley Blackwell.
2. American Diabetes Association. (2017). American Diabetes Association Standards of medical care in diabetes–2017. *Diabetes care*, *40*(Suppl. 1), S1.
3. American Diabetes Association. (2020). 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2020. *Diabetes care*, *43*(Supplement_1), S14-S31.
4. Amutha, A., & Mohan, V. (2013). Childhood and adolescent onset type 1 diabetes in India. *MGM Journal of Medical Sciences*.
5. Baker II, P. R., & Eisenbarth, G. S. (2009). Type 1 diabetes—pathogenesis, prediction, and prevention. *US Endocrinology*, *05*(01), 79. https://doi.org/10.17925/use.2009.05.1.79
6. Balasubramanian, K., Dabadghao, P., Bhatia, V., Colman, P. G., Gellert, S. A., Bharadwaj, U., ... & Bhatia, E. (2003). High frequency of type 1B (idiopathic) diabetes in North Indian children with recent-onset diabetes. *Diabetes Care*, *26*(9), 2697-2697.
7. Basu, M., Pandit, K., Banerjee, M., Mondal, S. A., Mukhopadhyay, P., & Ghosh, S. (2020). Profile of auto-antibodies (disease-related and other) in children with type 1 diabetes. *Indian Journal of Endocrinology and Metabolism*, *24*(3), 256-259.
8. Bronner, M. B., Peeters, M. A., Sattoe, J. N., & van Staa, A. (2020). The impact of type 1 diabetes on young adults' health-related quality of life. *Health and quality of life outcomes*, *18*, 1-7.
9. Cheng, B. W., Lo, F. S., Wang, A. M., Hung, C. M., Huang, C. Y., Ting, W. H., ... & Lee, Y. J. (2018). Autoantibodies against islet cell antigens in children with type 1 diabetes mellitus. *Oncotarget*, *9*(23), 16275.
10. Dabelea, D., Pihoker, C., Talton, J. W., D'Agostino Jr, R. B., Fujimoto, W., Klingensmith, G. J., ... & SEARCH for Diabetes in Youth Study. (2011). Etiological approach to characterization of diabetes type: the SEARCH for Diabetes in Youth Study. *Diabetes care*, *34*(7), 1628-1633.
11. Dayal, D., Samprati, M., Kaur, N., Minz, R. W., & Jayaraman, D. (2015). Prevalence of beta-cell, thyroid and celiac autoimmunity in North Indian children with recent-onset type 1 diabetes (T1D). *Journal of Clinical and Diagnostic Research: JCDR*, *9*(3), SM01.
12. Dhanwal, D. K., Agarwal, S., Garg, S., & Agarwal, P. (2014). Clinical & immunological profile of newly diagnosed patients with youth onset diabetes mellitus. *Indian Journal of Medical Research*, *140*(3), 356-360.
13. Endesfelder, D., Hagen, M., Winkler, C., Haupt, F., Zillmer, S., Knopff, A., ... & Achenbach, P. (2016). A novel approach for the analysis of longitudinal profiles reveals delayed progression to type 1 diabetes in a subgroup of multiple-islet-autoantibody-positive children. *Diabetologia*, *59*, 2172-2180.
14. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. (2003). Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes care*, *26*(suppl_1), s5-s20.
15. Goncalves, C. B. C. D., Silva, I. N., Tanure, M. G., & Bahia, M. (2013). Study of prevalence of celiac disease in children with type 1 diabetes mellitus: result of 10 years of follow-up. *Arquivos Brasileiros de Endocrinologia & Metabologia*, *57*, 375-380.
16. Gale, E. (2002). A missing link in the hygiene hypothesis?. *Diabetologia*, *45*, 588-594.
17. Kimpimaki, T., Kulmala, P., Savola, K., Kupila, A., Korhonen, S., Simell, T., ... & Knip, M. (2002). Natural history of β-cell autoimmunity in young children with increased genetic susceptibility to type 1 diabetes recruited from the general population. *The Journal of Clinical Endocrinology & Metabolism*, *87*(10), 4572-4579.
18. Kumar, K. M. P. (2015). Incidence trends for childhood type 1 diabetes in India. *Indian journal of endocrinology and metabolism*, *19*(Suppl 1), S34-S35.

19. Maahs, D. M., West, N. A., Lawrence, J. M., & Mayer-Davis, E. J. (2010). Epidemiology of type 1 diabetes. *Endocrinology and Metabolism Clinics*, *39*(3), 481-497.
20. Marita, A. R., Rane, S., Mokal, R. A., Nair, S. R., & Irani, A. (2004). Autoantibodies against GAD65 and IA-2 in recently diagnosed Type 1 diabetic children from Western India. *Diabetic medicine*, *21*(8), 956-957.
21. Mayer-Davis, E. J., Dabelea, D., Crandell, J. L., Crume, T., D'Agostino Jr, R. B., Dolan, L., & The, N. (2013). Nutritional factors and preservation of C-peptide in youth with recently diagnosed type 1 diabetes: SEARCH Nutrition Ancillary Study. *Diabetes Care*, *36*(7), 1842-1850
22. Naaraayan, S. A., Dhakshayani, R. V., & Chandramohan, R. (2021). Autoimmunity in south Indian children with recently diagnosed type 1 diabetes mellitus. *Journal of Diabetology*, *12*(2), 182-185.
23. Paudel, U., Paudyal, B. P., Kansakar, P., & Shakya, M. (2021). Clinical characteristics and perspectives of children and youths with type 1 diabetes attending Patan Hospital, Nepal. *Journal of Patan Academy of Health Sciences*, *8*(1), 113-120.
24. Sanyal, D. (2020). Current perspective on auto-antibodies in type 1 diabetes. *Indian Journal of Endocrinology and Metabolism*, *24*(3), 233-234.
25. Sanyal, D., Batabyal, S. K., Maity, S., & Chatterjee, S. (2019). Changing profile of GAD and IA-2 antibody positivity in Indian children with recently diagnosed type 1 diabetes mellitus. *Clinical Diabetology*, *8*(2), 116-120.
26. Sanyal, D., Majumder, A., Chaudhuri, S. R., & Chatterjee, S. (2017). Thyroid profile and autoantibodies in Type 1 diabetes subjects: A perspective from Eastern India. *Indian Journal of Endocrinology and Metabolism*, *21*(1), 45-50.
27. Scottish Diabetes Survey Monitoring Group (2016) Scottish Diabetes Survey 2016. http://www.diabetesinscotland.org.uk/Publications/Scottish%20Diabetes%20Survey%202016.pdf.
28. Shakya, B., Shrestha, N., & Shrestha, S. R. (2020). An epidemiological study of diabetes mellitus in productive aged urban Nepalese of Makalbari area of Gokarneshwor Municipality. *Nepal Medical College Journal*, *22*(3), 173-180.
29. Shrestha, S., Maurya, M., & Manandhar, K. D. (2021). An Investigation on the Detection of Human Leucocyte Antigen HLA Class I Loci (A, B, C) and Class II Loci (DR, DQ) Allele Frequency in Nepalese Population by Next Generation Sequencing. *Journal of Applied Pharmaceutical Sciences and Research*, *4*(1), 1-6.
30. Sorgjerd, E. P. (2019). Type 1 diabetes-related autoantibodies in different forms of diabetes. *Current diabetes reviews*, *15*(3), 199-204.
31. Tandon, N., Shtauvere-Brameus, A., Hagopian, W. A., & Sanjeevi, C. B. (2002). Prevalence of ICA-12 and other autoantibodies in north Indian patients with early-onset diabetes. *Annals of the New York Academy of Sciences*, *958*(1), 214-217.
32. Thomas, N. J., Jones, S. E., Weedon, M. N., Shields, B. M., Oram, R. A., & Hattersley, A. T. (2018). Frequency and phenotype of type 1 diabetes in the first six decades of life: a cross-sectional, genetically stratified survival analysis from UK Biobank. *The lancet Diabetes & endocrinology*, *6*(2), 122-129.
33. Tina-quant Hemoglobin A1c Gen. 3 package insert. Roche Diagnostics. 2022.
34. Tuladhar, A., Shrestha, S., Raut, P. P., Bhandari, P., & Shrestha, P. (2013). HLA antigen distribution in renal transplant recipients and donors. *Journal of Nepal Health Research Council*.
35. Wenzlau, J. M., Juhl, K., Yu, L., Moua, O., Sarkar, S. A., Gottlieb, P., ... & Hutton, J. C. (2007). The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. *Proceedings of the National Academy of Sciences*, *104*(43), 17040-17045.
36. Wenzlau, J. M., Frisch, L. M., Hutton, J. C., Fain, P. R., & Davidson, H. W. (2015). Changes in zinc transporter 8 autoantibodies following type 1 diabetes onset: the type 1 diabetes genetics consortium autoantibody workshop. *Diabetes Care*, *38*(Supplement_2), S14-S20.

37. Wenzlau, J. M., Walter, M., Gardner, T. J., Frisch, L. M., Yu, L., Eisenbarth, G. S., ... & Hutton, J. C. (2010). Kinetics of the post-onset decline in zinc transporter 8 autoantibodies in type 1 diabetic human subjects. *The Journal of Clinical Endocrinology & Metabolism*, *95*(10), 4712-4719.
38. WHO. (2023)10 facts on Diabetes. Available from: https://www.who.int/features/
39. Williams, C. L., Aitken, R. J., Wilson, I. V., Mortimer, G. L., Long, A. E., Williams, A. J., ... & Gillespie, K. M. (2022). The measurement of autoantibodies to insulin informs the diagnosis of diabetes in a childhood population negative for other autoantibodies. *Diabetic Medicine*, *39*(12), e14979.
40. Zamanfar, D., Aarabi, M., Amini, M., & Monajati, M. (2020). Prevalence of autoantibodies in type 1 diabetes mellitus pediatrics in Mazandaran, North of Iran. *Journal of Pediatric Endocrinology and Metabolism*, *33*(10), 1299-1305.
41. Zhang, M., Wang, X., Wang, R., Shu, J., Zhi, X., Gu, C., ... & Lv, L. (2022). Clinical study of autoantibodies in type 1 diabetes mellitus children with ketoacidosis or microalbuminuria. *Journal of Clinical Laboratory Analysis*, *36*(1), e24164.