Neonatal screening for congenital adrenal hyperplasia in Turkey: a pilot study with 38,935 infants

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Background-Aim

Congenital adrenal hyperplasia (CAH) is the most common form of primary adrenal insufficiency in children. 21-hydroxylase enzyme deficiency (21-OHD) occurs in 90 to 95% of all cases of CAH. Despite it being a treatable condition, if unrecognized, CAH may present with life-threatening cardiovascular collapse. Mortality in the first years is reported to be higher than in the general population. Neonatal screening for CAH is effective in detecting the salt-wasting form and thereby reducing mortality. This study describes the incidence of CAH in Turkey and analyses the results obtained from a pilot study of public CAH screening program of Turkish Directorate of Public Health comprising four cities of Turkey.

Method

A pilot newborn CAH screening study was carried out under the authority of Turkish Directorate of Public Health. Newborn babies ≥32 gestational weeks and ≥1500 gr birth weight from four cities between March 27- September 15, 2017 were included. Screening protocol included one sample two-tier testing. In the first step, 17α-hydroxyprogesterone (17-OHP) was measured by fluoroimmunoassay in dried blood spots obtained at 3-5th days of life. The cases with positive initial screening were tested by steroid profiling in dried blood spots using liquid chromatography-tandem mass spectrometry method to measure 17-OHP, 21-deoxycortisol, cortisol, 11-deoxycortisol and androstenedione as a second-tier test. The babies with steroid ratio of (21-deoxycortisol+17-OHP)/cortisol≥0.5 were referred to pediatric endocrinology clinics for diagnostic assessment (Figure 1).

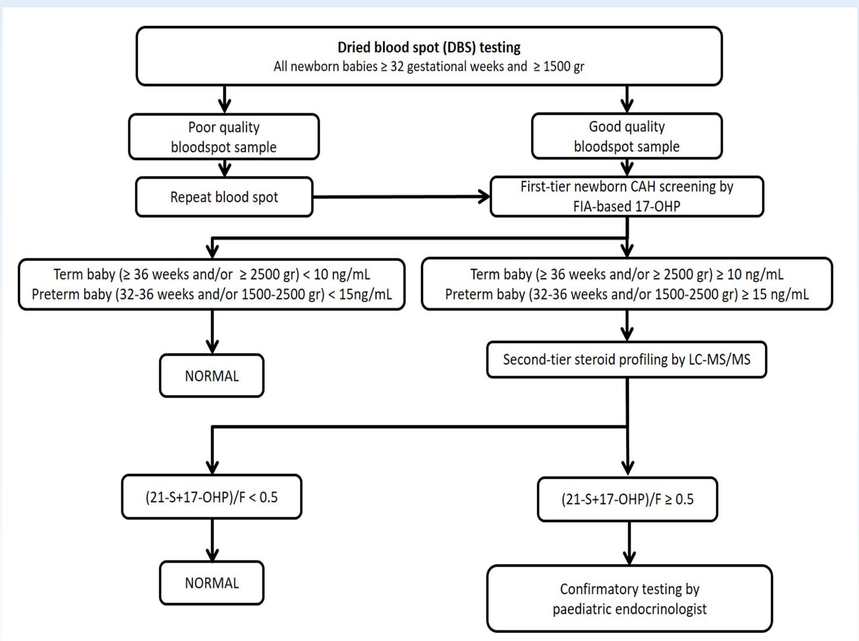

Figure 1. Flowchart for pilot neonatal CAH screening initiated by the Turkish Directorate of Public Health. Abbreviations: FIA= fluoroimmunoassay, LC-MS/MS= liquid chromatography-tandem mass spectrometry, 17-OHP= 17-hydroxyprogesterone, 21-S= 21-deoxycortisol, F= cortisol, 4AS= androstenedione and 11-S= 11-deoxycortisol. (17-Hydroxyprogesterone (17-OHP) conversion factor from ng/mL to nmol/L: Multiply by 3.02)

Results

38,935 infants were tested, 2265 (5.82%) had second-tier testing, and 212 (0.54%) were referred for clinical assessment, 6 of whom were diagnosed with CAH (four males,two females). Four cases were identified as salt-wasting 21-hydroxylase deficiency (21-OHD) (2 males,2 females), one male baby had simple virilizing 21-OHD, one male baby had 11-OHD CAH. The incidence of classical 21-OHD in the screened population was 1:7,787. Detailed analyses of screening data is given in Table 1-5.

Table 1. FIA based 17-OHP values of screened population according to birth weight and gestational age.

17-OHP (ng/mL) [nmol/L]	1500-2500 gr	≥ 2500 gr	32-36 gw	≥ 36 gw	32-36 gw+ 1500-2500 gr	≥ 36 gw + ≥ 2500 gr
(n)	(3,022)	(35,907)	(3,684)	(35,245)	(1,744)	(33,967)
Mean (SD)	8.29 (8.68) [25.08 (26.2)]	4.07 (2.75) [12.3 (8.3)]	8.60 (8.27) [26.02 (25.0)]	3.96 (2.53) [11.9 (7.6)]	10.80 (10.11) [32.6 (30.5)]	3.92 (2.43) [11.8 (7.3)]
Min-max	0.10-137.30 [0.3-415]	0.05-56.63 [0.15-171]	0.11-137.30 [0.33-415]	0.05-57.66 [0.15-174]	0.11-137.30 [0.33-415]	0.05-56.63 [0.15-171]
Median	5.33 [16.1]	3.53 [10.6]	5.92 [17.9]	3.48 [10.5]	7.36 [22.2]	3.47 [10.5]
IQR (25-75%)	3.40-9.96 [10.2-30.1]	2.49-4.89 [7.5-14.7]	3.84-10.52 [11.6-31.8]	2.47-4.80 [7.4-14.5]	4.58-13.72 [13.8-41.5]	2.47-4.77 [7.4-14.2]
99.5%	50.80 [17.5]	18.05 [54.6]	49.99 [151]	16.71 [50.5]	58.27 [176]	15.97 [48.1]
99.8%	63.64 [191]	23.48 [71]	59.95 [181]	21.38 [64]	77.21 [233]	20.21 [61]

Abbreviations: 17-OHP= 17-hydroxyprogesterone, FIA= fluoroimmunoassay
SI units are given in brackets.

2,265 (5.8%) babies had second-tier testing by LC-MS/MS steroid profiling of same DBS. During screening

Table 2. Rate of second-tier testing among babies based on birth weight and gestational weeks

	1500-2500 gr	≥ 2500 gr	32- 36 gw	≥ 36 gw	32-36 gw + 1500-2500 gr	≥ 36 gw + ≥ 2500 gr
Number of babies	3,022	35,907	3,684	35,245	1,744	33,967
Second-tier testing (number; %)	(722; 24)	(1,543; 4)	(973; 26)	(1,292; 4)	(607; 34)	(1,117; 3)

Table 3. Distribution of babies based on (21S+17-OHP)/F ratio adjusted for gestational age and birth weight

(21S+17-OHP)/F ratio	1500-2500 gr	≥ 2500 gr	32- 36 gw	≥ 36 gw	32-36 gw + 1500-2500 gr	≥ 36 gw + ≥ 2500 gr
0.5-1.0	54	107	68	93	45	84
1.0-2.0	19	20	22	17	18	16
2.0-5.0	8	1	9	0	8	0
> 5.0	1	2	2	1	1	1
Total (n)	82	130	101	111	72	101

Abbreviations: 21-S= 21-deoxycortisol, 17-OHP= 17-hydroxyprogesterone, F= cortisol

Table 4. Clinical characteristics and laboratory details of the patients with CAH diagnosed through NBS

Case No	Karyotype	Birth weight (gr)/ Gestational week	17-OHP by FIA (ng/mL)	Second-tier testing by LC-MS/MS (ng/mL)		Day of treatment initiation	Blood biochemistry at diagnosis		Diagnosis	Molecular defect
1	46, XX	3290/38	137.3	17-OHP	263.34	10th day	Na (mEq/L)	132	21-OHD (SW)	*CYP21A2* cluster E6 (c.707T>A, c.710T>A, c.716 T>A) homozygous
				21-S	40.65		K (mEq/L)	6.1		
				F	29.95					
				(21-S + 17-OHP)/F	10.14		17-OHP (ng/mL)	12.5		
				4AS	90.44					
				11-S	7.17					
2	46, XX	3139/38	137.3	17-OHP	262.59	28th day	Na (mEq/L)	113	21-OHD (SW)	ND
				21-S	36.52		K (mEq/L)	6.5		
				F	9.21					
				(21-S + 17-OHP)/F	32.47		17-OHP (ng/mL)	>128		
				4AS	23.10					
				11-S	0.83					
3	46, XY	2900/38	96	17-OHP	908.45	21st day	Na (mEq/L)	137	21-OHD (SW)	*CYP21A2* IVS2-13C>G (c.293-13C>G) homozygous and CYP21A2 p.Q319X (c.955C>T) homozygous
				21-S	0.49		K (mEq/L)	6.6		
				F	41.27					
				(21-S + 17-OHP)/F	22.02		17-OHP (ng/mL)	>20		
				4AS	346.9					
				11-S	27.84					
4	46, XY	3200/39	96	17-OHP	58.73	30th day	Na (mEq/L)	NA	21-OHD (SW)	*CYP21A2* IVS2-13C>G (c.293-13C>G) and p.R357W (c.1069C>T) Compound heterozygous
				21-S	0.02		K (mEq/L)	NA		
				F	12.69					
				(21-S + 17-OHP)/F	4.62		17-OHP (ng/mL)	>20		
				4AS	6.83					
				11-S	3.84					
5	46, XY	2950/39	44.29	17-OHP	19.66	19th day	Na (mEq/L)	134	21-OHD (SV)	*CYP21A2* p.I172N (c.518 T>A) homozygous
				21-S	0.05		K (mEq/L)	5.7		
				F	14.99					
				(21-S + 17-OHP)/F	1.31		17-OHP (ng/mL)	47.2		
				4AS	1.53					
				11-S	0.62					
6	46, XY	3250/36	16.33	17-OHP	4.82	71th day	Na (mEq/L)	137	11-OHD	*CYP11B1* p.Asn394Argfs X37 (c.1180_1181 insGA) and p.Phe487Cys (c.1460T>G) Compound heterozygous
				21-S	0.002		K (mEq/L)	5.3		
				F	31.31					
				(21-S + 17-OHP)/F	0.15		17-OHP (ng/mL)	NA		
				4AS	52.33					
				11-S	113.83					

Abbreviations: NBS= Newborn screening, FIA= fluoroimmunoassay, LC-MS/MS= liquid chromatography-tandem mass spectrometry, 17-OHP= 17-hydroxyprogesterone, 21-S= 21-deoxycortisol, F= cortisol, 4AS= androstenedione and 11-S= 11-deoxycortisol, 21-OHD=21-hydroxylase deficiency, 11-OHD= 11β- hydroxylase deficiency, SW=salt wasting,

Table 5. Comparison of first-tier 17-OHP levels and second-tier (21-S + 17-OHP)/F ratios between the 206 false-positive healthy recalled infants and 5 infants with 21-OHD.

	False-positive healthy recalled babies						21-OHD CAH babies	
	1500-2500 gr	≥ 2500 gr	32- 36 gw	≥ 36 gw	32-36 gw + 1500-2500 gr	≥ 36 gw + ≥ 2500 gr	≥ 36 gw + ≥ 2500 gr	*p* value*
(n)	(82)	(130)	(101)	(111)	(72)	(101)	(5)	
First-tier 17-OHP (ng/mL) [nmol/L]								
Mean±SD	29.52±18.68 [89.3±56.5]	15.11±6.52 [45.7±19.7]	28.00±17.73 [84.7±53.6]	14.02±4.69 [42.4±14.1]	31.09±19.33 [94±58.4]	13.61±4.42 [41.1±13.3]	302.6±357 [915±1080]	
Median (IQR)	24.9 (18-35) [75 (54-105)]	12.8 (10.3-17) [39 (31-51.5)]	24.2(16-33.5) [73 (49-101)]	12.5 (10.3-16) [38 (31-48)]	27 (18.5-35) 82 (56-106)]	12.3 (10-15.3) [37 (30-46)]	262 (39-586) [793 (118-1773)]	***<0.0001***
99.5%	117.8 [356]	39.8 [120.4]	112.8 [341]	28.9 [87.4]	120 [363]	27 [81.7]		
99.8%	129 [390]	45 [136]	127.5 [386]	29.8 [90.1]	130.3 [394]	27.5 [83.2]		
(21S+17-OHP)/F ratio								
Mean±SD	1.38±2.92	1.01±1.81	1.49±3.24	0.85±0.68	1.47±3.11	0.85± 0.70	14.1±12.94	
Median (IQR)	1.38 (0.7-1.1)	0.7 (0.6-0.9)	0.8 (0.65-1.2)	0.66 (0.6-0.85)	0.9 (0.7-1.15)	0.65 (0.6-0.86)	10.1 (3-27.2)	***<0.0001***
99.5%	17.9	11.6	23.3	4.2	19	4.5		
99.8%	23.17	16.64	25.35	5.89	23.61	5.99		

Abbreviations: 17-OHP= 17-hydroxyprogesterone, 21-S= 21-deoxycortisol, F= cortisol, 4AS= androstenedione and 11-S= 11-deoxycortisol, 21-OHD=21-hydroxylase deficiency
* *p* values indicate the comparison of the parameters in babies with 21-OHD with the term and ≥ 2500 gr birthweight babies (n=101) with false-positive second-tier screening results.
SI units are given in brackets.

Conclusion

The incidence of CAH due to classical 21-OHD is higher in Turkey in comparison to previous reports. Thus, it is suggested to add CAH to newborn screening panel in Turkey. The use of steroid profiling as a second-tier test improves the efficacy of the screening and reduces false-positives.