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TYPE 1 DIABETES IN THE PEDIATRIC AGE: FROM DUSK TILL DAWN

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CONTENT

PREFACE

This dissertation is intended as articles collection from indexed peer-review journals concerning diabetes in the pediatric age that I develop during my Ph.D. program from 2018 to date.

Diabetes (especially type 1) is the most frequent endocrinopathy in children and adolescents, but accounts for morbidity and mortality in all age groups. Short-term and long-term complications are well known between clinicians, and different studies directed towards primary, secondary and tertiary prevention have been running for several years. Prevalent therapy is insulin, injected more times during the day and necessary *quoad vitam*.

Incidence of diabetes is increasing in the U.S. (estimated +1,8% [1]), and in 2021 there was 1,5 millions of children and adolescents, which will be 80% more in 2040 (more than 2,7 millions) [2].

Basic research is of the outmost importance, but clinical care and diabetes management are able to improve outcomes and, at the same, reduce the burden at this moment, especially through applied technology [3]. Education, particularly, is fundamental to create knowledge and awareness around diabetes, and if we succeed, we can reduce long-term cost of this chronic disease today, while waiting for the cure tomorrow [4].

As university center, pursuing a better care for children and adolescents with diabetes using science and research is in our mandate, and we have the responsibility (but also the honor) to be part of the change in the way we manage and in how patients live diabetes.

Connection between glucose outcomes and quality of life, the use of technology to improve metabolic outcome, but also how to manage physical activity or if Sars-Cov-2 pandemic changed diabetes management are some of the topics we searched about. These topics will be analyzed through the articles published during my Ph.D.

CHAPTER I – TYPE 1 DIABETES DEVELOPMENT - FROM WHAT IS KNOWN TO NEW HYPOTHESIS

BACKGROUND

Type 1 diabetes, which develops most frequently in childhood but can also present in adult life, is a prime candidate to explore the relationships among risk loci, age at diagnosis, and genetic contribution to disease [5]. It has long been recognized that genetics plays a role in determining age at type 1 diabetes diagnosis. For monozygotic twins, concordance for type 1 diabetes increases with younger age at diagnosis in the index twin [6]. Combining information from type 1 diabetes–associated HLA and non-HLA loci, progression is predicted from single to multiple autoantibody positivity in individuals under age 35 years but not in older participants [7].

But genetic is not sufficient; a "second-hit" on the immune system is necessary to develop antibodies directed toward β-cells, with a progressive loss of insular mass and consequent insulin deficiency [8]. What is still debated is this immune activation, since culprit (or culprits) inducing CD8+ T-cells against β-cells are still unclear [9]. Epidemiological, clinical and pathological studies in humans support a role for viral infections, particularly *enteroviruses*, in type 1 diabetes [10]. There is evidence that infection in early life may may initiate the autoimmune process or later development of type 1 diabetes, but definitive proof is lacking [11].

Moreover, the envelope protein of HERV-W family, one of the endogenous retroviruses embedded in our DNA, named HERV-W-Env, was detected in pancreata from T1D patients and was shown to display pro-inflammatory properties and direct toxicity toward pancreatic beta cells [12]. We sought to determine RNA expression from endogenous retroviruses in subjects with T1D at the onset compared to control population, to confirm or discard this observation [13].

TITLE: ENHANCED EXPRESSION OF HUMAN ENDOGENOUS RETROVIRUSES IN NEW-ONSET TYPE 1 DIABETES: POTENTIAL PATHOGENETIC AND THERAPEUTIC IMPLICATIONS.

AUTHORS: Pier-Angelo Tovo, Ivana Rabbone, Davide Tinti, Ilaria Galliano, Michela Trada, Valentina Daprà, Franco Cerutti & Massimiliano Bergallo.

INTRODUCTION

Human endogenous retroviruses (HERVs) constitute about 8% of the human genome. They are the remnants of ancestral infections that led to their integration into the genome of primates over 25 millions of years ago with subsequent transmission to every future generation [1,2]. During evolution the accumulation of mutations, deletions and recombinations blocked the production of infectious virions. Most HERV elements are inactive, but some are transcribed and encode viral proteins. HERVs can stimulate or block the transcription of close cellular genes, acting directly as proviruses, or indirectly through novel insertions into the DNA by retro-transposition of HERV pseudogenes. For instance, they can act as promoters to trigger activation or suppression of genes involved in carcinogenesis [3]. HERV transcription can be modulated by epigenetic factors, such as DNA methylation and heterochromatin-silencing by histone modifications [1]. Given their potential pathologic effects, HERVs have been studied and proposed as possible cofactors in the

etiopathogenesis of various diseases, including several autoimmune disorders [4], and their proinflammatory and autoimmune properties have been demonstrated [5].
Type 1 diabetes (T1D) is considered an autoimmune disease caused by a combination of genetic and environmental factors inducing immune responses against pancreatic b-cells, ultimately resulting in their destruction [6]. Autoantibodies against b cell antigens are produced months or years before the clinical manifestations and depletion of b cells is associated with T cell infiltration. Indeed, not only adaptive immunity but also innate immunity is involved in the disease process [7]. Differential expression profiles of miRNAs were observed in autoimmune diseases, including T1D [8], and gag, pol, and env RNAs of endogenous retroviruses were detected in the pancreatic islets from non-obese diabetic mice [9]. At disease onset these animals develop a cellular and humoral response against a b cell retroviral antigen [10] and antibody titers to retroviral targets increase as the disease progresses [11,12]. Furthermore, hyperglycaemia, decreased levels of insulin, and pancreatic immune cell infiltrates were observed in a HERV-W-env transgenic mice model [13]. Several families of HERVs have been identified; among these, HERV-H, HERV-K, and HERV-W are those most widely studied. The HERV-K family has been reported to be associated with T1D in humans [13], though with antithetical results [14,15], while recent findings suggest a pathogenetic role of HERV-W [12]. However, no study evaluated HERV activation at the diagnosis of diabetes. In order to gather further insights into the underlying mechanisms leading to the development of T1D we assessed the transcription levels of pol genes of HERV-H, HERV-K, and HERV-W in peripheral leucocytes from children and adolescents with new-onset T1D. The results documented significantly higher expressions of HERV-H-pol and HERV-W-pol, not of HERV-K-pol, in diabetic patients as compared to age-matched control subjects, suggesting a pathogenetic role of retroviral elements.

MATERIALS AND METHODS

White blood cells (WBCs) were collected from diabetic children and adolescents at time of diagnosis. Age-matched, asymptomatic subjects with no family history of diabetes who were tested at the Regina Margherita Children's Hospital, Turin, Italy, for routine laboratory examinations and whose results were all within the normal reference range were the control group. Subjects with any confirmed or suspected disease, such as infections, cancer, autoimmune disorders, inflammatory diseases, neurological disturbances, or abnormal laboratory results were excluded from the study. The tests were performed using leftovers of laboratory samples after informed parent's consent; data were gathered anonymously. 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. Informed consent was obtained from all individual participants involved in the study.

Reverse transcription

Total RNA was extracted from WBCs using the automated extractor Maxwell (Promega, Madison, WI) using RNA Blood Kit protocol without modification. This kit provides treatment with DNase during the RNA extraction process. Four hundred nanogram of total RNA was reverse-transcribed with 2 µl of buffer 10X, 4.8 µl of MgCl2 25mM, 2 µl ImpromII (Promega), 1 µl of RNase inhibitor 20U/l, 0.4 µl random hexamers 250 µM (Promega), 2 µl mix dNTPs 100mM (Promega) and dd-water in a final volume of 20 µl. The reaction mix was carried out in a GeneAmp PCR system 9700 Thermal Cycle (Applied Biosystems, Foster City, CA, USA) under the following conditions: 5 min at 25 °C, 60 min at 42 °C and 15 min at 70 °C for the inactivation of enzyme; the cDNAs were stored at -80° until use. About control for genomic DNA contamination we amplify directly RNA extract without reverse transcription.

Relative quantification of HERV expressions by real- time PCR

Relative quantification of mRNA expression of HERV-H, -K -W was achieved by means of PCR real time Taqman amplification and normalisation to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the ABI PRISM 7500 real time system (Life technologies, Texas, USA). GAPDH was chosen as reference gene being the most stable among 9 reference genes [16] and previously used in our targeted studies [17–19]. 40 ng of cDNA were amplified using HERV-H, -K and -W mRNA expression kit PP-BioMole- 054, -055 and -056 (BioMole srl, Torino, Italy) in a 20 µl total volume reaction. The amplifications were run in a 96- well plate at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Furthermore, in order to confirm that there was no DNA genomic contamination, control PCR was performed with RNA before reverse transcription using the same primers and probe described above. Each sample was run in triplicate. Relative quantification of target genes expression was performed with the DCt method. All HERVs pol expression data were normalised with GAPDH (housekeeping) expression. Using 40 ng of cDNA in amplification we obtained Ct value from 26 to 30.4. These Ct value correspond to a good performance of real time PCR. Since we measured Ct for all target in all the samples tested, we argued that our method is suitable for HERV detection and quantification.

Statistical analysis

Mann-Whitney test was used to compare the transcriptional levels of every HERV family in diabetic patients and age- matched control subjects. Spearman's rho correlation coefficient was calculated to determine any correlation between HERV transcriptional levels and child's age. Statistical analyses were done using the Prism software (GraphPad Software, La Jolla, CA). In all analyses, $p<.05$ was taken to be statistically significant.

RESULTS

The characteristics of children and adolescents with new- onset T1D and of control subjects are reported in Table 1. Diabetic patients were tested for 5 typical autoantibodies (ICA, IA2, IAA, GADA, ZnT8) and all were positive for at least two; they were also tested for the predisposing HLA and all were DRB1*0301-DQA1*0501-DQ*B10201(DR3)- positive and/or DRB1*0401-DQA1*0301-DQB1*0301 (DR4- DQ8)-positive. The pol genes of HERV-H, HERV-K, and HERV-W were always transcriptionally active in both the study populations. Their transcripts did not show significant variations according to the age both in normal subjects (HERV-H-pol: $p = 0.476$, HERV-K-pol: $p = .7769$, and HERV-W-pol: $p = .5475$, Figure 1) and in T1D patients (HERV-H-pol: $p = .5074$, HERV-K-pol: $p = .8967$, and p 1⁄4 .8967, and HERV-W-pol: $p = .6434$, Figure 2).

As detailed in Figure 3, the expression levels of HERV- H-pol and HERV-W-pol genes were significantly higher in diabetic patients than in age-matched control subjects: mean HERV-H-pol values: 0.191 ± SD 0.02 vs. 0.172 ± SD 0.01, $p =.0001$; mean HERV-W-pol values 0.319 ± SD 0.05 vs. 0.278±0.03, $p<.0001$, respectively. In contrast, no significant difference emerged in the expression levels of HERV- K-pol gene between diabetic patients and normal controls (mean values: 0.165 ± 0.02 vs. 0.159 ± SD 0.01, $p = .1393$).

DISCUSSION

Present results show that the pol genes of HERV-H, HERV- K, and HERV-W are persistently transcribed in WBCs from healthy children and adolescents as well as in diabetic patients. This is in line with our previous findings that all the three HERV families are physiologically expressed without significant variations during infancy and childhood [18]. The most interesting result of our study was that HERV-H- pol and HERV-W-pol transcripts were significantly higher in subjects with new-onset T1D than in control subjects of comparable age. Conversely, HERV-K-pol RNAs were similar between T1D patients and healthy subjects.

HERV-H is the most represented retroviral family in the human genome [20]. Extremely high levels of its transcripts are present in embryonic stem cells [21] and HERV-H fragments were detected in several human tissues and cancer cells [22]. The increase in HERV-H-pol expression emerged from our study is the first report concerning this group of retroviruses in patients with T1D.
The enhanced transcriptional levels of HERV-W-pol are consistent with the higher prevalence of HERV-W-env RNAs observed by Levet et al. [13] in mononuclear cells from T1D patients after short or long periods of disease as compared to non-T1D individuals. In their brilliant study they also observed a higher presence of HERV-W-env proteins in diabetic sera and pancreata than in controls; furthermore, the HERV-W-env protein directly inhibited insulin secretion in human Langerhans islets. All these findings strongly support an involvement of HERV-W sequences in T1D and their activation at disease onset is a further relevant clue. HERV-W interacts with TLR4, a receptor present in pancreatic b-cells [23,24] and involved in the innate immune response [5]; its stimulation inhibits insulin secretion by blocking specific transcription factors [25]. Therefore, HERV-W upregulation may lead to hypoinsulinemia by both a direct cytotoxicity and an immune-mediated damage on b-islet cells. It is worth noting that high expression of HERVs has been documented in a large array of autoimmune diseases [4], and patients with the multiple sclerosis (MS) show a similar, combined overexpression of both HERV-H and HERV-W [26].
An association between human retroviruses and T1D has been reported for the HERV-K family too [14], but this was not confirmed by other targeted researches [15,27]. HERV- K-pol transcripts were higher in our diabetic patients than in the control group, but the difference was not statistically significant. Therefore, our results do not support an involvement of HERV-K expression in T1D. Recently a low number of HERV-K copies within the C4 gene cluster has been linked to an increased risk of developing T1D [28].
The reason of the upregulation of HERV-H and HERH- W in T1D remains to be elucidated. Accumulating evidence underlines the role of epigenetic factors in pathogenesis of diabetes [29]. Indeed, epigenetic changes, such as altered DNA methylation and histone modification, can modulate not only the expression of cellular genes but also of HERV sequences, which in turn can influence the former [30]. Several environmental factors, such pollution and nutritional changes due to lifestyle, are responsible for epigenetic alterations that can change HERV transcription [19,30]. The increasing incidence of T1D in recent years [31] might thus derive from the impact of environmental epigenetic variables leading to HERV activation.
Among the environmental factors, exogenous viral infections are the most frequently called into question in the T1D pathogenesis. Several viral infections can trigger the transactivation of retroviral genes [32–35], HERVs might thus represent the missing link between common viral infections and T1D onset in susceptible hosts.
Our study has some limits and raises some doubts. The primers and probes we used encompass all pol genes of HERV-H and HERV-W groups, therefore the higher transcripts cannot be ascribed to single loci. Furthermore, we did not assess their protein-coding capacity, although this does not undermine their potential involvement in T1D pathology. For instance, HERV-W transcripts can be mobilised by long interspersed nuclear elements (LINE-1) [36] providing potent stimuli for the induction of autoimmunity [37], and HERV proviruses can regulate the activation of adjacent cellular genes. Our findings show overexpressions of HERV-H-pol and HERV-W-pol at diagnosis of T1D; whether these persist at follow-up, their possible relations with the heterogeneity of T1D phenotypes or with the risk of complications need further targeted investigations. In addition, since both the extent and the immunologic profile of pancreatic insulitis show a distinct, more aggressive pat- tern in childhood as compared to adulthood [38], one wonders whether the HERV transcription levels have a similar or different picture in adults with new-onset T1D.
Most HERV elements are inactive, but a few genes have maintained their protein-coding capacity, such as Syncytin-1 and -2, that are involved in the syncytiotrophoblast formation during placenta development [39,40]. A HERV-W-env protein was identified in patients with MS [41] and it is upregulated also in T1D patients [13]. A humanised monoclonal IgG4 antibody, named GNbAC1,

against this protein has been developed and is currently studied in patients with MS or T1D as an innovative non-immunomodulatory disease-modifying treatment [42]. Our findings on one hand may support this therapeutic perspective, with the additional suggestion that treatment of T1D should start early, because HERV activation is already present at disease onset; on the other hand, since not only the HERV-W-env gene but also the HERV-H-pol and HERV-W-pol genes are overexpressed in T1D, an elective therapeutic action towards the HERV- W-env target might be insufficient to block the HERV- driven pathologic mechanisms leading to b cell destruction. Cocktails of antiretroviral drugs are used for years in HIV-positive subjects and their optimal doses and side effects are well-known. Some products can reduce the expression of endogenous retroviruses in vitro [43]. HERVs are particularly transactivated in HIV + individuals [44] and antiretroviral therapy reduces the viral load not only of HIV but also of HERVs [45,46]. Notably, HERV transcription is enhanced by inflammatory signals that through the ubiquitin-proteasome cascade activate NF-kB; following its passage into the nucleus this binds specific sequences of HERV pro-viruses triggering their transcription [47]. We demonstrated that antiretroviral drugs display an intrinsic anti-proteasome activity [48,49]. Consequently, these drugs can reduce HERV transcription not only through a direct specific action against retroviruses, but also through indirect effects on host cell components. Therefore, the administration of antiretroviral drugs in new-onset T1D appears an exciting speculation potentially heralding a new therapeutic strategy.

FIGURES

Table 1. Characteristics of patients with new-onset type 1 diabetes and of control subjects.

	Diabetic patients	Control subjects
Number	37	50
Males/females	22/15	27/23
Median age, years (range)	8.99 (0.95–15.79)	5.05 (1.3–12.55)
Glycemia, mg/dL range	222–460	78–96

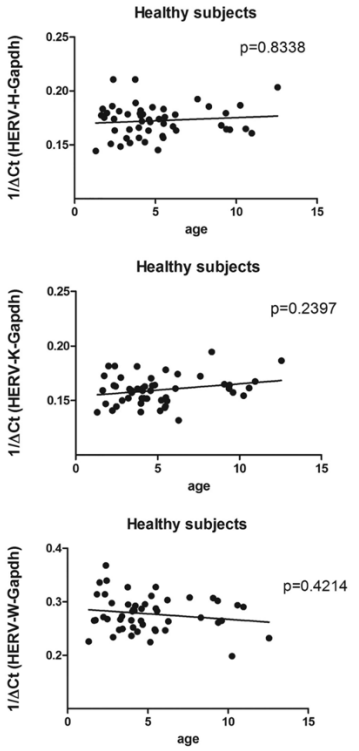

Figure 1. Correlation of pol gene transcripts of HERV-H, HERV-K, and HERV-W in WBCs from healthy subjects according to age. Footnote: Pol levels are represented by 1/ΔCt. See Results session for correlation Spearman analysis details.

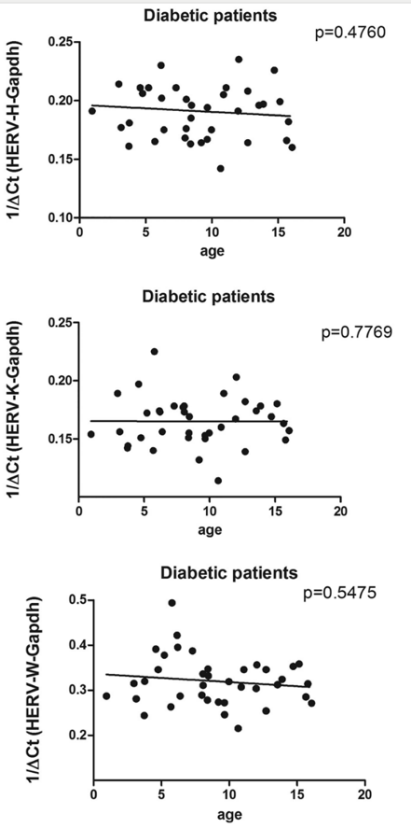

Figure 2. Correlation of pol gene transcripts of HERV-H, HERV-K, and HERV-W in WBCs from diabetic patients according to age. Footnote: Pol levels are represented by 1/ΔCt. See Results session for correlation Spearman analysis details.

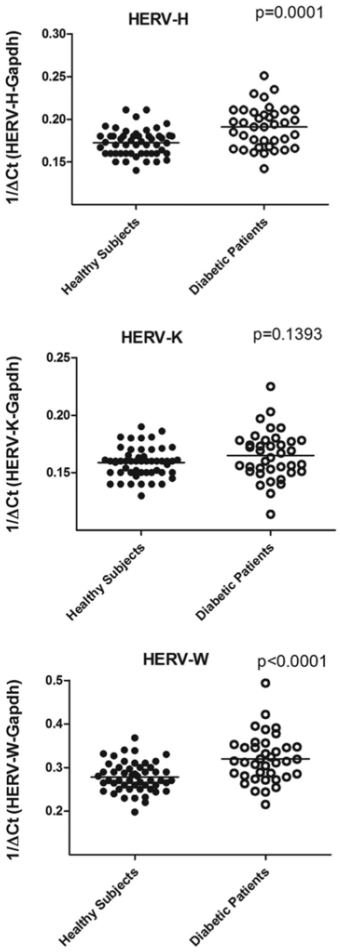

Figure 3. Transcriptional levels of pol genes of HERV-H, HERV-K, and HERV-W in WBCs from children and adolescents with new-onset type 1 diabetes and from control subjects of comparable age. Footnote: Pol levels are represented by 1/ΔCt. See Results session for statistical analysis through Mann-Whitney test.

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BACKGROUND

In the COVID-19 years, a global increase of new diagnosis of type 1 diabetes was observed [14]. In addition, an increase in new cases of diabetic keto-acidosis (for DKA definition please see the next chapter) was also evident [15]. Some authors speculated that infection from Sars-Cov-2 play a role in T1D development, and new studies were demanded to shed a light about a possible connection.

In our center, in the period between October 2020 and April 2021, we had a net increase of new onset T1D of 50-77%, while DKA increased of 45-128% with respect to previous years. Therefore, we conducted a study in which we investigated Sars-Cov-2 infection in new onset, to see if the infection itself is more prevalent in patients with T1D in the pediatric age [16].

Moreover, recently new data (not yet published, actually under review) from a broader area has been analyzed (Piemont and Marche), with a significant four-year cycle in incidence over the entire study period (1990-2021), while rate observed in 2021 (26.7, 95%CI 23.0-30.9) was significantly higher than expected (19.5. 95%CI 17.6-21.4; p = 0.010). This means something new is happening from 2021, soon after Sars-Cov-2 appearance under the spotlight. And, as a consequence, we observed across the Country more cases of severe DKA, with a proportion increased from 36.1% in 2019 to 44.3% in 2020 (p = 0.03) [17].

TITLE: INCREASE IN NEWLY DIAGNOSED TYPE 1 DIABETES AND SEROLOGICAL EVIDENCE OF RECENT SARS-COV-2 INFECTION: IS THERE A CONNECTION?

AUTHORS: Marco Denina, Michela Trada, Davide Tinti, Elisa Funiciello, Chiara Novara, Martina Moretto, Sergio Rosati, Silvia Garazzino, Claudia Bondone and Luisa de Sanctis

INTRODUCTION

Since the beginning of the COVID-19 outbreak, several studies have investigated the correlation between the pandemic and the onset of type 1 diabetes (T1D) in children, reporting an increased incidence of T1D and severe diabetic ketoacidosis (DKA) (1–4). Moreover, an increased incidence of T1D was also noted in a big population in the US (5). Given the disease's autoimmune nature, exogenous triggers (such as viruses) have been linked to T1D development in the past years (6). During the COVID-19 pandemic, lockdown reduced the viral spreading of different viruses (7). Therefore, a possible link between SARS- CoV-2 infection and the development of diabetes and DKA was hypothesized.

METHODS

The present study aimed to investigate the infection by SARS-CoV-2 in children with newly-diagnosed T1D during the second wave of the COVID-19 pandemic to explore a possible link between SARS-CoV-2 infection, T1D and DKA. The study includes all the 39 patients who presented to the emergency department for T1D new onset between October 15, 2020, and April 15, 2021, aged between 0 and 14 years, in the Turin area (Piedmont, Italy). We investigated an ongoing or recent SARS-CoV-2 infection through a polymerase chain reaction of nasal swab and dosage of SARS-CoV-2 specific antibodies in each patient enrolled in the study at admission. Due to the absence of seroprevalence data in our study population, we used nasal swab molecular analysis as a reference. In addition, each patient was subjected to a question form to evaluate medical history and, whenever applicable, to identify and to date SARS-CoV-2 infection. Demographic data, clinical presentation, and T1D characteristics at blood sample collection were also registered. Written informed consent was obtained from the parents of patients, and the Institutional Ethical Committee approved the study (protocol number 0000168). Molecular tests were performed with SimplexaTM COVID-19 Direct kit (Diasorin, Saluggia, Italy); the PCR assay targets two different regions of the SARS-CoV-2 genome, ORF1 and S (spike) genes. We investigated the presence of anti-SARS-CoV-2 antibodies using an ELISA assay (In3diagnostic Eradikit COVID-19, Turin, Italy) with a reported sensibility of 96% (8). Data were statistically analyzed using SPSS Statistic v23 (IBM, Armonk, NY). Continuous variables with non-normal distribution were expressed as medians (interquartile ranges), and categorical variables were expressed as numbers (percentages) and were compared with the χ 2 test or Fisher exact test. A 2-sided p-value <0.05 was considered statistically significant.

RESULTS

During the study period, 39 newly diagnosed T1D pediatric patients were enrolled, 20 (51.2%) males and 19 (48.8%) females, with a median age of 8.5 years (IQR 5.6–11.2 yrs.). In 9 (23%), antibodies directed toward SARS-CoV-2 were identified. Five patients had a history of recent SARS-CoV- 2 infection in themselves or in their family, but in the other 4 cases, no clinical or anamnestic infection evidence since March 2020 was disclosed. No molecular swabs were positive for SARS-CoV-2 collected upon admission of the patients to the emergency department.
Compared to the previous period, the incidence of COVID- 19 (resulting from a positive nasal swab) in the Piedmont pediatric population rose from 0.3 (on 15/10/2020) to 4.1% (on 15/04/2021) (9), resulting in an overall incidence at the end of the study 5.6 times higher in the T1D patient's group ($p < 0.00000001$).
Referring only to the cases in the area surrounding the hospital considering the variations in the pediatric population, we also analyzed the incidence of T1D in the 5 years preceding our study, finding a net increase (Table 1) in the cases which was statistically significant. In particular, the number of newly diagnosed T1D increased by 50% compared to the same months in 2016/2017 and

2017/2018, by 69% compared to 2018/2019 and by 77% compared to 2019/2020. The attributable risk to the pandemic in the 2020–2021 cohort compared to the previous year is 44%.
Finally, we analyzed the number of T1D presented with DKA in 2020/2021, comparing them with the previous year. As shown in Table 1, the increase in DKA cases was clear and significant, especially compared to 2017–2018 and 2018– 2019. Interestingly, preliminary data on the current season, from October 2021 to March 2022, overlap with those of our study period (Table 1).

DISCUSSION

A link between common viral infections and T1D in children is widely reported, acting through several mechanisms (10–12). Several viral infections are associated withT1D (13), with enterovirus being one of the most commonly associated, and Enteroviral major capsid protein VP1 and RNA have been detected in islets from people with recent-onset type 1 diabetes (14).
First, viruses can cause direct damage to pancreatic B-cells, resulting in the release of specific antigens that can act as a target for the immune response. Second, homologies between viral epitopes and autoantigens may stimulate the production of cross-reactive antibodies. Third, the immune activation accompanying a viral infection may reinforce an auto-immune response in individuals genetically predisposed (15), and viral infection may act as an accelerator in the immunological process which leads to T1D (16).
These mechanisms are common in all viral infections and are also described in SARS-CoV-2 infection: recent evidence suggests that SARS-CoV-2 can infect and replicate into pancreatic Beta cells and alter the pathway of insulin synthesis (17). Nevertheless, according to current knowledge, in-vitro models cannot fully explain the pathophysiological link between SARS-CoV-2 and T1D. However, in the last 2 years, epidemiological and clinical studies have extensively described the connection between COVID-19, newly diagnosed T1D, and DKA increase at clinical presentation (1–3).
We observed that 23% of T1D patients had a positive SARS-CoV-2 serology while only 4% of the pediatric population experienced COVID-19 in the same time period. Children were not vaccinated against SARS-CoV-2 at the moment of this observational study. We do not have epidemiological data about other viruses, but we observed less hospital admittance for any other cause during the study period. This abnormal disproportion between our children with T1D and the pediatric reference population, with a ratio of 5.6, appears to support the causative role of SARS-CoV-2 in triggering the immune response underlying diabetes, as often described for other viral infections. Additionally, we identified a 44% increased risk attributable to the pandemic years compared to previous years. Most of the patients investigated had precise exposure to the virus in the past month. As for patients with no known history of COVID-19, the higher antibody anti-SARS-CoV-2 title suggests that they developed a paucisymptomatic disease, followed by some immune response that caused or emerged T1D. While it seems unlikely that patients developed COVID-19 previously (the first lockdown, from March 9, 2020, to May 3, 2020, was extremely rigid), it is possible that the immune process leading to T1D could be ongoing at the moment of infection, and SARS-CoV-2 only accelerated T1D onset.
Although there are minor discrepancies based on the study's characteristics and the reference populations, the COVID-19 pandemic was characterized by an increase in the absolute number of cases of T1D onset and the severity (DKA) with which they occurred (18, 19). One of the reasons for understanding the DKA rise is the difficulty accessing care services during the pandemic during lockdown, with a consequent diagnosis delay, as demonstrated from an Italian survey which observed less cases of diabetes during first 2 months of pandemic but more DKA (20). Although partial, this explanation does not justify the increase in observed T1D cases, which appear to be directly linked to the pandemic. In this scenario, the acceleration of the immune process provoked by SARS-CoV-2 may seem more explicative in the development of T1D with DKA.

Different from Tittel et al. (1), which found a nominal increase in T1D rates among males but a nominal decrease in rates among females, in our study, we describe a percentage increase in the prevalence of females in the composition of the cohort of diabetic patients. This increase is not statistically significant, probably due to one of the major limitations of our study, namely the small size of the study population.
A major study limitation is the lack of seroprevalence data in the reference population, which is not available since no mass screening was done at the time of the study. If we use data from a study carried out in children referring to the hospital for any reason in an area with similar characteristics, the seroprevalence of SARS-CoV-2 antibodies was 9.5% (21). Given that, we can consider that value as a reference to evaluate the prevalence of Sar-Cov-2 infection and, although data are not comparable in a strict statistical modality, patients with T1D would have more than a double seroprevalence for SARS-CoV-2 as other patients, supporting the thesis proposed so far.
Additionally, as reported in other studies, we have identified a significant increase in the absolute number of T1D cases compared to previous years. The trend also persists when we analyze preliminary data about the ongoing winter season. Unfortunately, we have no serological data about this season, but the trend is still significant. Therefore, the rise of T1D during the two pandemic years compared with the previous 5 years cannot be split from the circulation of SARS-CoV-2 and appear to be attributable to the viral infection, as largely reported in the literature.

CONCLUSION

SARS-CoV-2 infection is potentially linked to a rise in newly diagnosed T1D and DKA in our population. The SARS-CoV- 2 virus can act as a trigger or accelerator in T1D development. Multicenter studies with precise dating of viral infection are needed to deepen and fully understand the pathophysiological link between SARS-CoV-2 and the onset of T1D in children.

TABLE

TABLE 1 Number of annual newly diagnosed T1D and DKA from 2016 to 2022.

Period	No of new onset T1D	Percentage increase in the study period	p	No of T1D presented with DKA	Percentage increase in the study period	p	Turin pediatric population—no
October 2016–April 2017	26	50.0%	**0.049**	8	100.0%	0.064	110,325
October 2017–April 2018	26	50.0%	0.056	7	128.6%	**0.039**	108,911
October 2018–April 2019	23	69.6%	**0.022**	7	128.6%	**0.041**	107,974
October 2019–April 2020	22	77.3%	**0.024**	11	45.5%	0.31	103,291
October 2020–April 2021	39			16			101,136
October 2021–March 2022 *(preliminary data)*	39			14			

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CHAPTER II - THE IMPORTANCE OF A PROMPT RECOGNITION AND MANAGEMENT OF DIABETES AT ONSET THE CASE OF DKA

BACKGROUND

There is a big difference in the way we manage diabetes at the onset. Usual presentation of diabetes is polydipsia, polyuria, and weight loss, but Diabetic Keto-Acidosis (DKA) and Hyperglycemic Hyperosmolar State (HHS) represent a life-threatening onset of the disease. While HHS is rare in the pediatric age [18], DKA represent less than 50% of all new diagnosis of type 1 diabetes, which is the commonest form of diabetes in that age group [19].

A diagnosis of DKA is confirmed when all of the three criteria are present - 'D', either elevated blood glucose levels or a family history of diabetes mellitus; 'K', the presence of high urinary or blood ketoacids; and 'A', a high anion gap metabolic acidosis.

Early diagnosis and management are paramount to improve patient outcomes. The mainstays of treatment include restoration of circulating volume, insulin therapy, electrolyte replacement and treatment of any underlying precipitating event. Without optimal treatment, DKA remains a condition with appreciable, although largely preventable, morbidity and mortality [20]. Estimated mortality is decreasing, but still a 0.4% of all cases of DKA die because of metabolic decompensation related to diabetes [21].

While evaluation of blood gas analysis (BGA) and glucose are usually carried out, ketones measurement is not always done since BGA does not contain ketone assay, and a point-of-care ketone device is not available in every Emergency Department (ED). Still, ketone evaluation represents a crucial value to define and monitor DKA. From an experience made in two referral centers for Pediatric Diabetes in Piedmont Region (Italy), we described three cases of kidney injury (a consecution of DKA at onset) in which ketone evaluation and monitoring were fundamental to treat related morbidity [22].

TITLE: CASE REPORT: ROLE OF KETONE MONITORING IN DIABETIC KETOACIDOSIS WITH ACUTE KIDNEY INJURY - BETTER SAFE THAN SORRY.

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INTRODUCTION

Type 1 diabetes (T1D) can present with diabetic ketoacidosis (DKA) in 29.9% of cases, with a different prevalence across Europe (1). After a slight decrease in the frequency of DKA in Italy in children diagnosed with T1D under 15 years of age between 2014 and 2016 (2), recent studies showed that DKA risk, especially that of severe DKA, has significantly increased during the SARS-CoV-2 pandemic (3). According to the International Society for Pediatric and Adolescent Diabetes (ISPAD) Guidelines (4), hyperglycemia (>11 mmol/L, >200 mg/dl), acidosis (venous pH < 7.3 or serum bicarbonate <15 mmol/L), and ketosis (>3 mmol/L in blood) are necessary for a diagnosis of DKA. Higher values of glycemia (>600 mg/dl, >33.3 mmol/L), with no or mild acidosis (venous

pH of >7.25, bicarbonate >15 mmol/L) and no ketonemia define the Hyperglycemic Hyperosmolar State (HHS) which can be observed, although rarely, in patients with T1D with consumption of high-carbohydrate beverages before diagnosis (5) or in young patients with other forms of diabetes (4). It is also possible to have a clinical presentation with mixed features of HHS and DKA, especially in those with severe dehydration with mild or moderate acidosis for other causes. Bicarbonate administration is not recommended except for the treatment of life-threatening hyperkalemia or unusually severe acidosis with evidence of compromised cardiac contractility (4).

We present three very similar case reports about adolescents with T1D at the onset with severe acidosis but low ketones to increase awareness of serum ketones (BOHB) monitoring.

CASE 1

A 40 kg 13- year-old female presented to be unresponsive in a peripheral Emergency Department (ED). She was unconscious, with a history of polydipsia and polyuria from a few days and vomit, rapid weak pulse, and deep labored breathing from the day before. Familiar and personal history was silent. Pediatric Glasgow Coma Scale (GCS) was 3, with arterial pressure of 90/60 mmHg. At venous blood gas analysis, a severe acidosis (pH 6.71, pCO2 17.3 mmHg, HCO3− 2.2 mmol/L) with hyperglycemia (550 mg/dl, 30.6 mmol/L) was evident. Hemoglobin was 13.4 g/dl, creatinine was 1.58 mg/dl (age normal values 0.4–0.7 mg/dl), and blood urea nitrogen (BUN) was 100 mg/dl (age normal values 6–20 mg/dl). Upon bladder catheterization, there were 1,000 ml of urine, of which analysis revealed glucose (3+), ketones (1+), and proteins (1+). A diagnosis of suspected DKA was performed and she received intubation and assisted ventilation followed by two boluses of crystalloid solution (normal saline 0.9%, 10 ml/kg each) through the intraosseous route. A saline solution at 5 ml/kg/h was commenced.

On arrival in our ED, she was still unresponsive, with acidosis (pH 6.775, HCO3- 4,3 mmol/L), Anion Gap (AG) of 22.7 mEq/L, persistent elevation of serum creatinine (1.2 mg/dl), and reduced estimated glomerular filtration rate (eGFR 48 ml/min/1.73 m2) with oliguria. Serum ketones were slightly increased (BOHB 1 mmol/L). A cranial tomography (CT) resulted to be normal. She was transferred to the Pediatric Intensive Care Unit (PICU) and received fluids, insulin (never exceeding 0.05 IU/kg/h), and potassium, but no bicarbonates, according to ISPAD Guidelines (4). Renal function progressively worsened, with increasing levels of creatinine (up to 4.21 mg/dl) with oligo-anuria despite the use of a diuretic (see Figure 1A). After 12 h, AG decreased to 7.1 mEq/L.

A diagnosis of acute kidney injury (AKI) (6) was made, and the persisting anuria required continuous veno-venous hemodiafiltration. Ketones always returned negative (<0.1 mmol/L). T1D-specific antibodies resulted positive (IA2 1.01 UA/ml, GADA 43.6 UA/ml, ZnT8 105 UA/ml).

CASE 2

A 54kg 10-year-old boy arrived in the ED with polyuria, polydipsia, and a weight loss of about 6kg in 4 weeks. He also reported vomiting and dyspnea since the previous day. He showed poor general condition, dehydration, stable vital signs, heart rate of 131 beats/min, GCS of 15, and Kussmaul breathing.

At venous blood gas, a severe acidosis was present (pH 6.9, adjusted K+ 1.5 mmol/L, HCO3- 4.3 mmol/L, AG 22,2 mmol/L, glucose 362 mg/dl, SBE−27.8 mmol/L; ketonemia was 5.1 mmol/L).

The rehydration treatment started with 2.5 L/m2/24h of saline supplemented with potassium (20 mEq/ L). After 2h, insulin therapy in continuous intravenous infusion was added at 0.04 U/Kg/h,

and gradually increased to 1.2 U/Kg/h. After the dropping of blood glucose <300 mg/dl, intravenous infusion was replaced with a solution made of 50% saline and 50% glucose- 10% solution supplemented with potassium (40 mEq/L), without showing an improvement of pH and electrolytes at venous blood gas.

Rehydration and insulin therapy were continued for 8h when the child worsened in his general condition, with a progressive reduction of GCS, persistence of dyselectrolytemia (especially hypokalemia, with 1.6 mmol/L), and respiratory fatigue. Moreover, he presented an episode of desaturation and respiratory acidosis (pH 6.7, PCO2 100%), probably due to respiratory exhaustion despite a clear reduction in ketonemia (1.2 mmol/L) and a reduction of AG to 12 mmol/L. He was immediately transferred to the PICU where endotracheal intubation was performed and a brain CT scan was carried out, which resulted negative for cerebral edema.

While he was under ventilatory support, the child showed a gradual increase in BUN and creatinine values (about four times initial values), reduction of eGFR, persistence of dyselectrolytemia (K+ 1.6 mEq/L), with a diagnosis of AKI (6) (renal function and metabolic parameters are shown in Figure 1B). Venous blood gas showed an absence of ketonemia without an improvement of pH.

Bicarbonate intravenous infusion was then started with an initial dosage of 0.5 mEq/h which was progressively increased to 17.5 mEq/h (0.31 mEq/kg/h) with an improvement in pH value until complete resolution. Furosemide was also administered in order to react to diuresis contraction, with gradual improvement in creatinine levels. K-Cl supplementation was continued to resolve dyselectrolytemia. The next day, as conditions improved, it was possible to stop sedation and ventilatory support and gradually resume oral nutrition.

Bicarbonate intravenous infusion and furosemide helped us to resolve acidosis and prevent further kidney damage. After 48 h, insulin infusion was interrupted, and it was replaced with a multi-injection subcutaneous treatment. T1D-specific antibodies resulted positive (ICA 17.2 UA/ml, GADA 221 UA/ml).

CASE 3

A 31.8 kg 11-year-old boy arrived in the ED with the suspicion of T1D for the presence of polydipsia, polyuria, and weight loss (5kg in 10 days) with obtundation and a tendency to sleep. Preceding his arrival to the ED, he received oral steroids and anti-histaminic drugs for urticaria, after which the child developed drowsiness.

On arrival in our ED, he had a modest general condition, with signs of dehydration, GCS 12, and accelerated breathing.

Medical history showed evidence of cardiopathy in the family (the mother was affected by Fallot tetralogy and retinal detachment). The child was otherwise healthy except for a cystic hygroma in the groin (diagnosed at the age of 3).

First blood gas analysis showed a pH of 7.07, pCO2 of 14.7 mmHg, Base Excess of−22.1, AG of 25.8 mEq/L, and HCO3- of 5.2 mmol/L with a glucose value of 609 mg/dl. Creatinine was 0.69 mg/dl (age normal values 0.4–0.7 mg/dl) with a K+ of 5.5 mEq/L and a sodium of 132 mEq/L. A normal saline solution was then commenced at 5 ml/kg/h according to guidelines (4). BOHB resulted to be modestly elevated (1 mmol/L).

After 2 h, values from blood gas analysis resulted comparable, with ketones slightly decreased (0.8 mmol/L), while AG resulted to still be elevated (24 mEq/L). Diuresis was reduced, with eGFR of 75 ml/min/1.73 m2, compatible with a diagnosis of AKI (6).

After nephrological consultation, a therapy with bicarbonate (0.375 mEq/kg/h), fluids (5 ml/kg/h), potassium (20 mEq/L), and insulin at 0.05 IU/kg/h was prescribed, with progressive resolution of acidosis and bicarbonate deficit. pH and bicarbonate returned to normal value after 10 h, while potassium normalized after 16 h. Diuresis returned normal (2.2 ml/kg/h) after 12 h (Figure 1C). T1D-specific antibodies resulted positive (GADA 1.64 UA/ml, ZnT8 37.1 UA/ml).

DISCUSSION

These are three similar case reports of adolescents at the onset of T1D with AKI, which commonly occurs in children with DKA (7). Despite severe acidosis and low bicarbonates, our patients had serum BOHB below 3 mmol/L, generally unassociated with DKA. In considering a DKA, it would always be important to take into account all differential diagnoses in children (lactic acidosis, metabolic acidosis, salicylate toxicity and septic shock). Also, the origin of metabolic acidosis could be different and/or seems to be very dynamic during the course of the condition.

These cases demonstrate that acidosis does not always arise from ketone-body accumulation in patients with hyperglycemia. In our cases, acidosis probably had a composite origin from defective bicarbonate reabsorption in the kidney and mild ketosis.

We hypothesized that massive polyuria induced severe dehydration with low volume and hypotension in association with metabolic decompensation that occurred in T1D at the onset. This situation might have initially led to hemodynamic damage with hypoperfusion and oligo-anuric AKI, followed by a more consistent ischemic tubular damage with the development of acute tubular necrosis. The altered tubular function did not allow bicarbonate reabsorption, and the buffering capacity was completely blunted, worsening underlying metabolic acidosis.

As previously reported (8), AG should be used to determine the moment of DKA resolution as opposed to using pH or bicarbonate. AKI can manifest with persisting non-AG acidosis, often self-limiting with conservative management. However, it would be important to intercept this complication early to avoid severe kidney damage.

In these presented cases, on arrival, AG were well above the upper limit (22.7, 22.2, and 25.8 mEq/L, respectively), which is suggestive for DKA. During the course of treatment, AG was not useful for all cases to differentiate AKI- from DKA- related acidosis, while serum ketone bodies were helpful early to intercept an acidosis unrelated to ketosis.

According to ISPAD guidelines (4), bicarbonate administration is not recommended except for treatment of life-threatening hyperkalemia or unusually severe acidosis with evidence of compromised cardiac contractility. This recommendation is due to possible side effects related to the infusion of bicarbonate, such as worsened hypokalemia, worsened intracellular acidosis due to increased carbon dioxide production, delay of keto-anion metabolism, and development of a paradoxical central nervous system acidosis.

However, in the second and third case, a rapid intervention in limiting the progression of AKI, with fluid infusion, better hemodynamic control, and through bicarbonate infusion, has helped to avoid a more consistent acute tubular damage as in the first case.

After the first hours, all patients demonstrated improvements that likewise happens for patients with classic T1D at the onset. All of them are now followed in the diabetes center of the hospital, and none have signs of organ damage (kidney, cardiovascular, brain) or worse glycemic control that could be related to the onset episode.

In conclusion, BOHB monitoring was helpful at first to rule out a suspicion of DKA and, in the following hours, was diriment to understand with great anticipation the acidosis origin and attribute it to tubular damage. We recommend serum BOHB measurement in every patient referring to ED with a suspicion of DKA to accordingly rule out other origins of acidosis and to treat patients.

In consideration of the increase in severe DKA cases reported during the pandemic (3), healthcare systems must be aware of the increase of possible DKA complications. In addition, pediatric emergency physicians must be prepared to manage very severe cases of DKA at the onset of T1D.

FIGURE

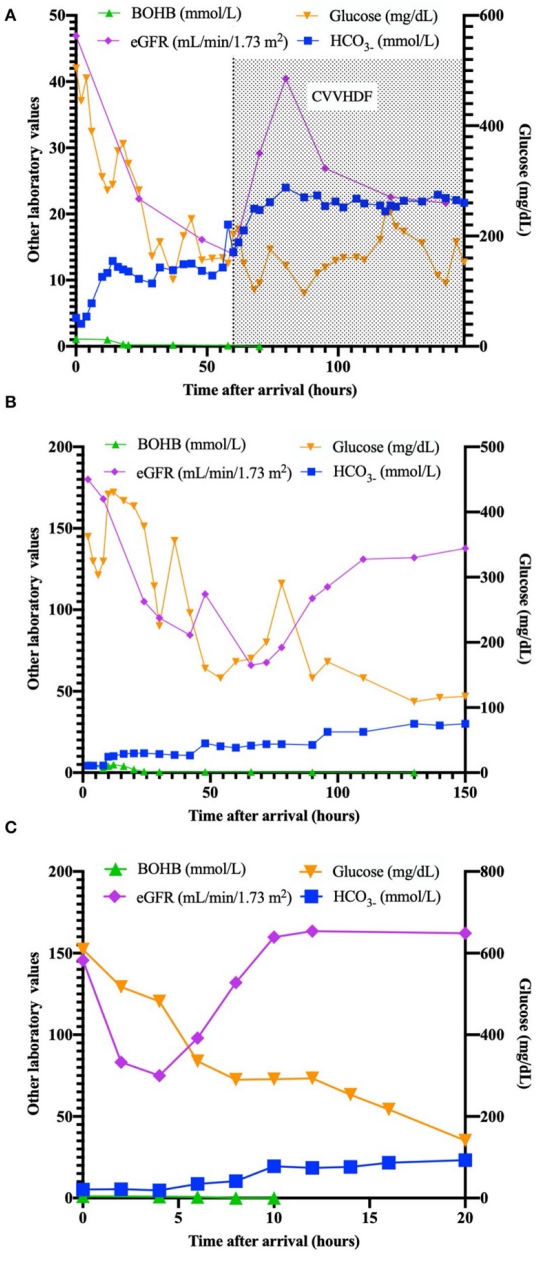

FIGURE 1 | Plasma values (ketones, eGFR, bicarbonates, glucose) in patients **(A–C)** in the time after arrival (hours).

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BACKGROUND

We know that DKA, which represent most worrying complication of diabetes at onset, is demonstrated to be preventable [23,24]. One important Italian paper from Vanelli *et al.* (the so-called "*Parma campaign*") showed a marked reduction (78%) of DKA through poster in schools and outpatient pediatrician clinics [25], underlining the role of social education about T1D, anticipating diagnosis and therefore preventing DKA. We also know that DKA prevalence is reversely related to T1D incidence, since the more knowledge about T1D, the earlier recognition of symptoms and a prompt diagnosis and management [26].

Prevention campaigns, targeted to physician, schools, are general population are therefore beneficial to reduce morbidity and mortality related to diabetes, especially in those Countries in which diabetes is less prevalent, or in those with higher DKA rates at T1D onset (and Italy has one of the highest in Europe, 41.2%) [26].

This is the reason why, while in some Countries DKA prevention showed benefits also in term of cost [27], others with lower prevalence did not show similar results [28]. From this observation, Italian Society for Pediatric Endocrinology and Diabetology (ISPED) sought to determine if a nationwide prevention campaign about T1D could replicate the effect observed with previous campaign, distributing a poster from 2015 until 2017 to pediatricians, evaluating rates of DKA and comparing them with previous years [29].

TITLE: DIABETIC KETOACIDOSIS AT THE ONSET OF DISEASE DURING A NATIONAL AWARENESS CAMPAIGN: A 2-YEAR OBSERVATIONAL STUDY IN CHILDREN AGED 0–18 YEARS

AUTHORS: Ivana Rabbone, Giulio Maltoni, Davide Tinti, Stefano Zucchini, Valentino Cherubini, Riccardo Bonfanti, Andrea Scaramuzza, On behalf of the Diabetes Study Group of the Italian Society for Pediatric Endocrinology and Diabetology (ISPED)

INTRODUCTION

In calendar years 2012 and 2013, paediatric endocrinologists belonging to the Italian Society for Pediatric Endocrinology and Diabetology (ISPED) conducted a multicenter retrospective survey,1 the aims of which were (1) to evaluate the incidence of diabetic ketoacidosis (DKA) at onset of type 1 diabetes in children and adolescents in Italy, and (2) to determine utilization of the International Society for Pediatric and Adolescent Diabetes (ISPAD) guidelines about DKA management in various centres. In more than 2400 new cases of type 1 diabetes, the overall incidence of DKA was 38.5%, and 10.3% of patients presented in severe DKA (pH <7.1). In preschool-aged children (aged 6 years or younger), DKA incidence reached more than 70% and was severe in 16.6% of cases. (1) Only 68% of centres reported adoption of the ISPAD guidelines to manage DKA, and there was great variation in the approach to management. (1) These data were consistent with a comprehensive study of DKA incidence during a 10-year follow-up in Italy by Cherubini et al, (2) and with studies published in the same period in other European (3–5) and Extra European countries. (6,7)
Despite several efforts to reduce the occurrence of DKA at onset of type 1 diabetes, there continues to be an unacceptably high burden of DKA, with an incidence rate often more than 30%. Inspired by the successful Parma campaign, which reduced DKA incidence at onset of type 1 diabetes (8) (even if the positive effect tended to disappear after some time (9)), in 2015 we decided to launch a nationwide awareness campaign intended to prevent DKA at diabetes onset and to pursue the

campaign for 2 years. In the meantime, the Diabetes Study Group of the ISPED published its recommendations for DKA treatment (10) partly based on the ISPAD guidelines, (11) with some differences, (12) with the aim of standardizing the management of DKA in all Italian paediatric diabetes centres.
The primary aim of the present survey was to prospectively evaluate the number of new cases of type 1 diabetes below the age of 19 during the awareness campaign (figure 1) throughout the calendar years 2016 and 2017. The secondary aim was to determine how many Italian centres reported to adhere to the Italian recommendations for DKA treatment. (10)

METHODS

A national publicity campaign to increase awareness about DKA has been launched in Italy in November 2015 and is still ongoing, and therefore continued alongside the period of data collection. In Italy, quite all children below the age of 6 years and most of the older ones (8–14 years of age) are under the care of family paediatricians. For this reason, the campaign was aimed to family paediatricians, and each of them received an advertising poster (figure 1) to exhibit in their ambulatory setting. Moreover, they received a monthly newsletter via email, highlighting various topics related to DKA. The same advertising poster was sent to many schools, asking the principal to exhibit it, as well as to families, who receive bimonthly by mail a magazine ("*Conoscere per Crescere"*, a magazine about growth issues) that features various aspects of paediatrics. In total the advertising poster and the newsletters were sent to 10000 family paediatricians, while the advertising poster was sent to 250 elementary, junior high or high schools.
The awareness campaign was also launched on ISPED's social media (ie, Facebook and Instagram) and was endorsed by two famous Italian comedians (Ale e Franz; official website: http:// www.alefranz.com/), who played a short commercial to advertise Italian awareness of DKA campaign and was shown on many national and regional television channels.
After 31 December 2017, all 68 centres that participated in our first survey (1) were invited to complete a survey to collect data and investigate DKA epidemiology and management in patients who presented with new-onset type 1 diabetes during the calendar years 2016 and 2017. The survey questions were identical to those included in the previous survey. (1) To facilitate data collection, the survey was web-based and data collection was centralised at the Turin centre. Each centre was asked to review all records of patients under 19 years of age who had diabetes onset between 1 January 2016 and 31 December 2017.
The following information was requested from each patient: date of birth, date of diabetes onset, pH value at diabetes onset (to evaluate DKA severity), if a published DKA treatment protocol was used (eg, ISPED protocol for DKA (10) or other) and any treatment complication (eg, cerebral oedema). In the final analysis the following data were analyzed: number of patients 0–18 years of age with diabetes onset during the observation period, number of patients <6 years of age, number of patients with DKA (either as a total number or only patients <6 years of age), number of patients with severe DKA (either as a total number or only patients <6 years of age), number of patients who developed cerebral oedema, and number of centres following any DKA protocol and which one. DKA was defined according to the ISPAD criteria (11) (e.g. blood glucose >11mmol/L (≈200mg/dL); venous pH <7.3or bicarbonate <15mmol/L; ketonaemia and ketonuria). The severity of DKA was categorized according to the degree of acidosis: (1) mild: venous pH <7.3 or bicarbonate <15mmol/L; (2) moderate: pH <7.2 or bicarbonate <10 mmol/L; and (3) severe: pH <7.1 or bicarbonate <5mmol/L.11. Data for the calendar years 2016 and 2017 were pooled for analysis, and we used the $\chi 2$ test to evaluate possible statistical differences between groups; statistical significance was deter- mined to be $p<0.05$.

RESULTS

Among the 68 centres belonging to the ISPED that participated in the first survey, (1) 58 (85.3%) completed the web-based survey and returned complete data records of patients with newly diagnosed diabetes. One hundred per cent of the tertiary referral centres (n=39) of the ISPED were included among the 58 centres that responded.
The results of the current survey compared with those of the previous one (1) are shown in table 1.
In 2016–2017, a total of 2361 children 0–18 years of age were diagnosed with type 1 diabetes in the 58 respondent centres, and their records were evaluated and reviewed for the present report.
DKA was observed in 1123 out of 2361 (47.6%) patients, which is a significant increase in DKA incidence compared with the 2012–2013 survey (1) (47.6% vs 38.5%, p=0.002). Similarly, the rate of severe DKA was significantly increased (table 1).
With an equal number of preschool-aged children in the two surveys (n=617, 26.1% in 2016–2017 vs n=650, 26.7% in 2012–2013), the incidence of DKA significantly decreased in preschool-aged children in 2016–2017 compared with 2012– 2013 (52.5% vs 73.8%, p=0.009) (table 1); however, the rate of severe DKA both in preschool-aged children and in the entire cohort increased significantly (table 1).
Cerebral oedema was observed in 4 out of 1123 patients with DKA in the current cohort (0.35% vs 0.53% in 2012–2013, p=0.548). Seven cases of mild DKA occurred in adolescents who subsequently were diagnosed with type 2 diabetes at disease onset. Among the 58 respondent centres, 95% reported following the ISPED DKA treatment recommendations. (10)

DISCUSSION

The present survey provides a comprehensive depiction of DKA epidemiology and management in 58 out of 68 centres that provided data about newly diagnosed children 0–18 years old with type 1 diabetes in Italy during the calendar years 2016 and 2017, during an awareness campaign, from November 2015 until the end of 2017. The data collected are considered to represent DKA at the national level because information was obtained from 100% of the tertiary-level centres that participated in the previous survey (n=39), (1) which provided coverage of approximately 90% of paediatric diabetes in Italy.
The DKA awareness campaign was directed to family paediatricians throughout Italy. In Italy, since 1978, each child 0–14 years of age has been assigned to a family paediatrician who is responsible for his/her health and disease management. Family paediatricians received by mail one or more advertising posters (figure 1) to exhibit in their ambulatory setting.
Unfortunately, the reach of the campaign has not been measured and only partial figures are available. We sent 10000 posters but we have no way to verify if all of them have been displayed in the ambulatory setting, as well as how many schools displayed the poster.
An earlier prevention campaign (8) succeeded in decreasing the occurrence of DKA in the Emilia Romagna region but not throughout Italy. We thought that an extra effort to reduce the occurrence of DKA in new-onset diabetes throughout Italy was needed. It is disappointing that the present survey shows the campaign coincided with an increase in the incidence of DKA in patients with new-onset diabetes. It is difficult to explain the reason for the higher incidence in 2016–2017 compared with 2012–2013. However, the SWEET consortium has presented similar data at the last annual conference of the ISPAD. (13)
Even if the observational study design does not allow for a definitive conclusion, it is interesting to note that the awareness of DKA campaign was associated with a significant 30% decrease in the incidence of DKA in preschool-aged children. In this respect, family paediatricians, the main target of the awareness campaign, might have played an important role in the early identification of new-onset diabetes in young children.

A previous national awareness campaign in Austria was not able to show a decrease in DKA incidence. (14) The authors described a DKA incidence of 37.2% for all new-onset diabetes in the period 1989–2011, and no difference before and after a prevention campaign that was similar to the Parma campaign. (14) By contrast, Vanelli et al (8) showed a decrease in DKA incidence from 78% to 12%, highlighting the fact that a similar prevention campaign might work better when applied to a small area (the Emilia Romagna region) instead of a national area (eg, Italy and Austria). Similarly, in a small area (Newcastle area of New South Wales, Australia), Bruce King and colleagues (15) reported a decrease in DKA by 64%.

It is also disappointing that the awareness of DKA campaign coincided with a higher incidence of severe DKA when compared with 2012–2013 both in the entire cohort and in preschool-aged children. The reason for this increase is unknown. DKA continues to be a threat to all children and adolescents with type 1 diabetes onset despite the campaign to prevent it. The length of the campaign (2 years) may be too short to obtain consistent results. The Austrian campaign took 20 years to attain a 13% decrease in the DKA incidence at diabetes onset in paediatric age. (14) Recent data from the USA showed a steadily increasing incidence of DKA in both children and adults, but associated with a decrease in DKA-associated mortality. (16) Moreover, it is interesting to note that a recent systematic review of publicity interventions to increase awareness among healthcare professionals and the public to promote earlier diagnosis of type 1 diabetes in children and young people does not reach a definitive conclusion on the effectiveness of the interventions reported. (17) The decrease in DKA incidence only in preschool-aged children and not in the entire cohort could also be explained by the ongoing shortage of family paediatricians observed in Italy in the past years, due to a large number of paediatricians retiring in the last 5 years and not being replaced by younger paediatricians. The lower number of family paediatricians is the reason why many families when their children are older than 6 years of age move to family physicians (ie, general practitioners), who were not the target of the awareness campaign. It is likely that general practitioners who care for some older children and adolescents may not have been reached by the campaign.

However, despite the increased number of patients with severe DKA, a slight, non-significant, decrease in the rate of cerebral oedema was observed during 2016–2017. We cannot conclude that this improvement could be due to a higher percentage of recommendation implementation. (10,11) Certainly, the release of the ISPED recommendations for the management of DKA in 2015 (10) enabled a standardization of the procedures for the management and treatment of DKA in the different Italian centres. While in the previous report only 68% of the centres claimed to follow a treatment protocol, based on the ISPAD guidelines, nearly all the centres responding to the present survey reported using the ISPED/ISPAD recommendations.

As a limitation of this study, we would like to note that despite our aim of evaluating whether the campaign was effective in decreasing DKA incidence, it was not possible to determine the effectiveness using our study design, as there was no control group or experimental design, and changes over time since the last study may have been caused by other changes unrelated to the campaign. Also, the data were collected while the campaign was ongoing, so there was no way of knowing whether the decrease would be sustained following the end of the campaign. We aim to collect data for the next calendar year to sort this out.

In conclusion the findings of this second nationwide survey in the calendar years 2016 and 2017 about the incidence of DKA in children and adolescents at the onset of type 1 diabetes show that DKA is still common at diabetes onset in children and adolescents. A continuous reinforcement of the awareness of DKA prevention campaign is needed and should probably be implemented. New strategies of communication should probably be promoted and supported by scientific societies and national health systems, targeting family paediatricians and general practitioners, as well as the whole population, via radio/television/ social media advertising. The Diabetes Study Group of the ISPED is currently working to implement a nationwide awareness DKA prevention campaign.

TABLE

Table 1 Diabetic ketoacidosis incidence in the paediatric population (0–18 years) in the calendar years 2016 and 2017 in Italy, compared with the previous survey in calendar years 2012 and 2013[1]

	2012–2013 survey	2016–2017 survey	P value
Patients with type 1 diabetes onset (n)	2453	2361	–
Preschool patients with type 1 diabetes onset, n (%)	618 (26.6)	617 (26.1)	–
Patients with DKA at onset, n (%)	945 (38.5)	1124 (47.6)	0.002
Preschool-aged patients with DKA at onset, n (%)	445 (73.8)	323 (52.5)	0.009
Patients with severe DKA at onset, n (%)	97 (10.3)	172 (15.3)	0.008
Preschool-aged patients with severe DKA at onset, n (%)	103 (16.6)	70 (21.7)	0.008

DKA, diabetic ketoacidosis.

FIGURE

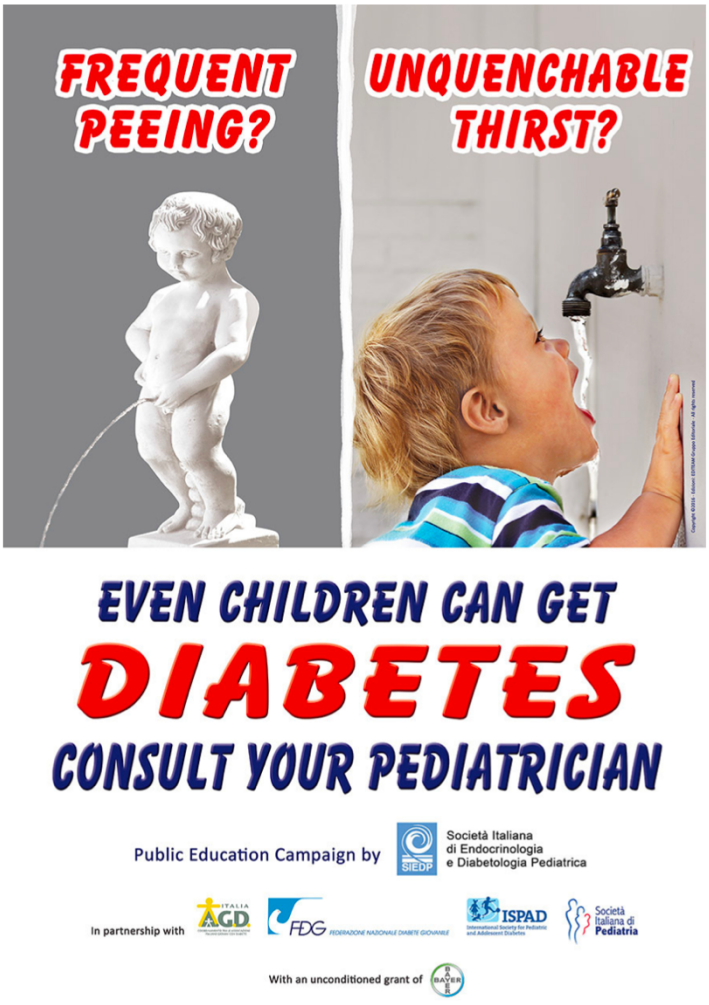

Figure 1 Advertising poster of the Italian campaign in English. This poster is also available in Italian, Arabic, Romanian and Chinese.

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BACKGROUND

DKA is a well-known complication of unrecognized diabetes [26]. Late recognition of diabetes symptoms may increase DKA rates especially in children affected from T1D.

In 2020, during COVID-19 pandemic, access to Emergency Services was reduced and/or delayed due healthcare professional shortages and fear SARS-CoV-2 infection in hospitals [30]. From March, 2020 a general lockdown was declared and all but necessary activities were closed or forced home [31]. In diabetes, this led to more severe presentation, such as DKA (especially for patients with T1D) and a higher mortality. In fact, during lockdown due to COVID-19, many centers observed an increased prevalence of DKA among new T1D onset, while some observed also more cases of T1D overall [17] with an increased mortality [32].

A study depicting the Italian situation was necessary, therefore data was sent to a coordination center for analyses, with a confirmation of an increased prevalence of DKA in new T1D onset [19].

TITLE: THE SILENT EPIDEMIC OF DIABETIC KETOACIDOSIS AT DIAGNOSIS OF TYPE 1 DIABETES IN CHILDREN AND ADOLESCENTS IN ITALY DURING THE COVID-19 PANDEMIC IN 2020

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INTRODUCTION

Italy witnessed a very high frequency of diabetic ketoacidosis (DKA) at onset of type 1 diabetes between 2006 and 2016 (1). However, thanks to awareness campaigns such as those promoted by the Italian Society of Pediatric Endocrinology and Diabetology (ISPED), these figures slowly but significant decreased and indeed became more evident between 2014 and 2016 (2). The COVID-19 pandemic has prompted rapid changes in the organization of healthcare systems and public behavior, and there were early reports of a large increase in the frequency of DKA at the time of diagnosis of type 1 diabetes during the pandemic (3–10); indeed, a German study reported that the observed versus predictive frequency of DKA was only higher in the early phase of the pandemic in 2020 (11).

In 2020, Italy suffered the highest total number of deaths since the Second World War, with a 15.6% excess in deaths in 2020 compared with the 2015-2019 period. Although there were fewer reported deaths in January and February 2020, March to December 2020 saw a 21% increase in mortality, with two peaks between March and April and October and December during the two waves of the pandemic (12).
With the aim of controlling the spread of the SARS-CoV-2 virus, the Italian government issued several legislative decrees to limit the movement of people and ensure physical distancing together with economic and social policies to support health and employment (13). Containment measures have alternated between extreme and partial based on the impact of COVID- 19 on the population. Extreme measures included limiting individual travel, closing schools and restaurants, banning gatherings, banning interregional, national, and international travel, cancelling public events, and shutting down workplaces, while partial measures included nationally controlled individual driving permits, opening takeaway restaurants, authorized outdoor public events, and opening workplaces. It is currently unclear whether these COVID disease severity and containment measures are related to the frequency of DKA at diabetes diagnosis. Therefore, the purpose of this study was to investigate whether the frequency of DKA at diagnosis of type 1 diabetes changed during the COVID-19 pandemic and was associated with COVID-19 disease severity or the measures taken by government to curb its spread.

RESEARCH DESIGN AND METHODS

Study Design and Data Sources

We analyzed data from the ISPED Network for DKA Study and Prevention (2). The Network was established in 2014 following observation of a very high frequency of DKA in Italy (14) with the aim of design studies to continuously analyze DKA frequency and discuss prevention measures. It includes all the Italian centers for childhood and adolescent diabetes, which prospectively record the data of all children at the time of diagnosis of type 1 diabetes according to a procedure agreed in 1997 (15). The Network has annual meetings with local collaborators to discuss and agree on methods and measures for the control and prevention of this harmful complication. In Italy, pediatric diabetic centers care for 100% of children and adolescents under 15 years with diabetes, over 80% between the 15 and 18 years, and <10% over those aged over 18.
Locally collected pseudonymized longitudinal data are transmitted annually to the coordinating center in Ancona for quality control, plausibility checking, and analysis. Inconsistent data are reported back to participating centers for validation and/ or correction. Since 2014, centers have transferred data on clinical, socio-economic, and immigration background to the Network, and since 2017 data on b-cell autoantibody test results from children and adolescents with newly diagnosed type 1 diabetes. Autoantibody measurements are used to confirm patient has type 1 diabetes.
Here we included children and adolescents aged <18 years from 47 diabetes centers in Italy newly diagnosed with type 1 diabetes between 1 January 2017 and 31 December 2020. The diagnosis was confirmed according to the presence of b-cell autoantibodies or the absence of MODY mutations and clinical criteria for the diagnosis of type 2 diabetes. DKA was defined as venous pH <7.3 or serum bicarbonate <15 mmol/L or a documented clinical diagnosis of DKA (yes/no) according to the treating physician. Severe DKA was defined as venous pH <7.1 or bicarbonate <5 mmol/L.
There were two epidemic waves of COVID-19 of different intensity in Italy in 2020: the first between March and April and the second between October and December. The containment measures were significantly changed between March and December 2020 based on the daily number of infections, number of hospital admissions, number of intensive care unit (ICU) admissions, number of deaths, and daily positivity rate. We therefore identified four periods in 2020 that differed in terms of containment measures but with clearly definable characteristics (Figure 1): (i) the pre-pandemic period included January and February and ended on March 9, when (ii) the

second period started during which the number of daily deaths in the general population increased dramatically and the government imposed extreme measures to contain the pandemic; (ii) the third period ran from May 24 to October 7, during which government-imposed restrictions eased following a sharp reduction in the number of daily deaths; and (iv) the fourth period between October and December was characterized by a rapid increase in daily deaths and the resumption of extreme containment measures.
The frequencies of DKA and severe DKA observed in the four periods of 2020 were compared with the mean frequency observed in the same periods of the calendar year in 2017-2019. Data quality control was performed as previously described (14).

Variables

Demographic data included age at type 1 diabetes diagnosis (0.5-4; 5-9; 10-14; and 15-18 years), sex, immigration background (defined as the patient and/or one of the parents born outside Italy), and family history of diabetes (defined as at least one parent with type 1 diabetes). Socioeconomic variables included household income (<15.000, 15.000-25.999, 26.000-54.999, 55.000-75.000, >75.000 euros), number of family members, home ownership, and parents' ages, occupations, and educational level. Clinical data included HbA1c (% and mmol/mol), presence of DKA and severe DKA, and presence of at least one b-cell autoantibody (islet cell antibody, anti-GAD, anti-IA2, IAA, or anti-ZnT8).

Statistical Analysis

Descriptive statistics were used to characterize children and adolescents at type 1 diabetes diagnosis based on year of disease onset (before COVID-19 pandemic, 2017-2019, and during pandemic in 2020). Household income and parents' educational level were dichotomized into ≥26,000 euros (high household income) and high school diploma or degree (high educational level), respectively. Variables were summarized using absolute and percentage frequencies for the categorical variables and the means and standard deviations (SD) for the quantitative variables; comparisons between the 2017-19 and 2020 were made using the chi-squared test and Student's t-test for independent samples for categorical and continuous variables, respectively.
Point estimates and 95% confidence intervals (95%CIs) for the frequency of DKA at diabetes onset before and during the COVID- 19 pandemic were calculated in each of the four subperiods in 2020 (January-February, March-May, June-September, October- December) using the binomial distributions. Comparisons between the DKA frequencies were performed using the z-test for two proportions.
Logistic regression analysis was used to evaluate the frequency of DKA at diagnosis of type 1 diabetes according to 2020 subperiods compared with the frequency observed in the same months of the preceding three years 2017-2019. Gender, age group at diagnosis, geographical area of residence at diagnosis, family history of type 1 diabetes, immigration background, and the interaction between the four subperiods and years at diagnosis were included in the multiple logistic regression model as covariates.
A multiple logistic regression model was used to estimate the association between DKA frequency and patients' socioeconomic characteristics in 2020. The final model was obtained using forward criteria and the likelihood ratio test.
Results are expressed as odds ratios (ORs) and 95%CIs. The Hosmer-Lemeshow test was used to assess the goodness of fit of data to the model. Significance was defined as a p-value <0.05 and confidence intervals were set at 95%. All analyses were performed with R-gnu version 4.0.5.

RESULTS

Overall, 4237 new cases of type 1 diabetes were diagnosed between 2017 and 2020: 3068 in 2017-2019 and 1169 in 2020. There were no missing values in demographic and diagnosis of diabetes, while completeness of DKA data was 99%, HbA1c 96%, beta/cell autoantibody test 92%, family history of diabetes 80%, immigration background 73% and socioeconomic variables between 45 and 55%.

Supplemental Table S1 shows the characteristics of the new cases of type 1 diabetes according to period of diagnosis. There were no significant differences between the two periods in terms of geographical distribution, gender, age at diagnosis, immigration background, family history of type 1 diabetes, or negative b-cell autoantibody test result. However, frequencies of DKA and severe DKA were significantly higher in 2020 than in the preceding three- year period. Additionally, pH was significantly lower and HbA1c values at type 1 diabetes diagnosis were significantly higher in 2020 than in 2017-2019.

Table 1 reports estimates of the frequency of DKA, severe DKA, and b-cell autoantibody negativity before and during the COVID-19 pandemic based on the restrictions imposed in 2020. DKA was significantly more frequent during the first period of extreme and partial restrictions in 2020 (10% and 7%, respectively) compared with the same subperiods before the pandemic. The 4% greater frequency observed during the second period of extreme restrictions in 2020 was significantly different to the same period in 2017-2019. During all periods of extreme and partial restrictions, the frequency of severe DKA was significantly higher in 2020 than in 2017-2019. Of note, the frequencies of DKA and severe DKA were significantly lower in January-February 2020 compared with the same months in the previous three-year period. Figure 1 illustrates the number of daily deaths due to COVID-19 in Italy in 2020 and the monthly frequency of DKA and severe DKA in 2020 compared with in 2017-2019 based on the subperiods marking imposition of national restrictions in 2020.

Seventy-eight percent of participating centers were able to measure at least four out five b-cell autoantibodies, and all the other centers measured more than one b-cell autoantibody. The frequency of b-cell autoantibody negativity was similar in 2020 and 2017-2019, with a significantly higher value observed in June-September 2020 than in the previous period (Table 1).

Table 2 reports the multiple logistic regression analyzing the factors associated with the frequency of DKA and severe DKA. Females were at higher risk (>20%) of DKA at diagnosis of type 1 diabetes than males, and children in the younger age group were at higher risk of DKA and severe DKA than older children. The probability of presenting with DKA was lower in the north of Italy than in central Italy, children with at least one first degree relative with type 1 diabetes were at lower risk of DKA and severe DKA, while children in families with an immigrant background were at higher risk of DKA and severe DKA. The probability of DKA at diagnosis was 2.31-times greater in the first period of extreme restrictions in 2020 compared with the same months in 2017-2019. Children were at significantly higher risk of severe DKA during both the extreme and partial restriction periods in 2020 than in the same months before the COVID-19 pandemic. Table 3 shows associations between the frequency of DKA and factors significant according to logistic regression, i.e., subperiods of restrictions, age at diagnosis, family history of type 1 diabetes, and household income. Due to the missing values the association was analyzed only in 2020. The probability of DKA at diagnosis of type 1 diabetes in the partial and extreme restriction periods was over twice that seen in January and February of 2020. The highest age group at diagnosis, presence of a first degree relative with type 1 diabetes, and family income significantly reduced the probability of DKA at type 1 diabetes diagnosis.

Only 14 of 815 (1.7%) children at type 1 diabetes presentation reported a COVID-19 infection before diabetes, and none required hospitalization. 90% of children were tested by PCR for SARS-CoV-2 at diagnosis of diabetes, and 2.5% were positive. Additionally, in 3.4% of cases, at least one

family member reported a COVID-19 infection confirmed before the child was diagnosed with diabetes. There were two DKA-related deaths during 2017-2019, and none during 2020.

DISCUSSION

This large national study identifies that there was a worrying increase in DKA and severe DKA in children newly diagnosed with type 1 diabetes during the COVID-19 pandemic in 2020 compared with the previous three years. However, there was no apparent association with COVID-19-related number of deaths or the containment measures imposed by the Italian government.

Description of the Increase in DKA in Italy and Comparisons with Other Countries

This observed increase is consistent with reports from other studies around the world (3–11). To date, except for the study conducted in Germany (11) and in the Lombardy Region of Italy (3), almost all other studies focused on part of 2020 (4–10), in particular the first wave of the pandemic. Furthermore, many were based on few incident cases of type 1 diabetes (4, 6, 7, 9), so consequently the increase in the frequency of DKA varied enormously.
The observed increase in DKA appears to be independent of the daily number of deaths from COVID-19, as the frequency of DKA remained high even in the third period of the year when the number of COVID-19-related deaths decreased and the frequency of severe DKA increased. Likewise, there was no direct relationship between DKA frequency and the government containment measures imposed by law. In particular, the easing of restrictions between June and September 2020 did not appear to directly reduce DKA frequency. It should be noted that, in line with the decreasing trend previously observed (2), in the first two months of 2020 the frequency of DKA was significantly lower than observed in the same months of the preceding three-year period. This might have been due to the awareness campaigns implemented in several areas of the country.

Possible Causes of Increased DKA and the Role of Socioeconomic Factors

The most compelling reason explaining the increased frequency of DKA is a delay in diabetes diagnosis, although the reasons for this are not fully understood. Parents' fear of accessing hospital or health services in general due to the risk of contracting SARS- CoV-2 is likely to have been a major cause of delayed diagnosis. However, delays in performing diagnostic tests or restricted access to health services committed to treating COVID-19 patients may have also contributed. Any delay in diagnosing life-threatening childhood diseases such as complicated diabetes with severe DKA must be seen as a wake-up call for national health systems. In a recent study (16), there was no evidence of a delay in the diagnosis or increased disease severity for childhood cancers or type 1 diabetes during the first wave of the COVID-19 pandemic in centers in the UK. Therefore, any delays in diagnosing diabetes are heterogeneous according to individual contexts and geography. If the increased frequency of DKA was solely due to parental fear, it might be expected that months of containment measures, drastic reductions in COVID-19 mortality, reduced pressure on hospitals, and the COVID-19 vaccination rollout would reduce the frequency of DKA. We did not assess a metric of parental fear; however, in the last three months of 2020 and over seven months after the start of the COVID-19 pandemic, the frequency of DKA increased.
This study confirms that DKA and severe DKA at diagnosis of type 1 diabetes are more likely in children with an immigrant background. Analysis of the role of socioeconomic factors showed that people with high household incomes were at lower risk of DKA, suggesting that high socioeconomic status is protective even in times of emergency, such as during the pandemic. We hypothesize that people with higher incomes may favor private health services, assuming that the private system may be easier to access and less exposed to the risk of infection.

Link Between COVID-19 and Diabetes

There is currently no convincing evidence of a change in the incidence of type 1 diabetes during the COVID-19 pandemic. In theory, COVID-19 could act as an environmental trigger for diabetes in individuals with high genetic risk and pre-existing b- cell autoimmunity. It has also been reported that COVID-19 may have a direct cytotoxic effect on b-cells by binding to the angiotensin converting enzyme 2 (ACE2) receptor or by proteolytic cleavage of the viral spike protein by the serine transmembrane protease 2 (TMPRSS2) (17). However, recent evidence suggests that SARS-CoV-2 is unlikely to directly infect b-cells in vivo (18). The increased frequency of DKA cases without b-cell autoantibodies observed in the third period of 2020 requires further in-depth study. It has been hypothesized that the virus could directly damage b-cells without immune system activation. However, in our series, most cases with b-cell- negative autoantibodies had a COVID-19 negative swab at the time of diabetes onset, and COVID-19 autoantibodies were only tested in a small number of patients. A negative PCR test for SARS-CoV-2 does not exclude viral infection, and indeed computed tomography-positive cases of COVID-19 infection in patients with SARS-CoV-2 RNA-negative tests have been described (19). To better understand this result, more high-quality, long-term data need to be collected and analyzed.

Limitations and Strengths

This study is strengthened of by the participation of most pediatric diabetes centers in Italy, all of which have been involved for some time in the study and management of DKA in newly diagnosed type 1 diabetes. The study is, however, limited by not considering potential confounding factors such as the presence of COVID-19 infection in family members of children newly diagnosed with type 1 diabetes and measurement of parental fear. Furthermore, most centers were only able to measure up to four b-cell autoantibodies.

SUMMARY

In summary, our results show that there was a silent epidemic of DKA in children in Italy during the pandemic in 2020. This phenomenon must be prevented in the event of any future generalized lockdowns or epidemics. Prevention information campaigns, which were proven to be effective in the pre- pandemic period in reducing this potentially life-threatening acute complication (20), could be a useful tool in the general population when health systems are stressed. Also, telemedicine should be considered when DKA is diagnosed locally and the transfer to specialized centers hindered by different factors. The continuous collection of high-quality, population-based data is essential for a better understanding of the association between COVID-19 and type 1 diabetes and to prevent DKA in children and adolescents.

TABLES

TABLE 1 | Frequency of DKA, severe DKA, and negative b-cell antibody status (95%CI) before and during the COVID-19 pandemic by periods of restrictions.

	2017-2019		2020		
	DKA Cases	**% DKA (95%CI)**	**DKA Cases**	**% DKA (95%CI)**	**p-value**
DKA	1071	35.7 (33.5-36.9)	460	39.6 (36.7-42.4)	0.008
Jan-Feb	216	34.8 (31.1-38.8)	57	27.0 (21.3-33.6)	0.045
Mar-May	235	32.1 (28.8-35.7)	104	42.4 (36.2-48.9)	0.004
Jun-Sep	291	36.6 (33.2-40.0)	152	44.3 (39.0-49.8)	0.017
Oct-Dec	329	35.7 (32.6-38.9)	147	39.7 (34.7-44.9)	0.199
Severe DKA	319	10.4 (9.4-11.5)	166	14.2 (12.3-16.4)	<0.001
Jan-Feb	62	10 (7.8-12.7)	7	3.3 (1.5-7.0)	0.004
Mar-May	76	10.4 (8.3-12.9)	45	18.4 (13.8-23.9)	0.002
Jun-Sep	78	9.8 (7.9-12.1)	52	15.2 (11.6-19.5)	0.012
Oct-Dec	103	11.2 (9.3-13.4)	62	16.8 (13.2-21)	0.009
b-cell autoantibody negativity	353	11.5 (10.4-12.7)	137	11.7 (9.9-13.7)	0.888
Jan-Feb	74	11.9 (9.5-14.8)	15	7.1 (4.2-11.7)	0.067
Mar-May	87	11.9 (9.7-14.5)	23	9.4 (6.2-13.9)	0.337
Jun-Sep	84	10.6 (8.5-12.9)	55	16.0 (12.4-20.4)	0.013
Oct-Dec	108	11.7 (9.8-14)	44	11.9 (8.9-15.7)	0.999

TABLE 2 | Factors associated with DKA and severe DKA at type 1 diabetes diagnosis.

	DKA			Severe DKA		
	OR	**95%CI**	**p-value**	**OR**	**95%CI**	**p-value**
Intercept	0.97	0.71-1.32	0.855	0.12	0.08-0.20	<0.001
2020 vs 2017-2019	0.89	0.67-1.18	0.154	0.36	0.12-0.86	0.037
Mar-May vs Jan-Feb	1.13	0.86-1.48	0.423	1.22	0.77-1.95	0.392
Jun-Sep vs Jan-Feb	1.07	0.82-1.39	0.385	1.36	0.87-2.15	0.183
Oct-Dec vs Jan-Feb	0.74	0.49-1.11	0.617	1.37	0.89-2.15	0.156
F vs M	1.21	1.03-1.41	0.019	1.21	0.95-1.53	0.117
5-9 vs 0-4 y	0.55	0.44-0.68	<0.001	0.49	0.36-0.66	<0.001
10-14 vs 0-4 y	0.72	0.58-0.89	0.002	0.60	0.45-0.81	<0.001
15-18 vs 0-4 y	0.64	0.45-0.90	0.010	0.31	0.16-0.56	<0.001
North vs central	0.69	0.56-0.84	<0.001	1.09	0.80-1.49	0.604
South vs central	0.95	0.77-1.18	0.635	1.08	0.77-1.53	0.642
Family history of type 1 diabetes	0.40	0.31-0.51	<0.001	0.57	0.37-0.85	0.008
Immigration background (yes vs no)	1.55	1.24-1.94	<0.001	1.80	1.32-2.44	<0.001
Mar-May 2020 vs Mar-May 2017-2019	2.31	1.34-4.01	0.003	5.61	2.02-18.4	0.002
Jun-Sep 2020 vs Jun-Sep 2017-2019	1.68	1.00-2.84	0.052	3.70	1.35-12.0	0.017
Oct-Dec 2020 vs Oct-Dec 2017-2019	1.54	0.93-2.57	0.097	4.90	1.84-15.57	0.003

DKA model: Likelihood ratio test c^2 with 15 degrees of freedom=124.9, p < 0.001; Hosmer-Lemeshow test c^2 with 8 degrees of freedom=5.29, p=0.726;
Severe DKA: Likelihood ratio test c^2 with 15 degrees of freedom=93.1, p < 0.001; Hosmer-Lemeshow test c^2 with 8 degrees of freedom=14.2, p=0.076.

TABLE 3 | Factors associated with DKA at type 1 diabetes diagnosis in 2020 during the COVID-19 pandemic.

	DKA		
	OR	**95%CI**	**p-value**
Intercept	0.56	0.29-1.05	0.076
Mar-May vs Jan-Feb	2.65	1.43-5.04	0.002
Jun-Sep vs Jan-Feb	2.43	1.33-4.56	0.005
Oct-Dec vs Jan-Feb	2.28	1.26-4.22	0.007
5-9 vs 0-4 y	0.68	0.39-1.18	0.165
10-14 vs 0-4 y	0.92	0.53-1.58	0.753
15-18 vs 0-4 y	0.39	0.15-0.97	0.049
Family history of type 1 diabetes	0.40	0.21-0.73	0.004
Household income, high vs low	0.60	0.41-0.88	0.010

DKA model: Likelihood Ratio test c^2 with 8 degrees of freedom=31.8, p < 0.001;
Hosmer-Lemeshow test c^2 with 8 degrees of freedom=8.75, p=0.364.

FIGURE

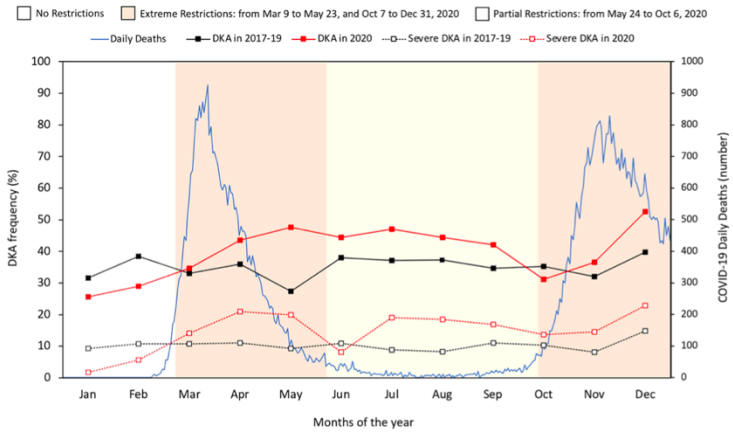

FIGURE 1 | Number of daily deaths from the COVID-19 pandemic in Italy in 2020, and monthly frequency of DKA and severe DKA based on government restriction periods in 2020 compared with the three-year period 2017-2019.

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CHAPTER III – DIABETES CARE IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES

BACKGROUND

Diabetes (both type 1 and type 2) is a chronic disease in which hyperglycemia and, in a less extent, hypoglycemia may undermine cardiovascular system. While acute hypoglycemia can expose patients to arrythmia, cardiac failure and death [33], recurrent hypoglycemia may alters brain function and growing structure, where children and adolescents are particularly exposed [34].

On the other edge, hyperglycemia leads to vascular complications such as chronic kidney disease (CKD), diabetic neuropathy (DN), diabetic retinopathy (DR), and cardio-vascular disease (CVD) [35], with an increased mortality and a decreased life expectancy of estimated 8-10 years compared to reference population [36].

Mainstays of diabetes care are a proper diet (similar to individuals without diabetes), a regular physical activity and a pharmacological therapy aiming for as normal as possible glucose values [37]. Since early 50' of the previous century, glycated hemoglobin (HbA1c) has been used as a marker to define morbidity and mortality outcomes [38]. In the last years, new markers from Continuous Glucose Monitoring (CGM) devices have been considered to define glucose outcomes in a more precise way [39].

TITLE: TIME IN RANGE IN CHILDREN WITH TYPE 1 DIABETES USING TREATMENT STRATEGIES BASED ON NON-AUTOMATED INSULIN DELIVERY SYSTEMS IN THE REAL-WORLD.

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INTRODUCTION

Continuous Glucose Monitoring (CGM) systems have been shown to reduce HbA1c levels (1), reduce hypoglycaemia (2), and provide essential glucose metric information (3) that is not available with traditional self-monitoring blood glucose systems (SMBG) in Type 1 Diabetes (TID). For these reasons, the use of CGM is rapidly increasing in many countries, despite the reported disparities in reimbursement (4). The term CGM includes rtCGM, which provides real-time numerical information about the current glucose level, glucose trends, alerts the user to lows and highs, and isCGM which provides the current glucose value only when the user chooses to scan the device, plus retrospective glucose data for a specified time period, without alarms for low or high glucose values. Even though these devices are often considered within the same category of CGM, recent clinically important differences were reported in the estimates of glycaemic indices (5). Downloading and analysing CGM data remain a barrier for both people with diabetes (PWD) and clinicians to use these devices as a basis for improving glucose control. Wong et

al. (6) reported that only 56% of caregivers of children with T1D ever downloaded device data and only 27% completing this task routinely. Despite the observed challenges in a real-world setting, there is increasing clinical evidence to support the importance of using CGM data for management of type 1 diabetes. A recent international consensus statement provided recommendations for CGM data utilization and reporting (7). There are four metrics from CGM that are of clinical value for people with diabetes and health care professionals as they are reflective of diabetes management in clinical practice. These metrics are: Time In Range (TIR), Time Below Range (TBR), Time Above Range (TAR), and coefficient of variation of glucose % CV. Specifically, the seven-point quarterly SMBG calculated TIR was strongly associated with diabetic complications in a new analysis of the data obtained from the diabetes control complications trial (8). Based on this evidence, TIR assumes a key role in clinical practice and it is necessary to have reference values to set the optimal therapeutic objectives. To date, there is a lack of information on TIR and other glucose metrics in the real-world setting for children with T1D. The aim of this study is to evaluate percentage of TIR in group of children under the age of 18 years, with T1D using glucose sensors with non-automated insulin delivery systems in a real-world setting.

METHODS

A multicentre study including children with T1D followed by 11 Italian centres for paediatric diabetes was conducted from January-May 2019. All centres have used CGM (9) and IP (10) technologies for the care of diabetes in children for more than 15 years and follow national recommendations on their use.

Inclusion criteria for the study were diagnosis of T1D and use of CGM for more than one year, age under 18 years, no changing of insulin administration (multiple daily injections, MDI or insulin pump, IP) within last three months, participants' willingness to enter study, centre's willingness to share anonymised clinical data and CGM downloaded information. We considered one year as a sufficient time to become confident with CGM as a tool to manage insulin therapy in clinical practice. Individuals with diabetes were excluded if they declared their unwillingness to participate, the centre could not share anonymised file data, and/or there was a lack of CGM downloaded data. The glucose sensors and insulin pumps are accessible to all children with T1D in Italy. The study was approved by the Local Ethics Committee of each centre. During a planned visit at the paediatric centre for diabetes care, children and their guardians were asked to participate in the study. Both children and their guardians provided written informed assent and consent.

For each participant, the following data were collected: date of birth, date of type 1 diagnosis, weight, height, number of weekly hours of physical activity, HbA1c, type of sensor used (rtCGM or isCGM), percentage of time CGM was active during the last two weeks, number of daily SBGM, type of therapy (MDI, or IP), type of IP, use of carbohydrate counting, number of severe hypoglycaemic episodes during the last 12 months, and number of DKA episodes requiring hospital admission during the last 12 months. The International Society for Paediatric and Adolescent Diabetes (ISPAD) guidelines state that hypoglycemia is an event associated with cognitive impairment, including coma or convulsions. Hypoglycemia was measured as blood glucose levels <70 mg/dl (11); DKA was defined as hyperglycaemia (> 200 mg/dl), venous pH <7.3 or serum bicarbonate <15 mmol/l (12). All centres used DCA Vantage® Analyzer to determine HbA1c.

Data from CGM of the 2 weeks adjacent to the HbA1c measurement were downloaded at the each centre using a dedicated software or the open source Tidepool software. Data 5 were anonymized at each centre and collected in a centralised database for the analysis.

Four glucose metrics data were included for the analysis. Time in range (TIR) was defined as the percentage of time with blood glucose between 70 and 180 mg/dl (3.9-10.0 mmol/l), TBR and TAR as the percentage of time below and above target range and glucose variability was determined using the percentage coefficient of variation of glucose (%CV).

Statistical analysis

A non-parametric approach was used, since variables were not normally distributed to the Shapiro test. Children were subdivided into groups according to four treatment strategies, as the combinations of non-automated insulin-delivery systems (IP and MDI) and CGM (isCM and rt(CGM), i.e. MDI-isCGM, MDI-rtCGM, IP-isCGM, IP-rtCGM. The Kruskal-Wallis test was used to perform comparisons between treatment strategies on quantitative variables; medians and interquartile range (IQR, 1st – 3rd quartiles) were used to summarize data. Chi-square or Fisher exact test were applied to categorical variables and results were expressed as absolute and percent frequencies. The absolute and percentage frequencies of children achieving CGM-based targets as suggested by recent international consensus recommendations (7) were evaluated according to treatment strategies; the Chi-square test was used to evaluate the association between CGM-based targets and treatment strategies.

Quantile regression analysis was performed to analyse the impact of insulin treatment strategies on TIR adjusted by personal and clinical characteristics collected on participants. Likelihood ratio test was used to identify the most parsimonious model. Quantile regression analysis allows the estimation of quantile-specific effects describing the impact of each independent variable (i.e. insulin-delivery system and subjects' personal and clinical characteristics) on each part of interest of the dependent variable (i.e. TIR). The nine deciles of the TIR distribution were considered in the analysis. Results were graphically summarized, the x-axis shows the values of the 9 deciles of the TIR distribution and the y-axis shows the effects of independent variables (regression coefficients) on TIR 6 for each decile (dotted lines) and 95% confidence bands (95%CI, grey area). If the zero line does not cross the grey area, the estimates significantly differ from 0. All the analyses were performed using the R statistical package; a level of probability of 0.05 was used to assess the statistical significance.

RESULTS

Overall, data from 666 children under the age of 18 years (51% males and 49% females) with T1D and disease duration more than one year, were analysed. Less than 2% of the total eligible participants were excluded due to the lack of all data downloaded from CGM. The median age and diabetes duration were respectively 12 years (IRQ: 10-15 years) and 5 years (IRQ: 3-7 years). The IP was used by 46% of participants, isCGM by 49%, rtCGM by 51%. All of the isCGM were Abbott FreeStyle LibreTM 1 (Abbott Diabetes Care, Inc, Alameda, CA); rtCGM were 2% DexcomTM G4, 20% DexcomTM G5, 19% DexcomTM G6 (Dexcom, Inc, San Diego, CA), 10% GuardianTM Connect (Medtronic). Children on MDI were using basal bolus therapy with glargine or degludec insulin analogue and Lispro, Aspart or Glulisine rapid-acting insulin analogue. Insulin Pumps were 8.5% Roche Accu-check insight (Roche Diagnostic Deutschland GmbH), 16.7% Tandem t: slim X2TM (Tandem Diabetes Care®, San Diego, CA), 3.9% Ypsopump® (Ypsomed AG, Burgdorf, Switzerland), and 62.4% Omnipod® (Insulet Corporation, Billerica, MA). Severe hypoglycaemia and DKA requiring hospitalization during last 12 months occurred in eleven (1.6%) and nine (1.3%) subjects, respectively.

Table 1 and Figure 1 show subjects' personal and clinical characteristics according to the treatment strategies. Subjects on IP treatment used carbohydrate counting system more frequently and had a

significant longer diabetes duration. Children using IP and rtCGM had significant lower HbA1c values than those on MDI and isCGM. Children on IP and rtCGM checked their capillary blood glucose values more frequently than all the other treatment strategies. Children using rtCGM, regardless of the insulin-delivery system, reported significant lower %CV values. In children using the IP the % time CGM active was higher than in those using MDI treatment. No significant differences between treatment 7 strategies were found in the distribution of gender, number of severe hypoglycaemia and DKA requiring hospitalization during the last year (Table 1).

Significant differences were found in the CGM metrics among the four treatment strategies (Figure 1). The group treated with IP & rtCGM had significantly higher median value of TIR (61, IQR: 50-71) than MDI and isCGM (49, IQR: 40-60), MDI and rtCGM (56, IQR: 39-66), and IP and isCGM (56, IQR: 42-65). The group treated with MDI and isCGM had a significantly higher median value of TBR (5, IQR: 2-8) than MDI and rtCGM (2, IQR: 1- 4) and IP and rtCGM (3, IQR: 1-6); group MDI and rtCGM had lower median value of TBR (2, IQR: 1-4) than IP and isCGM (5, IQR: 3-7) and IP and rtCGM (3, IQR; 1-6), and IP and isCGM had higher median value of TBR than IP and rtCGM. The groups treated by MDI and isCGM and MDI and rtCGM had significantly higher median values of TAR (44, IQR: 33-56; 42, IQR: 30-61, respectively) than IP and rtCGM group (35, IQR; 24-46). Subjects treated with IP and isCGM reported median value of TAR of 38 (IQR: 30-54).

Table 2 shows children and adolescents achieving CGM-based targets as suggested by recent international consensus recommendations (7) according to treatment strategies. All of the targets were achieved more frequently by subjects using rtCGM independently from the insulin-delivery system.

Figure 2 shows the results of quantile regression analysis, with TIR as dependent variable. Age, diabetes duration, treatment strategies, use of carbohydrate counting, percentage of time CGM was active during the last two weeks, were found significantly associated with TIR. A positive effect of age was found from the second decile of TIR distribution, while higher diabetes duration significantly decreased TIR and this effect was observed in all the 9 deciles of TIR distribution. The treatment strategy MDI-isCGM was considered as reference category and all the other strategies significantly increased TIR: MDI-rtCGM and IP-isCGM in the second part of TIR distribution, while IP-rtCGM in all the deciles of TIR distribution. The use of carbohydrate counting and the high percentage of time CGM was active during the last two weeks were significantly associated with high percentage of TIR in almost all the deciles of the distribution.

DISCUSSION

To the best of our knowledge there are no previous studies examining differences in glucose metrics in children and adolescents with T1D recorded in the real-world using different treatment strategies based on non-automated insulin-delivery systems. In this large cohort of children with T1D under 18 years of age, using CGM and non-automated insulin-delivery systems, the simultaneous use of rtCGM and IP was associated with higher percent values of TIR, lower TAR, and lower HbA1c values. Independently from the insulin- delivery system, lower values of TBR were associated with the use of rtCGM. Our study highlights the positive effect of rtCGM on TIR distribution compared to isCGM when associated to MDI, or IP compared to MDI when associated to isCGM and suggests that the combination of more advanced technological non-automated systems (IP-rtCGM) offers the best results in achieving time in range in the real-world. The Diamond study (13), that analysed TIR in a randomized control study of adults with diabetes using MDI, reported an improvement of TIR, reaching a median value of 52% after 6 months of the use of rtCGM. At the same time, TAR and TBR reduced to 44% and 2.7% respectively.

In our study, similar values were reported by children using isCGM and MDI, while rtCGM and MDI allowed them to obtain median TIR, TAR and TBR values of 56%, 42% and 2%, respectively. In a randomized study performed during a summer-camp (14), children using isCGM and IP reported TIR of 50.9%, TAR 45.2%, and TBR 1.3%. The 2014 Italian Society of Paediatric Endocrinology and Diabetes (ISPED) recommendations for self- monitoring blood glucose, including CGM, explicitly state that paediatric diabetologists must encourage and provide ongoing education for PWD and families on the importance of the use of CGM and download data to enhance self-management (9). These recommendations are widely applied throughout Italy, since coverage for CGM is provided by the National Health System and most paediatric centres are equipped with multidisciplinary teams. Thus, the better results on glucose metrics reported in our study could be related to education programs on the use of CGM for children and their parents, having involved paediatric centres with long-lasting experience on the use of technology for diabetes care. The HypoDE study (15) reported a median TBR value of 1.6% (IQR: 0.9- 3.7) in a trial of adults with T1D treated with MDI and rtCGM for 6 months, with impaired hypoglycaemia awareness, receiving instructions on optimal use of rtCGM. In our cohort of children using the same treatment strategy (n=119), the median TBR was comparable (2%, IQR: 1-4).

To date, research has focused on glucose metrics analysis comparing non-automated insulin-delivery systems, that are usually referred to as sensor augmented pump (SAP), and automated systems, commonly known as predictive low glucose management (PLGM), hybrid closed-loop (HCL), and full-closed loop (FCL) or artificial pancreas (16). A published randomised study (17), that analysed the effects of at-home use of the Tandem Control-IQ artificial pancreas in young children, reported a mean TIR of 52.8%, TBR of 2.1%, TAR of 44.7% with SAP, and mean TIR of 71.2%, TBR of 2.1%, TAR of 26.2% with Control-IQ system. In our study glucose metrics of children using SAP, showed a slightly higher median TIR of 56% or 61% if the IP was associated with the isCGM or rtCGM, respectively; at the same time, TAR was lower reaching median values of 38% and 35% with isCGM and rtCGM, respectively; TBR was superimposable. A systematic review and meta-analysis of outpatients randomised controlled trials evaluating efficacy of artificial pancreas systems (18) reported a weighted mean of TIR of 58.21% with SAP and 12.59% higher with artificial pancreas systems.

A recent consensus recommendation provided guidance (7) on targets for assessment of glycaemic control for people with T1D. These targets are goal values of TIR>70%, TBR<4%, TAR<25% for adults, and goal value of TIR> 60% for age <25years, if the HbA1c goal is 7.5%. In our analysis, performed on children under 18 years, the percentage of participants meeting TIR>70% was 8.3% with MDI-isCGM (Table 2) and 28.1% with IP-rtCGM. The percentage of participants with TIR>60% was 24.2% with MDI-isCGM and 52.5% with IP- rtCGM. Interestingly, the percentage of children reaching a TBR<4% was higher for both the treatment strategies using rtCGM. High glucose variability is considered a possible risk factor for diabetic vascular complications and is associated with increased risk of hypoglycaemic events. A threshold for % CV of 36% indicates which PWD had stable or unstable glucose homeostasis (19), with the lower values associated to low glucose variability. In our study, the lower %CV values were obtained by participants using rtCGM, suggesting that its use is the best

choice in non-automated insulin-delivery system to reduce the risk of hypoglycaemia.Beyond treatment strategy, the carbohydrate counting and the percentage of time CGM was active were modifiable factors associated with TIR. This observation demonstrates the impact of education in improving glucose control in children with T1D.

There were several limitations to this study. The variation in different CGM systems, could contribute to the differences in the accuracy of CGM-based glucose metrics. The recruitment

methods included only participants of the paediatric centres with specialized expertise in the use of technologies that could contribute to an overestimation of device use in comparison to the entire population of all Italian youth with type 1 diabetes. It is also assumed that recruited children have received education on the use of those devices, and that education resulted in a higher proportion of those reaching their targets for glucose metrics than the national population. Both of these factors impact generalizability of the results.

We did not collect information on the number of participants downloading data. We do not know if this activity is associated to TIR variation and this remains a gap in knowledge and a crucial challenge for PWD, nurses, educators and physicians when providing diabetes care. Systematically gathering information on downloaded and analysed data from PWD and health care providers could be useful to interpret the TIR variation in the real-world. In addition, we did not analyse socio-economic factors of the study population. Therefore, we do not know if these factors impacted CGM-based targets. Despite these limitations, the large sample of participants and the inclusion of many centres evenly distributed in North, Centre and South areas offer a country-wide picture of glucose results in the real- world.

Based on the glucose metrics reported with the four therapeutic strategies, few children with type 1 diabetes are able to reach a TIR> 70% while the number increases substantially if the target is considered at TIR> 60%. These results pose the question of what is the best clinical suggestion in this situation. Is it to settle for a lower TIR to avoid frustration for children and parents or discuss with them the reasons for not reaching the target and accept the challenge of improving glucose metrics? We suggest the latter as the DCCT

dataset analysed by Beck and co-authors (8) showed that TIR, calculated on the basis of quarterly seven-point glucose tests, has a strong association with the risk of developing retinopathy and microalbuminuria. In particular, the frequency of retinopathy was 9% with a TIR between 60 and 70% and 5% with a TIR> 70%; the frequency of microalbuminuria was 2% with TIR between 60 and 70% and 3% with TIR> 70%. Therefore, even with a TIR above 60%, the risk of chronic complications remains limited, however there is no doubt that the higher the TIR, the lower the risk of complications and the CGM-based target for TIR should be> 70% for all children with type 1 diabetes. These topics should be discussed with children and parents and, where there are no barriers, the use of a more advanced therapeutic strategy to improve glucose metrics should be considered. CGM-based goal setting in clinical practise is hard to establish and the stepwise approach suggested by the international consensus group (7) is deemed the best way to support children and families to achieve outcomes closest to suggested goals. We consider the third quartile of each treatment strategy of our analysis as a reasonable result that can be reached as first step for TIR and the first quartile for TBR and TBR. Therefore, for children using isCGM-MDI, rtCGM-MDI, isCGM-IP, TIR could be >60%, TBR<3%, TAR <30%; for children using rtCGM-IP as non-automated modality TIR could be >70%, TBR<3%, TBR<25%.

The study results showed that the best glucose metrics were achieved with the combination of rtCGM and the IP. Until automatic insulin-delivery systems are available on the market, the most advanced non-automated system and diabetes education services should be available to all children with type 1 diabetes. If there are no barriers, an upgrade of the treatment strategy with a higher performing technology should be offered to all children who do not achieve blood glucose metrics within the suggested range.

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TABLES

Table 1. Patients' clinical characteristics according to treatment strategy

		Treatment strategy				
		MDI-isCGM (G_1)	MDI-rtCGM (G_2)	IP-isCGM (G_3)	IP-rtCGM (G_4)	
		n=240	n=119	n=85	n=221	p
Gender*	M	122 (50.8)	64 (53.8)	42 (49.4)	114 (51.6)	0.932
Use of Carbo Counting System*	Yes	79 (33.1)	38 (31.7)	51 (60)	144 (65.2)	<0.001
Severe Hypoglycaemia (last 12 months)*	Yes	6 (2.5)	1 (0.8)	2 (2.4)	2 (0.9)	0.462
DKA requiring hospital admission (last 12 months)*	Yes	1 (0.4)	1 (0.8)	2 (2.4)	5 (2.3)	0.213
Age#	Year	12.9 (10.2-15.3)	12.2 (8.9-14.6)	12.4 (11.4-14.4)	12.1 (9-15.1)	0.110
Diabetes duration#	Year	4.4 (2.3-6.7) G_1 vs G_3 and vs G_4	3.5 (1.8-6) G_2 vs G_3 and vs G_4	5.5 (3.8-9.4)	5.1 (3.5-7.4)	<0.001
BMI#	kg/m^2	19.6 (17.5-22)	19.1 (16.7-21.3)	19.2 (17.5-21.5)	19.2 (16.9-21.5)	0.432

Physical activity[#]	hours/ week	3 (2-4)	3 (0.5-5)	4 (2-5)	3.5 (2-5)	0.110
HbA1c[#]	%	7.6 (6.9-8.1) G_1 vs G_4	7.5 (6.7-8.2)	7.3 (6.9-7.7)	7.3 (6.7-7.8)	0.002
SMBG/day[#]	n°	1 (0.8-2) G_1 vs G_4	1 (0-2) G_2 vs G_4	1 (0.5-2) G_3 vs G_4	2 (1-3)	<0.001
CV[#]	%	39.4 (37.1-43.4) G_1 vs G_2 and vs G_4	36.2 (32.8-40.8) G_2 vs G_3	40.5 (37.4-45.1) G_3 vs G_4	36.8 (34-39.9)	<0.001
% time CGM active in the past 2 weeks[#]		91 (84.5-96) G_1 vs G_3 and vs G_4	92.1 (81.6-96.9) G_2 vs G_3	95.6 (89.5-99)	94.1 (87.6-97)	<0.001

*Values are n (%), p-value refers to Fisher exact test

#Values are median (1^{st} – 3^{rd} quartiles); p-value refers to Kruskal-Wallis test

Significant multiple comparisons are indicated as G_i vs G_j, with i, j=1,…,4 and i≠j

Table 2. Children achieving CGM-based targets

Recommended CGM-based targets	MDI-isCGM (n=240) n (%)	MDI-rtCGM (n=120) n (%)	IP-isCGM (n=85) n (%)	IP-rtCGM (n=221) n (%)	p
TBR<4%	102 (42.5)	87 (72.5)	24 (28.2)	136 (61.5)	<0.001
TIR>60%	58 (24.2)	51 (42.5)	29 (34.1)	116 (52.5)	<0.001
TIR>70%	20 (8.3)	17 (14.2)	11 (12.9)	62 (28.1)	<0.001
TAR<25%	25 (10.4)	20 (16.7)	17 (20)	58 (26.2)	<0.001

p-values refer to Chi-square test

* as suggested by recent international consensus recommendations according to technology treatment.

FIGURES

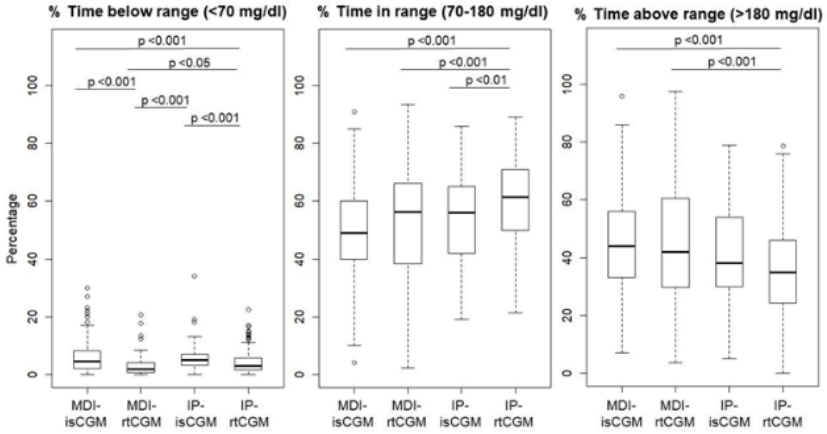

Figure 1. Time Below Range (TBR), Time in Range (TIR), Time Above Range (TAR) by treatment strategy

p-values refer to Kruskal-Wallis test

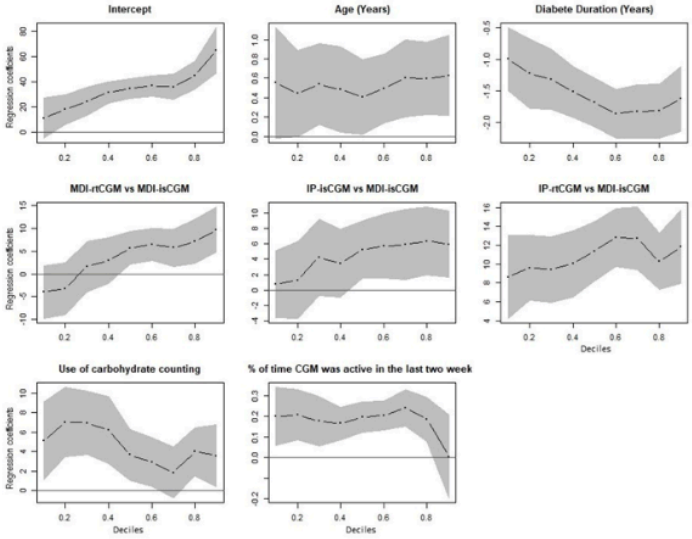

Figure 2. Factors associated to TIR, results of quantile regression analysis

y-axis shows the regression coefficients, i.e., the effects of independent variable on TIR deciles;

grey area shows 95%CI

CHAPTER IV - ISSUES AND PROBLEMS IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES

BACKGROUND

Spending time in the ambulatory with children and adolescents with diabetes, there is a behaviors spectrum in where motivation and education connect, and have an impact on diabetes care. While motivation is generally related to psychological status of the patient (and the family), education rely on a proper communication between the healthcare professional (physician or other) about technical issues that come across frequently (e.g. mild hypoglycemia, hyperglycemia) or may pose a risk and need to be addressed for a safety reason (e.g. severe hypoglycemia, DKA)[4]. Moreover, education need to be adapted also to specific characteristics (such as smaller children), and different protocols should be created according to specific needs. This is the case of smaller children with T1D who are more prone to gastrointestinal illness, and may have food refusal with consequent risk of severe hypoglycemia and hospital admittance. From some article in the literature [40,41], we created a protocol specific for this age group and we tested in our population with good results [42].

TITLE: MINI-DOSES OF GLUCAGON TO PREVENT HYPOGLYCEMIA IN CHILDREN WITH TYPE 1 DIABETES REFUSING FOOD: A CASE SERIES.

AUTHORS: Davide Tinti, Ivana Rabbone

INTRODUCTION

One of the major challenges to achieve optimum glycemic control in children with type 1 diabetes (T1D) is overcoming the risk of hypoglycemia.
Mild/moderate hypoglycemia (now called *hypoglycemia alert*) takes place in children when blood glucose level is less than 70 mg/dl (3.9 mmol/l) and is commonly considered as a threshold at which to intervene giving glucose avoiding events defined as severe hypoglycemia, when associated with important cognitive impairment (including seizure or loss of consciousness) [1].
Severe hypoglycemia (SH) is one of the major burdens in patients with T1D, especially in children and adolescents. In an observational study conducted in Germany between 2011 and 2013 on 31,300 patients 0.5–20 years, the rate for SH was 1.45/100 patient-years [2]. SH can lead to short-term (neuroglycopenic symptoms, seizures, coma or arrhythmias until death) and long-term complications (possible negative effects in long-term memory, attention, and verbal IQ) [3, 4]. Therefore, it generates fear in patients and their parents, especially if children are very young, and can be a deterrent to reach optimal metabolic control [5].
The best choice to treat SH consists in a dose of glucagon given subcutaneously or intramuscularly [6].
For these reasons, the hypoglycemia paradigm is now shifting from treatment toward prevention [1]. Some help can come from technology, using continuous glucose monitoring (CGM) sensors to track an impending hypoglycemia, or more sophisticated algorithm to suspend insulin before reaching the hypoglycemia threshold (such as predictive low glucose suspension), but the risk of hypoglycemia cannot be completely removed [7].
As yet, oral carbohydrates (most of all glucose, 0.3 g/kg in children) are the best option to treat or to avoid impending hypoglycemia. However, in the case of vomit, nausea or food refusal, if hypoglycemia occurs, oral carbohydrates are not accepted. Therefore, it has been suggested a modified smaller-than-usual glucagon injection protocol to reverse glycemic trend and enable oral fluid intake to be re-established (“mini glucagon treatment”) (Fig. 1) [8, 9].

In the past, the effectiveness of the latter treatment has been evaluated only with blood glucose measurements [10] or with continuous glucose monitoring (CGM) but only in adults [11]. The purpose of this paper is to evaluate the glycemic response after mini-doses of glucagon used to prevent SH or impending hypoglycemia, using a CGM in young children with T1D on insulin pump therapy.

METHODS

Educational program in T1D children and their families at the onset or during out-patient visits is based on explanation, recognition, treatment and prevention of hypoglycemia. Mini-doses of glucagon protocol instruction, for the management of mild hypoglycemia associated with illness or food refusal, according to International Society for Pediatric and Adolescent Diabetes (ISPAD) guide- lines [1], are included. Caregivers are also instructed to reconstitute glucagon (GlucaGen® HypoKit 1 mg, Novo Nordisk®A/S, Bagsvaerd, Denmark) according to package instructions and to use insulin syringes (U30) instead of the glucagon ones. If the patient is less than 2 years of age, the dose of glucagon is 2 marks on the syringe, equivalent to 20 μg or 2 "units." For children aged 2–15 years, the dose is 1 unit (10 μg) per year of age, to a maximum of 15 units (150 μg). If blood glucose remains < 70 mg/dl after 30 min, another injection of glucagon mini-dose is recommended. The blood glucose needs to be checked frequently (every 5–15 min), and repeated doses of glucagon can be given as required. The child is encouraged to have little sips of cool oral rehydrating or cold glucose solution, depending on availability.
Parents are always able to contact the Diabetes team for advice, and in case of further assistance, it directs the family to the Emergency Department.
The following day of a potentially severe hypoglycemic episode, we asked caregivers to upload data of meters, CGM and pump in Glooko–Diasend ® platform (a widespread internet-based tool for teleconsulting) and to send a detailed report of what happened before, during and after the hypoglycemic episode. From that, we were able to assess glucagon efficacy in a series of cases, to better evaluate clinical and glycemic response after giving mini- doses of glucagon.

RESULTS

Over a 3-month period, we collected CGM readings, blood glucose values and pump data of 3 children followed in our Centre who were treated with mini-doses of glucagon res- cue, for a total number of 4 episodes.
Patient 1 is a 3-year-old boy, with T1D from 1 year of age, using an insulin pump (Animas® Vibe ®, Johnson and Johnson) and a continuous glucose monitoring (Dexcom G4®, Dexcom) from diabetes onset. His last HbA1c is 6.6% (49 mmol/mol), with 7% of time spent below 70 mg/dl (< 3.9 mmol/l) and 67% of time spent in range (70–180 mg/ dl). Coefficient of variation (CV) is 44%. Patient 1 had an episode of vomit right after receiving the lunch insulin bolus. Glucose from CGM was 223 mg/dl. Since the child refused to eat, parents decided, after a phone call to the Diabetological Team, to suspend basal insulin infusion from the pump and to give 3 UI of glucagon. Glucose continued to drop and, after one hour, glycemia was 98 mg/dl, while after 90 min it reached the lowest value, 74 mg/dl. Two hours later basal rate was resumed, with a sensor value of 95 mg/dl (Fig. 2).
At dinner, the child had a meal of 40 g of carbohydrates, with a pre-meal blood glucose of 261 mg/dl. Due to a poor appetite, the father gave the insulin bolus 10 min after the meal; sensor value was not available due to the Dexcom ® new sensor warm-up period (2 h). After 60 min, the child had another episode of vomit. At that time, blood glucose was 176 mg/dl. Basal insulin was reduced by 30%, and glucose was offered to the child, who refused. After another hour, glucose dropped to 81 mg/dl, with persistence of food refusal; therefore, basal insulin was suspended and 3 UI of glucagon were given again. Glucose continued to drop until 60 mg/dl, then 30 min after injection,

glucose increased to 84 mg/dl and then to 118 mg/dl. After 1 h, basal insulin was resumed, halved, and after 2 h glucose sensor was 171 mg/ dl, while reaching 129 mg/dl after 3 (Fig. 2). Except nausea, no other symptoms were referred.
Patient 2 is a 3-year-old boy, with T1D from the age of 1, using an insulin pump (Animas® Vibe ®, Johnson and Johnson) and a continuous glucose monitoring (Dexcom G4®, Dexcom) from diabetes onset. His last HbA1c is 8.9% (74 mmol/mol), with 2% of time spent below 70 mg/dl (< 3.9 mmol/l) and 45% of time spent in range (70–180 mg/ dl). Coefficient of variation (CV) is 27%.
Thirty minutes after receiving the lunch insulin bolus, he vomited the meal. Glycemia was 128 mg/dl. Despite many attempts, parents were not able to feed him with sugar. After a phone consultation with the Diabetological team, when glycemia was 84 mg/dl, they injected 3 UI of glucagon subcutaneously. After 15 min, glycemia dropped to 64 mg/dl and after 20 min to 42 mg/dl, while values from CGM were higher (around 100 mg/dl). For that reason, a second dose of glucagon was administered, and 15 min later glycemia was 114 mg/dl. After 1 h, glucose reached 247 mg/dl (while values from CGM glucose were estimated still below 70 mg/ dl), and 2 h later glycemia was 271 mg/dl (confirmed by CGM). He did not lose consciousness, but showed tremors, irritability, pallor and had nausea (Fig. 3).
Patient 3 is a 7-year-old girl, with T1D from the age of 5, using an insulin pump (Animas® Vibe ®, Johnson and Johnson) and a continuous glucose monitoring (Dexcom G4®, Dexcom) from the age of 6. Her last HbA1c is 6.8% (51 mmol/mol), with 4% of time spent below 70 mg/dl (< 3.9 mmol/l) and 65% of time spent in range (70–180 mg/ dl). Coefficient of variation (CV) is 32%.
At 7:00 a.m., 10 min after receiving the breakfast insulin bolus, she felt nauseous and ate only a few grams of carbo- hydrates; glycemia was 198 mg/dl. The mother kept offering food, but after 40 min, when glucose reached 72 mg/dl, she set insulin basal rate to 0025 IU/h; then following a phone call to the Medical Team, 7 UI of glucagon was given; glycemia dropped to 59 mg/dl. Ten minutes later, blood glucose began to increase (97 mg/dl) reaching 139 mg/dl 20 min after the injection. In 1 h, glucose value from sensor had slightly risen to 163 mg/dl, while 2 h later it had decreased to 121 mg/dl (Fig. 4). The child felt nauseous for 1 h; then she recovered (blood glucose 113 mg/dl) and was able to eat lunch, afternoon snack and dinner normally.

DISCUSSION

There are no similar case series in the literature about glucagon given subcutaneously as mini-doses, to avoid impending, treat or prevent SH. In all these cases, glucagon was given after at least one attempt of other measures to prevent or treat hypoglycemia, such as glucose-containing solutions and/or basal insulin reduction, in order to avoid hospital admission in case of a hypoglycemic episode and food refusal (risk of severe hypoglycemia).
In all four cases, glucagon was shown to be effective in avoiding severe hypoglycemia, irrespective of subministration timing. However, mini-doses were not effective in avoiding impending hypoglycemia, if given near hypoglycemia threshold (70 mg/dl), with a good glycemic response at one (mean glycemia: 127 ± 80 mg/dl) and 2 h (mean glycemia: 165 ± 78 mg/dl) later. In all except one case, a single dose was sufficient to treat and prevent severe hypoglycemia.
The glucagon resulted effective in treating and preventing impending severe hypoglycemia both for low levels of blood glucose and food refusal after an insulin bolus. No major symptoms were reported, with the exception of nausea.
This approach has already been used in other studies to treat non-severe hypoglycemia during physical activity or after an insulin overdose to avoid hospitalization [11–13]. Given the fact that severe hypoglycemia is one of the major causes of Emergency Department access, in our opinion mini-doses of glucagon were useful to avoid hospital admission in children with T1D refusing food during illness leading to a remarkable social and economic burden in Italy, estimated in 23 million euros per year [2, 14].

Despite being included in ISPAD Guidelines, in our experience glucagon mini-doses protocol is not used widely across our Country, probably for a lack of knowledge both in parents and health-providers. This case series, focused on smaller children whose families received a full instruction on glucagon mini-doses, is one of the first real-life experience on the protocol, especially in preschoolers. In this specific population, mini-doses of glucagon resulted effective even in a non-physiological situation of hyperinsulinization (after an insulin bolus); this opens the road to the use of glucagon not only to treat severe hypoglycemia, but also in dual hormone-artificial pancreas devices (closed-loop systems) to prevent or to treat hypoglycemia more aggressively, together with insulin infusion [15].
We believe that, also thanks to other glucagon formulation such as nasal sprays or liquid stable forms (which are at present under development and are expected to be launched in the near future [16, 17]), glucagon will be used together with insulin to put a hold on severe hypoglycemia while reducing mild/moderate hypoglycemia.

CONCLUSION

Glucagon is the recommended treatment for severe hypoglycemia in both adults and children. Moreover, as ISPAD Guidelines state, glucagon given in mini-doses is helpful also in children with impending (≤ 80 mg/dl or 4.4 mmol/l) or mild/moderate hypoglycemia in case of gastrointestinal illness and/or poor oral carbohydrate intake, to avoid SH and hospitalization [1, 10].
This option paves the way to new utilization for glucagon, which now can be implemented in the care of children and adolescents with T1D. New formulations, such as nasal glucagon, will facilitate subministration preventing severe episodes, reducing fear of hypoglycemia and its complications in patients and for caregivers.

FIGURES

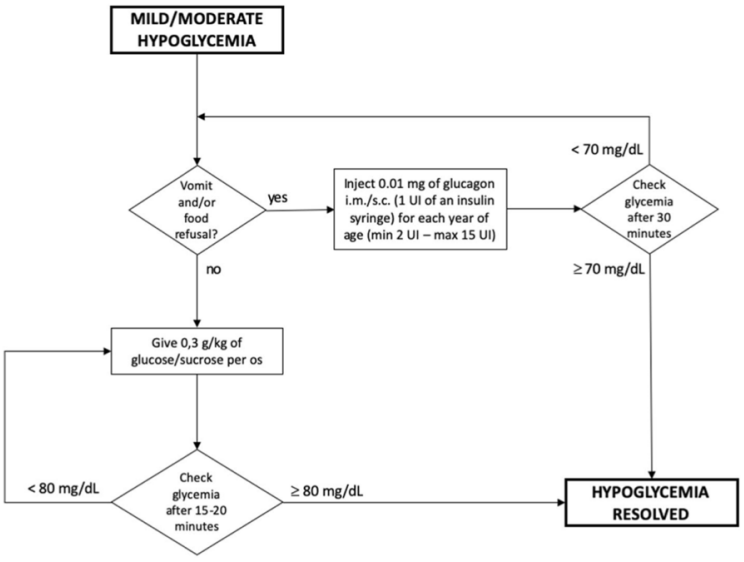

Fig. 1 Flowchart for treatment options in case of mild/moderate hypoglycemia in children with type 1 diabetes

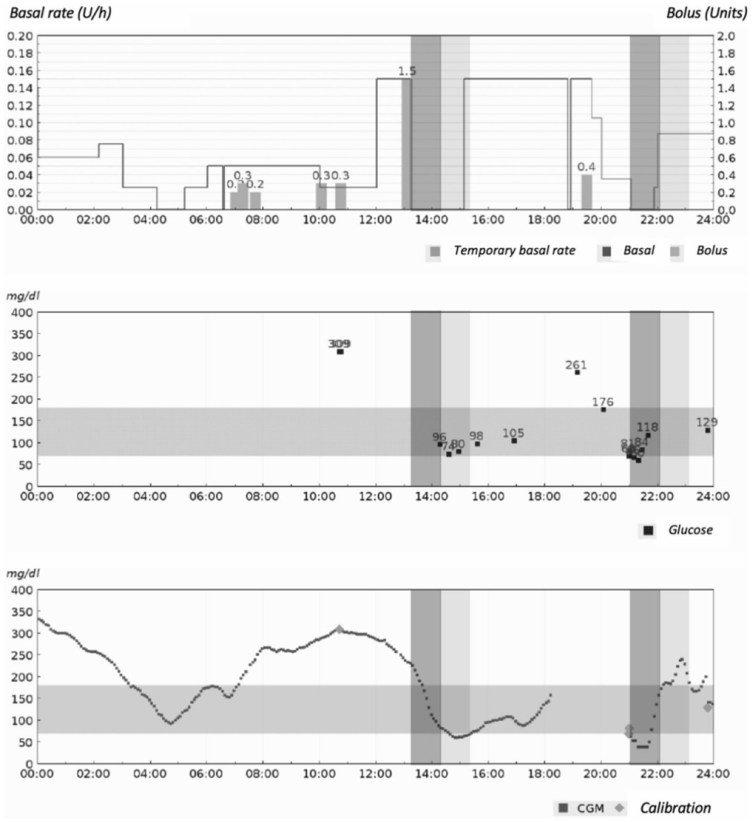

Fig. 2 Insulin pump, meter and CGM downloaded from Diasend ® personal account of Patient 1. The vertical dark gray bars are the first hour after glucagon subministration. The vertical light gray bars are the second hour after glucagon injection

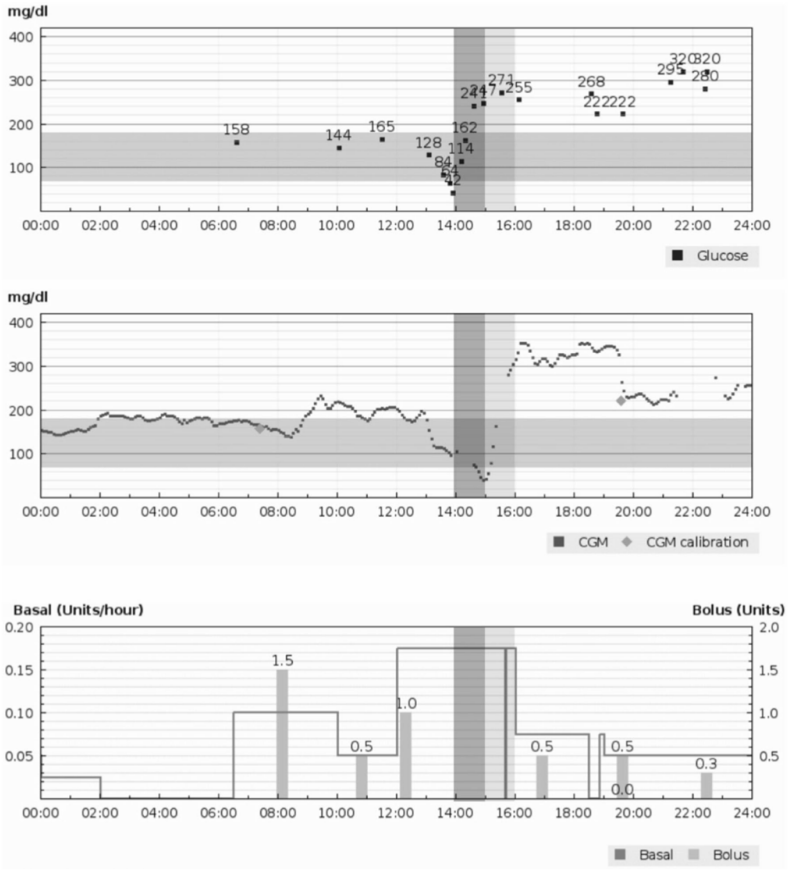

Fig. 3 Insulin pump, meter and CGM downloaded from Diasend ® personal account of Patient 2. The vertical dark gray bar is the first hour after the second dose of glucagon. The vertical light gray bar is the second hour after the second glucagon injection

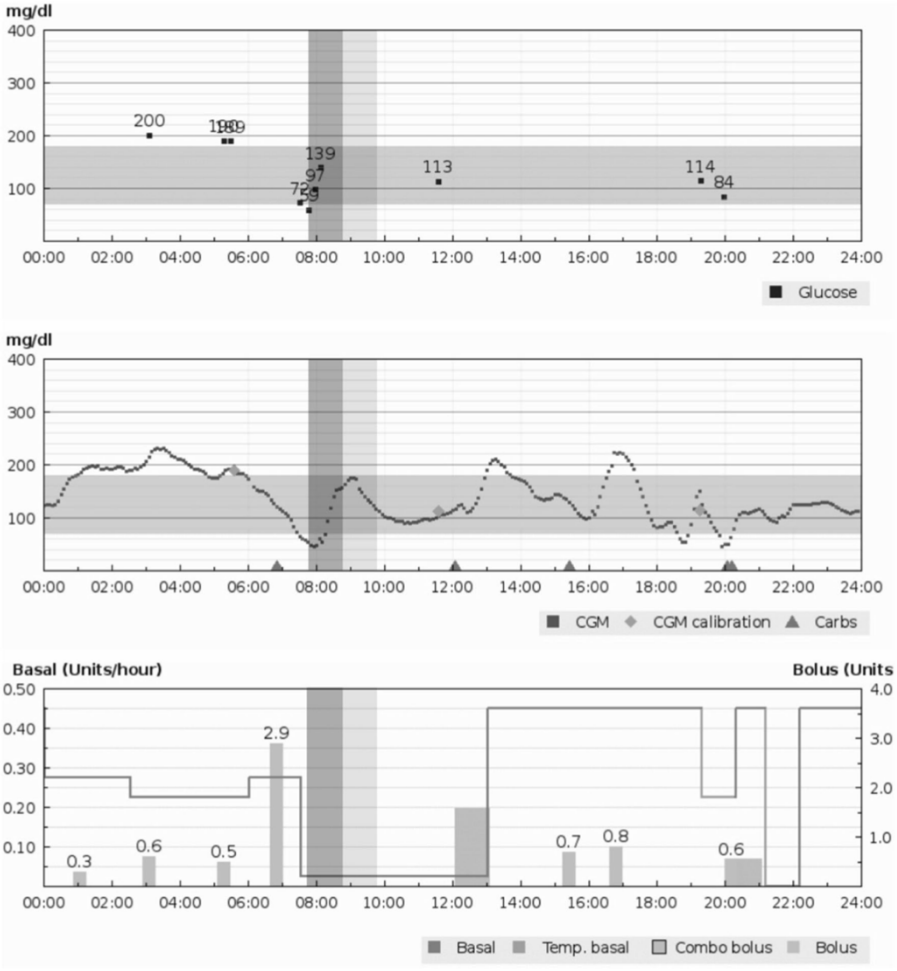

Fig. 4 Insulin pump, meter and CGM downloaded from Diasend ® personal account of Patient 3. The vertical dark gray bar is the first hour after glucagon subministration. The vertical light gray bar is the second hour after glucagon injection

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BACKGROUND

In reading an article concerning the use of technology in diabetes, there are frequently different comments on the many advantages on glucose outcomes, burden of disease and quality of life [43-45]. But, the more we use it, the further we know about the downsides: pump failures, cannula detachments, and skin issues, which finally can lead to discontinuation and glucose deterioration [46].

Skin issues are of major concern, since some of them are problematic and complicates devices prosecution (both pumps and sensors) [47]. We sought to determine the prevalence of skin issues in children and adolescents with T1D using technology using a widespread web-based survey [48].

TITLE: HIGH FREQUENCY OF DERMATOLOGICAL COMPLICATIONS IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES: A WEB-BASED SURVEY.

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INTRODUCTION

In the past two decades, technology has acquired a crucial role in the management of type 1 diabetes (T1D). It is estimated that about 75% of children and adolescents with T1D use medical devices such as continuous subcutaneous insulin infusion (CSII) pump or continuous glucose monitoring (CGM) systems.1 CSII allows to simulate the physiological insulin secretion with its circadian variations.2 Among others, a subtype of CSII is now recognized which is applied directly on the skin at the infusion site without external tube, also known as "patch pump".3 CGM system allows to monitor the current glucose value in real time. Two types of CGM systems are currently available: real-time CGM (rtCGM) and intermittently scanned CGM (iCGM), also called flash glucose monitoring (FGM).4 To maintain clear distinction throughout this article, we will refer to these devices as CGM and FGM. The impact of technology on adherence and glycemic control has been well demonstrated. Benefits due to the advance of technology include improved glycemic outcomes, fewer episodes of severe hypoglycemia, and reduction in the rate of diabetic ketoacidosis.5,6

Despite these benefits in the management of T1D, an alarmingly increasing rate of skin reactions related to the use of technological devices has been emerging.7 The recurrent application and the long wearing time (up to 14 days) of the adhesives used to ensure pumps and sensors to the skin are related to a high risk to have dermatological complications, which are barriers to continuous use. Case reports on skin reactions caused by devices used for the management of T1D have been increasingly described in the literature, as well as observational studies and intervention trials that discuss cutaneous complications due to patch pump or CGM/FGM have been recently conducted.8-11 The most reported skin reactions due to technological diabetes devices are scarring, lipodystrophies (including both lipohypertrophy and more rarely lipoatrophy), irritant contact dermatitis, and allergic contact dermatitis. These are usually characterized by intense itching and represent tricky dermatological issues since they are related to a certain risk of developing a secondary bacterial infection.

METHODS

We conducted a cross-sectional survey based on an online questionnaire filled out by patients' parents between June and December 2019.

Survey participants were patients with T1D who use technological diabetes devices followed-up at Pediatric Diabetes Center in Turin and Messina. Patients' parents gave their writ- ten informed consent through the online form before completing the questionnaire. Inclusion criteria were represented by the duration of T1D for at least 3 months and the use of technological devices for more than 1 month. Exclusion criteria were the inability to complete the questionnaire. Out of 180 patients who were randomly recruited and invited to take part in the study, 139 (77.2%) agreed to participate. The questionnaire included questions on the following items: demographic characteristics; diabetes duration; type of insulin treatment; duration of the use of patch pump, FGM, or CGM; occurrence, timing, and severity of dermatological complications if present; and measures taken in the event of skin reactions. Particularly, we focused on skin reactions typically characterized by erythematous lesions.

The severity of dermatological complications was divided into three classes: a mild reaction (faint homogenous erythema without infiltration), a moderate reaction (defined if erythematous lesions with infiltration and small vesicles were present), and a severe reaction (characterized by intense erythema with coalescing vesicles and bullous skin lesions). To facilitate the definition of severity, participants were asked to characterize the type of skin reaction in comparison with photographs of

various skin conditions due to pumps or glucose sensors (Figure 1). The photographs used as examples of different skin conditions were taken from our clinical experience. Demographic and clinical patients' characteristics and the results of questionnaire were statistically analyzed. The numerical data were expressed as mean and standard deviation and the categorical variables as absolute frequencies and percentages (Table 1). In order to compare
patients with and without skin reactions, we applied unpaired *t*-test for numerical parameters and ChiSquare test for categorical variables. A *P*-value <.050 was considered to be statistically significant.

RESULTS

Mean age of our study population was 11.1 years (range 4-17 years) with a homogenous distribution between male and female (48.2% and 51.8%, respectively). Mean duration of T1D was 5.7 years. Almost all patients presented a suboptimal glycemic control as demonstrated by the last year mean value of glycated hemoglobin (7.2 ± 0.6%, 55 ± 7 mmol/mol). Over half of patients (51.1%) experienced dermatological complications due to patch pumps or glucose sensors. Of these, 59.1% referred to mild reactions, 35.2% reported moderate reactions, and 5.6% of patients had severe skin reactions. Dermatological issues were mainly present in subjects wearing CGM (56.3% of total cases). FGM and patch pump users reported a skin lesion rate of 35.3% and 8.4%, respectively (Figure 2). The distribution of CSII, CGM, and FGM users, as well as the correct percentages of skin reactions due to the different techno- logical devices among our study participants, is shown in Table 2. The timing of appearance of dermatological reactions varied from a few days to several months after the introduction of these devices. The majority of patients solved the problem by applying hypoallergenic barrier patches and bandages (43.7% of the total cases). In accordance with the diabetes specialist's decision, 23.9% of patients replaced the model or brand of the "culprit" device. Ten subjects (14.1%) used barrier films for skin protection, while 12 patients (16.9%) adopted no solutions considering the problem as not very influential. One patient was forced to discontinue any type of continuous or flash monitoring system due to the persistence of skin lesions, and she was switched back to self-monitoring blood glucose. Finally, there were no significant differences in age, gender, dura- tion of T1D, and last year mean value of glycated hemoglobin between patients with reported skin reactions and other study participants (Table 3).

DISCUSSION

More than half of the pediatric patients wearing technological devices for the management of T1D experience dermatological complications. Our findings are in accordance with the results reported by other recent studies. Berg et al demonstrated a rate of 63% of cutaneous complications among CSII users and a rate of 46% of skin reactions among children who regularly used glucose sensors.12 Another study showed that 43% of patients experienced skin complications related to insulin infusion sets.13 These data are similar to those founded by Al Hayek who reported that 48.2% of adolescents on CSII therapy had dermatological unwanted effects.14 Allergic contact dermatitis (ACD) is the most insidious among skin-related complications. ACD is a delayed type allergic hypersensitivity reaction, which is caused by a T cell-mediated immune reaction to usually harmless allergens. Clinical manifestations of ACD include erythema, edema, vesicles, oozing, and intense pruritus.15 Although it is considered that ACD is less frequent than other milder dermatological conditions, there is a strong impression that the real prevalence of this condition in children and adolescents with T1D is higher than commonly expected.16 One of the main causes of the increasing prevalence of ACD is the exposition of a potent allergen. The longer insertion time than in the past may allow the allergen to forward sensitization.17,18 One of the worst consequences related to ACD is pruritus, which may also cause "bad school performance" with tiredness and impaired concentration due to itching during nights. The influence of pruritus on the

determination of the patient's tolerance for skin lesions is well known. Some patients even use antihistamines to relieve the itching sensation with the potential risk of adverse sedating effects.19 Several factors may promote the appearance of skin reactions in T1D pediatric patients. One of these is the compromised integrity of the skin that may be related to pre-existing skin diseases such as atopic dermatitis. Repeated taping of the same sites may be the leading cause of cutaneous damage due to the trauma of repeated insertions. Careless removal of adhesive tapes, if made with excessive force and energy, may increase the risk of tissue damage. Another crucial aspect is represented by the components contained in the adhesives of patch pumps and glucose sensors. Unfortunately, the exact composition and preparation of adhesives used by various manufacturers are rarely available, since single elements quantity may vary in the ratio in each device. However, several threatening compounds included in diabetes devices have been identified. Among these, isobornyl acrylate (IBOA), N,N-dimethylacrylamide (DMAA), and colophonium seem to be the most harmful agents. IBOA is a photopolymerizable acrylate monomer, and it is used, in its liquid form, in coat- ings, sealants, glues, adhesives, paints, and inks and also as a plasticizer in various plastic materials. 20 In 2016, the presence of IBOA was discovered in a popular glucose sensor. Several studies have since confirmed the role of IBOA as the culprit sensitizer causing ACD in patients using the same glucose sensor.21,22 DMAA is frequently used as a monomeric diluent in ultraviolet curing adhesives. Small amounts of DMAA were recently identified in the adhesive patch of the FGM sensor.23 Colophonium is a natural substance derived from pine trees, and it is used in both unmodified and modified forms as a fast-acting adhesive for industrial, medical, or other commercial uses.24 Colophonium was found in both glucose sensors and patch pumps. Positive patch test reactions to colophonium were observed in numerous patients wearing diabetes devices containing this substance. 9,21,25

To manage the emerging issue of skin reactions in young patients with T1D, several preventive measures have been put forward. Recently, Messer et al proposed a practical comprehensive guidance to preserve the skin integrity of patients who chronically use diabetes devices. The authors shared tips on the correct device placement, prophylactic skincare, accurate removal techniques, and promoting skin healing. In addition, they suggested the application of various barrier agents to minimize the risk of hypersensitivity reactions.26 This preventive solution appears to be the most frequent choice among pediatric patients with T1D, as demonstrated by our results. The majority of individuals apply hypoallergenic (hydrocolloid and/or silicone-based) plasters for blocking adhesives from sensors and pumps from the contact with the skin.27 For some patients, liquid barriers may offer sufficient protection from adhesive agents. Based on our experience, we consider that these skin protective devices are very useful and, in most cases, allow to solve dermatological complications so that patients may continue to use the potentially harmful device. These barrier films are mostly only useful in milder irritations but not in ACD. They are not able to prevent a potent allergen from migrating into the skin. Furthermore, they can be irritative to skin itself.28 Another solution that is increasingly being adopted is the off-label use of fluticasone propionate nasal spray. This nasal steroid is commonly used by patients with allergic rhinitis. Benefits of applying fluticasone propionate spray to the skin were recently reported by Paret et al who described their real-life experience with 12 patients suffering from local skin irritation due to CGM. The authors demonstrated that spraying two puffs of the steroid on the skin area prior to adhesion of glucose sensor significantly reduced the degree of cutaneous irritation.29 However, there are no studies on the long- term use of nasal steroids applied topically, and their use is widely debated. Finally, the recognition and avoidance of the sensitizing agent contained in the adhesives used to secure patch pumps and sensors to the skin remain the landmark of the management in the event of ACD.16

CONCLUSIONS

Our survey confirms the high frequency of dermatological complications in patients with type 1 diabetes. Skin reactions due to technological devices represent a clinical condition that diabetes specialists must learn to know and manage in a proper way. The physicians should also constantly report the occurrence of skin adverse reactions to device manufacturers. There is a compelling unmet need for companies to supply detailed information about allergens and irritants contained in their devices.
The persistence of dermatological concerns increases the risk of diabetes-related emotional distress. Although several preventive measures are available so far, there are no clear and universal recommendations on the most suitable management plan. We remark the need for well-designed studies to minimize the burden of diabetes devices and to optimize the quality of life for people with diabetes.

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FIGURES AND TABLES

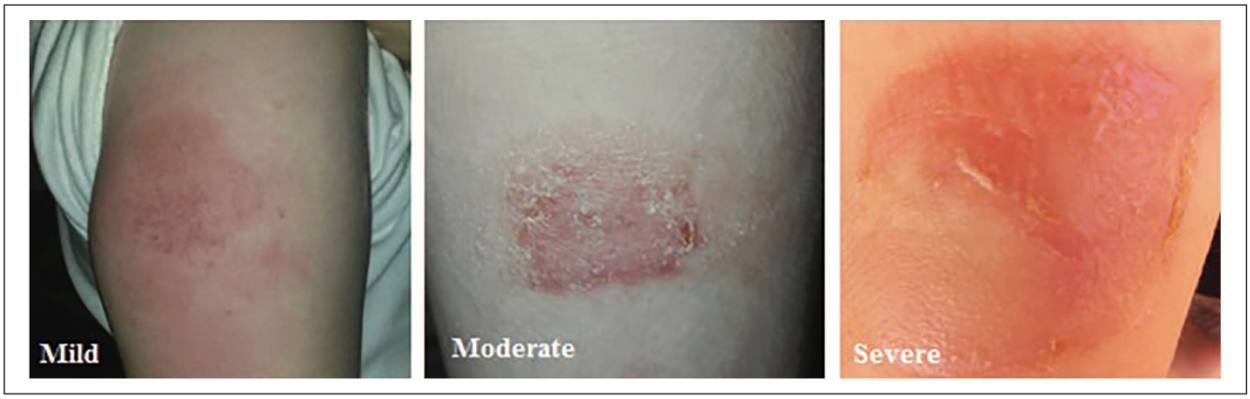

Figure 1. The three photographs characterizing the severity of skin lesions.

Table 1. Details on Demographic and Clinical Patients' Characteristics, and Results of Survey.

Age (years)	11.1 ± 3.3
Female (%)	67 (48.2%)
Duration of diabetes (years)	5.7 ± 4.0
Last year mean value of glycated hemoglobin (%)	7.2 ± 0.6
Type of insulin treatment	
Continuous subcutaneous insulin infusion	95 (68.3%)
Multiple daily insulin injections	44 (31.7%)
Appearance of dermatological complications	71 (51.1%)
Severity of skin reactions	
Mild	42 (59.1%)
Moderate	25 (35.2%)
Severe	4 (5.6%)
Time of appearance of skin manifestations	
0-1 month	29 (40.8%)
1-6 months	11 (15.5%)
6-12 months	17 (23.9%)
>12 months	14 (19.7%)
Solutions adopted	
Application of hypoallergenic barrier patches	31 (43.7%)
Replacement of the model or brand of device	17 (23.9%)
Application of protective skin barrier films	10 (14.1%)
None	12 (16.9%)

Table 2. Relationship between Skin Reactions and the Total Number of Patients Wearing Different Technological Devices among Our Study Participants.

Devices	Total users in our study	Mild reactions	Moderate reactions	Severe reactions
Patch pump*	30	4 (13.3%)	2 (6.7%)	0 (0.0%)
CGM	70	23 (32.9%)	14 (20.0%)	3 (4.3%)
FGM	64	15 (23.1%)	9 (14.1%)	1 (1.6%)

*Some patients wearing patch pump also used CGM or FGM to monitor glycemic levels.
CGM, continuous glucose monitoring; FGM, flash glucose monitoring.

Table 3. Comparison between Patients With and Without Skin Reactions.

Variables	Reported skin reactions	Not reported skin reactions	*P* value
Age (years)	11.1 ± 3.6	11.0 ± 2.9	.739
Female (%)	30 (42.3%)	37 (54.4%)	.151
Diabetes duration (years)	5.9 ± 3.6	5.4 ± 4.3	.421
Last year mean value glycated hemoglobin (%)	7.1 ± 0.6	7.3 ± 0.6	.167

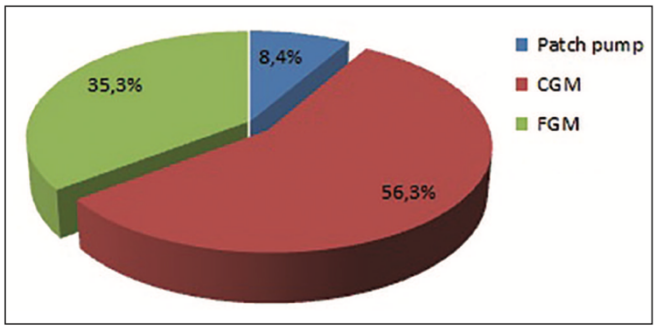

Figure 2. Distribution of different diabetes device responsible for dermatological complications among patients who reported skin issues.

CHAPTER V - INSIGHT ABOUT TYPE 1 DIABETES IN THE PEDIATRIC AGE

BACKGROUND

In this chapter I will try to give some insight about diabetes taste, as a new proposal to study the correlation between taste perception and type 1 diabetes. These two arguments, ideally far apart from each other, have a link under different perspectives: eating habits, diabetic complications, and genetics. In this multicentric study, coordinated from geneticists in Trieste, we evaluated taste perception in a cohort of children and adolescents with type 1 diabetes, finding something we did not expect at the beginning, giving some insight about diabetes in this age group [49].

TITLE: ALTERED TASTE FUNCTION IN YOUNG INDIVIDUALS WITH TYPE 1 DIABETES.

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INTRODUCTION

Taste perception is one of the most important factors influencing individual food preferences and eating habits with possible implications on health status (1–3). Recently, the study of taste function and its relationship with diseases, such as obesity or diabetes, has received increasing attention. Several reports have already described taste impairment in type 2 diabetes, suggesting a possible impact of this disorder on the ability to follow a controlled diet and thus reaching good glycemic control. Taste impairment in type 2 diabetes has also been related to micro- and macro-vascular complications of the disease (4–7).

Altered taste function is also reported in individuals affected by type 1 diabetes. Many years ago, hypogeusia involving all the four primary tastes (bitter, salty, sour, sweet) was described in adults with type 1 diabetes, significantly associated with type 1 diabetes duration and its complications such as peripheral neuropathy, proposing that the impairment could be a complication of the disease (8). Changes in electrogustometric taste thresholds and in the gustatory anatomical structures were also reported (9). More recently, a significantly increased threshold for bitter, salty, sour, and sweet tastes was observed in 70 participants with type 1 diabetes compared to controls (10). Another recent study in 31 pediatric participants with uncomplicated type 1 diabetes shows a significantly lower ability to correctly identify bitter and sour tastes compared to healthy controls (11).

However, other studies showed no significant differences in taste function between participants with uncomplicated type 1 diabetes and healthy subjects (12, 13). Furthermore, there are conflicting reports on the associations between taste impairment and metabolic control, disease duration, and the presence of diabetes-related complications (8, 12).

Therefore, in the present study, we evaluated taste perception in young individuals with type 1 diabetes and healthy controls. In participants with type 1 diabetes, we also investigated the possible influence of personal and disease characteristics (e.g., puberty, age at onset, onset with ketoacidosis, disease duration, etc.) on taste function.

MATERIALS AND METHODS

Subjects

We included 276 individuals with type 1 diabetes and 147 healthy controls.
Participants were recruited at Diabetes Units of IRCCS Burlo Garofolo (Trieste, Italy), Regina Margherita Children's Hospital (Torino, Italy), Santa Chiara Hospital (Trento, Italy), and UMC Ljubljana University Children's Hospital (Ljubljana, Slovenia) between October 2018 and December 2019.
Inclusion criteria were diagnosis of type 1 diabetes (14), age between 6 and 21 years, type 1 diabetes duration of at least 1 year. Participants with other types of diabetes (i.e., type 2 diabetes, monogenic diabetes, cystic fibrosis-related diabetes) were excluded.
Sex- and age-matched healthy controls were recruited from emergency department. They were not included if they had: type 1 or type 2 diabetes, obesity or other metabolic disorders, glycated hemoglobin (HbA1c) > 6% (>42 mmol/mol), family history of diabetes/obesity, as well as other diseases (e.g., respiratory infection) affecting smell or taste function. The ethics committee approved the protocol [CEUR-2018-Em-323-Burlo (Italy) and KME-0120-65/2019/4]. All participants and their parents (for participants aged < 18 years) gave written informed consent/assent prior to enrolment.

Personal and Clinical Data

In participants with type 1 diabetes, all data were collected during a follow-up visit (15). Personal information such as age, sex, and pubertal status was obtained. Puberty is defined as the presence of breast budding in girls and testicular volume of 4ml in boys (16). The following clinical measurements collected on the day of the taste analysis were available: blood glucose concentration, HbA1c, insulin daily requirement, disease duration, height, weight, and BMI. Moreover, the following data from the type 1 diabetes onset were collected: age, HbA1c, blood glucose concentration at admission, presence of ketoacidosis, and insulin daily requirement at discharge. In healthy controls, weight, height, and BMI were collected, and HbA1c was measured using DCA 2000 Analyzer System (Siemens, Munich, Germany).
In all participants, the standard deviation scores (SDS) of weight, height, and BMI were calculated according to WHO reference charts (17) using a software (Growth Calculator 3: http://www.weboriented.it/ghc3/).

Taste Evaluation

Using a filter paper method, we evaluated the capacity to recognize the following compounds: PROP (0.0085 g/ml), quinine (0.0024 g/ml), citric acid (0.165 g/ml), sucrose (0.2 g/ml) and sodium chloride (0.058 g/ml) (18). Specifically, after receiving instruction by an expert administrator, each participant was asked to rinse the mouth with bottled filtered water, place the paper on the tongue and recognize the correct taste among sweet, bitter, salt, sour (4-alternative forced choice). A possible choice was "I do not perceive any taste," and this answer was considered a missing value. A binary variable was used to define taste recognition for each compound: "yes" if the subject correctly identifies the compound and "no" if the subject does not correctly identify the compound.

Statistical Analysis

Descriptive statistics represent percentages, means, and standard deviations.
To evaluate taste recognition in type 1 diabetes participants and healthy controls, logistic regression analysis was performed. Gender, age, and standardized BMI were included as covariates in all models. Linear or logistic regression models with age, gender, standardized BMI, and disease duration were also performed in participants with type 1 diabetes to test the association between personal or clinical characteristics and taste recognition.

Glycemic control was evaluated grouping participants with type 1 diabetes based on HbA1c levels as follow: optimal control (OC) [HbA1c < 7.5% (<58 mmol/mol), given previously HbA1c target from ISPAD 2014], intermediate control (IC) [HbA1c 7.5– 8.5% (58–70 mmol/mol)], and poor control (PC) [HbA1c > 8.5% (>70 mmol/mol)] (19).
Statistical significance was set at a p-value ≤ 0.05. All statistical analyses were performed with R software (www.r-project.org).

RESULTS

Sample Characteristics
Sample characteristics of participants are reported in Table 1. No age and gender differences were found comparing healthy controls and type 1 diabetes participants. The age range was from 6 to 20 years with a mean and standard deviation of 12.3 ± 3.4 in controls and 12.8 ± 3.3 in type 1 diabetes subjects. A significant difference emerged among healthy subjects and participants with type 1 diabetes for standardized BMI (−0.36 ± 1.1 vs. 0.08 ± 1.1, p = 0.0001).
Mean HbA1c in participants with type 1 diabetes was 7.8 ± 1.1% - 62 ± 12 mmol/mol (range 5.5–13.4% - 37–123 mmol/mol); all healthy controls have A1c below 6%. 39.5% of type 1 diabetes individuals achieved OC, 37.5% IC and 23% PC. Of 276 type 1 diabetes participants, 76% were in puberty, and 43% presented ketoacidosis at the onset.

Taste Evaluation Among Participants with Type 1 Diabetes and Healthy Controls
Overall, 47% of participants with type 1 diabetes vs. 63.5% of healthy controls recognized all tastes (p = 0.0006) (Table 2). Moreover, PROP and citric acid recognition was less common in participants with type 1 diabetes (p = 0.014 and p = 0.003, respectively). No significant differences emerged for quinine and sodium chloride recognition. Since only six subjects (4 individuals with type 1 diabetes and two healthy controls) did not recognize sucrose, data on sucrose recognition were excluded.

Association of Taste Recognition with Personal and Health Parameters in Type 1 Participants
No significant differences in taste recognition were found among participants with type 1 diabetes divided by HbA1c values, and no significant effect of other tested variables (including puberty, ketoacidosis at onset, disease duration, median HbA1c over the last year) was detected.
Regression analysis showed that, irrespective of disease duration, earlier age at onset (defined as <6 years of age) was significantly associated with decreased overall taste recognition (p = 0.048), as well as PROP and quinine recognition (p = 0.027 and p = 0.015, respectively).
As reported in Table 3, the percentage of type 1 diabetes participants with type 1 diabetes recognizing tastes was lower among those with age at onset < 6 years.

DISCUSSION

In the present work, we compared taste recognition between young individuals with type 1 diabetes and healthy subjects. We identified significantly reduced overall taste perception, the bitterness of PROP, and the sour of citric acid in participants with type 1 diabetes. To date, findings on the relationship between type 1 diabetes and taste function are limited and controversial. Some studies report a difference in all four primary tastes (8, 10), others only in some taste qualities (11, 20), while others report no differences (12, 13). Our results substantiate findings that report an altered PROP and citric acid perception in individuals with type 1 diabetes (10, 11). However, we did not find differences in quinine and NaCl recognition, unlike past studies conducted in both adult (8, 10, 20) and pediatric participants (11). Age and clinical characteristics of the enrolled participants could

be among the reasons for the contradictory published results. Moreover, differences in the methods used to measure taste recognition across the studies may represent another possible confounder.
The mechanisms underlying taste dysfunction in diabetes are still unclear. Hyperglycemia has been indicated as one of the possible factors; it can induce a concentration-dependent impairment of taste perception (21), and long-standing hyperglycemia was associated with microvascular complications, such as neuropathy. In turn, peripheral nerve injury associated with neuropathy may involve lingual nerves, leading to gustatory impairments (8, 22). However, our data do not support this hypothesis in pediatric age with the mean disease duration of just over 5 years since HbA1c level at onset and at the time of the test was not associated with taste recognition.
Inflammation of oral mucosa may be another possible cause of the taste dysfunction observed in type 1 diabetes (23). As also described for dysgeusia associated with COVID-19 (24), inflammatory cytokines can trigger apoptosis and may cause abnormal turnover and loss in taste buds and ultimately the development of taste dysfunction.
Consistent with previous findings (12, 13), we did not find an association between taste perception and other type 1 diabetes characteristics (i.e., disease duration, puberty, ketoacidosis at onset, etc.). Otherwise, in the present work, we found an association between altered taste function and decreased age at onset, independently of diabetes duration. Although no guidelines consider the age of onset as a risk stratify, it could be a proxy for several important factors related to type 1 diabetes, such as variations in autoimmune mechanisms. For example, early-onset type 1 diabetes is associated with the presence of other autoimmune diseases, higher insulin antibodies value, lower initial insulin reservoir, and higher insulin requirements 1 year after diagnosis (25). In an extensive study of 27,195 individuals with type 1 diabetes, age at onset also presented a critical determinant of survival and cardiovascular outcomes (risk of coronary heart disease and acute myocardial infarction) (26). Therefore, early onset may be more harmful than late-onset disease, with the worst prognosis and higher risk of related complications (26). Additionally, children developing type 1 diabetes in early childhood are more likely to score relatively poorly on cognitive tests, independent of diabetes duration. Children with onset before the age of 7 years are found to have mild central brain atrophy and significant differences in intellectual performance in adulthood (27). Taste recognition is also thought to be associated with cognitive function, although studies have been focused on the elderly (28, 29). Based on this evidence, we can speculate that differences in cognitive ability may also contribute to taste recognition observed in type 1 diabetes.
This work has some limitations. Our study protocol precludes the possibility of evaluating the potential link between taste alteration and type 1 diabetes-associated complications. Moreover, the lack of information on inflammation data or cognitive function does not allow to confirm cited studies on the mechanism responsible for taste alteration. Furthermore, while most of the literature has focused on sweet taste impairment in diabetes (4, 10), we did not evaluate this taste modality. Moreover, we did not assess the possible role of other factors that may influence taste function, as well as genetic polymorphisms in taste receptor genes (30), oral microbiota (31) and hormonal fluctuation throughout the menstrual cycle (32). Despite these limitations, this study is, to our knowledge, the largest study so far documenting taste alteration in young individuals with type 1 diabetes.

In conclusion, taste impairment in individuals with type 1 diabetes was possibly related to age at onset. Further studies evaluating the actual mechanisms underlying taste changes in type 1 diabetes and its link with age at onset are warranted.

TABLES

TABLE 1 | Sample characteristics of type 1 diabetes subjects and healthy subjects.

	Healthy subjects ($n = 147$)	Type 1 diabetes subjects ($n = 276$)	*p*-value
Gender (% females)	55%	47%	0.14
Data at taste evaluation			
Age, years (mean ± sd)	12.3 ± 3.4	12.8 ± 3.3	0.15
Standardized BMI, SDS (mean ± sd)	−0.36 ± 1.1	0.08 ± 1.1	0.0001
Puberty (% yes)	61%	76%	0.13
HbA1c, % (mean ± sd)	5.5 ± 0.2	7.8 ± 1.1	$<2 \times 10^{-16}$
HbA1c, mmol/mol (mean ± sd)	37 ± 2	62 ± 12	
Glycemia, mg/dL (mean ± sd)	–	180 ± 88	–
Insulin daily requirement, U/kg/day (mean ± sd)	–	0.75 ± 0.25	–
Disease duration (years) (mean ± sd)	–	5.4 ± 3.6	–
Data at diabetes onset			
Age, years (mean ± sd)	–	7.5 ± 3.8	–
HbA1c, % (mean ± sd)	–	11.2 ± 2.3	–
HbA1c, mmol/mol (mean ± sd)		99 ± 24	
Glycemia at admission, mg/dL (mean ± sd)	–	457 ± 167	–
Ketoacidosis (% yes)	–	43%	–
Insulin daily requirement at discharge, U/kg/day (mean ± sd)	–	0.59 ± 0.29	–

As possible, significant differences among type 1 diabetes subjects and healthy subjects were assessed by T-test for quantitative variables and Chi-squared test for categorical variables.

TABLE 2 | Taste recognition in healthy subjects and type 1 diabetes subjects.

	Healthy subjects ($n = 147$)	Type 1 diabetes subjects ($n = 276$)	*p*-value* OR (CI)
Taste recognition (% yes)			
PROP	87%	77%	**0.014** 2.1 (1.2–3.9)
Citric acid	84%	72%	**0.003** 2.2 (1.3–3.7)
Quinine	75%	70%	0.244 1.4 (0.8–2.3)
NaCl	89%	85%	0.273 1.4 (0.7–2.7)
All	63.5%	47%	**0.0006** 2.1 (1.4–3.2)

"All" refers to an overall taste recognition in which subjects that correctly identify all compounds were compared to others.
**p-value from logistic regression analysis with sex, age, and standardized BMI as covariates.*
In bold are shown significant results.

TABLE 3 | Taste recognition accordingly age at onset.

	Age at onset of type 1 diabetes subjects		
	<6 years ($n = 100$)	≥6 years ($n = 174$)	*p*-value* OR (CI)
Taste recognition (% yes)			
PROP	65%	83%	**0.025** 3.4 (1.2–10.1)
Citric acid	68%	73%	0.144 2.1 (0.8–5.5)
Quinine	62%	74%	**0.015** 3.8 (1.3–11.6)
NaCl	82%	87%	0.275 0.5 (0.1–1.7)
All	38%	52%	**0.048** 2.3 (1.1–5.5)

**p-value from logistic regression analysis with sex, age, standardized BMI and disease duration as covariates.*

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CHAPTER VI - TYPE 1 DIABETES IN THE COVID-19 AGE

BACKGROUND

In this final chapter, I want to discuss the importance of glucose control also during a fragile situation like the COVID-19 pandemic. The idea of this study came during the first months of Sars-Cov-2 pandemic, where patients and families with a chronic disease were asked to stay home, unless in urgent need. For children and adolescents with type 1 diabetes happened the same, with a lot of in-person visit converted remotely (via videocall), but also with different appointment cancelled. Moreover, physical activity was strictly reduced, and school frequentation was suspended. From this, we imagined a large glucose decompensation, and we collected data from the Piedmont region to study the impact of lockdown in a single area.

Surprisingly, we observed an improvement of glucose metrics during the lockdown compared to the previous period, despite less physical activity, less leisure activities and less medical visits [50]. Our study has been recently included in a meta-analysis concluding similar results in what we observed in our population, warranting further investigation to inform future pediatric diabetes management [51]. What we speculated, from our observation, is the role of school, where glucose control is not the main target of teachers and educators, and from this observation there is actually a protocol between schools and our hospital to improve the way diabetes is managed in this setting.

Moreover, during COVID-19 pandemic we have learned that telemedicine may help in reaching patients when is impossible to have them in clinic. There is evidence that training for closed-loop onboarding was as effective as face-to-face training in achieving optimal blood glucose control, with a similar increase in Time In Range and a consistent improvement in Glucose Management Index and Time Above Range [52]. Overall, patients and families are overall very satisfied with the quality of the service offered through telemedicine, especially those living far from the hospital or using a technology (such as an insulin pump) [53]. Similarly, we observed a good adaptation from patients and families while at home, since medical team was available through e-mail or phone calls for those “in help”.

TITLE: IMPACT OF LOCKDOWN DURING COVID-19 EMERGENCY ON GLUCOSE METRICS OF CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES IN PIEDMONT, ITALY

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INTRODUCTION

From late December 2019, Severe Acute Respiratory Syndrome Coronavirus 2 has spread throughout the world, with Italy recording the first outbreak in Europe. To limit contagion, the Italian government implemented a national lockdown, which started in Piedmont (one of the most affected area in Italy) on February 23rd with schools. Complete lockdown, with sports and educational activities, started thereafter (9th of March 2020). Remote school lessons forced children and adolescents to stay home, with an overturned daily routine. Visits and scheduled checkups were canceled, further impacting physical and psychological health, with a potential negative outcome on

glycemic control and acute complications. In fact, from early reports, risk of Coronavirus Disease-19 (COVID-19) progression to severe disease was higher among adult with diabetes [1]. However, children and adolescents with type 1 diabetes mellitus (T1DM) do not show higher morbidity and mortality related to COVID-19 [2]. We aimed to determine the impact of lockdown on patients with T1DM using a continuous glucose monitoring (CGM), and the effect of remote consultations on glucose metrics.

SUBJECTS AND METHODS

We randomly selected children and adolescents (0–18 years) without comorbidities (such as celiac, thyroiditis, or other autoimmune diseases) with T1DM from at least 1 year, among patients followed in the four Centers of the Piedmont Pediatric Diabetes Network. Patients were randomly selected among those who had been using a CGM (Dexcom G5/G6) for at least 6 months. Selected patients were invited to participate through e-mail and were enrolled if informed consent was signed. Ethics approval was provided by a central institutional review board.
Time spent in range (TIR), (70–180 mg/dL, 3.9–10 mmol/L), below range (TBR) (< 70 mg/dL, < 3.9 mmol/L), above range (TAR) (> 180 mg/dL, > 10 mmol/L), as well as coefficient of variation (CV), sensor use, and glucose management index (GMI), were extracted during 90 days of lockdown (24th February 2020–24th May 2020) and compared with those 90 days before (25th November 2019–23rd February 2020). HbA1c values, measured at the last available visit before lockdown, were collected. Patients reported physical activity (hours per week) and total daily insulin dose (TDD) in the same study period, both before and during lockdown. Every patient was offered a remote visit and, if interested, was called back by the physician and obtained remote consultation via telephone. Primary end point was to evaluate changes in CGM metrics during lockdown in a cohort of children and adolescents with T1DM, compared to the previous period. Secondary end point was to evaluate remote consultation impact on glycemic control during lockdown. Demographic variables were summarized by mean ± standard deviation (SD) or percentage (%), and they were compared using a t test or a paired-sample t test for continuous variables, with an alpha value of 0.05. A multivariate regression was conducted to analyze relationship between TIR during lockdown with analyzed variables. Analyses were performed using SPSS ® statistical software (24th edition, IBM).

RESULTS

Of the patients invited to participate, 66 were enrolled. Mean age was 11.6 ± 4.5 years, subdivided in three age groups (8 children 0–6 years, 22 children 6–12 years, and 36 adolescents 12–18 years). The mean value of HbA1c was 7.2 ± 0.8% (53 ± 9 mmol/L) and BMI Z-score was 0.386 ± 1.13. Fifty-eight (88%) were carbohydrate counters, while 36 (55%) used an insulin pump (Table 1). No participant reported COVID-19 infection, diabetic ketoacidosis, or severe hypoglycemia during the study. Before lockdown, participants showed a mean glucose of 168 ± 61 mg/dL (9.3 ± 3.4 mmol/L), while during lock- down was 165 ± 58 mg/dL (9.2 ± 3.2 mmol/L) ($P < 0.05$). TIR increased from 59.7 ± 13% to 62.5 ± 14% ($P = 0.001$), while TAR decreased from 37.8 ± 14% to 35.2 ± 15% ($P = 0.004$), as shown in Fig. 1. No significant differences were detected for TBR (from 2.5 ± 2.3% to 2.3 ± 2.5%, $P = 0.177$) and GMI (from 7.5 ± 0.9% to 7.4 ± 0.8%, $P = 0.05$). CV decreased from 36 ± 5% to 35 ± 5% ($P = 0.003$). Physical activity spent per week reduced from 6.1 ± 3.3 h to 2.7 ± 3.1 h ($P < 0.001$), while TDD increased from 0.79 ± 0.25 UI/kg/day to 0.87 ± 0.31 UI/kg/day ($P = 0.004$). CGM use increased from 87 ± 17 to 92 ± 10% of time ($P = 0.006$). Multivariate regression about TIR during lockdown did not observe associations, except for HbA1c before lockdown (β −0.696).
We did not observe significant differences in TIR (61.5 ± 15% versus 63.7 ± 13%), TBR (2.1 ± 2.4% versus 2.5 ± 2.6%), TAR (36.4 ± 16% versus 33.8 ± 13%), and CV (35 ± 5 versus 35 ± 5%),

as well as TDD (0.85 ± 0.27 versus 0.89 ± 0.37 UI/kg/day) in patients who received remote consultations (n = 40) during lockdown.

DISCUSSION

Our study population was in good metabolic control, with roughly half of them using an insulin pump. Before lock- down, patients demonstrated a mean TIR of 59.7%, mean TBR of 2.5%, and mean TAR of 37.8%, approaching International Recommendation targets and similar to those observed in Italian children [3]. Enrolled subjects were physically active, with a mean of 6.1 h per week, which dropped during lockdown (−3.4 h/ week), probably leading to a higher TDD (around 0.1 UI/kg/ day). Despite this, patients did not demonstrate a worsening in CGM metrics, with a mild improvement of TIR (+ 2.8%), TAR (−2.6%), and CV (−1%) instead. A study has been recently published in a group of T1DM pediatric subjects, evaluating glucose metrics in a shorter period of time (2 weeks), with no deterioration or improvement of the glycemic balance [4].
We conclude that seeing the lockdown period as a whole, children and adolescents (and their caregivers) using a CGM were able to adjust insulin therapy despite less physical activity and a different lifestyle. On the other hand, the improvement in glucose metrics could be due to less stress related to school and diabetes management, as some authors also suggested [5]. Furthermore, in our observation, remote consultation was requested by 60% of patients but did not impact significantly glucose control.

CONCLUSION

Despite less physical activity, CGM metrics of children and adolescents with T1DM in the Piedmont Region did not worsen but, instead, slightly improved during lockdown due to COVID-19 emergency. Special attention should be paid to diabetes management at school for children and adolescents with T1DM.

TABLE AND FIGURE

Table 1 Characteristics of the study participants. Data are expressed as number ± SD unless otherwise indicated. BMI: Body Mass Index

	n = 66
Age (years)	11.6 ± 4.5
0 to < 6, *n (%)*	8 (12)
6 to < 12, n (%)	22 (33)
12 to < 18, n (%)	36 (55)
Sex, *n of males (%)*	46 (65)
BMI (kg/m^2)	18.6 ± 2.8
BMI Z-score	0.386 ± 1.13
Waist circumference (cm)	65 ± 8
Hip circumference (cm)	75 ± 12
Disease duration, (years)	4.5 ± 3
1 to < 2, *n (%)*	13 (20)
2 to < 5, *n (%)*	28 (43)
≥ 5, *n (%)*	24 (37)
HbA1c (%)	7.2 ± 0.8
HbA1c (mmol/mol)	55 ± 9
< 7% (53 mmol/mol), *n, %*	25 (38)
7% to < 7.5% (53 to < 58 mmol/mol), *n (%)*	18 (27)
7.5% to < 8% (58 to < 64 mmol/mol), *n (%)*	14 (21)
8% to < 10% (64 to < 86 mmol/mol), *n (%)*	9 (14)
Carbohydrate counters, *n (%)*	58 (88%)
Type of therapy, *n* of CSII (%)	36 (55%)

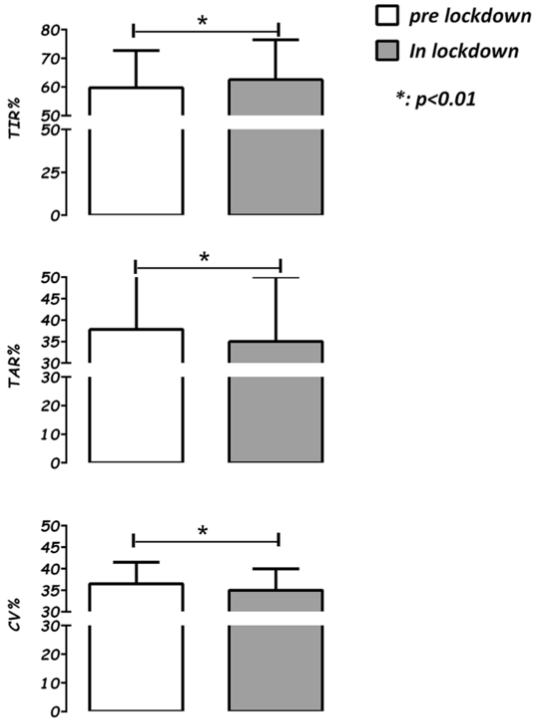

Fig. 1 Plots of time spent in range (TIR), above range (TAR) and coefficient of variation (CV) before (in white) and during lockdown (in grey)

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SUMMARY, PERSPECTIVES AND CONCLUSIVE REMARKS

In this collection of articles published during my Ph.D., from 2008 to date, there are some messages related to clinical management of type 1 diabetes in the pediatric age.

First of all, we still don't know the trigger which determine, in a predisposed subject, antibodies formation and β-cells destruction. During my Ph.D., with some colleagues in the University we tested two hypotheses about viral infectious (one from endogenous retroviruses and the other from Sar-Cov-2) on the development of T1D. These two studies, which are just a speculation about what we observed among our patients, may represent an advancement of what is the renowned "streetlight effect", where we are still wandering in the bright spot but the answer lies in the dark [54].

Secondly, Diabetic Keto-Acidosis (DKA) is still a major issue in type 1 diabetes (especially at the onset) and need to be addressed as a health problem, especially in children and adolescents. Measurement of pH, glycemia, bicarbonates and ketones are of utmost importance to diagnose and to properly manage DKA, as well as finding DKA comorbidities, such as acute kidney injury (AKI).

Thirdly, the use of technology (sensors, pumps, or both) gives better glucose outcomes, and should be offered to every child or adolescent with diabetes as soon as the patient can have a benefit from it, like the new guidelines from International Society for Pediatric and Adolescent Diabetes (ISPAD) states [55]. On the backside, technology bring some issues, such as skin problems, which need to be addressed and companies are actually studying new way to reduce allergic reaction (e.g. removing colophony from skin adhesives). Likewise, problem such as refusal to eat or drink in smaller children with T1D need to be addressed with specific protocols, using glucagon at a small dose, and we demonstrated this approach is effective and feasible.

Moreover, a new perspective on taste perception and diabetes was discussed, with new insight on the disease. Finally, we observed some surprising data about glucose outcomes during COVID-19 pandemic with some thoughts about school management.

As final remark, we are living in the era where pricking a finger and licking it afterwards (we called those people the "diabetes vampires") is beginning to be considered history, likewise the "dusk or dawn phenomena" which is no longer a problem thanks to the new insulin delivery algorithms. From this, comes the title of this thesis: "from dusk, till dawn". Which, apart from being a vampire movie of 1996 from Quentin Tarantino, is also for good auspice: from the dusk of a chronic autoimmune disease diagnosis, to a brighter dawn without diabetes.

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