NEWBORN AND CHILDHOOD
SCREENING PROGRAMMES

Zerrin ORBAK
Yusuf Kenan HASPOLAT

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Editors

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Editors
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PREFACE

Newborn and childhood screening programs play a critical role in helping our children live a healthy life and improve their quality of life. Healthy children form the basis of healthy generations and thus the foundations of our future are laid firmly.

Our wish is this: Let our children have a world full of peace and tranquility, where smiles never leave their faces, happiness is seen in their eyes.

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Chapter

1

Screening Tests Performed in the Newborn Period in our Country for Congenital Endocrine and Metabolic Diseases: Definition and Their Importance

İlknur SURUCU KARA, Zerrin ORBAK

Congenital anomalies, and genetic, metabolic, and endocrinological diseases are the most important causes of malformation and death in the first months of life (Shoraka et al., 2020). In the European Union and Turkey, diseases affecting 1 in 2000 people or less are called rare diseases. It is estimated that there are approximately 350 000 000 people with rare diseases in the world and approximately 5 000 000 in Turkey (Fidan et al., 2022). The prevalence of inherited endocrinologic and metabolic diseases is higher in our country and in countries like ours where consanguineous marriages are common. Early or presymptomatic detection of these rare diseases and early initiation of treatment significantly reduces morbidity and mortality. Patients diagnosed early may have a better prognosis and quality of life (Kılıç, 2021). Phenylpyruvic acid was detected in urine in the 1930s. In the 1960s, Guthrie developed a screening method

for phenylketonuria from dried blood spots using blood samples taken from the heel of newborn babies. Wilson and Jungner were first commissioned by the WHO (World Health Organization) in 1968 for a newborn screening program. Wilson and Jungner determined the disease to be screened, the test method, its applicability, and the cost of the test. Some principles and recommendations related to screening were determined by the World Health Organization (Table 1). Screening for diseases that can be treated with early diagnosis is practiced in some countries (Lüders et al., 2021; Shoraka et al., 2020; Wilson & Jungner, 1968). First, phenylketonuria was screened and over time, inherited metabolic diseases such as biotinidase enzyme deficiency, galactosemia, homocystinuria, maple syrup urine disease, endocrinological diseases such as congenital adrenal hyperplasia, congenital hypothyroidism, hematological diseases such as sickle cell anemia, G6PD (glucose-6-phosphate dehydrogenase deficiency), immunological diseases such as severe combined immunodeficiency, cardiovascular diseases, cystic fibrosis, and congenital hearing loss were added to the newborn screening program. Newborn screening tests are not diagnostic. Additional tests are required to confirm or exclude a suspected diagnosis (Kılıç, 2021; Tluczek et al., 2022). In the United States, local PKU screening has been done since the 1960s. It started later and became mandatory with the National Screening Program in 1975. The first expanded newborn screening program in the United States was adopted in 2006 and 54 diseases were screened in the United States. 42 of these 54 diseases can be treated with MS/MS technology. The other 12 diseases screened (congenital hypothyroidism, biotinidase deficiency, congenital adrenal hyperplasia, cystic fibrosis, some hemoglobinopathies, and galactosemia) were measured by other methods. In

Table 1. Wilson and Jungner's Classic Screening Criteria published in 1968

1	The disease being screened must be an important health (public health) problem.
2	The natural course of the disease (from the latent period until clinical findings appear) must be best known.
3	Diagnostic and treatment facilities must be available.
4	There must be a suitable test or inspection method.
5	The test must be socially acceptable.
6	There must be an accepted treatment for diagnosed patients.
7	There must be a recognizable latent or early symptomatic phase.
8	An agreed-upon decision about who will be treated as a patient should be policy
9	The cost of case detection (possible expenses for diagnosis, treatment, and medical care of diagnosed patients) must be balanced as a whole.
10	Scanning should be a continuous process and not a one-time project.

recent years, severe combined immunodeficiency has also been added. The diseases included in the screening program vary from country to country. Approximately 60 diseases are screened in the USA (depending on the state) and approximately 5-10 diseases are screened in the UK and France through the newborn extended screening program (Fabie et al., 2019; Kılıç, 2021). Of the 20 countries in Latin America, 8 were for galactosemia, 12 for congenital adrenal hyperplasia and cystic fibrosis, 14 for phenylketonuria, and 16 screened for congenital hypothyroidism (Borrajo, 2021). Currently, screening is performed in approximately 51 countries in Europe. Of these 51 countries, 47 are screened for PKU, 46 for congenital hypothyroidism, 25 for cystic fibrosis, 24 for congenital adrenal hyperplasia, and 7 for severe combined immunodeficiency (la Marca et al., 2023). In Austria, the Netherlands, Slovenia, and Norway, MS-MS technology is used in the National Newborn Screening Program for more than 19 diseases (Table 2, Table 3) (Kılıç, 2021; Lampret et al., 2020; Pollak & Kasper, 2014; Tangeraas et al., 2020).

In the 1970s, concentrations of thyroid hormones were measured from dried blood spots using radio-immunochemical methods. After 2000, tandem mass spectrometry (MS/MS, tandem mass spectrometry) made it possible to screen for more than 30 diseases from a single sample. Wilson and Jungner' criteria have also been revised over time and adapted to the present day. Some incurable diseases have also been included in the expanded newborn screening program for early diagnosis and planning of subsequent pregnancies. Over time, the diversity (panel) of diseases screened in newborn babies is gradually expanding according to country conditions and the prevalence of the disease in that country. Today, there are some centers in some countries working on the detection of rare diseases through genomic newborn screening (Kılıç, 2021; Loeber et al., 2021; Stark & Scott, 2023; Wilson & Jungner, 1968).

In Turkey

In a project conducted by the Scientific and Technological Research Council of Turkey in 1987, the incidence of phenylketonuria in newborns in Turkey was found to be higher than expected. Subsequently, by the Ministry of Health of the Republic of Turkey, the newborn screening program was started and expanded throughout Turkey in 1993. The National Newborn Screening Program was launched on 25.12.2006 with the addition of screening for Congenital Hypothyroidism to the screening for Phenylketonuria measured from dry blood. Biotinidase deficiency screening was added to the panel in October 2008 and Cystic Fibrosis screening in January 2015. Pilot screening for Congenital Adrenal Hyperplasia was initiated in four provinces (Adana, Kayseri, Konya, and Samsun) in 2017. Later, this screening was expanded every year

Table 2. Newborn Screening Program Information in Some Countries (Loeber et al., 2021)

Country	Approx, Population 2020 (Million)	Approx, Number of Infants	Number Screening Laboratories	Interval Birth-Sampling (hrs)	Interval Sampling-Analysis (Days)	% Infants Screened	Length Storage (yrs)
Russia	142	1 670 000	78 [1]	48–72	3–5	90-92	3
Turkey	84.3	1 300 000	2	48–72*	1–2	97	5
Germany	80	787 000	11	36–72	2–3	100	<1
France[2]	67	760 000	16	48–72	2–3	99.96	1
United Kingdom	66.6	760 000	16	120	3–4	96.5	>5
Italy	60.5	434 000	15	48–72	1–4	96.7	2–10
Spain	46.5	372 000	15	24–72	3–10	99.2	5- indefinite
Ukraine	43.7	393 000	7	48–72	3	n.d.	<0.5
Poland	38.4	373 000	6	48–96	3	99.8	5
Uzbekistan	31.3	760 600	14	72–96	10	95	1
Romania [3]	19.6	185 600	5	48–72	18	85	5
Kazakhstan	18.7	402 000	21	24–72	1–2	96.5	3
Netherlands	17.8	168 500	5	72–96	1–3	99.3	5
Czech Republic	10.6	113 000	4 [1]	48–72	2.5	100	5
Belgium	10.5	117 000	4	48–120	2	99.8	5
Greece	10.5	80 000	1	48–72	6–8	100	2
Portugal	10.3	87 300	1	48–72	1–3	99.5	5
Hungary	10	90 000	2	48–72	3–4 *	99.99	indefinite
Sweden	10	116 000	1	48–72	1–3	>99.5	indefinite
Belarus	9.8	108 000	1	72–120	1–5	n.d.	5

Table 2. Newborn Screening Program Information in Some Countries (Loeber et al., 2021)

Country	Approx, Population 2020 (Million)	Approx, Number of Infants	Number Screening Laboratories	Interval Birth-Sampling (hrs)	Interval Sampling-Analysis (Days)	% Infants Screened	Length Storage (yrs)
Azerbaijan	9.7	170 000	1	48–72	3–5	30	n.d.
Tajikistan (no screening)	9.4	291 000	n.a.	n.a.	n.a.	n.a.	n.a
Israel	9.2	194 000	1	36–72	1–3	99.8	5
Austria	8.8	87 000	1	36–72	1–3	>99.5	10
Switzerland	8.1	88 000	1	72–96	2	>99.9	indefinite
Bulgaria	7.4	61 000	2 [1]	72–120	5–10	n.d.	20
Kyrgyzstan	7	160 000	1	48–72	3–5	30	n.d.
Serbia	7	65 000	2	48–72	3–5	99	5
Turkmenistan	6	110 200	1	48–72	3–5	30	n.d.
Denmark	5.6	63 000	1	48–72	1–2	99.1	indefinite
Finland	5.5	45 000	1 [1]	48–120	1–5	99	varies
Slovakia	5.4	57 000	1	72–96	2–3	100	indefinite
Norway	5.3	55 500	1	48–72	1–3	>99	indefinite
Ireland	4.9	59 700	1	72–120	1–2	>99.5	10
Croatia	4.2	36 200	1	48–72	3–5	100	5
Georgia	3.7	48 500	1	48–72	14–15	100	15
Moldova	3.5	37 400	1	>48	30	92.3	0
Bosnia-Herzegovina	3.3	28 000	3 [1]	48–96	1–7	96	10
Armenia	3.1	36 000	2 [1]	48–96	1–5	99.8	indefinite
Albania (no screening)	3	36 000	n.a.	n.a.	n.a.	n.a.	n.a

Table 2. Newborn Screening Program Information in Some Countries (Loeber et al., 2021)

Country	Approx, Population 2020 (Million)	Approx, Number of Infants	Number Screening Laboratories	Interval Birth-Sampling (hrs)	Interval Sampling-Analysis (Days)	% Infants Screened	Length Storage (yrs)
Lithuania	2.8	24 600	1	48–96	2–9	99.6	20
North Macedonia	2.1	20 000	1	32–72	3	>98	3
Slovenia	2.07	20 000	1	48–72	1–2	>99	indefinite
Latvia	1.9	20 800	1	48–72	5–7	98.5	7
Kosovo	1.8	25 000	n.a.	n.a.	n.a.	n.a.	n.a
Estonia	1.3	13 500	1	48–72	2–5	99.55	>25
Cyprus [4]	1.1	9 500	1	48–168	5–10	>99.9	2
Montenegro	0.62	7 200	1	24–72	1–3	100	0.5
Luxembourg	0.6	7 200	1	48–72	4	>99	indefinite
Malta	0.48	4 400	2 [1]	72–120	5	99.7	indefinite
Iceland	0.35	4 500	1	48–72	3–5	100	indefinite

1 : not all laboratories carry out the whole screening panel, 2: France only consented to cystic fibrosis transmembrane conductance regulator analysis 3: Romania total newborns includes Romanian infants born elsewhere, 4: data regarding the northern part of the Cyprus island not included, % screened excludes private labs, n.d: no data, na: not applicable

Table 3. In Neonatal Screening Programs in Some Countries Diseases (2020) (Loeber et al., 2021)

	Endocrine Disorders			Amino Acidemias									Organic Acidemias									Fatty acid Oxidation Disorders									Other								
	CH	CAH	CF	PKU	MSUD	HCY	Tyr-1	Tyr-2	ASA	Cit.1/2	ARG	MAT I/III	GA1	IVA	3MCC	PA	MMA	BKT	HCSD	3HMG	MCD	GA2	MCAD	LCHAD/TFP	VLCAD	SCAD	CPT1	CPT2	CACT	CUD	RMD	GAL	BIOT	UDP	G6PD	xALD	SCID	SMA	SCD
Russia	x	x	x	X																											x							
Turkey	x	x	x	X																												x							
Germany	x	x	x	X	x		x																x	x	x		x	x	x			x	x				x	P	p
France	x	x	x	X																			x																x
United Kingdom	x		x	X	x	X																	x																x
Italy	x	p	x	x	x	X	x	X	x	X	x											x	x	x	x	x	x	x	x	x	x	x	x		p		p	P	
Spain	x	p	x	x	p	P	p	P	p	P	p											p	x	x	p	p	p	p		p	p	p	p				p		x
Ukraine	x	x	x	x	p	P	p	P	p	P	p											p	p	p	p		p	p	p	P		p	p						
Poland	x	x	x	x	x	X	x	X	x	X	x											x	x	x	x		x	x	x	X		p	x				p		
Uzbekistan	x		p	x	p	P	p		p	P	p												p	p	p		p	p		p			p						
Romania	x			x			x	x																															
Kazakhstan	x		p	x																																		
Nethelands	x	x	x	x	x		x																x	x	x		x					x	x			x	p		p
Czech Rep.	x	x	x	x	x	X				X	x												x	x	x		x	x	x		x		x						
Belgium	x	x	x	x	x	X	x	x														x	x		x							x	x		p			P	p
Greece	x			x																												x			x				
Portugal	x		x	x	x	X	x	x	x	X	x											x	x	x	x		x	x	x	x									
Hungary	x		p[1]	x	x	X	x	x	x	X												x	x	x	x	x	x	x	x			x	x						
Sweden	x	x		x	x	X	x		x	X	x											x	x	x	x		x	x	x	x		x	x				x		
Belarus	x			x																																			
Azerbaijan	x	x		x																												x			x				
Tajikistan																																							
Israel	x	x		x	x	x	x	x	x	X	x											x	x	x	x		x	x		x		x		x		p	x		
Armenia	x[2]			x																																			
Switzerland	x	x	x	x	x																		x									x	x				x		
Bulgaria	x	x		x																																			
Kyrgyzstan	x																																						
Serbia	x		x	x																																			
Turkmenistan	x	x	x	x																												x			x				
Denmark	x	x	x	x	x		x		x														x	x	x					x		x	x				x	p	
Finland	x	x		x	x	x	x		x	X	x											x	x	x	x		x	x	x	x							p		
Slovakia	x	x	x	x	x		x	x		X	x												x	x	x	x	x	x	x	x							p	p	
Norway	x	x	x	x	x	x	x																x	x			x	x	x	x			x				x		
Ireland	x		x	x	x	x																	x									x							
Croatia	x			x																			x	x	x					x									
Georgia	x		x	x																																			
Moldova				x																																			
Bosnia-Her.	x			x																																			

Table 3. In Neonatal Screening Programs in Some Countries Diseases (2020) (Loeber et al., 2021)

	Endocrine Disorders			Amino Acidemias									Organic Acidemias									Fatty acid Oxidation Disorders									Other								
Bosnia-Her.	x			x																																		
Austria	x	x	x	x	x	x	x	x	x	X	x											x	x	x	x		x	x	x	x	x	x	x					
Albania																																						
Lithuania	x	x		x																												x						
N. Macedonia	x		p	x	x	x	x		x	X	x											x	x	x	x	x	x	x	x	x								
Slovenia	x			x	x		x																x	x	x		x	x	x	x				p				
Latvia	x	x	x	x																												x	x					
Kosovo																																						
Estonia	x			x	x	x	x	x		X	x												x	x	x		x	x	x	x								
Cyprus	x			x																																		
Montenegro	x		x																																			
Luxembourg	x	x	x	x																			x															
Malta	x			x																																		x
Iceland	x	x		x	x	x			x	X	x											x	x	x	x	x	x	x	x	x				p	x			

3HMG: 3-Hydroxy-3-methylglutaric aciduria, 3MCC: 3-Methylcrotonyl-CoA carboxylase deficiency/3-Methylglutacon aciduria/2-methyl-3-OH-butyric aciduria, x-ALD: x-Adrenoleukodystrophy, ARG: Argininemia, ASA: Argininosuccinic aciduria, BIO: Biotinidase deficiency, BKT: Beta-ketothiolase deficiency, CACT: Carnitine acylcarnitine translocase deficiency, CAH: Congenital adrenal hyperplasia, CF: Cystic fibrosis, CH: Congenital hypothyroidism, CIT1: Citrullinaemia type I, CIT2: Citrullinaemia type II, CPT1: Carnitine palmitoyltransferase deficiency type I, CPT2: Carnitine palmitoyltransferase type II-/Carnitine acylcarnitine transporter deficiency, CUD: Carnitine uptake defect, G6PD: Glucose-6-phophate dehydrogenase deficiency, GA1: Glutaric acidaemia type I, GA2: Glutaric acidaemia type II or multiple acyl coA dehydrogenase deficiency, GAL: Classical galactosaemia, HCY: Homocystinuria (CBS deficiency), HCSD: Holocarboxylase synthetase deficiency, Hemo: Haemoglobinopathies, IVA: Isovaleric acidaemia (IVA)/2-Methylbutyrylglycinuria, LCHAD: Long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency/Trifunctional protein deficiency, MAT I/III: Methionine adenosyl transferase I/III deficiency, MCAD: Medium-chain acyl-CoA dehydrogenase deficiency, MCD: Multiple carboxylase deficiency, MMA: Methylmalonic acidaemia, MSUD: Maple Syrup Urine Disease, PA: Propionic acidaemia, PKU: Phenylketonuria, RMD: Remethylation disorders (methylenetetrahydrofolate reductase, methylcobalamine deficiencies), SCD: Sickle cell disease/thalassemia, SCID: Severe Combined Immunodeficiencies, SCAD: Short-chain acyl-CoA dehydrogenase deficiency, SMA: Spinal muscular atrophy, TFP: Trifunctional protein deficiency, TYR1: Tyrosinaemia type 1, TYR2: Tyrosinaemia type 2, UDP: UDP-galactose-4-epimerase deficiency, VLCAD: Very long-chain acyl-CoA dehydrogenase deficiency, p = pilot/regional screening

and since 2022, screening for congenital adrenal hyperplasia in newborns has been carried out throughout Turkey. Spinal Muscular Atrophy (SMA) started to be screened in our country as of 09.05.2022 (https://hsgm.saglik.gov.tr/tr/tarama-programlari/ntp.html#; İçke & Genç, 2017). Thanks to national newborn screening in Turkey, early treatment is provided for approximately 4500 children annually and disability is prevented. Newborn Hearing Screening Program, developmental hip dysplasia screening program, vision screenings, hearing screening in school-age children, premarital hemoglobinopathy screening, and premarital SMA carrier screening are other national screenings conducted by the Ministry of Health in our country (https://hsgm.saglik.gov.tr/tr/tarama-programlari/ntp.html#).

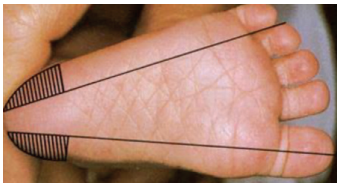
For the newborn metabolic and endocrine disease screening program, the heel blood of newborn babies is screened for diseases such as phenylketonuria, congenital hypothyroidism, biotinidase deficiency, cystic fibrosis, congenital adrenal hyperplasia, and spinal muscular atrophy. In our country, heel prick blood samples taken for the Newborn Screening Program in all health units across the provinces are collected at the provincial directorate of health. The collected blood is sent to screening laboratories in Ankara and Istanbul. Each disease is studied in the screening laboratory. These blood results are transmitted to the provinces via the Newborn Screening Program Web Application and babies with suspicious screening results are referred to the relevant clinics (https://hsgm.saglik.gov.tr/tr/tarama-programlari/ntp.html#). Extended newborn screening with MS/MS technology has not yet been initiated in Turkey.

Screening Principles and Points to Consider in Newborns

Newborn screening is a public health issue. Reaching every baby is the main goal. Blood samples are screened for diseases included in each country's health practices. There are rules that all relevant teams should pay attention to, such as proper planning, informing the family, taking the sample correctly with the appropriate technique, storing and transmitting it under appropriate conditions, recording it correctly, and working with the samples correctly. These rules are summarized in Table 4. It is recommended that these blood samples, which can be used for research purposes (taking into account ethical guidelines), investigation of the etiology of sudden infant death, investigation of criminal cases (DNA source), routine procedure repetitions, and quality control, be kept for at least three years. For research, storage periods can be much longer (Çoker, 2018).

If a false positive test is repeated from the same dried blood sample and the result is normal, this will invalidate the initial result. Reference intervals for newborn screening programs should be chosen carefully. A good balance must be struck between sensitivity

Table 4. Rules to consider in newborn screening

<table>
<tr><td colspan="2">Training parents, family members and healthcare personnel on the subject</td></tr>
<tr><td colspan="2">Reaching all newborns and babies discharged early from the hospital before their third day</td></tr>
<tr><td colspan="2">In newborn baby
• If the first sample was taken after the 24th hour, there is no need to repeat it.
• If taken before 24 hours, take it again after the 7th day
• If the newborn weighs less than 2500 grams, the first sample should be taken on the third day, there is no need to repeat it.
• If the transferred baby was taken during the transfer, it should be taken again when discharged.
• If blood was taken before blood transfusion, if it is repeated due to measurement of enzyme activity in erythrocytes in galactosemia, blood should be taken 2 months later.</td></tr>
<tr><td colspan="2">Informing the family about the procedure</td></tr>
<tr><td colspan="2">Writing introductory information about the newborn legibly and accurately</td></tr>
<tr><td>ID information
Prematurity
Medicines used</td><td>Nutritional status
Whether transfusion was applied or not
Transport information for babies</td></tr>
<tr><td colspan="2">Taking a technically appropriate blood sample</td></tr>
<tr><td>• The most suitable area for taking blood from newborn babies is the heel.
• The heel area is divided into three longitudinal imaginary lines.
• The heel is cleaned and waited for it to dry.
• In the heel area, one of the outer sections, except for the middle section, is pierced with a lancet.</td><td></td></tr>
<tr><td>• The first drop is wiped off, the second drop of blood is absorbed into the marked ring on one side of the previously prepared special paper (Guthrie paper).
• For the example to be sufficient, the blood stain must be seen continuously on the back of the paper.</td><td rowspan="2"></td></tr>
<tr><td>• Venous blood samples or blood samples taken from a hematocrit pipette should not be used.
• A blood sample that is not taken under appropriate conditions will cause the amount extracted to be more or less, negatively affecting the results.
• There should be no contamination of the blood sample from external factors such as milk, urine, feces and baby creams.</td></tr>
<tr><td colspan="2">Blood samples should be stored under appropriate conditions until sent to the relevant laboratory.</td></tr>
<tr><td colspan="2">Blood samples should be dried horizontally at room temperature.</td></tr>
<tr><td colspan="2">The monitoring process should be explained to the family again.</td></tr>
<tr><td colspan="2">The purpose of blood sampling is to accurately detect any disease-possible situation.
In cases of suspected illness, the family will be called.
• Patients will be referred to the relevant department for more detailed analysis.
• Following the necessary diagnostic procedures, disease-specific treatment will be arranged.</td></tr>
<tr><td colspan="2">Delivery of all collected samples to central laboratories as scheduled.
After proper drying, it should be transported quickly and under appropriate conditions to the center where the analysis will be carried out.</td></tr>
<tr><td colspan="2">Correct analysis of samples</td></tr>
</table>

and specificity. Positive results should be confirmed by further tests and repeated after several weeks. Low birth weight (<1500 g), prematurity, intensive care hospitalization, total parenteral nutrition, hemolysis, transfusion history and transport of the sample in an extremely hot or humid environment may cause false positive results. Taking a sample earlier than the recommended time, insufficient sample collection, neonatal jaundice, history of intensive care unit stay, or a history of prematurity may also cause false negative results. The test is not reliable if a blood transfusion has been performed. Therefore, amino acid and acylcarnitine evaluation is required at least 48 hours after transfusion; In hemoglobinopathies, testing should be done at least 60 days later (Table 5) (Çoker, 2018; Henderson et al., 2023; Kılıç, 2021; Sürücü Kara et al., 2023).

Table 5. Some limitations of expanded newborn screening programs

Ability to detect false negative and false positive cases	
False positive cases	Unnecessary anxiety in parents Additional diagnostic testing, unnecessary treatment
False negative results	Missing the case Delay in diagnosis and treatment
Inability to predict the clinical course of some diseases well	For example, short chain acyl CoA de-hydrogenase deficiency, histidynaemia, 3-methylcrotonyl CoA carboxylase deficiency, 2-methylbutyrylglycinuria, some forms of citrullinemia type 1 may be asymptomatic or symptomatic due to genetic and environmental factors.
Wide clinical spectrum of diseases	Indecision about whether to start treatment
Occurrence of symptoms in intoxication type and energy type metabolic diseases before the screening is completed	Organic acidemias Urea cycle defects
Incorrect results due to secondary causes	• Vitamin B12 deficiency in the mother, high C3 in the baby • Carnitine deficiency in the mother, carnitine deficiency in the baby • Carnitine deficiency in the mother can lead to carnitine deficiency in the baby • Carnitine levels may be very low in babies of mothers with malnutrition • If the mother has a carnitine metabolism disorder, the baby's carnitine level may be very low. • If the mother has excess carnitine, the baby may have normal carnitine levels (false negative). • In babies of mothers with preeclampsia; free and C2, C18:1, C18:2 carnitine levels may be high

Inherited Metabolic Diseases

Inherited metabolic diseases are a group of genetic disorders that develop as a result of a deficiency of an enzyme involved in a metabolic pathway or disruption of other mechanisms. Inherited metabolic diseases are usually inherited in an autosomal recessive inheritance pattern, but there are also autosomal dominant and X-linked inheritance types. inherited metabolic diseases are grouped according to the substrate affected or according to clinical manifestation (Table 6, Table 7) (Aslı İnci, 2021; Ezgu, 2016). Treatments for inherited metabolic diseases can be life-saving. Treatment modalities for inherited metabolic diseases include removal of toxic metabolites, dietary restrictions, enzyme replacement therapy, enhancement of enzyme activity with cofactors, chaperone therapies, stem cell or solid organ transplantation, and gene therapies (Fidan et al., 2022; Lampret et al., 2020; Lüders et al., 2021; Wilson & Jungner, 1968). Today, many types of metabolic diseases can be detected at an early stage from a dry blood sample and patients have the chance for early treatment.

Table 6. Inherited metabolic diseases according to the affected substrate (Aslı İnci, 2021; Ezgu, 2016)

Disorders of amino acid and peptide metabolism
Carbohydrate metabolism disorders
Disorders of fatty acid and ketone metabolism
Energy metabolism disorders
Disorders of lipid and lipoprotein metabolism
Lysosomal diseases
Sterol metabolism disorders
Porphyrin and heme metabolism disorders
Congenital glycosylation and other protein modification disorders
Neurotransmitter metabolism disorders
Peroxisomal diseases
Disorders of purine, pyrimidine and nucleotide metabolism
Disorders in the metabolism of vitamins and cofactors
Disorders in the metabolism of trace elements and metals
Disorders and variants in xenobiotic metabolism

Table 7. Inherited metabolic diseases according to clinical findings (Aslı İnci, 2021; Çoker, 2018; Ezgu, 2016; Kılıç, 2021; Loeber et al., 2021)

Intoxication type metabolic diseases	
After a symptom-free period, there are rapidly progressing clinical findings. It usually occurs as a result of accumulation of toxic substrate. The age at which symptoms begin varies depending on the degree of enzymatic activity. If not recognized and treated early, permanent damage or death may occur. Catabolic processes such as hunger, infection, and trauma may trigger the event. Some diseases may not cause acute symptoms.	**Organic acidemias** • Propionic acidemia • Methylmalonic acidemia • Isovaleric acidemia • Glutaric acidemia type I • Other **Urea cycle disorders** Ornithine transcarbamylase deficiency Argininosuccinate synthetase deficiency Carbamoyl phosphate synthetase deficiency type I Argininosuccinate lyase deficiency N-acetyl glutamate synthetase deficiency Other **Amino acid metabolism disorders** Phenylketonuria Maple syrup disease Tyrosinemia Homocystinuria Other **Carbohydrate metabolism disorders** Galactosemia Hereditary fructose intolerance Other
They are metabolic diseases caused by the inability to obtain or use energy	
They are basically diseases of mitochondria, the organelle where energy metabolism takes place. It usually occurs as a result of product deficiency. There is multisystem involvement	**Carbohydrate metabolism disorders** • Glycogen storage disease type Ia • Glycogen storage disease type Ia • Other **Mitochondrial diseases** • Respiratory chain disorders • Krebs cycle disorders • Other **Fatty acid oxidation disorders** • Medium-chain acyl-CoA dehydrogenase deficiency • Short-chain acyl-CoA dehydrogenase deficiency • Carnitine palmitoyltransferase deficiency type I • Carnitine palmitoyltransferase type II Other
Storage disorders of a complex molecule	
It usually occurs with chronic accumulation as a result of defects in the catabolism of complex molecules There may be signs of 'hydrops fetalis' in the newborn period May present with multiple system involvement	**Lysosomal diseases** • Mucopolysaccharidoses • Oligosaccharidoses • Sphingolipidoses • Lysosomal membrane carrier protein deficiencies **Peroxisomal diseases** • Peroxisomal biogenesis disorders • Peroxisomal single enzyme deficiencies • Peroxisomal membrane transporter protein deficiencies Congenital glycosylation disorders Other

Newborn Screening in Metabolic Diseases

Congenital metabolic diseases are highly heterogeneous in phenotype and genotype. Advances in genetic sequencing technologies and genomic studies have made it possible to identify rarer diseases (Kılıç, 2021; Stark & Scott, 2023). Partial or complete deficiency at the level of enzymes, cofactors, and transport in metabolic pathways occurs depending on the variant in the gene involved. Clinical findings are characterized by the accumulation of toxic substrates, formation of by-products, and reduction of the main product. It is mostly inherited in an autosomal recessive manner, but other forms of inheritance are also observed. Although the incidence varies between countries, the total incidence is more than 1:1500. It can be seen at any age and in any race. Clinical manifestations are non-specific. Different findings such as epilepsy, respiratory alkalosis, metabolic acidosis, hyperammonemia or hypoglycemia may occur. If left untreated, it can lead to irreversible physical and/or mental disabilities and even death. Therefore, newborn screening programs are crucial to recognize the disease before clinical signs appear (Kılıç, 2021).

Detection and treatment of diseases before they cause clinical symptoms and damage to the organism is very important in terms of preventive medicine. Clinical findings and neurodevelopmental evaluation results are better in patients who are diagnosed and treated early (Kılıç, 2021; Landau et al., 2017).

In screening, radioimmunoassay, enzyme-linked immunoassay, and fluoroimmunoassay are used for the diagnosis and screening of congenital hypothyroidism, congenital adrenal hyperplasia, glucose 6-phosphate dehydrogenase deficiency (G6PD), biotinidase deficiency and galactosemia. Immunoassays are used for congenital adrenal hyperplasia, congenital hypothyroidism, and cystic fibrosis. The enzymatic reaction 'fluoroimmunoassay' is used for phenylketonuria. MS/MS (tandem mass spectrometry) technique is used for fatty acid oxidation disorders, amino acid and organic acid levels, and acylcarnitine profile (can be used to diagnose more than 50 congenital metabolic disorders). There are recommendations to include Duchenne muscular dystrophy, some lysosomal storage diseases, peroxisomal diseases, severe combined immunodeficiency, guanidinoacetate methyl transferase (GAMT) deficiency, and Wilson's disease in screening programs. While metabolites, enzymes, and molecular analyses can be examined in screening tests, DNA-based tests such as next-generation sequencing and Sanger sequencing are mostly used for confirmatory purposes. Preconceptional carrier screening in Ashkenazi Jews includes Tay-Sachs disease, Gaucher, Canavan, Niemann-Pick type A, and Mucolipidosis type IV. Inherited metabolic diseases are most commonly diagnosed postnatally through expanded newborn screening (Kılıç, 2021; Landau et al., 2017; Loeber et al., 2021; Wang et al., 2019).

Newborn screening for phenylketonuria and congenital hypothyroidism is almost universal. Tandem mass spectrometry (MS/MS) was developed in the early 1990s. Many well-characterized genetic disorders can be screened in a dried blood sample using MS/MS at a lower cost. The number of disorders covered by expanded newborn screening has increased according to countries' level of development, disease incidence, and cost. In the United States, Europe, and some parts of Asia, many screenings can be performed with MS/MS (Kılıç, 2021; Tezel et al., 2014; Therrell et al., 2015).

The screening test should have high specificity, sensitivity, and predictive efficiency (true positive/false positive rate) for diseases. The screening laboratory should have reference ranges, cut-off values, and ratios for the metabolites screened (Kılıç, 2021). For example, studies conducted in Greece and China at different times identified some of the diseases that can be screened and the thresholds in their countries (Loukas et al., 2010; Wang et al., 2019).

Newborn screening programs and expanded newborn screening programs have some limitations. False positive results can lead to unnecessary further testing and unnecessary family anxiety. False negative results can cause cases to be missed, leading to late diagnosis and delayed treatment. There may be hesitation to start treatment due to the wide clinical spectrum or unpredictable clinical course of some metabolic diseases. There may be false positive and negative results due to secondary causes, or they may originate from the mother. Therefore, when abnormal values are obtained in the test results, the mother should also be evaluated. In some diseases, different tests may be required, e.g. in tyrosinemia type 1, succinylacetone level instead of tyrosine level, and in homocystinuria, homocysteine level instead of methionine level. In glutaric aciduria type 1, low excretion can lead to false negative results and missed cases. In diseases with acute intoxication, clinical findings occur before screening results are obtained (Table 2). For this reason, some benign diseases have been excluded from the expanded screening programs (Kılıç, 2021).

It is estimated that at least 25 million newborns are screened for MS/MS each year worldwide. In cases where positive results are obtained using newborn screening dry blood kits, second-stage tests with Mass Spectrometry are performed for confirmation. In people with high propionyl carnitine levels, free methylmalonic acid and propionic acid in the blood are checked. Homocysteine levels can be measured in patients with increased methionine (Homocystinuria) or low methionine (MTHFR); 17-hydroxyprogesterone, cortisol, and androstenedione levels can be measured in patients with high 17-hydroxyprogesterone levels. Many such tests can be performed as second-step tests with Mass Spectrometry (Kılıç, 2021; Millington, 2024; Schnabel et al., 2023).

Today, next-generation sequencing (NGS) techniques are used in newborn screening programs in many countries for validation purposes. With NGS tech-

niques, many metabolic and endocrinologic diseases can be screened with a single test. NGS techniques are independent of secondary events affecting metabolite levels. NGS techniques can also screen for diseases without screening biomarkers (spinal muscular atrophy, cystinosis, Wilson's disease, etc.). NGS is recommended as a first-line test in screening programs for diseases such as SMA, Fragile-X syndrome, and DMD. Variants of uncertain significance may also cause diagnostic difficulties. Therefore, genetic and metabolic screening is utilized for the most accurate and reliable results. It would be more useful to perform and evaluate the tests together (Hogner et al., 2023; Kılıç, 2021)

Newborn Screening Tests for Inherited Metabolic Diseases in Turkey

Phenylketonuria and Phenylketonuria Screening Test

Phenylketonuria is caused by a deficiency of the enzyme phenylalanine hydroxylase. It is an inherited metabolic disease. If untreated, phenylalanine accumulates in the blood, brain, and other body tissues. It results in neurocognitive and behavioral problems, severe intellectual disability, epilepsy, and movement disorders in children. Treatment for phenylketonuria is to limit the amount of phenylalanine in the diet. The goal of phenylalanine limitation is to maintain plasma phenylalanine levels in the range of 2-6 mg/dL (120-360 μmol/L) and to ensure normal growth. This phenylalanine-restricted diet also includes the consumption of medical formulas and foods that provide phenylalanine-free protein and other essential macro- and micronutrients in the form of L-amino acids. Other treatment options include the use of glycomacropeptides, large neutral amino acid supplementation, pharmacotherapy with sapropterin dihydrochloride, and enzyme substitution therapy with pegvaliase depending on the clinical condition, laboratory and PAH gene mutation (Burcu Öztürk Hişmi, 2021; Coşkun et al., 2022).

In 1934, Asbjörn Folling established the first knowledge about phenylketonuria (PKU). In 1953, Horst Bickel discovered effective diet therapy. PKU screening began in 1960 with a test developed by Robert Guthrie and Ada Susi. In Turkey, PKU screening was initiated in 1983 by the Metabolism Unit of Hacettepe University as part of a TUBITAK-funded project. In 1993, it became widespread and as of December 2006, a national newborn screening program was initiated across the country under the supervision of the Ministry of Health. The prevalence of PKU varies around the world. In Europe, the average prevalence of PKU in newborns is approximately 1:10000. The prevalence of PKU in Turkey is 1:6228 in newborns. Turkey is the country with the highest prevalence of PKU in the world. In Turkey,

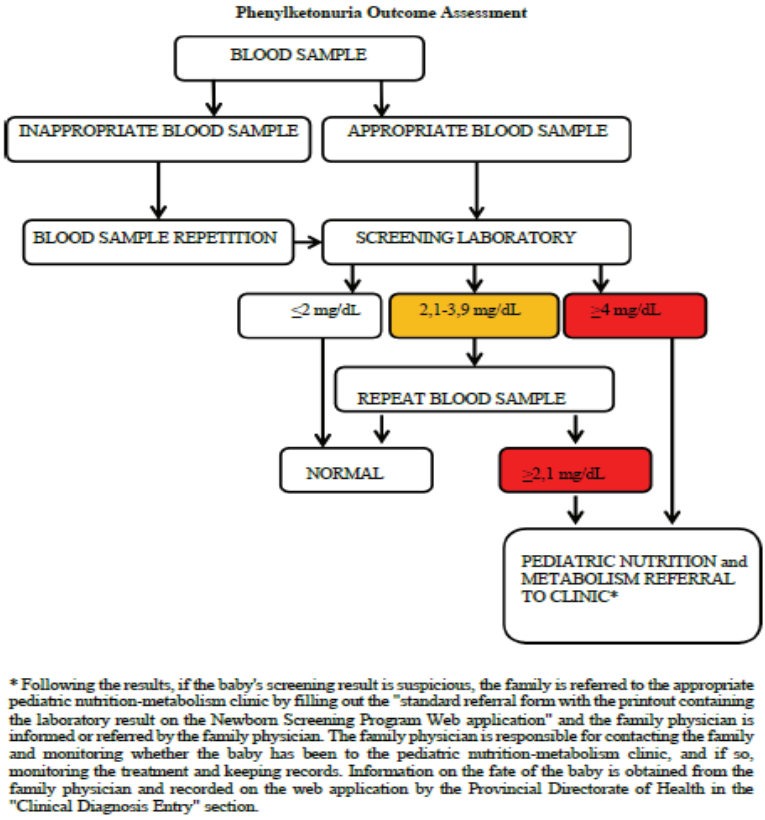


Figure 1. Phenylketonuria flow chart within the context of the Ministry of Health newborn screening program (https://hsgm.saglik.gov.tr/tr/tarama-programlari/ntp.html#)

4 out of every 100 people are carriers of PKU. The negative effects of PKU can be prevented with early diagnosis of the disease and appropriate dietary treatment. (Coşkun et al., 2022; https://hsgm.saglik.gov.tr/tr/tarama-programlari/ntp.html#; Kılıç, 2021). Phenylalanine level is measured in a heel blood sample. According to the Ministry of Health FKU flow chart (Figure 1). If the phenylalanine level is ≥4 mg/dl, the sample is not repeated and the patient is referred directly to the pediatric metabolism department. If the phenylalanine level is ≤2 mg/dl or 2.1-3.9 mg/dl, the sample is repeated, and if the repeated sample is ≥2.1 mg/dl, the patient is referred to the pediatric nutrition and metabolism clinic. In the pediatric nutrition and metabolism clinic, the patient's plasma phenylalanine level is checked (https://hsgm.saglik.gov.tr/tr/tarama-programlari/ntp.html#).

Biotinidase Deficiency and Biotinidase Deficiency Screening Test

Biotin is one of the B vitamins. It acts as a coenzyme in many places and is involved in fatty acid synthesis, gluconeogenesis, and amino acid catabolism. The biotinidase enzyme releases and recycles biotin. If biotinidase enzyme deficiency is not treated, permanent vision and hearing loss occur. There may be serious neurological, neurodevelopmental, dermatological, and immunological problems. In this metabolic disease, the best results are obtained with early treatment. The first newborn screening program for biotinidase deficiency was initiated as a pilot study in Virginia in 1984. Since 1984, it has been included in newborn screening programs in many countries around the world (Canda et al., 2020; Heard et al., 1986; Jay et al., 2015). The global prevalence of biotinidase deficiency is 1:61067 (Wolf, 1993). In Turkey, biotinidase deficiency has been included in the newborn screening program since 2008 (Canda et al., 2020). Its incidence in our country is approximately 1:7166. Biotinidase activity on dried blood spots is measured by a colorimetric method, the results are not related to dietary protein intake. Prematurity, incorrect sample collection, high albumin levels, high triglyceride levels, and valproate therapy cause false positive results. Sulfa drugs, biotinyl-6-aminoquinoline may cause false negative results. (Çoker, 2018; Sürücü Kara et al., 2023). Biotinidase enzyme activity is measured with a sample taken from the heel blood. In our country, screening results are evaluated by the biotinidase flow chart of the Ministry of Health. If the baby's biotinidase enzyme activity in dry blood is ≤ 65 U, a repeat blood sample is taken, and if the repeat blood sample is also ≤ 65 U, the baby is referred to the pediatric nutrition and metabolism clinic (Figure 2). The diagnosis is made by serum/plasma biotinidase follow-up enzyme measurement and confirmation by genetic analysis. Oral biotin 5-10 mg/day is recommended for those with serum biotinidase enzyme activity <10% and 2.5-10 mg/day for those with biotinidase enzyme activity 10-30% (https://hsgm.saglik.gov.tr/tr/tarama-programlari/ntp.html#).

Neonatal Screening Tests for Inherited Metabolic Diseases in different countries

In some countries, the screening test for tyrosinemia, branched-chain amino acid metabolism disorders, urea cycle disorders, the screening test for carbohydrate metabolism disorders (the most important disease included in the screening program in this group is galactosemia), the screening test for mitochondrial diseases, lysosomal storage diseases tests such as tests are applied as extended neonatal screening tests. Expanded neonatal screenings vary in parallel with the development level of countries in terms of disease subgroups and numbers. The screenings carried out in some countries are given in Table 6. GAMT enzyme screening (to detect creatine metabolism disorders), mutation screening in the SLC25A13 gene (to detect citrine deficiency), glutaryl carnitine screening (to screen for glutaric aciduria type 1 disease), very long chain fatty acid

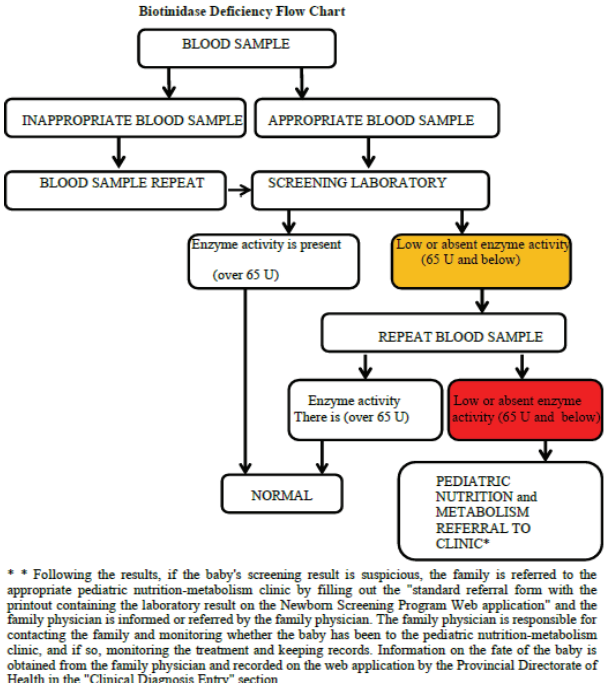


Figure 2. Biotinidase deficiency flow chart within the context of the Ministry of Health newborn screening program (https://hsgm.saglik.gov.tr/tr/tarama-programlari/ntp.html#)

(C26:0) screening (to detect adrenoleukodystrophy due to X linked). With the results obtained; It has been shown that with early diagnosis, disease management becomes easier and the prognosis shifts to a very positive direction (Çoker, 2018; Loeber et al., 2021; Sürücü Kara et al., 2023).

Newborn Screening in Endocrine Diseases

Congenital Hypothyroidism

Thyroid hormones play a role in the endocrine system, central nervous system, circulatory system, hematopoietic system, gastrointestinal system, skeletal system, sympathetic system, basal metabolic system, and thermogenesis. Thyroid hormones are important for normal growth and development in every period of life, starting from the fetal period. Congenital hypothyroidism is the most common endocrinopathy worldwide. The prevalence of congenital hypothyroidism in the population is approximately 1:2000-4000.

The incidence of congenital hypothyroidism varies according to national income levels and geographical regions. Diagnosis and treatment may be delayed because clinical findings are often unclear in the neonatal period. If untreated, permanent mental retardation and developmental delay occur (IQ: measured as 64-107 in those who started treatment before three months, 35-96 in those who started treatment between 3-6 months, and 25-80 in those who started after 6 months). More than 90% of congenital hypothyroidism is caused by primary congenital hypothyroidism (Doğuş Vurallı, 2021; Liu et al., 2023; Selim Kurtoğlu, 2017). By measuring blood thyroxine (T4) concentrations in nanomoles per liter by radioimmunoassays, congenital hypothyroidism was detected in a newborn blood sample. It then developed a method for measuring total T4 in dried blood spots. A few years later, a method for the measurement of Thyroid Stimulating Hormone was developed (Boelen et al., 2023). Measurement of T4 level is not specific for the detection of central congenital hypothyroidism. T4+TSH or T4-reflex TSH screening has been abandoned in most countries in the world due to false positive results and improvement in the quality of TSH measurement. Central congenital hypothyroidism cannot be detected with TSH level measurement. Today, in most countries in the world and our country, TSH is screened from heel blood for congenital hypothyroidism (Boelen et al., 2023; Doğuş Vurallı, 2021; Draznin et al., 2022; İçke & Genç, 2017; Murat Aydın, 2017; Selim Kurtoğlu, 2017). Immunoassay methods are used for T4 and TSH level measurement (ELISA, IUD, FIA, etc.). T4 levels can also be measured by MS/MS technology (Doğuş Vurallı, 2021). Screening for congenital hypothyroidism was started in the Province of Quebec in 1974, T4 analysis was performed on samples taken from PKU screening papers, and TSH was checked in the following period. Japan was the first country to implement national congenital hypothyroidism screening using the TSH value in 1979. Before heel scans, 10% of babies could be diagnosed with congenital hypothyroidism in the first month. 35% could be diagnosed with congenital hypothyroidism in the first three months. Unfortunately, a total of 70% could be diagnosed in the first year of life and 30% could be diagnosed at the age of 3-4 years. In our country, while 3% were diagnosed in the neonatal period, the age of diagnosis was over 24 months in 55.4%. Today, congenital hypothyroidism screening covers 29% of all babies in the world and approximately 800 000 newborns are screened annually in our country (Doğuş Vurallı, 2021; Draznin et al., 2022; Murat Aydın, 2017; van Trotsenburg et al., 2021). In our country, newborn screening for congenital hypothyroidism started as a local programs in the 1990s. In 2006, the National Newborn Screening Program conducted by the Ministry of Health aimed to reach newborns throughout the country. In Turkey today, 97% of live births are reached through the newborn screening program (Doğuş Vurallı, 2021).

The number of infants referred for newborn screening and diagnosed with the disease in 2022 by the Turkish Statistical Institute is shown in Table 8 (https://

Table 8. Number of babies referred by the newborn screening program and diagnosed with the disease according to years in the data of the Turkish Statistical Institute (https://hsgm.saglik.gov.tr/depo/birimler/cocuk-ergen-sagligi-db/Dokumanlar/Istatistikler/NTP.pdf).

Years	Phenylketonuria		Congenital Hypothyroidism		Biotinidase Deficiency		Cystic Fibrosis		Congenital Adrenal Hyperplasia		SMA+
	Number of referrals	Number of patients	Number of referrals	Number of patients	Number of referrals	Number of patients	Number of referrals	Number of patients	Number of referrals	Number of patients	Number of patients
2013	2039	188	19548	2702	580	369	-	-	-	-	-
2014	1824	190	13720	341	861	504	-	-	-	-	-
2015	1674	196	13634	3606	1881	864	3123	143	-	-	-
2016	2225	191	12068	3303	2060	991	5737	152	-	-	-
2017	2085	183	9646	2899	2441	970	3306	117	338	8	-
2018	2331	186	8824	3142	3722	1934	2674	107	367	27	-
2019	2714	168	8217	3116	3686	1824	2949	123	229	23	-
2020	2540	178	10454	3100	4321	1966	3534	114	668	59	-
2021	2508	196	11396	3316	3185	1637	3236	126	1853	104	-
2022	2237	164*	8489	2867*	2611	1156*	1712	63*	3065	123*	108

SMA+: Spinal Muscular Atrophy, *Patient numbers for 2022 are not exact figures as the diagnostic process is ongoing

hsgm.saglik.gov.tr/depo/birimler/cocuk-ergen-sagligi-db/Dokumanlar/Istatistikler/NTP.pdf). The measurement methods used for screening for congenital hypothyroidism are T4 measurement in heel blood followed by TSH measurement, simultaneous T4 and TSH measurement, T4 or TSH measurement in cord blood, and TSH measurement. Screening with more than one test can be done to capture both primary and central congenital hypothyroidism, but it also increases the false positive rate. The sensitivity and specificity of screening for congenital hypothyroidism with TSH measurement have been reported to be 97.5% and 99%, respectively (Doğuş Vurallı, 2021). False-negative results may occur in low birth weight babies, premature babies, and sick babies, in conditions that delay TSH elevation. Therefore, it is recommended to repeat the sample after 10-14 days. In babies with Down syndrome, TSH should be measured at the end of the neonatal period, a second scan should be considered in same-sex twins, and the unaffected two should be followed up due to elevated TSH in the later periods. Further evaluation for primary and central congenital hypothyroidism should be recommended in children with clinical suspicion of hypothyroidism and a family history of central congenital hypothyroidism, even if TSH is normal in the screening tests (Doğuş Vurallı, 2021; Draznin et al., 2022; Murat Aydın, 2017; van Trotsenburg et al., 2021). Within the scope of the Ministry of Health newborn screening program, screening results are evaluated according to the Congenital Hypothyroidism flow chart (Figure 3). Babies who screen positive for congenital hypothyroidism should be evaluated by a pediatric endocrinologist. Serum TSH and free T4 levels should be checked and the diagnosis confirmed. Thyroxine treatment (10-15 μg/kg) should be started immediately. Serum free-T4 and TSH should be repeated in the follow-up two weeks after starting treatment. The type of dysgenesis must be distinguished (by ultrasonography or technetium 99 or iodine 123) (Draznin et al., 2022; van Trotsenburg et al., 2021).

Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia is a group of autosomal recessive disorders leading to multiple hormonal imbalances. Congenital adrenal hyperplasia is a deficiency of some enzymes in the adrenal-steroidogenic pathway. Causes of congenital adrenal hyperplasia: 21 hydroxylase deficiency, 11 beta-hydroxylase deficiency, P450 oxidoreductase deficiency, 17α-hydroxylase/17,20 lyase deficiency, 3 beta-hydroxysteroid dehydrogenase deficiency, steroidogenic acute regulatory protein deficiency, P450 cholesterol side chain cleavage enzyme deficiency (Claahsen-van der Grinten et al., 2022). The most common cause of congenital adrenal hyperplasia is 21-hydroxylase deficiency. 21-hydroxylase

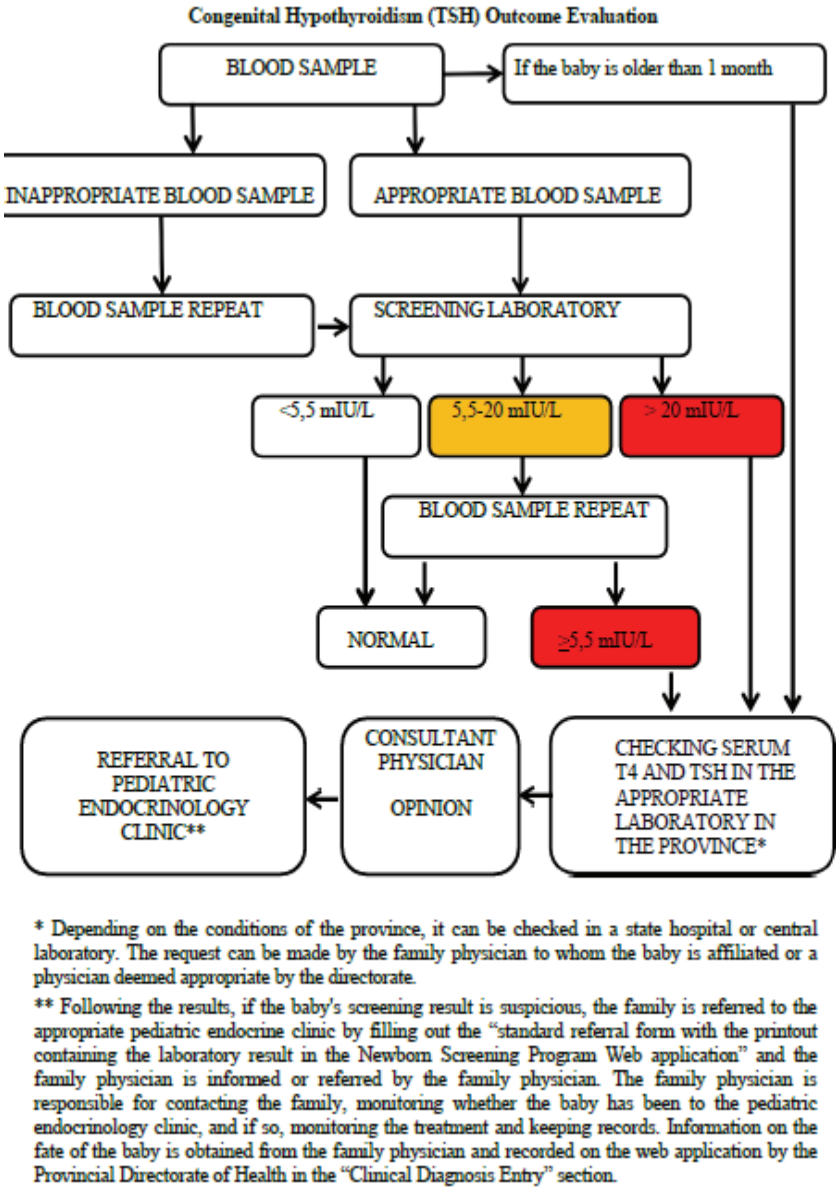


Figure 3. Congenital Hypothyroidism flow chart within the context of the Ministry of Health newborn screening program (https://hsgm.saglik.gov.tr/tr/tarama-programlari/ntp.html#)

deficiency is caused by mutations in the CYP21A2 gene. The clinical spectrum of 21-hydroxylase deficiency is broad. The classic (severe) form of 21OH deficiency causes adrenal crises (life-threatening), and atypical genitalia in 46 XX karyotypes. The mild form (non-classical form) is not characterized by glucocorticoid deficiency. The mild form is more common (Auer et al., 2023). Prevalence varies from country to country (Table 1) (Claahsen-van der Grinten et al., 2022). The first assumed case of congenital adrenal hyperplasia was reported in 1865. In 1950, cortisone was first used in the treatment of congenital adrenal hyperplasia. Congenital adrenal hyperplasia was first screened in newborn infants for adrenal crisis (life-threatening) in boys in 1977. It is now included in national newborn screening programs in many rich countries. 17-OH progesterone levels have been measured. In 1987, with newborn screening programs in France, Japan, Scotland, the United States of America, and New Zealand, the incidence of salt loss in this disease was reported as 1:18921, simple forms of virilization as 1:63068 live births, and the girl/boy ratio was found to be similar. In the United States of America, newborn babies have been screened since 2015 within the scope of the expanded newborn program since 2005. Currently, the incidence of classical congenital adrenal hyperplasia in most populations is 1:16000-18000. Neonatal survival rates in patients with congenital adrenal hyperplasia are currently high as a result of early detection through newborn screening programs (Auer et al., 2023; Claahsen-van der Grinten et al., 2022; Draznin et al., 2022). In first-line screening for congenital adrenal hyperplasia, 17-hydroxyprogesterone (17OHP) level is determined by immunoassay (mostly lanthanide fluoroimmunoassay). Limitations of this technical method: Poor diagnostic accuracy in the first two days (17OHP is high at birth in healthy babies and then decreases, low in sick babies and then increases). 17OHP is lower in girls than boys (salt-losing female babies are recognized because they are virilized), false positivity may occur in premature babies and sick babies. False negativity may occur if the mother used corticosteroids in the intrauterine period. Therefore, the test should be repeated after 72 hours and second-line tests should be performed. The second step test should be performed using liquid chromatography-mass spectrometry (LC-MS/MS). If the LC-MS/MS method is not available, an ACTH stimulation confirmation test should be performed. Due to consanguineous marriages in our country, it is common enough to be included in the screening program. For this reason, approximately 40000 babies were screened in Kayseri, Konya, Adana, and Samsun in the first pilot study in 2017. Two-step screening was performed in a single heel prick. In this pilot study in 2017, the frequency of congenital hyperplasia in infants with (21-decocycortisol+17 hydroxyprogesterone)/cortisol positivity ratio=0.5 or >0.5 values was determined as 1:7787. In the pilot study conducted in 14 provinces where 241083 infants were screened in 2018, the prevalence of classical congenital hyperplasia in infants with (21-decocycortisol+17 hydroxyprogesterone)/cortisol positivity ratio=0.7 or >0.7

was determined as 1:15067. Since 2022, all newborn babies in our country have been screened for congenital adrenal hyperplasia by the flow chart determined by the Ministry of Health. If the 17 hydroxyprogesterone level is >50ng/mL in the first-line screening, the patient is referred to the pediatric endocrinology department without waiting for the second-line screening results (Güran, 2021). Infants with a confirmed diagnosis of congenital adrenal hyperplasia are started on emergency treatment with cortisol 10-15 mg/m2/day, divided into three doses, mineralocorticoids (fludrocortisone) 0.05-0.2 mg/day and salt supplementation (1-2 g (17-24 mEq/day). The family is given adrenal crisis management training during stress and illness and 112 emergency call training. Imaging is performed in terms of internal genital anatomy and sexual differentiation disorders and specialist surgeons are consulted. Follow-up by endocrinology (Claahsen-van der Grinten et al., 2022; Draznin et al., 2022). The flow chart of the Ministry of Health's newborn screening program according to diseases is given in Figure 4.

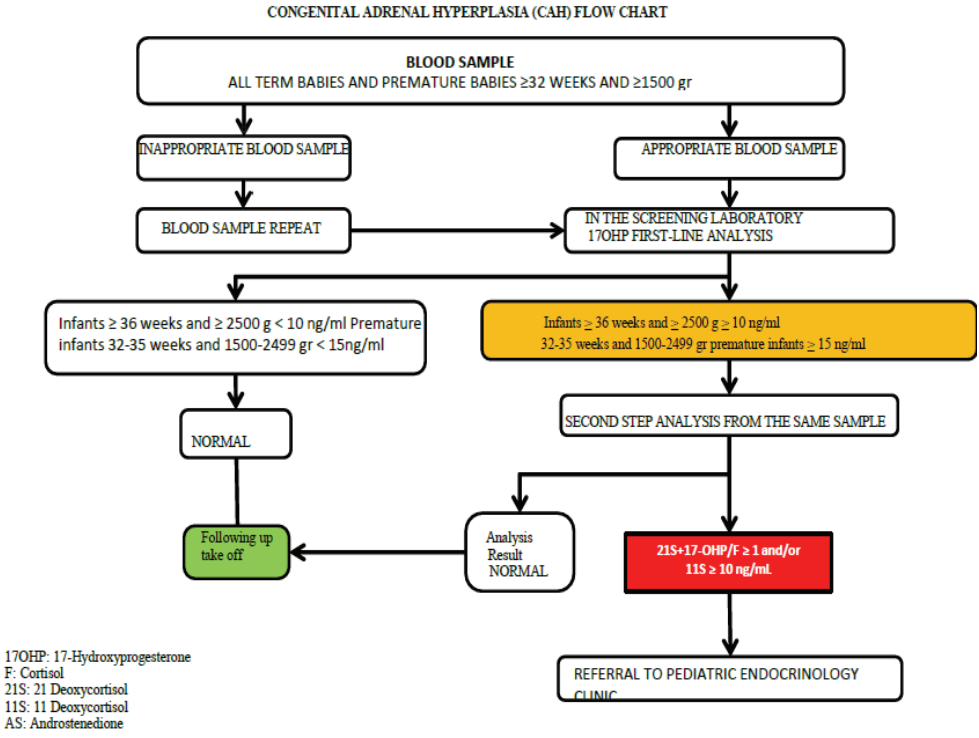


Figure 4. Congenital Adrenal Hyperplasia (CAH) Flow Chart flow chart within the context of the Ministry of Health newborn screening program (https://hsgm.saglik.gov.tr/tr/tarama-programlari/ntp.html#)

Conclusion

Thanks to newborn screening programs and advanced screening programs in our country and the world, thousands of children can be diagnosed and treated early. The number of screened diseases increases depending on the development and economic status of countries. Studies in some countries are promising for more accurate identification of patients through molecular analysis. In our country, the national newborn screening program should be sensitive to false positivity or false negativity in metabolic diseases and endocrinological diseases. Attention should be paid to all stages of the screening program to minimize inaccuracies. Since the incidence of rare diseases increases in consanguineous marriages, the public should be made aware of this issue through public service announcements. It is hoped that the diseases in the national newborn screening program in our country will be expanded to detect treatable rare diseases, thus reducing the mortality and morbidity of infants. Studies on diseases that can be added to the national screening panel are ongoing in our country.

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Chapter

2

Neonatal Screenings for Congenital Hypothyroidism

Burçe ORMAN, Semra ÇETİNKAYA

Primary congenital hypothyroidism (CH) is one of the endocrine emergency problems in which thyroid hormone (TH) synthesis of the thyroid gland in fetal life and at birth is affected, causing preventable mental retardation. The frequency of this situation is 1:2000-1:4000 in newborns (Ford & LaFranchi, 2014; Rastogi & LaFranchi, 2010). There are permanent (persistent) and temporary (transient) causes of CH. In some babies, thyroid hormone (TH) replacement treatment is started with the diagnosis of CH, but over time the need for treatment decreases and medication discontinuation may be possible (Grosse & Van Vliet, 2011; Nettore et al., 2013; Rastogi & LaFranchi, 2010; Rose et al., 2023). This temporary CH condition most often develops due to iodine deficiency, which is a common global problem. Transient CH is more common than permanent CH. Permanent CH is often seen and the cause is TH synthesis or gland development disorder. Less common than primary CH, central CH originating from the hypothalamus and pituitary gland is observed(Grosse & Van Vliet, 2011; Nettore et al., 2013; Rastogi & LaFranchi, 2010; Rose et al., 2023). Most babies with CH do not show clinical signs at birth because there is some thyroid reserve and there is hormone transfer from the mother through the placenta (Vulsma et al., 1989). For this reason, the diagnosis is usually made after three months, and in this case, the neurological effects on the brain due to TH deficiency may not be reversible. In a study conducted in Denmark

in the 1970s, 10% of patients were diagnosed in the first month of life and 70% in the first year of life (Jacobsen & Brandt, 1981). In a study from Turkey, the mean age of diagnosis was 49 months, 3.1% were diagnosed in the first month of life, and 55.4% were diagnosed after 2 years of age (Tarim & Yordam, 1992). Therefore, early diagnosis of CH and early initiation of TH replacement therapy are necessary to prevent mental retardation. CH is included in the scope of screening in the neonatal period.

Historical of Screening for CH

Primary CH has been known since ancient times. Statues of 'goitrous dwarfs' were found in South America in the 400s BC. Documents describing mental retardation and goitrous hypothyroidism in ancient Rome in the 1st century. A document about goiterous hypothyroidism belonging to Paracelsus was found in the 6th century. In newborns with CH, there are either no or very few clinical findings. Therefore, it is difficult to predict which newborns are affected by this disease. Due to this situation; The description of non-goitrous congenital hypothyroidism was made by Thomas Curling in 1850, at the beginning of the Industrial Revolution, long after the definition of goitrous CH. At the University of Toronto in the 1960s, Volpe studied fetal and neonatal thyroid physiology and laid the foundation for studies on the placental transfer of T3 in humans. During the same period, Chopra developed the RIA method for T3 and T4.In the same period, Raiti, Newns, Klein et al. treated a three-month-old newborn with CH and reported that he had normal intelligence. In 1973, the Quebec Genetics Department screened 30 thousand newborns in heel blood samples taken during phenylketonuria screening and detected 4 newborns with CH, which laid the foundation of the newborn screening program. Since April 1974, thyroxine (T4) or thyrotropin (TSH) measurement in heel blood has begun to be performed in Quebec to diagnose newborns with CH early and start treatment early (Auray-Blais et al., 2003). In the second half of the 20th century, enzymatic defects in hormoneogenesis were identified and different types of familial dyshormonogenesis were revealed. In the 1990s, the extracellular region of the thyrotropin receptor, the beta subunit of TSH, and transcription factors that regulate thyroid embryogenesis were identified; rarer causes of CH have been identified.

Newborn Screening Programs for Congenital Hypothyroidism

Thyroid function tests, which have been added to newborn screening programs (NBS) since the 1970s, have enabled the diagnosis and treatment of affected infants and reduced neurodevelopmental impairment (Dussault et al., 1975). Screening for

hypothyroidism is not available in all countries, with 70% of newborns worldwide not being screened(Rose et al., 2023). According to the data of the countries where hypothyroidism screening is performed (South America, North America, Europe, Australia, Africa, and parts of Asia), the incidence of CH is about 1 in 2000 to 4000 (Rogan et al., 2014). In the first years of NBS, the incidence was found to be higher (about 1 in 4000) because milder CH cases were detected due to the screening strategy(Aleksander et al., 2018; Schoelwer et al., 2017; Tzifi et al., 2022). In addition, genetic factors may also have an impact on this situation because CH is more common in some populations (Tuli et al., 2022).

NBS aims to identify all patients in the shortest time and at the least cost. Recently, more sensitive methods (chemiluminescence, fluoroimmunoassay, etc.) have begun to be used for the detection of TSH and T4 from dried blood spots (DBS) (Evans et al., 2011; John et al., 1997; Miyai et al., 1980; Najafi et al., 2011; Torresani & Scherz, 1986; Tuuminen et al., 1993). These new methods have enhanced sensitivity and specificity in the detection of CH. Despite more sensitive testing methods, approximately 5% of cases are still missed. Reasons for this may include failure to collect samples, inadequate samples, misinterpretation and inadequate recall of samples, subclinical status, or defect to detect babies with central CH for programs that measure TSH only(Evans et al., 2011; Fisher, 1983; LaFranchi et al., 1985; Najafi et al., 2011).

The dried blood spot is placed on a filter paper and transmitted to the responsible laboratory. The recommended sampling time is the first 48-72 hours. Taking samples before this time may cause false positivity due to physiological TSH increase (Meyer et al., 2020; Virili et al., 2016; Zeitler & Solberg, 2010). Early postpartum discharge approximately doubled the rate of false-positive TSH elevations (Walfish et al., 1979).

Test Strategies and Results

There are three testing methods used to screen for CH. These are primary TSH, primary T4, and combined T4 and TSH measurements. All methods determine moderate to severe primary CH at similar rates (Ford & LaFranchi, 2014). The primary TSH method is used in most countries. Some results are reported in whole blood units and some results are reported in serum units. The result reported in serum units is approximately 2.2 times the value of whole blood(Selva et al., 2005).

Primary TSH Screening

With this method, TSH is first measured from the sample, and if TSH is found to be higher than the threshold value (there are age-specific TSH thresholds), total T4 is

measured. If there is an abnormal result (high TSH or low T4), a confirmatory test is performed or a second sample is taken(Jacoba J Bongers-Schokking & de Muinck Keizer-Schrama, 2005; Weichsel, 1978). Primary TSH screening is more likely than primary T4 screening to detect mild cases of CH but cannot determine infants with delayed TSH elevation (premature or low birth weight) and infants with central CH. The recall rate is lower compared to primary T4 screening (Korada et al., 2010).

While the recall rate is 0.05% when 15 mU/mL is used as the cut-off point in the primary TSH method (immunofluorometric method), this rate increases to 3% since false positivity will increase in case of iodine deficiency. The sensitivity of the TSH method for CH is 97.5% and the specificity is 99%. Most screening programs use a threshold value above 20 to 25 mU/mL to report an increase in TSH. Infants with baseline TSH >50 mU/mL often experience permanent CH, while those with TSH levels between 20 and 49 mU/mL often experience false positivity or transient hypothyroidism(Büyükgebiz, 2003; Deladoëy et al., 2011). As a result, while the primary TSH method determines primary hypothyroidism, it overlooks infants with central hypothyroidism, thyroxine-binding globulin (TBG) deficiency, and delayed TSH increase (premature or low birth weight) (Büyükgebiz, 2013).

Primary T4 Screening

With this method, total T4 is measured from the sample. FT4 is generally not measured for technical problems. If the total T4 concentration is low (in the 10th percentile of T4 values), TSH is measured from the same sample. If there is an abnormal result (low T4 or high TSH), a confirmatory test is performed or a second sample is taken. Primary T4 screening is less sensitive than primary TSH screening in detecting cases of mild primary hypothyroidism (elevated TSH-low/normal T4) but has greater potential to detect both neonates with central CH and cases with delayed TSH elevation. Babies at high risk for delayed TSH elevation (e.g. premature, low birth weight or critically ill newborn babies) also have low T4 values(Hanna et al., 1986; Nebesio et al., 2010). Some cases of central hypothyroidism may be overlooked due to not being recalled from screening, either because the T4 value is at the lower limit of normal or because the low T4 value is not accompanied by high TSH. These cases occur with additional pituitary hormone deficiencies in the later period (Nebesio et al., 2010). Similar to primary TSH screening, the recall rate for the primary T4 program is 0.3% (Hanna et al., 1986) As a result, the primary T4 method detects apparent primary hypothyroidism, central hypothyroidism, TBG deficiency, hypothyroxinemia in sick and premature neonates. However, it overlooks compensated hypothyroidism, in which subnormal T4 and high TSH levels (temporary TSH elevation due to iodine deficiency) are observed (Büyükgebiz, 2013).

Combined T4 and TSH Screening

This program is used in some countries. Both TSH and T4 are measured from the sample (Carswell et al., 2013; Paone et al., 2016). A confirmatory test is performed in the presence of low T4 or high TSH. This method can determine primary hypothyroidism (mild/severe) and congenital central hypothyroidism (Akcay et al., 2010; Lichtenberger-Geslin et al., 2013). Combined screening can also determine infants with delayed TSH elevation when a second sample is requested. The recall rate with this method is higher than primary TSH or primary T4 methods(Rose et al., 2023).

False Positive and False Negative Results

Infants who are positive according to the screening test but whose confirmation test is normal are defined as false positives. False-positive results may result from the timing of the initial screening test and the cutoff value of the tests used(Vurallı & Özön, 2021). Cases with a negative screening test and not recalled but with congenital hypothyroidism are defined as false-negatives, that is, missed cases. False-negative results may be due to disease-related causes, test methods, biological factors, and screening errors(Vurallı & Özön, 2021). Among biological factors, an important factor is sampling time. Recommended sampling time is 2-5 days. Due to the physiological increase in TSH at birth, TSH will be found to be high in samples taken in the first 24 hours and the recall rate will increase.For infants discharged early (within the first 24 hours), it is recommended to take a DBS before discharge and then repeat a second sample("Revised guidelines for neonatal screening programmes for primary congenital hypothyroidism. Working Group on Neonatal Screening of the European Society for Paediatric Endocrinology.," 1999; Rose et al., 2006). For infants whose screening sample is taken after the 5th day, the TSH reference range is chosen according to the age of the baby because TSH drops sharply after the first week of life (Kilberg et al., 2018). Transient hypothyroidism is common in prematures, especially in the area of iodine deficiency, and these infants may be overlooked in screening due to the delayed TSH increase. It is recommended to take DBS sample again in these infants (Hunter et al., 1998). In critically ill patients receiving dopamine, the screening sample should be obtained after the dopamine infusion has been discontinued for 30 minutes (de Zegher et al., 1995). In babies who will receive a blood transfusion, a blood sample should be taken before the transfusion. In same-sex monozygotic twins, a second screening sample is recommended due to twin-to-twin transfusion (Vurallı & Özön, 2021).

According to the recommendations of the Working Group of Neonatal Screening of the European Society for Pediatric Endocrinology (ESPE), screening should be guided

in centralized laboratories covering 100 000 newborns per year (Fisher, 1987; Matsuura et al., 1980; "Revised guidelines for neonatal screening programmes for primary congenital hypothyroidism. Working Group on Neonatal Screening of the European Society for Paediatric Endocrinology.," 1999).

Low T4 with Normal TSH

This result is found in central hypothyroidism, prematurity, euthyroid sick syndrome, and TBG deficiency (Baysal et al., 2017; Connelly et al., 2017; van der Sluijs Veer et al., 2012). Central hypothyroidism may be suspected in the presence of midline development defects, vision problems, and other additional findings related to pituitary hormone deficiency. In multiple pituitary hormone deficiency, hypothalamic-pituitary development defect is considered (Cherella & Wassner, 2017; Persani & Bonomi, 2017).

Low T4 value with normal TSH is been in 50% of premature infants born less than 30 weeks ("Revised guidelines for neonatal screening programmes for primary congenital hypothyroidism. Working Group on Neonatal Screening of the European Society for Paediatric Endocrinology.," 1999). The causes of hypothyroxinemia due to prematurity are not clear. Inadequate hypothalamic-pituitary maturation, low TBG levels, and declined conversion of T4 to T3 are among some of the reasons. A correlation has been found between the degree of low T4, and adverse outcomes such as death and developmental problems. Systematic support is not currently recommended for all low birth weight babies (Briët et al., 2001; Büyükgebiz, 2013). Immature liver function, malnutrition and disease, low T4 and normal TSH levels in euthyroid sick syndrome are the reasons. Euthyroid sick syndrome can also be seen in term patient newborns (Büyükgebiz, 2013). TBG deficiency is an X-linked case and can only be detected by screening programs using the primary T4 approach. Since plasma levels of free thyroid hormones are normal and the patients are euthyroid, no treatment is required(Hulse, 1984). TBG deficiency should be investigated and TBG levels should be measured in male infants with low T4 levels and normal TSH levels (Büyükgebiz, 2013).

High TSH with Normal T4

High TSH with normal or low T4 indicates insufficient hormone production. It is most commonly seen in premature babies. In some babies, hypothyroidism is compensated. In babies discharged early (on the first or second day), TSH levels were found to be high due to the physiological TSH increase caused by cold. Temporary TSH elevation may occur due to goitrogens, iodine deficiency, or medications. Dysgenesis (especially ectopia) and dyshormonogenesis can cause elevated TSH. Excess iodine, especially due

to the use of iodine antiseptic, may cause temporary hypothyroxinemia in premature infants (Hudaoglu et al., 2009; Klein & Mitchell, 2000). Even if the TSH elevation is mild, if it is persistent, treatment may be required (Vurallı & Özön, 2021).

If the confirmatory serum TSH level is >20 mIU/L, L-T4 treatment should be started even if FT4 is normal. If confirmatory serum TSH level is high but <20 mIU/L, treatment may be initiated or serum TSH and FT4 may be closely monitored every 1 to 2 weeks without treatment. Persistent elevations of TSH levels above 10 mIU/L are associated with negative outcomes in children (Léger et al., 2001). Therefore, it has been suggested that permanent TSH elevations (>10 mIU/L) are an indication for starting L-T4 treatment (Rose et al., 2023).

High TSH with Low T4

The most frequent cause of this outcome is primary CH. Not uncommonly, transient CH cases may occur due to antithyroid drugs used by the mother, topical iodine exposure, maternal iodine deficiency or excess, antibodies blocking the TSH receptor in the mother, medications used by the baby (dopamines, steroids), or prematurity (<30 weeks)(Höpfner et al., 2005; Hudaoglu et al., 2009; Simşek et al., 2003)

Treatment

The treatment aims to normalize T4 within 2 weeks and TSH within 1 month (Vurallı & Özön, 2021). 10-15 μg/kg/dose oral sodium LT4 tablet is used once a day in the treatment (Carswell et al., 2013). Breastfeeding does not affect L-T4 treatment (Zeitler & Solberg, 2010). The intravenous dose is 75% of the oral dose(Jonklaas et al., 2014). The L-T4 treatment aims to ensure normal growth and neurocognitive development. Adequate L-T4 treatment should be started as early as possible (usually in the first two weeks of life) to achieve optimal results, especially in severe CH cases(Aleksander et al., 2018; Alexander et al., 2017).

The prognosis is worse in babies diagnosed at an older age, treated with insufficient L-T4 dose, or diagnosed with severe hypothyroidism (Aleksander et al., 2018; Baysal et al., 2017; van der Sluijs Veer et al., 2012). Rapid return of serum thyroid hormone levels to normal leads to improved neurocognitive outcomes (Jacoba J Bongers-Schokking & de Muinck Keizer-Schrama, 2005; "Characteristics of infantile hypothyroidism discovered on neonatal screening," 1984; Salerno et al., 2002; Selva et al., 2005; Weichsel, 1978). The starting L-T4 dose of 10-15 μg/kg/day increases FT4 levels and often requires a dose reduction in the 2nd week(Vaidyanathan et al., 2012). The required L-T4 dose may remain constant or change over time. L-T4 dose may be affected by chronic

diseases, organ dysfunctions, medications, changes in body weight, dietary soy intake, L-T4 absorption, and serum estrogen level (Rose et al., 2023).

Treatment aims to keep the ST4 level in the upper half of normal (Vurallı & Özön, 2021). Near laboratory monitoring is required during L-T4 treatment to keep serum TSH and FT4 within the target range and to prevent under or over-treatment (Vaidyanathan et al., 2012). Age and test specific reference ranges are used when interpreting FT4 values (Akcay et al., 2010; Balhara et al., 2011; Önsesveren et al., 2017; Zdraveska et al., 2016).

Due to the increased metabolic clearance of thyroid hormone and the relatively rapid growth period, serum TSH and free T4 should be measured monthly in the first 6 months of life, every 2-3 months in the second 6 months, and every 3-4 months until the age of 3 (Rose et al., 2023).

In babies who use the treatment properly, growth rate and adult height are within normal limits. The intelligence level, school success and neuropsychological tests of CH cases whose treatment was started early do not differ significantly from their peers (J J Bongers-Schokking, 2001; Grüters et al., 2002; Oerbeck et al., 2003). The main determinants of developmental outcomes are the degree of CH, the age at which treatment begins, and the drug dose at the beginning of treatment (Heyerdahl & Oerbeck, 2003). Insufficient drug döşe, as well as excessive drug dose, have negative effects (left ventricular diastolic dysfunction, decrease in exercise capacity, increase in intima-media thickness) (Salerno et al., 2008).

If it cannot be determined during the follow-up whether CH is transient or permanent, treatment should be discontinued for 4-6 weeks after the age of three to determine whether CH is permanent or not (Vurallı & Özön, 2021).

CH Screening Program In Our Country

The screenings, which started locally in the late 1980s, were launched at the national level in 2006. The recall rate in our country is 2.2%. This may be due to iodine deficiency in our country. According to the national program, the heel blood of newborns should ideally be taken on the 3rd-5th day in birth in a health institution. In our country, CH screening is carried out by TSH measurement. The TSH cut-off values used in the recall are 5.5 and 20 mIU/L.

Heel blood repeats are taken at values between 5.5-20 mIU/L. If TSH is >5.5 mIU/L in the repeat sample or TSH is >20 mIU/L in the first screening, the babies are recalled for serum T4 and TSH measurement. As a result of all examinations, babies with low T4 or high TSH are referred to a pediatric endocrinologist, and treatment is started (Vurallı & Özön, 2021).

Conclusion

As a result, although screening programs are successful, hypothyroidism should be considered if the patient has clinical symptoms as a result of a normal scan. Hypothyroidism, which develops in babies whose initial scan is normal but subsequently develops hypothyroidism, who has an incorrect scan result, or who has not been scanned at all, negatively affects neurodevelopment (Dubuis et al., 1996). Therefore, if there are clinical symptoms and signs of hypothyroidism (such as macroglossia, umbilical hernia, wide posterior fontanelle, prolonged jaundice, constipation, lethargy, or hypothermia) serum TSH and FT4 should be measured anyway during the screening result (Rose et al., 2023).

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Chapter

3

Neonatal Screening for Congenital Adrenal Hyperplasia

Betül ERSOY

A group of autosomal recessive diseases characterized by enzyme deficiency due to pathogenic variants in specific enzyme genes involved in adrenal steroidogenesis is known as congenital adrenal hyperplasia (CAH). Enzyme deficiencies due to gene defects cause multiple hormonal imbalances (1). 21hydroxylase (21OH) deficiency is the most common cause of CAD, accounting for approximately 95% of all cases. Classical and non-classical types are described in the literature, but recent thoughts are to create a continuum in the form of CYP21A2 allelic variants and their phenotypes (2). The classic form, which occurs in 1 in 14,000 to 18,000 based on newborn screenings, appears clinically in the neonatal period with impaired cortisol production due to severely decreased or absent enzyme activity (2). In the most severe, salt-wasting (SW) form of CAH, there is aldosterone and cortisol deficiency as well as androgen excess (2).

Purposes of Neonatal screening for CAH

There are two important problems in classical CAH. The first is life-threatening adrenal crises in both sexes, the second is virilization of external genitalia in 46 XX patients (2,3). Newborn screening (NBS) programs, especially for the classical form of CAH,

have been initiated in many countries to prevent life-threatening adrenal crisis in the newborn and to assist in gender determination in affected women (3). Preventing adrenal crisis is especially important for the male gender. Since the external genitalia are not affected in 21OH deficiency in males, diagnosis becomes difficult and delayed (3,4). The nonclassical form, in contrast to the classical form, has a milder phenotype, with clinical problems that develop in adolescence or adulthood and not in the neonatal period or childhood. Although some NBSs screened nonclassical forms, not all programs are designed to detect them (5). However, the methodologies of first-line screening tests lack specificity and sufficient sensitivity to recognize all patients with classic CAH (4).

Methods of Neonatal Screening for CAH

17-hydroxyprogesterone (17OHP) is an important identifier for newborns at risk for 21OH deficiency. First-tier screening tests for CAH use immunoassays to measure 17-OHP levels in dried blood spots on the same filter paper cards used for other newborn screenings, such as the Guthrie test. First, laboratories quantified 17OHP for screening by radioimmunoassay; However, this method was later replaced by dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA) in automated systems. Newborn screening laboratories set the 17OHP threshold values to lower thresholds (100% screening sensitivity) to detect more patients and to detect all newborns with classical 21OH deficiency. However, although many positive cases (cases with high 17OHP) are detected in the screening, very few of them are confirmed to have the disease (low positive predictive value (PPV) (6). 17OHP is elevated in both preterm and sick newborns, and 17OHP concentrations are generally higher in males than in females (7). Therefore, laboratories adjust cutoff values according to the baby's gender, birth weight, and/or gestational age (8). However, the PPV of first-tier immunoassay with samples collected within the first two days of life generally remains low (less than 10% on average).

In the screening program, examinations begin by obtaining dried blood spots (DBS) by taking heel prick blood using filter paper ("Guthrie" cards) between the 3rd and 5th days of life or as soon as possible after 48 hours. Direct biochemical analysis of steroid levels liquid chromatography then tandem mass spectrometry (LC–MS/MS) is more effective than the other method (9).

Factors such as the gradual increase in the 17OHP concentration in babies with 21OH deficiency after birth, the lower mean 17OHP concentration in female newborns compared to male newborns, and the high 17OHP levels in sick and stressed prema-

ture babies limit the first-tier screening tests. Therefore, there is no accepted standard for 17OHP levels and laboratories determine the threshold value adjusted for birth weight. 17OHP levels are related not only to birth weight but also to gestational age (10). Some 17-OHP immunoassays cross-react with other steroids such as 17-hydroxy pregnenolone sulfate and 15β-hydroxylated compounds; the latter is largely produced by intestinal bacteria and absorbed via the entero-hepatic circulation. To increase the specificity of immunoassays, removing cross-reacting substances can be enhanced by an organic extraction step (11).

To achieve near 100% sensitivity, most screening laboratories must use threshold levels for 17-OHP that are low enough that approximately 1% of all test results will be reported as positive. Higher threshold values can be used to increase the positive predictive value (PPV). These fully recognize the salt-wasting (SW) types, causing approximately 30% of the mildly simple virilizing type (SV) to be missed. In patients with positive first screening test results, the costs of the second screening can be reduced by using a more specific test. Additionally, because 17-OHP levels are increased in premature infants, sick or stressed infants, testing serial filter paper blood samples from such patients may increase the positive predictive value (10).

Direct biochemical analysis of steroid levels by liquid chromatography followed by tandem mass spectrometry (IC-MS/MS) is more effective than organic solvent extraction immunoassays for problems. Run times for individual samples in LC-MS/MS tests are 6-12 minutes. This period is too long for first-tier screening but is suitable for second-tier screening using original, dried blood samples. Approximately 40% of samples that tested positive in the first tier had normal 17-OHP levels as measured by LC-MS/MS; this is due to the suboptimal specificity of the antibodies used in the immunoassays. Measurement of steroid ratios further increases the specificity of LC-MS/MS (9,12).

After second-tier screening, molecular genetics workup for CYP21A2 mutations can be obtained from DNA samples taken from the same dried blood spots used for hormonal screening. Individuals who do not carry any of these mutations can be considered 99% unaffected. If at least one mutation is detected, additional evaluation is needed. The number of babies affected by the disease is about a quarter of the number of pregnant women at risk of having such babies, that is, the Mendelian ratio is intact. However, the negative impact of these mutations on fetal survival cannot be ignored. If it is assumed that 1% of all individuals will be positive for CAH in the first-tier screening when the apparent carrier frequency is 3.5%, CAH positivity will be expected to be 0.035% among all babies in the second-tier screening using molecular genetic techniques (10).

Genotype helps determine the degree of enzyme impairment and thus the severity of hormonal abnormalities can also be determined. Studies have shown genotypic-phenotypic correlation in 50% of cases. Mutation types can differentiate patients with SW, SV, and nonclassical CAH. However, patients with SV and SW cannot always be differentiated by evaluating their genotype (13,14). Therefore, genotyping has implications for prenatal diagnosis and genetic counseling for the next pregnancy. DNA analysis of a single sample is not used to diagnose CAH because an individual who is heterozygous for a known mutation may have a new mutation for the other allele. Heterozygous carriers may be mistakenly classified as homozygous. Additionally, many CAH alleles may contain more than one deleterious mutation, depending on the extent of recombination that produces each, and it is impossible to determine whether two mutations are on different alleles or within the same allele without determining the genotype of at least one parent (15). The approach of genotyping patients may aid hormonal measurements, but there are no studies showing the effect of genotyping such as second-tier tests. Genotyping remains more costly and time-consuming per sample than IC-MS/MS. If the previously reported success of second-tier screening with LC-MS/MS can be replicated, this method should become the method of choice (10).

Clinical features may be similar in other types of CAD, and baseline serum 17-OHP may not be fully discriminatory in all such cases. Precursor to product ratios in LC-MS/MS are important in discriminating various enzyme defects. Serum 17-OHP, cortisol, 11-deoxycortisol, 17-OH pregnenalone, dihydroepiandrostenedione, and andro-stenedione should be measured to distinguish various enzyme defects. Tests other than 17-OH pregnenalone are available on the LC-MS/MS platform. These tests can be performed with venous blood samples in the first 2 weeks of life (14).

Urinary steroid profile (USP) is a biochemical analytical technique used in the diagnosis of various steroidogenesis defects, including those leading to CAD. This technique can identify and quantify a range of steroid metabolites above and below the enzymatic block simultaneously in a single analysis. Sample preparation to create a urine steroid profile in GCMS requires a long time. Besides its technical difficulties, it is not possible to evaluate large numbers of samples in a high-throughput format (16).

A second blood sample is taken on filter paper from babies with moderately high 17-OHP levels and monitored. Electrolyte and serum 17-OHP levels of babies whose 17-OHP levels remain high are measured (Figure 1.) (10).

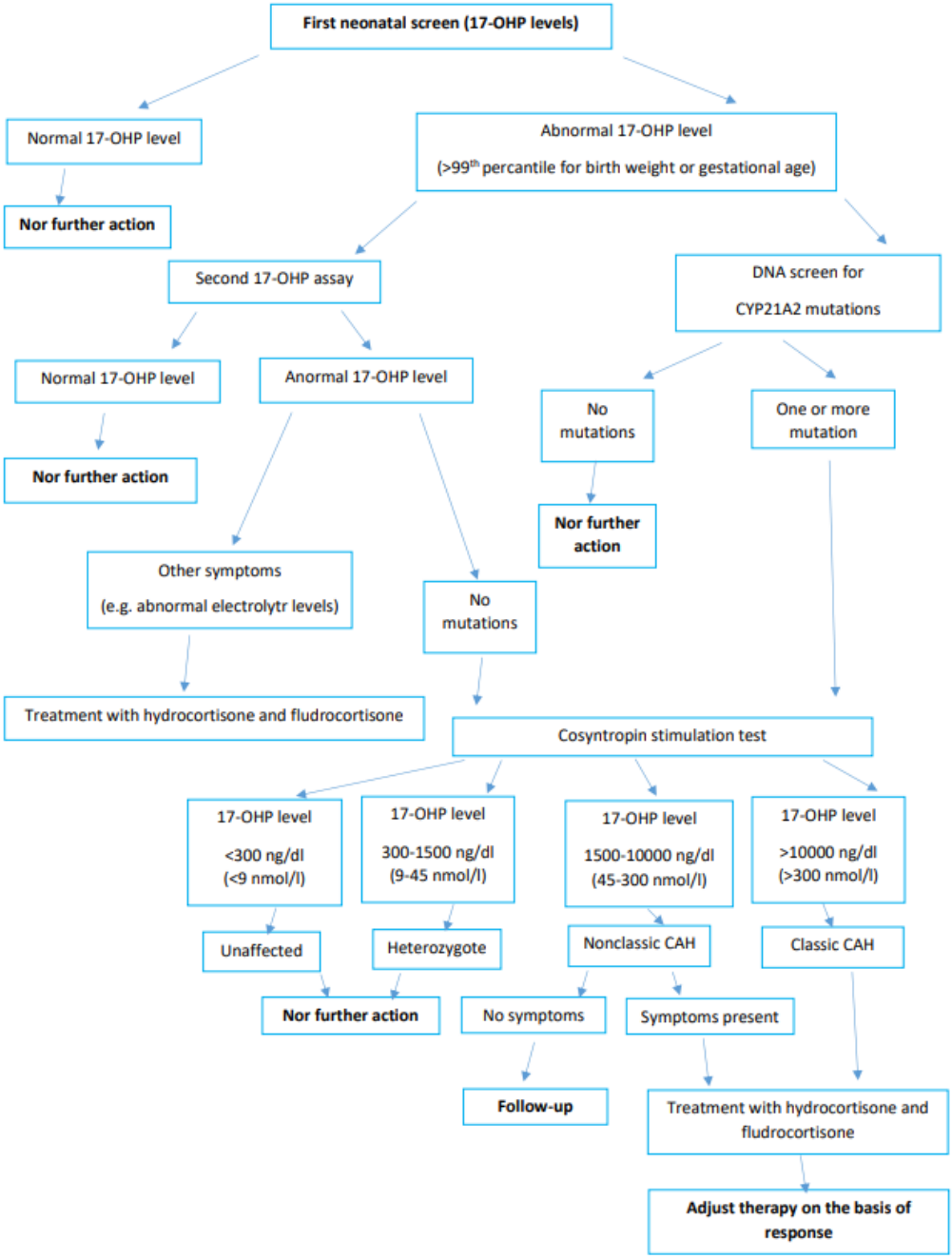


Figure 1. Algorithm for screening for CAD in newborns. Protocols vary between clinical centers. Initial screening is performed using dried capillary blood. A 17-OHP level above the 99th percentile for birth weight requires second-tier screening: either another 17-OHP assay (repeat immunoassay or liquid chromatography followed by tandem mass spectrometry) or genetics for common mutations in CYP21A2, which encodes 21-hydroxylase.

Worldwide Neonatal Screening for CAH

Screening for CAD was first initiated in the United States in 1977, and today CAD screening is performed in more than 40 countries (4). The timing of the blood sample is critical because screening results must be delivered before life-threatening adrenal crises occur (14). After 26 years of screening for 21OHD in Sweden, 100% sensitivity for SW-CAH CAD and 80% sensitivity for SV-CAH were reported. In this report, blood samples were collected 48 hours after birth and cut-offs were calculated based on gestational age. The positive predictive value (PPV) for screening was determined to be 13.4% (25% for full term babies and 1.4% for preterm babies) (4).

No cases of SW-CAH were missed in the Brazilian screening program. Cutoff values for 17OH P were also adjusted according to the baby's birth weight and the age at which the sample was taken. Authors recommended taking blood samples after 48 hours (17,18).

In a meta-analysis summarizing CAH screenings conducted in China, it is emphasized that the incidence of CAH in China was accurately determined for the first time with neonatal CAH screening. There are difficulties in CAH screening in China, such as difficulty in recalling positive cases, errors in setting the 17-OHP cut-off value, and the existence of low PPV values. Additionally, the authors emphasized that CAH screening provides interesting clinical information regarding the proportions of different types and genera and their different 17-OHP cut-off values (19).

Sample collection in the Netherlands took place between the 4th and 7th days. SW cases were not missed and the PPV was quite high. However, there are cases of SW presenting with adrenal crisis before receiving results from screening laboratories (17). In the Netherlands, measuring 21-deoxycortisol (21-DF) levels using LC-MS/MS from the first heel blood in inconclusive cases in recent practices reports that false positive cases have decreased (20).

In neonatal screening for CAH in Japan, samples were collected between days 4 and 7 after birth. The screening is divided into two procedures. The concentration of 17 OHP is initially determined by enzyme-linked immunosorbent assay (ELISA). Threshold values for 17OHP were adjusted for birth weight and gestational age (21).

The result of the pilot study conducted in Turkey suggested that the incidence of CAH was higher than previously reported. This study included 38,935 newborn babies with a gestational age of 32 weeks or more and a birth weight of 1500 g or more. The screening protocol included one sample and second-tier testing. Dried blood samples were collected between days 3 and 5 of life after birth, and 17-OHP levels were measured by fluoroimmunoassay. In those whose initial test was positive, 17-OHP, 21-deoxycortisol (21-S), cortisol (F), 11-deoxycortisol and androstenedione were measured.

Babies with steroid ratio (21-S+17-OHP)/F ≥0.5 referred to pediatric endocrinology clinics for diagnostic evaluation (22).

Screening has been carried out in New Zealand since 1984. Many false positive results were obtained in screenings using bloodspot immunoassay. Using LCMSMS in second stage tier test after 2017 improved the screening results. To improve CAH screening accuracy in New Zealand, the addition of LCMSMS markers to second-tier testing is suggested to increase both screening specificity and sensitivity. Use of the steroid rate marker compared to 17OHP alone reduces the number of false-positive tests and reduces the burden of repeated sample collection and follow-up (23).

When screening began in the United States, blood samples were collected 48–96 hours after birth. To minimize the risk of missing cases, some states offered a second chance at testing to find affected babies between days 8 and 14 of life. In 2015, it was decided that the optimal time to collect samples was between 24-48 hours of life. Although babies with SW were detected in the first screen, it was understood that the milder forms of SV and nonclassical types could be recognized in the second screen. Secondary screen increase screening accuracy, defined as high PPV, but do not eliminate false negative cases (17).

Review of False Positives and Negatives

False positives

One of the causes of false positivity is physiological changes in 17OHP concentration after birth. First-tier immunological analyzes for 17OHP, which is diagnostic of 21OH deficiency, produce a large number of false positive cases (20). This results in a low positive predictive value. One of these is that 17OHP values increase due to stressful events after birth. Since 17OHP levels decrease at the 24th hour after birth, the timing of blood collection is important. False positive results will be obtained if blood samples are taken before 48 hours. False positive results will be obtained if blood samples are taken before 48 hours (24).

Another cause for false positivity is premature newborns. 17OHP levels are high in premature newborns due to adrenal enzyme immaturity. 11β-hydroxylase enzyme and 21-hydroxylase activity are low in all premature babies, healthy or sick, born before 29 weeks. Steroid precursors increase in premature babies due to adrenal steroidogenesis not being fully active. Therefore, the 17OHP cut-off points of these babies should be adjusted according to birth weight and gestational age. However, PPV is low in these babies (8,25). Some authors recommend screening preterm newborns for CAH. In addition, since preterm newborns are under close monitoring, it does not seem possible

to miss the salt wasting crisis in these babies. In the United States, it has been recommended that premature babies be screened for CAH at 2 and 4 weeks of age. Some groups recommend final screening on day 28 of life (14,26). There is consensus that 17OHP concentrations in premature infants should be monitored 2-3 times during the first month of life (17).

If the newborn has 21OH deficiency and is not treated, 17OHP levels increase over time. Therefore, the probability of detecting the disease in second-tier tests increases. Second screening improves screening accuracy (26).

The third reason for false positivity is stressful situations experienced by the mother and the newborn. In cases of stress in the fetus, ACTH increases, leading to an increase in cortisol and its precursors. For example, respiratory distress syndrome is one of them. 17OHP concentration increases in conditions such as hydrops fetalis, intraventricular hemorrhage, septicemia, congenital pneumonia, perinatal asphyxia, major abdominal surgical conditions, and severe congenital heart disease. Maternal preeclampsia causes elevated 17 OHP concentration in the infant. These situations should be taken into consideration when interpreting the results (17).

Laboratory methods are also one of the factors that cause false positivity. The performance of the 17OHP screening immunoassay is limited by cross-reactivity of the antibody, particularly with the main interfering compound, 17-hydroxypregnenolone sulfate. This steroid is high in preterms and causes false positivity in preterms. Liquid chromatography-tandem mass spectrometry can be used to provide additional specificity in measuring 17OHP concentration, especially in premature newborn (2).

The 5th factor that causes false positivity is other forms of CAH. Other forms of CAH, such as 11-hydroxylase deficiency, 3β-HSD deficiency, also have high 17OHP levels. Physicians and screening laboratories should be aware of the differential diagnosis from other forms of CAH in the presence of elevated 17OHP (17).

False Negatives

While cases with SW are easily detected, there is less reliability in detecting SV types. Reasons that may cause false negativity can be listed as follows:

Early collection of newborn screening samples is one of the reasons that will produce false negatives. Especially in cases with SV, samples taken within the first two days may cause some cases to be missed. It is thought that the second screen will increase the accuracy (27).

One of the reasons for false negativity is the immaturity of adrenal functions in premature babies. Since 3β-hydroxysteroid dehydrogenase (3β-HSD) expression is low in the adrenal gland in the early fetal period, all steroid precursor synthesis is directed to

dehydroepiandrosterone (DHEA). Because the activity of 3β-HSD is the key enzyme of fetal adrenal development, there is a view that flux through the immature biosynthetic pathway of cortisol will not adequately increase 17OHP concentrations in 21OH-deficient preterm neonates (28).

Another reason for false negativity is increased exposure of the fetus to maternal cortisol. Due to maternal cortisol levels increasing approximately 10-fold throughout pregnancy, fetal exposure to cortisol is regulated via 11β-HSD through the placenta. Approximately 10% of maternal cortisol enters the fetal circulation and suppresses fetal ACTH (21). Thus, 17OHP production in the fetus also decreases due to the reduction of steroidogenic pathway and patients with 121OH deficiency may be missed (17).

The effect of antenatal glucocorticoid treatment on the fetal adrenal varies depending on factors such as the dose of single treatment, the number of doses, and the time of the last dose. A study also showed that administration of multiple synthetic steroids reduced 17OHP concentration in preterm infants (29). Therefore, maternal glucocorticoid treatment is a risk factor leading to false negativity in neonatal screening (30,17).

17OHP concentration reflects 21OH enzyme activity and disease severity in untreated individuals. Some authors claim that mild and moderate forms of 21OH deficiency are missed by 33%. In other words, in mild and moderate forms, the false negativity rate is one third. For this reason, physicians should be aware that neonatal screening may miss cases such as simple virilizing and nonclassical forms (31).

Cost-effectiveness

The most striking benefit of screening is that it reduces the time until cases are diagnosed and SW cases are identified earlier, reducing morbidity and mortality. Babies who die early and suddenly may not be diagnosed. It may be possible to determine the death of babies diagnosed with CAH through screening. Since the diagnosis of boys with SW is difficult, it may be possible to provide retrospective diagnosis of deaths that were not reported as salt wasting (32). While the mortality rate is 10% in populations without newborn screening, it is reported to be 4% in developed countries where screening is performed (33).

Screening also reduces morbidity from CAH. Less severe hyponatremia was reported in infants identified at screening than in those not screened. Learning disabilities have previously been reported in patients with SW. However, it is unclear whether screening reduces the severity and frequency of these disorders. Although screening seems to be beneficial in early recognition of the SW type, especially in boys, it also plays an important role in early correct gender assignment virilized girls. In the absence of screening in males with SV, rapid growth is detected in childhood, which negatively affects final height. Its usefulness in the diagnosis of nonclassical CAH is debatable (34).

False positive cases may increase costs and consume more time for the doctor. In addition, parents of babies with false positives are seriously worried and may incur a significant increase in costs and waste of time. Adopting screening methods with improved positive predictive values could significantly reduce these problems. Positive predictive values can be improved by second-tiers screening using IC-MS/MS or DNA-based methods. A conventional wisdom is that screening for a given disease is cost-effective at less than $50,000 per life year (or per quality-adjusted life year if quality of life is taken into account) (35).

Finally, the likelihood of medical transfer decreased in screened newborns. Hospital stay shortened. Hospital costs also decreased. Although screening is not cost-saving, it is considered cost-effective given the clinical benefits and increased cost-effectiveness ratio. Although screening is not cost-saving, it is considered cost-effective, given the clinical benefits and the increased cost-effectiveness ratio (36).

Conclusions

Neonatal screening for CAH is widely practiced around the world. Neonatal screening for CAH has significantly shortened the time to diagnosis, especially in babies with classic CAH. Therefore, it reduced morbidity and mortality. Diagnostic delay is greater in men due to lack of genital ambiguity. Accordingly, deaths occur without diagnosis in the presence of SW in men. However, due to first-tier tests with low specificity, a high false positive rate and low positive predictive value are obtained. Its limited sensitivity in detecting all children with classic CAH is also concerning.

False-negative results may be due to early collection of samples, immaturity of adrenal functions in premature infants, exposure of the fetus to the mother's maternal cortisol, antenatal glucocorticoid therapy, and the presence of infants with mild or mild forms of the disease. False positivity may occur due to reasons such as sample collection time, immaturity of adrenal functions in premature babies, and stress states of the mother and baby. Second-tier tests evaluated using the LC-MS/MS method increase PPV, which increases screening accuracy

Although neonatal screening for CAH has benefits such as reducing medical transfers, shortening hospital stays, and reducing hospital costs, its benefit will be more meaningful with the adoption of screening methods that will improve PPV.

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Chapter

4

Phenylketonuria

Sezai ARSLAN

Phenylketonuria is one of the most significant causes of dietary preventable mental retardation. This condition results from a partial or complete defect in the enzyme phenylalanine hydroxylase (PAH), which is responsible for converting phenylalanine to tyrosine. Consequently, the concentration of phenylalanine in plasma exceeds the normal range. Restricting dietary intake of phenylalanine results in excellent neuromotor development in patients.

Phenylketonuria was first described by Folling in 1934, when phenylketones were identified in the urine of two siblings with neuromotor developmental delay and behavioural problems (Centerwall & Centerwall, 2000). In 1953, Bickel reported the effectiveness of phenylalanine restriction in the treatment of a patient with phenylketonuria (Green, 2021). In 1960, the implementation of screening programs was proposed following the discovery of the Guthrie technique (based on bacterial inhibition) in dry blood samples for the measurement of phenylalanine. The first screening program for phenylketonuria was initiated in the U.S. state of Massachusetts in 1962 (Gonzalez & Willis, 2009; Levy, 2021).

In Europe, phenylketonuria is most common in Turkey and Ireland and least common in Finland (Van Wegberg et al., 2017). In Turkey, phenylketonuria screening was first started in 1987 in 7 provincial centers in cooperation with the Minis-

try of Health and Hacettepe University Faculty of Medicine. Later, this screening was extended to 26 provincial centers. In 1993, it became widespread throughout Turkey. In 2006, the "National Newborn Screening Program" was initiated by the Ministry of Health by adding hypothyroidism to phenylketonuria screening. As of 2023, hypothyroidism, biotinidase deficiency, cystic fibrosis, congenital adrenal hyperplasia and spinal muscular atrophy are being screened along with phenylketonuria screening in the national newborn screening program in Turkey (Ministry of Health, 2023).

Phenylketonuria is inherited in an autosomal recessive manner. Parents are often carriers. It is more prevalent in countries where consanguineous marriage is common. The global prevalence of phenylketonuria is estimated to be 1:23930 live births (Italy 1:4500, Japan 1:125000) (Hillert et al., 2020). Studies conducted in Turkey have shown that the prevalence of hyperphenylalaninemia is 1:2785 and the prevalence of phenylketonuria is 1:4370 (Ozalp et al., 1990). The most common cause of PAH deficiency is PAH gene mutations. A total of 1,500 pathogenic PAH gene mutations have been identified (Iuhas et al., 2023).

Ethiology /pathogenesis

Phenylalanine hydroxylase, an enzyme found in the liver, plays a crucial role in the metabolism of phenylalanine. This enzyme facilitates the conversion of phenylalanine to tyrosine, thereby enabling the formation of neurotransmitters such as dopamine, adrenaline, noradrenaline and melanin pigment (Fig. 1) (Saudubray et al., 2022). Mental retardation is a consequence of PAH enzyme deficiency, which occurs for a number of reasons. The initial hypothesis is that an excessive increase in phenylalanine levels leads to a reduction in glutamate synaptic transmission, low pyruvate kinase activity and low HMG-CoA activity in the brain. The second hypothesis postulates that an elevated phenylalanine level competes with other large neutral amino acids at the LAT1 (L-type amino acid carrier) level in the blood-brain barrier, resulting in a reduction in tyrosine and tryptophan amino acid levels in the brain (Fig. 2). Concurrently, an elevated phenylalanine level will result in a reduction in neurotransmitter levels, including dopamine and serotonin, due to a decline in tyrosine and tryptophan hydroxylase enzyme activities. As a consequence of these effects, oxidative stress, reduced protein synthesis, and alterations in brain cell microstructure, such as abnormalities in myelinisation, decreased axon length and dendrites, impaired signalling, and gliosis, appear to be the underlying causes of neurological symptoms. (Rovelli & Longo, 2023)

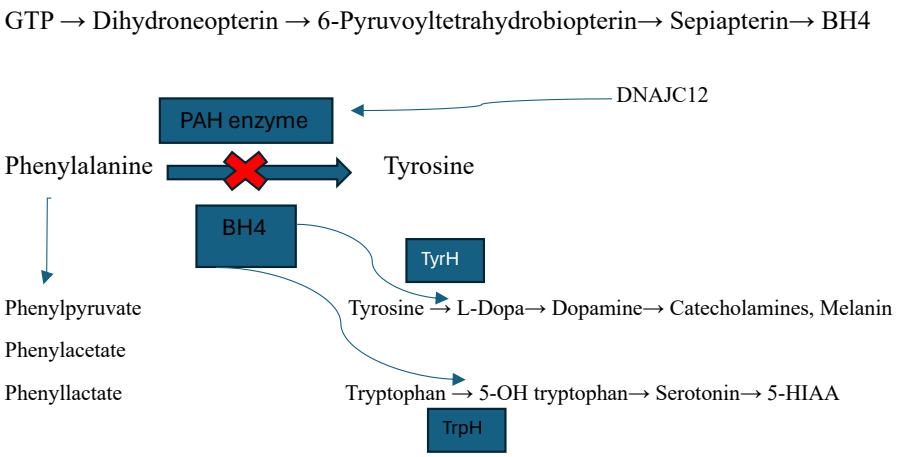


Figure 1. The phenylalanine hydroxylation system, including the synthesis and regeneration of pterins and other pterin-requiring enzymes

BH4, tetrahydrobiopterin; DNAJC12, heat shock protein of the HSP40 family; GTP, guanosine triphosphate;; 5HIAA, 5-hydroxyindoleacetic acid; PAH, PHE hydroxylase; TrpH, tryptophan hydroxylase; TyrH, tyrosine hydroxylase.

Burgard P., Lachmann R.H., Walter J.H. (2022). Hyperphenylalaninaemia. Saudubray JM, Baumgartner MR, García-Cazorla A.,Walter J.H. (Eds.) Inborn Metabolic Diseases Diagnosis and Treatment (p338).

It is also observed that elevated blood phenylalanine levels are present in disorders of tetrahydrobiopterin (BH4) metabolism, which is a cofactor of the enzyme phenylalanine hydroxylase (Fig.1). Also, BH4 is also a cofactor of tyrosine hydroxylase and tryptophan hydroxylase enzymes. Therefore, synthesis of neurotransmitters such as dopamine, noradrenaline and serotonin will be impaired in BH4 synthesis disorders. In these patients, neurologic findings related to neurotransmitter deficiency, such as hypotonicity, oculogyric crisis, drooling and sleep disturbance, will occur.

It has been demonstrated that hyperphenylalaninemia can also be observed in DNAJC12 deficiency, which is responsible for the proper folding of the recently identified PAH enzyme and other aromatic amino acid hydroxylases (TH, TRH) and works in conjunction with a heat shock protein (HSP70) (Blau et al., 2018). Neuropsychiatric findings such as autism-like symptoms, intellectual disorders and parkinsonism are observed in these patients.

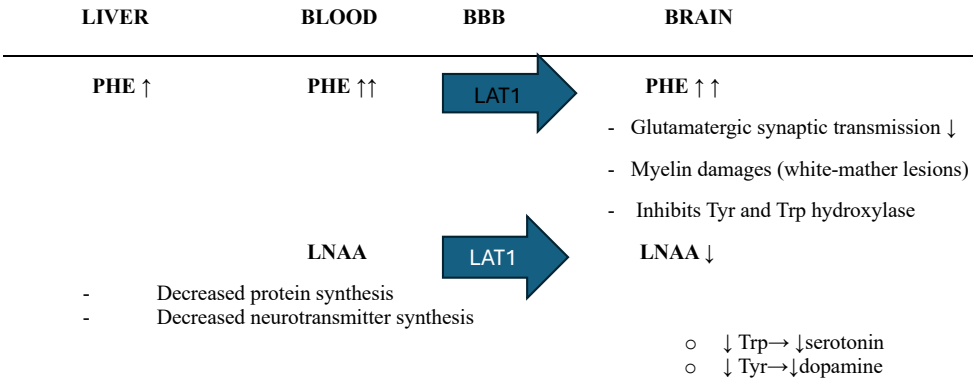


Figure 2. Pathophysiology of PKU: Summary of potential mechanisms of neurocognitive impairment by high phenylalanine concentrations.

Phe: phenylalanine; BBB, blood–brain barrier; LNAA: Large Neutral Amino Acids; LAT1, L-type amino acid carrier; Tyr, tyrosine; Trp, tryptophan

Van Wegberg AMJ, MacDonald A, Ahring K et al. The complete European guidelines on phenylketonuria: diagnosis and treatment. Orphanet Journal of Rare Diseases volume 12, Article number: 162 (2017)

Clinical findings

In classical phenylketonuria, a diet therapy that restricts phenylalanine intake is highly effective when initiated early. When blood phenyl alanine levels are kept within the target range (<360 μmol/L under 12 years of age, <600 μmol/L above 12 years of age), patients can complete their neuromotor development similar to their peers (Van Wegberg et al., 2017; Saudubray et al., 2022).

In untreated cases;

- Pyloric stenosis-like vomiting, usually normal at birth, after protein intake
- Microcephaly, intellectual disability, developmental delay
- Autism-like behavioral disorders, psychiatric problems
- Movement disorders, epilepsy
- Decreased pigmentation (light hair-skin and iris)
- Ezematous rash

Diagnosis

In the context of the National Newborn Screening Programme, the initial blood sample is collected from infants born in Turkey within the initial 48 hours following

oral feeding. The second sample is obtained within the first week of life and is sent to screening centres for analysis. The detection of a phenylalanine level >2 mg/dl in a dry blood sample necessitates further investigation. It is possible for false positives to occur in cases of tyrosine metabolism disorders, infection, prematurity, and liver disease (such as galactosemia). Patients with positive screening results should be referred to a paediatric metabolic diseases centre for further evaluation. The flow chart of the phenylketonuria screening process in Turkey is presented in Fig. 3.

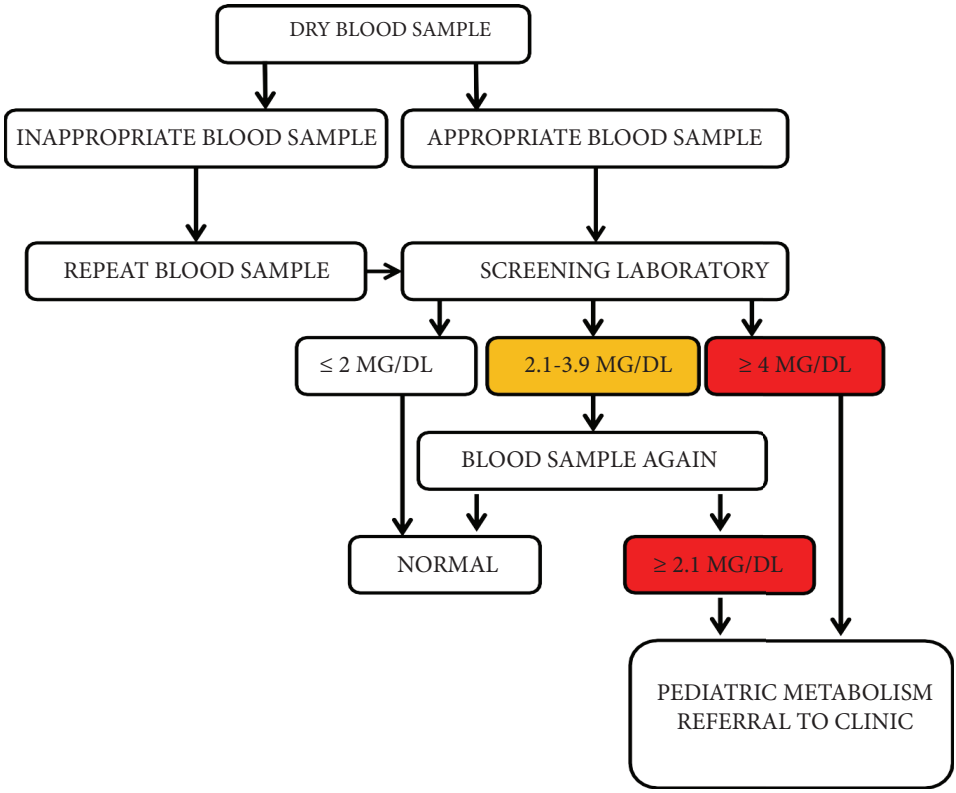


Figure 3. Phenylketonuria Outcome Assessment in Turkey

In patients referred with suspicion of phenylketonuria, an amino acid analysis reveals a significant increase in the likelihood of phenylketonuria if the phenylalanine:tyrosine ratio exceeds 2.5 (Alsharhan & Ficicioglu, 2020). Patients with a blood phenylalanine level of 2-6 mg/dl (120-360 µmol/L) are defined as hyperphenylalaninaemia, while pa-

tients with phenylalanine levels >6 mg/dl (>360 µmol/L) are defined as phenylketonuria (Van Wegberg et al., 2017; Saudubray et al., 2022). In patients diagnosed with Phenylketonuria, analysis of the PAH gene and demonstration of homozygous or combined heterozygous mutation is crucial for confirmation of the diagnosis.

In the differential diagnosis of hyperphenylalaninaemia, it is important to rule out BH4 metabolism disorders. Consequently, pterins (neopterin, biopterin) and dihydropterin reductase enzyme activity in dry blood or urine should be investigated in patients presenting with elevated phenylalanine. It has been demonstrated that neuromotor development in patients with pterin metabolism disorders is more effectively treated by early L-Dopa/carbidopa, 5-Hydroxytryptophan intervention. Furthermore, decreased levels of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF) may also indicate neurotransmitter deficiency. The differential diagnosis of pterin metabolism disorders is presented in Table 1.

Table 1. Interpretation of results of investigations in disorders of biopterin metabolism

Deficiency	Blood PHE µmol/L	Blood or urine biopterin	Blood or urine neopterin	Blood or urine primapterin	CSF 5-HIAA and HVA	Blood DHPR activity	Gene
PAH	>120	↑	↑	-	↓*	N	PAH
GTPCH	50-1200	↓↓	↓↓	-	↓	N	GTCH1
PTPS	240-2500	↓↓	↑↑	-	↓	N	PTS
DHPR	180-2500	↓↓	N or ↑	-	↓	↓	QDPR
PCD	180-1200	↓	↑	↑↑	N	N	PCBD1
DNAJC12	>120	N	N	-	↓	N	DNAJC12

CSF, cerebrospinal fluid; DHPR, dihydropterin reductase; GTPCH, guanosine triphosphate cyclohydrolase I; 5-HIAA, 5-hydroxyindole acetic acids; HVA, homovanillic acid; N, normal; PAH, phenylalanine hydroxylase; PCD, pterin-4a-carbinolamine dehydratase; PHE, phenylalanine; PTPS, 6-pyruvoyl-tetrahydropterin synthase. aIn PAH deficiency, as long as PHE concentrations remain elevated, there is a secondary inhibition of tyrosine and tryptophan hydroxylases causing depletion in CSF amines

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CSF, cerebrospinal fluid; DHPR, dihydropterin reductase; GTPCH, guanosine triphosphate cyclohydrolase I; 5-HIAA, 5-hydroxyindole acetic acids; HVA, homovanillic acid; N, normal; PAH, phenylalanine hydroxylase; PCD, pterin-4a-carbinolamine dehydratase; PHE, phenylalanine; PTPS, 6-pyruvoyl-tetrahydropterin synthase. [a]In PAH deficiency, as long as PHE concentrations remain elevated, there is a secondary inhibition of tyrosine and tryptophan hydroxylases causing depletion in CSF amines

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Treatment

In the treatment of classical phenylketonuria, it is essential to restrict dietary phenylalanine intake to a significant degree. It is recommended that animal protein sources with a high protein value, such as meat, chicken, fish, eggs, and milk and dairy products, be avoided. Nevertheless, phenylalanine is also an essential amino acid. Consequently, patients with this condition are permitted a daily intake of phenylalanine of between 200 and 400 mg. One gram of natural protein contains approximately 50 milligrams of phenylalanine. The remaining daily protein requirements of the patients are met with vegetable protein sources and special protein mixtures that do not contain phenylalanine. Furthermore, low-protein products, specifically manufactured for this purpose (e.g., milk, flour, and cereal), are employed to regulate the dietary intake of affected patients. As animal proteins are prohibited in these patients, it is crucial that they are regularly tested for B12 levels, essential fatty acid deficiency and osteopenia.

In patients with mild to moderate phenylketonuria (Phe>360 μmol/L), it is recommended that BH4 responsiveness be tested prior to initiating dietary therapy. In particular, patients with residual partial PAH enzyme activity are expected to respond. Following the administration of BH4 at a dose of 20 mg/kg over a period of 48 hours, the blood phenylalanine level is again measured. If the phenylalanine level falls below the target values according to age (i.e., below 360 μmol/L for individuals below 12 years of age and below 600 μmol/L for individuals above 12 years of age), or if a decrease of at least 30% is observed in the mean blood phenylalanine level, it is considered that the patient is BH4 responsive phenylketonuria (Anjema et al., 2013). In these patients, blood phenylalanine levels can be maintained within normal limits with only sapropterin treatment, obviating the need for a phenylalanine restricted diet. Furthermore, protein tolerance can be augmented in some patients undergoing dietary therapy. Sapropterin is administered orally at a dosage of 10 to 20 mg per kilogram of body weight per day.

In other cases of BH4 metabolism disorders and DNAJC12 deficiency, levodopa/carbidopa, 5-hydroxytryptophan treatment is typically administered in conjunction with BH4 treatment. Additionally, folinic acid is used in DHPR deficiency (Saudubray et al., 2022). LNAA (large neutral amino acids) can be used to treat patients who are diagnosed late or who are unable to adapt to a phenylalanine restricted diet in adulthood. LNAA preparations act by competing with phenylalanine, decreasing the absorption

of phenylalanine from the intestinal system and reducing the passage of phenylalanine into the brain by competing in the blood-brain barrier (Scala et al., 2020). In recent years, Pegvaliase enzyme therapy has commenced to be employed in Europe in the treatment of phenylketonuria, particularly in individuals over the age of 18 (Harding et al., 2024). Gene therapy research continues today.

Phenylketonuric patients who become pregnant are at risk of experiencing a range of adverse effects on the fetus due to elevated levels of phenylalanine in the maternal bloodstream. This condition is referred to as maternal phenylketonuria syndrome. In these patients, it is essential to maintain a blood phenylalanine level between 2 and 6 mg/dL throughout pregnancy, with strict monitoring. If the levels of phenylalanine remain elevated, there is a possibility that the offspring will exhibit symptoms of mental retardation and/or microcephaly, as well as congenital cardiac abnormalities and intrauterine growth restriction (Levy and Ghavami, 1996).

In conclusion, classical phenylketonuria is among the causes of mental retardation that can be prevented with a phenylalanine-restricted diet therapy. The assessment of BH4 responsiveness in patients with moderate and mild forms of phenylketonuria is of significant importance from the perspective of quality of life. In the differential diagnosis of hyperphenylalaninaemia, it is important to consider the possibility of tyrosine metabolism disorders, BH4 metabolism disorders and DNAJC12 deficiency.

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Chapter

5

Newborn Screening for Biotinidase Deficiency

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Introduction

Biotinidase deficiency is an inherited metabolic disorder with the most favorable outcome with early diagnosis and treatment. Thanks to national newborn screening programs, many babies have been diagnosed and treated early. Biotin treatment initiated in the early period provides complete prevention of clinical symptoms. When cost-effectiveness calculations were made, it was seen that the screening resulted in a significant economic gain in medical expenses that may be incurred due to biotinidase deficiency (Çoker, 2017). Cost-effectiveness has been demonstrated. therefore biotinidase deficiency is covered by newborn screening in many countries around the world (Vallejo-Torres et al., 2015).

Biotin

Biotin is a water-soluble vitamin B 7. Biotin is found in a protein-bound form in foods such as milk, yeast, liver, cereals (including whole wheat), soy, avocado, nuts, egg yolks and green leafy vegetables (León-Del-Río, 2019; Solvik & Strand, 2024). Biotin is synthesized by microorganisms and plants but not by animals and humans. Biotin

is normally taken into the human body with food (Solvik & Strand, 2024). If biotin is taken orally at pharmacological doses, absorption occurs by passive diffusion, and all biotin is absorbed. Protein-bound biotin taken into the human body with food is broken down into biocytin and biotin-oligopeptides in the digestive system. Biocytin and biotin oligopeptides are converted into free biotin by the biotinidase. It is then transported from the small intestine into the enterocyte by a sodium-dependent multivitamin transporter. It is then transported into the circulation. It is transported from circulation to tissues and cells by monocarboxylate transporter and sodium-dependent multivitamin transporter. Biotin taken into the cell is firstly proteolysed by holocarboxylase synthase and holocarboxylases and biotin bound to short biotinyl peptides is formed. Biotin is then liberated from biotin bound to biotinyl peptides by biotinidase enzyme (Göksoy, 2023; Semeraro et al., 2022). Intracellular free biotin acts as a cofactor for the enzymes methylcrotonyl-CoA carboxylase, acetyl-CoA carboxylase, pyruvate carboxylase and propionyl-CoA carboxylase. These enzymes are also involved in carboxylation reactions, degradation of branched amino acids and single chain fatty acids, synthesis of fatty acids, conversion of pyruvate to oxaloacetate (Solvik & Strand, 2024). Biotin is also involved in modification of the activity of transcription factors (gene regulation), histone modifications and cell signaling. The bioavailability of biotin in different foods may vary. Some compounds such as avidin in raw egg white bind biotin and prevent its absorption. Biotin and its metabolites are excreted in the urine. Biotin not absorbed in the intestine and produced by intestinal bacteria is excreted in the feces. Dietary biotin deficiency is rare as most foods contain biotin. Biotin deficiency affects the enzyme activity and transcription of the hepatic enzymes pyruvate kinase, glucokinase, ornithine transcarbomoylase, 6-phosphofructokinase, and guanylate cyclase. Biotin deficiency may cause conjunctivitis, hair loss, dermatitis, developmental delay, hypotonia, ataxia, and seizures (Göksoy, 2023; León-Del-Río, 2019; Solvik & Strand, 2024).

Biotinidase deficiency

Biotinidase deficiency is a congenital metabolic disorder caused by a mutation in the *BTD* gene. It is inherited as autosomal recessive. The enzyme biotinidase is involved in the endogenous and exogenous cycling of biotin, resulting in the formation of free biotin. In biotinidase deficiency, the function of all mitochondrial carboxylases is impaired. If biotinidase enzyme activity is <10%, it is called profound biotinidase deficiency, and if it is between 10-30%, it is called partial biotinidase deficiency. If left untreated, biotinidase deficiency may lead to clinical manifestations such

as neuromotor impairment (developmental delay, seizures, hypotonia, ataxia, hearing loss), respiratory problems (laryngeal stridor, hyperventilation, apnea, etc.), ophthalmologic problems, feeding difficulties, cellular immunologic dysfunction (candidiasis, alopecia, skin manifestations) or severe life-threatening metabolic acidosis (Göksoy, 2023; Porta et al., 2017; Wolf, 1993). Except for ophthalmologic, neurologic, and audiologic sequelae, other findings completely resolve with the administration of pharmacologic doses of biotin (Wolf, 1993). Profound biotinidase deficiency may present with severe neuromotor, skin, eye, and hearing symptoms or metabolic decompensation acidosis, usually between one week and ten years of age. Partial biotinidase deficiency may be asymptomatic or present with a wide clinical spectrum including skin rash, autism spectrum disorder, hair loss, and developmental delay (more serious symptoms may occur in stress situations). If left untreated, myelopathy and optic neuropathy are seen at older ages (adolescents or adults) and often these patients may be initially diagnosed as multiple sclerosis, brainstem encephalitis, or transverse myelitis. It is therefore essential to diagnose the disease in the neonatal period before symptoms develop (Canda et al., 2020; Solvik & Strand, 2024). The diagnosis is made by demonstrating low biotinidase enzyme activity in serum or plasma. If the results of enzymatic tests are uncertain, the biallelic pathogenic variants in the *BTD* gene can be identified by molecular genetic analysis (Sürücü Kara et al., 2023; Wolf, 1993). A common clinical diagnostic criteria for the diagnosis of biotinidase deficiency has not yet been published (Wolf, 1993).

Biotinidase deficiency treatment and follow-up

Treatment is 5-10 mg/day oral free biotin treatment in patients with profound biotinidase deficiency and 2.5-10 mg oral free biotin treatment in patients with partial biotinidase deficiency. Biotin treatment is lifelong. In patients with metabolic decompensation and acidosis, biotin therapy, hydration, and bicarbonate replacement can rapidly resolve decompensation (within hours or days). In symptomatic patients, symptom-oriented supportive therapies should be applied (developmental therapy, cochlear implant, hearing aid, etc.). Patients with profound biotinidase deficiency should be evaluated annually and those with partial biotinidase deficiency should be evaluated every two years by metabolic, genetic, ophthalmology, and audiology departments (Wolf, 1993). Biotinidase deficiency meets the main criteria for inclusion in neonatal screening programs because it causes serious morbidity and mortality if left untreated, can be detected early with rapid and economical methods, and can be effectively treated with cheap and simple treatment (Porta et al., 2017).

Newborn screening for biotinidase deficiency

Biotinidase deficiency was first described by Wolf et al. in 1983. The incidence of biotinidase deficiency is approximately 1:40,000-60,000 worldwide. The prevalence varies from country to country. The prevalence is higher in countries such as Turkey and Saudi Arabia where consanguineous marriages are more common. For biotinidase deficiency, Heard et al. in 1984 reported that the blood of normal babies was purple, while the sample of affected babies was straw-colored when analyzed by colorimetric method for biotinidase deficiency from dried whole blood samples dripped on the same filter papers used in newborn screening for phenylketonuria in Virginia, USA. (Heard et al., 1984). On January 24, 1984, a pilot study was initiated in Virginia and 81,243 newborns were screened in one year. Using a simple colorimetric screening procedure, this pilot study identified two newborns with biotinidase deficiency and, through secondary family screening, two affected siblings of one of the newborns. These results suggest that this disorder is as common as many other disorders for which newborn screening is performed (Heard et al., 1986). In 1987, 18,945 newborns were screened by a modified calorimetric method at Sapporo City Institute of Public Health in Japan and no infants with biotinidase deficiency were detected. (Yamaguchi et al., 1987). Newborn screening has been carried out in Canada, Italy and other countries (Burlina et al., 1988; Dunkel et al., 1989). Data from 36 newborn screening programs conducted in 14 countries (Australia, Austria, Canada, Germany, Hungary, Italy, Japan, Mexico, New Zealand, Scotland, Spain, Sweden, Switzerland, Switzerland, United State) between 1984 and 1990 were evaluated. 8,532,617 newborns were screened and the prevalence of biotinidase deficiency was reported to be 1:60,089 (profound biotinidase deficiency is 1:112, 271, partial biotinidase deficiency is 1:129,282) (Wolf, 1991). The longest retrospective evaluation reported that the incidence of biotinidase deficiency was 1:61,000 in Italy, where 1,097,894 newborn babies were screened in a 30-year newborn screening program (Porta et al., 2017). Some of the countries where newborns are screened for biotinidase deficiency and the prevalence of biotinidase deficiency in these countries are given in Table 1.

Table 1. Some of the countries where newborns are screened for biotinidase deficiency and the prevalence of biotinidase deficiency in these countries		
America Virginia	A pilot study was launched to screen newborns for biotinidase deficiency by colorimetric method.	(Heard et al., 1984)
America Virginia	Between 1984-1985 81,243 newborns screened 2 infants diagnosed with biotinidase deficiency one infant's two affected siblings diagnosed with Biotinidase deficiency (1:20,310)	(Heard et al., 1986)
Japan	18,945 newborns screened 0 biotinidase deficiency (0)	(Yamaguchi et al., 1987)
Italy	24,300 newborns screened 1 with biotinidase deficiency (1:24,300)	(Burlina et al., 1988)
Canada	163,000 newborns screened 15% biotinidase deficiency Three profound shortcomings 12 partial deficiencies	(Dunkel et al., 1989)
Australia, Austria, Canada, Germany, Hungary, Italy, Japan, Mexico, New Zealand, Scotland, Spain, Sweden, Switzerland, United State	Between 1984-1990 8,532,617 newborns screened Incidence of biotinidase deficiency 1:60,089 Profound biotinidase deficiency is 1:112,271 Partial biotinidase deficiency is 1:129,282	(Wolf, 1991)
Hungary	43,493 newborns screened 2 biotinidase deficiency (1: 21 747)	(Havass, 1991)
Germany/ Lower Saxony	Between 1985-1989 420,000 newborns screened 12 Biotinidase deficiency (1:35,000) 3 Profound biotinidase deficiency: 1:140,000 9 Partial biotinidase deficiency: 1:46,667	(Schweitzer et al., 1991)
Turkey	Between 1991-1998 371,654 newborns screened 32 biotinidase deficiency (1:11,614) 25 Profound biotinidase deficiency: 1: 14 866 7 Partial biotinidase deficiency: 1: 53 093	(Baykal et al., 1998)
Brazil/Paraná State	125,000 newborns screened 2 biotinidase deficiency (1:62,500) Profound biotinidase deficiency: 1:125,000 Partial biotinidase deficiency: 1:125,000	(Pinto et al., 1998)
Hungary	Between 1989-2001 1,336,145 newborns screened 58 biotinidase deficiency (1: 23,036) The most frequent *BTD* gene variant: c.1595C>T	(László et al., 2003)

Brazil	225,136 newborns screened Colorimetric method 21 biotinidase deficiency (1:9,000) 3 Profound biotinidase deficiency The most frequent *BTD* gene variant: 1330G>C	(Neto et al., 2004)
Brazil/ Maringá, Paraná	Between 2001-2006 20,529 newborns screened 3 biotinidase deficiency (1:6,843)	(Luz Gdos et al., 2008)
Somalia/ Minnesota (Somali and Asian migrants)	Between 2004-2008 264,727 newborns screened Colorimetric method 31 biotinidase deficiency (1: 8,540) Profound biotinidase deficiency: 1:52,945 Partial biotinidase deficiency: 137,401 The most frequent *BTD* gene variant: p.P497S in 4 Somali patients, other detected p.P142T, p.Y428Y	(Sarafoglou et al., 2009)
Turkey (four cities in Central Anatolia)	Between 2006-2007 34,378 newborns screened 1 biotinidase deficiency (1: 34,378) 0 Profound biotinidase deficiency (0) 1 Partial biotinidase deficiency (1: 34,378)	(Tanzer et al., 2009)
Greece/Athens	Between 2007-2009 45,000 newborns screened Colorimetric method 1 biotinidase deficiency (1:45,000)	(Loukas et al., 2010)
Austria	Between 2002-2009 622,489 newborns screened 0 biotinidase deficiency (0)	(Kasper et al., 2010)
Greece	Between 2010-2011 18,954 newborns screened 5 biotinidase deficiency (1:3,791) The most frequent *BTD* gene variant: p.Asp444His, p.Gln456His	(Thodi et al., 2011)
Spain -Murcia	Between 2007-2010 71,595 newborns screened Colorimetric method	(Juan-Fita et al., 2012)
Greece	63,119 newborns screened Colorimetric method 14 biotinidase deficiency (1:4,508) 0 Profound biotinidase deficiency (0) 14 Partial biotinidase deficiency (1:4,508) The most frequent *BTD* gene variant: D444H, Q456H, T532M, C186Y, R157H	(Thodi et al., 2013)

United States of America/Michigan	Between 1988-2012 Colorimetric method Biotinidase deficiency 1:14,539 32 Profound biotinidase deficiency: 1: 101,779 197 Partial biotinidase deficiency: 1: 16,533 The most frequent *BTD* gene variant: No data	(Jay et al., 2015)
India/ Uttar Pradesh	13,426 newborns Semiquantitative fluorometric assay Biotinidase deficiency: 0 The most frequent *BTD* gene variant: No data	(Agarwal et al., 2015)
Netherlands	Between 2007-2012 304,982 newborns screened Colorimetric method 50 biotinidase deficiency (1:6,099) 6 Profound biotinidase deficiency (1: 50,830) 44 Partial biotinidase deficiency (1: 6,931) The most frequent *BTD* gene variant: c.1330G>C (p.(Asp444His), c.1368A>C, c.1595C>T	(Wiltink et al., 2016)
Italy	Between 1987-2016 1,097,894 newborns screened Colorimetric method 18 biotinidase deficiency (1:61,000) The most frequent *BTD* gene variant: p.Q456H	(Porta et al., 2017)
China/Zhejiang	Between 2009-2016 1,861,262 newborns screened 3 biotinidase deficiency (1:620,400)	(Hong et al., 2017)
Czech Republic	Between 2010-2017 Fluorometric method 181,396 newborns screened 21 Biotinidase deficiency (1: 8,638)	(David et al., 2019)
Italy/ Tuscany and Umbria	Between 2007-2019 466,182 newborns screened Colorimetric method 75 Biotinidase deficiency (1:6 300) 10 Profound: 1:46182 65 Partial: 1:7172 The most frequent *BTD* gene variant: p.(Asp444His) amino acid substitution and the complex allele p.(Ala171Thr)/p.(Asp444His)	(Funghini et al., 2020)
Brazil/ Minas Gerais	Between 2013- 2018 1,168,385 newborns screened Colorimetric method 84 Biotinidase deficiency (1:13,909) Profound 1:194,731 Partial 1:14,979 The most frequent *BTD* gene variant: c.1330G>C (p.D444H);c.511G>A (p.D444H;A171T), p.D543E, c.310-15delT (intronic), p.V199M, p.H485Q.	(Carvalho et al., 2020)

Saudi Arabia/ Riyadh	Between 2012-2017 56,632 newborns screened 2 Biotinidase deficiency (1:28,316) The most frequent *BTD* gene variant: No data	(Mohamed et al., 2020)
Italy/Abruzzo	Between 2018-2021 26,393 newborns screened 3 Biotinidase deficiency (1:8,797) Partial: 1:8,797 The most frequent *BTD* gene variant: c.1330G>C (p.D444H)	(Semeraro et al., 2022)
Turkey/Diyarbakır	Between 2011-2020 417,525 newborns screened 177 Biotinidase deficiency (1:2,359) Profound: 1:4,175 Partial: 1:5,422 The most frequent *BTD* gene variant: No data	(Toktaş et al., 2022)
Brazil/ Mato Grosso	Between 2005-2019 599.534 newborns screened 10 Biotinidase deficiency (1:59,953) The most frequent *BTD* gene variant: No data	(Costa et al., 2023)

In Turkey

In our country, biotinidase deficiency has been screened in newborn babies since 2008 within the scope of the national newborn screening program (Göksoy, 2023; *https://hsgm.saglik.gov.tr/tr/tarama-programlari/ntp.html*). In our country, 371,654 infants were screened for biotinidase enzyme deficiency using blood samples taken for newborn phenylketonuria screening between 1991 and 1998, and the incidence of biotinidase enzyme deficiency was found to be 1:11,614 (deep deficiency: 1: 14,866 and partial deficiency 1: 53,093). It was also reported that consanguineous marriage was 52% in patients with biotinidase deficiency (Baykal et al., 1998). In the evaluation of the results of 84,044 newborns whose heel prick blood was taken within the scope of newborn screening between 2011-2021 in Sivas, the lowest and highest prevalence frequencies for biotinidase deficiency were reported as 1:6,815- 1:1,861 (Akova et al., 2022). It was reported that 15,848 newborn babies were screened between 2015 and 2020 in Kırşehir Province and seven biotinidase deficiencies were detected (Bayrak et al., 2023). Between 2011 and 2020, 417,525 newborn babies were screened in Diyarbakır province and 177 of them were reported to have biotinidase deficiency (incidence of biotinidase deficiency: 1:2,359) (Toktaş et al., 2022). The prevalence of biotinidase deficiency in Şanlıurfa was reported as 1:1,177 (Karaca & Kazanasmaz, 2019). A previous study has reported an incidence of biotinidase enzyme deficiency of 1:7,116 according to the data published by the Ministry

of Health. The number of babies born alive in 2021 reported in the Turkish Statistical Institute is 1 million 79 thousand 842 and the Ministry of Health page states that 97.2% of these babies were screened and the Ministry of Health page states that 1,637 babies were diagnosed with biotinidase deficiency. According to this calculation, the incidence of biotinidase deficiency in our country in 2021 is 1:1,063 (https://data.tuik.gov.tr/Search/Search?text=bebek%20say%C4%B1s%C4%B1&dil=1; "https://hsgm.saglik.gov.tr/depo/birimler/cocuk-ergen-sagligi-db/Dokumanlar/Istatistikler/NTP.pdf,").

In our country, all newborn babies are currently screened for phenylketonuria, congenital hypothyroidism, biotinidase deficiency, cystic fibrosis, congenital adrenal hyperplasia, spinal muscular atrophy (SMA) within the scope of the Newborn Metabolic and Endocrine Disease Screening Program. For this purpose, screening is done in two stages. The first blood sample is taken from each newborn within 48 hours after oral feeding following birth. This blood sample is tested for biotinidase deficiency, phenylketonuria, and SMA. In addition, a second blood sample is taken within the first week at a family medicine unit or migrant health center. The second sample is tested for congenital adrenal hyperplasia, congenital hypothyroidism, and cystic fibrosis, and re-analyzed for phenylketonuria. In the first 48 hours, heel prick blood samples are collected from sick babies who are hospitalized in neonatal wards and entered into the web system for screening. A second sample is taken 48 hours after the baby is switched to oral feeding. It should be stated in the discharge summary given from the hospital that the heel blood sample of the infants who are subject to transfer is taken (https://hsgm.saglik.gov.tr/depo/birimler/cocuk-ergen-sagligi-db/Dokumanlar/Egitim_Dokumanlari/Saglik_Personeli/NTP_Saglik_Personeli.pdf). In the hospital where the sick baby is transferred, the discharge summary should be checked first, if there is no indication that a heel blood sample has been taken, a heel blood sample should be taken for screening and the discharge summary should indicate that a sample has been taken when the baby is discharged from the hospital. In all health units across the province, heel prick blood samples for the Newborn Screening Program are collected at the Provincial Directorate of Health and sent to Ankara and Istanbul Screening Laboratories. The blood results of the screening laboratories for each disease are transmitted to the provinces via the Newborn Screening Program Web Application, and infants with suspicious screening results are referred to the relevant clinics. Scientific commissions established for each disease in the screening program support monitoring and evaluation studies. In this way, approximately 4500 children per year can be protected from the consequences of existing diseases, and disability is prevented. Children should be followed up and treated according to the Ministry of Health's screening flow chart (Figure 1) (*https://hsgm.saglik.gov.tr/tr/tarama-programlari/ntp.html*).

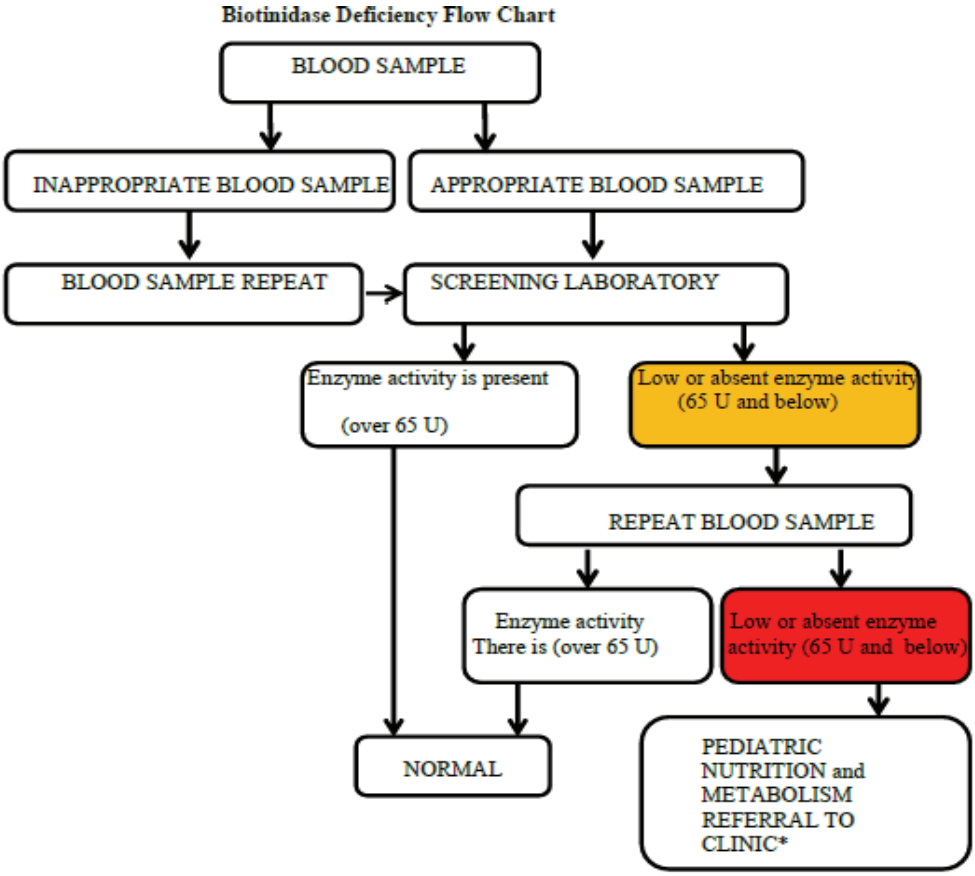


Figure 1. Biotinidase deficiency flow chart within the context of the Ministry of Health newborn screening program(https://hsgm.saglik.gov.tr/tr/tarama-programlari/ntp.html)

Biotinidase deficiency detection methods

Newborn screening includes fluorescent or colorimetric tests for biotinidase activity in dried blood spots on Guthrie paper. If presumptive positive samples have biotinidase activities below the threshold values set by the relevant screening laboratory, biotinidase deficiency requires measurement of biotinidase activity in serum or plasma. If there is low enzyme activity consistent with biotinidase deficiency, the patient's diagnosis is confirmed by molecular genetic testing (Wolf, 1993).

Various methods have been tried to detect biotinidase deficiency. The calorimetric method reported by Heard et al. in 1984 is the most commonly used. With this

method, the enzyme biotinidase hydrolyzes biotinyl-p- aminobenzoate (B-PABA) to p-aminobenzoic acid. It does not require special laboratory equipment and is suitable for screening for neonatal biotinidase deficiency (González et al., 2006; Heard et al., 1984). A method for biotinidase deficiency reported in 1963 but nowadays both laborious and unreliable, is the microbiological detection of biotin separated from its biocide. It is not used today (Khan et al., 2024). Fluorometric velocity assay with biotinyl-6-aminoquinoline substrate by fluorometric method. It has problems such as long incubation time (2 hours), sensitivity between tests , etc. Not much preferred. Several liquid chromatographic methods combined with mass spectrometry (LC/MS and LC-MS/MS)/ (a method based on liquid chromatography-tandem mass spectrometry) have been developed for activity measurement of biotinidase in dry blood spots and serum samples with high accuracy, precision, sensitivity, and selectivity. It is costly, requires special expertise, and is a complex method and therefore not preferred. Two HPLC methods using B-PABA and biotinyl-6-aminoquinoline as substrates in milk and serum are complex, laborious, require 1 ml of serum, long run time, not preferred (Khan et al., 2024).

False positivity- false negativity. Since direct enzyme analysis is performed in biotinidase deficiency, the biotinidase enzyme is not affected by food. If the baby is premature, if there is neonatal health, if the baby's albumin levels are above normal, if the triglyceride levels are high, or if the baby or mother is on valproic acid treatment, it causes a false positive test. Water content in the filter paper, chemicals used in the printing process, high temperature, and humidity can reduce biotinidase activity. Causes false positives (Henderson et al., 2023; Strovel et al., 2017; Sürücü Kara et al., 2023). If treated with sulfa drugs, false negative results may occur when biotinyl-6-aminoquinoline is used as substrate (sulfa drugs are contraindicated in pregnant women and infants) (Canda et al., 2020).

Genetic of biotinidase deficiency

Biotinidase deficiency is caused by biallelic pathogenic variants in the *BTD* gene (OMIM 253260). The diagnosis of infants with low serum biotinidase activity should be confirmed by genetic analysis. Genetic analysis may also be required to identify family members at risk. The relationship between genotype and phenotype is not fully established. Insertions, deletions or nonsense variants usually cause a complete loss of biotinidase activity. In the ClinVar database for the *BTD* gene, 150 pathogenic, 125 likely and 218 uncertain significance pathogenic variants have been reported (https://www.ncbi.nlm.nih.gov/clinvar/?term=btd%5Bgene%5D&redir=gene). In the United

States, the most common *BTD* gene mutations in infants with symptomatic profound biotinidase enzyme deficiency are c.98-104del7ins3 and p.Arg538Cys. In addition, p.Asp444His (c.1330G>C), p.Gln436His (c.1308A>C), p.Ala151Thr (c.451G>A), and p.Asp232Gly (c.695A>G) mutations have been reported in most patients with profound biotinidase deficiency in the USA. While the most common *BTD* gene variation in Europe is p.Asp444His, the most common *BTD* gene variants in Turkey are p.Asp444His (c.1330G>C), p.Arg157His (c.470G>A), c.98-104del7ins3 (p.C33Ffs*36) and p.Thr532Met. The most frequently detected *BTD* gene in profound biotinidase enzyme deficiency is the p.Arg157His (c.470G>A) variation, the most frequently detected *BTD* gene in partial biotinidase enzyme deficiency is the p.Asp444His (c.1330G>C) variation in Turkey (Canda et al., 2020; Sürücü Kara et al., 2023; Wolf, 1993).

Conclusion

It is important to screen for biotinidase deficiency, which is diagnosed in the neonatal period and promising when treatment is initiated. Attention should be paid to situations that will affect the analysis result in terms of false positive and negative values. In infants with positive screening results, serum/plasma biotinidase activity should be checked and the diagnosis should be confirmed by molecular genetic analysis. Early diagnosis and initiation of treatment prevent the development of the disease and reduce the economic cost of the disease.

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Chapter

6

Newborn Screening for Cystic Fibrosis

Ismail GUZELKAS

Cystic fibrosis (CF) is a multisystem genetic disease with autosomal recessive inheritance. Cystic fibrosis develops as a result of pathogenic mutations in the genes encoding cystic fibrosis transmembrane conductance regulatory protein (CFTR). It is a progressive and life-shortening disease characterized by recurrent lung infection, exocrine pancreatic insufficiency, malnutrition, growth and developmental delay(Rowe et al., 2005). Since studies have shown that the course of the disease is better in cases diagnosed in the asymptomatic period, it has been included in newborn screening (NBS) programs in many countries for early diagnosis.

In the past years, the diagnosis of CF was made through a sweat test performed after clinical findings compatible with CF developed. With the demonstration that immunoreactive trypsinogen (IRT) could be used in CF screening in the late 1970s, a new era in the diagnosis of CF began and CF NBS programs began to be formed. With this development, the opportunity has arisen to compare patients diagnosed with screening programs in the following years with patients presenting with clinical findings. The study by Farrell et al. showed that patients diagnosed through a NBS program had better growth parameters at diagnosis and during follow-up than patients diagnosed late(Farrell et al., 1997). In the Sydney cohort, which is an important study on long-term results, it was observed that the survival of CF patients diagnosed by screening at

the age of 25 was higher than that of patients diagnosed late(Dijk et al., 2011). Indicating that it is a cost-effective method, it has been shown in the United Kingdom registry cohort that the treatment costs of CF patients diagnosed with a screening program are lower than those diagnosed with clinical findings(Sims et al., 2007).

For the first time, the underlying pathology has been targeted in treatment with CFTR modulators, which are effective in the treatment of CF and are increasingly used. In individuals carrying the variant indicated for treatment, CFTR functions increase, the individual's symptoms regress, and the quality of life and life expectancy increase(Middleton & Taylor-Cousar, 2021). The value of the NBS program has increased even more with these treatments. Prevention of organ damage and longer life spans are aimed with CFTR modulators to be started from the neonatal period. Today, ivacaftor can only be used for 1 month or more in CF cases carrying the variant compatible with treatment. Once the effectiveness and safety of other CFTR modulators are demonstrated in the neonatal period, it will be possible to start them on patients diagnosed through screening.

Cystic fibrosis screening programs are carried out with various strategies. Countries use different strategies depending on economic conditions and CF variant frequencies. The most commonly used biomarkers and analysis methods are IRT, pancreatitis-associated protein (PAP), CFTR DNA analysis (DNA), and extended CFTR gene analysis (EGA). The first step of all CF screening programs is IRT measurement. Frequently used CF screening programs are given in Table 1.

Due to the nature of screening programs, false positives and false negatives may occur. In order to minimize this situation, countries should frequently optimize the program with the data they have. It should be kept in mind that babies of mothers diagnosed with CF and using CFTR modulator therapy during pregnancy may not be detected in screening programs. These cases should be monitored and referred for sweat

Table 1. Cystic Fibrosis Screening Programs

1.	IRT/IRT
2.	IRT/PAP
3.	IRT/DNA
4.	IRT/DNA/EGA
5.	IRT/PAP/DNA

IRT: Immunoreactive trypsinogen
PAP: Pancreatitis-associated protein
DNA: CFTR pathogenic variant panel
EGA: Extended CFTR gene analysis

testing. Although NBS programs are not diagnostic tests for CF, they aim to ensure early diagnosis of suspicious cases by directing them to sweat testing. For the European Cystic Fibrosis Society (ECFS) CF screening program to be acceptable;

1. The total number of samples to be repeated due to inappropriate blood sample is 0.5% less
2. The positive predictive value(PPV) must be at least 30%
3. The program must have at least 95% sensitivity
4. It recommends that the case detected by the program be evaluated by a CF team within 58 days at the latest(Castellani et al., 2018).

Newborn Screening Programs For Cystic Fibrosis

IRT/IRT

Immunoreactive trypsinogen increases in serum at pancreatic damage. High IRT level can be detected in CF, perinatal stress, prematurity, and sometimes in healthy newborns. Although its levels decrease after birth in cases other than cystic fibrosis, it remains high for up to 4 weeks in CF(Wilcken et al., 1983). Although IRT measurement at the 1st week of birth is a sensitive method in CF screening, it is not specific. IRT levels may be detected as low in meconium ileus, which may cause false negativity in the screening program. In this protocol, blood is collected from capillary blood onto Guthrie papers within the first week after birth. Cases above the determined cut-off value (usually >99 percentile) are called to health centers for a second IRT measurement. In the second example, if the IRT is above the determined cut-off level, the CF screening program is considered positive and the patient is referred to the CF center for a sweat test. It is thought that this program may cause delay in diagnosis since a second blood sample must be taken. It can be used in populations of different ethnicities with unknown CFTR variant frequencies. While the advantages of this method include low cost and not detecting CF carrier individuals, its disadvantages are high false negative and false positive rates and delays in diagnosis(Farrell et al., 2017). The IRT/IRT program has been implemented in Turkey since 2015. According to this program, the cut-off for the first IRT was 90ng/ml, while in the second example, it was determined as 70ng/ml(Refika Ersu, 2015)(Figure 1). In a study conducted in Turkey evaluating the specificity and sensitivity of the IRT/IRT protocol, it was observed that the sensitivity of the protocol was over 96% while the PPV was 7.1%(Ramasli Gursoy et al., 2023). False positive cases detected by the screening program cause both increased costs and anxiety in their families(Gokdemir et al., 2021). For this reason, false positive rates are being tried to be reduced.

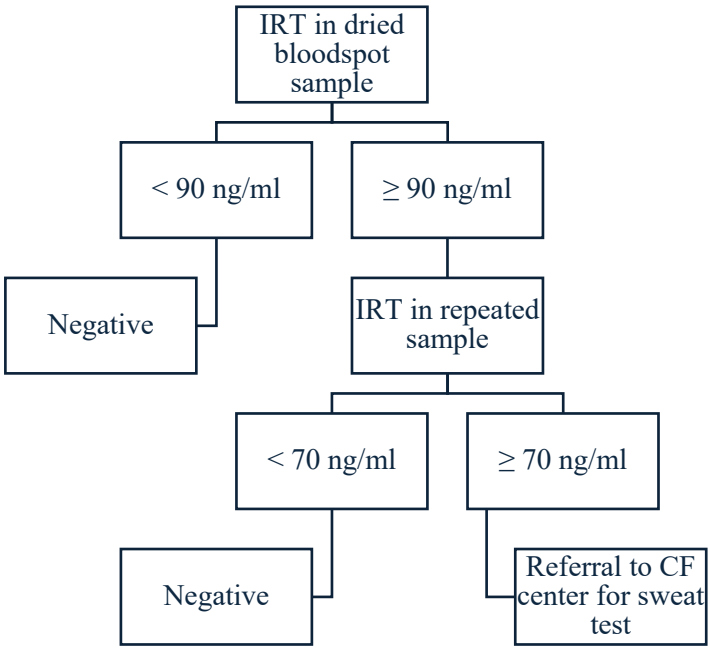


Figure 1. CF screening program implemented in Turkey

IRT/PAP

Pankeratitis-associated protein is a protein that is not normally detected in the blood but is released in case of pancreatic damage. Although blood levels increase in CF, it is not specific to CF(Weidler et al., 2016). In this program, if the initial IRT is above the determined cut-off, PAP is studied from the same sample. If it is detected above the specified cut-off, the patient is referred to the CF center for a sweat test. Since it is not necessary to take a second sample, it does not cause a delay in diagnosis. It is a less costly screening method than the IRT/DNA program. Another advantage of the program is that it does not detect individuals who are CF carriers. A study conducted in Argentina showed that the IRT/PAP program is more sensitive and has a lower false-positive rate than IRT/IRT(Teper et al., 2021). If the genetic analysis facilities of the screened population are limited and the CFTR variant frequency is unknown, the IRT/PAP program may be preferred. The IRT/PAP protocol is given in Figure 2. By adding 3rd step DNA to this program, it is aimed to increase the PPV. Germany has been using the IRT/PAP/DNA program since 2016. In a study evaluating this program, the PPV was found to be 23%(Gesenhues et al., 2024). EGA can be added to the program to prevent false negatives. When the case data scanned according to the IRT/PAP/DNA/

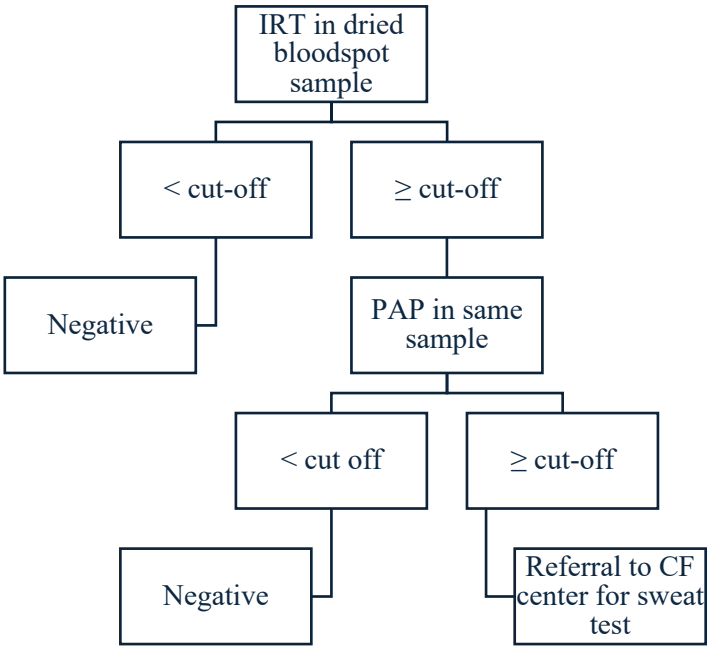


Figure 2. IRT/PAP CF screening program

EGA program in the Netherlands between 2011 and 2014 were evaluated, it was seen that the positive predictive value of the program was 63%(Bouva et al., 2024).

IRT/DNA

With the identification of the CFTR gene in CF, it began to be used in both diagnosis and screening. As in other programs, the first step is the measurement of IRT in blood taken within the first week of the newborn. In cases above the specified value, the CFTR variant panel is studied from the same sample. The IRT/DNA program is given in Figure 3. Here, the variant panel may be only F508del, or it may be a variant panel containing the most common CFTR variants in that population. It is the most frequently applied CF screening program in the world due to its low false negative rate and the fact that it does not cause any delay in diagnosis. It is more costly than other methods. In this program, the diagnosis of CF can also be made by demonstrating two known pathogenic variants. False negatives may occur due to rare variants not being detected. To prevent this situation, EGA can be added to the program. The name of this program is called IRT/DNA/EGA. It can be studied from the same sample without the need for a new blood sample. While false negative cases are rarely observed in

this program, cases that do not meet the diagnostic criteria for CF can be detected due to the detection of variants of unknown clinical significance. These asymptomatic cases, whose screening program was found positive but did not meet the CF diagnostic criteria, are called "Cystic fibrosis screening positive, inconclusive diagnosis" (CFSPID) or "CFTR-related metabolic syndrome" (CRMS)(Munck et al., 2015). These situations can be summarized as follows;

1. NBS positive and the sweat test is intermediate and 1 or less CF-related CFTR variant is detected
2. NBS positive and the sweat test is negative and 1 or more variant are not known to be related to CF in an individual with 2 CFTR variants

It is recommended to follow up these undiagnosed cases and repeat the sweat test. Since individuals with rare CFTR variants may be missed in the IRT/DNA program, the 3rd step, IRT measurement, can be added to this program. In this program, which is IRT/DNA/IRT, specificity increases. When the 15-year screening program data of a large center in the United Kingdom was evaluated, it was seen that PPV was quite successful with 66.7%(Driscoll et al., 2024).

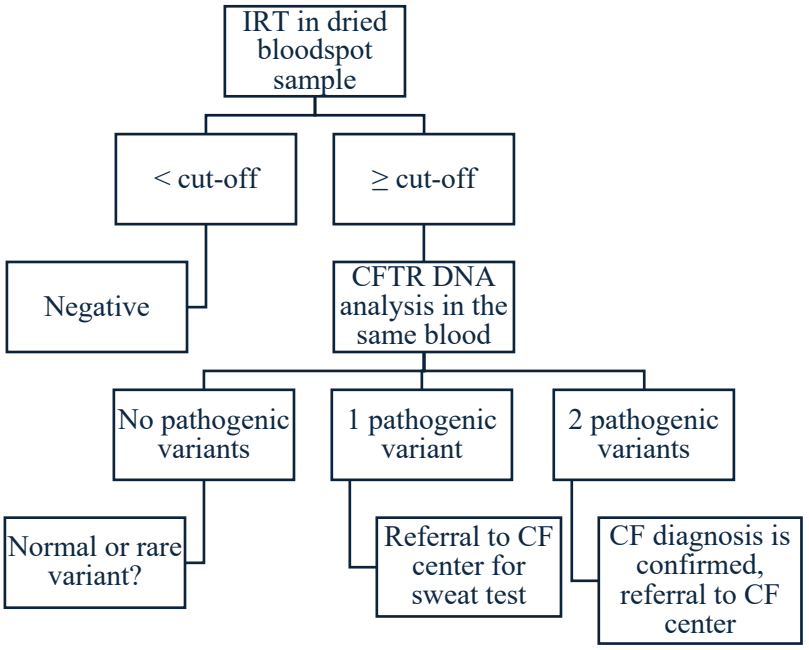


Figure 3. IRT/DNA CF screening program

As a result, no matter which program is applied, positive cases should be directed to CF centers and the diagnosis should be confirmed by sweat test. Cases in which the diagnosis of CF cannot be excluded should be followed up in CF centers and sweat tests should be repeated. No implemented program is perfect, patient records should be evaluated annually and the program should be optimized to improve the program.

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Chapter

7

Newborn Screening for Spinal Muscular Atrophy

Elif YILDIRIM, Hüseyin TAN

Spinal muscular atrophy (SMA), an autosomal recessive hereditary condition that damages the anterior horn cells in the spinal cord and the motor nuclei of the brainstem. Progressive muscle weakness and atrophy are the hallmarks of the disease. (Hashimoto et al., 2023) Usually, the lower extremities are primarily affected, followed by the upper extremities, spine, and neck muscles. In more severe cases, the pulmonary and masticatory muscles may also be affected. The five subtypes of SMA are as follows: type 0 (the most severe form), type 1 (Werdnig-Hoffmann disease), type 2 (Dubowitz disease), type 3 (Kugelberg-Welander disease), and type 4 (adult form, the mildest form), based on the age of onset and maximal degree of function. (Nishio et al., 2023) With an estimated global frequency of 1 in 6,000–11,000 live births, SMA is a major hereditary cause of infant death. 1 in 60 individuals is a carrier of a mutation in the SMN1 gene. (Díaz et al., 2023) Although SMA usually shows an autosomal recessive inheritance pattern, de novo mutation occurs in 2-4% of cases.(Hashimoto et al., 2023) The disease is caused by mutations in the motor neuron survival gene 1 (SMN1) on chromosome 5. (Butterfield, 2021) In healthy individuals, the SMN1 gene produces a protein crucial for motor neuron survival. Both the SMN1 and SMN2 genes have nine exons and are highly similar, sharing over 99% nucleotide identity. The crucial difference lies in a single nucleotide substitution within exon 7 of SMN2, which leads to exon skipping

during mRNA processing, resulting in the production of predominantly truncated and unstable SMN protein. This discrepancy in protein production between SMN1 and SMN2 accounts for the varying severity of SMA types, as SMN2-derived transcripts are largely ineffective in compensating for the loss of functional SMN protein. (Eissa et al., 2022) Individuals who are healthy typically have two copies of SMN2, whereas those with SMA may have between 1 and 5 (or more) copies. Each SMN2 copy can only produce approximately 10% of functional SMN protein. (Hashimoto et al., 2023)

The severity of SMA is linked to the number of SMN2 copies present, with a higher number of copies usually associated with a milder form of the condition. However, it is not only the SMN2 gene copy number that determines disease severity in SMA; other genetic modifiers are also thought to be involved. (Aragon-Gawinska et al., 2023)

The widespread implementation of neonatal screening for SMA marks a significant advancement in early detection and treatment strategies for the disease. This approach has been adopted in various countries, including Italy, Canada, Germany, Belgium, Australia , the United States, Japan, and Taiwan, as reported by several studies.(Oliveira et al., 2023)

In Turkey, this screening program was started in 2022, additionally, to determine the carrier individual, a pre-marital screening test is applied too. Absolutely, having an established system of NBS for SMA is crucial for early diagnosis and subsequent effective therapy.(Glascock et al., 2018) Typically, NBS for SMA involves screening dried blood spots (DBS) from newborns. Real-time quantitative polymerase chain reaction (qPCR) has been widely employed as the primary method for initial screening, while the Multiplex Ligation-dependent Probe Amplification (MLPA) technique is commonly used for diagnostic confirmation in many studies. This comprehensive approach ensures accurate and timely detection of SMA, facilitating prompt intervention and management. In comparison to type 3 SMA (86%), types 1 and 2 have a slightly greater frequency of homozygous deletion of SMN1 (96% and 94%, respectively). (Oliveira et al., 2023) While newborn screening (NBS) for SMA has significantly improved early detection rates, it's important to note that current methods may not identify around 5% of SMA cases. This subset of cases typically involves individuals who are compound heterozygotes, meaning they have one deleted SMN1 allele and a second allele with a point mutation. These cases can present challenges for detection using standard NBS methods, highlighting the need for ongoing refinement and improvement in SMA screening protocols to capture a broader spectrum of genetic variations associated with the disease. (Hashimoto et al., 2023)

Before NBS programs, SMA was classified according to the patient's age at onset and maximal motor function. Today, since SMA is diagnosed in the pre-symptomatic

period with NBS, the classification is trying to be predicted by the SMN2 gene copy number. Most patients with type 2 have three SMN2 copies, milder individuals with type 3 have three or four SMN2 copies; and most patients with severe type 1 only have one or two SMN2 copies.

As can be seen, the SMN2 gene copy number does not always accurately predict SMA types. (Aragon-Gawinska et al., 2023; Glascock et al., 2018) SMA Carrier Screening (SCS) is performed on couples in the pre- marital phase and on couples who are already married and considering starting a family. It is necessary for at least one parent to undergo the SCS. Blood samples are taken for screening, typically about 2- 3cc in a single EDTA tube, and DNA is isolated from the blood sample in a laboratory. The isolated DNA is subsequently examined to identify any mutations in the SMN1 gene, a process commonly carried out through the polymerase chain reaction (PCR) technique.(Glascock et al., 2018) The average duration of the whole process is 10 days. It is crucial to consider the partner of the individual identified as a carrier in the SCS. If neither parent carries the SMA gene mutation, there is no risk of the child having SMA. However, there is still a 50% chance that the child could be a carrier of the SMA gene mutation inherited from one of the parents. The prospective spouse should also undergo SCS. If an individual is confirmed to be a SMA carrier, this increases the likelihood of their offspring inheriting SMA. When both parents are carriers of the SMA gene mutation, there is a 25% chance with each pregnancy that their child will have SMA. Couples facing this situation have several options, including genetic counseling to help them understand their risks and options for family planning. Preimplantation genetic diagnosis(PGD) combined with in vitro fertilization (IVF) is another option, allowing for the screening of embryos to identify those free of the SMA mutation before implantation. If a pregnancy occurs without prior genetic screening, diagnostic procedures such as chorionic villus sampling (CVS) at 12 weeks, amniocentesis (AS) at 16 weeks, or cordocentesis (umbilical cord blood sampling) at 20 weeks can be performed to detect SMA in the unborn child. These diagnostic tests provide valuable information to expectant parents, enabling them to make informed decisions about their pregnancy and prepare for the potential challenges associated with SMA.

The approval of Nusinersen by the US Food and Drug Administration (FDA) in December 2016 marked a significant milestone in SMA treatment. It's an antisense oligonucleotide SMN2 modulator administered intrathecally with an initial series of loading doses over two months followed by maintenance doses every four months. Notably, its approval encompasses all SMA patients, regardless of age, based on the positive outcomes observed in randomized, blinded clinical trials.(Butterfield, 2021; Łusakowska et al., 2023) Onasemnogene abeparvovec,employs adeno-associated virus (AAV9)

vector to deliver a functional copy of SMN1 gene to motor neurons. It's administered intravenously with a specified dosage during an hour-long infusion. Both treatments offer promising options for SMA patients, addressing the underlying genetic mechanisms associated with the disease. In order to handle major possible problems, such as liver inflammation, this treatment method is a complex application that requires expertise. In August 2020, the FDA authorized risdiplam, an oral SMN2 modulator, for patients with SMA who were older than two months. In 2018, a therapy algorithm for SMA was created based on expert consensus. Newborns with SMN2 copy number 2 or 3 and diagnosed by SMA through NBS, should be started treatment as early as possible, at the latest in the first 6 weeks.(Butterfield, 2021)

With the newborn screening program, SMA patients can be diagnosed at an asymptomatic stage. Infants diagnosed with SMA through NBS have lower medical costs and societal burdens compared to those diagnosed and treated after symptom onset.

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Chapter

8

Developmental Dysplasia of the HIP

Mustafa Kemal YENİDUNYA

Developmental dysplasia of the hip (DDH) is a common condition in newborns resulting from structural abnormalities of the structures that form the hip, despite being normal or near-normal during intrauterine development, due to various reasons during the later stages of pregnancy and early newborn period. A study by Dunn et al. demonstrated that signs of hip dysplasia were not observed in fetuses undergoing abortion at 20 weeks of gestation, indicating that DDH is a condition relevant to the late intrauterine period (1). Additionally, the condition exhibits a dynamic process and may progress or regress during the newborn period due to external factors affecting the baby.

The hip joint begins to develop with a cartilage-like structure around the 7th week of gestation. As pregnancy progresses, the femoral head develops faster than the acetabulum. At birth, the femoral head is surrounded by the acetabulum in less than 50% of cases. During this period, the acetabulum is at its loosest and most superficial state, facilitating childbirth. After birth, the development of the femoral head and acetabulum is closely related. Acetabular cartilage begins to develop and grow rapidly. This development allows the acetabulum to encompass the femoral head and establish normal hip anatomy. If the femoral head and acetabulum do not come into contact, these two structures cannot develop fully. Growth and development depend on two factors: proper alignment of the femoral head within the acetabular cavity and parallel, synchronized growth of the

triradiate and acetabular cartilage. When these factors are in place, as the bone grows, the femoral head transforms into a spherical structure, while the acetabulum acquires a socket-like feature. The femoral head is securely fixed to the acetabulum by various soft tissues and ligaments (capsule, ligamentum teres, transverse ligament). This results in the formation of a highly mobile, weight-bearing hip joint.

During the development of the hip joint, if the acetabulum fails to properly cover the femoral head, the acetabular cavity begins to flatten. Structures such as the labrum, capsule, and transverse ligament undergo hypertrophy. As a result of this flattening and hypertrophy, the percentage of coverage of the femoral head by the acetabulum decreases further. In later stages, this condition results in an increase in dysplasia. While it is traditionally believed that acetabular anteversion increases in classic hip dysplasia due to orientation, some studies have also shown no difference in anteversion between affected and unaffected sides (2). Increased valgus, shortening of the neck, and increased anteversion are also observed in the proximal femur (3).

The term DDH encompasses a wide range of hip changes, ranging from neonatal instability, dysplasia, sublucation, dislocation and even teratologic hips. In dysplasia, the asetabulum is shallow and underdeveloped. In more advanced stages of subluxation, there is a slight displacement of the joint with minimal contact between the joint surface. Dislocation refers to complete lack of contact between joint surfaces due to displacement. The term of teratologic hip is an advanced pathology characterized by a hip joint that cannot be reduced upon the examination, resulting in the formation of pseudoacetabulum. It is crucial to differentiate between these pathologies as their clinical courses, diagnosis, and treatments are entirely different. Early-stage pathologies may be challenging to detect but are easier to treat. Diagnosis becomes easier in later stage, but treatment becomes more challenging.

Various theories have been proposed regarding the cause of DDH. One of these theories is hormonal theory, which suggests that estrogen is protective against dislocation while progesterone may contribute to dislocation, as demonstrated experimentally (4). Another theory is the mechanical theory, which proposes that the fetus's flexible structure and rapid growth may lead to external deforming forces that can result in DDH. Factors such as first pregnancy, female gender, breech presentation, oligohydramnios, macrosomia, multiple pregnancies, and hyperlaxity can also be considered as risk factors for DDH during the intrauterine period. A normal newborn baby has knee and hip flexion posture that spontaneously resolves in the following weeks. Tightly swaddling, such as in bundling, can hinder the development of the hip joint, thus predisposing to DDH. Nevertheless, familial predisposition has also been identifying as a risk factor for DDH in the literature. When DDH is present in first-degree family members, the risk

increases by 12 times, whereas it increases by 1.7 times when present in second-degree relatives (5). A meta-analysis by De Hundt et al. demonstrated that familial predisposition, breech presentation, and female gender increase the risk of DDH (6). In a study by Bache CE et al., the most significant risk factors were identified as female gender, breech presentation, family history, and hip instability (7). In a study conducted in Turkey by H. Ömeroğlu et al. using ultrasound, positive family history and swaddling were found to be involved in the etiology of DDH patients, and it was also understood that swaddling exacerbated dysplasia in DDH patients (8).

Congenital dislocation of the knee, metatarsus adductus, congenital muscular torticollis, and other conditions often coexist with developmental dysplasia of the hip (DDH) (9). It is beneficial to screen and examine newborns with these conditions for the presence of DDH as well.

For the diagnosis of developmental dysplasia of the hip (DDH) in a newborn, collaboration among family physicians, pediatricians, radiologists, and orthopedic surgeons is necessary. These healthcare professionals need to be familiar with the clinical presentation, physical examination, and radiology of DDH to make the diagnosis.

In the newborn examination for DDD, the Ortolani and Barlow tests are important. In both tests, the baby's hip is grasped with thumb placed on the medial aspect of the thigh and the second and third fingers over the greater trochanter. The hip and knees are flexed to 90 degrees. In the Ortolani test, the hips are abducted, and gentle pressure is applied inferiorly to the hip. This maneuver reduces the subluxated femoral head. If this reduction is felt by hand, the Ortolani test is considered positive (Figure 1.a). In the Barlow test, the hip is adducted with slight posterior pressure applied from the knee. In this test, the hip joint subluxes from its place. If this subluxation is palpated with fingers, the Barlow test is considered positive (Figure 1.b) (10). These tests are nonspecific and can resolve spontaneously by the 6th week of life.

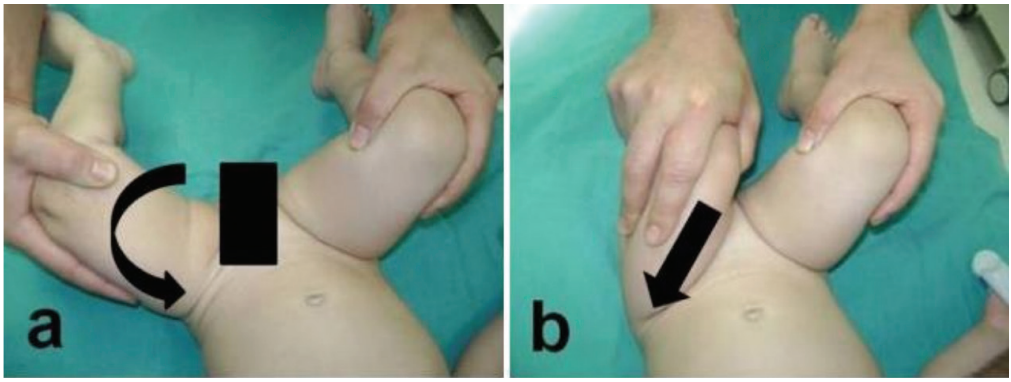


Figure 1. a. The Ortolani test b. The Barlow test

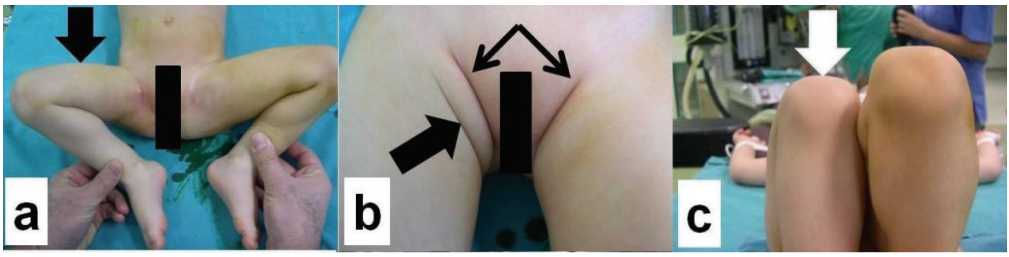


Figure 2. a. Abduction limitation b. Asymmetry in thigh folds c. Galeazzi sign

Abduction limitation is another important parameter in the diagnosis of DDH, and it is specific in unilateral DDH diagnosis. (Figure 2.a). Another physical examination finding is asymmetry observed in thigh folds. (Figure 2.b). Another specific sign and test for DDH is the measurement of the difference in length caused by the shortened femur when the hip is flexed to 90 degrees. This measurement result is referred to as Galeazzi sign. (Image 2.c) (10)

In the diagnosis of DDH, radiological parameters are also utilized. Since the pediatric femoral head begins ossification around the 4^{th}-6^{th} month, ultrasound is the most reliable parameter for diagnosis until these months. Although ultrasound is the most significant adjunctive method in diagnosis, an accurate diagnosis can be made when performed by experienced radiologists and orthopedic surgeons. In ultrasound, many anatomical structures such as bone acetabular anatomy, femoral head, and hip capsule can bu visualized, and measurements between these structures are taken. The measurements are classified using the Graf classification to determine the level of dysplasia, and appropriate treatment is applied based on this level. In later months, diagnosis can be made using pelvic anteroposterior and lateral radiographs. Various measurements are taken in these radiographs to determine the level of dysplasia. Computed Tomography (CT) and magnetic resonance imaging (MRI).

Due to the ease of early diagnosis with ultrasound and the prevalence of hip instability as one of the most common newborn conditions, various screening programs are implemented worldwide during the neonatal period. The prevalence varies, with rates of 3.6 in the United Kingdom, 25.5 in Spain, 35.8 in Eastern Europe (11), and between 5-15 in Turkey. Early recognition and treatment of this condition, which is prevalent at such frequencies in newborns, reduce both patient health risks and costs for countries.

Early diagnosis and treatment programs can be implemented differently in each country. All babies need to be screened in these programs. In many countries, all babies undergo ultrasound screening. However, it has not been proven to be more

cost-effective. Some examples of screening programs in various countries are as follows:

- In Austria, all newborns are screened. The first examination and ultrasound performed in the first week, and the second examination is conducted at 6-8 weeks.
- In the Czech Republic, a triple screening system is applied. According to this system, the first ultrasound is done in the first week, the second ultrasound is performed at 6-9 weeks, and the third ultrasound is carried out at 9-12 weeks. If there is suspicion radiography is added.
- In Denmark, selective ultrasound screening is conducted for DDH. After birth, the baby is examined by a midwife. This examination is repeated by a general practitioner at 5 weeks. If there is a positive finding or a positive family history, patients undergo ultrasound using the Graf technique.
- In France, babies with positive clinical examinations or negative clinical examinations but with at least one positive family history are recommended to undergo ultrasound using the Graf technique at 4 weeks.
- In Greece, the screening program for babies follows the recommendations of the American Academy of Pediatrics. Ultrasound screening is recommended at 3-4 months for babies with positive clinical examination findings and at 6 months for babies with positive family histories.
- In Norway, clinical and ultrasound examinations are performed within the first three days of life. If abnormal findings are detected, ultrasound is repeated 2-3 weeks later.
- In Turkey, within the first 48 hours after birth, the responsible healthcare personnel provide information to the family about DDH diagnosis and risk factors through face-to-face interaction. If there is a positive family history of DDH, an appointment is made for ultrasound screening. If there is no family history, the family is referred to the family physician for examination. After the examination by the family physician, if there is a positive finding, the baby is referred for ultrasound with a prepared document.

Although screening programs for DDH are implemented rigorously, as previously mentioned, the high frequency of occurrence and the inability of some patients to access screening programs can lead to a failure in diagnosis. This can result in disability. If diagnosed early, DDH can be treated with simple and inexpensive treatments such as bracing and swaddling, which do not pose complications for the patient. In such cases, facilitating the compliance of both the family and the baby with current treatment makes more feasible. In moderate to advanced

stages, if these diagnoses are made, the disease can be treated with simple immobilization techniques that do not require surgical intervention. In advanced stages, if the disease is diagnosed in the neonatal period, surgical treatments such as hip reductions and anatomical reconstructions can be performed to restore hip joint anatomy. However, if these patients are not recognized in the neonatal period, a more difficult and challenging recovery period ensues, involving surgical procedures with large soft tissue incisions and bone osteotomies, resulting in decreased compliance of patients and families.

Undiagnosed hip instabilities can lead to femoral and acetabular osteoarthritis in later stages and ages. Consequently, patients may experience leg length discrepancies, limping, and chronic pain. Due to anatomical deformities, the femoral head may articulate with the iliac bone rather than the acetabulum, resulting in a significant increase in leg length discrepancy. In such cases, treatment typically involves total hip replacement and osteotomies, which are major surgeries that increase patient mortality and morbidity.

In conclusion, early diagnosis, and treatment of DDH are important for ensuring the anatomical integrity of the hip, preserving natural structures, enhancing patient and family compliance, and reducing the burden and expenditures of the healthcare sector. With increasing diagnostic parameters in the evolving world, the diagnosis and treatment of DDH are expected to become easier and more widespread.

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Chapter

9

Vision Screening and Ophthalmic Evaluation

Fatma Tugce YAVUZ OZTURK, Emine CINICI

1. Normal Growth and Development of the Eye

Most of the growth of the eye occurs throughout infancy and early childhood. Axial length increases by 3 mm from birth to 1 year of age. Corneal diameter also increases rapidly during the first year of life. Accordingly, the cornea flattens and becomes thinner gradually.

Refractive state

While the eye's axial length increases and the lens and cornea flatten with aging, the refractive state of the eye changes. Generally, the eyes are hyperopic at birth, and become slightly hyperopic until 6-8 years of age, followed by a myopic shift toward emmetropia. These changes during eye development are called emmetropization. It has been shown that myopia developing before 10 years of age is associated with progression to adult myopia of 6.00d or more. Increased outdoor activities and topical lo-dose atropin therapy may delay the onset and progression of myopia in children.

Orbit and ocular adnexa

Orbital volume increases and orbital opening becomes horizontally oval during childhood. Horizontal growth of palpebral fissure is more than vertical growth. At the end of these changes, it reaches the elliptical adult shape.

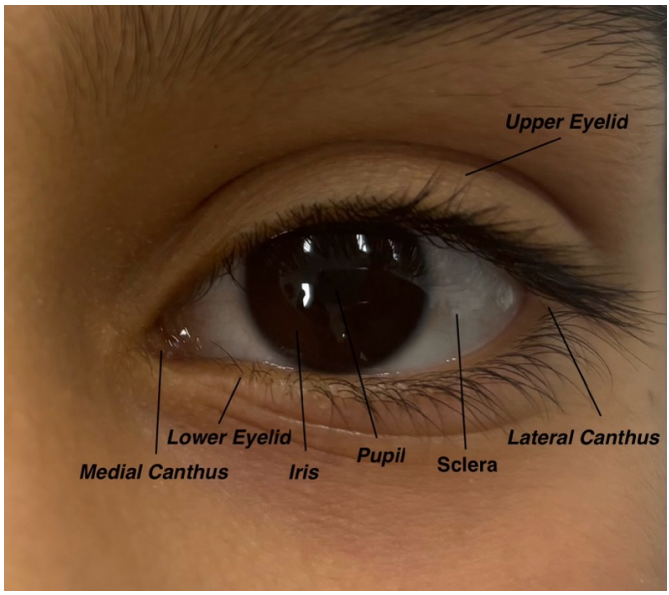


Figure 1. External structures of the eye

Cornea, Iris, Pupil, and Anterior Chamber

The corneal endothelial cell density (ECD) is high at birth and diminishes as the corneal diameter increases. In pathological situations like congenital glaucoma, an increase in corneal diameter causes greater ECD reduction.

Because of pigment accumulation in the iris stroma, changes in iris color may occur over 6-12 months of age. The infant pupil is relatively small according to the adult pupil.

Intraocular Pressure

The normal intraocular pressure (IOP) is lower in infants than in adults, therefore IOP levels higher than 21mmhg should be taken abnormally in infants.

Retina

Retinal vascularization begins at the optic nerve head at 16 weeks of gestational age and reaches the temporal ora serrata by 40 weeks of gestational age. The macula development is poor at birth. Significant changes like macular pigmentation, and cone photoreceptor differentiation take place during the first 4 years of age.

2. General Considerations

Since the eye structures and ophthalmic needs of children differ greatly from those of adults, early diagnosis and treatment are of great importance. Untreated vision disorders can affect the child's normal development, school performance, and social interactions and lead to problems in adulthood.

Examinations are more successful when children are relaxed and feel safe. Because children may be afraid of white coats, many practitioners forego it. When the practitioner enters the examination room, he/she collects data about the child's visual behavior, any abnormal head position, any dysmorphic features, and familial disorders. A parent's phone may contain old photos and videos that provide useful information about progressive disorders. Asking children easy questions can make them feel confident. For a child who has difficulty in the examination, it may be helpful to take some rest periods, persuasion, persistence, patience, and rewards. The examiner should sit at the same eye level as the child. Since the child's attention can be easily distracted, the most critical part of the examination should be done first.

3. Patient History

The patient's history is an initial and crucial component of the examination. It helps identify and evaluate possible causes of the problem. A comprehensive patient history for pediatric population may include, but is not limited to:

- History and nature of the current complaint
- Visual and ocular history
- Prenatal (gestational diabetes, intrauterine growth retardation, in utero exposure to infection), perinatal (fetal distress, oxygen deprivation), postnatal (trauma, seizures) problems
- Prematurity history (retinopathy of prematurity, optic nerve hypoplasia)
- Systemic abnormalities
- Family history (an inherited retinal, optic nerve, or neurologic disease, family history of strabismus, refractive status of parents)

- Developmental and school performance history of the child
- Time spent outdoors and near work

The American Academy of Ophthalmology and the American Academy of Pediatrics recommend assessment of the visual system from birth and at all routine health visits. The child's eye anatomy and visual functions should be rechecked at every visit furthermore, visual acuity should be evaluated at the preschool child as well as when there is a complaint. Normal visual development stages of a child are shown in Table 1. Infants with a strong family history of strabismus, congenital glaucoma, congenital cataracts, and known risk factors like retinopathy of prematurity should be referred for further evaluation.

Table 1. Normal visual development of a child

NORMAL VİSUAL DEVELOPMENT STEPS	
Light reflex	Gestational 30th week
Blink reflex	2-5 months
Eye contact	4-6 weeks
Fixation	2 months
Follow for objects (central foveal fixation)	6-8 weeks
Accommodation	2-4 months
Stereopsis	3-7 months
Recognize faces	9 months
Well hand-eye coordination	3 years

4. Ocular Examination

The ocular examination components include external eye structures examination, pupil and light reflex examination, red reflex testing, fundus examination by using direct or indirect ophthalmoscopy, and assessment of visual acuity.

For the newborn, it is mandatory to check for glaucoma, cataracts, corneal opacity, and ocular infections. Preterm neonates and those taken oxygen for a prolonged time need special attention in terms of retinopathy of prematurity and oxygen-induced retinopathy. According to the American Academy of Ophthalmology guideline for retinopathy of prematurity, all preterms with gestational age before 34 weeks, birth weight under 1700gr, and those with oxygen supplementation must be referred to an ophthalmologist for further evaluation.

For the visit at 1 month, the red reflex must be assessed to evaluate ocular media. From 2 to 18 months of age, pupil light reflexes and ocular mobility should be evaluated in addition to red reflexes.

The tests for check-ups at 2 to 5-year-old children consist of the red reflex, light reflex, cover-uncover test, and visual acuity testing.

External Examination

External examination of the eye consists of eyelids, conjunctiva, sclera, cornea, and iris. All asymmetries between the eyes should be noted. Abnormal examination findings such as ptosis, conjunctivitis, enlarged or cloudy corneas, and dacryocystocele require referral to an eye care specialist.



Figure 2. An infant with bilateral corneal opacities



Figure 3. Left dacryocystocele

Red Reflex Testing

The normal red reflex is the reflection of red light from the retina through the pupil, seen from a distance of 0.5 m with a direct ophthalmoscope set to a '2' diopter. After maximum pupil dilation is achieved, red reflex should be evaluated in the darkroom. The red reflex should be equal in brightness and color in both eyes. The light must fill the pupil.

Leukocoria is a term used when white pupillary reflex from the pupil. Leukocoria may first be noticed by the family and described as a 'cat's-eye appearance'. The differential diagnosis of leukocoria is shown in Table 2.

Table 2. Differential Diagnosis of Leukocoria

Cataract
Corneal opacity
Retinoblastoma
Persistent fetal vasculature
Retinopathy of prematurity
Toxocariasis
Familial exudative retinopathy
Coloboma of choroid
Coats disease (xanthocoria)
Myelinated nerve fibers
Retinal detachment
Uveitis
High myopia

Pupil Examination

Pupils are usually miotic in newborns because of the immaturity of the dilatator pupilla muscle. Therefore, the pupillary light reflex is not reliable until the 30th gestational age. Both pupils should be regular, round, and equal in diameter. Direct and indirect light responses should be checked in both eyes. The afferent limb of the pupillary light reflex is the optic nerve (CN 2) whereas the efferent limb is the oculomotor nerve (CN 3). The pupillary light reflex consists of 4 groups of neurons:

1st Neuron: Afferent pupillary fibers starting from the retinal ganglion nerve layer extend to the pretectal nucleus at the level of the superior colliculus.**1st Neuron:** Afferent pupillary fibers starting from retinal ganglion nerve layer extend to pretectal nucleus at the level of superior colliculus.

2nd Neuron: These second-order neurons connect the pretectal nucleus to both Edinger-Westphal nucleus (parasympathetic nucleus of CN 3). Therefore, light stimulation given to one eye leads to a bilateral light reaction.

3rd Neuron: Efferent parasympathetic preganglionic fibers originating from the Edinger-Westphal nucleus synapse in the ciliary ganglion.

4th Neuron: This order of neurons located within the short posterior ciliary nerves, connects the ciliary ganglion and the sphincter pupillae. These groups of neurons are shown in Figure 4.

A Marcus Gunn Pupil, also known as a relative afferent pupillary defect (RAPD) can be detected by swinging a flashlight between both eyes. In this test, both pupils are stimulated consecutively with light. When light is shined on the healthy eye, bilateral miosis occurs. When light is shined on the other eye, the pupil dilates instead of narrowing because the afferent pathway is defective. RAPD may occur in some optic nerve lesions (glaucoma, optic neuritis, anterior ischemic optic neuropathy...) and retinal diseases.

Anisocoria is a term used when there is a difference between the diameters of both pupils. Differences up to 1 mm diameter between both pupil size may be normal unless there is concomitant ptosis or ocular motility defect.

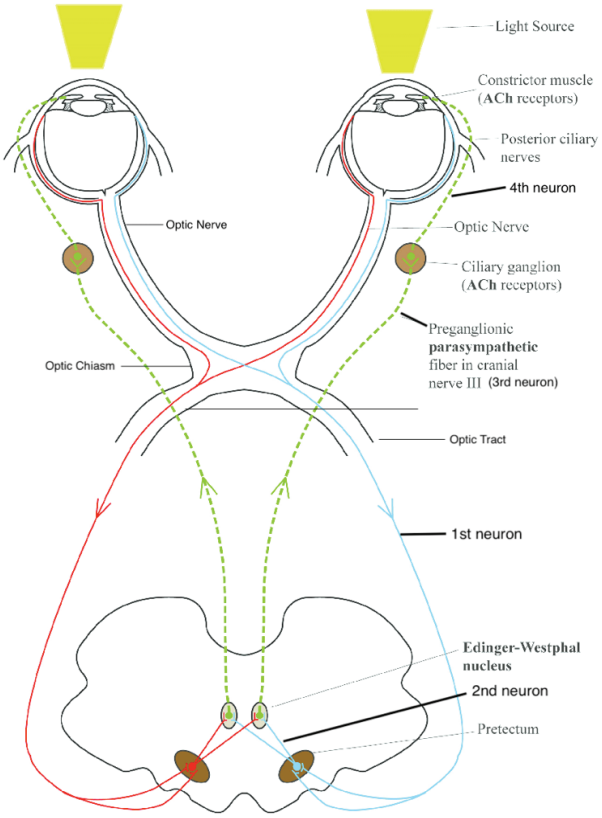
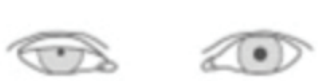


Figure 4. Light reflex pathway. The afferent pathway is shown with red and blue lines, efferent pathway is shown with green lines.

If anisocoria increases in light and is accompanied by ptosis, abnormal pupil light reflex, and ocular motility, we should suspect possible CN 3 palsy. In CN 3 palsy, ptosis and ophthalmoplegia are not always accompanied by pupillary involvement. When the pupillary dysfunction is involved, an intracranial aneurysm, trauma, and tumor must be excluded. If the pupil is spared, microvasculopathy due to diabetes and hypertension is more probable. In such cases, it would be appropriate to refer a pediatric neurologist. If anisocoria increases in dim light with normal pupil light reflex responses, we must look for ptosis, , and face anhydrosis to exclude possible Horner Syndrome. Additional pupil abnormalities are shown in Table 3.

Table 3. Pupil Abnormalities

Unilateral (Right eye)		**Reaction to Light**	**Signs**
Third Nerve Palsy		Negative	Ptosis, Ocular motility defect
Horner's Syndrome		Poor reaction to dimlight, Light reaction is normal	Ptosis(partial,reverse) Anhydrosis Pseudoenophthalmos, Heterochromia(Congenital cases)
Holmes-Adie Syndrome		Slow reaction	Constriction to dosage of 0.1% pilocarpine, Absent or poor tendon reflexes, Viral etiologies
Bilateral			
Argyll Robertson		Negative	Normal accommodation reflex, Neurosyphilis, Bilateral, asymmetric involvement
Midbrain Compression		Negative	Coma, Lateralizing signs
Pontine Stroke		Negative	Coma Hyperpyrexia

Vision Assessment

The child's age, cooperation status, and developmental level determine the practitioner's approach to vision evaluation.

Fixation and the following behavior

The assessment of visual acuity in preverbal children (infants and toddlers) is accomplished by evaluating the ability of children to fixate, maintain fixation for some time, and follow an object held. Assessment of visual behavior should be performed binocularly first and then repeated with each eye covered by turns. Fixation behavior should be central, steady, and maintained (CSM). Central means foveal fixation during monocular viewing. Noncentral corresponds to eccentric fixation. Steady refers to stable eye position while nonsteady refers to roving eye movement or nystagmus. 'Maintained' corresponds to if fixation is held during binocular view after the opposite eye is uncovered. A strong fixation preference of one eye may indicate amblyopia. If any disability to fixate on and follow and maintain objects after 3-4 months of age is noticed, the practitioner must further evaluate whether the decreased vision is due to an ocular or neurologic abnormality. This topic will be discussed later in this chapter.

Preferential looking tests

In infants and young children, quantitative vision assessment can be made by using preferential-looking tests. These tests can be accomplished by using Teller Acuity and Cardiff Acuity cards. In the Teller acuity test, the child is shown a card with a black and white grating on one side. The examiner notes where the child gazes upon the card and controls whether it is toward the stimulus.

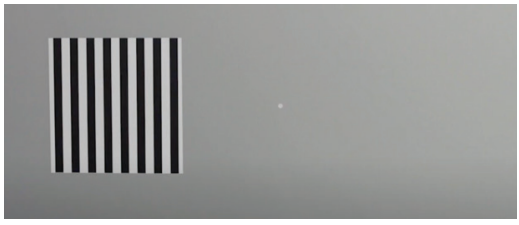


Figure 5. Teller Acuity Card

Recognition visual acuity

In older, verbal, and cooperative children (older than four years of age mostly), visual acuity can be tested by using linear letter optotypes. It has been shown that Sloan letters, consisting of C, D, H, K, N, O, R, S, V, and Z are favorable since they are easily and equally recognizable. Common charts used:

- HOTV chart (set of four-letter optotypes)
- Logarithm of the minimum angle of resolution (LogMAR), 'ETDRS' chart
- The Allen figures (pictograms) are not well standardized, thought to be culturally specific.

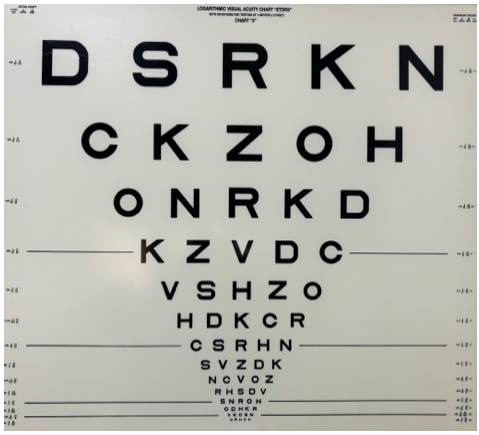


Figure 6. ETDRS' Chart

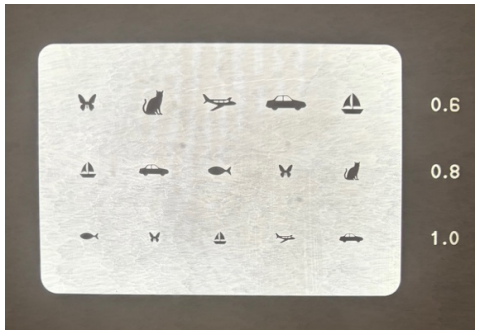


Figure 7. Visual Acuity Pictograms

Visual evoked potential

When the examiner does not rely on the child's verbal and motor responses, the visual evoked potential (VEP) may be used for evaluating visual function. Electrodes placed over the occipital lobe measure cortical electrical signals produced result of visual stimulus. Preverbal children's visual acuity can be estimated with the sweep VEP method.

Ophthalmoscopy

The fundus examination can be challenging in infants and toddlers. It is important to visualize structures such as optic nerve, macula, and retinal vessels by using a direct ophthalmoscope. This examination is accomplished by attracting the child's attention to a distant target or a toy held near to examiner. The foveal reflex can be viewed making the child look through the light of the ophthalmoscope. The normal optic disc looks yellow-pink and mostly flat whereas a swollen optic nerve head may be an indicator of increased intracranial pressure. Examination needed to visualize the far periphery like retinopathy of prematurity, retinal dystrophies, and eye involvement in systemic disorders should be performed by using indirect ophthalmoscopy, eyelid speculum, and scleral depression.

In fundus examination, tropicamide (0.5% or 1%), cyclopentolate hydrochloride (1%), and phenylephrine (2.5%) are mostly preferred agents for pupil dilation. Tropicamide and cyclopentolate hydrochloride provide additional cycloplegia while phenylephrine does not. Cycloplegic agents may be associated with some adverse effects. Local adverse effects may include allergic reactions like conjunctivitis, eyelid edema, and dermatitis. Nonallergic systemic symptoms include dry mouth, fever, flushing, tachycardia, nausea, urinary retention, dizziness, and delirium. Treatment should be discontinued when these adverse effects are seen. Severe adverse effects must be managed in emergency care settings.



Figure 8. Indirect Ophthalmoscope

5. Decreased Vision in Infants

As mentioned earlier, the fovea and central cone photoreceptors are immature at birth and differentiation occurs in the first 6 months of life. The maturation process of the fovea continues until 3-4 years of age. When an infant does not fixate and follow an object after 3-4 months of life, the clinician must decide whether the low vision is due to an ocular or pregeniculate (optic nerve) condition, retrogeniculate (cerebral) impairment, or delayed visual maturation. Dysconjugate eye movements, sunsetting, and skew deviations may be normal in healthy newborns up to 4 weeks of life while searching eye movements (visual acuity <1/10), eye-poking (oculo-digital reflex), and nystagmus point out poor visual development of an infant.

Pregeniculate visual pathways state pathways anterior to the lateral geniculate nucleus. Pregeniculate visual impairment causes include corneal opacity, lens condensation, glaucoma, retinal disorders, optic nerve and tract diseases. Strabismus and nystagmus may be seen in infants with pregeniculate visual impairments. If suspicion is high for pregeniculate visual impairment despite normal ocular examination, optical coherence tomography (OCT) and electroretinography (ERG) may help diagnose possible optic atrophy, foveal hypoplasia, and retinal diseases.

Cerebral visual impairment (CVI) may be congenital or acquired. The most common cause is periventricular leukomalacia due to perinatal hypoxia or prematurity. If CVI is isolated, ocular examination may be normal. Neuroimaging may be helpful for diagnosis.

If an infant does not develop normal fixation behavior within the first 3-4 months of age, delayed visual maturation may be thought in existance of normal pupillary light reaction, normal ocular structures and absence of nystagmus, and history of neurological disorder. In such a case, it may be necessary to re-evaluate the infant at 6 months of age before further investigation.

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Chapter

10

Hearing Screening Programs for Children

Abdulkadir SAHIN

Hearing loss in children has a direct impact on speech and language acquisition. As a result, there may be problems with the child's education, cognitive level, and social development. It would not be correct to consider it as only a personal impact, this situation has consequences with social effects. Early evaluation and identification of children with hearing loss is the most important step. The most effective way to achieve this is through a multidisciplinary approach of pediatricians, otolaryngologists, and audiologists and national screening programs. The earlier children with hearing loss are identified and rehabilitated, the better the child's language acquisition and social development (Sahlı 2018).

The concept of newborn refers to the first 4-week period after birth in mature and premature births. Newborn hearing screening (NHS) is not performed immediately after birth, but usually in the first 72 hours before discharge. There have been national screening programs for many diseases such as phenylketonuria and congenital hypothyroidism for years, but the national NHS program implemented in our country (2007) is the "universal newborn hearing screening" program, which was first implemented in the USA in 1993. This program includes objective electrophysiological tests such as

otoacoustic emission (OAE) and auditory brainstem response (ABR). It includes referral to a higher center if hearing loss is detected as a result of the test or if there is a risky situation (https://hsgm.saglik.gov.tr/tr/mevzuat/genelgeler/122-2014-27-yenidoğan-işitme-tarama-programı-genelgesi.html ; National Institutes of Health Consensus Development Conference Statement. Early identification of hearing impairment in infants and young children, 1993).

In its 2010 report, the World Health Organization (WHO) recommended that all countries with adequate infrastructure implement NHS programs (World Health Organization (WHO). (2010) Newborn and infant hearing screening: current issues and guiding principles for action. Outcome of a WHO informal consultation held at WHO Headquarters, Geneva, Switzerland, 09–10,2009).

Newborn Hearing Screening

Previously, with the NHS program in our country, screening-OAE (s-OAE) and screening ABR (s-ABR) were evaluated for babies who failed the test. As of 2017, only evaluation with s-ABR is performed. The main purpose of this program is to perform the first hearing screening in the first 72 hours with s-ABR and to refer newborns who fail the tests and have risk factors to the reference center within 30 days at the latest and to start their treatment and rehabilitation (https://hsgm.saglik.gov.tr/depo/birimler/cocuk-ergen-sagligi-db/Programlar/Guncel_Test_Protokolu_.pdf). (Figure-1 and 2.)

Risk factors for hearing loss in the newborn were first described by the "Joint Committee On İnfant Hearing" in the USA in 1973 and this was later expanded (Joint Committee on Infant Hearing., 2007), (Table 1).

Table 1. Risk factors for hearing loss in the newborn

RISK FACTORS FOR HEARING LOSS
1. Syndromes or diseases in the infant and child • Syndromic-nonsyndromic diseases Down, Turner, waardenburg, Usher, Alport syndrome etc. • Neurodegenerative diseases Neurofibromatosis, white and gray matter diseases
2. Family history of childhood permanent hearing loss
3. Craniofacial anomalies in Infant / Child Auricle anomalies, Ear pit, ear canal anomalies, Temporal Bone Anomalies, Ear Tag, Cleft Palate/Lip Anomalies
4. Conditions in the Infant/Child Low Birth Weight (< 1500 g), Intensive Care Unit Stay for more than 5 days, Mechanical Ventilation Therapy for 5 days or more, Ototoxic Drug Use, Hyperbilirubinemia Requiring Blood Exchange, Bacterial Fungal Sepsis, Meningitis, Treatment with ECMO (Extracorporeal Membrane Oxygenation), Asphyxia, Difficult Delivery (Ex: APGAR Score ≤6 at 5. Minute APGAR Score ≤6) or Infants Receiving Therapeutic Hypothermia, Cerebral Complications (Cerebral Hemorrhage, Cerebellar Hypoplasia, Stroke, Hydrocephalus, Cerebral Hematoma), Microtia or External Ears Tract Atresia, Head Trauma
5. Conditions in the mother during pregnancy Toxoplasma, Cytomegalovirus (CMV), Herpes, Syphilis, Mumps, Measles, Rubella, Varicella, Other Febrile Diseases, Maternal Diseases (Hypothyroidism, Hypertension, Gestational Diabetes, etc.), Antibiotic Use (Aminoglycosides, Isotretinoin, etc.)

(https://hsgm.saglik.gov.tr/depo/birimler/cocuk-ergen-sagligi-db/Programlar/Gncel_Yenidoan_itme_Kayb_Risk_Faktrleri.pdf)

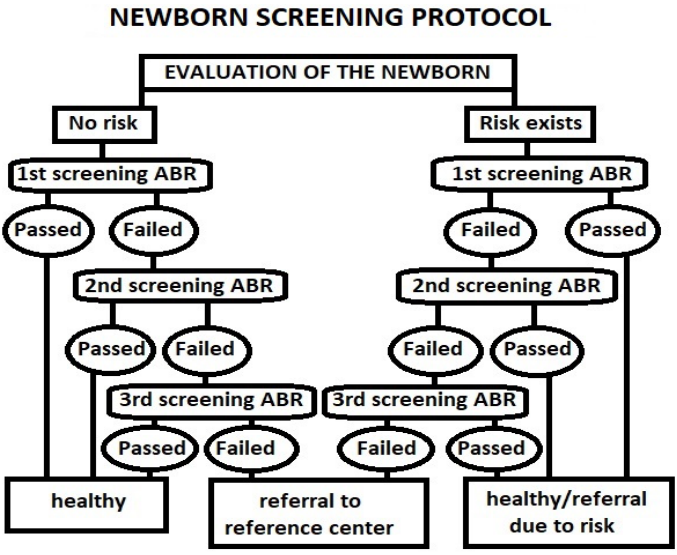


Figure 1. National newborn hearing screening protocol

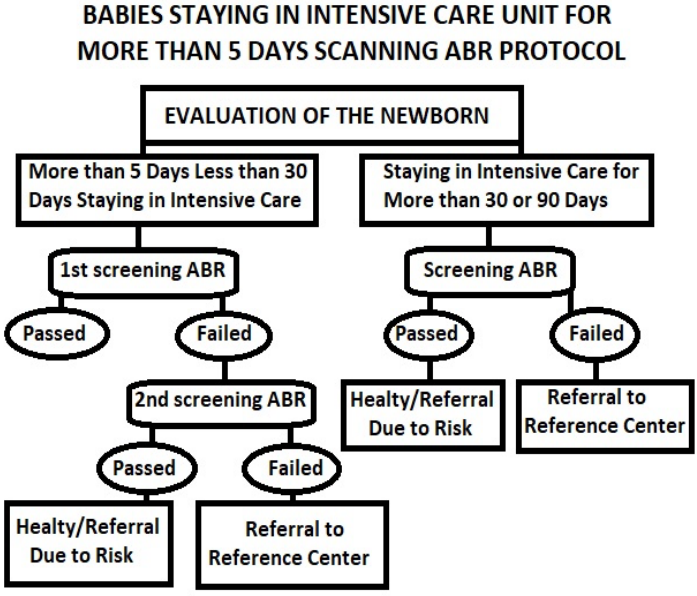


Figure 2. Hearing screening protocol for infants in intensive care for more than 5 days

Hearing Screening in School Age Children

It is also aimed to include preschool and school-age children in the hearing screening program. Hearing is the most important component of education and communication. It becomes even more important during the school period. By the end of 2015, it was decided to start a screening program again for school-age children in our country with parental consent. Hearing loss can also occur in the postnatal period due to infections, traumas, and genetic diseases that cause progressive hearing loss. The prevalence of permanent hearing loss is reported to rise to nine per thousand in the school-age population. Permanent or temporary hearing loss in one or both ears affects more than 14% (one in seven) of school-age children. Even with unilateral hearing loss, the grade repetition rate is reported to be 37%. Hearing loss increases difficulties in attention, learning, and social functioning. The prevalence of hearing loss in children causes significant economic losses by negatively affecting school success if the necessary medical and educational support is not provided. For this reason, the hearing screening program for 1st-grade children has been ongoing since 2015. This program includes the referral and rehabilitation of children with hearing loss detected as a result of screening audiometry without interrupting their education (https://hsgm.saglik.gov.tr/tr/tarama-programlari/okul-cagi-cocuklarda-isitme-taramasi-programi.html). Figure 3.

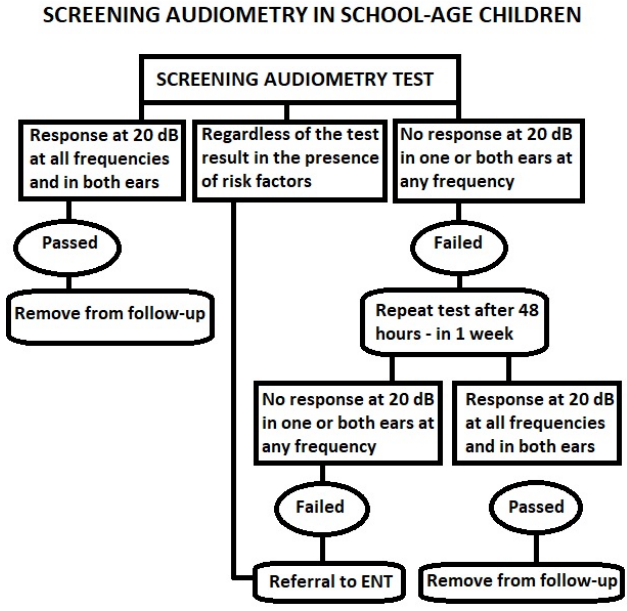


Figure 3. Screening audiometry protocol in school-age children

Tests Used for Hearing Screening in Children

Children who fall at any stage of hearing screening programs should undergo a complete audiological examination and evaluation at a specialized center. In addition to the chronological age of the child, behavioral and cognitive age should also be taken into consideration in the selection of the tests to be performed in this evaluation. *Electrophysiologic tests* such as OAE and ABR are used to determine the hearing threshold in children up to 6 months of age. In infants 6 months and older, *behavioral audiological tests* should be included in these tests. In hearing screening, the entire test battery can be used to detect hearing pathology, especially in infants or children who fail the test, and several test repetitions may be required. Children who have developmentally reached the age of 5 years can be evaluated for hearing with *audiometry* conventionally used in adults.

Electrophysiological Tests

These are tests that provide an objective measurement of hearing level. The patient being tested does not actively participate in the test but is screened for hearing or a part of the hearing organ is assessed while awake or asleep. Especially children younger than 6 months, infants, and children with mental and developmental retardation or autism are evaluated with objective electrophysiological hearing screening tests because they cannot cooperate in behavioral tests (Yoshinaga, 2004).

The electrophysiological hearing tests we use in clinical screening are acoustic immittance test, otoacoustic emission OAE, auditory evoked potentials; ABR, Pediatric Cortical Potentials, and Auditory Steady State Response (ASSR).

Acoustic Immittance Test

It is a non-invasive test that provides information about middle ear functions. It is based on the principle that the pressure in the external auditory canal is changed and the response from the middle ear is obtained with the help of a microphone placed in the external auditory canal. Tympanometric measurements provide information about acoustic reflex evaluation, outer ear, and middle ear volume. In infants over 4 months of age, tympanometric measurements can be interpreted as in adults. Acoustic immitansmetry measurements include measurements of the transmission of sound from the middle ear to the inner ear and then to the upper centers via the cochlear nerve.

With acoustic immittancemetry, tympanometric evaluations are performed first, and if no problems with the middle ear and tympanic membrane are detected, a standard pressure peak is obtained. This is followed by the evaluation of acoustic reflexes.

Tympanometric Curves:

Type A tympanogram curve: It is observed in healthy ears, occurs at normal ear pressures between -50 and +50 daPa and has mean compliance values between 0.5-1. Type As tympanogram curve is observed when the compliance drops below 0.6 in the same pressure range. Type As is usually associated with pathologies such as tympanosclerosis and otosclerosis leading to ossicular chain fixation. The Type Ad tympanogram curve is also seen in the same pressure range and the amplitude of the compliance amplitude usually exceeds 1.5. It is usually observed in ossicular chain dislocations.
Type B tympanogram: Nearly flat tympanogram curves with no peak.
Type C tympanogram: Curves with a peak but a pressure value below -100 daPa.

Acoustic Immittance tests are objective, inexpensive, and practical tests used in children. The test battery is generally preferred first in the order of application. The results may be affected by external auditory canal pathologies and the incorrect position of the probe placed in the external auditory canal. The test must be performed by an experienced clinician after otoscopic evaluation (Lilly, D. J., & Margolis, R. H. 2013; Sanford et al., 2013; Yoshinaga, 2004; Belgin & Şahlı, 2015).

Otoacoustic Emission

OAE, an objective test, provides information about outer hairy cell functions. In the absence of any sound stimulus, the microphones in the probe placed in the external auditory canal record the sound transmitted from the otoacoustic functions of the outer hairy cells in the cochlea. It is a simple, rapid, and primary test in the evaluation of cochlear functions. It provides information about hearing losses below 30-40 dB on average. It is not useful for hearing losses of more than 30-40 dB and also does not give information about the type of hearing loss (Kemp, 2002; Prieve & Fitzgerald 2015).

The OAE test, which was used in the national NHS program until 2017, was then completely replaced by the ABR test. The OAE test has several disadvantages and margins of error. Various software is used to determine whether the sounds recorded from the external auditory canal belong only to the outer hairy cells. Various internal sounds and ambient noises, such as the baby's swallowing and sucking, can interfere with these software errors and cause differences in the measurement results. OAE is also affected

by the tester not placing the probe in the external auditory canal correctly or by the presence of mucus and fluid. The OAE test only tests the part of the cochlea up to the outer hairy cells, which is a small area of the hearing system. For all these reasons, it was a good decision to base our current National NHS program on the ABR center (Belgin & Şahlı, 2015; Bayat et al. 2024; https://hsgm.saglik.gov.tr/depo/birimler/cocuk-ergen-sagligi-db/Programlar/Guncel_Test_Protokolu_.pdf)

Auditory Evoked Potentials

It is based on the principle that auditory stimuli are given through the auditory pathways and activities are recorded with the help of electrodes placed on the head. Auditory evoked potentials mainly include ABR, Middle latency response (MLR), and Cortical Auditory Evoked potentials (CAEP) (Barrozo et al., 2024; de la Torre et al., 2020).

Auditory Brainstem Response: ABR is the most important and effective method used for hearing screening in the first 6 months of life. In young children, audiogram and hearing levels are also evaluated by correlating them with ABR.

Usually, 4 electrodes are placed on the head for recording. Click or tonal stimuli are given and rhythmic EEG recordings are taken. Waveforms are obtained by averaging a large number of early stimulations occurring in milliseconds. Generally, 5 or 6 waveforms are formed. Hearing thresholds between 500hz and 4000hz can be determined with ABR. It is an important advantage that the ABR test is not affected by sleep. However, patient movement may cause artifacts in the test. ABR testing with click stimuli can provide information on the level of hearing loss and the diagnosis of conductive or neural type. It also provides a comparative evaluation of both ears (Eggermont, 2019; Mohammed et al., 2021; Reynard et al., 2021).

Cortical Auditory Evoked potentials: CAEP is the most effective method by which we evaluate the degree of gains achieved by children who have been equipped and rehabilitated after diagnosis. CAEP is affected by sleep, so the patient should be awake and active at the time of the test (Gardner-Berry et al., 2016; Tremblay & Clinard, 2015).

Auditory Steady State Response

It is a test based on obtaining potentials separated by continuous modulated stimulation. The conduction can be electrical, as in children with bony or cochlear implants. Hearing threshold assessment can be performed objectively with ASSR as a free-field test. With these measurements, the benefit of the cochlear implant can be objectively evaluated.

However, various artifacts may occur when evaluating patients with cochlear implants with ASSR. Acoustic stimulation is used to determine whether the recordings are distorted due to artifact formation (Torres-Fortuny et al., 2018; David et al., 2023).

Behavioral Tests

Behavioral hearing tests are based on the principle of conditioned assessment of hearing sensitivity in an environment completely isolated from external sound. Sound stimuli can be delivered through air or bone. Both ears can be evaluated separately thanks to the inserted headphones. These tests are a subjective screening method that may vary according to the success of the patient and the practitioner. To reach more accurate results in test selection, the developmental and cognitive age of the patient should be taken into consideration, regardless of chronological age. Behavioral hearing tests include 3 different testing techniques: behavioral observation audiometry, visual reinforcement audiometry, and conditioned play audiometry.

Behavioral Observation Audiometry

The test is performed by observing infants' reclexive responses to sudden sound stimuli, such as stopping or accelerating sucking, startling, or opening their eyes suddenly. This test can be administered to infants up to 5 months of age and younger or to children of younger developmental age. The results obtained are imprecise and subjective. Erroneous results can be obtained by quickly adapting to the stimuli and the type of stimuli. With this test, only the hearing threshold can be determined, not the hearing assessment. Therefore, electrophysiologic tests such as OAE and ABR should be used for definitive diagnosis (Allan, 2015; Delaroche et al., 2011).

Visual Reinforcement Audiometry

Children older than 5 months can be assessed with visual reinforcement audiometry. Developmentally appropriate children naturally return to the auditory stimulus or are reinforced to return to the stimulus with a moving or lighted stimulus. Free-field stimuli generally elicit the response of the better ear. However, with the insertion of headphones and masking, both ears can be evaluated separately. The auditory stimulus must be positioned equidistant and 90° to the left and right side of the patient. Different toys can be used to help the child get used to the test and avoid distraction, and too many repetitions of the test should be avoided. (Figure 4) This test is more commonly used to determine the "lowest response level" rather than the hearing threshold (Belgin & Şahlı, 2015; Johnson, 2002).

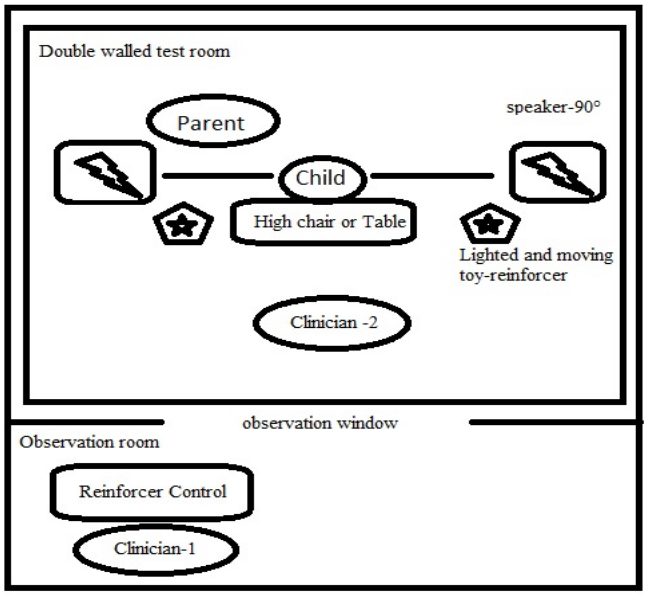


Figure 4. Test room layout for visual reinforcement audiometry

Conditioned Play Audiometry

Developmentally and cognitively, children who reach the age of 2 years are considered to have their hearing threshold determined through play activity. The child is trained to perform behaviors such as sorting toys or throwing them in a basket in response to auditory stimuli. The most difficult part of this test is to adapt the child to this test. During the test, hearing threshold levels can be determined with sound intensity changes of 5 or 10dB2 . Conditioned play audiometry and similar applications are used in both therapy and hearing threshold level control in children with cochlear implants who have reached the age of 2 years developmentally (AAA: American Academy of Audiology, Audiologic guidelines for assessment of hearing infants and young children, 2012; Goehring & Hughes, 2017).

Speech Audiometry

It is generally accepted that children over the developmental age of 5 or 6 can undergo pure tone and speech audiometry tests, which can be performed in adults. In speech audiometry, the patient is asked to repeat what is spoken during the test to determine perception, while hearing levels are determined with pure tone. This evaluation provides information about middle ear function, the cochlea, auditory pathways, and the auditory center (Favier et al., 2018).

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Chapter

11

Developmental and Behavioral Screening

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Development is a dynamic and sequential process encompassing the acquisition of various functions across cognitive, language, fine and gross motor, social-emotional, sensory (vision and hearing), and behavioral domains for the full independence of the individual from conception to adulthood (Galliher et al., 2017). Improvements in neonatal intensive care, public health interventions, effective vaccination campaigns, and advanced treatments for infectious diseases have resulted in a decline in childhood mortality rates. However, this has been accompanied by a rise in morbidity. For these reasons, the prevalence of developmental disabilities has increased in recent years, and while it varies across studies and regions, affecting around 16.65% of children, as highlighted in the National Health Interview Survey (2018–2021) from the USA (Li et al., 2023). Childhood developmental disabilities are often accompanied by behavioral problems, with up to 23% of children in the United States affected by behavioral and emotional disorders, and 6% experiencing severe conditions (Simon et al., 2015). Given the high prevalence of developmental and behavioral disorders in children, primary pediatric care faces challenges in early identification within a medical home setting. Implementing a systematic approach incorporating surveillance, screening, and evaluation during preventive healthcare visits has proven effective (Barger et al., 2018).

The American Academy of Pediatrics offers guidelines for healthcare providers to screen and evaluate children, stressing the integration of developmental and behavioral surveillance into routine pediatric care (American Academy of Pediatrics [AAP], 2006; Hagan et al., 2017; Marks, 2020;

Weitzman et al.,2015; Zuckerbrot et al., 2018). In the realm of pediatric healthcare, behavioral and developmental screenings, and surveillance play a crucial role in identifying and supporting children with developmental and behavioral difficulties. They play a crucial role in enabling early diagnosis and interventions, thereby preventing long-term consequences. These practices provide healthcare professionals with the opportunity to address parental concerns, and offer specialized support, ultimately promoting the child's optimal development and enhancing the quality of life for both children and their families.

This chapter emphasizes the significance of pediatricians enhancing their interviewing skills and knowledge of child development and behavior during early childhood screening and surveillance, particularly in the first five years, while also discussing principles and techniques for screening and surveillance that foster effective communication about child development and behavior between healthcare professionals and parents.

1. Definitions

1.1. Developmental difficulties

Developmental difficulties refer to conditions that could potentially impede children's developmental progress in the areas of physical, learning, language, or behavior, deviations from anticipated developmental norms, as well as delays or disabilities (Ertem, I. O., & World Health Organization [WHO], 2012).

1.2. Developmental Screening and Surveillance

Developmental screening is a systematic process that utilizes standardized tools to identify potential developmental concerns in children, such as delays or atypical progress in motor, language, social, and cognitive abilities. It involves assessing a child's development and comparing it to expected milestones, often through questions to caregivers or demonstrations of skills (Lipkin et al., 2020; Marks, 2020). Developmental screening is distinct from other types of developmental assessments in early childhood, which may focus on specific areas like Autism Spectrum Disorder (ASD) (Hyman et al., 2020) or social-emotional development (Hagan et al., 2017; Weitzman et al.,2015; Bright Futures Periodicity Schedule Workgroup, 2021). Autism Spectrum Disorder

(ASD) screening is now integral to healthcare screening due to the significance of early identification (Hyman et al., 2020). Social-emotional screening, endorsed by the AAP is also crucial (Hagan et al., 2017; Weitzman et al.,2015; Zuckerbrot et al., 2018). Screening for social risk factors and providing corresponding resources to interested families can be beneficial due to the long-term impact of social adversity on development. These early detections allow for the provision of targeted interventions, support services, and resources to families, empowering them to address challenges and promote their child's optimal development (Hagan et al., 2017; Bright Futures Periodicity Schedule Workgroup, 2021; Zuckerbrot et al., 2018).

Developmental surveillance is an ongoing process integrated into every pediatric care visit, where clinicians identify children at risk for delays or developmental issues. It aims to promote healthy development, educate parents, and guide interventions. Components include addressing parental concerns, maintaining developmental history, observing the child, documenting findings, and collaborating with relevant professionals (Hagan et al., 2017; Lipkin et al., 2020; Marks, 2020). Unlike developmental surveillance, which is a continuous process of monitoring a child's development, screening is typically conducted at specific ages or intervals. Additionally, developmental screening differs from surveillance in its use of formal, standardized tests with known reliability, validity, sensitivity, and specificity, and is typically administered at specific ages based on key times for identifying developmental disorders. The screening test is typically ordered and interpreted by the clinician, potentially administered by healthcare staff, while surveillance is usually directly performed by the primary pediatric healthcare professional during preventive care visits (Marks, 2020).

1.3. Behavioral Screening and Surveillance

The 2015 AAP Clinical Report "Promoting Optimal Development: Screening for Behavioral and Emotional Problems" recommends conducting behavioral and emotional problem surveillance at all health supervision visits and using formal screening tools when concerns are identified (Weitzman et al., 2015). Parents' concerns about their child's behavior and social skills are crucial for identifying significant problems, especially in children over 4 years old. Family and social histories may highlight additional areas of concern, such as familial mental health issues including anxiety disorders, and mood disorders. Environmental, familial, and psychosocial risk factors can disproportionately affect a child's behavioral and emotional development. Increasing attention has been given to the impact of "toxic stress" on development, which can arise from adverse childhood experiences (ACEs) like maternal depression, poverty, substance abuse, and family disorganization. Regular surveillance of these

psychosocial risk factors is recommended (Garner & Shonkoff, 2012). Behavior history, obtained during surveillance, focuses on symptoms rather than developmental milestones. Healthcare professionals should inquire about the child's interactions with parents, siblings, peers, and unfamiliar individuals, as well as their behavior during daily activities like eating, sleeping, and playing. Specific concerns such as compliance, tantrums, attention, activity level, impulsivity, and aggression should be addressed. Additionally, unusual behavior patterns, such as repetitive speech, excessive preoccupation with objects, unusual visual gaze, hand-flapping, or self-injurious behaviors, should be noted.

Additionally, AAP advises developmental surveillance and psychosocial/behavioral assessments at specific intervals from birth to 21 years. They recommend annual assessments for tobacco, alcohol, or drug use in children aged 11 and older, and annual depression screenings for those aged 12 and above (Hagan et al., 2017). The AAP's Guidelines for Adolescent Depression in Primary Care set the standard for screening and initial management of adolescent depression (Zuckerbrot et al., 2018).

2. Timing of screening and surveillance

During pediatric preventive care visits in the first 5 years of life, developmental concerns should be addressed alongside other health topics. Developmental surveillance, a flexible and continuous process, involves professionals identifying children at risk of developmental issues through parental concerns, developmental history documentation, accurate observations, risk assessment, and record-keeping. If surveillance indicates potential developmental delays based on parental or professional concerns, targeted developmental screening should be conducted (AAP, 2006). Standardized developmental screening is recommended at 9, 18, and 30 months (Lipkin et al., 2020), with autism-specific screening at 18 and 24 months (Hyman et al., 2020). Positive screening results prompt further evaluation, including developmental and medical assessments, leading to early intervention if needed. Children identified with developmental disorders should receive chronic condition management, even without a specific diagnosis, to ensure appropriate care and monitoring within the medical home (AAP, 2006; Weitzman et al.,2015).

Developmental screening should ideally be administered at regular intervals during routine healthcare visits, providing an opportunity for healthcare providers to systematically assess developmental milestones and behavioral patterns as part of comprehensive pediatric care. This approach enhances the detection of concerns during critical early stages. In its 2020 clinical report, The American Academy of Pe-

diatrics (AAP) recommends standard developmental screening in the first 3 years of life at 9, 18, and 30 months of age, in addition to developmental surveillance at every health supervision visit for children without identified developmental concerns (Lipkin et al., 2020; Marks, 2020). These stages in a child's early development represent a time when specific areas of developmental achievement can be accurately assessed, such as gross/fine motor skills and early social skills at 9 months, early language and emerging social skills at 18 months (including potential signs of autistic spectrum disorder), and expressive/receptive language at 30 months, particularly in the 15% to 20% of "late talkers" at 24 months, many of whom may have reached normal language development by 30 months. If children miss their 9, 18, and 30-month healthcare visits, it is recommended to perform developmental screening at their next visit. At 9 months, screening targets undetected hearing loss, visual disorders, and motor delays like cerebral palsy. By 18 months, clinicians can identify milder motor problems, gait abnormalities, signs of neuromuscular disorders, early ASD indicators, and language or intellectual issues. The 30-month screening emphasizes language, social, and intellectual development, potentially revealing ASD, intellectual disabilities, or speech-language disorders. Neuromuscular disorders may also manifest motor or developmental issues at this stage (AAP, 2006). The report also emphasizes assessing children for school readiness at ages 4 to 5 (Lipkin et al., 2020) and reaffirms screening for Autism Spectrum Disorder (ASD) at 18 and 24 months using standardized tools, emphasizing the importance of early intervention (Hyman et al., 2020) The AAP's Bright Futures Recommendations for preventive child health care include behavioral and social-emotional surveillance at every well-child visit from infancy and a formal behavioral screening when surveillance raises concerns, emphasizing the interconnectedness of social-emotional development with other developmental aspects during early childhood (Weitzman et al.,2015). While a comprehensive system for behavioral screening has not been developed, the existing developmental screening framework can be extended to identify behavioral concerns and disorders at key ages throughout childhood and adolescence, such as the 30-month visit, 4- or 5-year visit (before school entry), 8-year visit (early elementary), and other preadolescent and adolescent milestones (Hagan et al., 2017).

3. Implementation of the Screening

Given the lifelong impact of developmental and behavioral difficulties, primary pediatric healthcare professionals should establish medical homes in a family-centered approach for affected children. These medical homes provide coordinated and monitored care and form reliable alliances with families for support and advocacy

from diagnosis through adulthood (AAP, 2014; AAP & Duby, 2007; Tamburlini & Ertem, 2019). The AAP's guidelines on developmental and behavioral surveillance and screening emphasize their importance in the medical home for early detection of behavior disorders (AAP, 2006). Additionally, a transdisciplinary care coordination system should be developed, linked to local community-based health, developmental, and educational professionals, to collaboratively manage the special needs of the child (Ramaswamy et al., 2022).

Moreover, identifying children at risk for developmental delays and disabilities necessitates a family-centered and strengthened-based approach, characterized by collaborative interactions between parents and healthcare providers. Social stigma, historical injustices, and cultural perceptions of mental health significantly shape parental attitudes toward child development, requiring clinicians to comprehensively evaluate and counsel families (AAP, 2014; AAP & Duby, 2007; Hagan et al.,2017). Successful implementation of routine developmental screenings demands a structured workflow that includes defined staff roles, appropriate tool selection, effective use of electronic health records, and continuous quality improvement monitoring. Key planning steps involve evaluating the needs of both staff and patients, anticipating potential challenges, securing support from stakeholders, choosing suitable screening tools, addressing language barriers, ensuring compliance with protocols, establishing follow-up procedures, and soliciting feedback to enhance efficiency and team collaboration, ultimately leading to better outcomes for children and families (Silver et al., 2017).

4. The importance of using a screening tool

The use of validated screening tools is essential in developmental and behavioral screening, as it provides a standardized approach to assessing developmental and behavioral domains, ensuring accurate and reliable identification of potential concerns while minimizing subjectivity and bias. These tools also educate parents by highlighting previously unconsidered concerns, enhancing their ability to observe and communicate their child's developmental strengths and needs. They also, when administered before clinical interviews and examinations, help focus the evaluation on areas of concern, guide clinicians in their observations, and ensure thorough data collection. While screening tests are not diagnostic, they prompt relevant questions, highlight potential issues, and indicate the need for further evaluation (Guevara et al., 2013; Lipkin et al., 2020). Studies, such as those by Brothers et al.(2008) and Goevera et al. (2013) indicate that using validated tools increases detection sensitivity and yields more comprehensive results.

The American Academy of Pediatrics recommends the use of standardized tools for developmental screening and surveillance (AAP, 2006; Lipkin et al., 2020; Marks, 2020), and their adoption has become increasingly widespread, with a significant increase in pediatricians' use of these tools from 21% in 2002 to 63% in 2016 (Lipkin et al., 2020).

5. The selection of a developmental and behavioral screening test

Developmental and behavioral screening in infants and beyond requires the selection of validated screening tools with high sensitivity, reliability, and specificity. These tools cover various categories such as general developmental, language/cognitive development, motor development, and autism screening, often involving parental inquiries or direct assessment of the child's progress (AAP, 2006). It is recommended for primary care pediatricians to specialize in one test per category to ensure thorough screening. The choice of a developmental screening test should adhere to established guidelines, considering factors like sensitivity, specificity, feasibility, cultural and linguistic appropriateness, and comprehensiveness to effectively detect potential delays or concerns across developmental domains. The chosen test should be suitable for the specific age group, standardized psychometrically, and align with clinical workflows. Considerations also include the ease of administration, scoring, training requirements, cost, and reimbursement options. The trend in developmental screening has shifted towards parent-completed questionnaires, or checklists, which supplement clinical interviews effectively. These checklists can be completed at home, in the waiting room, on paper, or electronically. Some pediatric practices utilize trained staff to systematically screen for developmental and behavioral issues during well-child visits (AAP, 2006; Weitzman et al.,2015).

Considering all these factors, some of the widely used current developmental screening tests encompass several domains. Some of the general developmental screening tests based on parent reports are Ages and Stages Questionnaires (ASQ), Parents' Evaluation of Developmental Status (PEDS), Parents' Evaluation of Developmental Status: Developmental Milestones (PEDS: DM), and the Survey of Well-Being of Young Children (SWYC). Language screening can be conducted using the Communication and Symbolic Behavior Scales Developmental Profile Infant-Toddler Checklist (CSBS DP ITC). Some autism spectrum disorder (ASD) screening are the Modified Checklist for Autism in Toddlers, Revised with Follow-Up (M-CHAT-R/F), the Social Communication Questionnaire (SCQ), and the Early Screening for Autism and Communication Disorders (ESAC) (AAP, 2006).

A variety of commonly used general behavioral screening tools are the Ages and Stages: Social-Emotional-2 (ASQ:SE-2) for children 2 months to 6 years, the Brief Infant-Toddler Social and Emotional Assessment (BITSEA) for ages 12-36 months, the Eyberg Child Behavior Inventory for ages 2-16 years, the Pediatric Symptom Checklist for ages 4-16 years, the Strengths and Difficulties Questionnaire for ages 3-17 years, and the Survey for Well-Being in Childhood (Weitzman et al., 2015). For condition-specific behavioral screening, depression can be assessed using the Patient Health Questionnaire-9 and Patient Health Questionnaire-2. Anxiety can be screened with the Generalized Anxiety Disorder 7-item (GAD-7) scale and the Screen for Child Anxiety Related Disorders (SCARED) for children 8 years and older. ADHD can be evaluated using the ADHD-IV Rating Scale for ages 6-18 years and preschool children 3-5 years, as well as the Vanderbilt ADHD Diagnostic Rating Scales for ages 4-18 years (Weitzman et al.,2015).

6. The importance of integrating screening and surveillance

Regular developmental and behavioral surveillance, complemented by standardized screening tests at specific ages, enhances the sensitivity and specificity in distinguishing children with these disorders, enabling a holistic understanding of a child's development and capturing both immediate and subtle changes for effective intervention. Integrated surveillance and screening in primary care are more strongly associated with access to early intervention services compared to using either approach alone (Barger et al., 2018). Early detection and intervention for children with developmental challenges in the early years not only help them reach their developmental potential but also reduce healthcare costs for at-risk children. Supporting development in early childhood has an estimated cost of $0.2-$0.7 per child annually. Early childhood interventions yield significant returns on investment, ranging from 3 to 18 times the cost. Lack of investment in early childhood can lead to a doubling of gross national product spent on health in countries (Richter et al., 2017).

Monitoring and promoting early child development is a crucial task for pediatric primary care services. There is consensus that the most effective approach includes both longitudinal surveillance and monitoring rather than one-time screening tests for promoting early child development (Tamburlini & Ertem, 2019; WHO, 2020). The International Guide for Monitoring Child Development (GMCD) was developed based on these principles, providing a comprehensive tool for assessing, monitoring, supporting, and offering individualized early intervention plans for child development worldwide (Ertem, 2017; Ozalp Akin et al., 2022). Developed by Ertem and Galip at

Ankara University School of Medicine, Department of Pediatrics Developmental Pediatrics Division (AUDPD) (Galip & Ertem, 2010), the GMCD integrates bioecological theory (Bronfenbrenner & Ceci, 1994), strength-based and family-centered approaches (Brewer Jr et al., 1989), the World Health Organization, the International Classification of Functioning, Disability and Health (ICF) (WHO, 2001), and the Nurturing Care Framework (NCF) (WHO, 2018) to guide its approach.

7. Post-Screening Evaluation

Following developmental and behavioral screening, clinicians should promptly discuss results with families during the same clinical encounter using a family-centered and strength-based approach (Brewer Jr et al., 1989), emphasizing that screening identifies potential delays rather than diagnoses. Referral to specialized services (e.g., developmental pediatrics, child psychiatry) should be carefully considered for children with identified concerns (AAP, 2014).

A comprehensive evaluation should be initiated immediately in the relevant specialty to which the child is referred. This evaluation should include a thorough analysis of screening results, comprehensive developmental and medical histories, and a physical examination to investigate potential medical causes, while simultaneously providing early intervention services and exploring the underlying etiology (AAP, 2006, 2014). If no concerns are identified through screening and surveillance, and the child is not at risk for developmental, behavioral, or chronic health problems, the next health supervision visit can be scheduled after the completion of the current examination (Weitzman et al.,2015).

Children with positive behavioral screening results should undergo further evaluation by medical or mental health professionals within the medical home or through referral to specialists, to determine a diagnosis and develope an effective treatment plan incorporating psychotherapy and/or psychopharmacology, while also addressing any comorbid medical or developmental conditions (AAP, 2014; AAP & Duby, 2007; Simon et al., 2015; Weitzman et al.,2015).

8. Challenges in Developmental and Behavioral Screening

Implementing developmental and behavioral screening in pediatric healthcare settings presents several challenges. Addressing these challenges and promoting a collaborative approach can enhance the effectiveness and sustainability of these screening programs. However, it is essential to recognize potential limitations, such as overlooking

individual concerns, cultural differences, and the risk of false-positive results leading to unnecessary anxiety or mislabeling (Lipkin et al., 2020; Perrin & Stancin, 2002; Silver et al., 2017).

While developmental screening tools provide valuable insights, over-reliance on them can overlook important considerations. Solely focusing on standardized assessments may neglect specific parental concerns and lack individualized focus tailored to each child's unique needs. Excessive emphasis on checklists can create a false sense of thoroughness, potentially missing cultural differences that impact developmental trajectories (Perrin & Stancin, 2002). Healthcare providers must balance using validated screening tools with considering parental input, cultural factors, and individual differences. By recognizing the limitations of screening tools and adopting a holistic approach to developmental assessment, healthcare professionals can ensure that each child receives appropriate and effective support, promoting optimal developmental outcomes. Some of the common implementation challenges include (Lipkin et al., 2020; Silver et al., 2017):

Time Constraints: Limited time for screening due to patient volume can hinder comprehensive assessments, but using brief screening tools and reimbursement strategies can help mitigate this issue.

Incomplete Tool Usage: Delays in scoring and interpretation can occur when parents fail to complete screening tools before visits, highlighting the need for improved previsit completion methods.

Inconsistent Tool Distribution: Ensuring proper distribution of screening tools to eligible children at the right visits can be challenging without automated reminders, necessitating manual tracking methods.

Clinician Buy-In: Securing support from all clinicians is crucial, as some may question the utility of screening tools compared to clinical judgment, emphasizing the importance of early engagement and education.

Staff Turnover: High turnover rates can disrupt screening initiatives, underscoring the need for consistent training and buy-in across all clinic roles.

Cost of Screening Tools: Expenses related to copyrighted screening tools may hinder their integration into electronic health records, requiring negotiation with publishers for local modifications.

While the American Academy of Pediatrics recommends developmental screening in the U.S., challenges in resource-limited settings may necessitate a longitudinal devel-

opmental monitoring approach recommended by the World Health Organization. Standardized assessments are advised in cases of clinical suspicion, emphasizing the need for tailored strategies in diverse healthcare settings (World Health Organization, 2020).

9. Conclusion

Developmental and behavioral screenings are crucial in pediatric care for the detection and intervention of developmental and behavioral issues in the early years. American Academy of Pediatrics (AAP) offers guidelines for healthcare providers to screen and evaluate children, stressing the integration of developmental and behavioral surveillance into routine pediatric care. When screenings indicate potential risks, children should immediately undergo comprehensive developmental and medical assessments and be enrolled in early intervention programs without waiting for the results. By early diagnosis and intervention, children at risk will reach their developmental potential, and healthcare costs will be reduced.

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Chapter

12

The Importance of Hypertension Screening in Children

Ragıp Afşin ALAY

High blood pressure (BP), more commonly known as hypertension (HT), is one of the significant public health issues in today's world. Although it is considered primarily an adult disease, its prevalence in children has been increasing significantly in recent years. Decreased physical activity, obesity, sleep disorders, and depressive personality disorders, which are consequences of increased technology dependency, are known to play a major role in the etiology of hypertension. (Baranowski et al., 2011; Flynn et al., 2017).

Prevalence

In studies conducted, when evaluated with a one-time BP measurement, the frequency of HT in children was found to be higher in boys than girls and at an M/F ratio of 15-17/7-12%. In repeated measurements, it was observed that the frequency of HT decreased, although it was still higher in boys than in girls. The estimated frequency of HT in children is thought to be 3-5%. This shows us that a definitive assessment cannot be made with a single measurement in childhood and that two or more checks are required with correct methods (Flynn et al., 2017; Göknar & Çalışkan, 2020; Lurbe et al., 2016).

There are few studies on the epidemiology of childhood HT in our country. Studies have reported that the frequency of HT in children between the ages of 6 and 15 is

8.5%-15%. (Demirci et al., 2013; Önsüz & Demir, 2015). The frequency of hypertension increases in conditions such as obesity, sleep apnea syndrome, chronic kidney disease, and prematurity. While the prevalence of hypertension is 4–14% in overweight children, it increases to 11–23% in obese children (Flynn et al., 2017; Freedman et al., 1999; Sorof et al., 2004). High amounts of salt intake in the diet are also an important risk factor in the development of HT, especially for obese and overweight children (Lo et al., 2013; Yang et al., 2012). The coexistence of hypertension and obesity also brings cardiovascular risk factors (e.g. dyslipidemia, impaired glucose tolerance) with it (Brian K Kit et al., 2015).

The most important purpose of detecting HT in childhood is to prevent end-organ damage. In addition, it also aims to prevent long-term and high-cost treatment because childhood-onset HT affects morbidity and mortality in adulthood (Flynn et al., 2017; Roulet et al., 2017).

There are two guidelines for hypertension definition, monitoring, and treatment in children. The first one was written by the European Society of Hypertension (ESH) in 2006 and updated in 2016. The second is the guideline created by the American Academy of Pediatrics (AAP) and took its final and recent form in 2017 (Flynn et al., 2017; Lurbe et al., 2016).

Hypertension definition, measurement, and classification

The American Academy of Pediatrics recommends that all children aged 3 and over should have their BP measured once a year at every health checkup in the presence of risk factors such as obesity, diabetes mellitus, decreased physical activity, sleep disorder-snoring, poor eating habits (consumption of salty food-consumption of ready-made food). It recommends BP measurement (Flynn et al., 2017). When making a diagnostic approach, anamnesis should be taken carefully and medical history, family history, and symptoms should be carefully questioned. Situations that may appear as risk factors in the development of HT are listed in Table 1 (Flynn et al., 2017; Göknar & Çalışkan, 2020).

Table 1. Situations to be considered in anamnesis

Perinatal history	Gestational age, birth weight, neonatal intensive care unit admission history, catheter history, asphyxia, renal artery vein thrombosis history
Current disease history	Urological, cardiological, endocrine, and rheumatological diseases
Drug Use History	Steroids, antidepressants, birth control pills
Family History	such as hypertension, obesity, diabetes mellitus, hereditary diseases, Polycystic kidney, Alport Syndrome
Symptoms	Dysuria, nocturia, hematuria, cold sweats, inability to gain weight, edema, palpitations, headache, dizziness, nosebleeds, visual impairment, paralysis, stroke symptoms

Table 2. HT classification and recommendations

Classification	<13 years	≥13 years	Recommendations
Normal blood pressure	<90th percentile	<120/80 mmHg	If blood pressure is normal or found to be normal with repeated measurements, it is recommended to measure it at the next healthy child evaluation one year later.
High blood pressure	≥90th percentile to <95th percentile or 120/80 mmHg to <95th percentile (whichever is lower)	120/80 to 129/80 mmHg arası	**Step 1:** Lifestyle changes (healthy diet, sleep, and physical activity) should be recommended. If it is necessary, she/he may be referred to a nutrition or weight unit. **Step 2:** If there is still high BP in the first year of admission after the end of 6-month interval checks, ABPM and diagnostic tests should be performed and the child should be referred to pediatric nephrology.
Stage 1 hypertension	≥95th percentile to <95th percentile+12 mmHg or 130/80 to 139/89 mmHg (whichever is lower)	130/80 to 139/89 mmHg	**Step 1:** If the blood pressure is detected at stage 1 HT and the patient is asymptomatic, he/she is invited for follow-up visits every one to two weeks. **Step 2:** Blood pressure is measured in the right arm, left arm, and one leg (right or left). Nutrition and weight control recommendations are given. **Step 3:** If it is still high after three visits, ABPM should be inserted and medical examinations and required tests should be performed. The child should be referred to pediatric nephrology or pediatric cardiology.
Stage 2 hypertension	≥95th p+12 mmHg or ≥140/90 mmHg	≥140/90 mmHg	**Step 1:** If blood pressure is detected as HT stage 2 and symptomatic, blood pressure is measured in the right arm, left arm, and one leg. Life Standard recommendations are made. **Step 2**: If blood pressure is still detected high within a week, the patient is referred to the relevant center. It is recommended to use ABPM.

BP: Blood Pressure, HT: Hypertension, ABPM: Ambulatory Blood Pressure Measurement
Note: However, if the patient is symptomatic at first admission or is above the 95th percentile +30 mmHg or 180/120 mmHg, it is recommended to be referred to the emergency department.

In children, BP increases in direct proportion to age and body size. For normal BP evaluation, BP was classified according to age, gender, and percentile values in both guidelines. In both guidelines, a measured BP value of 90th percentile and below is considered normal BP, and a BP value of 95th percentile and above is considered HT. The ESH guideline recommends that BP be evaluated according to the percentile up to the age of 16 and that blood pressure values used in adults should be used for those over the age of 16. According to the AAP guideline, BP evaluation is taken at 13 years of age according to the percentile. HT percentile tables were created according to age, gender, and height. Classification and recommendations according to measured values are given in Table 2 (Göknar & Çalışkan, 2020).

Who Should Have Blood Pressure Measurement?

In children aged three years and older, ESH recommends BP measurement at each healthcare visit and APP recommends BP measurement annually. ESH recommends BP measurement at every health care application, and APP recommends annual BP measurement. BP measurement in children under 3 years of age should be performed in the presence of risk factors for HT (Neonatal ICU, renal disease, congenital heart disease, increased ICP, taking medication that may increase BP).

Blood Pressure Measurement

Blood pressure measurement in children is frequently performed with Office BP (OBP), home BP measurement (HBPM), and ambulatory BP measurement (ABPM) methods. However, due to the problem of child compliance, both guidelines emphasized that evaluation with ABPM would be more accurate in selected cases (white coat hypertension, inconsistency in home and office measurements, diabetes) (Başaran & Demir, 2021; Flynn et al., 2017; Lurbe et al., 2016).

Although ABPM is the gold standard, it may not be applicable for all settings or patients. It requires the purchase of ABPM equipment and the need for training personnel to use and interpret the results. ABPM is not recommended in children under 5 years of age due to a lack of normative data and feasibility. It may not be tolerated in some special populations of children over the age of five. In such cases, Home Blood Pressure (HBP) monitoring may be helpful for diagnosis and treatment (Haseler & Sinha, 2022).

Home BP monitoring can help obtain information about out-of-office BP. Normative data for HBP monitoring with automatic or Doppler devices has not been available. Publications on HBP have been limited. In a recent systematic review on HBP, it has been recommended that a 7-day, morning, and evening (minimum 12) measurement series for HBP can be used until more data are available (Stergiou et al., 2019).

Once the diagnosis of hypertension is confirmed, a targeted assessment should be performed and secondary causes that may cause hypertension should be investigated. Table 3 summarizes the secondary causes that should be considered (Singh et al., 2017).

Table 3. Important Secondary Causes of HT (adapted from reference Singh et al., 2017)

Category	Specific Causes
Obesity	
Renal parenchymal disease	Glomerulonephritis Focal and segmental glomerulosclerosis Pyelonephritis-associated renal scarring Acute kidney injury with salt and water overload Polycystic kidney disease Chronic kidney disease Obstructive uropathy
Renovascular	Renal artery stenosis (Idiopathic, Fibromuscular dysplasia, Neurofibromatosis type 1, Williams syndrome) Middle aortic syndrome Renal artery or vein thrombosis Acute or post-hemolytic uremic syndrome Fistula External compression
Endocrine	Cortisol/glucocorticoid excess Aldosterone/mineralocorticoid excess Catecholamine excess Congenital adrenal hyperplasia Thyroid disease
Cardiovascular	Coarctation of the aorta Takayasu arteritis
Central Nervous System	Pain Convulsions Increased intracranial pressure Guillain-Barré syndrome Dysautonomia
Malignancies	Wilms tumor (nephroblastoma) neuroblastoma pheochromocytoma
Drugs	Amphetamine or sympathomimetics Acute vitamin D intoxication, hypercalcemia Calcineurin inhibitors (cyclosporine/tacrolimus) Decongestants Erythropoietin Oral birth control pills Steroids
Other	Obstructive sleep apnea Bronchopulmonary dysplasia Single gene defects causing hypertension (e.g. Liddle syndrome)

The 2016 ESH guideline recommends an echocardiogram for all patients with hypertension, and the 2017 AAP guideline recommends an echocardiogram to assess hypertension-mediated organ damage during the evaluation of pharmacological treatment (Flynn et al., 2017; Lurbe et al., 2016).

In light of these studies and guidelines, the prevalence of HT in children is increasing and HT in children, if not detected and controlled, can lead to extremely adverse cardiovascular events in middle age or earlier. A single measurement is not sufficient for the diagnosis of HT in children. Annual blood pressure monitoring should be performed for every child over a certain age and children with risk factors should be followed up more frequently.

There are no common blood pressure assessment and monitoring charts that cover all children worldwide. This study aims to emphasize that the detection of HT in childhood in the light of current guidelines is to prevent early end-stage organ damage and to prevent morbidities and early mortality that may occur in adulthood. Risk factors of each society should be determined according to the life, nutrition, and development level, and follow-up and screening algorithms should be established.

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Chapter

13

Dyslipidemia Screening

Turgay ARAS

Atherosclerosis and the role of lipids:

The development of atherosclerosis is thought to start with the thickening of the vascular intima as a result of the accumulation of lipid-laden macrophages. The conversion of LDL-C to oxidized LDL-C due to exposure to cigarette smoke, hypertension, and diabetes leads to endothelial dysfunction, inflammation of the vascular bed, and subsequent fatty streaking. This process can be reversed by HDL-C (Sata et al., 2002). The next step is the damage to arterial smooth muscle cells by the interaction of certain cytokines and growth factors. The resulting atherosclerotic plaque grows and narrows the vascular bed and/or ruptures, causing ischemia in another area. As a result, it causes atherosclerotic cardiovascular disease (ASCVD), most commonly involving the coronary arteries, but can also affect the aorta, lower extremity arteries, and carotid arteries (Neal & John, 2016). There is a relationship between dietary fat intake and elevated plasma cholesterol levels and thus the risk of ASCVD (Neal & John, 2016). Studies have shown a relationship between increased abdominal fat and increased risk of atherosclerosis (McMahan et al., 2006). Risk factors for the development of ASCVD include familial predisposition, obesity, exposure to tobacco smoke, diabetes, and familial hypercholesterolemia (FH). Dyslipidemias are among the important risk

Table 1. Risk factors for ASCVD in children (De Ferranti et al., 2019; Health et al., 2011; Sarah D de Ferranti, 2024)
Dyslipidemia
Obesity
Diabetes mellitus (type 1 or 2)
Hypertension
Family history of premature ASCVD
Smoke exposure
Familial hypercholesterolemia
Chronic kidney disease
Kawasaki disease
Childhood cancer
Transplant vasculopathy
Certain congenital heart disease defects: • coarctation of the aorta • aortic stenosis • transposition of the great arteries • congenital coronary artery anomalies)
Cardiomyopathy
Chronic inflammatory disorders : • SLE • Systemic JIA
HIV infection
Adolescent depressive and bipolar disorders

groups with a prevalence of approximately 20% (Health et al., 2011; B. K. Kit et al., 2015). Risk factors for ASCVD in children are given in Table 1.

Dyslipidemias

Lipoprotein metabolism disorders that result in high total cholesterol (TC), LDL-C, non-HDL-C, triglyceride (TG) levels and low HDL-C levels are called dyslipidemia. Since dyslipidemia begins in childhood, early diagnosis and treatment will improve the quality of life of these children. Screenings based on lipid and lipoprotein results are important to reduce or prevent the risk of atherosclerosis and subsequent ASCVD in children (Neal & John, 2016; TÜMER & KASAPKARA, 2013). Although lipid and lipoprotein normal values vary according to age and gender (Table 2), acceptable, borderline high and high values for children are given in Table 3 (Daniels et al., 2008; Health et al., 2011)

Table 2. Lipid and lipoprotein distributions in subjects aged 5 to 19 years (Daniels et al., 2008)

	Males			Females		
	5-9 years	**10-14 years**	**15-19 years**	**5-9 years**	**10-14 years**	**15- 19 years**
Total cholesterol, mg/dL						
50th percentile	153	161	152	164	159	157
75th percentile	168	173	168	177	171	176
90th percentile	183	191	183	189	191	198
95th percentile	186	201	191	197	205	208
Triglyceride, mg/dL						
50th percentile	48	58	68	57	68	64
75th percentile	58	74	88	74	85	85
90th percentile	70	94	125	103	104	112
95th percentile	85	111	143	120	120	126
LDL, mg/dL						
50th percentile	90	94	93	98	94	93
75th percentile	103	109	109	115	110	110
90th percentile	117	123	123	125	126	129
95th percentile	129	133	130	140	136	137
HDL, mg/dL						
5th percentile	38	37	30	36	37	35
10th percentile	43	40	34	38	40	38
25th percentile	49	46	39	48	45	43
50th percentile	55	55	46	52	52	51

Normal fasting lipoprotein profiles from the United States National Health and Nutrition Examination Surveys (NHANES).

Table 3. Acceptable, Borderline High, and High Plasma Lipid, Lipoprotein, and Apolipoprotein Concentrations (mg/dL) for Children and Adolescents. (Health et al., 2011; Sarah D de Ferranti, 2024)

Category	**Accetable (mg/dL)**	**Borderline high (mg/dL)**	**High (mg/dL)**
LDL-C	<110	110-129	≥130
Non -HDL-C	<120	120-144	≥145
ApoB	<90	90-109	≥110
TG			
• 0- 9 years	<75	75-99	≥100
• 10-19 years	<90	90-129	≥130
Category	**Accetable (mg/dL)**	**Borderline low (mg/dL)**	**Low (mg/dL)**
HDL-C	>45	40-45	<40
ApoA-1	>120	115-120	<115

Excessive intake of saturated and trans fats in the diet causes an increase in total cholesterol and LDL, and excessive consumption of refined carbohydrates and sugars causes an increase in triglyceride levels. In addition, secondary causes such as alcohol, drugs, endocrine, metabolic, renal problems, infections, and genetic factors play a role in the etiology of dyslipidemia (Table 4) (Health et al., 2011; Neal & John, 2016).

Tablo 4. The etiology of dyslipidemia (Health et al., 2011; Neal & John, 2016; Sarah D de Ferranti, 2024)

Table 4. The etiology of dyslipidemia (Health et al., 2011; Neal & John, 2016; Sarah D de Ferranti, 2024)	
Dietary causes	
Excessive consumption of saturated and trans fats in the diet, excessive consumption of refined carbohydrates and sugars	
Secondary causes	
Exogenous	
	Alcohol
Drug therapy	
	Corticosteroids
	Isotretinoin
	Some oral contraceptives
	Select chemotherapeutic agents
	Select antiretroviral agents
Endocrine/Metabolic	
	Hypothyroidism/hypopituitarism
	Diabetes mellitus types 1 and 2
	Pregnancy
	Polycystic ovary syndrome
	Lipodystrophy
	Acute intermittent porphyria
Renal	
	Chronic renal disease
	Hemolytic uremic syndrome
	Nephrotic syndrome
Infectious	

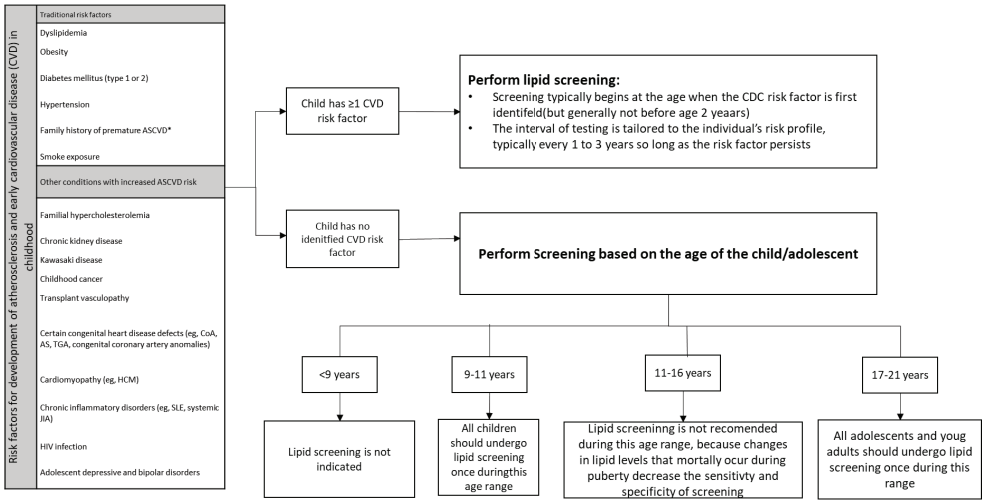
	Acute viral/bacterial infection*
	HIV infection
	Hepatitis
Hepatic	
	Obstructive liver disease/cholestatic conditions
	Biliary cirrhosis
	Alagille syndrome
Inflammatory disease	
	Systemic lupus erythematosus
	Juvenile rheumatoid arthritis
Storage disease	
	Glycogen storage disease
	Gaucher disease
	Cystine storage disease
	Juvenile Tay-Sachs disease
	Niemann-Pick disease
Other	
	Kawasaki disease
	Anorexia nervosa
	Solid organ transplantation
	Childhood cancer survivor
	Progeria
	Idiopathic hypercalcemia
	Klinefelter syndrome
	Werner syndrome
Genetic causes	
Heterozygous familial hypercholesterolemia	
Homozygous familial hypercholesterolemia	
Familial combined hyperlipidemia	
Familial hyperapobetalipoproteinemia	
Polygenic hypercholesterolemia	
Familial dysbetalipoproteinemia	

Screening

Age and underlying risk factors for early ASCVD determine the frequency and duration of screening (Health et al., 2011; Neal & John, 2016). If the child has early ASCVD risk factors, screening starts at the age at which the risk factors are first detected and screening is performed at intervals of 1 to 3 years depending on the status of the risk factors. However, if there is a family history of hypercholesterolemia or early ASCVD, such as a history of heart attack, treated angina, history of intervention for coronary artery disease, sudden cardiac death, or a history of ischemic cerebrovascular events in male family members before the age of 55 or female family members before the age of 65, screening should start at the age of 2 years in case of a known familial history of hypercholesterolemia.

For children without risk factors for early ASCVD, screening for dyslipidemia should be performed twice, first at 9-11 years of age and second at 17-21 years of age (Table 5) (De Ferranti et al., 2019; Health et al., 2011).

Table 5. Approach to lipid screening in children and adolescents (De Ferranti et al., 2019; Health et al., 2011(Sarah D de Ferranti, 2024)



Screenings are planned based on lipid profile levels measured in fasting or postprandial state (Neal & John, 2016) (Table 3). Lipid profiles include directly measured TC, HDL-C, Triglycerides, and LDL-C, non-HDL-C calculated by formulas. If there are abnormalities in the results measured in satiety, fasting lipid profiles can be checked for follow-up. However, treatment decisions and medical treatment follow-up are planned

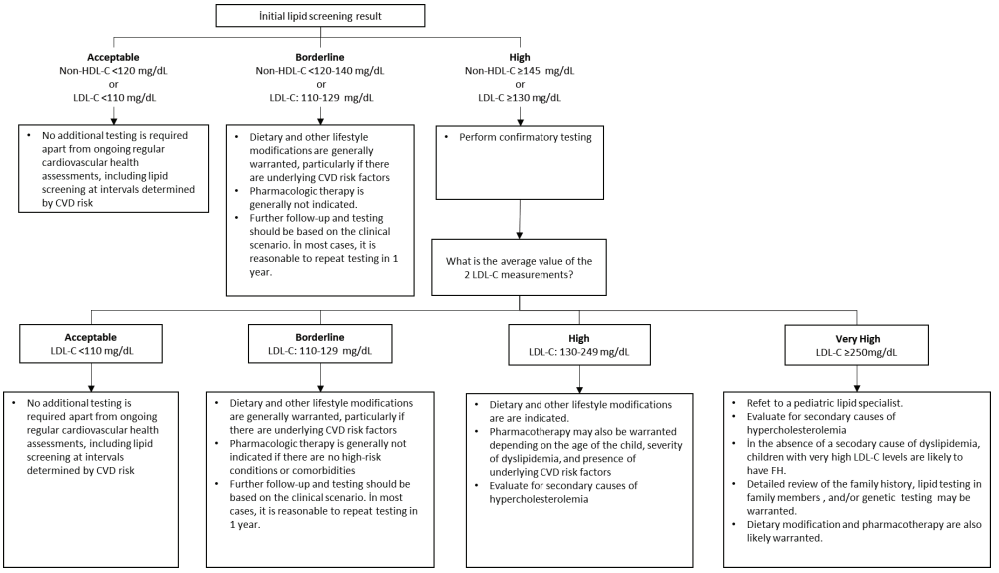
according to fasting lipid levels (Steiner et al., 2011). Non-HDL-C is calculated as total cholesterol minus HDL-C and includes all lipoproteins considered atherogenic. It is as good a marker for dyslipidemia screening as other lipid profiles (Frontini et al., 2007; Srinivasan et al., 2006). Studies have shown that non-HDL-C elevation detected in childhood persists into adulthood. Therefore, non-HDL-C elevation detected in childhood may be a harbinger of dyslipidemias that may occur in adulthood (Srinivasan et al., 2006).

Initial screening results are evaluated by looking at non-HDL-C and LDL-C levels. Children with acceptable levels of Non-HDL-C or LDL-C do not need further evaluation and are recommended to continue lipid screening in accordance with the ages mentioned above. Children with borderline high-test results should be directed to diet and a healthy lifestyle. Medical treatment is usually not necessary. Additional diagnostic tests may be planned according to the underlying risk factors. It may often be necessary to repeat screening tests within a year. Children with abnormally high-test results should have their fasting lipid profile reconfirmed. Confirmatory test results should be decided by averaging fasting LDL-C values taken at least twice between 2 weeks and 3 months. Dietary and lifestyle changes should be initiated as a result of the first abnormal test result. Medical treatment may be required depending on the child's age, severity of dyslipidemia, and the presence of underlying ASCVD risk factors. In patients with high LDL-C values (> 130 mg/dL), the causes of secondary hypercholesterolemia should be investigated. Children with very high LDL-C values (≥ 250 mg/dL) should be referred to the relevant specialist. Dietary changes and medication are probably necessary. Secondary causes of hypercholesterolemia should be investigated. If there is no secondary cause, children with very high LDL-C levels are likely to have Familial Hypercholesterolemia (FH). Family history should be investigated and lipid testing and/or genetic testing should be planned for other family members (Table 6) (Sarah D de Ferranti, 2024).

Atherosclerotic heart disease is one of the most common causes of mortality and morbidity in adults. One of the factors involved in the pathogenesis of atherosclerotic heart disease is atherosclerotic plaque formation. One of the most common causes of atherosclerotic plaque formation is dyslipidemias which start in childhood and are usually silent (Neal & John, 2016).

Screening only children with underlying predisposing conditions such as familial hypercholesterolemia, obesity, diabetes, etc. leads to a significant number of children with dyslipidemia going unrecognized. Therefore, screening for dyslipidemia in childhood will contribute to controlling dyslipidemia with early diagnosis and reducing the risk and/or severity of ASCVD in later life (Health et al., 2011; Neal & John, 2016).

Table 6. Overview of follow-up of children undergoing lipid screening(Health et al., 2011; Neal & John, 2016; Sarah D de Ferranti, 2024)



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Chapter

14

The Effects Of Oral Screenıngs in Children And Adolescents On Oral Health

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Introduction

Children and adolescents are among the most valuable assets of societies. A healthy childhood and adolescence are vital for an individual's physical, mental, and emotional development. However, oral health should not be overlooked to sustain a healthy developmental process. This is because oral health is an integral part of overall health and a fundamental factor affecting individuals' quality of life.

Oral screenings in children and adolescents play a critical role in maintaining and improving oral health. These screenings help in early detection of serious diseases such as dental caries, periodontal diseases, malocclusions, other oral health problems that may arise in early childhood, and oral cancer. Early diagnosis is crucial for initiating treatment early and maintaining a healthy oral environment.

Oral screenings are also important in helping children and adolescents develop healthy oral hygiene habits that they can maintain throughout their lives. Giving importance to oral health at an early age leads children to adopt healthy habits such as brushing teeth, using dental floss, and regular dental visits. These habits contribute to maintaining oral health in later years and continuing the healthy habits acquired during childhood into adulthood.

This book chapter is prepared to emphasize the importance of oral screenings in children and adolescents and to provide guidance on how these screenings can be effectively conducted. Understanding the effects of oral screenings on the overall health of children and adolescents will provide health professionals and parents with an important perspective on paying more attention to the oral health of children and adolescents.

Critical Periods for Oral-Dental Screening in Children and Adolescents

It is important to conduct oral and dental screenings at specific age intervals related to oral health. The World Health Organization (WHO) has determined the ages of 5, 12, and 15 as index ages for oral-dental screenings for children and adolescents (Petersen & Baez, 2013). These index ages ensure more reliable epidemiological studies worldwide and comparison according to specific standards. This section will discuss examinations specific to different dental developmental stages in children and adolescents, as well as the particular aspects that need to be considered for each index age within each period.

Critical age intervals for oral and dental health in children and adolescents are as follows:

Infancy Period (0-2 Years)

The first dental evaluation should be performed during infancy (Bulut & Bulut, 2020). After assessing the general health status of newborns, an intraoral examination should be conducted. In newborns, it is necessary to examine the edentulous alveolar arches known as *gum pads*. Parents should be informed about the necessity of cleaning the baby's mouth after each feeding with disposable special oral cleaning wipes designed for infants (Goswami & Chawla, 2023). This regular cleaning of the infant's mouth will lay the foundation for toothbrushing habits.

The structures that should be examined in the intraoral assessment of newborns include:

Lip-Palate Deformities: Lip and palate deformities should be carefully examined during intraoral examination. Cleft lip and palate may sometimes involve only one jaw, while in other cases, it may affect both jaws. Non-obvious cleft palates may be overlooked during the baby's initial examination if not carefully examined. This situation will prevent the vacuum effect that is necessary during sucking and will negatively affect the baby's feeding. (Allori et al., 2017; McGuire, 2017; AAPD guideline, 2023, p.576-7).

Evaluation of Tongue Tie and Breastfeeding: Early assessment of tongue tie is important during intraoral examination. Almost all babies are born with a tissue fold called the lingual frenulum under their tongue. Sometimes, the lingual frenulum may restrict the movement and function of the tongue. This is known as *tongue tie or ankyloglossia* (Marcione et al., 2016; Martinelli et al., 2020; Araujo et al., 2020). This condition can affect the baby's breastfeeding function partially or completely. Proper positioning and attachment of the baby to the breast are important for optimal breastfeeding function (Araujo et al., 2020). When the baby is properly positioned and attached, the mother's nipple will be positioned deep in the baby's mouth, ideally at the junction of the hard and soft palate. In this position, the baby feeds better (Bhandari & Sashikala, 2016). Optimal breastfeeding is important for the development of the baby's jaw and facial structures (Page, 2001). Therefore, failure to intervene in babies with tongue tie in a timely manner will not only affect the child's ability to feed but also negatively impact jaw and facial development. This is related to the stimulation effect of correct breastfeeding on the growth of the muscles and bones of the jaw and face during sucking. In later stages, tongue tie that is not properly positioned will also negatively affect the child's speech function because the child cannot produce correct speech sounds (AAPD guideline, 2023, p. 71-76).

In newborns with tongue tie, they cannot lift their tongues upward when crying, and their tongue tips may appear heart-shaped or V-shaped. The baby cannot grasp the mother's nipple and becomes easily tired during feeding. Weight gain is minimal or nonexistent. Noisy or clicking sounds may be heard during feeding. The mother may experience nipple pain while breastfeeding. When the baby releases the breast, the nipple may appear compressed or wrinkled. Insufficient and improper breastfeeding leads to a lack of increase in milk supply, blocked milk ducts, or mastitis. If the baby is bottle-fed, they may swallow a lot of air and become quickly tired during feeding. Milk may leak from the corners of their mouths (Cordray, 2023; Griffiths, 2004). When the child begins to speak, tongue tie can hinder the ability to produce specific sounds such as "t", "d", "z", "s", "th", "r", and "l" (Marchesan, 2004; Marchesan, 2005). In addition to all these problems, tongue tie also leads to poor oral hygiene and spacing between the lower incisor teeth. Moreover, tongue tie restricts the child from activities such as licking ice cream, playing wind instruments, and kissing.

Various scales are used in the clinical assessment of tongue tie. Among these, the *Kotlow, Tabby, Coryllos, and Modified Coryllos Classifications* are the most well-known (Marcione et al., 2016; Walsh & McKenna, 2019).

Newborn Keratin Cysts: Newborn keratin cysts are inclusion cysts that can be observed on the palatal mucosa of newborns. They are small, white, and free-moving, typically ranging from 1-3 mm in diameter, and they may appear in multiples. These cysts are superficial.

Newborn keratin cysts are named according to the presence of keratin content and their localization as follows:

Epstein Pearls: These are seen along the midline of the palate and contain squamous epithelium.

Dental Lamina Cysts: Observed as part of the tooth eruption phenomenon, these cysts are located on the crest of the alveolar ridge and contain odontogenic epithelium.

Bohn's Nodules: These are seen on the buccal or lingual surfaces of the alveolar ridge and contain remnants of mucous gland tissue (Perez et al., 2018).

Assessment of Nasal Breathing in Newborns: It is important for the newborn to breathe through the nose and to prevent the habit of mouth breathing for proper jaw and facial development. Regular nasal cleaning in newborns is important to prevent the development of mouth breathing habits. When mouth breathing is noticed in babies, gently touching the lower jaw to encourage lip closure is recommended. If associated with nasal congestion, nasal cleaning with saline solution should be performed (Torre & Guilleminault, 2018).

Natal and Neonatal Teeth: *Natal teeth* are those seen in the baby's mouth at birth, while *neonatal teeth* are those seen within the first 30 days after birth. Although rare, they are important due to their potential to cause sublingual traumatic ulceration (Riga fede), aspiration, and ulceration on the mother's nipple during breastfeeding (Cunha et al., 2001; Meij et al., 2012). If these teeth are causing traumatic ulceration, the sharp edges should be smoothened. If they are excessively mobile, they should be extracted (Leung, & Robson, 2006).

Monitoring the Process of Primary Dentition: During infancy, the process of tooth eruption typically begins around the 6th month and continues until the 30th month, although it varies from baby to baby. Therefore, during infancy, it is important to inquire whether teeth are erupting and if so, whether dental hygiene practices are being implemented. If teeth have erupted, they should be evaluated. Normally, a 30-month-old child should have 20 primary teeth. During examination, the number, shape, and color of the teeth are evaluated. It is important to assess plaque accumulation, hypoplasia, hypomineralization, and dental caries in the upper incisors by holding the upper lip with the thumb and index fingers during the examination (AAPD guidelines, 2023, p. 288-300).

Oral health problems that should be monitored during primary dentition include:
Early Childhood Caries (ECC): Early childhood caries is a severe form of tooth decay affecting infants and young children. It is defined as the presence of one or more

decayed (cavitated or non-cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child 71 months of age or younger (AAPD guideline, 2023, p. 88-91).

Babies are fed with breast milk or formula milk (bottle feeding) during the initial teething period. Falling asleep with breast milk or formula milk in the mouth, especially during nighttime feedings, exposes the teeth to the risk of decay. Therefore, it is necessary to give the baby water and clean the teeth after such feedings (AAPD guideline, 2023, p. 79-81; Goswami & Chawla, 2023). To prevent early childhood caries, basic oral hygiene practices such as toothbrushing should be initiated during this period. Regular cleaning of the palate and tongue before the eruption of teeth lays the foundation for the baby to develop a habit of brushing teeth. Silicone finger brushes can be used for this purpose. These brushes have a brush-like surface on one side and small round protrusions on the other side. These protrusions can also be used for massaging the baby's palate during teething to relieve itching.

Eruption Cyst or Hematoma: An eruption cyst is a soft tissue cyst formed as a result of the separation of the dental follicle extending from the coronal to the root portion of an erupting tooth. Fluid accumulation occurs in this follicular area. They are usually found in the mandibular molar region. The color of these lesions may vary from blue-black to brown depending on the amount of blood in the cyst. Blood accumulation within the follicle usually occurs due to secondary trauma. In such cases, the eruption cyst is called an eruption hematoma. Since the tooth will erupt through the lesion, no treatment is necessary. If the cyst does not rupture spontaneously or if the lesion becomes infected, it can be surgically opened from the top (AAPD guideline, 2023, p.527-36).

Early Childhood Period (3-6 Years)

During the early childhood period, all primary teeth are present in the mouth, and towards the end of this period, permanent teeth begin to replace primary teeth. During oral screenings, it is essential to evaluate early childhood caries, periodontal health, occlusal relationships, poor oral habits affecting occlusion (such as pacifier and baby-bottle use, thumb sucking, nail biting, mouth breathing, etc.), and other intraoral issues. The oral health problems that require special attention during this period and the conditions leading to these problems will be discussed below under headings.

WHO recommends that children should be examined for oral health between their fifth and sixth birthdays whenever feasible. Considering 5 years as the index age is associated with the rapid development of caries due to the closure of physiological

diastemas between primary teeth at this age. The increased caries during this period can adversely affect the health of permanent teeth erupting at age 6. In some countries, starting school at the age of 5 allows access to children of this age group in a school environment. When the transition to permanent dentition has not yet occurred, the dmft/dmfs index should be used for caries assessment. However, in screenings conducted at age 6 or 7, the difficulty in distinguishing between missing primary incisors due to caries or trauma and those that have naturally exfoliated necessitates the use of the dft/dfs indices instead (Petersen & Baez, 2013).

ECC: The most common problem seen in children during early childhood is ECC. Due to the pain caused by ECC, a child may refuse to eat. As a result of eating on the side without pain, they may develop a habit of unilateral chewing. Unilateral chewing can lead to the formation of dental plaque and periodontal problems in the unused areas. In the long term, unilateral chewing can cause asymmetry in the jaw. ECC has been reported to negatively impact children's growth and development, quality of life, and academic success (AAPD guideline, 2023, p.88-91, p.92-93; Bağış et al., 2023).

Mouth Breathing: The child's breathing pattern should be assessed during the early childhood period. Children who breathe through their mouth due to chronic respiratory problems (such as adenoids, hypertrophic tonsils, and allergic rhinitis) may develop periodontal problems in the lower anterior teeth region. Prolonged mouth breathing, with the tongue not being in its normal position within the palatal vault, causes the deepening of the palatal vault and narrowing of the crest. This situation can lead to unilateral or bilateral posterior crossbite. (Linder-Aronson, 1969; Torre & Guilleminault, 2018).

Dental Erosion: Due to the increasing prevalence of dental erosion, it is important to evaluate for dental erosion in early childhood as well. Dental erosions seen at this stage may have developed due to gastroesophageal reflux (Wild et al., 2011; Taji & Seow, 2010). In children, complications such as tooth sensitivity, aesthetic problems, pulp exposure in severe cases, dental abscess, and vertical dimension losses can be observed due to dental erosion. The less inorganic structure of primary teeth enamel and morphological differences cause erosion to be more severe (Linnett & Seow, 2001).

Oral Bad Habits: Habits considered normal during infancy that persist into early childhood can negatively affect jaw-facial development and dental occlusion. In this age group, habits such as pacifier and bottle use, thumb sucking, and infantile swallowing are frequently observed. Discontinuing these habits during early childhood, before the transition to permanent dentition, is important for the proper development of dental occlusion (Gartika, 2008).

Periodontal Problems: Although rare, periodontal problems can be seen in children. Especially in the presence of systemic diseases, childhood illnesses, and certain syndromes, periodontal health should be evaluated in early childhood. Early tooth loss in early childhood is observed due to periodontitis associated with systemic diseases and syndromes (AAPD guideline, 2023, p. 493-507).

Primary School Age (7-12 Years)

In addition to the screenings conducted in early childhood, some assessments specific to this period need to be conducted during primary school age. This period, known as the mixed dentition phase, involves the eruption of permanent teeth while primary teeth are still present. This phase lasts approximately 6-8 years, during which the craniofacial skeleton grows rapidly. Preventing functional problems that could negatively affect jaw and facial development during this period will ensure proper arch and occlusion formation (Andley et al., 2019).

It is important for children to continue brushing their teeth regularly during primary school age. The first permanent molars, which emerge around the age of 6, erupt behind the primary molars into the oral cavity. These teeth may cause mild pain during eruption or may erupt without any pain, leading parents to be unaware of their children's permanent teeth emergence. Without adequate attention to oral hygiene, these teeth can decay rapidly (Heydari et al., 2018). Additionally, since children at this age are often unable to brush their teeth effectively, parents should assist with brushing, especially in the molar region (Manchanda et al., 2014; Chang et al., 2018). Effective tooth brushing skills are typically developed by the age of 8-9. In other words, for effective brushing, children should have the motor skills to tie shoelaces, write legibly, cut their food with a knife, and use scissors to cut shapes (Kerr et al., 2019).

During the 7-12-year period, oral screenings should focus on ensuring a healthy transition to permanent dentition, including early detection of caries, assessment of ectopic tooth eruptions, arch growth, space needs, and space excesses. Additionally, habits such as thumb sucking, nail biting, persistent infantile swallowing, mouth breathing, and crossbites should be evaluated to support ideal occlusion relationships. Moreover, structural abnormalities of teeth, increasingly referred to as molar-incisor hypomineralization (MIH), should be examined.

The second index age for oral health assessment after age 5 is 12 years. This age is particularly significant as it coincides with the end of primary school for many children. In many countries, it is the last age at which a reliable sample can be easily obtained through the school system. By this age, all permanent teeth except for the third molars are likely to have erupted. Therefore, the 12-year index age group is selected as

a global indicator for international comparisons and tracking disease trends (Petersen & Baez, 2013).

In addition to dental caries, occlusal relationships, and periodontal health, the conditions that require specific evaluation during the primary school age period are as follows:

Ectopic Tooth Eruption: Ectopic eruption occurs when a tooth does not follow its normal eruption path or loses its way, emerging in an unexpected area of the oral cavity. Although the exact etiology is not fully understood, trauma and infections might be responsible for this displacement, or genetic factors might play a significant role. Ectopic eruption is commonly observed in permanent first molars and upper canines. Lack of early intervention can lead to severe space loss, crowding, and tooth loss due to resorption in adjacent teeth (AAPD guideline, 2023, p. 466-83). Therefore, during the transition from primary to permanent dentition, ectopic eruptions should be evaluated radiographically.

Molar-Incisor Hypomineralization (MIH): MIH is a widespread developmental condition primarily affecting one or more first permanent molars and incisors. It is less common for central incisors to be affected. Additionally, hypomineralization of the second primary molar (HSPM) or canines may also be observed. Children with HSPM have a fivefold higher likelihood of MIH in permanent dentition. MIH can range from mild opacities to severe post-eruptive enamel breakdown. The exact etiology of MIH remains unclear. There are some correlations with prenatal, perinatal, and postnatal illnesses, exposure to antibiotics, and chemicals like dioxins, bisphenol A, and polychlorinated biphenyls, but there is still insufficient evidence (Lygidakis et al., 2022).

Assessment of Occlusion Relationships: The mixed dentition period is also known as the *ugly duckling stage,* where anterior teeth appear spaced and misaligned (Manjunatha, 2013). During this time, it is common for permanent incisors to erupt lingually while the primary incisors are still in place. This happens because the permanent teeth cannot perform the necessary rotational movements during eruption. In such cases, the primary tooth's root is resorbed by palatal pressure, leaving a thin vestibular plaque, preventing the primary tooth from falling out. Consequently, the permanent tooth appears lingual to the primary tooth in the oral cavity. Some researchers advocate for the extraction of these primary teeth, arguing that lingual eruption of the permanent tooth may cause arch dimension loss. Contrary to this belief, this dimension loss often corrects itself with tongue pressure and alveolar crest growth after the primary

tooth falls out (Aminabadi et al., 2009). It is important to remember that any surgical procedure may be a child's first dental experience, especially if they have no prior caries.

These conditions, alongside the usual concerns of dental caries, occlusion, and periodontal health, are crucial for maintaining optimal oral health during primary school age.

Adolescence (13-18 Years)

During adolescence, specific oral health issues that are unique to this developmental stage can emerge. The risk of dental caries may increase, and orthodontic problems can become more prominent. Hormonal changes can impact periodontal health. Oral screenings are crucial during this period to monitor oral health and plan necessary treatments.

The age of 15, which corresponds to the adolescent period, is the third index age group. Fifteen years old is considered the third index age group in oral health assessments. By this age, permanent teeth have been present in the oral environment for three to nine years, making it important to evaluate the prevalence of caries in adolescents. Additionally, assessing periodontal disease in the 15-18 age group is vital. In countries where obtaining reliable samples is challenging, assessments are typically conducted in regions including the capital city, another major city, and a rural area (Petersen & Baez, 2013).

During adolescence, in addition to dental caries, occlusal relationships, and periodontal health, the conditions that require specific evaluation are as follows:

Substance Abuse: The impact of substance abuse on oral health is significant during adolescence. It is crucial to evaluate findings related to substance abuse during dental examinations, as these can negatively affect oral and dental health (AAPD Guideline 2023, p. 143-4).

Oral Piercings: The popularity of intraoral jewelry and piercings among adolescents and young adults has led to an increase in related oral health problems. Intraoral jewelry or other oral accessories can cause elevated plaque levels, pathogenic periodontal bacteria, gingival inflammation and/or recession, caries, decreased speech ability, and metal allergies. Oral piercings, which can include the tongue, lips, cheeks, and uvula, are associated with pain, infections, scarring, tooth fractures, metal allergy reactions, localized periodontal disease, speech impairment, Ludwig's angina, hepatitis, and nerve damage. Specifically, gingival recession and permanent tooth injuries have been

observed in individuals with lip and tongue piercings. Severe complications such as bleeding, edema, endocarditis, airway obstruction, and other serious issues have also been reported due to oral piercings. Additionally, the use of dental ornaments, such as grills, has been documented to cause dental caries and periodontal problems (AAPD Guideline 2023, p. 143-4).

In conclusion, while dental caries, occlusion relationships, and periodontal health remain critical areas of focus during adolescence, special attention should be given to the unique challenges of substance abuse and oral piercings. Addressing these issues through regular screenings and appropriate interventions can help ensure better oral health outcomes for adolescents.

Conditions to Examine in Oral Screenings for Children and Adolescents of All Ages

Oral screenings for children and adolescents are typically conducted to evaluate oral and dental health. During these screenings, the following aspects are generally considered:

Condition of the Teeth: The alignment of the teeth, the number of teeth, and the condition of the enamel are examined.

Periodontal Health: The color of the gums, signs of swelling or bleeding, periodontal tissues, tartar and plaque buildup, and the degree of tooth mobility are assessed to detect early signs of gum diseases.

Caries Screening: Surfaces of the teeth are checked for caries and caries-prone areas, discoloration, and other conditions predisposing to decay such as defective enamel formation or stains.

Oral Hygiene: Tooth brushing habits and other oral hygiene practices are evaluated. The adequacy of oral cleanliness and the correct brushing technique are assessed. Improper and harsh brushing can lead to abrasion of the teeth and gum recession.

Condition of Oral Soft Tissues: The health and appearance of the soft tissues inside the mouth, such as the tongue, inside of the cheeks, and the palate, are evaluated. Signs of wounds, lesions, or infections are sought. Common oral lesions in children and adolescents include Aphthae, Mucocele, Morsicatio buccarum, Hairy tongue, Fibroma, Geographic tongue, Papilloma, Lip-tie, Herpes Simplex, Candidiasis, and Pyogenic granuloma (Owczarek-Drabinska et al., 2022; Porter & Kolokotronis, 2022).

Indices Used in Oral Screenings for Children and Adolescents

Oral screenings in children help maintain general oral health and assist in the early detection and treatment of potential issues. Therefore, regular oral screenings are

important for preserving healthy smiles in children. Several dental indices are used to evaluate caries, periodontal health, and occlusion relationships in oral screenings for children.

The commonly used indices in oral screenings for children include:

1. ***DMFT/dmft/dft/deft Indexes:*** This index was developed by Henry Klein, Carrole E Palmer and Knutson JW in 1938. Widely used to assess the status of dental caries in both primary and permanent dentition. This index represents the number of decayed, missing due to caries, and filled teeth in the permanent, primary and mixed dentition (Table 1) (Petersen & Baez, WHO, 2013).

Table 1. Descriptions of Commonly Used Caries Indices

Caries Indexes	
DMFT	Number of Decayed, Missing due to caries, and Filled Teeth in the permanent dentition
dmft	Number of decayed, missing due to caries, and filled teeth in the primary dentition
DMFS	Number of Decayed, Missing due to caries, and Filled Surfaces in the permanent dentition
dmfs	Number of decayed, missing due to caries, and filled surfaces in the primary dentition
dft	Number of decayed, and filled surfaces in the mix dentition
deft/defs	Number of decayed, extracted tooth/indicated for extraction (due to caries) and filled teeth/surfaces in the mix dentition

2. ***PUFA/pufa Index:*** This index was developed by Monse et al. in 2010.Used in both primary and mixed dentition periods. It records the presence of severely decayed teeth with visible pulpal involvement (P/p), ulceration caused by dislocated tooth fragments (U/u), fistula (F/f), and abscess (A/a). Uppercase letters are used for the permanent dentition and lowercase letters for the primary dentition (Table 2) (Monse et al., 2010). It is used to assess the clinical consequences of untreated caries in terms of pulpal involvement and associated conditions. In numerous low- and middle-income nations, decayed teeth often go untreated due to a variety of factors (Baelum et al. 2007).

Table 2. The Codes and Interpretations of the PUFA/pufa Index

Code	Interpretation
Pulpal involvement (P/p)	the opening of the pulp chamber is visible or the coronal tooth structures have been destroyed by the carious process and only roots or root fragments are left
Ulceration due to trauma (U/u)	sharp edges of a dislocated tooth with pulpal involvement or root fragments have caused traumatic ulceration of the surrounding soft tissues, e.g., tongue or buccal mucosa
Fistula (F/f)	a pus releasing sinus tract related to a tooth with pulpal involvement is present
Abscess (A/a)	a pus containing swelling related to a tooth with pulpal involvement is present

3. ***Oral Hygiene Index-Simplified (OHI-S):*** This index was given by John C. Greene and Jack R.

Vermillion in 1964. Commonly used to assess oral health in children and detect oral problems (Petersen & Baez, WHO, 2013). It includes the examination of teeth and gums for debris buildup or calculus. The six surfaces examined for the OHI-S are selected from four posterior and two anterior teeth. In this index, the lingual surfaces of teeth 46 and 36, as well as the buccal surfaces of the other teeth, are evaluated (Figure 1).

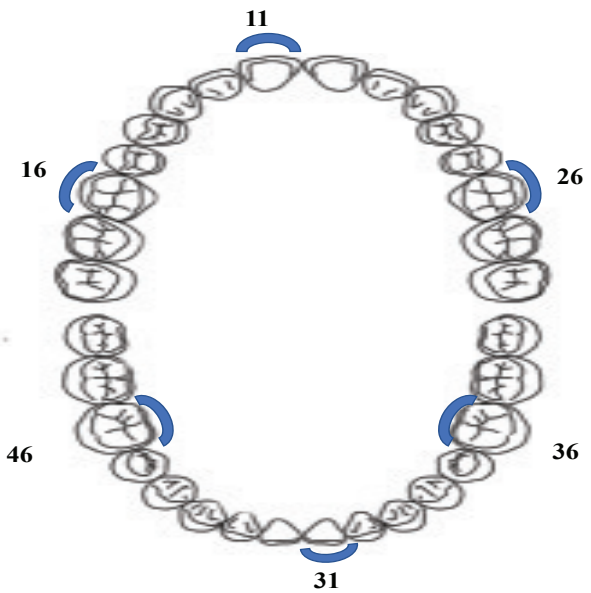


Figure 1. Oral Hygiene Index-Simplified

To calculate the debris score, sum the debris scores for all teeth and divide by the number of teeth scored. To calculate the calculus score, sum the calculus scores for all teeth and divide by the number of teeth scored. To calculate the OHI-S score, add the debris score to the calculus score. This total is the OHI-S score (Table 3 and 4).

Table 3. Debris and Calculus Scores

Scores	Debris score criteria	Calculus score criteria
0 	No debris or stain present.	No calculus present.
1 	Soft debris covering not more than one third of the tooth surface being examined or presence of extrinsic stains without debris regardless of surface area covered.	Supragingival calculus covering not more than one-third of the exposed tooth surface being examined.
2 	Soft debris covering more than one third, but not more than two thirds, of the exposed tooth surface.	Supragingival calculus covering more than one-third, but not more than two thirds of the exposed tooth surface and/or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth.
3 	Soft debris covering more than two thirds of the exposed tooth surface.	Supragingival calculus covering more than two thirds of the exposed tooth surface or a continuous heavy band of sungingival calculus around the cervical portion of the tooth.

Table 4. Interpretation of Debris/Calculus and OHI-S Indexes

Interpretation of Debris /Calculus Index	Interpretation OHI-S
0.0 to 0.6 = Good oral hygiene 0.7 to 1.8 = Fair oral hygiene 1.9 to 3.0 = Poor oral hygiene	0.0–1.2 = Good oral hygiene 1.3–3.0 = Fair oral hygiene 3.1–6.0 = Poor oral hygiene

4. ***Aine Erosion Index:*** Frequently observed in children with gastroesophageal reflux disease

   (GERD). The Aine erosion index is used in studies assessing dental erosion in both temporary and permanent teeth affected by GERD. Erosion lesions are classified based on clinical appearance, and this classification ranges from 0 (no erosion) to 3 (dentin exposure on occlusal surfaces or affected dentin on other surfaces) (Table 5) (Aine et al., 1993; Aine et al. 2000).

Table 5. The Aine Erosion Index

Code	Erosion scale
0	No erosion
1	Mild opacities or white spots/etched appearance
2	Occlusal surface filled with small holes (punched-out appearance), incisal edges thinned and flattening of cusps
3	Dentin exposure at the bottom of the holes on occlusal surfaces or dentin affected in other surfaces

5. ***Modified Developmental Defect of Enamel Index (Modified DDE Index):*** This index categorizes enamel defects as hypoplasia, demarcated opacity, and diffuse opacity.

Hypoplasia is a defect involving the enamel surface and associated with a reduction in enamel thickness, manifesting as pits, grooves, or large areas of missing enamel (Suckling, 1989). *Demarcated opacity* is characterized by a change in enamel translucency, with varying degrees. The defective enamel is of normal thickness and has a smooth surface. It has a clear boundary with adjacent normal enamel and can be white, cream, yellow, or brown (Suckling, 1989).

Diffuse opacity is characterized by a change in enamel translucency, with varying degrees. The defective enamel is of normal thickness and has a smooth surface. It is white at emergence and may be linear, patchy, or diffuse, with no clear boundary with adjacent normal enamel. Part or all of the tooth surface may be affected (Suckling, 1989). Other defects include staining caused by tetracycline, which does not fit into the categories of demarcated opacity, diffuse opacity, or hypoplasia (Suckling, 1989).

The combination of defects refers to the presence of both opacity and hypoplasia on the same tooth surface (Suckling, 1989). Modified Developmental Defect of Enamel Index is given in Table 6.

Table 6. Modified Developmental Defect of Enamel Index	
Defect	**Code**
Normal	0
Demarcated opacity	1
Diffuse opacity	2
Hypoplasia	3
Other defects * Demarcated and diffuse * Demarcated and hypoplasia * Diffuse and hypoplasia * All three defects	4 5 6 7 8

6. ***Index of Orthodontic Treatment Need (IOTN):*** This index was proposed by Peter H. Brook and William C. Shaw in 1989. Widely used to determine the orthodontic problems and treatment needs in children or adolescents. This index, which has established validity and reliability, is widely used in epidemiological studies. It evaluates factors such as tooth crowding, missing teeth, and misaligned tooth positions (Mandall et al., 2000; Marya 2012). This index consists of two components: dental health and aesthetics (Table 7).

Table 7. Index of Orthodontic Treatment Need	
Dental Health Component (5 grades from none to very great)	
Grade 1 None	Extremely minor malocclusions including displacements < 1 mm.
Grade 2 Little	• Increased overjet 3.6 to 6.0 mm, with competent lips • Reverse overjet 0.1 to 1.0 mm • Anterior to posterior cross bite with up to 1 mm discrepancy between retruded contact position and intercuspal position • Displacement of teeth 1.1 to 2.0 mm • Anterior or posterior openbite 1.1 to 2.0 mm • Increased overbite 3.5 mm, without gingival contact • Prenormal or postnormal occlusions with no other anomalies. Includes up to half a unit discrepancy.
Grade 3 Moderate (Borderline need)	• Increased overjet 3.6 to 6.0 mm, with incompetent lips • Reverse overjet 1.1 to 3.5 mm • Anterior or posterior cross bites with 1.1 to 2.0 mm discrepancy. • Displacement of teeth 2.1 to 4.0 mm • Lateral or anterior cross bite 2.1 to 4.0 mm • Increased and complete overbite without gingival trauma.

Grade 4 Treatment Required	• Increased overjet 6.1 to 9.0 mm • Reversed overjet > 3.5 mm with no masticatory or speech difficulties • Anterior or posterior cross bites with > 2 mm discrepancy between retruded contact position and intercuspal position • Severe displacement of teeth, > 4 mm • Extreme lateral or anterior openbites, > 4 mm • Increased and complete overbite with gingival or palatal trauma • Less extensive hypodontia requiring prerestorative orthodontic space closure to obivate the need for a prosthesis • Posterior lingual crossbite with no functional occlusal contact in one or both buccal segments • Reverse overjet 1.1 to 3.5 mm with recorded masticatory and speech difficulties • Partially erupted teeth, tipped and impacted against adjacent teeth • Supplemental teeth.
Grade 5 Treatment Required	• Increased overjet > 9 mm • Extensive hypodontia with restorative implications (more than 1 tooth missing in any quadrant) requiring prerestorative orthodontics • Impeded eruptions of teeth (with the exception of the third molars) due to crowding, displacement, the presence of supernumerary teeth, retained deciduous teeth, and any pathological cause • Reverse overjet > 3.5 mm with reported masticatory and speech difficulties • Defects of cleft lip and palate • Submerged deciduous teeth.
Aesthetic Component (attractiveness of the patient's labial aspect ranked from 1 (close to normal) to 10.)	
A patient's score is based on matching his or her dental appearance with one of a series of 10 photographs showing the labial aspect of different Class I or Class II malocclusions ranked according to their attractiveness.	

Conclusion

In this section, the importance of oral screenings for children and adolescents, the critical ages at which these screenings should be particularly emphasized, and the vital role these screenings play in the overall health of children and adolescents have been highlighted. Early diagnosis, timely initiation of treatment, and the maintenance of a healthy oral environment are of crucial importance. Oral screenings for children have a significant impact on establishing and maintaining oral health habits. Children who learn oral hygiene habits at an early age tend to maintain these habits as they grow older, which helps them achieve lifelong oral health.

However, more effort is needed to enhance the effectiveness of oral screenings. It is important to emphasize oral health more among healthcare professionals and parents and to encourage regular screenings. Additionally, increasing education and resources is necessary to make oral screenings more effective in early diagnosis.

In the future, it is necessary to further develop and expand oral screenings for children and adolescents. Technological advancements provide opportunities to improve screening methods and develop more precise diagnostic techniques. Moreover, a greater focus on oral health in health policies and programs can contribute to the oral health of children.

In conclusion, oral screenings for children and adolescents are crucial for a healthy future. Increasing the effectiveness of these screenings and striving to ensure that children and adolescents adopt lifelong healthy oral hygiene habits are critical steps toward improving public health.

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Chapter

15

Screening of Fetal Aneuploidies Using Fetal Cell-Free DNA

Nevin SAĞSÖZ, Zerrin ORBAK

In the 1970's, only two screening tests were used; one was measuring the mother's serum alpha fetoprotein (MSAFP) level to detect open neural tube defects (NTD), and the other was maternal age over 35 as the cut-off point to detect Down syndrome (Gilner et al., 2021). Today, with the combined use of biochemical markers and ultrasound in the first trimester, the detection rate of Down syndrome is 85% with a false positive rate of 5% (Gilner et al., 2021; Palomaki G. E, Messerlian G. M, 2022). However, with new genomic developments and fetal imaging methods, prenatal diagnosis has become more important. Nowadays, all pregnant women need to be offered prenatal screening and diagnosis opportunities to detect genetic anomalies and birth defects (Gilner et al., 2021).

Fetal cell-free DNA (cf-DNA) screening, which is a non-invasive method and called NIPT (Non-Invasive Prenatal Diagnosis), is the screening method that best detects fetal aneuploidy. It does. It was first described in 1997 and entered clinical use in 2011 (Gilner et al., 2021; Lo et al., 1997).

Cell-free DNA origins and fetal fraction: In fact, free DNA particles in the maternal circulation come from both the mother and the fetal-placental unit and disappear from the maternal circulation immediately after birth. While maternal DNA fragments

originate mostly from hematopoietic cells, fetal sources arise mostly from the apoptosis of placental syncytiotrophoblasts and, to a lesser extent, from the apoptosis of fetal erythroblasts (Lo et al., 1997; Lui et al., 2002; Sekizawa et al., 2000; Tjoa et al., 2006).

Fetal DNA fragments are shorter than maternal DNA fragments and thus discrimination is possible on examination (Chan et al., 2004).

For the test to give accurate results, the fetal fraction must be at least 4%. Otherwise, screening test results will not be reliable and will be reported as "no call" or "no result". Additionally, false negative results are also high in patients with low fetal fraction. Fetal fraction is inversely related to maternal body mass index, and fetal fraction may be low in extremely obese patients (8,9). On the other hand, since fetal DNA fraction rates increase as pregnancy progresses, it is appropriate to perform cf-DNA screening after the 9th and 10th gestational age. (Norton et al., 2012). Additionally, low fetal DNA fraction rates may occur in various situations. These are shown in Table 1.

Table 1. Causes in which the fetal DNA fraction decreases (Palomaki G. E, Messerlian G. M, 2022)

Causes
Early gestational age (It is appropriate to take blood after the 10th week of gestational age).
Suboptimal blood sample collection
Maternal obesity
Fetal karyotype
Maternal use of low molecular weight heparin
Conception by in vitro fertilization
Twin gestation

Performance of the cf-DNA in screened aneuploidies

The cf-DNA testing is often used to screen for trisomy 21, trisomy 18, trisomy 13, and sex chromosome trisomies (47,XXX; 47,XXY; and 47,XYY) and monosomy X (Turner syndrome) (Gilner et al., 2021). Screening success for these diseases is quite good, with the highest being in especially trisomy 21. In trisomy 21, detection rate (DR) is 99.5 with 0.05% false positive rate (FPR). Although, DR and FPR in trisomies do not differ in high-risk groups than general population, the positive predictive value (PPV) of the test is higher in the high-risk group than in the general population (95% vs 85%) (Table 2), (Gil et al., 2015; Iwarsson et al., 2017; Mackie et al., 2017; Palomaki G. E, Messerlian G. M, 2022; Taylor-Phillips et al., 2016).

As a result, CF-DNA is currently the most sensitive and specific test to screen for common trisomies. Therefore, ACOG (American College of Obstetricians and Gynecologists) recommends that all prenatal genetic screening and testing options should

be discussed and offered to all patients. After information and discussion, the patient has the right to undergo or refuse prenatal screening and diagnostic tests ("Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin, Number 226.," 2020).

Other possible applications of cf-DNA include fetal Rh determination, screening for microdeletions and other single gene diseases (Palomaki G. E, Messerlian G. M, 2022). However, routine screening is not recommended by various associations because there is limited data on the accuracy and positive and negative predictive values of cf-DNA screening in the general population in microdeletions and single gene diseases (Palomaki G. E, Messerlian G. M, 2022; "Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin, Number 226.," 2020). Nevertheless, American College of Medical Genetics and Genomics (ACMG) recommends screening with cf-DNA only for the 22q11.2 deletion syndrome, the most common microdeletion syndrome (Dungan et al., 2023).

One of the most important problems in using cf-DNA is cost (Palomaki G. E, Messerlian G. M, 2022).

Table 2. Performance of Cell-Free DNA Screening in Fetal Aneuploidies (Gil et al., 2015; Iwarsson et al., 2017; Mackie et al., 2017; Palomaki G. E, Messerlian G. M, 2022; Taylor-Phillips et al., 2016)

Test	Aneuploidies		Prevalence 1: N	DR %	FPR %	PPV % (95% CI)	NPV %	Odds:1 True positive/ False positive
Combined T (NT,PAPP-A, free β-hCG)	Trisomy 21		340	85	5	5	99.8	
cf-DNA	Trisomy 21		340	99.5	0.05	85	>99.9	5.8:1
	Trisomy 18		1130	97.5	0.04	68	>99.9	2.1:1
	Trisomy 13		3500	95	0.06	32	>99.9	1.0:2.1
	All Trisomy			98.5	0.15	73		2.7:1.0
	Trisomy 21 (High Risk)		100	99.5	0.05	95	>99.9	20:1
cf-DNA (Sex chromosome Aneuploidies) Total prevalence 1/400	45 X0			90.3	0.23	24.6		
	Sex chromosome trisomies	(47,XXX		93	0.14	61.6 (37.6 - 95.4)		
		47,XXY				100 (76.5 - 100)		
		47,XYY						

PAPP-A: Pregnancy-associated plasma protein A, NT: nuchal translucency

Cell-free DNA methodologies used:

Various methods for cf-DNA screening have been validated by different laboratories. Different techniques may provide advantages or disadvantages in different patients (Dugoff & Wapner, 2024; Palomaki G. E, Messerlian G. M, 2022):

1. **Whole genome sequencing:** The first technique reported was massively parallel sequencing, in which all extracted DNA was sequenced. The technique is also known as Shotgun sequencing. After the extracted chromosomes are sequenced according to their characteristics, the amount of each chromosome in normal non-pregnant patients is taken as a criterion and aneuploidies are tried to be determined according to the increase in the amount of DNA belonging to that chromosome (Dugoff & Wapner, 2024; Palomaki G. E, Messerlian G. M, 2022).
2. **Targeted techniques:** In these techniques, studies are focused on the chromosomes of interest. Therefore, the cost is lower than whole genome sequencing (Dugoff & Wapner, 2024).
   - **Single nucleotide polymorphisms (SNPs):** It is a method based on reading tens of thousands of highly polymorphic SNPs located on the chromosomes of interest. Since this method is based on SNP polymorphism, it provides advantages in detecting zygosity, vanishing dizygotic twins and aneuploidy in twin pregnancies. This method cannot be applied in pregnancies where egg donation has been made, in pregnancies with bone marrow or organ transplantation, and in carrier pregnancies. The amount of fetal fraction is important (Dugoff & Wapner, 2024; Palomaki G. E, Messerlian G. M, 2022).
   - **Microarrays**
   - **Rolling circle amplification (RCA):** RCA-based screening methods are equivalent to those reported for other methods and may provide a lower test failure rate after repeated testing (Palomaki et al., 2023).

Interpreting and discussing test results:

Posttest genetic counseling should be provided in addition to pretest counseling.

1. **Screen positive:** Although the test has high sensitivity and low false positive rates, it is not yet a diagnostic test and if the test result is positive, an invasive procedure (cordocentesis or amniocentesis) must be recommended for diagnosis ("Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin, Number 226.," 2020). Performing traditional G-banded karyotyping in invasive tests is especially important in these patients. Situations in which the test may be false positive are shown in Table 3 (Palomaki G. E, Messerlian G. M, 2022).

2. **Screen negative:** Patients who test negative should be informed that there may still be residual aneuploidy. Invasive testing is generally not recommended for this group of patients. However, if a structural anomaly is detected on ultrasound, it would be appropriate to recommend invasive testing ("Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin, Number 226.," 2020).
3. **No call or no result:** This condition may be present in approximately 1 to 5% of patients who undergo cf-DNA screening test. Management of this can be done in 3 ways (Palomaki G. E, Messerlian G. M, 2022)
   - Repeat testing 7 days or more after initial test
   - If the standard combined test and ultrasound have not been performed, it may be recommended to do these, but information about the screening success of the test should be given here.
   - Invasive tests and diagnostic tests may be recommended.

Since the risk of aneuploidy may increase especially in this group, it is important to provide the patient with detailed information and genetic counseling on this issue ("Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin, Number 226.," 2020)

Table 3. Situations where cf-DNA is false positive and false negative (Palomaki G. E, Messerlian G. M, 2022).

False positive cf-DNA	False-negative cf-DNA
Confined placental mosaicism	Confined placental mosaicism
Maternal mosaicism	Maternal copy number variants
Demised twin	Borderline low fetal fraction
Technical issues	Technical issues
Recent blood transfusion	
Maternal cancer	
Transplant recipient	
Maternal copy number variant	
Chance	

As a result, all pregnant women should be informed about prenatal screening for fetal aneuploidies and recommendations for screening and diagnostic tests should be offered. Fetal cf-DNA is the most powerful screening test for the detection of prenatal aneuploidies today. It will be used more widely as costs decrease.

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Chapter

16

Screening Cytomegalovirus Infection during Pregnancy

Nevin SAĞSÖZ

Cytomegalovirus (CMV), which is widespread throughout the world, belongs to the herpes virus family and has similar latency and reactivation properties. Primary infection occurs after the initial encounter in patients who have not previously been infected with CMV, while non-primary infection may occur through reactivation of latent infection or reinfection with new strains in patients who have been previously infected (Megli & Beigi, 2023). CMV is transmitted through contact with infected body fluids and typically manifests with asymptomatic or mild flu-like symptoms during pregnancy. Transmission of the virus to the fetus usually occurs transplacental and leads congenital CMV (cCMV) infection. Congenital CMV infection incidence varies between 0.2% and 1% in the population (Megli & Beigi, 2023; Wen & Wang, 2007).

While 20-50% of primary infections are transmitted to the fetus, only 0.5-1% of non-primary infections are transmitted. If the fetus is infected, particularly in the first trimester, serious developmental problems may occur. Overall, 85-90 % of congenital CMV infections are asymptomatic, while 5-15% are symptomatic. Of the symptomatic cases, 10-30% may die in the perinatal period due to severe infection, and 80% of survivors may experience significant morbidity. Long-term neurological problems, such as hearing loss and chorionic retinitis, may develop in 10-20% of asymptomatic cases (Megli & Beigi, 2023; Wen & Wang, 2007).

Although congenital CMV can lead to serious morbidity and mortality, most major associations do not recommend universal screening due to the lack of an ideal immunization system and treatment options (Xie et al., 2023).

Screening Cytomegalovirus during Pregnancy

A. Disadvantages of CMV screening during pregnancy

Reasons against universal screening include the following (Boppana SB & Wilkins-Haug L, 2024).

1. No vaccine is available for seronegative individuals.
2. Seropositive patients are at risk of reactivation, potentially causing fetal disease.
3. Even if fetal infection is detected, predicting the outcomes is difficult.
4. Routine screening may cause parental anxiety and unnecessary pregnancy terminations.

B. Advantages of CMV screening during pregnancy

Screening makes it possible to diagnose and treat CMV in the fetus, but opinions are unclear about which groups should be screened. Screening can be recommended in different groups (Walker et al., 2013).

1. **Universal scanning:** Universal scanning offers two important advantages.
   a. Identifying seronegative women and recommending them methods of protection against virus transmission,
   b. Identifying seropositive women and ensuring that they are not at risk of primary infection
2. **Screening high-risk women:** Those who come into contact with nursery children or provide day care are at especially high risk for CMV infection.
3. **'Once off' Serology**: At 20 weeks of gestational age, the patient is scanned once and managed. Thus, it is aimed to determine whether there is a primary infection in early pregnancy.
4. **Targeted scanning:** Screening is recommended in cases where the mother has a flu-like infection or if there are findings on ultrasonographic imaging of the fetus that suggest CMV. In fact, currently this type of scanning is especially recommended (Boucoiran et al., 2021; Megli & Beigi, 2023; Walker et al., 2013; Xie et al., 2023). Fetal ultrasonographic markers suggestive of cCMV are listed Table 1 (Boppana SB & Wilkins-Haug L, 2024; Boucoiran et al., 2021).

Table 1. Fetal ultrasonographic markers suggestive of cCMV (Boppana SB & Wilkins-Haug L, 2024; Boucoiran et al., 2021)	
Fetal Cranium	Periventricular calcifications Cerebral ventriculomegaly Microcephaly Pseudocysts, periventricular or adjacent to the occipital or temporal horn Polymicrogyria Cerebellar hypoplasia Large cisterna magna Porencephaly Lissencephaly
Fetal thorax	Pleural effusion Pericardial effusion Cardiomegaly Cardiac calcifications
Feta Abdomen	Hyperechogenic fetal bowel Ascites Hepatosplenomegaly Hepatic calcifications
Other	Fetal growth restriction Hydrops Death Polyhydramnios/ oligohydramnios Placental thickening and enlargement, heterogeneous appearance, calcifications

Maternal Infection- Diagnosis

The gold standard in diagnosis is that a pregnant woman who is immunoglobulin G negative, that is, seronegative, becomes seropositive (Boucoiran et al., 2021). However, this is not always possible, in which case the CMV IgM, CMV IgG and CMV IgG avidity test can be used in combination. CMV IgM positivity usually lasts for 1-3 months, but may persist for up to 12-18 months (Boucoiran et al., 2021). Therefore, in cases where CMV IgM is high, the test should be interpreted carefully together with CMV IgG levels. If CMV IgG is negative, primary infection or false positivity is considered. If CMV IgG is positive, avidity should be checked. Avidity determines how tightly CMV IgG binds to CMV antigen. Avidity is low in the early stages of the disease, however increases over time. Therefore, high avidity indicates that the disease was experienced 5-6 months ago. High IgG avidity indicates infection before 6 months or reinfection. The Avidity test should be checked in the presence of IgM and IgG positivity (Boucoiran et al., 2021; Megli & Beigi, 2023).

Additionally, a 4-fold or greater increase in IgG within 2 weeks is usually consistent with a recent acute infection (Megli & Beigi, 2023).

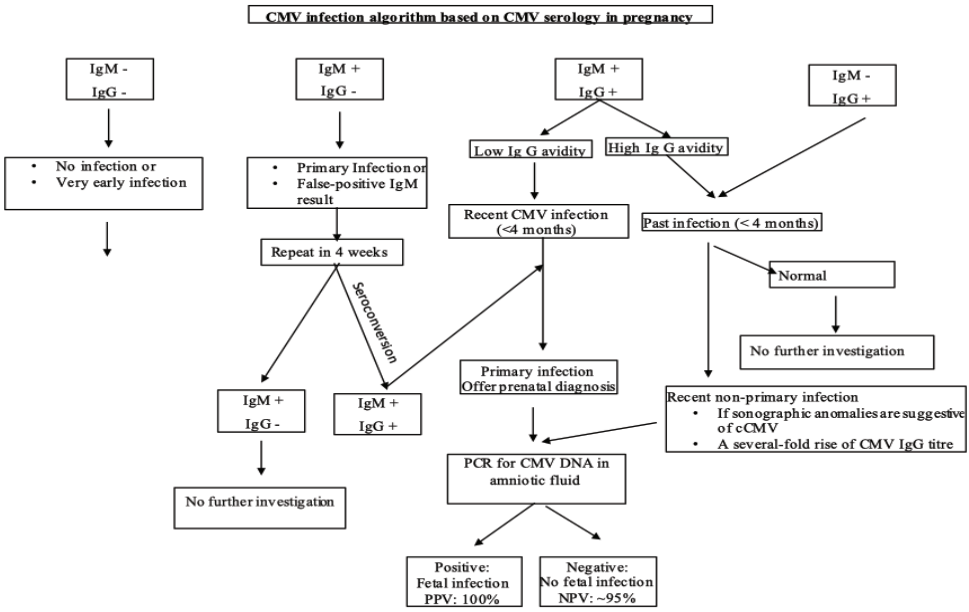


Figure 1. Evaluation according to serology (Boucoiran et al., 2021; Lazzarotto et al., 2011; Megli & Beigi, 2023).

Evaluation according to serology is summarized in in Figure 1 (Boucoiran et al., 2021; Lazzarotto et al., 2011; Megli & Beigi, 2023)

Fetal Infection: Diagnosis and treatment

The gold standard for diagnosing congenital CMV infection is a positive result of CMV PCR testing in amniotic fluid. It is recommended to be performed after the 21st week of pregnancy and at least 6-8 weeks after the mother suspects infection (Boucoiran et al., 2021; Lazzarotto et al., 2011). Congenital CMV can be diagnosed with amniocentesis CMV PCR with 86% (57-98) sensitivity, 100% (91-100) specificity, 100% (74-100) positive predictive value, 95% (83-99) negative predictive value (Dinsmoor et al., 2023).

In recent years, it has been shown that valacyclovir treatment can be effective in fetuses diagnosed with cCMV (Chatzakis et al., 2024; D'Antonio et al., 2023). However, new studies and protocols are needed regarding this regard.

Conclusion

Currently, universal screening is not recommended by most major associations and registries. However, screening of high-risk patients or targeted screening appears appropriate. With ongoing developments in treatments and cost-effectiveness analysis studies, universal screening may be appropriate in the future.

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NEWBORN AND CHILDHOOD

SCREENING PROGRAMMES

Zerrin ORBAK
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Modern medical practices have made great progress in the prevention and management of diseases through early diagnosis and treatment methods. At the center of these developments are newborn and childhood screening programs. These screening programs have been developed in order to contribute to children living a healthy life starting from birth, to identify them at an early stage before signs and symptoms of disease appear, and to make the necessary interventions in a timely manner.

This book in your hands aims to comprehensively cover the most up-to-date information and practices on newborn and childhood screening programs. This work, which has been meticulously prepared by authors who are experts in the field, covers a wide range of topics such as clinical practices, scientific foundations of screening tests, organization of programs and ethical dimensions.

We believe this book will be a valuable resource for pediatricians, family physicians, public health professionals, nurses, and medical students on their professional journeys. It will help health professionals improve their knowledge and skills and contribute to our children having a healthy future. We hope that your interest in newborn and childhood screening programs and your devoted work on this subject will lead to the raising of healthier generations.

With love and respect,

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