Submitted: 05.08.2024
Accepted: 29.09.2024
Early publication date: 11.12.2024

Endokrynologia Polska
DOI: 10.5603/ep.101940
ISSN 0423–104X, e-ISSN 2299–8306
Volume/Tom 75; Number/Numer 6/2024

Adrenal tumours and subclinical adrenal hyperfunction

Lucyna Siemińska [1], ***Katarzyna Siemińska*** [2], ***Bogdan Marek*** [1], ***Beata Kos-Kudła*** [3], ***Mariusz Nowak*** [1], ***Joanna Głogowska-Szeląg*** [1], ***Dariusz Kajdaniuk*** [1]

[1]*Pathophysiology Division, Department of Pathophysiology and Endocrinology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice, Poland*
[2]*Science Students' Association, Pathophysiology Division, Department of Pathophysiology and Endocrinology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice, Poland*
[3]*Department of Endocrinology and Neuroendocrine Tumours, Department of Pathophysiology and Endocrinology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice, Poland*

Abstract

There is a significantly higher prevalence of obesity, hypertension, impaired glucose metabolism, and dyslipidaemia in patients with adrenal incidentalomas. The role of excess adipose tissue in the pathogenesis of adrenal adenomas has not been explored in depth. The study reviews the knowledge about different mechanisms that are related to adrenal tumourigenesis and steroidogenesis. The next objective of this paper is to provide an overview of subclinical types of adrenal hormonal hyperfunction. Recent research has challenged the bimodal diagnosis of hypercortisolism and primary hyperaldosteronism as categorical diseases. Currently we know that milder and subclinical forms are very common, and their involvement in cardiovascular disease is well characterised. Mild autonomous cortisol secretion and mild primary aldosteronism lead to the development of hypertension and metabolic disturbances. The clinical picture of pheochromocytoma is extremely variable and depends on the synthesis and release of catecholamines. The term "subclinical" does not apply fully to pheochromocytoma. In this case, it would be appropriate to describe the tumour as clinically silent. In addition, the term "biochemically silent" is used, based on biochemistry, when plasma and urinary metanephrine levels are below the upper cut-offs of the reference intervals. **(Endokrynol Pol 2024; 75 (6): 630–642)**

Key words: *adrenal incidentaloma; obesity; mild autonomous cortisol secretion; subclinical primary aldosteronism; silent pheochromocytoma*

Adrenal incidentaloma

Adrenal incidentaloma (AI) is a common endocrine disorder that occurs in approximately 2% of the general population and is defined as a clinically inapparent adrenal mass greater than 1 cm in diameter detected in imaging studies performed for reasons other than suspicion of adrenal disease [1]. In the case of an AI, 2 issues arise: whether the lesion is potentially malignant and whether it is functionally active, as in both cases surgery is necessary. It is estimated that 10% of incidentally detected adrenal tumours are hormonally active, and up to 2% are malignant [1].

The therapeutic dilemma concerns tumours of uncertain malignant potential and tumours presenting subclinical hormone hypersecretion.

The following are forms of adrenal tumours with subclinical hyperfunction: mild autonomic cortisol secretion (MACS), mild autonomic aldosterone secretion, and clinically silent forms of phaeochromocytomas. The pathophysiology of hypercortisolism and primary aldosteronism (PA) manifests itself throughout a continuum of severity, corresponding to an increased risk of cardiovascular disease. However, an increased risk of cardiometabolic complications has also been reported even in patients with nonfunctioning adrenal incidentalomas (NFAI), compared to healthy controls [2–5]. In those patients, obesity, hypertension, and diabetes mellitus were more common than in the general population [2–5]. Several mechanisms could explain this link. In recent years, the concept of the 'adipose-adrenal axis', which describes the interconnections between adipose tissue and adrenal glands, has been created [6, 7]. Adipocytokines produced by abdominal adipose tissue and fat surrounding the adrenal gland are involved in steroidogenesis and proliferation of adrenocortical cells [8]. A relationship has been confirmed between obesity, insulin resistance, and adrenal tumourigenesis [5, 9–11]. It is considered that links are bidirectional because NFAI may release hormones in subtle larger amounts, not detected in laboratory tests, which results in accumulation of abdominal adipose tissue.

Lucyna Siemińska, Pathophysiology Division, Department of Pathophysiology and Endocrinology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice, ul. Jordana 19, 41–800 Zabrze, Poland; e-mail: LSieminska@sum.edu.pl

It is now obvious that increased cortisol and aldosterone secretion should be considered as a progressive continuum, suggesting that some tumours defined as hormonally inactive can produce excess hormones to a lesser degree. It can be assumed that subclinical hypercortisolaemia can contribute to the development of insulin resistance, obesity, and hypertension, while a mild excess of aldosterone can lead to hypertension and insulin resistance. This hypothesis is supported by clinical evidence of improved insulin sensitivity after NFAI resection [11]. Analysis of different studies conducted among patients with NFAI after adrenalectomy reveals a reduction in blood pressure and an improvement in metabolic parameters. Consistent with these results, some authors have shown a significant reduction in body weight and glucose levels after adrenalectomy [12, 13].

Different genetic factors are involved in the development of adrenal tumours. In a study that included 100 adrenocortical adenomas, a somatic mutation of the beta-catenin gene (*CTNNB1*) has been found in 61% of non-secreting adenomas, 22% of MACS, and 16% of cortisol-producing tumours [14]. Beta-catenin is an E-cadherin binding protein that promotes cell-to-cell adhesion and activates the WNT signalling pathway that controls cells proliferation. Somatic genetic alterations in cyclic adenosine monophosphate (cAMP) protein kinase A (PKA) signalling have been identified in unilateral cortisol-secreting adenomas. Sporadic cases of primary hyperaldosteronism in most cases are due to somatic mutations, mainly in ion channels and pumps, and rare cases of familial hyperaldosteronism are caused by germline mutations in an overlapping set of genes [15].

Adrenal tumours and obesity

There is evidence that excess body weight is associated with a higher risk of adrenocortical tumours. In the study by Cyranska-Chudek et al. conducted among 2005 patients with adrenal tumours, the mean body mass index (BMI) was 28.8 ± 5.1 kg/m^2, 37.5% subjects were overweight, while 38.3% were obese [16]. Elhassan et al. [17] revealed a similar finding. The researchers found that about 40% of patients with adrenal tumours were obese. This frequency is higher than the statistical data for the entire adult population indicate. In 2016, the global prevalence of obesity (BMI ≥ 30 kg/m^2) was 12% in men and 16% in women [18]. In 2013–2014, the prevalence of obesity in Poland was 24.4% in men and 25.0% in women [19]. The World Obesity Federation reported that in 2022, the percentage of obesity in the world did not exceed 18.5% in women and 14.0% in men [20].

The underlying mechanisms that might explain the relationships between obesity and adrenal tumours remain unclear. Cross-sectional studies suggest the role of insulin resistance, which is common in obese people [5, 9–11]. Insulin is an anabolic hormone with mitogenic effects that stimulates the proliferation of adrenal cortex cells [4]. Hyperinsulinaemia was shown to influence the expression of adrenocortical steroidogenic factor-1 (*SF-1*). The critical role of *SF-1* in adrenal gland development, excessive proliferation of adrenocortical cells, and tumourigenesis has been demonstrated [21]. The concept that there is a link between hyperinsulinemia and adrenal tumours is supported by the results of Muscogiuri et al., who reported the relationships between insulin resistance and the size of the adrenal tumour [9].

Not only high insulin, but also high free insulin-like growth factor 1 (IGF-1), create a favourable environment for adrenal tumourigenesis. Most circulating IGF-1 is of hepatic origin. In visceral obesity, the concentration of free IGF-1 increases as a consequence of the reduction in circulating insulin-like growth factor binding protein 1 (IGFBP-1), caused by hyperinsulinaemia. Insulin and IGF-1 act through specific receptors that have been shown in the adrenal cortex [22, 23] (Fig. 1).

Observation of the influence of the high-fat diet (HFD) on the development of adrenocortical adenomas provides additional information on the basic mechanisms of tumour formation. Swierczynska et al. [24] investigated the adrenal glands of HFD-fed mice. The authors found that feeding increased the expression of many genes involved in steroidogenesis, and thus plasma corticosterone and aldosterone levels. At the same time, the mice developed adrenal hyperplasia. These results indicate that HFD contributes not only to adrenocortical steroidogenesis, but also to the growth of the adrenal cortex [24]. One of the crucial enzymes of the adrenal cortex, implicated in steroidogenesis, is adrenal fatty acid desaturase 2 (FADS2), the expression of which in the adrenal gland is higher than in other organs. In experimental animals, Witt et al. evaluated changes in *FADS2* expression in mice after HFD feeding [25]. They found that diet-induced obesity was associated with elevated expression of adrenocortical *FADS2* and increased plasma corticosterone levels. Furthermore, researchers determined the expression of *FADS2* in human samples. The authors showed a higher expression of *FADS2* in nonfunctional adrenocortical tumours (n = 10 patients) compared to normal adrenal tissue (n = 6 individuals) and an increased expression of *FADS2* in aldosterone-producing adenomas (n = 30 patients) than in NFAI. In addition, *FADS2* expression positively correlated with the expression of other

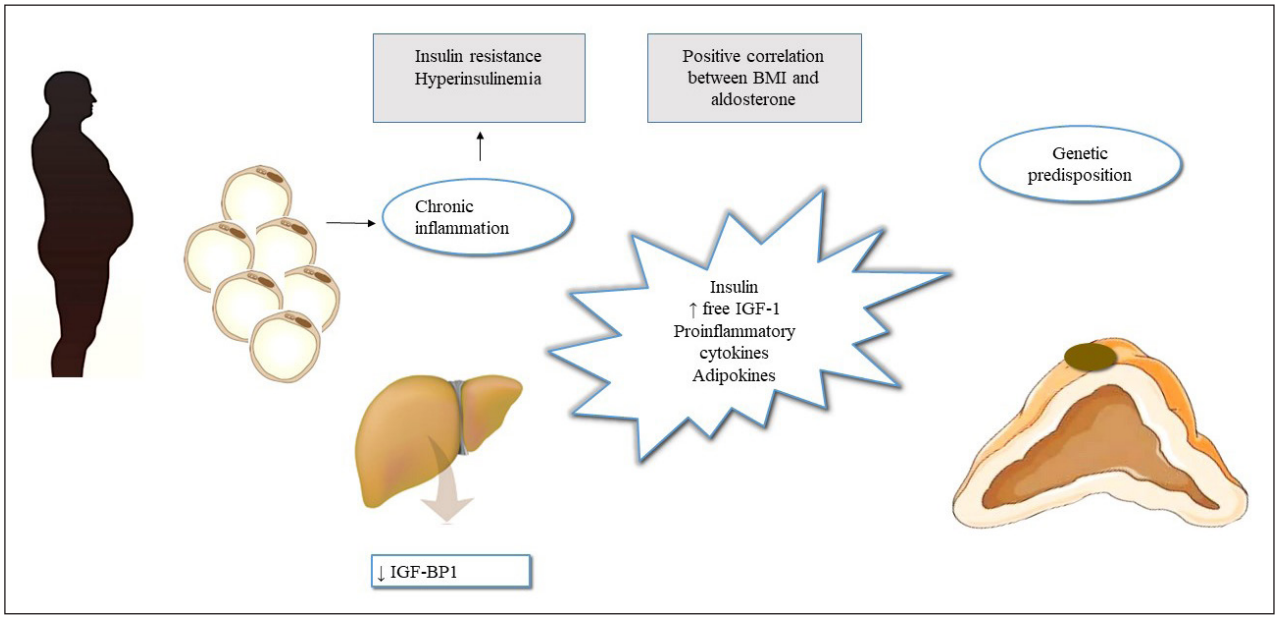

Figure 1. *Mechanisms that link obesity to the development of adrenal tumours. BMI — body mass index; IGF-1 — insulin-like growth factor; IGFBP-1 — insulin-like growth factor binding protein 1*

REVIEW

steroidogenic genes: acute regulatory steroidogenic protein (*STAR*), cholesterol side-chain cleavage enzyme (CYP11A1), *SF-1*, and *PKA* in the human adrenal gland. It should be mentioned that increased expression of *STAR* has been identified in human adrenocortical adenomas producing aldosterone and cortisol [25]. In conclusion, HFD through steroidogenic genes determines the release of steroid hormones and the mass of the adrenal cortex. Interestingly, the volume of the adrenal glands measured by magnetic resonance imaging (MRI) was significantly higher in people with prediabetes and diabetes than in healthy controls [26].

In obesity, hypersecretion of pro-inflammatory cytokines, such as tumour necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), contributes to the development of insulin resistance. In a study by Ermetici et al., higher concentrations of IL-6 were demonstrated in patients with AI, compared to controls, suggesting a potential role of IL-6 in adrenocortical tumourigenesis [27]. Kushlinskii et al. [28] and Babinska et al. [29] revealed similar findings. The latter group of researchers documented higher circulating levels of IL-6, monocyte chemoattractant protein-1 (MCP-1), and TNF-α in patients with adrenal tumours, while a decrease in adiponectin concentration was observed [29].

In addition, other humoral agents associated with obesity could also contribute to adrenal tumourigenesis. It is generally accepted that adipose tissue is an endocrine organ that secretes various adipokines such as leptin, adiponectin, resistin, and visfatin. Because adipokines are recognised as a link between obesity and insulin resistance, their involvement in the pathogenesis of adrenal tumours is also considered [8] (Fig. 1). The leptin receptor Ob-R and adiponectin receptors 1 and 2 are present in the human adrenal glands [30, 31]. High levels of leptin and low adiponectin, as seen in obesity, increase the growth and proliferation of tumour cells and are positively associated with the risk of developing multiple cancers [8, 32]. However, the underlying pathomechanisms connecting leptin and adiponectin with adrenal neoplasia are still poorly understood, and further prospective research is required.

In addition to leptin and adiponectin, other adipokines are believed to promote adrenocortical tumours. Higher visfatin concentrations were observed in patients with NFAI by Atasoy et al. [33]. It is well known that visfatin activates inflammatory pathways and, in this way, may be responsible for adrenal tumourigenesis. Higher resistin and lower adiponectin concentrations were detected in NFAI patients [34]. It is widely recognized that elevated resistin levels stimulate the production of many pro-inflammatory cytokines [35]. A study by Can et al. [36] showed that serum irisin concentration was higher and nesfatin-1 concentration was lower in patients with NFAI than in healthy subjects. However, their contribution to the pathogenesis of adrenal tumours is unclear. In addition to the proliferative effect, adipocytokines can regulate adrenal steroidogenesis [37, 38]. An excess of adipose tissue in obese people leads to secretion of large amounts of leptin that can reach the adrenals in an endocrine manner. Additionally, periadrenal fat can also release leptin that directly affects steroid-producing adrenocortical cells. Leptin has been shown to stimulate

aldosterone secretion in the glomerulosa zone [6, 38]; therefore, it can be considered a regulator of adrenal aldosterone secretion, independent of renin, potassium, and sodium. Interestingly, in obese patients, aldosterone concentrations are elevated, regardless of the presence of a pathological mass in the adrenal glands [39]. The role of adipocyte-derived factors in cortisol secretion has not been clarified.

Non-functioning adrenocortical adenomas and hypertension

Hormonally active adrenal tumours, such as PA, pheochromocytoma, and adrenocorticotropic hormone-independent Cushing syndrome, are the most common causes of secondary hypertension. However, the incidence of hypertension in patients with NFAI is also significantly higher than in the general population [40–42]. The potential mechanism underlying this phenomenon may be subtle hormone overproduction, within a range that is considered normal by accepted standards. Obesity may also contribute to the pathogenesis of hypertension. Arruda et al. found that hypertensive patients with NFAI had higher cortisol levels after the dexamethasone suppression test (DST) than NFAI patients without hypertension [40]. In a recent study by Kim et al., the prevalence of hypertension in patients with NFAI (n = 154), compared to controls without adrenal tumours (n = 462), was significantly higher [37% *vs*. 20.1%, adjusted odds ratio (OR) of 2.26] [43]. In another study, Araujo-Castro et al. reported that patients with NFAI and cortisol after DST > 0.9 ug/dl had a higher prevalence of hypertension compared to patients with NFAI and cortisol after DST ≤ 0.9 ug/dl [44].

Non-functioning adrenocortical adenomas and diabetes

A longitudinal retrospective study conducted by Lopez et al. in patients with NFAI (n = 110) and those without an adrenal tumour (n = 116) showed that even non-functioning adrenal tumours can predict significantly higher risk of diabetes. During a 7-year follow-up, diabetes developed in 27.3% of the participants with NFAI and in 11.7% of those without adrenal tumours [3]. It can be speculated that the higher risk of diabetes in NFAI subjects is associated with obesity and subtle abnormality in cortisol secretion with the subsequent development of insulin resistance.

Subclinical hypercortisolaemia

The 2023 European Society of Endocrinology (ESE) guidelines [42] recommend that all patients with adrenal incidentaloma should be clinically evaluated for symptoms consistent with the presence of a functionally active adrenal mass, such as hypertension, diabetes mellitus, and hypokalaemia. A 1-mg DST, also known as the overnight test, is recommended in all patients with AI, measurement of plasma or urinary metanephrines in patients with > 10 Hounsfield units (HU) on unenhanced computed tomography (CT), and plasma aldosterone/renin ratio in patients with hypertension or hypokalaemia [42]. A cortisol level after DST of 1.8 μg/dL (50 nmol/L) or less is considered normal, and higher values indicate a lack of appropriate negative feedback. Subclinical hypercortisolism, previously called subclinical Cushing syndrome, is defined as a lack of classical external features of Cushing syndrome and a failure to adequately suppress cortisol after DST. In the 2023 ESE guidelines, this condition is defined as MACS [42]. MACS is estimated to affect up to 50% of patients with AI [45, 46]. A recent observational study by Elhassan et al. [17] showed that the probability of developing MACS from NFAI was 4.3%, while the probability of resolution of preexisting MACS was 0%, during a mean follow-up period of 50.2 months. In another study, Araujo-Castro et al. [47] documented the development of autonomous cortisol secretion in 73 of 331 patients with NFAI (22.1%), during 35.7 months of follow-up. The lowest risk of developing MACS was found in patients under 50 years of age with unilateral NFAI, whose serum cortisol concentration after DST is < 0.45 μg/dL at the time of diagnosis.

MACS is associated with an increased risk of cardiovascular and metabolic morbidity, and therefore a correct diagnosis is very important [42, 45, 46]. The overnight test with 1 mg dexamethasone is highly specific and sensitive; however, certain limitations exist and several factors may affect the DST results [48, 49]. False positive results are possible in patients with elevated serum levels of cortisol binding globulin (due to oral oestrogens, pregnancy, or chronic active hepatitis), alcohol abuse, or in patients taking a strong inducer CYP3A4 that reduces the bioavailability of dexamethasone (i.e. carbamazepine, diltiazem, phenobarbital, phenytoin). As people age, receptor responses change, the hypothalamic–pituitary–adrenal (HPA) axis becomes less sensitive, and the feedback mechanism of the HPA axis weakens. With ageing, reduced surface area for absorption and slower gastric emptying can lead to an insufficient concentration of dexamethasone in the blood and therefore false positive DST results. The positive association between age and cortisol after DST was described in 1129 patients with AI between 55 and 74 years of age. A negative association between cortisol after DST and eGFR was also found. The authors

also revealed a linear increase in post-dexamethasone cortisol with BMI [49]. History of gastrointestinal diseases, such as Crohn's disease, celiac disease, or bariatric surgeries, can also lead to false positive DST results [48]. In patients with neuropsychiatric disorders, with or without metabolic syndrome, elevated cortisol levels in the morning and unsuppressed cortisol after DST can also be observed. Sharma et al. revealed that non-suppression rates were significantly higher in schizophrenic patients and patients with a major depression than in healthy controls [50].

In all patients with a serum cortisol level post dexamethasone > 1.8 μg/dL, conditions that alter the results of the overnight test should be considered. Furthermore, a repeat DST should be performed. Low early morning plasma ACTH and DHEAS reflect suppression of the HPA axis. Carafone et al. demonstrated that the combination of serum concentrations of DHEAS < 40 mcg/dL and ACTH < 10 pg/mL diagnosed MACS with the highest precision, with 91.6% specificity and 86.6% positive predictive value [51]. Additional biochemical tests may also be useful to assess the extent of hypercortisolaemia.

Although typical signs of overt hypercortisolism are absent, mild cortisol hypersecretion is associated with a variety of adverse clinical outcomes, including visceral obesity, an increased incidence of impaired glucose tolerance or type 2 diabetes, and a higher prevalence of hypertension and dyslipidaemia (Fig. 2). Furthermore, a higher probability of fractures and osteoporosis has also been reported compared to the general population [42].

There are several mechanisms for glucocorticoid-induced obesity in patients with MACS. The connections involve direct effects on adipocytes, glucose and lipid metabolism, stimulation of appetite, control of eating behaviour, thermogenesis, and energy balance. Delivanis et al. [45] found that excess of glucocorticoids induces changes in body composition. In a cross-sectional study of 227 patients with adrenal adenomas, cortisol concentrations after DST were positively correlated with the visceral/total fat area and the visceral/subcutaneous area, as assessed by CT. Contrary to an increase in adipose tissue, a decrease in total muscle mass was also found. The results are in line with the study of Debono et al. [46] that reported that higher post-DST cortisol was associated with a higher visceral:subcutaneous and visceral:total volume fat ratio. Chronically elevated glucocorticoids can induce lipogenesis and adipogenesis in visceral depots. Glucocorticoid receptors (GR) are found to be more abundant in visceral adipose tissue than in subcutaneous fat.

The relationships between fat centralisation and glucocorticoids are bidirectional. It is well known that individuals with central obesity have a higher responsiveness to HPA activity, compared to people with peripheral fat distribution. Increased HPA activation leads to functional hypercortisolism. Morning cortisol concentration is generally normal; however, impaired peripheral cortisol metabolism is often observed. Over-

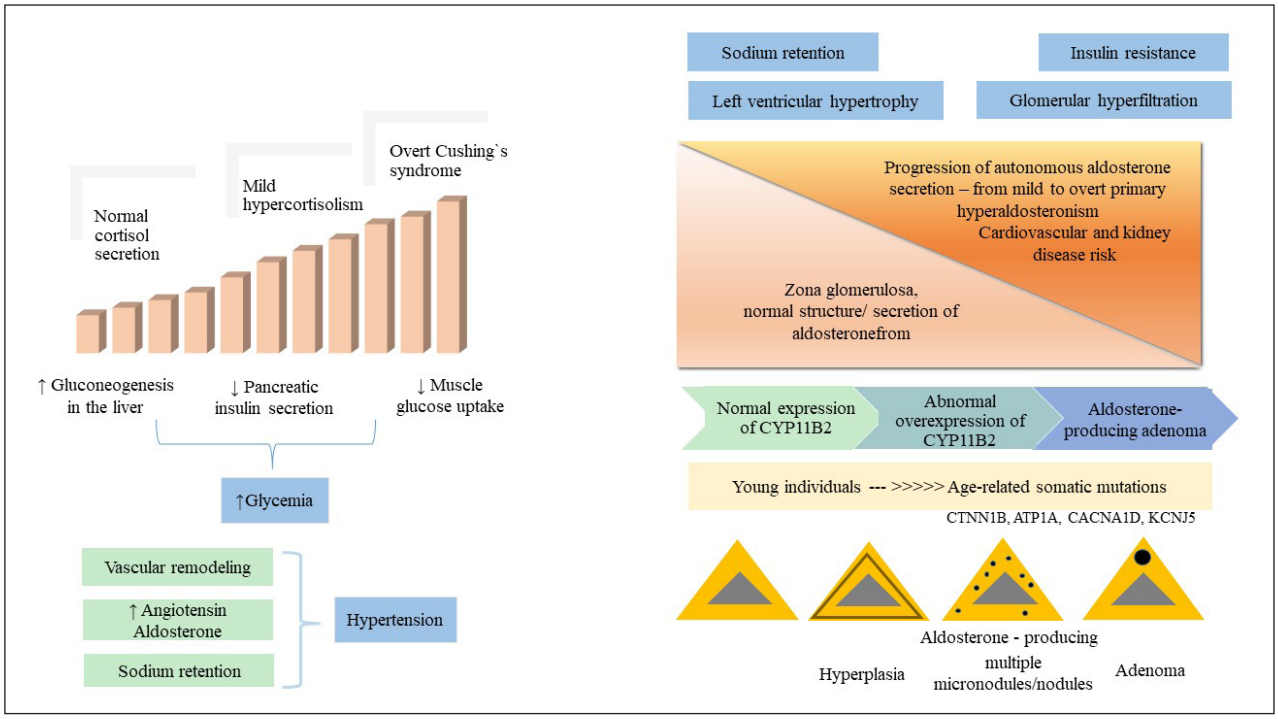

Figure 2. *Mild hypercortisolism. Mild hyperaldosteronism*

expression of 11 beta-hydroxysteroid dehydrogenase (*11βHSD*) in adipose tissue stimulates the peripheral conversion of cortisone to cortisol; therefore, the local tissue cortisol level increases. Furthermore, in obesity hyperinsulinaemia can trigger HPA activity. Additionally, insulin may directly influence adrenal cortisol production by activating *SF-1* [26].

Arterial hypertension is the most common clinical feature, present in more than 60% of patients with MACS. The incidence of hypertension in MACS is higher compared to NFAI [42, 52, 53]. In a study by Patrova et al. [52] among 204 patients with NFAI and 128 with MACS, individuals with MACS were more frequently affected by arterial hypertension (39.2% in NFAI *vs*. 64.8% in MACS). It agrees with the results of the meta-analysis that included more than 17,000 patients with AI. Subjects with post-DST cortisol greater than 1.8 μg/dL had a higher prevalence of hypertension compared to patients with NFAI (OR = 1.24) [53]. The main mechanism involved in the pathogenesis of hypertension in patients with MACS is increased activity of the renin angiotensin aldosterone system (RAAS), which causes sodium retention, increased plasma volume, and vascular remodelling. Cortisol is a potent mineralocorticoid receptor (MR) agonist. Furthermore, glucocorticoids exacerbate the vascular response to catecholamines and increase the synthesis of central neurotransmitters. The usual coexistence of visceral obesity may contribute to the development of hypertension. In addition, the coincidence of aldosterone and cortisol hypersecretion has been reported in about 30% of patients with PA. This cosecretion is called Connshing syndrome [54].

In a recent large-scale study [55] it was demonstrated that the probability of developing diabetes in patients with adrenocortical adenomas increased as a continuum from non-functioning adenoma (18.2%), through possible autonomous cortisol secretion (23%), to autonomous cortisol secretion (26.7%). Di Dalmazi et al. reported that a higher concentration of cortisol after an overnight test was associated with a higher risk of diabetes, in a mean follow-up of 7.5 years. In patients with autonomous cortisol secretion the prevalence was 40%, in patients with possible autonomous cortisol secretion it was 29%, and in patients with NFAI it was 14% [56]. The probability of developing diabetes is due to the deleterious effects of excess cortisol on glucose metabolism, mainly due to reduced insulin-dependent glucose uptake in peripheral tissues, increased gluconeogenesis, and impaired pancreatic insulin secretion (Fig. 2).

Some authors emphasise the poor diagnostic precision of the 1.8 μg/dL cut-off point, to exclude adrenal gland glucocorticoid hyperactivity [57]. In a recent multicentre retrospective analysis of 593 people with AI, a higher incidence of cardiovascular disease (OR = 2.58), hypertension (OR = 1.75), diabetes (OR = 1.90), and dyslipidaemia (OR = 1.6) was found in subjects with post-DST cortisol greater than 0.9 μg/dL, when compared to those with post-DST cortisol less than 0.9 μg/dL [44].

For the physician, the greatest problem is selecting patients with MACS who could benefit from adrenalectomy. The most important point is the evaluation of cardiometabolic comorbidities that are potentially associated with excess cortisol. Surgical removal of the adrenal mass that causes MACS can lead to improved glycaemic control, blood pressure, and dyslipidaemia. The benefits of adrenalectomy in patients with MACS were demonstrated in 2 randomised controlled trials and 9 observational cohort studies [42]. If surgery is contraindicated, impossible, or there are bilateral masses in the adrenal glands, pharmacological treatment of comorbidities should be introduced. Studies on the use of steroidogenic inhibitors in the treatment of MACS have not shown definite benefits, and further observations are required [57].

Cortisol-producing adrenocortical adenomas are frequently connected with aberrant cAMP-PKA pathway. Activating somatic mutations in cAMP-PKA catalytic subunit A (PRKACA) account for up to 42% of patients [15].-

Subclinical primary aldosteronism

In recent years, it has become clear that not only hypercortisolaemia but also PA constitute a broad continuum of autonomous aldosterone secretion, from mild forms to overt PA [58, 59]. There are many studies that have identified the biochemical characteristics of PA in normotensive individuals who later developed hypertension [60, 61].

Subtle forms, also called subclinical PA, are associated with abnormal MR activation, which leads to unfavourable cardiac and blood vessel remodelling. Initially, patients may be asymptomatic, without hypertension or hypokalaemia, but later overt manifestations develop. The thickening of the left ventricle wall and diastolic dysfunction develop, even in patients without hypertension. The subtle forms of renin-independent aldosteronism are also associated with accelerated arterial stiffness. Excess aldosterone increases volume and blood pressure and may cause hypokalaemia. In the early stages, hypertension may be mild but then progresses to severe forms that can be resistant to 3 or more drugs. The more severe hypertension is believed to be related to the greater likelihood of diagnosing PA [58].

Autonomous aldosterone production, in the presence of sufficient sodium intake, is associated with continuous renal sodium reabsorption, despite suppression of renin. Excess sodium retention can lead to kidney damage. In addition, excess aldosterone can stimulate the development of insulin resistance in skeletal muscles and fat cells (Fig. 2).

Progress in genetic and histopathological research has resulted in a significant change in understanding the pathophysiology of PA. Immunohistochemical studies using specific monoclonal antibodies have shown that human adrenal glands undergo significant remodelling with age. Aldosterone synthase, an enzyme involved in aldosterone biosynthesis, is encoded by the *CYP11B2* gene. The diffuse expression of *CYP11B2*, observed in childhood and youth, is usually absent in adults. Instead, *CYP11B2*-positive cells create areas of 1 to 3 mm in diameter located under the adrenal capsule. Consequently, a progressive pattern of abnormal expression of *CYP11B2* develops with ageing and leads to dysregulated aldosterone secretion [62, 63]. Autonomic areas can increase in size with age, and micronodules or nodules producing aldosterone appear (Fig. 2). As molecular changes increase, clinical symptoms gradually become more advanced.

It is speculated that the reason for this phenomenon is the modern high sodium diet that, by inhibiting renin production, suppresses the physiological production of aldosterone. In addition to environmental factors, adrenal lesions can carry somatic mutations that contribute to variable degrees of renin-independent hyperaldosteronism [58].

In 2021, an international consensus on the nomenclature of adrenal lesions in patients with PA was published, and according to these recommendations, several subforms of PA are distinguished: aldosterone-producing adenomas (APAs), aldosterone-producing nodules (APNs), aldosterone-producing micronodules (APMs), diffuse aldosterone-producing hyperplasia, and adrenocortical carcinoma (ACC) producing aldosterone [64]. The most common causes of PA are APMs, APNs, and APAs, less common is bilateral adrenocortical hyperplasia, and rarely the cause is ACC. Traditionally, familial hyperaldosteronism is also distinguished [65, 66].

Studies conducted in recent years have shown that PA is, in most cases, a genetic disease. Sporadic cases of APMs, APNs, or APAs are due to somatic mutations, mainly in *CACNA1D*, *KCNJ5*, *ATP1A1*, and *CTNN1B*, which are responsible for ion channels. These mutations cause abnormal sodium permeability, depolarisation of cell membranes, and loss of pump function. All these processes lead to calcium influx, increased calcium signalling, high expression of *CYP11B2*, and aldosterone production [54]. Whether somatic mutations are responsible for cellular proliferation has been debated. Molecular studies have shown that even 95% of APAs harbour a somatic mutation, and the mutations detected most frequently are in the *KCNJ5* and *CACNA1D* genes [65]. The somatic genotype may predict the subsequent development and prognosis of PA. Patients with *KCNJ5* mutations are more likely to have complete therapeutic success compared to patients with *CACNA1D* mutations.

Small micronodules or nodules with increased expression of aldosterone synthase are also associated with the same mutations as APAs: *CACNA1D*, *ATP1A1*, *ATP2B3*, and *KCNJ5*. Bilateral lesions often carry *CACNA1D* mutations. Rare cases of familial hyperaldosteronism are caused by germline mutations in an aldosterone synthase, *KCNJ5*, *CLCN2*, *CACNA1H*, and *CACNA1D* [65].

According to the guideline for the diagnosis of primary hyperaldosteronism, the most sensitive screening test for the detection of PA is the aldosterone-renin ratio (ARR) [67, 68]. However, there is no clear guideline on the cut-off value that should be used to define a result as positive. It is important that each laboratory establishes reference values for these parameters based on the methods and units used. The multitude of ARO and ALDO assay kits used, their low repeatability, and the multitude of factors that influence RAAS mean that the ARR cut-off level adopted in individual centres differs significantly [66]. Plasma renin can be determined as activity (plasma renin activity PRA) or by quantifying the concentration of active renin (direct renin concentration DRC). If aldosterone is measured in ng/dl and PRA in ng/ml/h, the ARR cut-off point mainly ranges between 20 and 30. If DRC is measured in mIU/L, the ARR cut-off point ranges between 1.12 and 2.7 [50]. PRA or DRC should be measured under standardised conditions in the morning, at least 2 hours after the patient wakes up, after 5 to 15 minutes in a sitting position, after unlimited salt intake, and with normal serum potassium concentration. To avoid false negative results, hypokalaemia must be corrected. The goal of potassium supplementation is a serum concentration of 4 mmol/L or higher. It is important to remember that low potassium levels are a strong inhibitor of aldosterone secretion and can cause false negative results. MRs antagonists (e.g. spironolactone, eplerenone, amiloride) should be discontinued 4 weeks before ARR testing. It is recommended to discontinue all drugs that interfere with the activity of the RAAS (β-adrenergic blockers, central α-2 agonists [e.g. clonidine, α-methyldopa], angiotensin-converting enzyme [(ACE) inhibitors, angiotensin receptor blockers (ARB), calcium channel blockers such as amlodipine] for at least

2 weeks before testing. In the treatment of hypertension, these drugs can be replaced with others that have a minimal effect on ARR (e.g. doxazosin, long-acting verapamil form). Non-steroidal anti-inflammatory drugs (NSAIs) can reduce PRA by inhibiting prostaglandin synthesis, and consequently increase ARR. Furthermore, NSAIDs promote sodium and water retention, which may also inhibit ARO. Therefore, their use should be discontinued for 2 weeks before testing. To confirm PA in patients who have obtained a positive result of the screening test, it is recommended that at least one of the following 4 tests be performed: saline infusion test, oral salt load test, fludrocortisone suppression test, or captopril challenge test [67, 68]. All tests except the captopril test carry the risk of fluid overload, increased blood pressure, development of heart failure, and hypokalaemia.

The guidelines of the Endocrine and Cardiological Society recommend the screening for PA not only in individuals with AI and hypertension, but also in patients with hypertension resistant to 3 antihypertensive drugs, in patients with hypertension and spontaneous or diuretic-induced hypokalaemia, in subjects with hypertension and sleep apnoea, in patients with hypertension and a family history of early onset hypertension or cerebrovascular accident at a young age (< 40 years), and in all patients with first-degree relatives of PA [67, 68]. However, it is estimated that less than 10% of patients with indications for the evaluation of RAAS have tests performed in both primary care and speciality facilities. The main reason is the need to modify antihypertensive therapy to limit the effect of drugs on RAAS. However, in severe PA, discontinuation is often unnecessary because, in patients with normal RAAS, a constellation of suppressed renin and unsuppressed aldosterone should not occur, regardless of the use of drugs [59]. Measurement of renin is suggested in all patients with hypertension, regardless of the medications taken. The most commonly used antihypertensive drugs, with the exception of beta-blockers, tend to increase renin levels. If renin remains suppressed, despite these interfering medications, further investigation will be warranted.

It should be noted that the biochemical diagnosis does not distinguish the causes of PA; therefore, further diagnostic procedures must be performed. Imaging tests such as CT or MRI are believed to not allow for a final definition of PA. This failure is due to the small size of most adrenal lesions and the high incidence of NFAIs in the general population. Therefore, expensive and invasive selective adrenal venous sampling (AVS) remains the gold standard for subtyping patients with PA and selecting patients for surgery. Catheterisation is technically difficult and may be sequential or simultaneous (bilateral); under basal conditions (without stimulation) or with cosyntropin stimulation. In the case of confirmed unilateral disease, treatment is simple and non-controversial; however, in the case of bilateral hyperaldosteronism, therapeutic decisions are more complex.

"Clinically silent" and "biochemically silent" pheochromocytoma

The term pheochromocytoma is reserved for tumours derived from adrenal medulla chromaffin cells that produce catecholamines. A paraganglioma is a tumour that arises from extra-adrenal chromaffin cells of the sympathetic or parasympathetic paraganglia. Pheochromocytomas and paragangliomas are commonly known as PPGL, to include both entities [69].

The term "subclinical" refers to a disease that is not serious enough to present with specific or easily observable symptoms. However, this statement does not fully apply to pheochromocytoma, where the subclinical form may depend not only on the amount, but also on the type of hormones released. Additionally, given the variability of catecholamine secretion, symptoms can occur sporadically during the course of the disorder. In the case of pheochromocytoma, it would be more appropriate to describe the tumour as asymptomatic or clinically silent [69].

There is a growing trend for incidental detection of pheochromocytomas. This often occurs during imaging studies (USG, CT, or MRI) for various reasons. The radiological characteristics of pheochromocytomas include homogeneity or heterogeneity, solid or cystic appearance, fat-containing areas, or the presence of necrosis and calcification. Due to its broad spectrum of radiological features, pheochromocytomas can be mistakenly identified as cysts, adrenocortical carcinoma, metastasis, adrenal lipid-poor adenomas, and retroperitoneal lymphomas. The prevalence of pheochromocytomas in AI cases is estimated to be up to 7% [71]. Among 130 patients who underwent adrenalectomy for AI, pheochromocytoma was found in 8 cases (6.1%) [71].

The clinical presentation of pheochromocytoma is extremely variable and depends mainly on the type of catecholamines released (norepinephrine, epinephrine, and dopamine) and the pattern of release (continuous / sporadic) (Fig. 3). Signs are also related to stimulation of adrenergic receptors. The enzyme dopamine hydroxylase converts dopamine into norepinephrine, while epinephrine is synthesised from norepinephrine by the enzyme phenylethanolamine N-methyltransferase (PNMT). Once synthesised in the cytoplasm, epinephrine is repackaged into small vesicles via the vesicular monoamine transporter.

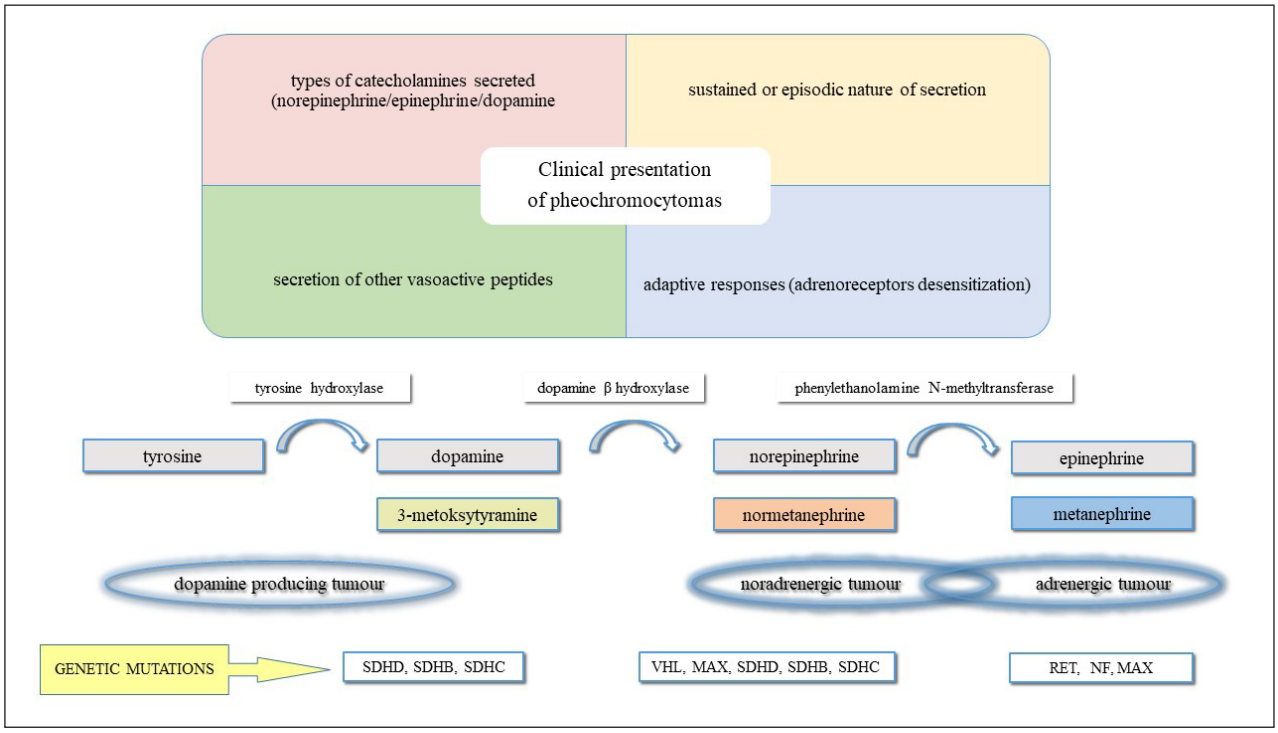

Figure 3. *Clinical presentation of pheochromocytomas*

Catecholamines regulate their own synthesis by inhibiting the activity of tyrosine hydroxylase. Lack of dopamine hydroxylase expression can result in a defect in norepinephrine and epinephrine synthesis, which can predispose to the development of clinically and biochemically silent pheochromocytoma [69] (Fig. 3). More than 80% of norepinephrine in the blood comes from sympathetic nerves; therefore, the increase in plasma norepinephrine concentration is not necessarily the result of the secretion of norepinephrine by the adrenal medulla tumour. Catecholamines are metabolised by enzymes: monoamine oxidase (MAO), catechol-O methyltransferase (COMT), and sulfotransferase. Epinephrine, norepinephrine, and dopamine are converted to metanephrine, normetanephrine, and 3-methoxytyramine. Biochemical evaluation of hypersecretion of catecholamine metabolites is crucial in the diagnosis of pheochromocytoma. Excessive release of catecholamines may cause typical symptoms. The most common include paroxysmal headaches, sweating, and tachycardia. However, this classic triad occurs in 60% to 52% of patients. Hypertension, persistent or episodic, is present in up to 65–80% of all cases [58]. In addition to typical symptoms, others can occur: tremor, weight loss, nausea, anxiety, and abdominal pain. In these cases, the clinical picture may suggest migraine, hyperthyroidism, or hypoglycaemia. Although most pheochromocytomas have severe or mild adrenergic and noradrenergic symptoms, almost 50% of tumours are detected incidentally. In a multicentre prospective study of 245 patients with PPGL, typical signs and symptoms allowed diagnosis in only 37% of the patients [73]. Rarely, the occurrence of hypertensive crisis leads to the search for endocrine causes of hypertension. However, it should be noted that among hypertensive patients, the incidence of catecholamine-producing tumours ranges from 0.2% to 0.6%. Interestingly, up to 9% of patients with adrenal pheochromocytomas are asymptomatic [74].

There are adrenergic and noradrenergic phenotypes of pheochromocytomas (Fig. 3). Tumours with an adrenergic phenotype secrete both norepinephrine and epinephrine in varying proportions. Epinephrine production depends on the expression of PNMT. Tumours lacking PNMT activity do not produce large amounts of epinephrine and generally have a noradrenergic phenotype. Epinephrine is a stronger agonist of α-adrenergic receptors, but it is often released sporadically. However, norepinephrine is generally secreted continuously. About half of all biochemically functioning pheochromocytomas secrete both epinephrine and norepinephrine. Approximately half of tumours secrete nearly exclusively of norepinephrine. A small percentage of pheochromocytomas secrete dopamine [69].

The most appropriate test for initial screening and confirmation is the measurement of 24-hour urinary excretion of fractionated metanephrines

and normetanephrines. Plasma levels of metanephrine/normetanephrine are also recommended. The significant predominance of daily urinary excretion of 3-methoxytyramine or an increase in plasma 3-methoxytyramine suggests excessive dopamine production [69, 75].

Approximately 15 to 20% of patients with catecholamine-secreting tumours have a germline mutation in genes such as *RET* (MEN2 A and B syndrome), *NF-1* (neurofibromatosis), *VHL* (von Hippel-Lindau syndrome), *SDHB*, *SDHC*, and *SDHD*. As genetic testing becomes more widely available, part of the diagnosis of pheochromocytomas occurs due to routine screening of patients with MEN2 syndrome/VHL or NF1 [69]. In these cases, pheochromocytomas are often diagnosed early, when the tumour secretes small amounts of catecholamines and the clinical picture may be silent.

Some pheochromocytomas may produce significant amounts of catecholamines, but if receptor desensitisation develops, catecholamines will not be effective and the clinical picture may be mild. Norepinephrine-producing tumours can be expected to contribute to tachyphylaxis more than sporadic tumours secreting epinephrine. The noradrenergic phenotype is associated with the VHL mutation. The adrenergic type is connected to a mutation of the *RET* gene, so it often occurs in MEN syndrome. Patients with VHL, with more sustained norepinephrine secretion, are often normotensive and asymptomatic [76].

In the case of MEN2, tumours secrete only epinephrine or epinephrine together with norepinephrine, and clinical signs are related to the tumour adrenergic secretory pattern. Episodic bursts of catecholamines cause paroxysmal symptoms, such as hypertensive crises, which are frequently associated with episodes of hypotension [77].

The clinical manifestation of pheochromocytoma depends on the size of the tumour and the type of co-released peptides. The amount of catecholamines secreted into the circulation is positively correlated with the tumour size; therefore, smaller tumours will cause fewer symptoms. However, pheochromocytomas are highly vascularised lesions, and large tumours can develop haemorrhagic or necrotic areas. For this reason, giant tumours can be associated with a silent clinical picture [78].

Sometimes large tumours present with few symptoms, even in the absence of necrosis. These forms have been observed in patients with *SDHB* mutations, and in these cases the malignant form occurs in 40% of patients.

Tachyphylaxis, i.e. physiological adaptation, can lead to a clinically silent course of the disease, even in the case of high concentrations of circulating catecholamines. The most common reason is a reduced response of adrenergic receptors to catecholamine activation after long-term adrenergic stimulation. In these patients, hypovolaemia may occur.

In addition to the term "clinically silent", which describes the absence of symptoms of catecholamine excess, the term "biochemically silent" is used based on biochemistry. In these cases, plasma and urinary metanephrine/normetanephrine levels are below the upper cut-off values of the reference intervals [69]. A large proportion of patients classified as "biochemically silent" present false negative results of biochemical tests because of inappropriate methodology or incorrect reference intervals. Laboratories should establish their own reference ranges because they vary depending on the analytical method used. High-performance liquid chromatography (HPLC) is considered by the Endocrine Society as a method with high sensitivity and specificity [42, 75]. The evaluation of independent markers of neuroendocrine tumours, such as chromogranin A (CgA), may be helpful in the diagnosis of pheochromocytoma. The other reason for the negative biochemical result may be the small size of the tumour.

False positive elevations are much more common than false negative ones. Various drugs can alter catecholamine secretion and metabolism. Ephedrine, amphetamine, nicotine, and caffeine are sympathomimetic agents that can increase the release of norepinephrine and epinephrine. Serotonin and noradrenaline reuptake inhibitors and tricyclic antidepressants can increase norepinephrine and normetanephrine levels. Calcium channel blockers and β-adrenoreceptor blockers are associated with a high frequency of false positive results. In contrast to the antihypertensive drugs listed above, angiotensin converting enzyme inhibitors, angiotensin-1 receptor blockers, and diuretics appear to have little effect on the incidence of false positive results. Different medications such as paracetamol, sulfasalazine, certain antibiotics, antihistamines, and iodine contrast used during radiological examinations may interfere with analytical methods. The test results may also be false as a result of the consumption of various foods, for example, nuts, bananas, black tea, and some fruits [79].

According to current guidelines, PPGL testing is recommended in patients with characteristic symptoms, signs of cardiovascular instability suggestive of PPGL, and newly diagnosed DM2 in lean people. A density of ≥ 10 HU on CT imaging of adrenal incidentaloma is also associated with a higher risk of pheochromocytoma. A separate group includes carriers of mutations in one of the susceptibility genes and patients with a family history of PPGL. Proper laboratory techniques, minimising sympathetic activity, and withdrawing different drugs that can cause analytical interference

are essential for diagnostic accuracy. After appropriate laboratory tests, anatomical imaging of adrenal tumours using CT and MRI, as well as functional imaging [scintigraphy ^{123}I-MIGB or ^{131}I-MIGB, PET imaging using: ^{18}F fluorodeoxyglucose (18F-FDG), ^{18}F fluoro L dihydroxyphenylalanine (^{18}F-DOPA), ^{68}Ga-labelled somatostatin receptor analogues (^{68}Ga-DOTA-SSA)] are the next diagnostic steps to confirm pheochromocytoma.

Silent tumours that produce catecholamines are rare but require the same radical treatment as in all cases of pheochromocytomas.

Conclusion

Recent studies showed a significantly higher prevalence of obesity, hypertension, impaired glucose metabolism, and dyslipidaemia in patients with NFAI and MACS. Progress in understanding the pathophysiology of NFAI and MACS allows appropriate management and treatment. Subclinical primary aldosteronism constitutes a broad spectrum of autonomous aldosterone secretion, from mild forms to overt primary aldosteronism. Several subforms of primary aldosteronism are included: aldosterone-producing adenomas, aldosterone-producing nodules, aldosterone-producing micronodules, diffuse aldosterone-producing hyperplasia, and adrenocortical carcinoma-producing aldosterone. The clinical picture of pheochromocytoma is extremely variable and depends on the synthesis and release of catecholamines. Signs and symptoms are related to stimulation of adrenergic receptors. The term "subclinical" does not fully apply to pheochromocytoma. In this case, it would be appropriate to describe the tumour as clinically silent. In addition, the term 'biochemically silent' is used, based on biochemistry, when plasma and urinary metanephrine levels are below the upper cut-offs of the reference intervals.

Author contributions

L.S.: conception of the paper, collection of the bibliography and its analysis, the main role in the preparation of the article text; K.S.: preparation of the article text, collection of the bibliography, editing and design; B.M.: preparation of the article text; B.K.K.: preparation of the article text; M.N.: preparation of the article text; J.G-Sz.: preparation of the article text; D.K.: preparation of the article text.

Conflict of interest

Authors declare no conflict of interests.

Funding

This work was supported by the Medical University of Silesia.

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