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Antigastric parietal cell antibody as a screening test for Autoimmune gastritis in Egyptian children and adolescents with juvenile autoimmune thyroid disease and those with type 1 diabetes, single center experience

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Abstract---Purpose: The diagnosis of pediatric autoimmune gastritis (AIG) is critical due to the poor outcome and risk of malignancy. Therefore, we evaluated the prevalence of autoimmune gastritis in children with autoimmune thyroid disease (ATD) and type 1 diabetes mellitus (T1D), using parietal cell antibody (PCA) to identify its use as a screening test. Methods: PCA was measured in 90 patients; 45 patients with ATD (Hashimoto Thyroiditis and Graves' Disease) and 45 patients with T1D. Their ages ranged from 5 to 18 years. Hemoglobin, ferritin, and HbA1c (in diabetic patients) were measured. Results: PCA demonstrated a statistically significant difference between the

two groups of patients with a p-value (0.024). The mean value of PCA in patients with T1D was 196.45, while it reached 148.58 in patients with ATD, with a p-value (0.003). Thirty-one (68.9%) and twelve (26.7%) of patients with T1D had high (30-200) and extremely high (> 200) values of PCA, respectively, compared to twenty-six (57.8%) and eight (17.8%) of patients with ATD who had high (30-200) and extremely high (> 200) values of PCA, respectively. Seven (87.5%) of patients with ATD with extremely high PCA(above 200) also had normocytic anemia. ATD patients with high and extremely high PCA levels had more gastritis symptoms than those with normal PCA levels, who were asymptomatic, and this was statistically significant where p-value (0.05). Conclusion: Patients with T1D had higher levels of PCA compared to those with ATD.. So PCA can be used as a screening test for autoimmune gastritis especially in limited resource settings. Its level is related directly to symptoms of gastritis.

Keywords---parietal cell antibody, autoimmune gastritis, autoimmune thyroid disease, type 1 diabetes mellitus.

Introduction

Autoimmune gastritis (AIG) is one of the most pervasive organ-specific autoimmune diseases in children, which may present alone or associated with other autoimmune diseases, most notably Autoimmune thyroid disease (ATD) or Type 1 diabetes mellitus (T1D).[1] In addition, it is a chronic inflammatory condition in which parietal cells of the corpus and fundus of the stomach are destroyed, leading to mucosal metaplasia and atrophy. It is also linked to a higher risk of adenocarcinoma and neuroendocrine tumors.[2]. Serum autoantibodies directed against gastric parietal cells (anti-parietal cell antibodies, PCA) have been associated with AIG and have become an effective tool for AIG diagnosis. PCA positivity has been reported in 80% to 90% of patients with AIG and subjects free from clinical gastric disease. In detecting PCA, the enzyme-linked immunosorbent assay (ELISA) is believed to be consistently more sensitive than indirect immunofluorescence (IIF). [1] In T1D, the autoimmune process that leads to the destruction of β-cells is not limited to pancreatic islets but also affects other tissues. In children, the most prevalent organ-specific autoimmune diseases associated with T1D are (ATD). [3]

However, it may be associated with other autoimmune disorders such as autoimmune gastritis (AIG) and celiac disease**.** ATD is the most common etiology of acquired thyroid dysfunction in pediatrics. ATD is a multifactorial disease with a genetic predisposition and environmental risk factors. Graves' disease (GD) and autoimmune thyroiditis (AT) are the two most common ATDs, both of which are characterized by the presence of circulating thyroid autoantibodies and infiltration by autoreactive lymphocytes of the thyroid gland and abnormal thyroid function (hyperthyroidism in GD and hypothyroidism in AT). [2] There have been few studies on children and adolescents that demonstrated the prevalence of AIG. Moreover, gastritis symptoms are typically non-specific and overlap with symptoms of other diseases among the pediatric age group. In this study, we

investigated the prevalence of AIG in children and adolescents with T1D and those with AID using PCA as a screening test for early detection of AIG.

Materials and Methods

Patients

This cross-sectional analytical study included 90 patients from 5 to 18 years attending the outpatient clinic. The study was conducted during the period from December 2019 to August 2021. They were divided into two groups; the first group included 45 patients with AID, either Autoimmune thyroiditis (AT) or Graves' Disease. Diagnosis of AT was based on finding one or more thyroid autoantibodies (thyroid peroxidase or thyroglobulin autoantibody) and thyroid ultrasound characterized by a lack of homogeneity, with a hypogenic or mixed echo pattern. Graves' Disease was primarily diagnosed clinically based upon symptoms of hyperthyroidism (excessive sweating, heat intolerance, tremors, palpitation, weight loss). The second group included 45 patients with T1D either presenting with diabetic ketoacidosis or accidentally diagnosed with hyperglycemia. All patients who fulfilled the inclusion criteria were subjected to full history, including (demographic data, onset of diabetes and thyroid disease, presence of any gastrointestinal symptoms in the form of chronic abdominal pain, nausea, vomiting, hiccough, family history of similar condition). Thorough physical examination including (anthropometric data: height, weight, BMI (SDS), pubertal assessment using Tanner staging , detection of signs of poor glycemic control as lipodystrophy, limited joint mobility, and thyroid examination). Subjects underwent the following:

- Laboratory evaluation in the form of:
  - Complete blood count done on CELL-Dyn 3700 using kits supplied by spectra group.
  - Serum ferritin done by chemiluminescence on Architect using kits supplied by Abbott.
  - Thyroid profile (TSH –Free T3 –Free T4) and Thyroid antibodies (Thyroglobulin and Thyroid peroxidase) done by electrochemiluminescence on Immulite using kits supplied by Roche.
  - Glycosylated hemoglobin (HbA1C) done on Dimention using kits from Siemens. For following up glycemic control in patients with T1D, which was done at diagnosis and then for three months later.
  - Detection of parietal cell antibody (PCA) in serum test using Enzyme-Linked Immunosorbent Technique (ELISA) method.
  - Helicobacter pylori stool antigen for symptomatic patients with epigastric pain.
- Imaging: Thyroid ultrasound, Thyroid scan, abdominopelvic ultrasound

Methods

Human Gastric Parietal Cell Antibody (PCA) was determined qualitatively in serum, plasma, and other biological fluids.

Principle of the assay

In order to determine the presence or absence of human anti-PC in samples, the kit employs a sandwich enzyme-linked immunosorbent assay (ELISA).

Determine the result

- Test validity: the average of positive control well ≥1.00; the average of negative control well ≤0.15.
- Calculate Critical (CUT OFF): Critical= the negative control average well + 0.15.
- Negative Result: sample OD< Calculate Critical (CUT OFF) is Negative.
- Positive Result: sample OD≥ Calculate Critical (CUT OFF) is Positive.
- PCA level classified into: a) Normal value < 30, b) High values: 30-200, c) Extreme high values: > 200.
- Positive cut off > 30, Negative cut off < 30

Statistical analysis

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Data were expressed as mean, standard deviation, median, minimum, and maximum, whereas quantitative data were expressed as frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were made using the non-parametric Mann-Whitney test. [4] The Chi-square (2) test was performed to compare categorical data, and the same test was used instead when the expected frequency was less than 5. [5] P-values less than 0.05 were considered statistically significant.

Results

The mean age of the patients with ATD and T1D was 9.58 (± 3.53 SDS) and 8.36 (± 2.7 SDS), respectively. In addition, 37 (82%) of patients with ATD were females, while 8 (17.8%) were males. It was found that 24 (46%) of patients with T1D were males, while 24 (53.3%) were females, who were more affected by ATD and T1D compared to males, with a statistically significant p-value (0.003). Older ATD patients were more likely than newly diagnosed patients to attend the outpatient clinic for follow-up. In contrast, newly diagnosed patients with T1D were more than old diabetic patients coming for follow-up 57.8% and 42.2%, respectively, with a statistically significant p -value< 0.001, as demonstrated in Fig (1). As depicted in Table (1), the two groups of patients were comparable in terms of gastritis symptoms such as (epigastric pain, nausea, vomiting, and hiccough).

Table 1
Comparison between clinical symptoms of gastritis in the two studied groups

		ATD (n:45)		T1DM (n:45)		p-value
		count	%	count	%	
Epigastric	Yes	13	28.9%	7	15.6%	0.128

pain	No	32	71.1%	38	84.4%	
Nausea and vomiting	Yes	5	11.1%	2	4.4%	0.434
	No	40	88.9%	43	95.6%	
Hiccough	Yes	2	4.4%	6	13.3%	0.266
	No	43	95.6%	39	86.7%	

No statistical significance in the symptoms of gastritis between the two study groups p- value>0.05

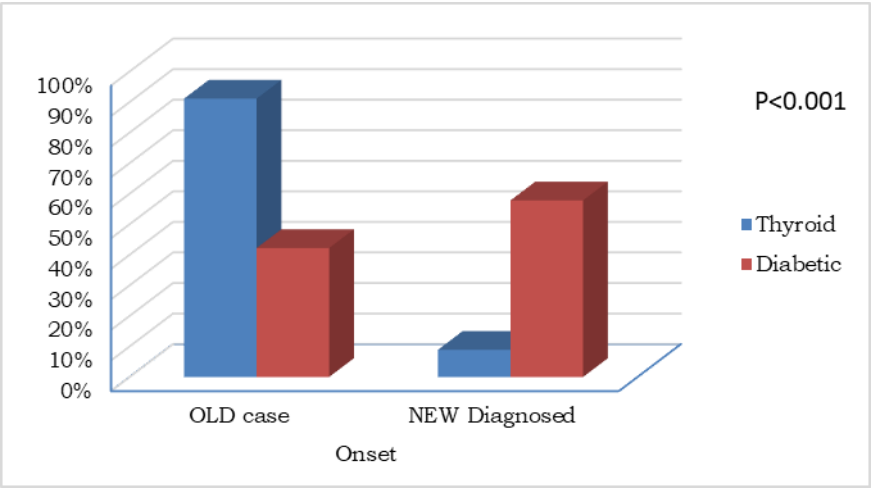

Fig 1. Comparison of onset of the disease among the two studied groups

Thirty-one (68.9%) patients with ATD were diagnosed with Hashimoto thyroiditis, while 14 (31.1%) were diagnosed with Grave's disease. During routine follow-up, we found that 28 (62.2%) of patients with T1D were anemic despite the normal celiac screening, while 35 (77.8%) of patients with ATD had normal hemoglobin levels, with a statistically significant p-value < 0.05. Fourteen (31.1%) of patients with T1D had a subnormal level of ferritin, and ten (22.2%) had a normal level. Nonetheless, this association did not demonstrate a statistically significant difference. Regarding glycemic control of patients with T1D, 27 subjects (60%) were well controlled. (HbA_{1C} < 7%, <53 mmol/mol), whereas 18 (40%) were poorly controlled (HbA_{1C} > 7%, > 53 mmol/mol) according to the international society of pediatric and adolescent diabetes (ISPAD) guidelines. [6]

PCA was measured in all patients who met the inclusion criteria, and the mean value in T1D patients was 196.45 and 148.58 in ATD patients, with a statistically significant p-value (0.003). Eleven patients (24.4%) of ATD patients, while two (4.4%) of the T1D patients had a normal value of PCA (PCA< 30). Furthermore, 31 subjects (68.9%) with T1D and 26 (57.8%) of ATD patients had high values of PCA (PCA 30-200). Twelve (26.7%) of T1D patients and 8 (17.8%) of patients with ATD had extremely high values of PCA (PCA> 200), with a p-value of 0.024, as depicted in Fig (2).

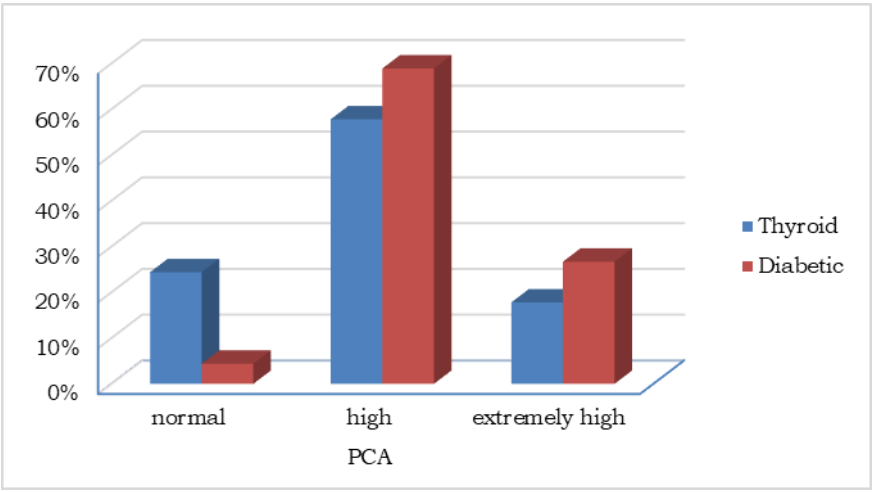

Fig 2. Comparison between Parietal cell antibody level in the two studied groups

There was no statistically significant correlation between the presence of PCA, whether normal, high, or extremely high, and age of patients, neither sex nor onset of the disease or diagnosis. Gastritis symptoms such as epigastric pain, nausea, vomiting, and hiccough were more prevalent in patients with ATD with high and extremely high PCA values, while patients with normal PCA values were asymptomatic, with a statistically significant p-value< 0.05, as displayed in Table (2).

Table 2
Relation between PCA and gastric symptoms in patients with ATD

ATD(n:45)		PCA						p-value
		Normal		High		Extremely high		
		Count	%	Count	%	Count	%	
Epigastric pain	Yes	0	0.0%	9	34.6%	4	50.0%	0.023
	No	11	100.0%	17	65.4%	4	50.0%	
Nausea / vomiting	Yes	0	0.0%	2	7.7%	3	37.5%	0.046
	No	11	100.0%	24	92.3%	5	62.5%	
Hiccough	Yes	0	0.0%	0	0.0%	2	25.0%	0.028
	No	11	100.0%	26	100.0%	6	75.0%	

Gastritis symptoms were more in patients with ATD with high and extreme high values of PCA, while patients with normal values of PCA level were asymptomatic, this was statistically significant where p- value< 0.05. In patients with T1D, symptoms of gastritis varied in different groups, patients with normal PCA levels were asymptomatic, while three (9.7%) of patients with high and four (33%) with extremely high PCA levels developed epigastric pain. Three (9.7%) patients with high and three (25%) with extremely high PCA levels developed hiccoughs. Nausea and vomiting were present in two (6.5%) of the patients with high PCA levels; however, it was not reported in patients with extremely high levels of PCA, with a statistically insignificant p-value > 0.05.

In patients with ATD, three (11.5%) with high and seven (87.5%) with an extremely high level of PCA had anemia (Normocytic) that was accidentally detected during a routine checkup, with a statistically significant difference p-value <0.001. On the contrary, evaluation of the correlation between PCA different levels and the presence of anemia in patients with T1D revealed that one (50%) of patients had normal PCA level, 17 (54.8%) had high levels, and ten (83.3%) had extremely high PCA levels developed anemia (Normocytic), with statistically insignificant p-value > 0.05. Hashimoto thyroiditis was diagnosed in nine (81.8%) of patients with normal PCA levels, in eighteen (69.2%) with high and four (50%) with extremely high PCA levels. Graves' disease was diagnosed in two (18%) of patients with normal PCA levels, in eight (30%) with high PCA levels, and four (50%) with extremely high PCA levels, with a non-statistically significant difference p-value 0.373. Regarding glycemic control and PCA levels among patients with T1D, all patients with T1D with normal PCA levels were well controlled, 17 (54.8%) with high PCA levels, and eight (66.7%) with extremely high PCA levels were well controlled, respectively, where HbA_{1C} was (< 7%, < 53mmol/mol). Fourteen patients (45.1%) with high PCA levels and four (33.3%) with extremely high PCA levels were poorly controlled, respectively, where HbA_{1C} was (> 7%, > 53mmol/mol), with non-statistically significant difference, p-value 0.75.

Discussion

There is an age-related increase in AIG prevalence in the general population, from 2.5% in the third decade to 12% in the eighth decade. [7, 8] Moreover, there are gaps in epidemiology and the incidence of gastric autoimmunity, particularly in the pediatric age. [2] Autoimmune gastritis is a common autoimmune disorder in up to 2% of the general population. The prevalence is 3- to 5-fold higher in type 1 diabetes or autoimmune thyroid disease patients. [9] In the current study, the female to male ratio in the current study was 4.8:1 and 1:1 in patients with T1D. Thirty-seven (82.2%) of patients with ATD were females with a mean age of nine years and SD (±3.5), while 24 (35%) of patients with T1D were females with a mean age of eight years and SD (±2.7). This finding is consistent with the study of Manjiri et al. (2017), who demonstrated that Hashimoto's thyroiditis affects 1.3% of children and has a female predominance with a 9:1 female to male ratio. [10] It also aligns with the cohort study conducted by Calcaterra et al. (2019) on 220 children with ATD (184 female and 36 male), with a mean (±SD) age at diagnosis was 11.28±6.37 years. [2]

Females are more frequently affected than males by the majority of autoimmune diseases. There are different mechanisms for sex-specific differences, including gender differences in immune response and organ vulnerability, sex hormones, genetic predisposition, parental inheritance, and epigenetics. [11] In this study, 68.9% of patients with ATD were diagnosed with Hashimoto thyroiditis, while 31% were diagnosed with Graves' disease; this finding is consistent with the study of Calcaterra et al. (2019), which revealed that the majority of patients had Hashimoto's thyroiditis (186/220) while Graves' disease patients (34/220). In addition, there was no significant difference in age at detection between patients with Hashimoto's thyroiditis and those with Graves' disease. [2]. In the current study, 91% of ATD patients were previously diagnosed, while 57% of patients with

T1D were newly diagnosed. The increasing incidence of diabetes can explain this among children and increased awareness about its symptoms and diagnosis. This finding was concluded from the meta-analysis conducted by M Mobasseri et al. (2020), which showed that the incidence of T1D was 15 per 100,000 people, and the prevalence was 9.5% (95% CI: 0.07 to 0.12) worldwide, which was statistically significant. [12] Also, according to the Egyptian study conducted by El-Ziny et al. (2014), the incidence of T1D is 8/100 000 per year in Egyptian children under the age of 15 years. [13]. Regarding gastritis symptoms in patients with ATD and T1D, most of the patients did not demonstrate any symptoms (Epigastric pain, nausea, vomiting, hiccough) in their presentation. Anxin et al. (2020) discussed that common gastrointestinal symptoms were nausea, vomiting, diarrhea, and abdominal pain. [14] Diarrhea was the most common gastrointestinal symptom in thyrotoxicosis as well as in Thyroid storm (TS). The most common gastrointestinal symptoms in patients with TS were nausea, and the most common cause of TS is Graves' disease, which is characterized by unknown or poor compliance with anti-thyroid drugs. [14] In this study, PCA was measured in all patients within the inclusion criteria, a statistically significant difference was found between the mean value of PCA in T1D patients, and ATD patients was 196.45 and 148.58, respectively. This finding is consistent with the study by Besançon et al. (2017), who demonstrated that autoimmune gastritis is common in children and adolescents with T1D but is rarely symptomatic. [3]

In the current study, there was no statistically significant correlation between PCA, either normal, high, or extremely high, and age, sex, the onset of the disease in both study groups. In contrast, the study of Besançon et al. (2017) on children under the age of 15 years revealed that the antibody-positive rate seemed more common in girls, although this was a non-significant trend. [3]. In this study, there was a statistically significant correlation between the presence of symptoms of gastritis in the form of (epigastric pain, nausea, vomiting, hiccough) and the value of PCA (either high or extremely high) in patients with ATD, while patients with normal PCA value were asymptomatic. While in patients with T1D, this correlation was statistically insignificant. This finding agrees with the study of Besançon et al. (2017), who illustrated that no significant differences were found in gastric symptoms between autoantibody-positive and negative patients with T1D, which aligns with the hypothesis that epigastric pain and dyspepsia are common symptoms in children. [3] Although anemia was more prevalent in patients with T1D than in patients with ATD, there was a statistically significant correlation between PCA level and subnormal hemoglobin in patients with ATD. Seven (87%) of patients with ATD with high PCA (more than 200) had anemia (normocytic), while the relation between PCA and anemia in patients with T1D was statistically insignificant. This finding is consistent with the study of Besançon et al. (2017) on children with T1D, where the patients screened positive PCA and those who were screened negative did not show a difference in mean values for hemoglobin level hematocrit, corpuscular volume, serum iron, and ferritin. [3]

In the present study, there was no statistically significant difference between the two groups of ATD patients (Hashimoto thyroiditis and Graves' disease) regarding PCA. This finding aligns with the study of Calcaterra et al. (2019), who demonstrated that the prevalence of PCA detection was not different based on the

underlying thyroid diagnosis: 8 of 186 (4.3%) in Hashimoto thyroiditis and 2 of 34 (5.8%) in Graves' disease. [2]. In this study, the correlation between PCA and glycemic control was statistically insignificant, based on HbA_{1C}. All patients with T1D with normal PCA levels were well controlled, 17 (54.8%) with high PCA levels, and eight (66.7%) with significantly high PCA levels were well controlled, where HbA_{1C} was (< 7%, < 53mmol/mol). Fourteen patients (45.1%) with high PCA levels and four (33.3%) with extremely high PCA levels were poorly controlled, where HbA1C was (> 7%, > 53mmol/mol), which was statistically insignificant.

In the study of Zoltan et al. (2020), HbA_{1C} levels in the high Chromogranin A (CgA) group as a predictor of autoimmune gastritis were significantly higher than those in the normal CgA group. However, based on the HbA_{1C} values, 69.6% of the patients in this study had poor glycemic control (HbA_{1C} ≥7.0%, which equals ≥53 mmol/mol) in the normal CgA group, whereas this figure rose to 96.4% of patients in the high CgA group. [15]. In conclusion, the present study demonstrated that, while the mean value of PCA as a marker of autoimmune gastritis was more in patients with T1D than patients with ATD, the gastritis symptoms were statistically significant in ATD patients with high and extremely high PCA levels compared to patients with T1D. Anemia was more prevalent in patients with T1D than patients with ATD. Nonetheless, 87.5% of patients with ATD with substantially high PCA levels were anemic. PCA is a useful marker for identifying patients at risk of developing AIG. Due to the longer life expectancy of the pediatric age group, early monitoring is mandatory for children and adolescents with T1D and ATD.

Limitations of the study

- PCA is a useful marker to screen for AIG but its high variability prevents its use as a single diagnostic and prognostic test.
- It should be combined with other parameters in a clinical laboratory index to diagnose AIG to guide clinical and therapeutic decisions.
- Esophagogastroduodenal endoscopy is indicated to confirm the diagnosis of autoimmune gastritis, as gastritis symptoms are non-specific, particularly in children.
- Further studies are recommended to assess the prevalence of autoimmune gastritis in patients with autoimmune thyroid disease and T1D on a larger scale.

Statements and Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Nancy Samir Subhy, Walaa Abdelfattah, and Radwa A. Shamma. The first draft of the manuscript was written by Radwa A. Shamma and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics Approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Cairo University Code: MD-34-2020 on 2nd February 2020.

References

1. Ariani, R. P., Darmawan, D. P., Atmaja, N. B., & Wijaya, I. M. A. S. (2018). Balinese traditional culinary promotes food skills and its positive impact on tourism vocational school. International Journal of Life Sciences, 2(1), 50–62. https://doi.org/10.29332/ijls.v2n1.107
2. Bagnasco M, Saverino D, Pupo F, Marchiano M, Alessio MG, Schlumberger W, Antico A, Pesce G, Bizzaro N. Estimate of the Prevalence of Anti-Gastric Parietal Cell Autoantibodies in Healthy Individuals Is Method Dependent. Am J Clin Pathol. 2018 Aug 30;150(4):285-292. doi: https://doi.org/10.1093/ajcp/aqy061. PMID: 30007281.
3. Besançon A, Michaud B, Beltrand J, Goncalves T, Jais JP, Polak M, Chatenoud L, Robert JJ. Revisiting autoimmune gastritis in children and adolescents with type 1 diabetes. Pediatr Diabetes. 2017 Dec;18(8):772-776. doi: https://doi.org/10.1111/pedi.12482. Epub 2016 Dec 22. PMID: 28004477.
4. Calcaterra V, Montalbano C, Miceli E, Luinetti O, Albertini R, Vinci F, Regalbuto C, Larizza D. Anti-gastric parietal cell antibodies for autoimmune gastritis screening in juvenile autoimmune thyroid disease. J Endocrinol Invest. 2020 Jan;43(1):81-86. doi: https://doi.org/10.1007/s40618-019-01081-y. Epub 2019 Jul 1. PMID: 31264142.
5. Chan YH. Biostatistics 102: quantitative data--parametric & non-parametric tests. Singapore Med J. 2003 Aug;44(8):391-6. PMID: 14700417.
6. Chan YH. Biostatistics 103: qualitative data - tests of independence. Singapore Med J. 2003 Oct;44(10):498-503. PMID: 15024452
7. De Block CE, De Leeuw IH, Van Gaal LF. Autoimmune gastritis in type 1 diabetes: a clinically oriented review. J Clin Endocrinol Metab. 2008 Feb;93(2):363-71. doi: https://doi.org/10.1210/jc.2007-2134. Epub 2007 Nov 20. PMID: 18029461.
8. Dighe M, Barr R, Bojunga J, Cantisani V, Chammas MC, Cosgrove D, Cui XW, Dong Y, Fenner F, Radzina M, Vinayak S, Xu JM, Dietrich CF. Thyroid Ultrasound: State of the Art Part 1 - Thyroid Ultrasound reporting and Diffuse Thyroid Diseases. Med Ultrason. 2017 Jan 31;19(1):79-93. doi: https://doi.org/10.11152/mu-980. PMID: 28180201
9. DiMeglio LA, Acerini CL, Codner E, Craig ME, Hofer SE, Pillay K, Maahs DM. ISPAD Clinical Practice Consensus Guidelines 2018: Glycemic control targets

and glucose monitoring for children, adolescents, and young adults with diabetes. Pediatr Diabetes. 2018 Oct;19 Suppl 27:105-114. doi: https://doi.org/10.1111/pedi.12737. PMID: 30058221.
10. El-Ziny MA, Salem NA, El-Hawary AK, Chalaby NM, Elsharkawy AA. Epidemiology of childhood type 1 diabetes mellitus in Nile Delta, northern Egypt - a retrospective study. J Clin Res Pediatr Endocrinol. 2014;6(1):9-15. doi: https://doi.org/10.4274/jcrpe.1171. PMID: 24637304; PMCID: PMC3986743.
11. Herold Z, Herold M, Nagy P, Patocs A, Doleschall M, Somogyi A. Serum chromogranin A level continuously rises with the progression of type 1 diabetes, and indicates the presence of both enterochromaffin-like cell hyperplasia and autoimmune gastritis. J Diabetes Investig. 2020 Jul;11(4):865-873. doi: https://doi.org/10.1111/jdi.13203. Epub 2020 Feb 3. PMID: 31883432; PMCID: PMC7378417.
12. Jacobson DL, Gange SJ, Rose NR, Graham NM. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. Clin Immunol Immunopathol. 1997 Sep;84(3):223-43. doi: https://doi.org/10.1006/clin.1997.4412. PMID: 9281381.
13. Karthik, G., Nataraj, N., Shantha Kumari, N., & Srinivasa, K. V. (2022). Comparative study of copper, zinc levels in uncontrolled type 2 diabetes mellitus with normal subjects. International Journal of Health Sciences, 6(S4), 11748–11753. https://doi.org/10.53730/ijhs.v6nS4.11321
14. Li A, Jiang X, Zhong M, Li N, Tao Y, Wu W, Yang C, Wang H, Min L, Ma Y, Deng W. Intractable hiccups as a rare gastrointestinal manifestation in severe endocrine and metabolic crisis: case report and review of the literature. Ther Adv Endocrinol Metab. 2020 Jun 20;11:2042018820934307. doi: https://doi.org/10.1177/2042018820934307. PMID: 32612802; PMCID: PMC7307285.
15. Mobasseri M, Shirmohammadi M, Amiri T, Vahed N, Hosseini Fard H, Ghojazadeh M. Prevalence and incidence of type 1 diabetes in the world: a systematic review and meta-analysis. Health Promot Perspect. 2020 Mar 30;10(2):98-115. doi: https://doi.org/10.34172/hpp.2020.18. PMID: 32296622; PMCID: PMC7146037.
16. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. Front Neuroendocrinol. 2014 Aug;35(3):347-69. doi: https://doi.org/10.1016/j.yfrne.2014.04.004. Epub 2014 May 2. PMID: 24793874.
17. Whittingham S, Mackay IR (1985) Pernicious anemia and gastric atrophy. In: Rose NR, Mackay IR (eds) The autoimmune diseases. Academic Press, New York, pp 243–266. doi: https://doi.org/10.1016/B978-0-12-596920-8.50017-2
18. Widana, I.K., Sumetri, N.W., Sutapa, I.K., Suryasa, W. (2021). Anthropometric measures for better cardiovascular and musculoskeletal health. *Computer Applications in Engineering Education*, *29*(3), 550–561. https://doi.org/10.1002/cae.22202