Primary adrenal insufficiency

Phillip, Shir

UNIVERSITY OF ZAGREB

SCHOOL OF MEDICINE

Shir Phillip

PRIMARY ADRENAL INSUFFICIENCY

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Abbreviations

ACTH- Adrenocorticotrophin Hormone

AIRE - Autoimmune Regulator

APECED - Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dystrophy

CAH - Congenital Adrenal Hyperplasia

CIRCI - Critical Illness-Related Corticosteroid Insufficiency

CLAH - Congenital Lipoid Adrenal Hyperplasia

CRH- Corticotrophin-Releasing Hormone

DHEA - Dehydroepiandrosterone

DMD- Duchenne Muscular Dystrophy

GKD- Glycerol Kinase Deficiency

HPA- Hypothalamic-Pituitary-Adrenal axis

OTC- Ornithine Transcarbamylase Deficiency

POMC- Proopiomelanocortin

MSH- Melanocyte Stimulating Hormone

WES - Whole-Exome Sequencing

WGS- Whole-Genome Sequencing

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1. Abstract

Primary adrenal insufficiency is a rare condition caused by pathology of the adrenal gland itself, often due to autoimmune adrenalitis or inborn error of steroidogenesis.

The adrenal cortex contains three zones, and each zone produces a different hormone - the zona glomerulosa (aldosterone), the zona fasciculata (cortisol), and the zona reticularis (sex steroids). The renin-angiotensin system regulates the production and synthesis of aldosterone, while cortisol is regulated by the hypothalamic-pituitary-adrenal axis.

Clinical symptoms depend on whether patient has glucocorticoid and/or mineralocorticoid deficiency, or sometimes androgen deficiency or excess. In patients with glucocorticoid deficiency weakness, weight loss, and anorexia are typically found, while patients with mineralocorticoid deficiency present with hyponatremia, hyperkalemia, acidosis, tachycardia, hypotension, and salt craving.

More than 30 different monogenic disorders can cause primary adrenal insufficiency with varying patterns of inheritance. The most common is congenital adrenal hyperplasia due to 21-hydroxylase deficiency.

The signs and symptoms of primary adrenal insufficiency might not be specific. Thus, clinicians have a considerable challenge in diagnosing the disease and should have a high index of suspicion. An electrolyte disbalance can provide a clue for diagnosis, but ACTH stimulating test is the most used diagnostic test for adrenal gland dysfunction.

As a result of a deficiency in glucocorticoids and mineralocorticoids, the patients require long-term replacement therapy and salt intake as needed. Patients with primary adrenal insufficiency also have androgen deficiency, but the benefits of androgen replacement are less clearly defined, and guidelines do not recommend androgen replacement.

2. Sažetak

Primarna adrenalna insuficijencija je rijetka. Uzrokovana je oštećenjem ili poremećajem funkcije nadbubrežne žlijezde, najčešće u sklopu autoimunog adrenalitisa ili poremećaja steroidogeneze.

Kora nadbubrežne žlijezde se sastoji od tri zone od kojih je svaka odgovorna za sintezu različitih hormona, zona glomerulosa (aldosteron), zona fasciculata (kortizol) i zona reticularis (spolni hormoni). Sustav renin-angiotenzin regulira proizvodnju i sintezu aldosterona, dok sintezu kortizola regulira osovina hipotalamus-hipofiza-nadbubrežna žlijezda.

Klinička slika može biti rezultat manjka glukokortikoida i/ili mineralokortikoida, a ponekad i hipo- ili hipersekrecije adrenalnih androgena. Kod manjka glukokortikoida tipično se javljaju slabost, gubitak težine i anoreksija, dok se manjak mineralokortikoida očituje hiponatremijom, hiperkalijemijom, acidozom, tahikardijom, hipotenzijom i žudnjom za soli.

Više od 30 različitih monogenih poremećaja koji se i različito nasljeđuju mogu uzrokovati primarnu adrenalnu insuficijenciju. Najčešća je kongenitalna adrenalna hiperplazija zbog manjka enzima 21-hidroksilaze.

Znakovi i simptomi primarne adrenalne insuficijencije često nisu specifični te zbog čega dijagnoza često predstavlja izazov. Elektrolitski disbalans može pobuditi sumnju na primarnu adrenalnu insuficijenciju. Stimulacijski test ACTH-om je najčešće korišten test u dijagnostici primarne adrenalne insuficijencije.

Zbog manjka glukokortikoida i/ili mineralokortikoida, bolesnicima je potrebna dugotrajna nadomjesna terapija i po potrebi dodatan unos soli. Bolesnici s primarnom adrenalnom insuficijencijom također mogu imati i manjak androgena, no kako prednosti nadomjesne terapije androgenima nisu jasno definirane, smjernice ne preporučuju supstituciju.

3. Introduction

3.1 Adrenal insufficiency

Adrenal insufficiency is a rare, potentially life-threatening condition that can be caused by wide variety of congenital or acquired disorders that interrupt production of adrenocortical hormones. Late diagnosis and treatment of adrenal insufficiency is associated with considerable mortality and morbidity. Adrenal insufficiency is classified into three sub-categories, primary (adrenal), secondary (pituitary), and tertiary (hypothalamus) (1).

Primary adrenal insufficiency occurs less frequently than secondary or tertiary adrenal insufficiency. It is caused by pathology of the adrenal gland itself, often due to autoimmune adrenalitis or inborn errors of steroidogenesis (1).

A secondary adrenal insufficiency occurs due to dysfunction of the pituitary gland, which manifests as a decrease in adrenocorticotrophin hormone (ACTH) release. This condition is commonly due to pituitary tumors, pituitary radiation, or drug-induced pituitary dysfunction (1,2).

Tertiary adrenal insufficiency is characterized by a decreased corticotrophin-releasing hormone (CRH) production from the hypothalamus. Usually, it occurs due to steroid treatment and is a common form of adrenal insufficiency. Unfortunately, this form of adrenal insufficiency can be easily missed due to non-specific signs and symptoms that may mimic or be indistinguishable from the underlying condition (1,2)

This article will review primary adrenal insufficiency in pediatric population.

4. Physiology of the Adrenal gland

The adrenal cortex contains three zones: the zona glomerulosa, the zona fasciculata, and the zona reticularis. The zona glomerulosa is responsible for aldosterone synthesis, the zona fasciculata for cortisol synthesis, and the zona reticularis for sex steroid synthesis (3).

The renin-angiotensin system regulates the production and synthesis of aldosterone, while cortisol and sex steroid synthesis are regulated by the hypothalamic-pituitary-adrenal axis (HPA) (figure 1). These facts explain why patients with central adrenal insufficiency will manifest with glucocorticoid deficiency while mineralocorticoid function will not be affected (4).

Secretion of CRH from the hypothalamus into the hypophyseal-portal venous system occurs in response to light, stress, and other stimulants. CRH binds to melanocortin 2, a specific cell surface receptor. This binding stimulates the release of preformed ACTH and de-novo transcription of the precursor molecule proopiomelanocortin (POMC). ACTH is produced due to the cleavage of POMC by the proprotein convertase 1 (5). Then, ACTH binds to steroidogenic cells found in the zona fasciculata and reticularis. This binding will cause the activation of adrenal steroidogenesis (5).

Moreover, ACTH has a trophic effect on adrenal tissue. Therefore, ACTH deficiency will result in adrenal cortex atrophy and a reduction in the capacity of glucocorticoid secretion. About 75% of circulating cortisol is bound to corticosteroid-binding protein, 15% to albumin, and 10% is free. Cortisol production rate is estimated between 6 and 10 $mg/m^2/day$, but it depends on multiple parameters varying between individuals, for example age, gender, and developmental stage. Glucocorticoids play a significant role in many processes, for instance: regulation of the immune system, affecting the circulatory and renal function, influencing growth and development, energy and bone metabolism, and central nervous system activity (3,4,6)

ACTH is secreted in pulses, and it affects the secretion of cortisol. Cortisol is secreted in a fashion of circadian and ultradian rhythm according to changes in the amplitudes of ACTH (figure 1). ACTH and cortisol are secreted in pulses every 30–120 minutes, and they reach a peak level during waking and decrease throughout the day to the lowest level overnight (3,5,6). This pattern is not constant and may be affected by the presence of serious illness, major surgery, and sleep deprivation. During stress, glucocorticoid secretion can increase up to

10 times the average level to allow survival; it occurs by increasing cardiac output and contractility, catecholamines sensitivity, work capacity of the skeletal muscles, and availability of energy stores (3). The interaction between the hypothalamus, pituitary gland, and adrenal gland is essential to homeostatic plasma cortisol levels. Cortisol decreases the secretion of both CRH from the hypothalamus and ACTH from the corticotrophin cells of the anterior pituitary gland (7). ACTH inhibition occurs at the level of the hypothalamus by a negative-feedback mechanism (figure 1). Androgen production may increase in a case of deficiency of enzymes involved in cortisol biosynthesis (3).

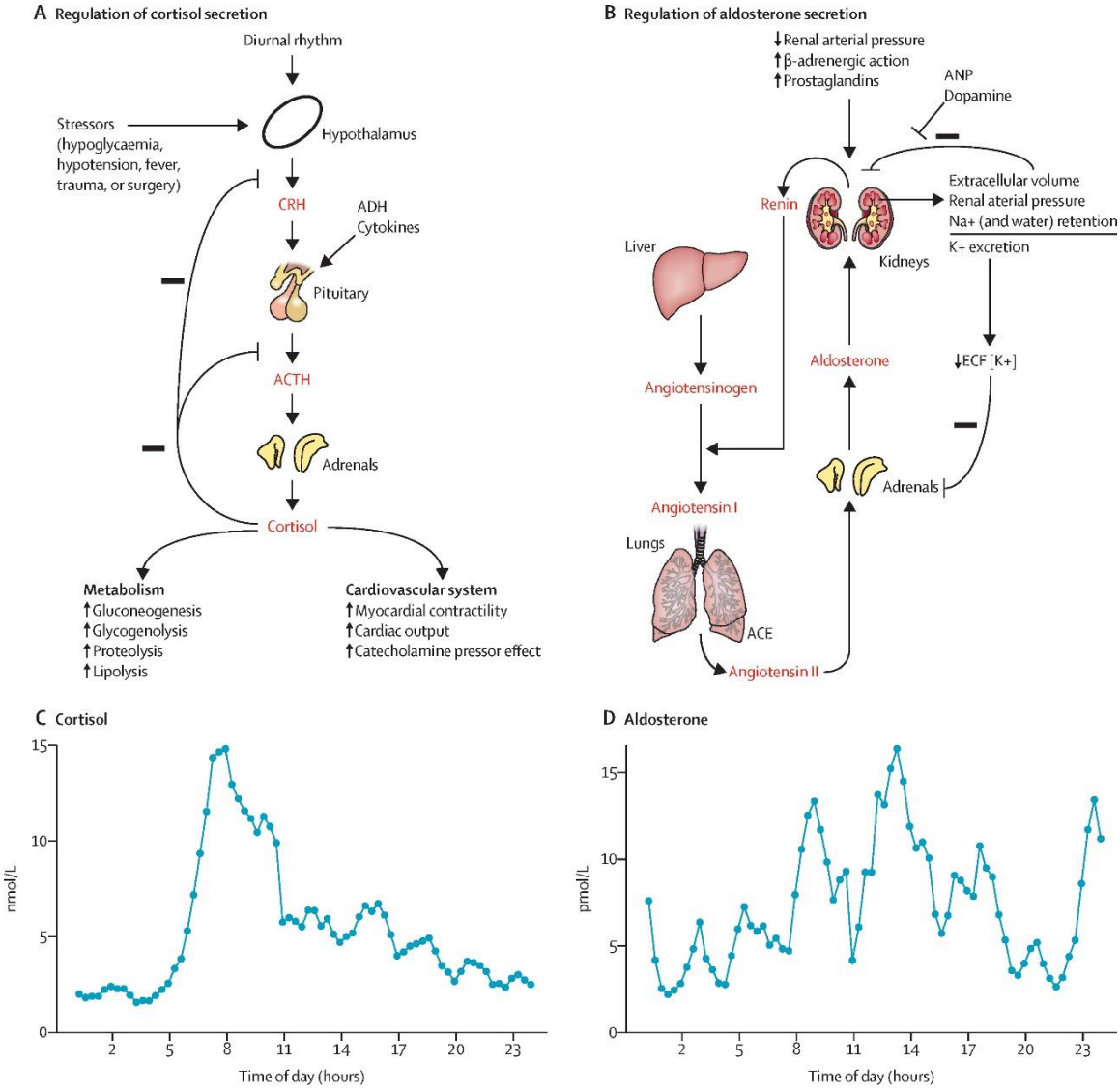

Figure 1 - **Regulation of cortisol and aldosterone secretion** Husebye, Eystein S, Prof, Lancet, The, Volume 397, Issue 10274, 613-629 Copyright © 2021 Elsevier Ltd

5. Primary adrenal insufficiency

5.1 Epidemiology and etiology

Primary adrenal insufficiency is less common than secondary adrenal insufficiency. The prevalence was estimated to be around 40-70 per million in the 1960s, but it increased in the 2000s to about 80-140 per million (1).

The most frequent etiology in adults is autoimmune adrenalitis. The incidence in females appears to be higher than in men, representing around 60% of the cases with a peak incidence between 30-50 years of age (8). Until the beginning of the 20th century, tuberculosis was the most frequent etiology for primary adrenal insufficiency in adults, but after the beginning of 2000, the incidence decreased to only 3%. Several studies have reported a significant reduction in adrenal insufficiency due to tuberculosis. Thus, the increasing prevalence is linked to the increase in the incidence of autoimmune adrenalitis, which may be connected to the rise in life expectancy of a patient diagnosed with adrenal insufficiency (9).

On the other hand, the incidence in children is 1:10,000 to 1:18,000. The etiology is substantially different in pediatrics population than adults, with a high predominance of genetic causes and congenital adrenal hyperplasia (CAH) being the most common cause. CAH comprises a group of autosomal recessive disorders caused by deficiency of one of several enzymes involved in cortisol synthesis in the adrenal cortex (figure 2) (8).

In a Canadian study that examine the etiology in 102 children (age 0-18), in 94% of the patients, the etiology for primary adrenal insufficiency could be found. Two thirds of patients had classical form of CAH due to 21-hydroxylase deficiency, 25% had autoimmune adrenalitis, and the rest were identified having different genetic diseases (10).

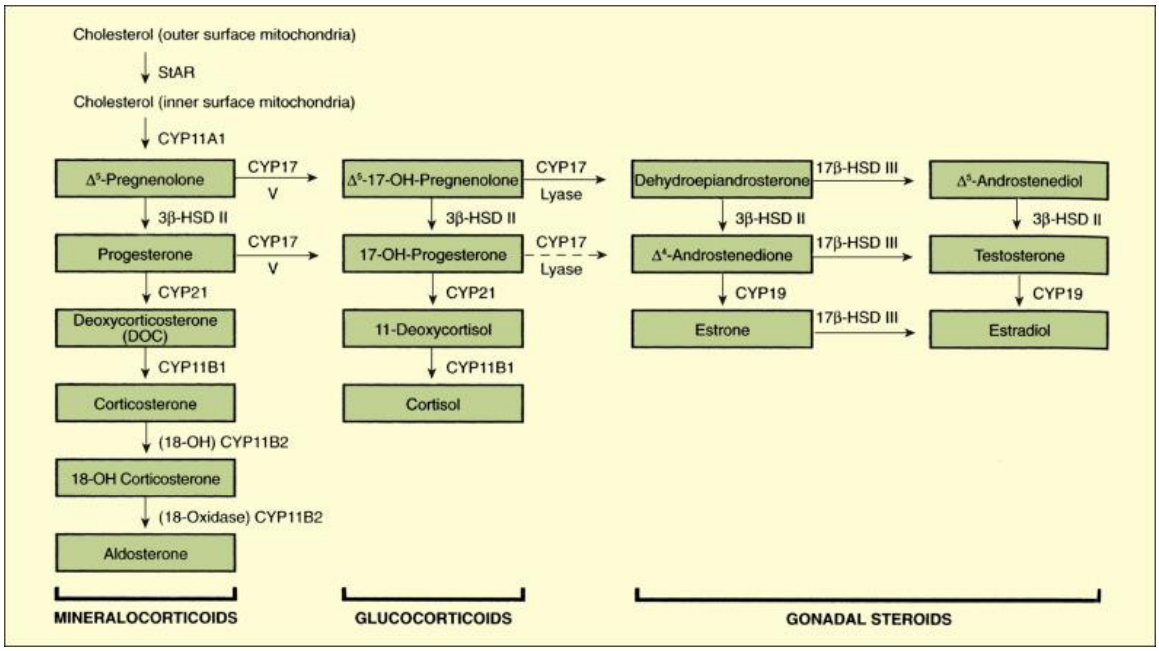

Figure 2 - steroidogenesis Anxiety Disorders, Marcdante, Karen J., MD, Nelson Essentials of Pediatrics, Chapter 17, 61-64 Copyright © 2019

5.2 Clinical manifestations

Adrenal insufficiency can be easily missed due to non-specific symptoms and mimic other life-threatening conditions. In the beginning, children can accidentally be diagnosed with sepsis, metabolic disorders, and cardiovascular disease, emphasizing the importance of considering adrenal dysfunction as a differential diagnosis (11). Depending on the etiology clinical course of adrenal insufficiency can as well be gradual and chronic or manifest as an acute adrenal crisis (5).

Clinical symptoms (Table 1) depend on underlying cause of primary adrenal insufficiency and can present with glucocorticoid and/or mineralocorticoid deficiency and sometimes adrenal androgen deficiency or excess. When glucocorticoids are deficient- weakness, weight loss, and anorexia are predominant. Severe hypoglycemia with average/low insulin levels is very common (5). When mineralocorticoids are deficient, the patient presents with hyponatremia, hyperkalemia, acidosis, tachycardia, hypotension, and salt craving. The negative feedback of glucocorticoid does not work in this situation; therefore, the ACTH level is elevated. High levels of ACTH and POMC peptides, including melanocyte stimulating hormone (MSH), result in over secretion of

melanin, stimulating hyperpigmentation of the mucosal and cutaneous surfaces. Hyperpigmentation can be a leader in diagnosing primary adrenal insufficiency since the other symptoms are non-specific. Nevertheless, we should bear in mind the difference in ethnicity, which affects the patient's skin color (12). In the case of autoimmune primary adrenal insufficiency, vitiligo may be present (5).

Clinical manifestations in CAH patients depend on the concentrations of intermediary and final products of adrenal steroid synthesis, which can result in a variety of phenotypes, such as virilization in females but also insufficient masculinization in males, hypoglycemia, renal salt wasting and hypotension, as well as the opposite, hypernatremia, and hypertension. Each of the aforementioned symptoms may appear alone or in various combinations (5).

In neonates, the classic symptoms of primary adrenal insufficiency include failure to thrive, and hypoglycemia associated with seizures. This condition can be tricky and misdiagnosed, resulting in neonatal death. In newborns, lack of cortisol will prolong cholestatic jaundice with elevated liver enzymes due to delayed synthesis of bile acids and transport maturation (13). In the classic CAH, the lack of mineralocorticoid presents as an adrenal crisis at 10-20 days of life. Female neonates present with atypical genitalia, with enlargement of the clitoris, a fusion of the labia, and urogenital sinus. Male neonates will not present any atypical genitalia, a part of hyperpigmentation of the scrotum and enlargement of the penis (8,14,15).

Children and adolescents that suffer from autoimmune primary adrenal insufficiency mainly develop a chronic adrenal insufficiency, which slowly progresses to an acute adrenal crisis in months to years. Initially, the patient can present with various symptoms like losing appetite, anorexia, nausea, abdominal pain, weight loss, fatigue, headache, generalized weakness, muscle and joint pain. As a result of salt wasting, blood pressure decreases, and orthostatic hypotension will present. In addition, the patient may complain about salt cravings (5).

Table 1- Features of Isolated Adrenal Insufficiency in Pediatric Population

	Newborn Infants	**Children and Adolescents**
Symptoms	Failure to thrive Vomits Seizures	Failure to thrive/weight loss Nausea/vomits, abdominal pain Anorexia Frequent infections Fatigue and lack of energy Headache, dizziness Myalgia, arthralgia
Physical examination	Prolonged jaundice Hypotension Tachycardia Dehydration	Hyperpigmentation (chronic high ACTH levels) Hypotension Tachycardia Dehydration
Baseline biochemistry	Hypoglycemia Hyponatremia Hyperkalemia (primary adrenal insufficiency only)	Hypoglycemia Hyponatremia Hyperkalemia Anemia Lymphocytosis Eosinophilia

5.1 Genetic causes of primary adrenal insufficiency

During the last two decades, significant progress has been made in understanding the genetic causes of primary adrenal insufficiency, especially for children and young adults. More than 30 different monogenic disorders can cause primary adrenal insufficiency with varying patterns of inheritance (16).

5.1.1 Congenital adrenal hyperplasia

The CAH comprise a group of autosomal recessive disorders caused by mutations of the genes coding for enzymes responsible for cortisol biosynthesis in the adrenal cortex. Decreased enzyme activity leads to impaired cortisol production, subsequent chronic adrenal cortex overstimulation and accumulation of precursors proximal to the blocked enzymatic step (13). The most common form, found in more than 95% patients with CAH is 21-hydroxylase deficiency due to mutations in the *CYP21A2* gene. Different mutations of *CYP21A2* gene lead to different residual activity of 21-hydroxylase enzyme and spectrum of phenotypes (17). The classical form is defined by severely reduced or absent 21-hydroxylase activity with impaired cortisol production and manifesting clinically in the neonatal period (18). The salt-wasting and simple virilizing forms are considered to be the classic forms. Impaired cortisol synthesis is found both in salt-wasting and simple virilizing patients. Additionally, salt-wasting type patients who carry the most severe *CYP21A2* gene mutations leading to complete 21-hydroxylase deficiency are unable to produce aldosterone, which results in the development of a life-threatening adrenal crisis (18). Impaired cortisol biosynthesis leads to lack of negative feedback on the HPA and rise in ACTH levels which in turn stimulates adrenal gland, causes hyperplasia, accumulation of the precursors proximal to 21-hydroxylase enzyme and stimulates adrenal androgen production. These androgens cause in utero virilization of the external genitalia in a female fetus with the classical form of CAH. Male newborns with 21-hydroxylase deficiency have normal genitalia, yet, like female infants, they may later show other symptoms of androgen excess (18). Newborn screening worldwide of almost 6.5 million babies has demonstrated an overall incidence of 1:15,000 live births for the classical form of 21-hydroxylase deficiency (17).

Non-classical or so called “late-onset” 21-hydroxylase deficiency is caused by mild mutations of *CYP21A2* gene leading to partial enzyme deficiency and milder

phenotype with postnatal androgen excess. Females with non-classical 21-hydroxylase deficiency do not have genital ambiguity at birth. Both males and females present with signs of androgen excess later in childhood, adolescence, or adulthood (18,19).

Diagnosis of classical 21-hydroxylase deficiency is established upon clinical symptoms and measurement of high levels of progesterone, 17-Hydroxyprogesterone, androstenedione and testosterone. Patients with salt-wasting forms have low levels of aldosterone, hyponatremia, hyperkalemia and increased plasma renin activity. In newborns with classical 21-hydroxylase levels of 17-Hydroxyprogesterone, a substrate of 21-hydroxylase enzyme, as well as adrenal androgen androstenedione are significantly elevated. In milder forms of 21-hydroxylase deficiency, to confirm the hormonal diagnosis ACTH stimulation test is needed. During this test, baseline, and serum concentration of 17-Hydroxyprogesterone and cortisol 60 minutes after intravenous administration of synthetic ACTH (Cortrosyn) are measured. Final confirmation of diagnosis is made by molecular testing of *CYP21A2* gene. Treatment includes substitution of glucocorticoids and if needed mineralocorticoids (14,19)

CAH due to 11-beta hydroxylase deficiency is a second most common cause of CAH that is found in about 3-5% of the patients. It is caused by mutations of *CYP11A2* gene leading to 11-beta hydroxylase enzyme impairment, reduced cortisol biosynthesis, increased ACTH secretion, and overproduction of steroid precursors and adrenal androgens. Phenotype is similar to simple virilizing 21-hydroxylase deficiency, including ambiguous genitalia in newborn females and signs of androgen excess in both males and females (18). Furthermore, these patients have signs of mineralocorticoid excess and develop low renin hypertension due to accumulation of 11-deoxycorticosterone and its metabolites. Treatment is glucocorticoid replacement and sometimes antihypertensives (20)

Human 3 beta-hydroxysteroid dehydrogenase deficiency is a very rare form of CAH caused by *HSD3B2* gene mutations. The 3 beta-hydroxysteroid dehydrogenase is an essential enzyme for the biosynthesis of glucocorticoids and mineralocorticoids and as well sex steroids. Patients with this condition have steroidogenesis impairment in both adrenals and gonads and present as disorders of sexual development in both sexes - genetic females have clitoromegaly and mild virilization while genetic males have insufficient masculinization of external genitalia. Complete enzyme defect causes salt wasting (21)

Congenital lipoid adrenal hyperplasia (CLAH) is the most severe form of CAH and it is caused by mutations in steroidogenic acute regulatory protein, a mitochondrial protein which is essential for movement of cholesterol form outer to inner mitochondrial membrane. Function of steroidogenic acute regulatory protein is crucial for initiation of steroidogenesis and therefore patients with CLAH are usually unable to produce any adrenal steroids. Both males and females present with adrenal crises in infancy. Genetic males can present with female external genitalia at birth (17).

The gene *CYP17A1* codes for two different enzymes 17α-hydroxylase and 17,20-lyase, key branching enzymes in steroidogenesis. The 17α-hydroxylase converts pregnenolone to 17α-hydroxypregnenolone and progesterone to 17α-hydroxyprogesterone, while 17,20-lyase converts 17α-hydroxypregnenolone to Dehydroepiandrosterone (DHEA). Patients with mutations of *CYP17A1* gene have impaired glucocorticoid and sex steroid production. Rise of ACTH will cause overproduction of deoxycorticosterone leading to low renin hypertension. The classic presentation of children with complete forms of combined 17α-hydroxylase/17,20-lyase deficiency is a phenotypic girl (either 46, XX or 46, XY) who has absent puberty and who is found to be hypertensive, hypernatremic, hypokalemic with suppressed plasma renin activity (22).

5.1.2 Adrenal hypoplasia

Adrenal hypoplasia often presents with primary adrenal insufficiency early in life. Usually transmitted as an X-linked condition, or less commonly can be associated with intrauterine growth restriction (16).

X-linked congenital adrenal hypoplasia mainly affects boys and disrupts the nuclear receptor, DAX-1, encoded by the *NR0B1* gene (23). It manifests as salt-wasting primary adrenal insufficiency during the first two months of life (24). The main features of X-linked congenital adrenal hypoplasia are- primary adrenal insufficiency, hypogonadotropic hypogonadism in adolescence, and impaired spermatogenesis (25). Pathogenic missense or loss-of-function (stop gain, frameshift) mutations in DAX-1/NR0B1 were found in around two-thirds of patients with X-linked congenital adrenal hypoplasia, localized deletion of this gene on the X-chromosome (Xp21) was found in one-sixth, and larger Xp contiguous gene deletion syndrome was found in another one-sixth of the patients. This syndrome can involve genes causing glycerol kinase deficiency

(GKD), ornithine transcarbamylase deficiency (OTC), and Duchenne Muscular Dystrophy (DMD) (26). Seldom, skewed x-inactivation can cause X-linked congenital adrenal hypoplasia in girls (27). Early diagnosis and recognition of the disease can help manage primary adrenal insufficiency and associated condition (16).

Primary adrenal insufficiency due to adrenal hypoplasia can be one of the manifestations of IMAGe Syndrome, intrauterine growth restriction, metaphyseal dysplasia, and genitourinary anomalies (28,29). Children with this syndrome usually present with salt-wasting primary adrenal insufficiency early in life. Other characteristic features can be frontal bossing, hearing loss, and glucose intolerance (29). The classic form of this syndrome is caused by a gain of function mutation in Cyclin-Dependent Kinase Inhibitor 1C, a cell cycle repressor (28). This mutation is caused by paternal imprinting or a de novo mutation. "IMAGe-like" syndrome was recently discovered instead of the classic syndrome; this syndrome also includes immunodeficiency and adrenal insufficiency. Clinical manifestation has distinctive facial features, hip dysplasia, postnatal growth restriction, and hypoplastic patella. This condition is caused by a mutation in polymerase epsilon-1, a DNA polymerase that interacts with Proliferating cell nuclear antigen during S-phase in DNA replication (30).

MIRAGE syndrome is another syndrome that causes adrenal hypoplasia associated with multisystemic growth restriction. This syndrome includes myelodysplasia, infections, growth restriction, adrenal hypoplasia, genital phenotypes, and enteropathy (31,32). Sever form of the syndrome appears in premature infants with salt-wasting primary adrenal insufficiency early in life. Patients with this syndrome frequently present with recurrent viral, bacterial, and fungal infections, esophageal reflux, aspiration, severe nephropathy, and anemia. 46,XY children have hypospadias or testicular dysfunction, and 46,XX children have gonadal dysfunction (16). MIRAGE syndrome has a high mortality rate, and those children that survive present with growth restrictions. MIRAGE syndrome results from heterozygous gain of function missense mutations in the growth repressor, sterile alpha domain containing 9 (31,32). This mutation usually occurs as a de-novo mutation, leading to cellular growth restriction and division (16).

5.1.3 ACTH Resistance-Like Conditions

Familial Glucocorticoid Deficiency Type 1 is an autosomal recessive condition in which there is a mutation in the ACTH receptor (melanocortin 2 receptor) (33,34). During the first weeks of life, children present with signs of cortisol insufficiency and severe hyperpigmentation. This condition can present with hyperpigmentation, lethargy, and recurrent infections later in childhood. Usually, patients respond to glucocorticoid replacement, but suppression of ACTH is complex (33).

Familial Glucocorticoid Deficiency Type 2 is also an autosomal recessive condition that occurs due to mutation in the gene encoding for melanocortin 2 receptor accessory protein (35). Melanocortin 2 receptor accessory protein traffics melanocortin 2 receptor to the cell membrane of the adrenal gland; thus, any disruption in its function will result in impairment of ACTH signaling (36,37). Children with this condition present with severe glucocorticoid insufficiency and hyperpigmentation during the first few weeks of life (36).

A defect in nicotinamide nucleotide transhydrogenase can cause an isolated form of primary adrenal insufficiency in children. An additional feature is an early puberty (34,38). Usually, a patient presents after one year of life, but it can also occur as early as four months (38).

Triple-A syndrome, also known as Allgrove syndrome, is a rare disease with the autosomal recessive transmission. The condition is multisystemic and clinical presentation includes alacrima, which is challenging to diagnose, achalasia of the esophagus, and primary adrenal insufficiency (39,40). Additionally, progressive neurological and autonomic dysfunction may also occur. The syndrome is caused by a pathogenic mutation in the *AAAS* gene, leading to the disruption of the Aladin protein (41).

5.1.4 Metabolic Conditions

Smith-Lemli-Opitz syndrome is a defect in cholesterol biosynthesis due to disruption of the enzyme 7-dehydrocholesterol reductase (42). Typical findings in infancy are microcephaly, cleft palate, syndactyly of the second and third toes, post-axial polydactyly, congenital heart defects, gastrointestinal disorders,

atypical genitalia, cryptorchidism, and characteristic facial features (42). A high level of 7-dehydrocholesterol and genetic testing can confirm the diagnosis.

Sphingosine-1-Phosphate Lyase deficiency is a newly described condition associated with primary adrenal insufficiency. It occurs due to the impairment breakdown of sphingosine 1-phosphate, which accumulates sphingolipids and ceramides (43–45). Clinical features of the condition are- primary adrenal insufficiency that may present with adrenal calcification, steroid-resistant nephrotic syndrome, neurologic dysfunction, ichthyosis, primary hypothyroidism, lymphopenia, and cryptorchidism (44,45).

A significant cause of primary adrenal insufficiency associated with progressive neurological symptoms is adrenoleukodystrophy. Adrenoleukodystrophy is a rare disorder. The X-linked form of the disease occurs due to mutation in the *ABCD1* gene. This mutation leads to the accumulation of very-long-chain fatty acids. These fatty acids accumulate in the adrenal cortex, the brain's white matter, spinal cord, and testes. The accumulation results in adrenal insufficiency that can present with or without aldosterone deficiency (46). Due to the disease progression, it is recommended that all boys with primary adrenal insufficiency with unknown etiology will undergo long-chain fatty acids measurement (47).

A fatal lysosomal disease known as Wolman disease occurs due to the disruption of lysosomal acid lipase. The condition is associated with adrenal insufficiency, usually presenting with adrenal calcification (16). Patients present with failure to thrive, anemia, and hepatosplenomegaly during the first few months of life (16).

5.1.5 Autoimmune Conditions

Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED), also known as autoimmune polyglandular syndrome type 1 , is a rare monogenetic autosomal recessive disorder caused by a mutation in the autoimmune regulator (*AIRE*) gene. The first manifestation of APECED is most commonly mucocutaneous candidiasis. The most common autoimmune endocrine involvement is hypoparathyroidism that develops in first decade, while the second most common is primary adrenal insufficiency that usually occurs after hypoparathyroidism. Some patients develop type 1 diabetes mellitus, autoimmune thyroid disease, and primary ovarian failure. Other non-endocrine autoimmune manifestations such as intestinal malabsorption, atrophic gastritis,

autoimmune hepatitis, alopecia, vitiligo, ungual dystrophy, keratoconjunctivitis and arthritis are commonly found. There is high phenotypic variability even within the same families (48, 49). Autoimmune polyglandular syndrome type 2 is rare in children and includes Addison's' disease, type 1 diabetes mellitus and autoimmune thyroid disease (48).

5.2 Diagnosis

The signs and symptoms of primary adrenal insufficiency might not be specific. Thus, clinicians have a considerable challenge in diagnosing the disease and should have a high index of suspicion before the diagnosis procedure (49).

An electrolyte disbalance can provide a clue for diagnosis; for example, a low sodium level in the serum and a high level of potassium are common manifestations in patients with primary adrenal insufficiency (49). On the other hand, a patient who suffered from protracted vomiting can present with a low serum sodium level but without a high potassium level (49). Hyponatremia is common in primary adrenal insufficiency due to aldosterone deficiency, and hypoglycemia may be another symptom of primary adrenal insufficiency. Thus, hypoglycemic investigation needs to include laboratory testing of serum cortisol levels during hypoglycemia. Hypoglycemia can be manifested with ketosis due to impairment of gluconeogenesis and hepatic glycogenesis in a patient with adrenal insufficiency. Patients with primary adrenal insufficiency have increased renin levels in the serum but low aldosterone levels; thus salt-losing crisis can occur. These patients will have increased sodium and chloride level in the urine and decreased potassium excretion (49). Thus, hyponatremia should always raise the clinician's suspicion of adrenal insufficiency (1).

Once a patient is suspected of having adrenal insufficiency, a measurement of serum cortisol level should be done. Serum cortisol level is the most reliable test. During morning cortisol measurement, a result of cortisol <3 ug/dL is sufficient for diagnosing adrenal insufficiency (49). In the case of cortisol >18 mcg/dL, adrenal insufficiency can be safely ruled out. Primary adrenal insufficiency can be diagnosed with cortisol <18 mcg/dL and significantly elevated ACTH and plasma renin. Before concluding the diagnosis, a few parameters should be carefully checked. It is crucial to notice that different laboratories have different cutoff levels of cortisol (50).

Some exceptional circumstances that can alter cortisol levels are needed to be considered, especially in pediatric patients. Many other factors can hide early morning cortisol levels in infants, like immaturity of the HPA and the circadian rhythm, variation of sleep patterns, or cortisol binding globulin (51). Stress and acute diseases can also affect cortisol levels. A false-negative test can occur in the case of a very ill or stressed child at the testing time.

Critical illness, such as sepsis, is a well-known cause of primary adrenal insufficiency, referred to as critical illness-related corticosteroid insufficiency (CIRCI). As a result, there should be a high index of suspicion for adrenal insufficiency in these individuals, and a normal baseline blood cortisol result is insufficient to rule out CIRCI (51).

The presence of adrenal autoantibodies confirmed the diagnosis of autoimmune adrenal insufficiency. Plasma level of long-chain fatty acids should be measured in every male patient diagnosed with primary adrenal insufficiency without evidence of autoimmunity etiology to exclude X-linked adrenoleukodystrophy (49). If CAH is suspected in newborns with ambiguous genitalia or salt-losing crisis, random cortisol levels and 17-hydroxy progesterone should be checked to confirm or exclude the diagnosis (49).

ACTH stimulating test is the most used diagnostic test for adrenal gland dysfunction. The test is done by artificial ACTH called cosyntropin administration. Administration of cosyntropin should stimulate the release of cortisol from the adrenal gland. Baseline levels of ACTH and cortisol are checked initially, together with plasma renin activity, aldosterone, and androgen hormones as needed. After baseline testing, administration of 250 μg cosyntropin intravenously is done, followed by drawing cortisol levels 30 and 60 minutes later. In the case of cortisol level ≥ 18 μg/dL with normal baseline ACTH level will exclude the diagnosis of primary adrenal insufficiency. The test will be inaccurate in the case of mild adrenal insufficiency or new onset of secondary adrenal insufficiency due to adrenal reserve (52).

Cortisol is a hydrophobic molecule; thus, cortisol is mainly bound to corticosteroid-binding globulin. Masking of adrenal insufficiency can occur by a potential bias during the diagnosis of adrenal insufficiency. Many clinicians measure the total serum cortisol level and neglect the possibility of abnormal corticosteroid-binding globulin levels and high affinity to cortisol. The corticosteroid-binding globulin concentration can be affected by a pregnancy, oral contraceptive pills, fever, infection, sepsis, cirrhosis, and mutation in the serine

protease inhibitor A6 gene. Thus, cortisol level measurement in some circumstances might not be the best way to assess adrenal function (53).

5.2.1 Genetic testing

Finding the genetic causes of primary adrenal insufficiency in childhood impacts the child and family's future. Diagnosing the disease's genetic cause will help manage the disease course, especially when inheritance patterns can be different. Early detection of the genetic cause will affect the other family members and help diagnose prior to clinical manifestation (54).

When a child is presented with a newly diagnosed primary adrenal insufficiency, thorough history, and clinical features can hint at the underlying cause. Some common causes of primary adrenal insufficiency can be diagnosed by specific biochemical testing and single-gene testing. Additionally, family history can point to an X-linked disease (like adrenoleukodystrophy) if males are affected in the maternal family or recessive cause if there are known blood relationships. The geographical background of the family is critical since it can give information about founder effects and geographical hotspots for some specific conditions (54).

The diagnosis of primary adrenal insufficiency can be very complicated since many forms of the disease can have a similar biochemical profile and clinical presentation. Additional information such as the patient's age at presentation and whether the patient presented with salt loss can help find the diagnosis, but it is not specific on their own. Thus, the next-level investigation approach using targeted panels of known genes or exomes is being used in clinical practice (16).

Previously, genetic testing depended on one-by-one Sanger sequencing of candidate genes. In typical cases like 21-hydroxylase deficiency, this technique may still be helpful. In these cases, there is a particular biochemical profile, well-established pathogenic mutations, and a pseudogene that might confound analysis or well-established correlations or inheritance patterns like in X-linked adrenal hypoplasia congenita. However, when a newborn appears with primary adrenal insufficiency, related characteristics or pathognomonic biochemical patterns are frequently absent; thus, using the "next-generation" sequencing techniques is increasingly cost-effective (16).

Access to services varies by nation. However, targeted "panels" that simultaneously evaluate all-important primary adrenal insufficiency genes are becoming more widely available as a clinical service. Furthermore, multiple studies have indicated how "trio" whole-exome sequencing (WES) can aid in the diagnosis of sick infants and children, particularly when complicated, multisystem characteristics are present, and exome analysis has been found to aid in the identification of children with primary adrenal insufficiency (55). Whole-genome sequencing (WGS) will become more widely available and has advantages and disadvantages over targeted panels or WES.

Genetic testing for primary adrenal insufficiency has a high detection rate compared to other pediatric endocrine disorders, including congenital hypothyroidism and hypothalamic-pituitary hormone deficiencies. For example, a precise genetic diagnosis was established in 80–90% of children in a nationwide cohort study of uncommon, undiagnosed primary adrenal insufficiency in Turkey (with CAH and evident metabolic causes omitted) (56).

Furthermore, founder effects and genetic "hotspots" can be crucial in determining a specific genetic etiology of primary adrenal insufficiency, and ancestral family history is essential. Founder effects are observed in children born in various nations as families migrate worldwide. Knowing a specific hotspot provides targeted, and cost-effective screening of "at-risk" family members before symptoms of primary adrenal insufficiency will develop (16).

5.3 Treatment

Patients with primary adrenal insufficiency are deficient in glucocorticoids and mineralocorticoids and require long-term replacement therapy and salt intake as needed. Patients with primary adrenal insufficiency also have androgen deficiency, but the benefits of androgen replacement are less clearly defined, and guidelines do not recommend androgen replacement (1,57).

5.3.1 Glucocorticoid replacement

In primary adrenal insufficiency, glucocorticoid replacement is the basis of treatment. The primary therapy aims are to reduce the signs and symptoms of

adrenal insufficiency, avoid the development of adrenal crisis, and achieve normal physical and pubertal growth. Moreover, adequate glucocorticoid replacement should match the physiologic pattern of cortisol secretion. Growth suppression, obesity, metabolic syndrome, diabetes, and osteoporosis are linked to non-physiologic and excessive glucocorticoid replacement, leading to poor long-term health effects (58).

Oral hydrocortisone or cortisone acetate is the treatment of choice in pediatrics and is preferred over other options. Cortisone acetate needs to be activated by 11β-hydroxysteroid dehydrogenase type 1 in the liver, and its action might be delayed (1). According to the European Adrenal Insufficiency Registry data, hydrocortisone is prioritized over cortisone acetate (59). Because of their powerful growth suppressant effects, prednisolone and dexamethasone should be avoided in children (58).

According to the primary diagnosis, daily hydrocortisone doses of 7.5- 15 mg/m² are given in 3–4 divided doses, with the first and slightly higher dose taken in the morning and the last dose taken up to 4 hours before bedtime. In individuals with primary adrenal insufficiency that does not involve CAH, an 8 mg/m²/day hydrocortisone dose is considered sufficient for replacement therapy, whereas different doses are necessary for patients with CAH to decrease ACTH-dependent adrenal androgen secretion. In order to prevent growth dysfunction of the final adult height in patients with CAH, the total daily hydrocortisone should not exceed 20 mg/m²/day (60).

Hydrocortisone replacement is monitored using clinical assessments like growth, weight gain, and general well-being and is titrated to the lowest level necessary to control symptoms. In primary adrenal insufficiency, ACTH cannot be considered a criterion for glucocorticoid dose adjustment. Attempts to keep ACTH levels within the reference range result in over-replacement therapy. Polymorphisms in the glucocorticoid receptor or CYP3A4, the primary drug-metabolizing enzyme influencing hydrocortisone clearance, may cause wide interindividual variability in doses (58).

Children given hydrocortisone have multiple cortisol spikes during the day, typically to increased concentrations, followed by protracted periods of hypocortisolemia in between doses (61). As a result, new technologies such as the subcutaneous hydrocortisone pump, novel glucocorticoid formulations, and adjuvant treatments are being explored to provide more physiological glucocorticoid replacement (58).

5.3.2 Mineralocorticoid replacement

Most patients with primary adrenal insufficiency will require mineralocorticoid and salt replacement to alleviate sodium depletion, which presents as lightheadedness and salt cravings, hypotension, hyponatremia, and hyperkalemia (1). According to new research, mineralocorticoid deficiency has been linked to low mood and poor cognition (62).

The availability of mineralocorticoids is problematic, and the only one available nowadays is Fludrocortisone. The drug is taken orally in the morning as a single dose of 0.05-0.2 mg. Fludrocortisone treatment is only effective if enough salt is consumed (1-2 g daily). Treatment adequacy is monitored using blood pressure, electrolytes, and plasma renin activity to maintain normotension, normokalemia, and plasma renin activity in the normal upper range (58).

Overuse of glucocorticoids may minimize the requirement for mineralocorticoid replacement. Since hydrocortisone has a mineralocorticoid effect, patients treated by this drug may need less mineralocorticoid replacement than patients who are receiving prednisone or dexamethasone. During the summer, especially for patients exposed to temperatures higher than 29°C, the mineralocorticoid dose needs to be raised. In patients with primary adrenal insufficiency, insufficient mineralocorticoid substitution may cause poor cardiometabolic outcomes and decreased well-being (63).

5.3.3 Acute adrenal crisis

Acute adrenal crisis can be triggered by severe infections, especially gastrointestinal infections, trauma, surgery, intense physical and emotional stress, and abrupt discontinuation of hydrocortisone medication without titration (58).

Patients with an adrenal crisis should be treated in the intensive care unit under close monitoring. Blood pressure, hydration, clinical status, and serum glucose and electrolyte concentrations should be closely monitored. Prior to treatment, blood and urine samples should be collected to test for cortisol, electrolyte, glucose, ACTH levels, additional steroid compounds, plasma renin activity, and urinary sodium and potassium concentrations should all be tested to determine

glucocorticoid and mineralocorticoid functions. It is essential to mention that none of the laboratory evaluations should delay the treatment (58).

In most cases, saline, glucose, and hydrocortisone are administered intravenously to treat an adrenal crisis. Fluid resuscitation entails treating dehydration rapidly with isotonic sodium chloride, including dextrose, replenishing deficits, ongoing losses, and maintenance fluids. In order to treat hypoglycemia, more dextrose may be required (58).

Together with the administration of intravenous fluids, stress doses of glucocorticoids should be given. Hydrocortisone is the preferred treatment for acute adrenal crisis; the first dose should be 50-75 mg/m² intravenously, followed by the same dose as a continuous infusion or four doses spread out over 24 hours. If intravenous access fails, a hydrocortisone injection can be given intramuscularly. If hydrocortisone is unavailable, methylprednisolone 10-15 mg/m² or dexamethasone 1.5-2 mg/m² might be used instead. Because of its first-pass metabolism in the liver, prednisone is not recommended in cases of adrenal crisis (64).

In most cases, intravenous sodium chloride combined with large doses of hydrocortisone is enough to correct electrolyte abnormalities.
Thus, mineralocorticoids are not needed in the first few hours of treatment, and potassium-lowering agents are only considered if severe symptomatic and persistent hyperkalemia continues despite stress dose hydrocortisone and rehydration therapy is started. Once the patient's blood pressure, blood glucose, and electrolytes are normal and they are feeling well, the stress dose of hydrocortisone can be reduced to the maintenance dose (58,64).

A stress dosage of 50 mg/m² hydrocortisone is indicated 30–60 minutes before induction, repeated every 6 hours, or provided as a continuous infusion for major surgical procedures. Reduce this amount in half on the second postoperative day and resume the maintenance dose on the third postoperative day (64).

It is critical to establish the medical and behavioral causes of each adrenal crisis, including treatment compliance and salt consumption, to prevent future crises. Patients should also be urged to vaccinate their children for influenza annually and alert healthcare providers about steroid dependency during medical or dental treatments (1).

5.3.4 Dose adjustment

Minor infections with low-grade fever may not necessitate a dose adjustment of hydrocortisone. Patients should increase the dose of glucocorticoids twice or even triple their glucocorticoids dose if they have a febrile illness with a fever greater than 38°C, diarrhea, vomiting, or poor oral intake.

If the child cannot tolerate oral medication, the caregiver should be able to provide 50 mg/m² of intramuscular hydrocortisone at home and seek medical assistance within six hours.

The value of education in improving patient compliance, preventing adrenal crises, and lowering the morbidity and mortality associated with adrenal insufficiency cannot be overstated. Written instructions on how and when to increase glucocorticoid therapy should be given to all patients, and this information should be reviewed at each visit or at least once a year so that the dose can be appropriately increased as the child develops. It is essential to educate the caregivers on performing intramuscular injection of hydrocortisone in case of persistent vomiting or severe stress. The patient's dosage should be reviewed every year to ensure that the child is treated correctly according to its growth rate. Using a medical alert bracelet or necklace with the diagnosis of adrenal insufficiency and the necessity to administer hydrocortisone should be recommended. Patients and parents should have an emergency glucocorticoid injection kit and thorough self-injection instructions. Patients' confidence and self-efficacy in self-management of adrenal insufficiency should be enhanced by repeated training about sick day rules and intramuscular injections as part of their usual follow-up care (58,64,65).

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8. Biography

Shir Phillip was born on the 8th of July 1992 in Haifa, Israel.

During the years 2004-2010, Shir studied at Western Galilee high school and took biology and chemistry majors.

Between the years 2010-2013, she served in the IDF as a medical organization officer at the Artillery corps.

Shir started her medical school in 2015 in the international medical program in the faculty of medicine, University of Zagreb, Croatia. During six years in Croatia, she passed all her exams with excellence and is ready to begin a new chapter in her medical future.