Original Article

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Comparison of 17-hydroxyprogesterone Levels in Infants with ELISA and LC-MS/MS Measurement Methods

İnfantlarda 17-hidroksiprogesteron Düzeyinin ELISA ve LC-MS/MS Ölçüm Yöntemleriyle Karşılaştırılması

Nazife DOĞAN, Alper GÜMÜŞ

University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital, Department of Medical Biochemistry, İstanbul, Türkiye

ABSTRACT

Objective: Although enzyme-linked immunosorbent assay (ELISA) is frequently used in the routine measurement of the steroid hormone 17-hydroxyprogesterone (17-OHP) level, there are studies on other methods. In our study, we compared the discordance of 17-OHP level in ELISA with liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods: 17-OHP level was serum samples up to 5 months of age at University of Health Sciences Türkiye, Başakşehir Çam and Sakura Hospital between May 1, 2022 and September 10, 2022. One hundred and thirty four sera had 17-OHP level above the reference range measured by ELISA, and 17-OHP level was measured with LC-MS/MS. After examining 17-OHP level in these sera with 2 different measurement methods, the obtained data were statistically analyzed using the SPSS 20.0 software package.

Results: 17-OHP level was detected above the reference range in 134 out of 134 sera (100%) by ELISA. 17-OHP level in 106 (79.1%) of the sera was detected within the reference range by LC-MS/MS. In statistical analysis, comparison of 17-OHP level with 2 different measurement methods was performed by chi-square test. A significant difference ($p<0.0001$) was observed in the 2x2 table in the chi-square test.

Conclusion: ELISA is widely used as a cost-effective method in measuring 17-OHP level, a steroid hormone. Differences that may occur due to the metabolism of 17-OHP in infants may lead to a false increase in ELISA. Due to this increase in routine, a more expensive and sensitive method, LC-MS/MS can also be used. In our study, we emphasized the importance of confirming falsely increased 17-OHP levels measured by ELISA with LC-MS/MS in infants younger than 3 months.

Keywords: Immunoassay, chromatography, infant

ÖZ

Amaç: Steroid hormon olan 17-hidroksiprogesteronun (17-OHP) düzeyinin rutin ölçümünde enzim bağlantılı immünosorbent ölçümü (ELISA) sıklıkla kullanılsa da başka yöntemler üzerinde çalışmalar vardır. Çalışmamızda ELISA'daki 17-OHP'nin ölçüm belirsizliğini sıvı kromatografi kütle spektrometrisi (LC-MS/MS) ile karşılaştırdık.

Yöntemler: 17-OHP düzeyleri, 1 Mayıs 2022 ile 10 Eylül 2022 tarihleri arasında Sağlık Bilimleri Üniversitesi, Başakşehir Çam ve Sakura Hastanesi'nde 0-5 aylık infantların serumunda ölçüldü. 17-OHP düzeyi ELISA ile referans aralığının üzerinde saptanan 134 serumda17-OHP düzeyi LC-MS/MS ile ölçüldü. Bu serumlarda 17-OHP düzeyleri 2 farklı ölçüm yöntemi ile incelendikten sonra elde edilen verilerin SPSS 20.0 yazılım paketi kullanılarak istatistiksel analizi yapıldı.

Bulgular: 17-OHP düzeyini ELISA ile 134 serumun 134'ünde (%100) referans aralığının üzerinde tespit ettik. Aynı örneklerin 106'sında (%79,1) 17-OHP düzeyi LC-MS/MS ile referans aralığında tespit edildi. İstatistiksel analizde 17-OHP'nin 2 farklı ölçüm yöntemi ile karşılaştırılması ki-kare testi ile yapıldı. Ki-kare testinde 2x2 tablosundaki p-değeri için anlamlı bir fark ($p<0,0001$) gözlendi.

Sonuç: Steroid yapıdaki hormonlardan 17-OHP'nin düzeyinin ölçümünde ELISA maliyet-etkin bir yöntem olarak yaygın kullanılmaktadır. İnfantlarda 17-OHP'nin metabolizmasına bağlı oluşabilecek farklılıklar ELISA'da yalancı artışa yol açabilmektedir. Rutindeki bu artmış kullanım nedeniyle daha pahalı ve hassas LC-MS/MS yöntemi de kullanılabilir. Çalışmamızda 3 aydan küçük infantlarda ELISA ile yalancı artmış 17-OHP düzeylerinin LC-MS/MS ile doğrulanmasının önemini vurguladık.

Anahtar Kelimeler: İmmün ölçüm, kromatografi, infant

Address for Correspondence: Nazife Doğan MD, University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital, Department of Medical Biochemistry, İstanbul, Türkiye
E-mail: drnazifedogan@gmail.com
ORCID IDs of the authors: N.D.: 0009-0008-6737-0416, A.G.: 0000-0002-4453-6339

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Introduction

Steroid hormones play crucial roles in processes such as differentiation, development, growth, and various physiological functions in living organisms. These hormones are structurally characterized by the presence of a cyclopentanoperhydrophenanthrene ring. During circulation, steroid hormones bind with high affinity to specific transport proteins, whereas they bind more loosely to non-specific carriers such as albumin. Steroid hormones are synthesized in various tissues including the adrenal cortex, gonads, and placenta. The initial step in their biosynthesis involves the enzymatic conversion of cholesterol to pregnenolone (1).

In the zona fasciculata and zona reticularis of the adrenal cortex, 17 alpha-hydroxylase/17,20-lyase enzyme systems located in the endoplasmic reticulum convert pregnenolone to 17-hydroxypregnenolone. In the zona fasciculata, 3 beta-hydroxysteroid dehydrogenase (3β-HSD) catalyzes the conversion of 17-hydroxypregnenolone to 17-hydroxyprogesterone (17-OHP). This intermediate is subsequently converted into 11-deoxycortisol by the enzyme 21-hydroxylase (21-OH). Finally, 11 beta-hydroxylase, a mitochondrial enzyme, catalyzes the formation of cortisol from 11-deoxycortisol (1,2).

During fetal life and the early postnatal period, serum 17-OHP levels are elevated. These levels gradually decline in both sexes until puberty. 17-OHP secretion exhibits a diurnal rhythm, with peak levels occurring in the early morning hours. Additionally, ovarian secretion of 17-OHP increases during the luteal phase of the menstrual cycle. The most common pathological cause of elevated 17-OHP levels is 21-OH deficiency, which underlies most cases of congenital adrenal hyperplasia (CAH). Therefore, 17-OHP measurement is essential in the diagnosis and monitoring of CAH and other disorders involving mineralocorticoid and androgen synthesis (2,3) (Figure 1).

In cases of CAH, which is an autosomal recessive disease group, genetic 46,XX and complete androgen insensitivity 46,XY can be observed. With an incidence of approximately 1 in 15,000 live births and a carrier rate of 1 in 60, CAH is the most common cause of ambiguous external genitalia in neonates. 21-OH deficiency accounts for 90-95% of CAH cases (4). Depending on the degree of enzymatic deficiency, CAH is classified into classical and non-classical forms. The classical form includes the salt-wasting and simple virilizing types. The salt-wasting form may present with hypovolemia, shock, and death, whereas adrenal crisis is not typically observed in the simple virilizing type. In contrast, the non-classical form often presents with mild symptoms and may not be recognized until later in life. Accurate classification of CAH subtypes is crucial for prenatal diagnosis and therapeutic decision-making. In newborn screening (NBS) programs, 17-OHP levels are measured in heel-prick blood samples. This screening is particularly valuable for early diagnosis in salt-wasting males, non-symptomatic males, and severely virilized females who may be misidentified as male at birth (5).

Elevated 17-OHP levels are also observed in 11 beta-hydroxylase and 3β-HSD deficiencies (2,3). However, transient elevations of 17-OHP can occur in preterm infants, those with low birth weight, neonatal jaundice, neonatal stress, due to cross-reactivity with conjugated steroid metabolites, low T4 levels, or antenatal corticosteroid exposure. Accurate interpretation of these transient elevations is essential to prevent misdiagnosis and unnecessary treatment (6).

Measurement of 17-OHP level can be performed by radioimmunoassay (RIA) and high-performance liquid chromatography (HPLC) after extraction from serum or plasma. In liquid chromatography-tandem mass spectrometry (LC-MS/MS), extraction is performed with the addition of an internal standard such as d8-17-OHP. In enzyme-linked immunosorbent assay (ELISA), enzymes like alkaline phosphatase or horseradish

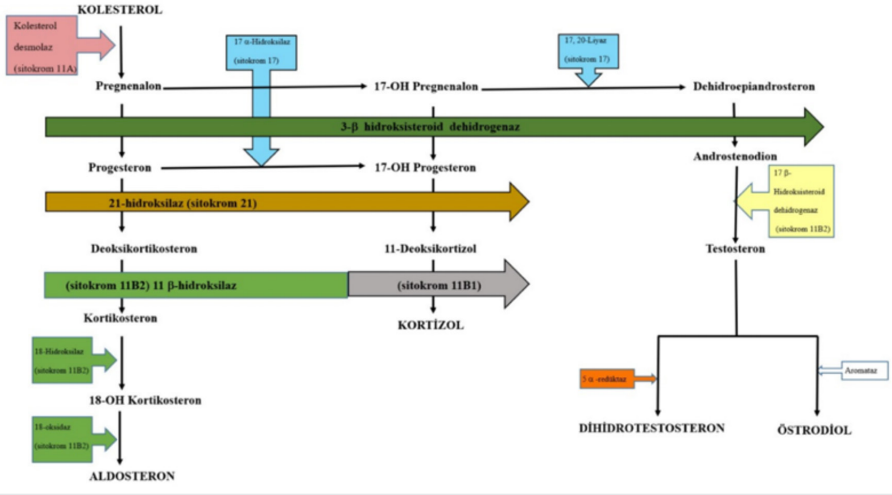


Figure 1. Adrenal cortical hormone biosynthesis pathway

OH: Hydroxy progesterone

peroxidase bind to specific antigen-antibody complexes and convert substrates into colored products, allowing quantification of 17-OHP in serum or plasma. Although immunoassays such as ELISA rely on highly specific antigen-antibody interactions, cross-reactivity with structurally similar compounds may still occur (7-10).

The reliability of 17-OHP measurements in biochemical laboratories is influenced by pre-analytical, analytical, and post-analytical factors. Variations in sample handling procedures and differences between analytical methods may affect measurement outcomes. A comprehensive interpretation that considers potential interferences along with the demographic and clinical context of the patient is essential. Final result approval should be conducted by a clinical biochemist as part of the post-analytical review process to ensure accurate reporting (11).

Methods

In our hospital, 17-OHP levels in serum were analyzed using an immunoassay method (Diameter kit, Alisei ELISA Analyzer) in a total of 1,013 patients between May 1, 2022, and September 10, 2022. Among these, 134 infants under the age of 5 months who had 17-OHP results exceeding the age-specific reference range were re-evaluated using a different method and analytical system: the LC-MS/MS method (Agilent 6460 Triple Quadrupole Mass Spectrometer).

The LC-MS/MS enables the separation and quantification of 17-OHP from complex biological matrices. The process begins with the extraction of 17-OHP from serum using an appropriate solvent system. In this study, d8-17-OHP was added as an internal standard to each sample prior to extraction in order to correct for variability during the analytical process and to ensure accurate quantification (12). Following extraction, the samples were liquid chromatographic system, where 17-OHP was separated based on its interaction with the stationary phase. The eluted analyte was then introduced into the tandem mass spectrometer, which provides enhanced sensitivity and specificity through a two-step process. In the first stage, precursor ions corresponding to 17-OHP were selected based on their mass-to-charge (m/z) ratio. In the second stage, these ions were fragmented into product ions, enabling precise identification based on their unique fragmentation patterns (12,13). Exclusion criteria included hemolyzed, lipemic, and clotted samples, which were omitted from further analysis. The data obtained were classified based on patient age and sex. Permission was obtained from the Ethics Committee of University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital with the subject (decision no: 14, date: 11.01.2023), and informed consent was also obtained from the patients' guardians.

Statistical Analysis

All statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including mean, standard deviation, and standard error, were used to summarize the data. Group comparisons were assessed using either analysis of variance or the chi-square test, as appropriate. All statistical results were evaluated at a 95% confidence interval, and a $p<0.05$ was considered statistically significant.

Results

The distribution of the 134 infants under 5 months of age included in the study is presented according to gender (Graphic 1a) and age (Graphic 1b). In all 134 infants, serum 17-OHP levels measured by ELISA method were above the age-specific reference range. However, when the same samples were reanalyzed using LC-MS/MS method, 79.10% (n=106) were reported to be within the reference range and 20.90% (n=28) were reported to be above the reference range according to the data in Table 1.

In addition, Table 2 reported 17-OHP levels above the quantifiable upper limit (>16 ng/mL) in 16.41% (n=22) of infants when tested with ELISA. When these samples were studied with LC-MS/MS, 77.27% (n=17) (group 1) were reported within the reference range, and 22.73% (n=5) (group 2) were reported above the reference range. Table 2 shows the distribution of data according to age and gender in these two groups. A chi-square test was conducted to compare the two measurement methods (ELISA vs. LC-MS/MS) in a 2x2 contingency table. The comparison revealed a statistically significant difference between the two methods ($p<0.0001$).

Table 1. Distribution of the study according to gender and age when studied with the LC-MS/MS method

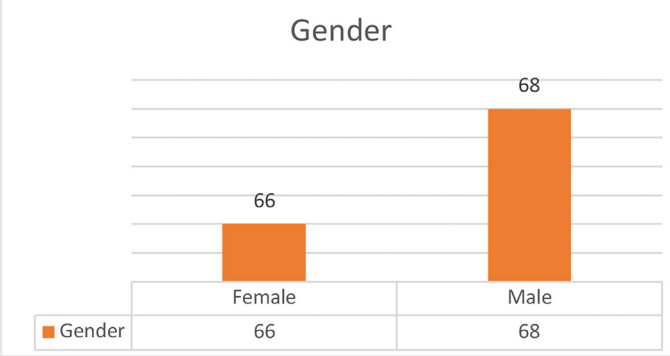
		Group 1		Group 2	
		Number (n)	Percentage (%)	Number (n)	Percentage (%)
Gender	Female	50	47.17	16	57.14
	Male	56	52.83	12	42.86
Age	<1 month	48	45.29	11	39.29
	1-2 months	37	34.9	11	39.29
	2-3 months	14	13.2	5	17.85
	3-4 months	5	4.72	1	3.57
	4-5 months	2	1.89	-	-

LC-MS/MS: Liquid chromatography-tandem mass spectrometry

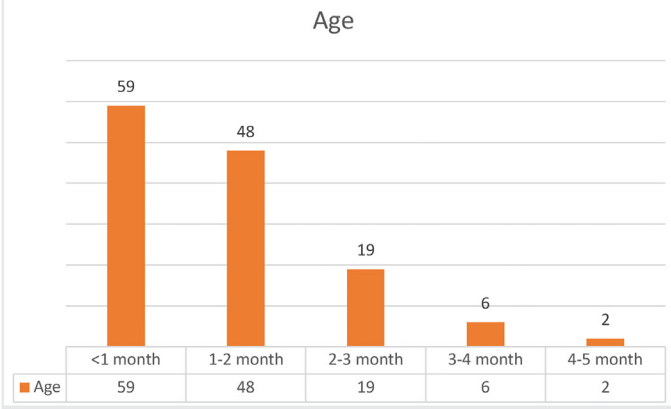
Table 2. Distribution of those higher than the upper limit (>16 ng/mL) by the ELISA method in the study according to gender and age when evaluated by the LC-MS/MS method

		Group 1		Group 2	
		Number (n)	Percentage (%)	Number (n)	Percentage (%)
Gender	Female	6	35.29	5	100
	Male	11	64.71	-	-
Age	<1 month	4	23.53	2	40
	1-2 months	11	64.71	3	60
	2-3 months	2	11.76		
	3-4 months	-			
	4-5 months	-			

LC-MS/MS: Liquid chromatography-tandem mass spectrometry, ELISA: Enzyme-linked immunosorbent assay



Graphic 1a. Distribution of the study by gender



Graphic 1b. Distribution of the study by age

Discussion

17-OHP is a 21-carbon steroid hormone that is produced as an intermediate during the biosynthesis of glucocorticoids and sex steroids. Physiologically, elevated serum 17-OHP levels are observed during fetal development and the early postnatal period. A slight postnatal increase in 17-OHP levels may occur in male infants between days 30 and 60. In both sexes, serum 17-OHP concentrations decline gradually until puberty, after which levels become comparable between males and females. Serum 17-OHP testing is commonly used for the diagnosis of CAH, particularly in cases of 21-OH or 11 beta-hydroxylase enzyme deficiencies. It also plays an important role in the diagnosis and monitoring of mineralocorticoid and androgen synthesis disorders (14). In circulation, approximately 55% of serum 17-OHP binds to albumin, 41% to corticosteroid-binding globulin, and a small proportion to sex hormone-binding globulin. In its protein-bound form, 17-OHP is considered biologically inactive (15).

In routine laboratory practice, 17-OHP level is measured using immunoassays such as ELISA and RIA, as well as the more advanced LC-MS/MS technique. Although immunoassays are convenient and widely used, they are subject to non-specific binding and cross-reactivity with structurally related steroid hormones, which can lead to false elevations inconsistent with the patient's clinical picture. As a result, the use of LC-MS/MS has become increasingly preferred for its superior specificity and accuracy in 17-OHP quantification (16). Although correlations have been reported between LC-MS/MS and immunoassay results in the measurement of steroid hormones like 17-OHP, methodological discrepancies may exist. Each technique has its own advantages and limitations, which must be considered in both clinical and research settings (17,18).

CAH is a rare but potentially life-threatening autosomal recessive endocrine disorder that affects both sexes equally. The disorder is categorized into classical and non-classical forms based on the severity of 21-OH deficiency. In its classical form, CAH is further subdivided into salt-wasting and simple virilizing types. The salt-wasting type, characterized by adrenal insufficiency, dehydration, hyponatremia, and shock, can be fatal if left undiagnosed in the neonatal period. In contrast, the non-classical type typically presents with milder symptoms that manifest later in life (19). In recognition of the critical importance of early diagnosis, the Ministry of Health has incorporated serum 17-OHP measurement into national NBS programs. CAH may present with adrenal crises, which are characterized by symptoms such as vomiting, diarrhea, dehydration, hyponatremia, hyperkalemia, and hypoglycemia. Approximately one-third of neonates with ambiguous genitalia are ultimately diagnosed with CAH. In affected female newborns, virilization and ambiguous genitalia are the predominant clinical features, whereas male newborns may present with hyperpigmentation, enlarged phallus, and scrotal enlargement (20).

In our study, serum 17-OHP levels measured by the ELISA method of infants whose gender (Graph 1a) and age (Graph 1b) data are given in were reported above the reference range. In newborns with ambiguous genitalia or salt-wasting symptoms, 17-OHP is commonly measured using ELISA as part of CAH screening protocols. In male infants, clinical suspicion of CAH is raised in the presence of scrotal hyperpigmentation, enlarged penis, and large scrotum. However, since these findings may be qualitatively interpreted based on the clinician's experience, early diagnosis and treatment may be delayed. In 21-OH deficiency (21-OHD) screening, when 17-OHP is reported by immunoassay, cross-reactivity to other steroids may cause false-positive results. A study from Japan evaluated false-positive results above the reference range of 17-OHP ELISA in preterm infants by LC-MS/MS (21). In the literature, in a study conducted on 328 preterm infants with immunoassay (ELISA) in NBS, 33 were false positives, which was confirmed by comparison with LC-MS/MS (22).

Female infants with classical CAH are typically diagnosed at birth due to abnormalities in external genitalia, which result from excess androgen exposure during intrauterine life. In contrast, male infants with classical salt-wasting CAH generally appear phenotypically normal at birth and are diagnosed between days 7 and 14 based on the development of clinical symptoms, including weight loss, vomiting, lethargy, hyponatremia, hyperkalemia, dehydration, and in some cases, shock. Less commonly, classical non-salt-wasting CAH cases are identified in children aged 2-4 years, often during investigation for virilization. These cases may be missed in NBS programs due to the absence of salt-wasting symptoms in the neonatal period (23). In our laboratory, the upper detection limit for serum 17-OHP using the Diameter kit (Alisei ELISA Analyzer) was 16 ng/mL. A noteworthy finding is that according to the data obtained from our study, more 17-OHP was detected in male infants above the upper reading value measured by ELISA than in female infants in Table 2. However, when the same male infant samples in Table 2 were reanalyzed by LC-MS/MS, it was observed that 17-OHP level was measured within the normal reference range. When the same samples of 6 female infants whose 17-OHP level measured by ELISA and found above the reference range were examined by LC-MS/MS, 5 female infants (n=5) were detected having 17-OHP above the reference range. According to the data obtained from our study, CAH with high 17-OHP level was reported more in female infants. This situation emphasizes the importance of confirmatory tests and method selection to prevent misdiagnosis and unnecessary treatment, especially in borderline or discordant cases.

Serum 17-OHP measurement remains a widely used diagnostic and monitoring tool in the detection and management of CAH, especially during the newborn period. In our study, the same serum samples from infants under 5 months of age with 17-OHP levels exceeding the upper limit measured by ELISA were also reanalyzed using the LC-MS/MS method. Importantly, elevated 17-OHP levels were confirmed by LC-MS/MS primarily in infants younger than 2 months. This finding emphasizes the importance of age-specific interpretation of 17-OHP levels and suggests that biochemical confirmation using LC-MS/MS is especially valuable in the early neonatal period to reduce the risk of false-positive results and avoid unnecessary interventions.

Study Limitations

In the late 1990s, in the reports of immunological analyses of steroid hormones such as 17-OHP, many analytical problems were evaluated with gas chromatography-mass spectrometry (MS) and techniques such as LC-MS/MS were more suitable for the analysis of steroid hormones in body fluids. In this technique, steroid hormones that can be visualized structurally with analytical selectivity can be measured with the multiple reaction monitoring mode of MS. In the literature, LC-MS/MS is considered a reliable reference method for the evaluation of less selective immunoassay techniques (ELISA, RIA, etc.) (13-15). This study has several potential limitations. A key methodological consideration is the inherent differences between the LC-MS/MS and ELISA techniques, each of which presents distinct advantages and disadvantages. The LC-MS/MS method operates on the principle of separating biological components based on their differential binding affinities to the stationary phase, allowing for high-resolution characterization of trace compounds, steroid hormones such as 17-OHP, toxins, metabolites, and proteins. The advantages of LC-MS/MS include its ability to:

- Use very small sample volumes
- Achieve high selectivity for structurally similar molecules
- Provide high reproducibility and low detection limits
- Simultaneously quantify multiple analytes

However, this method also presents limitations. The complexity of the instrumentation requires specialized training for both operation and data interpretation. Additionally, the high initial equipment cost, ongoing maintenance expenses, and sample preparation steps—especially for protein analysis—can be challenging. When analyzing proteins, HPLC is often used prior to MS, and this process typically involves enzymatic digestion of peptides to enhance chromatographic resolution. However, such enzymatic pretreatment may affect MS sensitivity and reproducibility (17,18). Another limitation of this study is its single-center design. Due to sample volume constraints in infants under 5 months of age, some patients had to be excluded from the analysis because of insufficient sample availability.

Since 17-OHP levels can be increased in premature infants due to cross-reactivity in immunoassay (ELISA), second-tier markers are recommended for this limitation. In CAH, the enzyme 11 beta-hydroxylase catalyzes the conversion of 17-OHP to 21-deoxycortisol (21-DF). In 21-OHD, 21-DF increases due to the increased presence of elevated 17-OHP. Although 21-DF is a specific biochemical marker for 21-OHD, it is used as a useful second-tier marker for CAH due to the long analysis time (24).

Conclusion

The ELISA method, on the other hand, is widely used in routine practice due to its low cost, practical application, and suitability for high-throughput screening. Despite its widespread use, ELISA is subject to non-specific interactions and cross-reactivity with structurally related steroids, which may result in false elevations of 17-OHP levels. These limitations have raised concerns regarding the clinical reliability of ELISA, especially in the measurement of steroid hormones like 17-OHP. In our study, we evaluated the discrepancies in serum 17-OHP levels measured by ELISA compared to LC-MS/MS in infants up to 5 months of age. We emphasized that the use of LC-MS/MS to confirm false positive elevations detected by ELISA may be important, especially in infants younger than 3 months, where physiological variations are common and the risk of misdiagnosis is higher. Although LC-MS/MS is more costly, it offers superior sensitivity and specificity.

Based on our findings, we propose that ELISA may remain a practical first-line method for measuring serum 17-OHP levels in children older than 3 months and adults, with LC-MS/MS reserved for confirming results that are clinically incongruent. This approach may offer a cost-effective yet clinically reliable strategy for routine laboratory use.

Ethics

Ethics Committee Approval: Permission was obtained from the Ethics Committee of University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital with the subject (decision no: 14, date: 11.01.2023).

Informed Consent: Informed consent was also obtained from the patients' guardians.

Footnotes

Authorship Contributions

Surgical and Medical Practices: N.D., A.G., Concept: N.D., A.G., Design: N.D., A.G., Data Collection or Processing: N.D., Analysis or Interpretation: N.D., A.G., Literature Search: N.D., A.G., Writing: N.D., A.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. White PC. Neonatal screening for congenital adrenal hyperplasia. Nat Rev Endocrinol. 2009;5:490-8.
2. Saka HN, Baş F. Konjenital adrenal hiperplazi. In: Kurtoğlu S, editor. Yenidoğan dönemi endokrin hastalıkları. İstanbul: Nobel Tıp Kitabevleri; 2013. p. 293-308.
3. Stanczyk FZ. Production, clearance, and measurement of steroid hormones. T Glob Libr Women's Med. 2009;10.
4. Merke DP, Bornstein SR. Congenital adrenal hyperplasia. Lancet. 2005;365:2125-36.
5. Honour JW. 17-hydroxyprogesterone in children, adolescents and adults. Ann Clin Biochem. 2014;51:424-40.
6. Miller WL. The adrenal cortex and its disorders. In: Brook CGD, Clayton PE, Brown RS, editors. Brook's clinical pediatric endocrinology. 6th ed. Oxford: Wiley-Blackwell; 2009. p. 283-326.
7. Apter D, Jánne O, Karvonen P, Vihko R. Simultaneous determination of five sex hormones in human serum by radioimmunoassay after chromatography on Lipidex-5000. Clin Chem. 1976;22:32-8.
8. Debeljak Ž, Marković I, Pavela J, Lukić I, Mandić D, Mandić S, et al. Analytical bias of automated immunoassays for six serum steroid hormones assessed by LC-MS/MS. Biochem Med (Zagreb). 2020;30:030701.
9. Ambroziak U, Kępczyńska-Nyk A, Kuryłowicz A, Wysłouch-Cieszyńska A, Małunowicz EM, Bartoszewicz Z, et al. LC-MS/MS improves screening towards 21-hydroxylase deficiency. Gynecol Endocrinol. 2015;31:296-300.
10. Emon JMV, Chuang JC, Trejo RM, Durnford J. Integrating bioanalytical capability in an environmental analytical laboratory. Immunoassay and other bioanalytical techniques. New York: CRC Press, Taylor&Francis; 2007. p. 1-43.
11. Alataş Ö, Çolak Ö, Köseoğlu M, Orçun A, Türkmen S. Laboratuvar organizasyonu yönetimi ve kalite. Türk Klinik Biyokimya Derneği. 2004:102-3.
12. Nimkarn S, Lin-Su K, New MI. Steroid 21 hydroxylase deficiency congenital adrenal hyperplasia. Endocrinol Metab Clin North Am. 2009;38:699-718.
13. Keevil BG. LC-MS/MS analysis of steroids in the clinical laboratory. Clin Biochem. 2016;49:989-97.
14. Riad-Fahmy D, Read GF, Walker RF, Griffiths K. Steroids in saliva for assessing endocrine function. Endocr Rev. 1982;3:367-95.
15. Turpeinen U, Itkonen O, Ahola L, Stenman UH. Determination of 17alpha-hydroxyprogesterone in serum by liquid chromatography-tandem mass spectrometry and immunoassay. Scand J Clin Lab Invest. 2005;65:3-12.
16. Kao PC, Machacek DA, Magera MJ, Lacey JM, Rinaldo P. Diagnosis of adrenal cortical dysfunction by liquid chromatography-tandem mass spectrometry. Ann Clin Lab Sci. 2001;31:199-204.
17. Murphy RE, Kinhikar AG, Shields MJ, Del Rosario J, Preston R, Levin N, et al. Combined use of immunoassay and two-dimensional liquid chromatography mass spectrometry for the detection and identification of metabolites from biotherapeutic pharmacokinetic samples. J Pharm Biomed Anal. 2010;53:221-7.
18. Karpievitch YV, Polpitiya AD, Anderson GA, Smith RD, Dabney AR. Liquid chromatography mass spectrometry-based proteomics: biological and technological aspects. Ann Appl Stat. 2010;4:1797-823.
19. Speiser PW, Arlt W, Auchus RJ, Baskin LS, Conway GS, Merke DP, et al. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2018;103:4043-88.

20. Turcu AF, Auchus RJ. Adrenal steroidogenesis and congenital adrenal hyperplasia. Endocrinol Metab Clin North Am. 2015;44:275-96.
21. Watanabe K, Tsuji-Hosokawa A, Hashimoto A, Konishi K, Ishige N, Yajima H, et al. The high relevance of 21-deoxycortisol, (androstenedione + 17α-hydroxyprogesterone)/cortisol, and 11-deoxycortisol/17α-hydroxyprogesterone for newborn screening of 21-hydroxylase deficiency. J Clin Endocrinol Metab. 2022;107:3341-52.
22. Stroek K, Ruiter A, van der Linde A, Ackermans M, Bouva MJ, Engel H, et al. Second-tier testing for 21-hydroxylase deficiency in the Netherlands: a newborn screening pilot study. J Clin Endocrinol Metab. 2021;106:e4487-96.
23. Livadas S, Bothou C. Management of the female with non-classical congenital adrenal hyperplasia (NCCAH): a patient-oriented approach. Front Endocrinol (Lausanne). 2019;10:366.
24. Janzen N, Peter M, Sander S, Steuerwald U, Terhardt M, Holtkamp U, et al. Newborn screening for congenital adrenal hyperplasia: additional steroid profile using liquid chromatography-tandem mass spectrometry. J Clin Endocrinol Metab. 2007;92:2581-9.