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Clinical and Biochemical Characterizations of Pediatric Patients with Urea Cycle Disorders in Upper Egypt: A Case- Control Study

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Abstract

Background: The diagnosis of inborn errors of metabolism is generally challenging. We aimed to explore various types of urea cycle disorders (UCDs), and their clinical presentations and biochemical findings among Egyptian pediatric patients.

Materials and Methods: This case-control study was conducted on 86 participants categorized into 43 pediatric patients suspected to have UCDs and 43 age- and sex-matched healthy controls, recruited from the Pediatric Outpatient Clinics, Inpatients Pediatric Departments, PICU and Neonatal Intensive Care Units of Qena, Assiut and Aswan University Hospitals, Egypt. In addition to the clinical assessments and routine laboratory investigations, colorimetric assays of blood lactate and ammonia, and plasma free amino acids assays using high performance liquid chromatography (HPLC), were performed for all included children. For patients with abnormal aminograms, the five enzymes of the urea cycle were measured in their liver tissue homogenates, using chemical methods.

Results: The results showed that 25 out of 43 suspected patients were confirmed to have UCDs. The most frequent type of UCDs was Ornithine transcarbamylase (OTC) deficiency (48%), followed by Argininosuccinate synthase (ASS) deficiency (36%) and the least frequent was arginase (ARG) deficiency (16%). The main clinical presentations were poor oral intake (100%), followed by lethargy (96%), hypotonia (68%), vomiting (64%), and hepatomegaly (48%). There were normal glucose and ABG values with significantly higher ammonia; lactate and the measured plasma free amino acids among patients with UCDs vs. the controls ($p<0.05$).

Conclusion: The most frequent types of UCDs among pediatric patients in Upper Egypt were OTC and ASS deficiencies. In addition to clinical suspicion, assays of lactate, ABG, glucose, ammonia and aminogram may be helpful biochemical tests in diagnosing UCDs.

Key Words: Argininosuccinate synthase, Hyperammonemia, Ornithine transcarbamylase, Arginase, Urea cycle disorders, Pediatric, Upper Egypt.

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1- INTRODUCTION

Urea cycle disorders (UCDs) are a set of rare inherited metabolic disorders that result in defects in urea cycle proteins which are responsible for removing excess ammonia from the body (1). The estimated overall incidence of UCDs in the United States and parts of Europe is 1:35 000 births, or approximately 113 new patients with UCDs per year (2). Hyperammonemia resulting from UCDs causes damage to the developing brain, with presenting symptoms such as posturing, cognitive impairment, seizures, and cerebral palsy (3, 4).

Within the Gastrointestinal (GI) tract, ammonia is a byproduct of protein digestion and bacterial metabolism. Within the kidney, ammonia is essential for the renal handling of acid. Ammonia in the venous system is metabolized to urea through the urea cycle. Several enzymes are required for the urea cycle, including the rate-limiting enzyme carbamyl phosphate synthetase (CPS), ornithine transcarbamylase (OTC), argininosuccinatesynthetase (ASS), argininosuccinic acid lyase (ASL), and arginase (ARG) (5). Variation of symptoms of UCDs depends on patient age and ammonia level. During the neonatal period, most affected neonates presented with non-specific symptoms such as (poor feeding, vomiting, somnolence, irritability, tachypnea, and lethargy) (6).

Clinical suspicion of hyperammonemia is considered the key step in diagnosis of urea cycle disorders. Once suspected, the next step used in evaluation is assaying of blood ammonia level. Other helpful laboratory investigations used in the diagnosis of UCDs include pH, CO_2, the anion gap and plasma amino acids. Increases or decreases in the intermediate amino-containing molecules arginine, citrulline, and argininosuccinate provide clues to the point of defect in the cycle (7). Since maps for inborn errors of metabolism in Upper Egypt are deficient, we aimed in the present study to identify the relative frequency of UCDs among pediatric patients and to establish the characterization of inherited hyperammonemia due to urea cycle disorders regarding the type and clinical presentations among such patients in Upper Egypt.

2- MATERIALS AND METHODS

2-1. Study design

The current case-control study was conducted on 43 pediatric patients, their ages ranged from three days to twelve years. The included patients were taken from the Pediatric Outpatient Clinics, Inpatients Pediatric Departments, Pediatric Intensive Care Unit and Neonatal Intensive Care Units (NICU) of Qena, Assiut and Aswan University Hospitals, Egypt, through the period from March 2017 to February 2018. This was in addition to 43 children who referred to Pediatric clinics and NICU for routine care and matched the patients in age and sex and served as control group.

2-2. Patients' selection criteria

Inclusion criteria included any pediatric patient presenting with any one or more of the following: lethargy, irritability, persistent vomiting, poor feeding, tachypnea with or without respiratory or cardiac manifestations, seizures and/or hepatomegaly together with elevated plasma ammonia level, normal arterial blood gases and normal blood glucose levels (8). Any pediatric patient who had the previous inclusion criteria but his or her parents refused to participate in the study, or had the previous inclusion criteria but was proved to have organic acidemias or mitochondrial diseases or other diagnosis, were excluded from the study.

2-3. Routine laboratory investigations

Routine investigation including complete blood count (CBC); arterial blood gases (ABG); random blood glucose; serum electrolytes; C- reactive protein (CRP); liver function tests including alanine transaminase (ALT), aspartate transaminase (AST), bilirubin (total and direct); kidney function tests (urea and creatinine), were performed for all participants included in the study.

2-4. Specific laboratory investigation

2-4-1. Blood assays

Six ml of venous blood was drawn from the included participants and was divided into two tubes: 3ml of blood was drawn on heparinized tubes and centrifuged at 3000 rpm for 15 minutes. The separated plasma from each tube was divided into aliquots, using 1 ml cryotubes for immediate lactate assays; while the remaining plasma samples were stored at -20°C till assays of the plasma free amino acids which were performed within 3 days of collection. The remaining 3 ml was drawn on EDTA tubes and centrifuged at 3000 rpm for 15 minutes. The separated plasma from each tube was divided into aliquots, using 1 ml cryotubes for immediate measurement of ammonia.

A) Lactate assay was done using commercially available colorimetric assay kit supplied by Spectrum Diagnostics, Cairo Egypt, Catalog No: 274001(9-11).

B) Plasma free amino acids (ornithine, citrulline, aspartate, and arginine amino acids) were measured using automatic amino acids analyzer (SYKAMS433, Germany, Catalog No: 1120001). This analyzer combines the classical method of ion exchange separation with derivatizationusing ninhydin with the modern technique of high liquid chromatography as described in previously published works (12, 13).

C) Ammonia was estimated using commercially available colorimetric assay kit supplied by Spectrum Diagnostics, Cairo Egypt, Catalog No. 220 001.

2-4-2. Liver biopsies and assays of urea cycle enzymes

Liver biopsies were approached only for pediatric patients suspected to have UCDs and had abnormal amino acid profiles according to the corresponding reference values. The biopsy samples underwent homogenization. The five enzymes of urea cycle (carbamyl phosphate synthetase-I, ornithine transcarbamylase, arginosuccinate synthase, arginosuccinase, and arginase) were measured in every sample using biochemical methods according to Brown and Cohen (14), and Schimke (15), at the Metabolic and Genetic Disorders Unit, Faculty of Medicine, Assiut University, Egypt (16), to confirm or exclude the diagnosis of inherited hyperammonemia and to determine the type of urea cycle defect.

2-5. Ethics approval and consent to participate

The study has been conducted in accordance with the Declaration of Helsinki and after approval of the Ethics Committees of Faculties of Medicine, South Valley, Assiut and Aswan Universities, Egypt. Written informed consents from the patients' parents were obtained before involvement.

2-6. Statistical analysis

Data was analyzed utilizing IBM SPSS Statistics for Windows version 20.0 (Armonk, NY: IBM Corp), and the data was tested for normality using Kolmogorov–Smirnov and Shapiro-Wilk tests. Data was presented as number, percentage, mean and standard deviation for parametric data and median and interquartile range for non-parametric data. Chi-square test ($\chi 2$) was used to study the comparison between two qualitative variables. The student's *t*-test was used for comparison between two groups having

quantitative variables with a normal distribution. ANOVA (f) test was used for comparison between three or more groups having quantitative variables that were normally distributed. A two-tailed test was considered significant when *P* was < 0.05.

3- RESULTS

3-1. Baseline characteristics of the study groups

The current study was conducted on 43 patients (24 males and 19 females) suspected to have inherited UCDs and 43 unrelated healthy controls (25 males and 18 females). The range of age of the total included participants was (3 days - 12.16 years) with non-significant differences between cases and controls regarding age and sex (p>0.05), indicating matching.

The main clinical presentations of the included 43 pediatric patients suspected of having UCDs were: poor oral intake (97.7%), followed by respiratory distress (60.5%), delayed physical development (60.5%), and delayed mental development (55.8%). Vomiting was also present in 51.2% of the total studied patients. Seizures were present in 48.8% of the total included patients. On clinical examination, (44.2%) of the included patients had hepatomegaly. The mean± SD plasma levels of ammonia (µg/dL) showed statistically significant higher levels (152.67 ± 69.47) when compared with controls (40.78 ± 10.78) (p<0.001). Their plasma lactate levels (mg/dL) showed statistically significant higher levels (22.83 ± 9.25) when compared with the controls (5.50 ± .95) (p <0.001).

Out of a total 43 clinically and laboratory suspected pediatric patients, 25 cases had been confirmed to have inherited UCDs, based on the abnormal aminograms and the low activities of some of the urea cycle enzymes that were measured in their liver tissue homogenates. Twelve cases (48%) were proved to have OTC deficiency, 9 cases (36%) have ASS deficiency, while the remaining 4 patients (16%) were proved to have ARGdeficiency (**Figure.1 and Figure.2)**.

The demographic data of these 25cases proved to have UCDs was presented in (**Table.1**). The frequency percentage of consanguineous marriage among the parents of the UCDs patients and history of sibling deaths were 76% and 64%, respectively, while among the included healthy controls were (4.6% and zero%, respectively). As regards the age at presentation (disease onset) of the included pediatric patients with UCDs, late onset (infancy and children) UCDs were more frequent (56%) than those presented with early (neonatal) onset UCDs (44%). Patients with OTC or ARG deficiency exhibited male predominance and frequently late presentation. ASS deficiency patients showed higher female frequency with frequently early (neonatal onset) manifestations (**Table.1**).

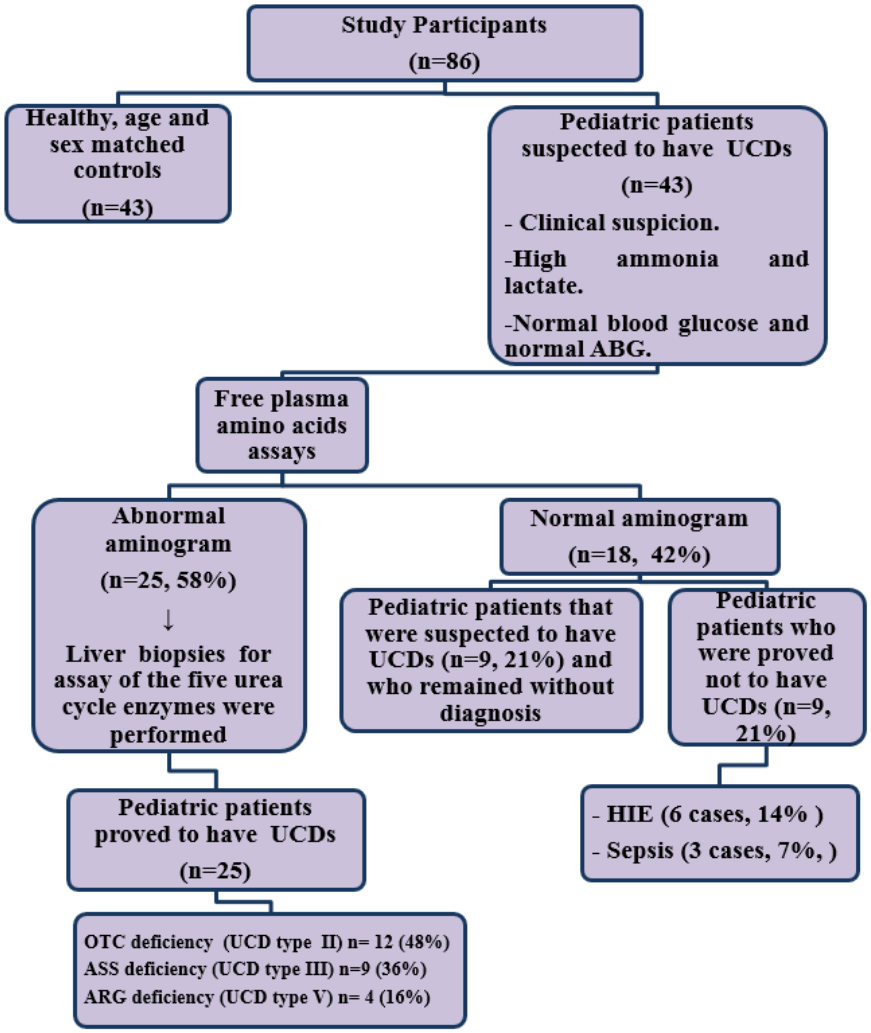

Fig.1: Algorithm of the study design and final diagnoses.

UCDs: Urea cycle disorders; OTC: OTC: Ornithine transcarbamylase; ASS: Argininosuccinate synthetase; ARG: Arginase; ABG: Arterial blood gases; HIE: Hypoxic ischemic encephalopathy.

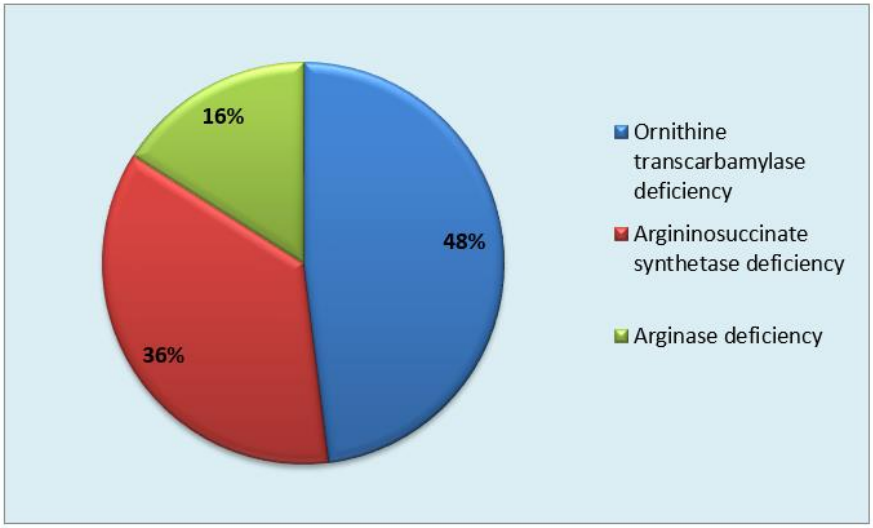

Fig.2: Frequency of different types of UCDs detected in pediatric patients in Upper Egypt.
UCDs: Urea cycle disorders.

Table-1: Demographic data of included pediatric patients with inherited urea cycle disorders.

Variables	OTC deficiency (n= 12)		ASS deficiency (n= 9)		ARG deficiency (n= 4)	
	No.	%	No.	%	No.	%
Pediatric age groups						
Neonates	3	25.0	5	55.6	0	0.0
Infants	6	50.0	0	0.0	0	0.0
Children	3	25.0	4	44.4	4	100.0
Age: Median (range)						
Neonate (day)	10 (5-12)		9 (3-29)		--	
Infants (Month)	9.5(1.5-12)		--		--	
Children (Year)	5.06(1.5-12.16)		3.04 (2.02-5.06)		3.54 (2.02-5.06)	
Gender						
Male	8	66.7	4	44.4	3	75.0
Female	4	33.3	5	55.6	1	25.0
Governorate						
Qena	4	33.3	1	11.1	1	25.0
Aswan	7	58.3	6	66.7	3	75.0
Assiut	1	8.3	2	22.2	0	0.0
Age onset at presentation						
Neonates	4	33.3	7	77.8	0	0.0
Infants	6	50.0	2	22.2	0	0.0
Children	2	16.7	0	0.0	4	100.0
Consanguinity	9	75.0	6	66.7	4	100.0
Sibling death	7	58.3	6	66.7	3	75.0

Abbreviations: OTC: Ornithine transcarbamylase; ASS: Argininosuccinate synthetase; ARG: Arginase.

3-2. Clinical data of the included pediatric patients with inherited UCDs

Concerning the clinical presentation of the included pediatric patients confirmed to have urea cycle defects, the most frequent clinical presentation of the UCDs patients was poor oral intake (100%), followed by lethargy (96%), hypotonia (68%), vomiting (64%), and delayed mental development (60%), and seizures (60%), respiratory distress (48%) and hepatomegaly was present in 48% of cases (**Table. 2**). According to the type of UCDs, lethargy (91.7%), delayed mental development (66.7%), and hypotonia (66.7%) were the most frequent manifestations among OTC deficiency cases. Among the ASS deficiency cases, lethargy (100%), respiratory distress and seizures were present in 66.7% of cases. While in ARG deficiency cases, lethargy (100%), vomiting, delayed physical and delayed mental development was observed incomprised (75%) of each case. Hepatomegaly was present in 75% of ARG deficiency, 44.4% of ASS deficiency and 41.7% of OTC deficiency cases (**Table. 2**).

Table-2: Clinical presentations of pediatric patients with inherited urea cycle disorders.

*Variables/ (No., %)	Total (n= 25)	OTC deficiency (n= 12)	ASS deficiency (n= 9)	ARG deficiency (n= 4)
Delayed development:				
Delayed physical development	14 (56)	7(58.3)	4(44.4)	3(75)
Delayed mental development	15(60)	8(66.7)	4(44.4)	3(75)
Poor oral intake	25(100)	12(100)	9(100)	4(100)
Vomiting	16(64)	9(75)	4(44.4)	3(75)
Respiratory distress	12(48)	6(50)	6(66.7)	2(50)
Seizures	15 (60)	7(58.3)	6(66.7)	2(50)
Lethargy	24(96)	11(91.7)	9(100)	4(100)
Hypotonia	17 (68)	8(66.7)	5(55.6)	4(100)
Hepatomegaly	12 (48)	5(41.7)	4(44.4)	3(75)

*Patient may have presented with more than one manifestation. Abbreviations: OTC: Ornithine transcarbamylase; ASS: Argininosuccinate synthetase; ARG: Arginase.

3-3. Biochemical findings of the studied pediatric patients with inherited UCDs and the controls

There were higher levels of alanine transaminase (ALT), aspartate transaminase (AST) among UCDs patients as regards normal references, with normal other routine investigations. Plasma levels of ammonia and lactate showed statistically significant higher levels among pediatric patients proved to have UCDs when compared with the controls, ($p<0.001$ for both) (**Table. 3**). Plasma levels of ornithine amino acid, citrulline, aspartate and arginine showed statistically significant higher levels among cases when compared with controls ($p <0.001$ for all) (**Table. 3**). Regarding the aminogram, plasma levels of ornithine showed statistically significant higher levels among OTC deficiency group when compared with ASS deficiency group ($p <0.05$), but with non-significant difference of the plasma levels of ornithine between ASS deficiency group and ARG deficiency group (**Table. 4**). Plasma levels of citrulline showed statistically significant higher levels among ASS deficiency group when compared with OTC deficiency group and ARG deficiency group (P-value was <0.001 and <0.05, respectively), but with non-significant difference between OTC deficiency group and ARG deficiency group (**Table. 4**). Plasma levels of arginine showed statistically significant higher levels among ARG deficiency patients when compared with OTC deficiency group ($p<0.05$), but no significant difference when compared with ASS deficiency group. OTC deficiency group and ASS deficiency group showed non-significant difference between each other regarding plasma arginine levels (**Table. 4**).

Table-3: Mean of various free plasma amino acids levels, ammonia and lactate among the included UCDs patients in comparison with the controls.

Measured biochemical parameters	Pediatric patients with UCDs (n= 25)	Controls (n= 43)	P-value
Ammonia(µg/dL)	176.26 ± 61.01	40.78 ± 10.78	0.000
Lactate(mg/dL)	21.14 ± 11.01	5.50 ± 0.95	0.000
Plasma Ornithine(µmol/L)	86.34 ± 21.67	40.78 ± 10.01	0.000
Plasma Citrulline (µmol/L)	39.24 ± 14.43	21.44 ± 5.28	0.000
Plasma Aspartate(µmol/L)	13.35 ± 4.13	5.00 ± 2.02	0.000
Plasma Arginine(µmol/L)	96.92 ± 35.07	53.56 ± 19.02	0.000

Abbreviations: UCDs: Urea cycle disorders.

Table-4: Mean and ranges of various free plasma amino acids involved in urea cycle according to type of UCDs.

Free plasma amino acid profile (µmol/L)	OTC deficiency (n= 12)	ASS deficiency (n= 9)	ARG deficiency (n= 4)	P-value[1]	P-value[2]	P-value[3]
Ornithine	101.78 ± 17.17 (80-125)	71.89 ± 12.02 (53-90)	72.51 ± 21.37 (47-93)	0.001	0.069	0.877
Citrulline	32.33 ± 9.94 (15-52)	54.89 ± 4.11 (50-61)	24.75 ± 7.37 (16-33)	0.000	0.202	0.04
Aspartate	12.40 ± 2.41 (9-15.94)	13.56 ± 5.61 (8-16)	15.73 ± 4.45 (12-21.9)	0.943	0.088	0.393
Arginine	85.11 ± 25.62 (30-107.82)	94.00 ± 33.81 (33-147)	138.89 ± 38.10 (105- 193.54)	0.619	0.011	0.076

P-value 1: OTC vs. ASS deficiency. P-value 2: OTC. Vs. ARG deficiency. P-value 3: ASS vs. ARG deficiency.
UCDs: Urea cycle disorders; OTC: Ornithine transcarbamylase; ASS: Argininosuccinate synthetase; ARG: Arginase.

3-4. Final diagnosis of the remaining included pediatric patients suspected to have UCDs

The remaining 18 out of a total 43 clinically and laboratory suspected cases exhibited normal aminograms and were diagnosed as follows: neonatal sepsis was diagnosed in 3 cases (7%), hypoxic ischemic encephalopathy (HIE) was diagnosed in 6 cases (14%), and 9 cases (21%) were undetermined diagnosis (which could be attributed to other undiagnosed metabolic disorders) (**Figure.1**).

4- DISCUSSION

There are relatively few reports of urea cycle disorders from Egypt. This research represents a summary of a significant effort to illustrate the demographic, clinical and biochemical characteristics of pediatric patients affected by inherited UCDs in Upper Egypt. Inborn errors of metabolism (IEMs) are considered relatively common disorders in some populations as Middle East and Arabic populations, this is mainly attributed to the high rates of parental consanguinity in the region (total

consanguinity up to 67% and first cousin consanguinity up to 49% in some Arab populations) (17). Demographic data of our study showed that the frequency percentage of consanguinity among the patients' parents was 76% and history of previous sibling deaths was encountered in 64% of the included patients who were proved to have UCDs, which was in line with Saleem et al. (18), and Abd El Sattaret al. (19), both studies reported on 50 and 40 patients respectively with inborn errors of metabolisms; a strong, statistically significant correlation between the consanguinity and the study group compared to control group.

Shawky et al. (20), revealed a high overall frequency of consanguinity in Egypt and attributed this high consanguinity rate to the fact that a lot of Egyptian families in the rural communities prefer marriage among first cousins to preserve family structure, links and provide social, economic and cultural benefits. Kumar et al. (21), reported that if there is a case history of a sibling's death of unknown cause within the first week of life, a pediatrician should suspect metabolic disorder. In many such instances, it is erroneously classified as sepsis. However, urea cycle defect could be a possible cause and leads to hyperammonemia and death. In the current study, history of previous sibling deaths was encountered in 64% of the included UCDs patients and this was in line with Shawky et al. (22). As regards different types of urea cycle disorders, the most frequent type was OTC deficiency followed by ASS deficiency and the least frequent one was ARG deficiency. Many studies were in line with our findings (23, 24). In disagreement with our findings, a study by Ibarra-González et al. (25), conducted on 37 patients in 33 families revealed that the most frequent type of UCDs was ASS deficiency (17 patients; 46%), followed by OTC deficiency (10 patients; 27%), ARG deficiency (7 patients; 19%), which could be explained by the geographic and regional differences and differences in the sample size. Concerning the age at presentation (disease onset) of the included pediatric patients with UCDs, late onset (infancy and children) UCDs were more frequent than those presented with early (neonatal) onset UCDs which was consistent with many research studies (23, 26). Patients with OTC or ARG deficiency exhibited male predominance and frequently late presentation. These were in agreement with many research studies (27-30). In accordance, a study was performed by Brassier et al. (31) on 90 patients with OTC deficiency proved that 53% of the patients were males, while 47% were females and 30% of patients had early-onset neonatal presentation while (58%) had late-onset presentation.

Conversely, ASS deficiency patients showed higher female frequency with frequently early (neonatal onset) manifestations which were in line with many studies (24, 26, 28). Our findings were in accordance with Waisbren et al. (30) who revealed higher female affectation (55%), but disagreed with the disease onset as they reported frequent late onset (73%) rather than the neonatal onset (27%). The studies which were in disagreement with our findings may be attributed to the smaller sample size of included patients in comparison with such studies. Regarding the frequency of the clinical presentations of the included UCDs patients, poor oral intake was present in all cases, followed by lethargy, hypotonia, vomiting, delayed mental development, seizures, respiratory distress and hepatomegaly. These findings were in accordance with Nettesheim et al. (32). Our findings revealed that cases with OTC or ARG deficiency exhibited frequently occurring neurological manifestations than gastrointestinal symptoms.These findings were in accordance with Huemer et al.

(29), and Brassier et al. (31).As regards the routine investigations that were done to the included patients with UCDs, complete blood counts, random blood glucose, serum electrolytes, renal function tests, C-reactive protein and arterial blood gases were within normal reference ranges except for mild increase in liver function tests. These data were in line with Martín-Hernández et al. (27). Any child with unexplained symptoms such as vomiting, lethargy, or other evidence of an encephalopathy should be investigated early for plasma ammonia level before any brain damage (33).Our results showed statistically significant high differences of plasma ammonia levels among the included UCDs patients. Iyer et al. (34) found that the hyperammonemia was considered to be the most likely consequence of UCDs which was in line with our results.

Regarding the plasma levels of various amino acids in the included UCDs cases, significantly higher plasma levels of ornithine were present among OTC deficiency group than ASS deficiency and ARG deficiency groups, while plasma levels of citrulline showed statistically significant higher levels among ASS deficiency group than OTC deficiency and ARG deficiency groups. Plasma levels of arginine showed statistically significant higher levels among ARG deficiency than OTC deficiency and ASS deficiency patients. These findings were in agreement with a study done by Ibarra-González et al. (25), Tuchman et al. (35), and Waisbren et al. (30). Martín-Hernández et al. (27) have examined specific urea cycle amino acids (citrulline and arginine), and reported that ASS deficiency had the highest level of citrulline and traces of it were found in OTC and ARG deficiencies; besides, arginine level was higher in ARG deficiency than ASS and OTC deficiency groups. In the present study, the final diagnoses of the total included patients were the urea cycle disorders which had the highest frequency followed by undetermined diagnosis which could be attributed to other undiagnosed metabolic disorders. This data was in accordance with Rao et al. (36), who reported that the most frequent diagnosis among pediatric patients with inherited hyperammonemia was UCDs followed by other metabolic disorders like organic acidemias. These findings were contrary to Multi et al. (37) who reported higher frequency of organic academia among those with congenital hyperammonemias and acidosis than UCDs, which could be attributed to the difference in the number and the patients' selection criteria. Inborn errors of metabolism may present in neonates with features of hypoxic ischemic encephalopathy (HIE).

Marked hyperammonemia due to hepatic dysfunction may occur in HIE in infants who have a clear history of perinatal distress. In UCDs, brain MRI in the sub-acute phase (3 to 7 days afterbirth) may resemble HIE. Damage to the cortex and underlying white matter, similar to ischemic injury, may be present in the chronic phase (38). Neonatal sepsis is the most common misdiagnosis in UCDs patients with early manifestations (39). This is in line with our study findings, where HIE and neonatal sepsis were among the other diagnoses of the remaining included patients suspected to have UCDs.

4-1. Study Limitations

Lack of genetic and molecular analysis, due to the high cost of such investigations, and the limited number of included individuals were the main study limitations.

5- CONCLUSION

OTC and ASS deficiencies are the most frequent types of UCDs among pediatric patients in Upper Egypt with high

frequency percentage of consanguineous marriage among the patients' parents. Poor oral intake, lethargy, hypotonia, vomiting, and delayed mental development, seizures and hepatomegaly were the most frequent clinical presentations of the UCDs patients. Together with clinical suspicion, high lactate, ammonia and amino acids involved in urea cycle with normal ABG and normal glucose levels are highly suggestive of UCDs diagnosis before the invasive liver biopsies used for enzymes assays.

6- ABBREVIATIONS

UCDs: Urea cycle disorders, OTC: Ornithine transcarbamylase; ASS: Argininosuccinatesynthetase); ARG (arginase); CPS (carbamyl phosphate synthetase; ASL: Argininosuccinic acid lyase; HPLC: High performance liquid chromatography; HIE: Hypoxic ischemic encephalopathy; CBC: Complete blood count; ABG: Arterial blood gases; CRP: C- reactive protein; ALT: Alanine transaminase; AST: Aspartate transaminase.

7- AUTHORS' CONTRIBUTIONS

Study concept and design: THS and MHH; Clinical evaluation of the cases: NIR, EAA and BH; Literature research: MHH, MEMA, DA-ES and NAE, Sample collections: MHH, DA-ES, EAA, BH, MEMA and NIR; Biochemical and laboratory assays: MHH and NAE; Data analysis: MHH, NAE, MEMA, MHH, DA-ES, NIR, EAA and BH; drafting the manuscript: MHH; All authors revised and approved the final version of the manuscript.

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9- CONFLICT OF INTEREST

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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