Original article

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New-onset diabetes in children during the COVID-19 Pandemic: an assessment of biomarkers and psychosocial risk factors at play in Mississippi

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Purpose: The coronavirus disease 2019 (COVID-19) pandemic has led to an association between COVID-19 and pediatric diabetes. Studies have indicated the increased likelihood of children with COVID-19 infection developing diabetes. Our objective was to assess not only the increase in pediatric diabetes at our hospital and identify possible risk factors, but also to correlate the psychosocial changes resulting from the pandemic with new-onset diabetes.
Methods: We analyzed data from 58 children aged 1 to 18 years admitted to our hospital with new-onset diabetes between March 2020 and December 2021. The data included inflammatory biomarkers and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies (Abs), as well as the results of a lifestyle questionnaire.
Results: The average number of hospital admissions per month for new-onset diabetes increased from 10 to 18 with the start of the pandemic. Of the 58 children in our analysis, 33% had positive SARS-CoV-2 IgG Ab, 31% had type 1 diabetes mellitus, and 62% had type 2 diabetes mellitus (T2DM). More than half (54%) were experiencing diabetic ketoacidosis. Those with T2DM were older, majority African American, had higher median body mass index (BMI) percentiles, and lower vitamin D levels. There were no significant correlations between any psychosocial risk factors and either diabetes type or SARS-CoV2 Ab status.
Conclusion: Despite the increased incidence of new-onset diabetes among children in Mississippi during the pandemic, this study was unable to demonstrate a significant correlation between COVID-19 infection and new-onset diabetes. The findings of this study highlighted the correlation between increased BMI and type 2 diabetes, underscoring the significant problems of obesity and diabetes in our study region. Further research is warranted.

Keywords: Pediatric, Type 1 diabetes mellitus, Type 2 diabetes mellitus, COVID-19, SARS-CoV-2, Pediatric obesity

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Highlights

- This study found an increase in type 2 diabetes, especially in children with higher body mass index and lower vitamin D levels, but no direct link to coronavirus disease 2019 in Mississippi.

Introduction

Since the start of the coronavirus disease 2019 (COVID-19) pandemic, the incidence of pediatric diabetes has increased worldwide. This increase has been accompanied by an

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apparent rise in the frequency and severity of individuals presenting with diabetic ketoacidosis at the time of COVID-19 diagnosis.[1-3] Studies have indicated the possibility of an increased likelihood of children with COVID-19 developing diabetes in the 30 days postinfection compared to children without COVID-19.[4] One theory for this occurrence is the direct infection of pancreatic cells by the SARS-CoV-2 virus through binding to the angiotensin-converting enzyme 2 (ACE-2) receptor, which is highly expressed in pancreatic beta cells.5) During the previous SARS-CoV-1 (SARS) pandemic in 2003, this same mechanism was implicated in the development of acute diabetes,[5] as both SARS-CoV-1 and SARS-CoV-2 utilize the same ACE-2 receptor binding as a means of cellular infection.[6]

There remains a paucity of data regarding possible predisposing factors that affected the pediatric population during the pandemic, such as obesity, vitamin D deficiency, and behavioral changes that developed when children were mostly homebound owing to lockdowns and virtual learning. Much of the previous research in this area has been related to type 1 diabetes. However, studies have also shown an increase in childhood obesity during the pandemic, particularly among minority populations and those with low socioeconomic status.[7] It can thus be theorized that behavioral and social changes associated with pandemic lockdowns, such as increased sedentary activities and increased snacking, contributed to the rise in obesity, which may have led to an increased incidence of type 2 diabetes in children.

Obesity is an established risk factor for severe COVID-19, at least in part to the increased circulating leptin levels in obese patients.[8] Although we traditionally think of leptin as an appetite regulator, it also plays a significant role in immunity, with increased leptin levels stimulating the proliferation of multiple immune cells that produce proinflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), as well as acute phase reactants such as C-reactive protein (CRP).[8]

While the increased severity of COVID-19 has been observed in adults with diabetes,[9] extensive data regarding this issue is lacking for the pediatric population. Although researchers may be quick to extrapolate adult data to children, such comparisons are frequently not reliable.

Our objective in this study was to investigate increased rates of diabetes at our hospital during the pandemic and to identify possible risk factors. We also aimed to correlate psychosocial changes resulting from the pandemic, such as increased snacking and decreased physical activity, with new-onset diabetes during the given time period.

Materials and methods

1. Population and Enrollment

This study was based on a cohort of children admitted to the University of Mississippi Medical Center (UMMC). It included 405 patients aged 1 to 18 years with new-onset diabetes admitted between March 2020 and December 2021 and who were screened for eligibility. Exclusion criteria included age greater than 18 years or less than 1 year at the time of diagnosis, prior diagnosis of diabetes, no longer receiving care at the enrolling institution, and/or confirmed infection with SARS-CoV-2 after the diagnosis of diabetes. Study enrollment began in December 2020. The enrollment is outlined in Fig. 1.

For the 68 patients enrolled in the study, a general lifestyle survey was completed at the time of enrollment and a blood sample was collected by nursing staff. These samples were spun and stored (at 4℃). The samples were then deidentified and assigned a study ID number. They were then taken to the research lab where assays were performed for leptin, CRP, cytokines IL-1b, IL-6, TNF-α, SARS-CoV-2 IgM/IgG antibodies to receptor binding domain (RBD), nuclear protein (NP), and RBD surrogate neutralizing antibodies. The following were gathered from the electronic medical record: demographic data; vital signs at the time of admission, including BMI and BMI percentile (measurement used in pediatrics to compare a child's BMI to that of their age-matched peers; it was used here used because appropriate BMI ranges vary based on age for children ages 2 to 18); and other laboratory data, including hemoglobin A1c, liver function, lipid profile, and vitamin D levels. All study data were compiled and managed using Research Electronic Data Capture (REDCap) electronic data capture tools hosted at the University of Mississippi Medical Center.[10,11]

2. Laboratory methods

1) SARS-CoV2 antibody ELISA

To enable the assays to detect SARS-CoV-2-specific antibodies, 384-well Maxi-Sorp enzyme-linked immunosorbent assay (ELISA) plates (Thermo Fisher Scientific, Waltham, MA, USA). Nunc Cell Culture were coated with a recombinantly expressed protein at a concentration of 3 μg per mL and

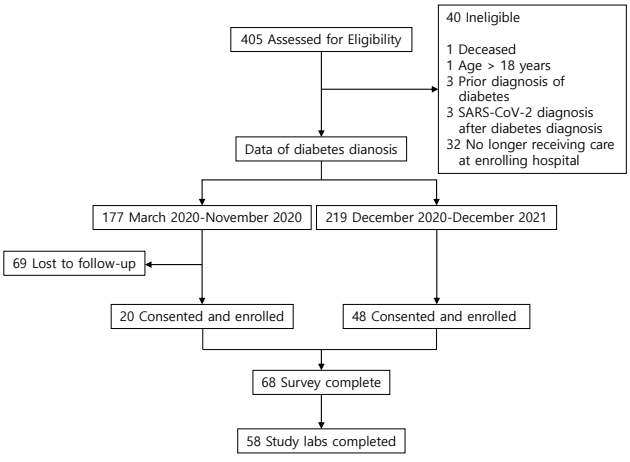

Fig. 1. Research subject selection and enrollment.

incubated overnight at 4℃. The plates were washed 4× with a BioTek 405TS plate washer and blocked with 1% powdered milk solution in phosphate-buffered saline (PBS) for 1 h at room temperature. After blocking, the samples were serially diluted in 1% powdered milk/PBS/Tween and applied to the plate. The plates were incubated for 2 hours at room temperature before washing 4×. Horseradish peroxidase (HRP)-conjugated anti-human IgG (Southern Biotech, Birmingham, AL, USA) was applied to the plates and incubated for 1 hour at room temperature. The plates were washed 6× and developed with tetramethyl benzidine (Sigma-Aldrich, St. Louis, MO, USA) for 30 minutes. The reaction was stopped by the addition of 2N H_2SO_4 and absorbance was read at 450 nm. The endpoint titer was calculated as the inverse of the dilution of the sample that produced an absorbance of 0.2 absorbance units above the background. All assays were performed in triplicate.

2) SARS-CoV-2 surrogate neutralizing assay

Titers of antibodies that block binding of the spike RBD with recombinant hACE2 protein were measured as a surrogate of virus-neutralizing activity as previously described (PMID 32704169). Reactions were performed in triplicate. Recombinant antigens for this assay and for the ELISA described above were transiently expressed in Expi293 (ThermoFisher) and purified via the passage of HiTrap HP columns (Cytiva, Marlborough, MA, USA). Recombinant RBD was conjugated to HRP using a HRP conjugation kit (Abcam, Cambridge, UK).

3) Multiplexed serum cytokine measurements

The cytokine level in serum was measured using a Bio-Plex Pro Human Cytokine Kit from Bio-Rad (Hercules, CA, USA) according to the manufacturer's instructions. Briefly, serum samples are added to beads labeled with cytokine-specific capture antibodies. The samples were incubated with the beads for 30 minutes while shaking. The beads were then washed and

Table 1. Demographic and laboratory features of children with and without antibodies (Ab) to SARS-CoV-2 at the time of hospitalization with new-onset diabetes mellitus, March 2020 to December 2021

Variable	No.	All (n=58)	SARS-CoV2 Ab negative (n=39)	SARS-CoV2 Ab positive[¶] (n=19)	*P*-value
Demographic variables					
Age (yr)	58	13.5 (11.0–15.0)	13.0 (10.5–14.0)	14.0 (11.5–15.0)	0.245
Male sex	58	33 (56.9)	24 (61.5)	9 (47.4)	0.459
African American race	58	45 (77.6)	32 (82.1)	13 (68.4)	0.318
Resident of county with above average poverty[†]	55	47 (85.5)	33 (89.2)	14 (77.8)	0.416
BMI percentile (percentile)	58	98.0 (89.4–99.4)	96.0 (84.8–99.3)	98.8 (95.1–99.6)	0.172
DKA on admission	58	31 (53.4)	26 (66.7)	5 (26.3)	0.009*
Diabetes type[‡]	54				0.99
Type 1		18 (33.3)	12 (34.3)	6 (31.6)	
Type 2		36 (66.7)	23 (65.7)	13 (68.4)	
Laboratory variables					
Hemoglobin A1c[§] (%)	58	12.9 (11.2–14.0)	13.0 (11.5–14.0)	11.7 (10.9–13.8)	0.226
Vitamin D[§] (ng/mL)	56	17.2 (12.8–25.3)	17.6 (12.8–23.4)	12.8 (12.8–25.8)	0.566
AST (IU/L)	45	30.0 (22.0–37.0)	29.5 (22.0–36.8)	31.0 (24.0–40.5)	0.210
ALT (IU/L)	45	16.0 (12.0–29.0)	15.5 (12.0–28.8)	23.0 (15.0–32.5)	0.117
Total cholesterol[§] (mg/dL)	55	172 (152–200)	175 (152–198)	169 (134–201)	0.853
LDL cholesterol[§] (mg/dL)	54	100 (84–128)	106.0 (85.5–138.0)	97.0 (67.5–114.0)	0.239
HDL cholesterol (mg/dL)	55	34.0 (27.5–44.0)	36.0 (28.8–43.0)	30.0 (26.5–46.0)	0.524
Triglycerides (mg/dL)	55	136 (87–212)	119 (81–205)	149 (110–222)	0.232
Leptin[§] (ng/mL)	58	12.2 (5.12–30.2)	9.30 (4.80–23.4)	14.1 (5.15–42.6)	0.308
CRP[§] (mg/L)	57	1.5 (0.4–5.5)	1.29 (0.37–5.13)	4.1 (0.6–7.1)	0.741
Cytokine IL-6[§] (pg/mL)	58	4.1 (1.4–8.3)	4.8 (1.4–10.1)	2.5 (1.5–4.2)	0.108

Values are presented as median (interquartile range) or number (%).
No. is the available sample size between the predictor and outcome variable.
SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; BMI, body mass index; DKA, diabetic ketoacidosis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CRP, C-reactive protein; IL-6, interleukin 6.
*$P<0.05$, statistically significant differences. [†]Defined as >20.3% of the population living below the poverty line based on income and family size. The cutoff was chosen at 20.3% because that is the percentage of people in Mississippi as a whole living in poverty). [‡]Defined as type 1 if any or all the following were positive: islet cell antibodies, antibodies to glutamic acid decarboxylase 65, and/or insulin autoantibodies. [§]For variables with laboratory values reported as > or <, such values were changed to the nearest defined value for the purpose of analysis (i.e., <12.5 = 12.5, >100 = 100). [¶]Positive defined as titer > 1:5000 to SARS-CoV-2 receptor binding domain IgG Ab and/or nuclear protein IgG Ab.

subsequently incubated with a biotinylated detection antibody for 10 minutes while shaking. Unbound detection antibody was washed away, and streptavidin-conjugated phycoerythrin was added to the beads before running the samples on a Bio-Plex MAGPIX instrument.

4) Leptin and CRP assays

Leptin levels were measured using a leptin ELISA kit from Bertin Bioreagent (Montigny-le-Bretonneux, France). Human CRP was measured using a Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

3. Statistical Analysis

Welch's *t*-test for independent samples was conducted to compare average monthly new diabetes admissions pre- and postpandemic. For continuous variables, differences in the data were tested using the Mann-Whitney *U*-test or the Kruskal-Wallis test. Categorical data were summarized as counts with relative frequencies as percentages. Differences in the groups were analyzed using the chi-square test or Fisher exact test (when the cell count was less than 5). Finally, binary logistic regression was used to assess the relationship between patient diabetes status and relevant predictors.

The predictors were chosen for the model based on the significance of the bivariate analysis (Table 1). First, an unadjusted model was carried out and the univariate odd ratios along a 95% confidence interval [CI] and *P*-value were calculated for age, race, and BMI percentile, as well as for leptin, CRP, and vitamin D levels. Finally, an adjusted model that included all the variables was used for analysis. A 2-sided *P*-value <0.05 was considered statistically significant for all analyses. All the statistical analyses were performed in R ver. 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

4. Ethical statement

This research was approved by the Institutional Review Board (IRB) of University of Mississippi Medical Center (IRB No. 2020-1268). Written informed consent was obtained from the participant's legal guardian prior to enrollment.

Table 2. Demographic and laboratory features of children hospitalized with new-onset type 1 and type 2 diabetes mellitus, March 2020 to December 2021

Variable	No.	All types (n=54)	Type 1 (n=18)	Type 2 (n=36)	*P*-vale
Demographic variables					
Age (yr)	54	14.0 (11.0–15.0)	10.5 (9.00–14.0)	14.0 (13.0–15.0)	0.004*
Male sex	54	30 (55.6)	8 (44.4)	22 (61.1)	0.384
African American race	54	42 (77.8)	8 (44.4)	34 (94.4)	<0.001*
Resident of county with above average poverty[†]	51	43 (84.3)	12 (75.0)	31 (88.6)	0.24
BMI percentile (percentile)	54	98.1 (89.4–99.4)	67.6 (30.7–94.4)	99.2 (97.3–99.6)	<0.001*
DKA on admission	54	29 (53.7)	10 (55.6)	19 (52.8)	0.99
Laboratory variables					
Hemoglobin A1c[‡] (%)	54	12.7 (11.2–14.0)	12.2 (10.4–14.0)	12.9 (11.5–14.0)	0.732
Vitamin D[‡] (ng/mL)	52	15.2 (12.8–23.0)	24.5 (18.4–32.6)	12.8 (12.8–18.4)	<0.001*
AST (IU/L)	41	30.0 (22.0–38.0)	25.5 (21.2–31.8)	33.0 (25.0–40.5)	0.076
ALT (IU/L)	41	19.0 (13.0–30.0)	15.5 (12.0–25.5)	22.0 (14.0–33.5)	0.177
Total cholesterol[‡] (mg/dL)	51	172 (152–200)	172 (153–191)	176 (148–225)	0.682
LDL cholesterol[‡] (mg/dL)	50	99.5 (84.0–127.0)	99.5 (84.2–111.0)	99.0 (84.0–138)	0.454
HDL cholesterol (mg/dL)	51	34.0 (27.5–45.5)	43.0 (33.0–47.0)	32.5 (27.0–39.0)	0.082
Triglycerides (mg/dL)	51	138 (100–227)	136 (81–228)	140 (106–219)	0.66
Leptin[‡] (ng/mL)	54	12.5 (5.12–31.5)	3.45 (2.05–7.63)	19.9 (10.8–44.3)	<0.001*
CRP[‡] (mg/L)	53	2.4 (0.4–5.5)	0.4 (0.1–1.0)	4.9 (1.0–17.2)	<0.001*
Cytokine IL-6[‡] (pg/mL)	54	4.1 (1.5–9.0)	4.2 (2.0–8.4)	4.0 (1.0–8.8)	0.645
SARS-CoV-2 Ab[§] (positive)	54	19 (35.2)	6 (33.3)	13 (36.1)	0.99

Values are presented as median (interquartile range) or number (%).
No. is the available sample size between the predictor and outcome variable.
Ab, antibody; BMI, body mass index; DKA, diabetic ketoacidosis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CRP, C-reactive protein; IL-6, interleukin 6; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
*P<0.05, statistically significant differences. [†]Defined as >20.3% of the population living below the poverty line based on income and family size. The cutoff was chosen at 20.3% because that is the percentage of people in Mississippi as a whole living in poverty). [‡]For variables with laboratory values reported as > or <, such values were changed to the nearest defined value for the purpose of analysis (i.e., <12.5 = 12.5, >100 = 100). [§]Positive defined as titer > 1:5000 to SARS-CoV-2 receptor binding domain IgG Ab and/or nuclear protein IgG Ab.

Results

Prior to the start of the COVID-19 pandemic in March 2020, an average of 10 pediatric admissions per month to the University of Mississippi Medical Center for new-onset diabetes were documented. With the start of the pandemic, this number increased to an average of 18 children per month admitted with new-onset diabetes. This figure represented a statistically significant increase in monthly new-onset diabetes admissions from March 2020 to December 2021 versus prior to March 2020 (18.4 vs. 10.3, $P<0.05$).

The study cohort (n=58) had a median age of 13.5 years (interquartile range [IQR], 11.0–15.0) and was 57% male and 78% African American. The median BMI percentile was 98.0 (IQR, 89.4–99.4), meaning that the subjects weighed more than 98% of children their age. A minority of the cohort lived in the Jackson metropolitan area (29.3%), where the hospital is located, and the majority (85%) of our cohort lived in counties with a higher-than-average percentage of the population living in poverty compared to the 20.3% population of Mississippi living in poverty. Approximately half of the patients were in DKA on presentation (53.4%).

The median hemoglobin A1C on presentation was 12.9% (IQR, 11.2%–14.0%) and the median vitamin D level was 17.2 ng/mL (IQR, 12.8–25.3 ng/mL). The median leptin level was 12.2 ng/mL (IQR, 5.12–30.2 ng/mL), the median CRP level was 1.56 mg/L (IQR, 0.38–5.49 mg/L), and the median cytokine IL-6 level was 4.12 pg/mL (IQR, 1.40–8.26 pg/mL).

Of the 68 patients enrolled in the study, 58 had SARS-CoV-2 antibody testing performed. Of those, 19 (33%) were positive (titer greater than 1:5,000) for IgG antibodies (Abs) to the SARS-CoV2 RBD and/or the SARS-CoV-2 NP, while 39 (67%) were negative for IgG antibodies to SARS-CoV2.

Table 1 compares the demographic characteristics and laboratory values of patients who were COVID Ab positive or negative, respectively. In our study, the patients who were COVID IgG Ab positive were less likely to be in DKA on presentation (26.3% vs. 66.7%, $P=0.009$). Those that were Ab positive tended to have higher leptin, CRP, and cytokine IL-6 levels. However, the differences were not statistically significant in this group.

Of the 58 patients who underwent laboratory testing 18 (31%) had type 1 diabetes, 36 (62%) had type 2 diabetes and 4 (7%) had insufficient data for classification. Patients were defined as having type 1 if any or all the following were positive: islet cell antibodies to glutamic acid decarboxylase 65, and/or insulin autoantibodies. Patients who were negative for all these antibodies were defined as having type 2 diabetes for the purposes of this study. This approach differed from other classification methods, where 2 or more of the antibodies had to be positive for a diagnosis of type 1 diabetes.

Table 2 compares the demographic characteristics and laboratory values of patients with type 1 and type 2 diabetes. The patients with type 2 diabetes were older (14.0 years vs. 10.5 years, $P=0.004$), more likely to be of African American race (94.4% vs. 44.4%, $P<0.001$), and had a higher BMI percentile (99.2% vs. 67.6 %, $P<0.001$). Those with type 2 diabetes had lower vitamin D levels (12.8 ng/mL vs. 24.5 ng/mL, $P<0.001$), higher leptin levels (19.9 ng/mL vs. 3.45 ng/mL, $P<0.001$), and higher CRP levels (4.9 mg/L vs. 0.40 mg/L, $P<0.001$) levels compared to those with type 1 diabetes. No statistically significant difference was evident in IL-6 levels or SARS-CoV-2 Ab status.

Table 3 shows the unadjusted and adjusted odds of developing type 2 diabetes. In the unadjusted analysis, older age, African American race, higher BMI percentile, and higher leptin levels were associated with higher odds of type 2 diabetes. After including all the variables in the same model, only an elevated BMI percentile was associated with increased odds of type 2 diabetes (adjusted odds ratio, 1.07; 95% CI, 1.02–1.17; $P=0.047$).

Based on the results of the survey completed by the study participants and their family members, the majority (39, 57.4%) of patients lived in a household of 2 to 4 members. In most families, the primary wage earner had not lost their job during the pandemic (49, 72.1%) and reported no increase in financial stress during the pandemic (38, 55.9%). Most participants reported that the local closures during the pandemic caused changes in their daily routine (40, 58.8%). One main concern of the study was to assess if the parents of the patients delayed seeking care once symptoms of diabetes arose owing to fear of contracting COVID-19, which they themselves did not have (15, 22.4%). The highest percentage of children reported spending 5 to 7 hours per day watching television or playing sedentary video games (27, 39.7%). Most of the families that agreed to

Table 3. Association between Type 2 diabetes and predictors using binomial logistic regression

Variable	Unadjusted MODEL			Adjusted MODEl		
	OR	95% CI	*P*-value	aOR	95% CI	*P*-value
Age	1.46	1.14–1.88	0.003*	1.30	0.89–2.06	0.202
African American race	18.7	3.39–155	<0.001*	7.42	0.64–112.27	0.111
BMI percentile	1.06	1.02–1.11	0.002*	1.07	1.02–1.17	0.047*
Leptin†	1.08	1.02–1.14	0.009*	0.99	0.92–1.07	0.806
CRP†	1.19	0.99–1.44	0.068	1.01	0.93–1.22	0.86
Vitamin D†	0.84	0.76–0.93	0.001*	0.87	0.71–1.00	0.123

OR, odd ratio; CI, confidence interval; aOR, adjusted OR.
*$P<0.05$, statistically significant differences. †For variables with laboratory values reported as > or <, such values were changed to the nearest defined value for the purpose of analysis (i.e., <12.5 = 12.5, >100 = 100).

participate in this study reported an increase in snacking in their household throughout the pandemic (50, 73.5%). Most children reported having been worried that they may get sick with COVID-19 (50, 74.6%). From the statistical analysis, no significant correlations were evident between any of the survey responses with regards to either diabetes type or SARS-CoV2 Ab status (Table 4).

Discussion

The primary contribution of this study was its attempt to find connections between biomarkers influenced by COVID-19 infection and new-onset diabetes, as well as correlations between pandemic-related psychosocial behaviors, BMI, and diabetes incidence. All of these factors are important in understanding how COVID-19 has and will continue to impact the health of children in Mississippi (the state with the second-highest rate of childhood obesity in the United State) and in implementing appropriate approaches to counseling children and families on lifestyle modifications that can decrease their risk of developing diabetes.

Although we investigated many biochemical markers, the

Table 4. Results of the survey administered to children/families to assess the psychosocial and behavioral impacts of the COVID-19 pandemic

Variable	By diabetes type					By COVID-19 status				
	No.	All types (n=54)	Type 1† (n=18)	Type 2 (n=36)	*P*-value	No.	All (n=58)	SARS-CoV2 Ab negative (n=39)	SARS-CoV2 Ab positive‡ (n=19)	*P*-value
How many people live with you?	54				0.384	58				0.180
≤4		30 (55.6)	8 (44.4)	22 (61.1)			34 (58.6)	20 (51.3)	14 (73.7)	
≥5		24 (44.4)	10 (55.6)	14 (38.9)			24 (41.4)	19 (48.7)	5 (26.3)	
Has the primary breadwinner lost his/her job during the pandemic?	54				0.751	58				0.99
No		40 (74.1)	14 (77.8)	26 (72.2)			42 (72.4)	28 (71.8)	14 (73.7)	
Yes		14 (25.9)	4 (22.2)	10 (27.8)			16 (27.6)	11 (28.2)	5 (26.3)	
Any increase in financial stressed during the pandemic?	54				0.923	58				0.992
No		28 (51.9)	10 (55.6)	18 (50.0)			32 (55.2)	21 (53.8)	11 (57.9)	
Yes		26 (48.1)	8 (44.4)	18 (50.0)			26 (44.8)	18 (46.2)	8 (42.1)	
Did local closures cause a change in your daily routine?	54				0.374	58				0.697
No		21 (38.9)	5 (27.8)	16 (44.4)			25 (43.1)	18 (46.2)	7 (36.8)	
Yes		33 (61.1)	13 (72.2)	20 (55.6)			33 (56.9)	21 (53.8)	12 (63.2)	
How many hours did you spend watching TV or playing video games (sedentary) during the pandemic?	54				1.000	58				0.502
≤4		12 (22.2)	4 (22.2)	8 (22.2)			12 (20.7)	7 (17.9)	5 (26.3)	
≥5		42 (77.8)	14 (77.8)	28 (77.8)			46 (79.3)	32 (82.1)	14 (73.7)	
Was there an increase in snacking?	54				0.512	58				0.871
No		14 (25.9)	6 (33.3)	8 (22.2)			16 (27.6)	10 (25.6)	6 (31.6)	
Yes		40 (74.1)	12 (66.7)	28 (77.8)			42 (72.4)	29 (74.4)	13 (68.4)	
Were you worried that you may get sick with COVID-19?	54				0.179	58				0.213
No		13 (24.1)	2 (11.1)	11 (30.6)			15 (25.9)	8 (20.5)	7 (36.8)	
Yes		41 (75.9)	16 (88.9)	25 (69.4)			43 (74.1)	31 (79.5)	12 (63.2)	
For parents - did you feel your child was getting sick but did not to bring them to the doctor because of the fear of COVID-19?	53				0.102	57				0.192
No		39 (73.6)	16 (88.9)	23 (65.7)			43 (75.4)	31 (81.6)	12 (63.2)	
Yes		14 (26.4)	2 (11.1)	12 (34.3)			14 (24.6)	7 (18.4)	7 (36.8)	

Values are presented as number (%).
No. is the available sample size between the predictor and outcome variable.
Ab, antibody; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
P-values are based on chi-square test (or Fisher exact test when the cell counts are below 5).
†Defined as type 1 if any or all the following were positive: islet cell antibodies, antibodies to glutamic acid decarboxylase 65, and/or insulin autoantibodies. ‡Positive defined as titer > 1:5000 to SARS-CoV-2 receptor binding domain IgG Ab and/or nuclear protein IgG Ab.

small sample size posed significant limitations. Therefore, the study demonstrated few significant differences in patient characteristics or laboratory data between those who were SARS-CoV-2 Ab positive, suggesting COVID-19 infection prior to diabetes onset, and those who were SARS-CoV-2 Ab negative. The only factor of statistical significance was that those patients with positive SARS-CoV-2 Ab appeared less likely to be in DKA when they presented with new-onset diabetes. This finding is contrary to those of multiple previous studies, which showed increased rates of DKA among children with new-onset diabetes of both types during the pandemic.[12,13] The time of diabetes diagnosis, the inability to use polymerase chain reaction (PCR) testing to identify COVID-19 infection, and the small sample size may have been the major limiting factors in our assessment. IgG antibodies to SARS-CoV-2 persist for at least 6 to 12 months postinfection, with titers decreasing over this time.[14] It is possible that a child could have had COVID-19 in previous months and had cleared antibodies prior to developing diabetes. Conversely, they may have been infected with COVID-19 at the time of developing diabetes and may have not yet mounted an antibody response. Given these factors, the exact timing of exposure/infection in relation to diabetes diagnosis could not be accurately determined for our patients based on the available data.

Although not statistically significant, several trends in the laboratory data merit discussion, as they may suggest factors that could prove significant with more intensive study. These include high leptin and CRP levels and low cytokine IL-6 levels in those patients who were SARS-CoV-2 Ab positive. As these findings were not statistically significant, further analysis was not conducted to ascertain if these increased levels may have been confounded by differences in BMI between the 2 groups (which would have been likely given that leptin levels are associated with increased BMI). However, prior studies with adults have shown increased leptin levels in patients with COVID-19 compared to their BMI-matched controls,[15] suggesting that leptin levels may be elevated in patients with COVID-19 independent of BMI. Given that leptin is involved in multiple proinflammatory pathways,[8] this suggestion is not surprising. Similarly, CRP is an acute phase reactant that is elevated during times of illness or inflammation. Thus, we would expect it to be elevated in patients with an active or recent history of COVID-19 infection. Interestingly, in this study, IL-6 levels tended to be lower in SARS-CoV-2 Ab-positive patients. This was likely on account of the insufficient study power, which conflicted with a large body of evidence in the literature showing elevated levels of IL-6 in critically ill patients with COVID-19. High IL-6 levels are strongly associated with severe COVID-19 and are consequently a target for multiple targeted drug therapies for COVID-19.[16]

The comparison of patients with type 1 versus type 2 diabetes in this study yielded more significant results. However, most of the results were merely supportive of previous findings. Those patients with type 2 diabetes were older, more likely to be African American, had higher BMI and leptin levels, and had lower vitamin D levels. These associations were consistent with previously demonstrated findings in the medical literature, with race and obesity being established risk factors for the development of type 2 diabetes.[17] Moreover, low vitamin D levels have been previously implicated in the elevation of proinflammatory adipokines (including leptin) and the development of obesity in children.[18] Given the complex interrelationship of these variables, it was no surprise that, when an analysis was conducted that controls for interactions, the only factor that remained independently associated with risk for type 2 diabetes was BMI.

We had hoped that the lifestyle survey given to patients at the time of enrollment would elucidate any COVID-19 pandemic-related lifestyle changes that may have impacted the increased levels of diabetes noted during the pandemic, particularly among the children that developed type 2 diabetes. However, no significant differences were evident between the survey results regarding either diabetes type or COVID-19 Ab status. This was likely mostly due to the underpowered nature of this study; however, it may also have occurred because some lifestyle changes during the pandemic, such as snacking and changes in daily routine, affected many of our study participants equally, regardless of diabetes type or COVID-19 infection status.

The primary limitation of this study was the small enrollment number, leading to a small sample size and an underpowered study. Several factors made it difficult to enroll patients. Among families with young children, there was reluctance to have the children undergo extra blood collection for a laboratory assay, as it could be difficult or traumatic for the child. Families of older children and teens frequently allowed the children's assent to determine enrollment in the study, which was appropriate. However, teens were frequently uninterested in enrolling given the lack of monetary incentives. Among the eligible study population, there was also a large subset of families who were distrustful of medicine or of clinical studies in general, and they did not want to participate in a study associated with COVID-19. Lastly, a significant proportion of eligible patients in the retrospective group were either lost to follow up or opted to follow up with their local providers instead of traveling to UMMC facilities. They were therefore unable to be approached for enrollment, which had to be conducted in person.

Another limitation of this study was the lack of financial support to include PCR testing of each patient for SARS-CoV-2 at the time of admission. Knowing the infection status at the time of admission, in addition to the antibody status of the patients, may have enabled us to identify further connections between acute infection versus more remote infection and the related biomarkers in the setting of new-onset diabetes.

Despite the notably increased levels of new-onset diabetes among children in Mississippi during the pandemic, this study was unable to demonstrate significant correlations between COVID-19 infection and demographic factors, laboratory findings, and pandemic-related behaviors in the children with new-onset diabetes included in our study. Although we noted some differences in characteristics between children with

type 1 versus type 2 diabetes in our study population, they appeared unrelated to COVID-19 in this sample size and had been previously described in the literature. Nevertheless, this study highlighted the strong correlation between increased BMI and type 2 diabetes. That finding emphasizes the major problem of obesity and diabetes in Mississippi. Future work in this area using a larger study population and stronger statistical power may more effectively elucidate the correlations between psychosocial factors and biomarkers relating to SARS-CoV-2 infection and the incidence of diabetes in children.

Notes

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Data availability: The data that support the findings of this study can be provided by the corresponding author upon reasonable request.

Author contribution: Conceptualization: JS, ND, SP, AS, AKT, PG, CK, FK; Data curation: JS, SP, AS, AKT, MKA, JTB, FK; Formal analysis: JTB, MAYA; Funding acquisition: ND, FK; Methodology: JS, ND, SP, AS, AKT, PG, CK, JTB, FK; Project administration: JS, ND, FK; Visualization: JS, ND, FK; Writing - original draft: JS, JTB, MAYA, FK; Writing - review & editing: JS, ND, SP, AS, PG, CK, JTB, MAYA, FK

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References

1. Gottesman BL, Yu J, Tanaka C, Longhurst CA, Kim JJ. Incidence of new-onset type 1 diabetes among US children during the COVID-19 global pandemic. JAMA Pediatr 2022;176:414-5.
2. Vlad A, Serban V, Timar R, Sima A, Botea V, Albai O, et al. Increased incidence of type 1 diabetes during the COVID-19 pandemic in Romanian children. Medicina (Kaunas) 2021;57:973.
3. Unsworth R, Wallace S, Oliver NS, Yeung S, Kshirsagar A, Naidu H, et al. New-onset type 1 diabetes in children during COVID-19: multicenter regional findings in the U.K. Diabetes Care 2020;43:e170-1.
4. Barrett CE, Koyama AK, Alvarez P, Chow W, Lundeen EA, Perrine CG, et al. Risk for newly diagnosed diabetes >30 days after SARS-CoV-2 infection among persons aged <18 years - United States, March 1, 2020-June 28, 2021. MMWR Morb Mortal Wkly Rep 2022;71:59-65.
5. Yang JK, Lin SS, Ji XJ, Guo LM. Binding of SARS coronavirus to its receptor damages islets and causes acute diabetes. Acta Diabetol 2010;47:193-9.
6. Magrone T, Magrone M, Jirillo E. Focus on receptors for coronaviruses with special reference to angiotensin-converting enzyme 2 as a potential drug target - a perspective. Endocr Metab Immune Disord Drug Targets 2020;20:807-11.
7. Jenssen BP, Kelly MK, Powell M, Bouchelle Z, Mayne SL, Fiks AG. COVID-19 and changes in child obesity. Pediatrics 2021;147:e2021050123.
8. Maurya R, Sebastian P, Namdeo M, Devender M, Gertler A. COVID-19 severity in obesity: leptin and inflammatory cytokine interplay in the link between high morbidity and mortality. Front Immunol 2021;12:649359.
9. Bode B, Garrett V, Messler J, McFarland R, Crowe J, Booth R, et al. Glycemic characteristics and clinical outcomes of COVID-19 patients hospitalized in the United States. J Diabetes Sci Technol 2020;14:813-21.
10. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 2009;42:377-81.
11. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap consortium: building an international community of software platform partners. J Biomed Inform 2019;95:103208.
12. Ho J, Rosolowsky E, Pacaud D, Huang C, Lemay JA, Brockman N, et al. Diabetic ketoacidosis at type 1 diabetes diagnosis in children during the COVID-19 pandemic. Pediatr Diabetes 2021;22:552-7.
13. Chambers MA, Mecham C, Arreola EV, Sinha M. Increase in the number of pediatric new-onset diabetes and diabetic ketoacidosis cases during the COVID-19 pandemic. Endocr Pract 2022;28:479-85.
14. Xiao K, Yang H, Liu B, Pang X, Du J, Liu M, et al. Antibodies can last for more than 1 year after SARS-CoV-2 infection: a follow-up study from survivors of COVID-19. Front Med (Lausanne) 2021;8:684864.
15. van der Voort PHJ, Moser J, Zandstra DF, Muller Kobold AC, Knoester M, Calkhoven CF, et al. Leptin levels in SARS-CoV-2 infection related respiratory failure: a cross-sectional study and a pathophysiological framework on the role of fat tissue. Heliyon 2020;6:e04696.
16. Jones SA, Hunter CA. Is IL-6 a key cytokine target for therapy in COVID-19? Nat Rev Immunol 2021;21:337-9.
17. Shaw J. Epidemiology of childhood type 2 diabetes and obesity. Pediatr Diabetes 2007;8 Suppl 9:7-15.
18. Zakharova I, Klimov L, Kuryaninova V, Nikitina I, Malyavskaya S, Dolbnya S, et al. Vitamin D insufficiency in overweight and obese children and adolescents. Front Endocrinol (Lausanne) 2019;10:103.