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A retrospective analysis of metabolic control in children with PKU in the COVID-19 era

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ABSTRACT

Background: Patients with phenylketonuria (PKU) must maintain a lifelong natural protein-restricted diet to prevent neuro-cognitive damage. Early diagnosis is established with newborn screening, with diet subsequently controlled by regular phenylalanine (Phe) monitoring. During the COVID-19 pandemic, significant lockdown measures were introduced that may have influenced the above.

Aim of our study: To establish whether the diagnosis was delayed in neonates during the pandemic. In addition, metabolic control was further assessed during the COVID-19 pandemic era (CE) compared to the same period a year prior (non-COVID-19 era, NCE). The lockdown periods (LD) were also compared with unrestricted periods (URP).

Patients, methods: Six neonates born during the CE and eight neonates born during NCE were included in the newborn screening analysis. Seventy-two classical PKU patients aged 2–18 years and categorized as children (2–12 years; 51 patients) and adolescents (>13 years; 21 patients) were included in the metabolic control analysis. The frequency of dried blood spot (DBS) sampling and Phe levels were assessed according to the different periods.

Results: There was no diagnostic or therapeutic delay in reaching the recommended Phe range in neonates born during CE compared to those born in NCE (median [interquartile range, IQR]: 23.5 [22.5–24] vs. 22 [18.0–27] days, p = NS). The cumulative DBS sampling frequency in children increased by 9.9% in the CE while no change was noted in the adolescent group. The median Phe level increased significantly in both age groups in the CE, but remained within the recommended target range. During CE, changes in Phe levels differed in the two age groups: children had the highest median Phe in the second lockdown period (LD2), while the adolescents had an increased Phe in URP.

There were significant negative correlations between DBS sampling frequencies and Phe levels in both age groups in NCE (children: r − 0.43, $p = 0.002$; adolescents $r = -0.37$, $p = 0.012$), and in adolescents in CE ($r = -0.62$, $p = 0.006$).

Conclusion: The pandemic did not impact newborn metabolic screening. The increased frequency of DBS sampling in CE and good target Phe levels suggest a better compliance in a very sensitive period. Since many factors may impact metabolic control in the different age groups, further studies are needed to analyse their respective role.

1. Background

Phenylketonuria (PKU, OMIM 261600) is an inherited metabolic disorder, described by Asbjorn Folling in 1934 [1]. It is caused by mutations in the gene encoding the hepatic enzyme phenylalanine hydroxylase (PAH, EC 1.14.16.1), resulting in accumulation of Phe and

Abbreviations: CE, COVID-19 era; CLD1, control to lockdown period 1; CLD2, control to lockdown period 2; COVID, coronavirus disease; CURP, control to unrestricted period; DBS, dried blood spot; IQR, interquartile range; LD, lockdown; MS/MS, tandem mass spectrometry; NCE, non-COVID-19 era; SARS, Severe Acute Respiratory Syndrome,; URP, unrestricted period.

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toxic metabolites in the blood and brain [2]. In undiagnosed or poorly controlled patients, PKU is characterised by mental retardation, epilepsy, as well as neuropsychologic and psychiatric repercussions [1–6]. The prevalence of PKU is approximately 1:8500 in Hungary [7].

Due to the implementation of neonatal screening programmes and early treatment, the psychomotor development of PKU patients has improved substantially. PKU is screened along with other 27 metabolic diseases in Hungary in the first 48–72 h of life using the tandem mass spectrometry (MS/MS) method. After the positive confirmatory test, the patients are managed by the metabolic centre. The cornerstone of therapy to date is a lifelong natural protein-restricted diet, supplemented with Phe-free medical formulas [8,9]. The patients' Phe levels are monitored by regular dried blood spot (DBS) self-sampling and clinical visits to a physician and dietician. The European guideline recommends target Phe concentrations of 120–360 μmol/l under 12 years of age and 120–600 μmol/l above this age [10].

Due to the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), many countries implemented quarantine measures ("lockdown", LD) to reduce the numbers of new cases of COVID-19 [11,12].

On March 11, 2020, a health emergency was declared in Hungary, after which a strict closure was introduced [13,14]. Restrictions were lifted on June 16th. Following another increase in the number of COVID-19 cases, the rules on partial closure were reintroduced on September 1st of the same year. As the spread decreased (partly due to the vaccination program), the restrictions were lifted on April 19, 2021.

The measures implemented during the lockdown periods differed. Initially, there was a total ban on leaving home and attending school. At the second closure, upper secondary education was conducted online and community programs were suspended.

Such COVID-19 lockdowns may impact the metabolic control of patients with chronic disorders. To the best of our knowledge, only three studies to date assessed metabolic control during the COVID-19 pandemic in patients with PKU [15]–[17].

2. Aims of the study

Our aim was to: 1) assess whether there was any delay in the diagnosis and early initiation of treatment in the PKU neonates who were born in the COVID-19 era (CE); (2) compare metabolic control during the CE (2020.03.11 to 2021.04.19) and a similar period of one year prior (2019.02.01 to 2020.03.10 (non-COVID-19 era, NCE); and 3) assess metabolic control in both lockdown periods (LD1: 2020.03.25–2020.06.16 and LD2: 2020.09.01–2021.04.19) vs. unrestricted period (URP, 2020.06.17–2020.08.31).

Given possible seasonal changes, metabolic control was also analysed in similar periods of NCE: 1) control period to LD1 (CLD1 2019.03.15–2019.06.16), 2) control period to LD2 (CLD2, 2019.06.17–2019.08.31) and 3) control period to URP (CURP, 2019.09.01–2020.03.10). Lastly, the correlations between frequency of DBS sampling and Phe levels were analysed.

3. Methods

For the current retrospective study, all PKU patients who were referred to or cared for during the studied periods were eligible.

Approximately 50% of the neonatal DBS samples in Hungary are analysed in our regional metabolic centre. For newborn screening, population-based cut-off values were) calculated at different percentiles. The whole data sets (approx. 650,000 newborn samples between 2011 and 2021) were systematically reviewed, and the statistical parameters calculated. All DBS samples that fulfilled pre-analytical and analytical requirements (i.e., sample collection, storage, transportation, pass quality control) were included. Phe levels above 179.64 uM (>99.98 pc) were considered abnormal. In order to reach the therapeutic target range, the day of the third Phe measurement within the target limit (120–360 μmol / l) was taken.

3.1. Ethical aspects

The study followed the principles of the guidelines of the World Medical Association Declaration of Helsinki of 1975. The study was approved by the institutional Ethics Committee (registration number: 36230–3/2018/EKU).

3.2. Inclusion and exclusion criteria

In the neonate study assessing an eventual therapeutic delay, patients were included with pathological Phe levels and completed the neonatal BH4 loading test during the CE (6 patients) vs. the NCE (8 patients). Patient enrolment for the neonatal study is summarized in Fig. 1. If Phe levels in the first DBS sample (taken between the first 48–72 h of life) was out of range, the patient was called to urgently send another DBS sample. If the Phe level was still elevated, the patient was admitted for 48 h to an infancy unit for a BH4-loading test after which a diet therapy was immediately started. After initiation of a low natural protein diet, the treatment goal was reached if three consecutive Phe levels were within the target range (120–360 umol/l).

In the second part analysing metabolic control, the following exclusion criteria were used: switch in therapy ($n = 3$), death ($n = 1$) and moved abroad ($n = 7$). The selection of the children is depicted in Fig. 2. Given the different recommended target Phe ranges for children and adolescents, patients were divided into two age subgroups: 2–12 years (51 patients) and 13 to 18 years (21 patients). Children younger than two years of age were excluded since a comparison of NCE and CE could not be made in these cases.

3.3. Data collection for Phe measurement

Phe levels were measured from DBS using MS/MS [18] (API2000; Perkin-Elmer Sciex, Toronto, ON, Canada).

In the second portion of the study, the frequency of DBS sampling and serum Phe concentrations were analysed according to the different study periods (as described in the aims of the study).

3.4. Statistical analysis

Data were tabulated using Microsoft Excel 2016 and analysed with the IBM SPSS 23 statistical software package. The normality of the data was tested by the Kolmogorov-Smirnov and Shapiro–Wilk tests, with $p > 0.05$ accepted as a normal distribution. Non-normally distributed data are reported as the median and interquartile range (IQR). Wilcoxon signed-rank tests were performed to assess related groups according to different time points, with level of significance set at $p < 0.05$. A Mann-Whitney test was performed for analysis of unrelated groups.

4. Results

4.1. Time needed to reach the therapeutic Phe range (neonatal study)

Six children were born with classical PKU during the CE. Data of eight classical PKU patients who were born during the NCE were analysed for the time required to reach the therapeutic Phe target range. Neonates born during CE necessitated a similar time to reach the therapeutic Phe range (median 25 days, IQR: 23.25–26) to that of patients born during NCE (median 23.5 days, IQR 22.5–24, $p = 0.59$). There was no clear trend between initial Phe level and days to reach the target metabolic range, although earlier confirmation of the diagnoses had some positive effect on reaching the aimed Phe range (Table 1).

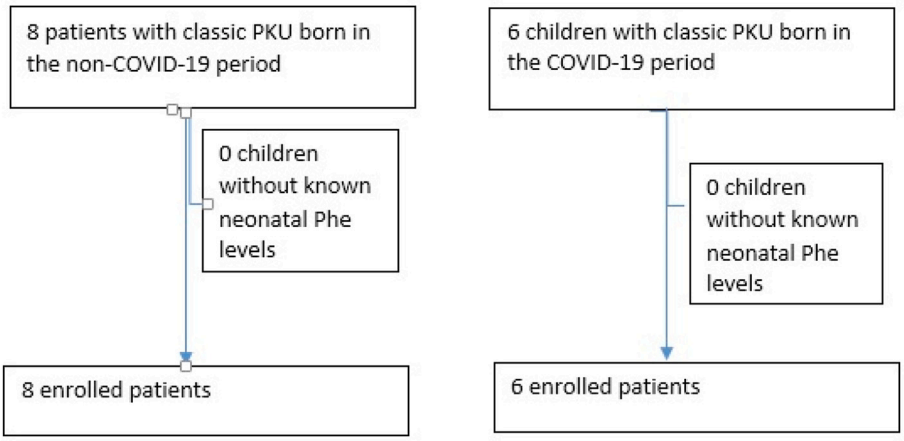

Fig. 1. The enrolled children of the neonatal study.

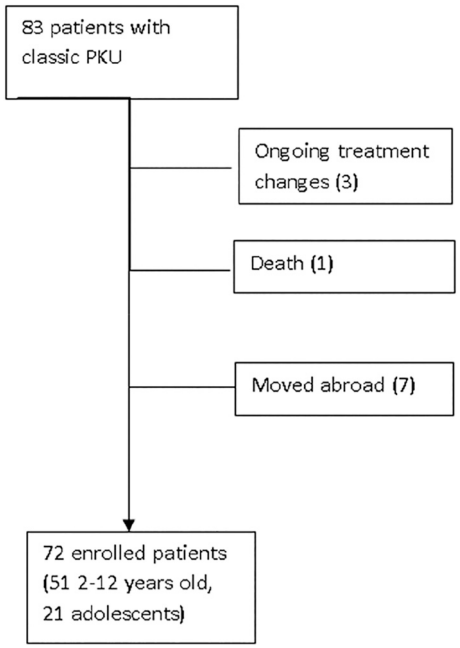

Fig. 2. Patient enrolment in the metabolic study.

4.2. Frequency of DBS sampling

Sampling frequency of the 51 children aged 2–12 years and 21 adolescents (13–18 years old) were analysed during a 13-month normal (NCE) and COVID-19 pandemic period (CE). The younger age group showed an increasing tendency in their cumulative and median DBS sampling frequency (9% vs. 15%) during the CE. Four percent of patients sent fewer samples, 35.2% sent the same number of samples, while 60.8% of the children sent more samples than during a similar unaffected period (Table 2).

In the older age group, the cumulative frequencies were similar in the two study periods. The incidence of DBS sampling remained relatively the same in most patients (40.9%), although two presented no samples at all. Eight (36.4%) adolescents sent more samples compared to the previous year and 5 (22.7%) decreased their frequency (Table 1) during the CE period. None of the differences were significant.

4.3. Phe levels: NCE vs. CE

The median Phe level significantly increased in both age groups (Table 3). The younger age group had a 5.3% higher Phe level during CE, with an even higher level (7.5%) in the adolescent group. Thirty of the 51 younger patients had Phe levels in the recommended range while 20 results were outside the range. One patient who did not complete Phe tests during NCE submitted 14 samples during the CE, each of these samples were within the reference range. Twelve of 21 adolescents

Table 1
Detailed values of the first DBS sample, as well as age in days of diagnosis confirmation and in reaching the therapeutic Phe range in the (studied) newborns. CE: COVID-19 era, DBS: dried blood spot, NCE: non-COVID-19 era.

Patient list	Diagnosis confirmed (age in days)	Therapeutic Phe range reached (age in days)	Phe level of the first DBS (umol/l)
Patients who were born in the CE ($n = 6$)			
P1	4	12	606.28
P2	16	23	615.83
P3	11	24	473.29
P4	13	26	544.85
P5	10	26	359.91
P6	20	33	260.77
Patients who were born in the NCE ($n = 8$)			
P1	5	19	606.28
P2	11	21	579.61
P3	19	23	699.48
P4	9	23	884.27
P5	13	24	600.11
P6	16	24	615.88
P7	10	24	359.91
P8	11	29	890.06

Table 2
DBS monitoring frequencies in children with classical PKU DBS: dried blood spot, NCE: non-COVID-19 era, IQR: interquartile range, CE: COVID-19 era.

DBS sampling frequencies in children		
Parameter	2–12 years old ($n = 51$)	>13 years old ($n = 21$)
Median DBS NCE [IQR]	17 [12.5–26.5]	11.5 [7–16.3]
Median DBS CE [IQR]	20 [11–28]	11.5 [8.8–14.5]
p value	0.158	0.917
Patients, with increased frequency (%)	31 (60.8%)	8 (36.4%)
Patients, with stable frequency (%)	18 (35.2%)	9 (40.9%)
Patients, with decreased frequency (%)	2 (4%)	5 (22.7%)

Table 3
DBS Phe levels (umol/l) of the studied patients according to age group.

Phe levels		
Parameters	2–12 years old (n = 51)	>13 years old (n = 21)
Median Phe [IQR] in NCE	321.3 [237.5–461.7 g	505.8 [377.2–659.7]
Median Phe [IQR] in CE	338.6 [247–453.1]	544.0 [438.5–724.8]
p value (NCE vs. CE)	0.036	0.009
Patients in rec. Phe range in NCE	30 (58.8%)	12 (57.1%)
Patients in rec. Phe range in CE	26 (51.0%)	11 (52.3%)
Patients out of the rec. Phe range in NCE	20 (39.2%)	7 (33.3%)
patients out of the rec. Phe range in CEP	25 (49.0%)	8 (38.1%)

Phe: phenylalanine, IQR: interquartile range, NCE: non-COVID-19 era, CE: COVID-19 era, rec.: recommended.

presented samples in the recommended range during NCE, while 7 had Phe values outside the range. In both periods studied, two patients did not submit DBS samples for Phe testing.

There was a decreasing tendency in the number of patients within the target Phe range in both age groups during CE.

4.4. Changes in Phe levels during CE (LDP vs. URP)

The Phe levels during the CE in the two age groups are presented in Fig. 3.

There was a considerable overlap between Phe values in each analysed time period. Median Phe levels remained in the normal range, although some minor differences could be observed. The median Phe levels varied differently in the two subgroups when separated according to the two lockdown periods based on the government's restrictions. The 2–12-year-old children had the highest median Phe (386.5 μmol/l) in the second lockdown (LD2) period with the strictest lockdown rules. LD2 Phe was significantly higher than the unrestricted period (URP) Phe level (312.2 μmol/l) in the younger group ($p = 0.001$), although did not differ from LD1 Phe (345.3 μmol/l, $p = 0.196$). LD1 and URP Phe levels were also similar ($p = 0.414$).

The adolescents' highest median Phe level (553.3 μmol/l) was measured in URP, without any lockdown regulations. It was significantly higher than the median LD1 Phe level (529.0, $p = 0.049$), but not significantly higher than the LD2 Phe level (525.1, $p = 0.8$). The Phe values were also examined in the corresponding NCE periods, although the differences were not significant.

4.5. Correlations of DBS sampling frequencies and median Phe levels

Correlations between DBS sampling frequencies and Phe levels were also analysed. There were significant negative correlations between the DBS frequencies and Phe levels in both age groups during NCE (children $r = -0.43$, $p = 0.002$; adolescents $r = -0.37$, $p = 0.012$), and in the adolescent group during CE ($r = -0.6$, $p = 0.006$).

5. Discussion

The COVID-19 pandemic has emerged as a new healthcare challenge in the routine follow-up care of patients with chronic illnesses [19–21] and PKU [15]–[17]. Patients were asked not to travel for regular outpatient visits and/or to choose telemedicine visits instead. This new context brought about many changes that can have both positive and negative effects on the effectiveness of newborn screening and the metabolic stability of the treated patients.

5.1. No delay in treatment

Our metabolic screening centre functioned without interruption during the COVID-19 pandemic. Postal delivery of DBS samples may have been an external limiting factor, although neither the postal service provider nor the patients indicated a delay. According to our results, PKU infants born during the COVID-19 period reached their therapeutic Phe range within the same timeframe as newborns born before the pandemic. This is consistent with reports from other countries [17]. However, no previous studies reported the necessary period to reach the therapeutic Phe range in neonates born during the COVID-19 era. To the best of our best knowledge, the current study is the first to compare the effectiveness of PKU screening in neonates born during the COVID era vs. neonates born pre-pandemic. It is also important to note) that there was no clear trend between the initial Phe level and the required days to reach the therapeutic range. This may also be due to the fact that the Phe target range for neonates is adjusted by titration of breast milk and Phe-free formula, tailored to the individual. Thus, in the case of very high Phe, e.g., infants initially fed by formula only, a marked decrease in Phe levels was observed in such instances. The earlier diagnosis appeared to have some positive effect on the achievement of the therapeutic level.

5.2. Frequencies of DBS sampling

Rovelli et al. reported an overall decrease (5.9%) in monitoring frequency during CE, although 39% of their patients sent more DBS samples during CE. Among those who increased their sampling frequency, 85% were older than 12 years. Whereas their study included data on adults, our study solely focused on patients aged 2–18 years old [15]. Herle et al. also reported that more school-aged patients sent fewer samples during CE, and patients $\geq$ 16 years sent significantly less DBS samples in 2020 [16].

During CE, our patients forwarded 1397 DBS samples, i.e. numerically 8% more than the previous year, although the increase was not significant. This indicates that our patients were well-prepared to collect blood samples at home, and that the vast majority (70 out of 72) did not neglect metabolic monitoring during the pandemic. Furthermore, we registered one child who began to send DBS samples during CE after three years without any Phe monitoring.

5.3. Changes in Phe levels during NCE vs. CE and the year of COVID-19

In Rovelli et al.'s study, the 4-12-year-old group had similar Phe levels in the COVID-19 era (median: 278.8 μmol/l) than the previous year (median 315.4 μmol/l, $p = 0.771$). However, in the older age group including adolescents and adults, the median Phe level decreased by 22.5% during the pandemic ($p < 0.0001$) [15]. In the present study, the median Phe level increased significantly in both age groups (5.4% and 7.5%) from NCE to CE; however, the median remained in the recommended range, and the number of patients out of target range did not change significantly.

In a study by Walkoviak et al., the median Phe levels during the lockdown and non-lockdown periods did not differ between different age groups, the latter of which were divided into three age subgroups (0–6 years, 7–12 years and 13–18 years) [17]. In our patients, the 2–12-year-old group had a significantly higher median Phe level in LD2 (with the strictest regulatory measures) than in the unrestricted period (URP) (368.8 umol/l vs. 281.8 umol/l, $p = 0{,}001$). In contrast, the 13–18-year-old group presented the highest median Phe level in the URP.

Metabolic control can be impacted by numerous factors such as reduction in physical activity [22–25] or changes in eating habits. Increased consumption of junk food and sweets, continuous snacking and a decreased consumption of fresh foods has also been highlighted [26,27]. Lopez-Moreno et al. reported an increase in emotional eating (the desire to consume a specific kind of food) and eating to compensate for boredom in adults during the pandemic [22]. Similar changes may also occur in childhood. Home isolation may further impact daily routine, such as shifting of sleeping time [24], with a possible effect on blood sampling and Phe levels (daily blood Phe levels typically vary by

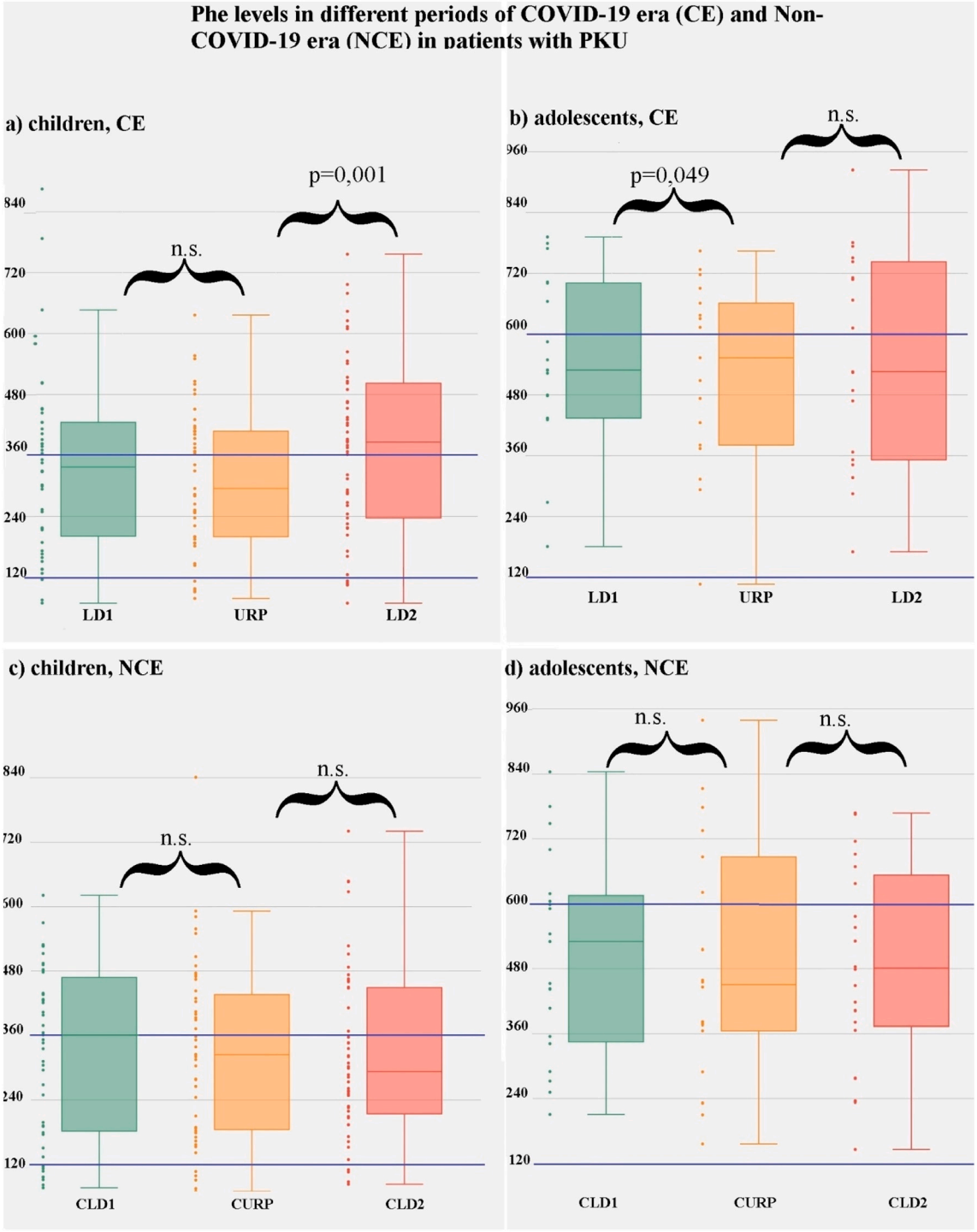

Fig. 3. Phe levels of PKU patients during CE and NCE. CE: COVID-19 era (2020/21), NCE: Non-COVID-19 era (2019/20). CLD1: control to lockdown period 1, CLD2: control to lockdown period 2, CURP: control to unrestricted period, LD1: lockdown period one, UPR: unrestricted period, LD2: lockdown period two. Blue horizontal lines indicate the recommended Phe ranges in PKU. n.s.: not significant. The single dots represent the median Phe levels of each patient.

≤50% in healthy subjects although the variation can be much higher in PKU patients [28]). Symptoms of anxiety, depression or ADHD exacerbation as well as other mental problems have also been reported by many authors [28]. The pandemic situation can also negatively impact the economic status of the families affected [29,30]. While the metabolic formulas (Phe-free protein substitutions) are supplied free of charge in Hungary, the low-Phe dietary products on the other hand are typically not reimbursed, expensive, and not readily available. Certain stores also had to change their opening hours and experienced delays in supply. All of these factors presumably played different roles in the observed two age-groups. Further studies are needed to assess the leading factor underpinning the changes in metabolic compliance in each age group. As stated in the European guidelines, various life events may necessitate a higher frequency of blood Phe testing and/or visits. If another COVID-19 lockout period should occur, a higher required DBS sampling may be recommended.

Similar periods of the NCE years were also analysed herein, namely CLD1: control to lockdown periods 1 and 2 (CLD1, CLD2) and control to

unrestricted period (CURP). There were no significant changes observed during these periods, in contrast to CE. Since no significant changes were observed in NCE, the differences between URP and LD periods during the pandemic period are likely independent of seasonal fluctuations and confirms the effect of the pandemic situation.

5.4. Correlations of DBS sampling with Phe levels

In both years examined, DBS sampling frequencies correlated negatively with Phe levels (more DBS samples resulted in better compliance). Thus, if a child or adolescent sends fewer DBS samples than before, closer monitoring and an outpatient visit may be recommended.

5.5. Limitations and strengths

Strengths: We conducted a single-centre study in the central Hungarian newborn screening and metabolic centre and included all available DBS Phe data of neonates and 2–18-year-old patients diagnosed with classical PKU respectively during the pandemic. Given potential seasonal fluctuations, we also compared similar periods of the preceding year prior to the pandemic (NCE).

6. Limitations

1) Despite the fact that we are a national screening centre, where more than half of Hungarian children with PKU are under our care, the current number of cases is still relatively low due to the low prevalence. The relatively small sample size can have a distorting effect on the results obtained.
2) The current study focused only on the effect of the pandemic on newborn screening and on metabolic control of patients with PKU. Therefore, the prevalence, effect and long-term consequences of COVID-19 disease with its psychological, physical and potential quality of life changes were not assessed.
3) Although our patients have not reported COVID-19 disease or long-COVID symptoms, the prevalence of COVID-19 disease was not formally assessed.
4) Our centre only monitors Phe on a regular basis from DBS, as this is a much lesser burden on the child. In reviewing the international literature, this can be considered routine. Plasma amino acid measurements are performed annually as advised by the current European guidelines [10]. During the COVID-19 era, in order to minimise human contact, outpatient visits were postponed if our PKU patients had no complaints and their Phe levels were in the recommended range. Due to these circumstances, a comparison of plasma Phe levels during CE and NCE was not possible.
5) The type and exact amount of low-protein diet products consumed were not recorded during the study.

7. Conclusion

Our PKU neonates born during the COVID-19 pandemic were effectively screened and there was no delay in the initiation and efficiency of dietary management. The median Phe level increased significantly in both studied age groups over the COVID-19 period, although still remained in the recommended range and the number of patients outside the range did not change significantly. In the COVID-19 era, the Phe levels changed differentially in the two age groups. Further studies are needed to analyse the role of the factors influencing compliance age-specifically. Significant negative correlations were found between the frequency of sent DBS samples and Phe levels in the examined two years, which underscore the importance of special attention in instances of decreased DBS frequency.

Author statement

DB, EK, ISz, JB, and PZs designed the project, providing clinical and scientific expertise about PKU. JB and PZs were in charge of overall direction and planning. DB, EK, ISz, JB and PZs contributed to the project administration. DB was involved in patient selection, data collection, interpreting the results and drafting the manuscript. EK, ISz, AA, GyR, AJSz, BJ and PZs critically reviewed and edited the manuscript. All authors discussed the results, read and approved to the final manuscript.

Financial disclosures

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Ethics approval

The study followed the principles of the guidelines in the World Medical Association Declaration of Helsinki of 1975. The local ethics committee approved the study (registration Manuscript File Click here to view linked References number: 36230/2018/EKU). All patients or their legally authorized representatives provided written informed consent before participation in the study

Declaration of Competing Interest

The authors have no conflict of interest to report.

Data availability

Data will be made available on request.

Acknowledgments

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