Medical University - Varna
"Prof. Dr. Paraskev Stoyanov"

Faculty of Medicine
Department "Second Department of Internal Medicine"

Dr. Evelina Boykova Zlatanova

Metabolic profile of patients with hormone-inactive adrenal adenomas

ABSTRACT

dissertation for the award of the scientific and educational degree

"Doctor"

Supervisor:

Assoc. Prof. Dr. Mira Siderova, Ph.

Varna, 2021

The dissertation contains 171 standard pages and is illustrated with 31 tables, 47 figures and 1 appendix. The reference list includes 250 references, 13 in Cyrillic and 237 in Latin.

The dissertation was discussed and directed for defense at the Departmental Council of the Second Department of Internal Medicine at Medical University "Prof. Dr. Paraskev Stoyanov" - Varna.

The formal defense of the dissertation will take place at ..... from ..... hours at ..... - gr. Varna at an open meeting of the Scientific Jury.

The defense materials are available in the Scientific Department of Medical University - Varna and are published on the website of Medical University - Varna.

Note: In the abstract the numbers of tables and figures do not correspond to the numbers in the thesis.

CONTENTS

ABBREVIATIONS USED

ACP	Adrenocorticotropic hormone
ALAT	Alanine aminotransferase
ASAT	Aspartate aminotransferase
AH	Arterial hypertension
GGT	Gammaglutamyl transferase
GT	Glucose tolerance
KG	Blood glucose
ITM	Body mass index
CT	Computed tomography
MetSyn	Metabolic syndrome
NGH	Impaired fasting glycaemia
NGT	Impaired glucose tolerance
NI	Adrenal incidentalomas
OGTT	Oral glucose tolerance test
PC	Uric acid
CMO	Free cortisol in urine
TG	Triglycerides
NMR	Magnetic resonance imaging
CSI	Chemical shift imaging
FLI	Fatty Liver Index
HDL	HDL-cholesterol
HOMA- IR	Homeostasis model assessment insulin resistance
HU	Hounsfield units, Hounsfield units
LDL	LDL-cholesterol
NAFLD	Non-Alcoholic Fatty Liver Disease
QUICKI	Quantitative insulin sensitivity check index
SII	Signal intensity index

I. INTRODUCTION

Adrenal incidentalomas (AIs) are defined as formations arising from the adrenal glands that are discovered incidentally during imaging in conditions unrelated to suspected adrenal disease. The introduction of ultrasound, CT and MRI into clinical practice has increased the ability to detect adrenal tumours. In recent decades, the incidence of these tumors has been found to be relatively high and represent a common adrenal pathology seen by clinicians. In most cases, they are completely asymptomatic and are detected incidentally. Common reasons for imaging studies are abdominal or low back pain, various gastroenterological, hepatic and renal pathology. The term "adrenal incidentalomas" has been adopted because these adrenal tumors are "incidentally discovered." Not surprisingly, with the widespread use of modern imaging techniques, these "diseases of modern technology" are being diagnosed with increasing frequency in modern medicine and have serious economic implications.

The onset and natural course of adrenal incidentalomas are unknown. The cardiometabolic manifestations of these adrenal tumors are also unclear. A growing body of clinical and experimental evidence supports the idea of a fully or partially resolved metabolic syndrome in patients with adrenal incidentalomas. The latter are associated with cardiometabolic health, with an unresolved causal relationship to metabolic syndrome (MS), hyperinsulinaemia and cardiovascular comorbidity. The emerging association between the presence of adrenal incidentalomas and metabolic syndrome raises the question of optimal clinical management of these patients, as well as the therapeutic benefit/risk ratio in the treatment of this indolent pathology.

The incidence of adrenal incidentalomas ranges from 1.4 to 8.7%, increasing with age. The probability of detecting an adrenal adenoma on CT scan of a patient between the ages of 20 and 29 is approximately 0.2%, whereas it is now about 7% in a patient over 70 years of age. The incidence shows no gender dependence, but is higher in Caucasian compared to African race as well as in obese, diabetic and hypertensive individuals. According to autopsy data, the overall incidence of subclinical adrenal tumors is about 2.0%.

Although various criteria have been used to define adrenal incidentalomas over the years, most experts agree on a collective term including incidentally detected adrenal masses ≥10 mm in size, which may be hormone-secreting or non-secreting (hormonally inactive), malignant or benign. Although common, adrenal incidentalomas present diagnostic and therapeutic challenges to the clinician. They must undergo detailed imaging and endocrine evaluation to determine whether they are benign or malignant, functionally active or functionally inactive in terms of hormone secretion.

According to the literature, hormone-secreting adrenal incidentalomas are associated with some of the components of MS, such as atherogenic dyslipidemia, arterial hypertension, abdominal obesity, insulin resistance, as well as increased thrombogenicity and hepatic steatosis, probably through the adverse effects of excess adrenal hormones on various metabolic pathways. A similar relationship has been demonstrated with respect to subclinical cortisol-secreting NIs. The potential relationship between hormone-secreting NIs and MS parameters remains a relatively unexplored topic that is particularly intriguing from a clinical and pathophysiological perspective because of the steadily increasing prevalence of NIs in the aging

population. This dissertation focuses specifically on incidentally detected hormonally inactive benign adrenal adenomas and their metabolic consequences.

II. AIM AND OBJECTIVES

2.1. Objective

To analyze the diagnostic, metabolic, biochemical and hormonal aspects of patients with hormone-reactive adrenal adenomas. To make a contemporary assessment of some additional markers associated with metabolic syndrome,
nonalcoholic steatohepatosis and cardiovascular risk in patients with hormone-
Inactive adrenal adenomas that have passed through a university specialized endocrinology clinic.

2.2. Tasks

1. To determine the frequency of metabolic syndrome and its individual components in patients with adrenal hormonally inactive adenomas and their relationship with anthropometric indices.
2. To investigate some hormonal parameters (serum cortisol - rhythm, SCU, express blockade with 1 mg dexamethasone, aldosterone/renin ratio) and their relationship with age, anthropometry and size in non-secreting adenomas.
3. To compare the frequency of metabolic disorders between patients with hormone-secreting adrenal adenomas and the general Bulgarian population.
4. To determine the relative proportion of newly diagnosed glycemic disorders and the role of insulin resistance in patients with adrenal hormone-reactive adenomas.
5. To investigate fatty liver index (FLI), liver enzymes in patients with hormone-reactive adrenal adenomas and to look for association with adenoma size.
6. To develop a diagnostic algorithm for the search of metabolic disorders in patients with hormonally inactive adrenal adenomas.

III. MATERIAL AND METHODS

3.1. Subject of study

The diagnostic, metabolic, biochemical and hormonal aspects of patients with hormone-reactive adrenal adenomas were studied.

3.2.Object of study

The subject of the study were 105 patients with hormonally inactive adrenal adenomas who underwent the Clinic of Endocrinology and Metabolic Diseases at St. Marina Hospital.

Patients were selected according to well-defined inclusion and exclusion criteria.

Inclusion criteria:

- Persons over 18.

- The presence of unilateral, clearly adrenal-derived tumors
- CT scan performed for a reason other than the tumor formation
- Completed informed consent

Exclusion Criteria:
- Persons under 18
- Presence of malignant oncological diseases
- History of malignancy for a period of 10 years prior to the diagnosis of the adrenal tumour
- Metastatic lesions
- Known type 1 or 2 diabetes mellitus
- Taking antidiabetic medication
- Taking biguanides (metformin)
- Presence of hormonal activity proven clinico-laboratory
- Presence of endocrine hyperony
- Persons who have not completed informed consent

3.2.1.Specific studies

In order to achieve the scientific research goal and to solve the formulated tasks, the data of patients with hormonally inactive adrenal adenomas were studied and analyzed, who were examined according to the standard protocol: history of the disease and concomitant diseases, physical examination, abdominal computed tomography with consideration of the size of the adenoma and its CT characteristics, laboratory studies of biochemical and hormonal indicators. Patients are detailed by sex, age, risk factors.

3.3.Methods

3.3.1. Questionnaire method - All participants were questioned about age, family history of socially significant diseases, presence of incidentally detected adrenal adenoma, its age and medication intake, concomitant diseases and their therapy; smoking.

3.3.2. Anthropometry - Weight was determined to the nearest 0.1 kg using a calibrated digital weighing instrument. Height was measured to the nearest 1mm using a height gauge with the patient standing upright and head held. Waist circumference was measured to the nearest 1 mm with a non-stretchable tape measure placed in a horizontal line dividing the right mid axillary line between the inferior edge of the X rib and the iliac wing. BMI was calculated using a standard formula for each participant: BMI=weight(kg)/height2 (m2)

3.3.3. Clinical Examination - Each participant had a complete clinical examination with a seated blood pressure measurement. Blood pressure measured twice with a mercury manometer on the right arm in the sitting position after a minimum of 5 min rest (European Socieaty of Hypertension Guideline 2014).

3.3.4. Biochemical tests

Blood is drawn from a brachial vein from each person examined. The sampling was performed between 8.00-9.30 am after a 12 hour fast. Clinical chemistry parameters were worked out on an Olympus AU 400 automatic biochemistry analyzer. Plasma glucose was determined by hexokinase method. Serum insulin determined by chemiluminescent immunoassay.

3.3.5. Oral glucose tolerance test

The study was performed after a 12-hour fast. Using was 75 grams of glucose powder dissolved in 300 ml of water taken for 3 to 5 minutes. Biochemical parameters (blood glucose and insulin) were studied at zero(0) and 120 minutes.

3.3.6 .Insulin resistance was assessed by HOMA-IR and **QUICKI** formulas:

1.HOMA-IR(homeostasis model assessment insulin resistance) = (fasting blood glucose in mmol/l x fasting insulin in IU/ml) /22.5.

2. QUICKI (quantitative insulin sensitivity check index)

QUICKI = 1/(log(fasting insulin μU/mL) + log(fasting glucose mg/dL)

3.3.7 Laboratory parameters

Laboratory parameters for hepatic steatosis were determined in appropriate units - ACAT, ALT, GGT, and FLI(fatty liver index).

FLI = (e0.953*loge (triglycerides) + 0.139*BMI + 0.718*loge (ggt) + 0.053*waist circumference - 15.745) / (1 + e0.953*loge (triglycerides) + 0.139*BMI + 0.718*loge (ggt) + 0.053*waist circumference - 15.745) x 100

3.3.8. Hormonal analysis

- Cortisol rhythm 08.00 and 22.00 hours
- Express blockade with 1 mg dexamethasone
- Metanephrines in plasma
- Aldosterone/renin ratio
- SCU-Free cortisol in urine /24 hours

All blood and hormone samples were made in the Central Clinical Laboratory of the University Hospital "St. Marina" Varna

3.3.9. Nutritional status

Participants' nutritional status was assessed based on the WHO classification and stratified into categories:

- underweight (BMI<18.5 kg/m2),
- normal weight (BMI 18.5-24.99 kg/m2),
- overweight (BMI 25-29.99 kg/m2),
- obesity I st. (BMI 30-34.99 kg/m2),
- obesity II st. (BMI 35-39.99 kg/m2),
- obesity III st. (BMI > 40 kg/m2)

3.3.10. Imaging diagnostics - The method used is contrast computed tomography. It shows detailed characterization of the adrenal mass. On CT the typical appearance of adenomas is homogeneous. A density below 2 HU speaks in favor of a benign nature of the adenoma.

3.3.11. Statistical methods

- Analysis of variance
- Variance analysis
- Correlation analysis
- Regression analysis
- Risk assessment analysis
- ROC curve analysis
- Comparative analysis

A graphical and tabular method was used to visualize the results.

For all analyses performed, a significance level of $p<0.05$ was assumed at the 95% confidence interval.

Data were processed using SPSS v.20.0 for Windows.

The clinical study was conducted after obtaining permission from the Research Ethics Committee at the Medical University - Varna - protocol № 108/25.11.2021. All participants or their relatives in the study completed informed consent.

IV. RESULTS AND DISCUSSION

4.1. Incidence of metabolic syndrome, its individual components in patients with adrenal hormonally inactive adenomas and their relationship with anthropometric indices.

The subject of the study were 105 patients with hormonally inactive adrenal adenomas who underwent the Clinic of Endocrinology and Metabolic Diseases at St. Marina Hospital. Over 60 years of age were 41.0%, under 60 years of age were 59.0%. Of the total number of individuals, 39.0% were male and 61.0% were female.

Tab. 1. Characteristics of patients

Indicator		Number/%
Gender	Men	41/39.0 %
	Women	64/61.0 %
Age (years)	mean±SD (range)	53.9±12.3 (24-76)
	< 60 r.	62/59.0 %
	> 60 r.	43/41.0 %

Table 2 presents the characteristics of metabolic syndrome and obesity in patients with adrenal hormone-reactive adenomas.

Table 2. Indicators of metabolic syndrome and BMI, number and percentage of patients fulfilling each of the MetSyn criteria.

Indicators		Number/ %
Waist circumference	mean±SD (range)	94.55±12.13 (68-138)
	men and women with increased waist circumference	86/81.9%
	Men > 94 cm	28/68.3 %
	Women > 80 cm	58/90.6 %
ITM	mean±SD (range)	30.02±5.79 (16.50-58.00)
	> 25 kg/m ^{2}	89/84.7 %
Arterial pressure	> 130/85 mmHg	98/ 93.3 %
Serum HDL	mean±SD (range)	1.44±1.10 (0.7-11.8)
	Men and women with elevated HDL	55/52.4%
	men <1.03 mmol/l	15/36.6 %
	women <1.3 mmol/l	40/62.5 %
Triglycerides	mean±SD (range)	1.79±0.69 (0.39-4.82)
	> 1.7 mmol/l	72/68.6 %
Fasting blood glucose	mean±SD (range)	6.46±1.22 (4.10-10.20)
	> 5.6 mmol/l	80/76.2 %

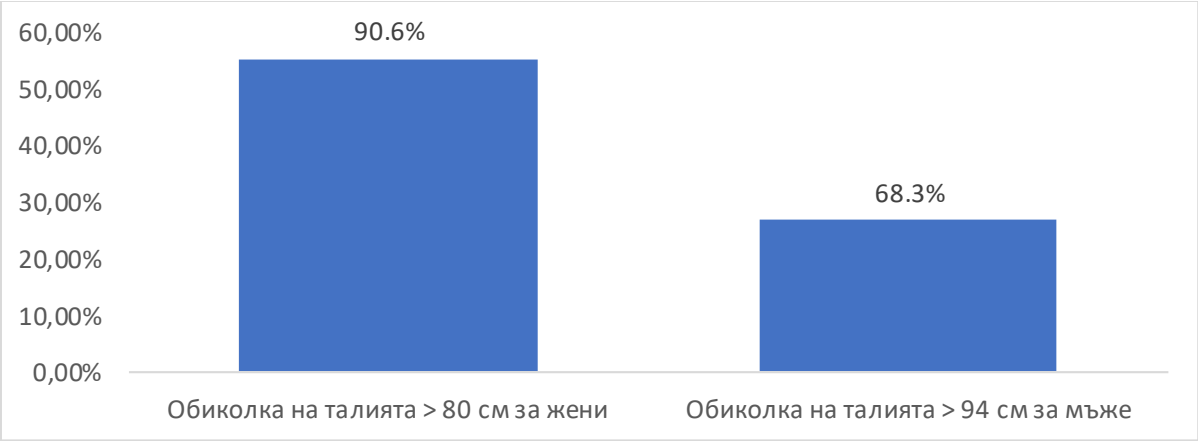


Fig. 1. Proportion of men and women with waist circumference above the norm

There was a correlation between gender and waist circumference. There was a significant difference in the relative proportion of men and women with waist circumference above normal in favour of women with NI ($p<0.001$) (Fig. 1), suggesting that female sex is a risk factor for waist circumference obesity in concomitant adrenal hormonally inactive incidentaloma.

Waist circumference correlated weakly in direct proportion to sex (Table 3).

Tab. 3. Univariate regression analysis of the relationship between waist circumference and sex

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	,376	,366		1,027	,307
	Waist circumference	,011	,004	,266	2,797	,006

a. **Dependent Variable: sex**

Waist circumference was accepted as the first screening sign to determine the possible presence of MetSyn. According to Fernández (80), 73.5% of the study population with adrenal hormone-reactive adenomas had an increased waist circumference.(80) Reincke (195) et al. found in over 55.6% of c NI patients had an increased waist circumference and an increased waist-to-hip ratio.(195) In our sample, an increased waist circumference according to generally accepted criteria for Caucasian and Caucasian race > 94 cm-male, > 80 cm-female (99) was found in 81.9% of our study subjects.

For men 68.3 % , for women against the accepted criteria 90.6 %. Significant difference was found among females compared to males($p<0.001$). In contrast to our results in individua ls with NI, the study by Borisova et al (2) in the general population found no significant difference between women and men in terms of increased waist circumference

Among the examined persons, those with overweight and obesity predominated, with an overall proportion of 84.7% (Table 2). A detailed estimate of BMI in the study cohort is presented in Fig. 2. Our results showed that obese patients were the most frequent (49.52%),

followed by overweight (35.24%), followed by those with normal BMI (13.33%) and least frequent underweight with BMI < 18.5 kg/m (^{2}1.905%).

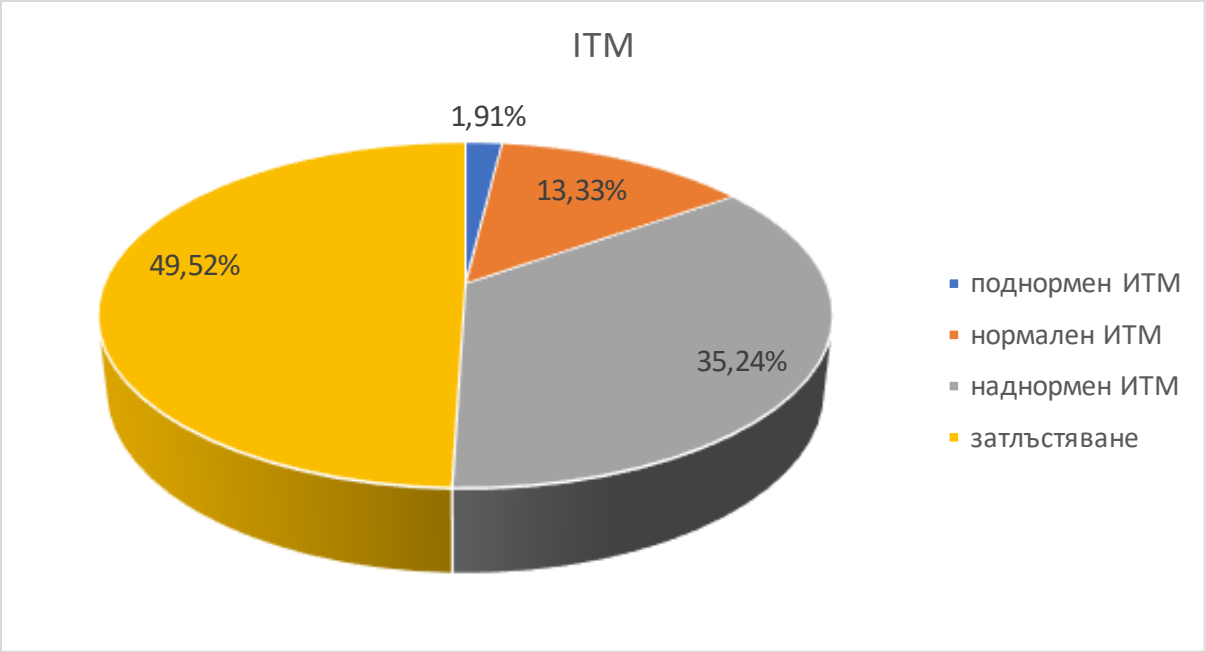


Fig. 2. Distribution of NCD patients by BMI

Similar data were observed in a study by Legierska et al. in which 48.5% of the population with adrenal incidentalomas were obese and another 30.3% were overweight (similar to the results of our study. (132).

In an analysis by Kolańska et al, the mean body mass index in patients studied with adrenal incidentalomas was 28.77 kg/m^2 (SD=4.71), with a 40% prevalence of obesity in the study group. They demonstrated a statistically significant higher prevalence of obesity among NI compared to the general population of Poland (120). In the analysis of data from the study by Vasilev et al. (2014), 33.0% of the studied NI patients were obese. (6)

Arterial hypertension with blood pressure values of 130/85 mmHg or intake of antihypertensive medication was found in the majority of the patients we studied - 93.3%. Only one man and 6 women had normal blood pressure. When the results of a study by Gomibuchi (89) were analyzed, a high prevalence of arterial hypertension was observed in 78.5% of the studied group of persons with NI. Comlekci (51) et al found a significantly lower incidence of AH(54.9%)compared to our results. A Bulgarian study by Vasliev et al (6) also found a high incidence of AH (78%). It found no statistically significant difference in the incidence of AH between patients with subclinical Cushing's and hormonally inactive adenomas.

In addition to being a component of cardiovascular risk, arterial hypertension has also been suggested as a strong predictor of MetSyn in the Bulgarian population. AH as a component of MetSyn was observed in 85% of individuals diagnosed with MetSyn by Borisova et al, a proportion that is similar to that we found in patients with hormone-reactive adrenal adenomas. A population-based study from Eastern India (182) also noted that the criterion with the highest prevalence among MetSyn was arterial hypertension, 63.1%.

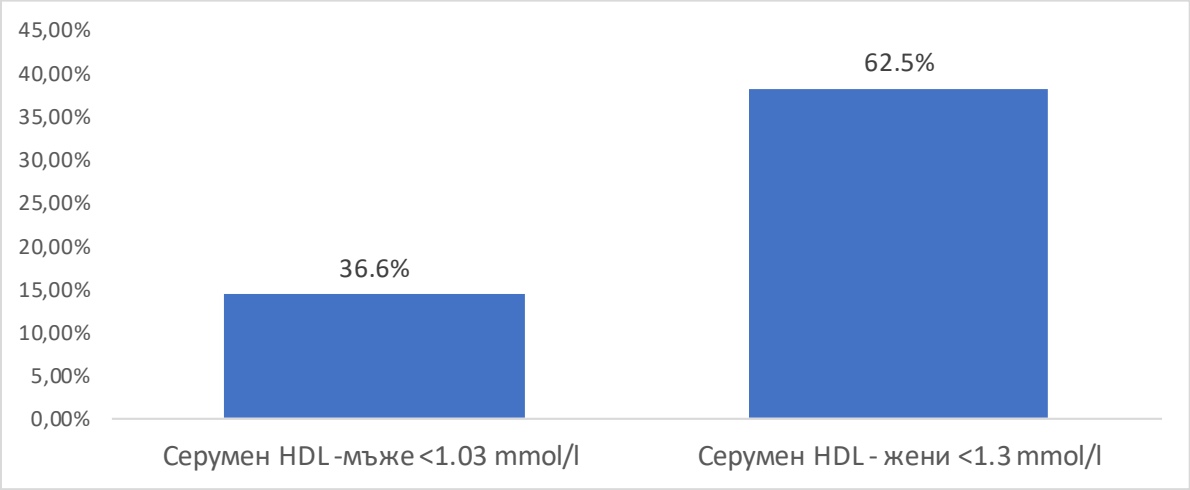


Fig. 3. Relative proportion of men and women with HDL above the norm

The metabolic criterion with the lowest prevalence among those we studied was a decreased HDL-cholesterol level (below 1.03 mmol/l for men and below 1.3 mmol/l for women). In women with adrenal hormonally inactive adenomas we found this lipid disorder more frequently compared to men (Women 62.5%, Men 36.6%, ($p<0.001$)) (Fig 3). Similar results were reported in a Spanish study (209) - women with MetSyn were more often diagnosed with low HDL compared to men with MetSyn ($p<0.001$).

Among the general Bulgarian population, a low HDL level was found in 32.9% (Anna-Maria Borisova and collaborators). Terzolo et al. obtained results similar to ours with a female sex predominance with low HDL values accompanying NI (215). The same collective showed the role of elevated triglycerides in shaping the metabolic profile among the 58.7% NI population (215).

Hypertriglyceridemia (TG > 1.7 mmol/l) was present in 63.8% of MetSin subjects in Bulgaria (Borisova et al.) From our results, similar data were observed, 68.6% for triglyceride levels above 1.7 mmol/l. There was no significant difference between general population and selected patients with adrenal hormonally inactive adenomas.

Longitudinal studies with at least 1 year of follow-up (156, 201) as well as a recent meta-analysis comprising 32 studies (65) have shown an increased prevalence of dyslipidemia in patients with adrenal hormone-reactive adenomas.

Elevated triglyceride levels similar to those in our study (1.79 mmol/l ±0.69 mmol/l) have been reported (215) in addition to elevated total cholesterol, LDL cholesterol, and decreased high-density lipoprotein (HDL) cholesterol levels (195, 213). The accumulating literature on lipid abnormalities in persons with NI suggests that triglyceride and HDL levels set as components of MetSyn are complemented by other lipid profile abnormalities. Our analysis showed a prevalence of hypercholesterolemia of 53.3% among patients with NI , and a significant relative proportion of patients with elevated LDL values of 49.5%.

Fasting blood glucose above 5.6 mmol/l was recorded in 76.2% of subjects with adrenal hormone-reactive adenomas. The involvement of this parameter was essential in the formation of the metabolic profile of the patients in our study. Carbohydrate disorders are discussed in detail in a separate chapter (4.5) because of their significant social and economic role in the

formation of MetSyn. Reimondo (189) et al. demonstrated by multiregression analysis the increased risk of type 2 diabetes mellitus in patients with NI.

From the statistical analysis performed, sex-related BMI, arterial hypertension, total cholesterol, and fasting blood glucose were not correlated among our group of NI patients. Regarding triglycerides, a weak correlation with gender was found.

Table 4. Regression analysis between serum triglyceride levels and sex

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1,158	,132		8,779	,000
	Serum triglyceride levels	,130	,069	,183	1,889	,062

a. Dependent Variable: sex

None of the metabolic syndrome components considered correlated with age.

We found the largest mean NI size in underweight patients, but it should be noted that these were two patientsand an error could be made by a small number of participants. Next, the mean size of the NI in the obese was 32.8 mm, larger than the size of the NI in the normal and overweight, 30.7% and 20.9%, respectively

Table 5. Adenoma size according to BMI

ITM	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Subdimensionally	2	42,50	3,536	2,500	10,73	74,27	40	45
Normal	14	30,71	16,007	4,278	21,47	39,96	10	57
Overweight	37	29,92	9,673	1,590	26,69	33,14	10	51
Obesity	52	32,85	14,753	2,046	28,74	36,95	12	86
Total	105	31,71	13,245	1,293	29,15	34,28	10	86

Table 6. Adenoma size according to waist circumference in men

Waist circumference	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Waist circumference men < 94	77	32,19	13,839	1,577	29,05	35,34	10	86

Waist circumferenc e men > 94 cm	28	30,39	11,583	2,189	25,90	34,88	12	56
Total	105	31,71	13,245	1,293	29,15	34,28	10	86

Tab. 7. Adenoma size according to waist circumference in women

Waist circumference	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Waist circumferenc e women < 80 cm	47	33,55	14,217	2,074	29,38	37,73	12	86
Waist circumferenc e women > 80 cm	58	30,22	12,326	1,618	26,98	33,47	10	71
Total	105	31,71	13,245	1,293	29,15	34,28	10	86

Adenoma size in both sexes was smaller in those with waist circumference above the established norm ($p<0.05$), with no significant difference between men and women.

Table 8 shows the lipid profile characteristics of patients with hormonally inactive adrenal adenomas, with more than half having elevated total cholesterol (53.3%) and triglyceride (68.6%) levels. Serum LDL levels above 3.4 mol/L were observed in 49.5% of the NI patients in our study. Yener et al (238) reported an incidence of LDL-cholesterol-related dyslipidemia of 59% among patients with hormone-reactive adenomas (238) , similar to Comlekci (51) et al 59.6%

Table 8. Lipid profile characteristics

Indicators		Number/ %
Total cholesterol	mean±SD (range)	5.33±1.01 (3.1-9.7)
	> 5.2 mmol/l	56/53.3 %
Serum HDL	mean±SD (range)	1.44±1.10 (0.7-11.8)
	men <1.03 mmol/l	15/36.6%
	women <1.3 mmol/l	40/62.5%

Triglycerides	mean±SD (range)	1.79±0.69 (0.39-4.82)
	≥ 1.7 mmol/l	72/68.6 %
Serum LDL	mean±SD (range)	3.47±0.81 (1.44-6.20)
	> 3.4 mmol/l	52/ 49.5 %

Tab. 9. Characteristics of patients with hormonally inactive adrenal adenomas according to the criteria for evaluation of metabolic syndrome

Indicator	Men with waist circumference > 94 cm (n=28)		Women with waist circumference > 80 cm (n=58)	
Triglycerides (TG)	≥ 1.7 mmol/l	21/75.0 %	≥ 1.7 mmol/l	40/ 69.0 %
Serum HDL	< 1,03 mmol/l	10/35.7 %	< 1,3 mmol/l	14/24.1 %
Blood pressure (BP)	≥ 130/85 mmHg	27/96.4 %	≥ 130/85 mmHg	53/91.4 %
Fasting blood glucose	≥ 5.6 mmol/l	22/78.6 %	≥ 5.6 mmol/l	43/74.1 %

We found a significant difference in the relative proportion of men with serum HDL levels<1.03 mmol/l with waist circumference below and above 94 cm (p=0.001), and there was an inverse moderate relationship between waist circumference in men with inactive adrenal adenomas and serum HDL levels (r=-0.369; p<0.001) (Fig. 4). In men with hormonally inactive adrenal adenomas, a waist circumference >94 cm was found to carry an 8-fold higher risk of lowering serum HDL levels (OR=8.0 (2.43-26.33); p<0.001).

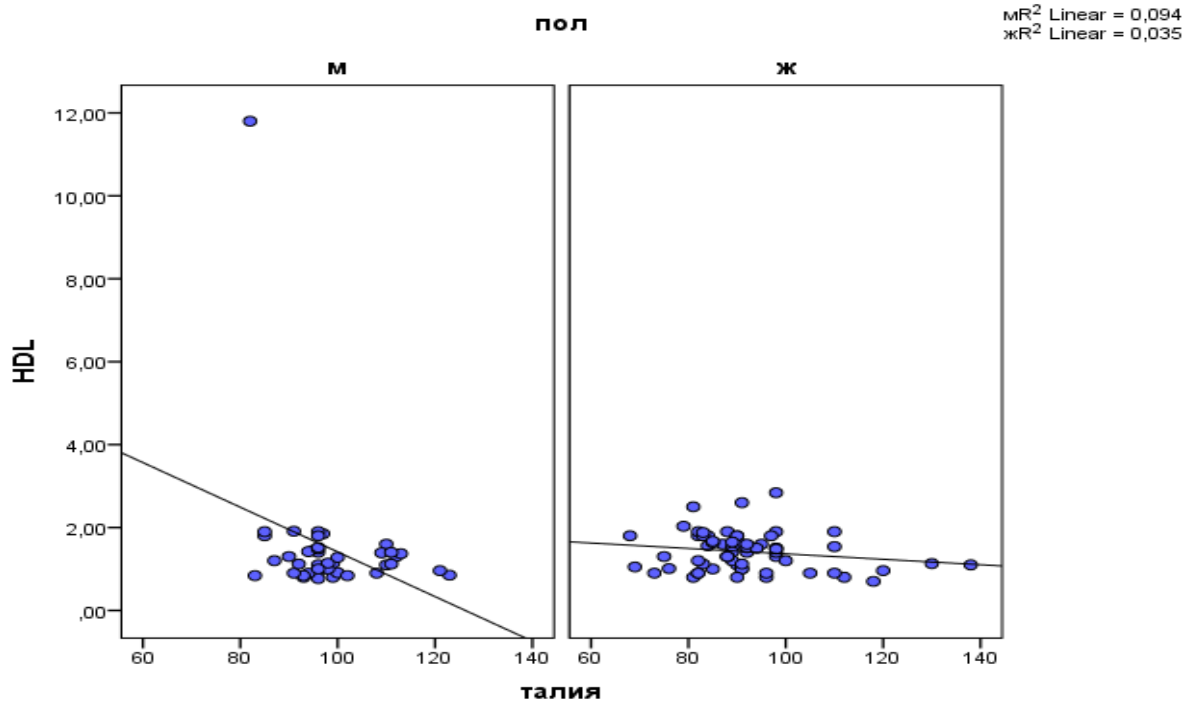


Fig. 4. Correlation analysis between waist circumference and serum HDL levels according to gender

A significant proportion of men with hormonally inactive adrenal adenomas and waist circumference greater than 94 cm had hypertension (96.4%); 78.6% had elevated fasting blood glucose levels. Elevated TG levels were reported in 69.0% of women with waist circumference above 80 cm. In contrast to men, in women decreased HDL levels did not correlate with waist circumference. A significant proportion (91.4%) of women with a waist circumference greater than 80 cm had hypertension. Slightly more than 3/4 (76.8%) of women with hormonally inactive adrenal adenomas with waist circumference greater than 80 cm had elevated fasting blood glucose levels, and there was a linear weak, tending to moderate relationship between the two parameters (r=0.282; p=0.024).

Uric acid is not routinely tested as an indicator in patients with NI.

Monitoring of serum uric acid level along with that of inflammatory and atherogenic markers in patients with metabolic syndrome is particularly important for cardiovascular risk assessment. A number of epidemiological studies have demonstrated an association between serum uric acid levels and a large number of cardiovascular diseases (CVDs), such as hypertension (47) and metabolic syndrome (84); moreover, this correlation has been found not only in hyperuricemia (defined as uric acid values above 464 μmol/l , but also at uric acid levels considered normal, 310-330 μmol/l (77, 161).

To complete the metabolic profile of NI patients, we calculated the mean uric acid value for patients in our study - 423.06±213.52 (111.0-980.0) With values above 464 μmol/l (laboratory norm used for hyperuricemia)) were 30.We found a positive moderate correlation between serum uric acid levels with GGT (r=0.397; p<0.001) (Fig. 5) and with FLI (r=0.293; p=0.002) (Fig. 6).

No correlation was found between serum uric acid levels with BMI nor with adenoma size.

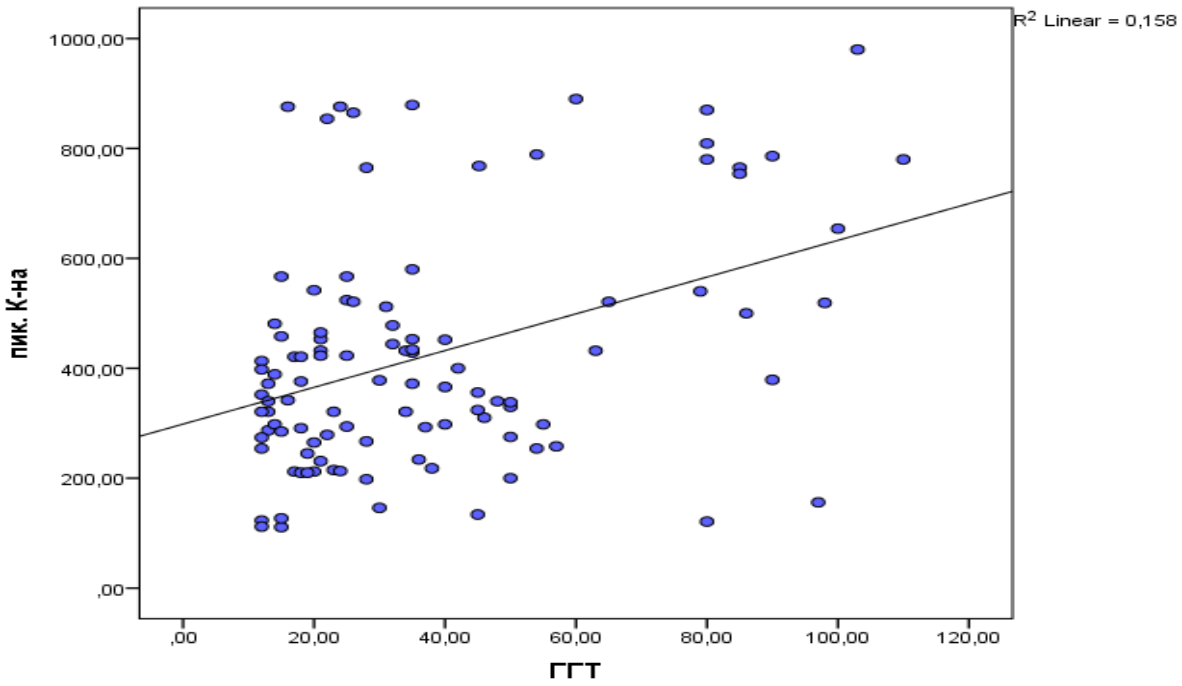


Fig. 5. Correlation between GGT and uric acid

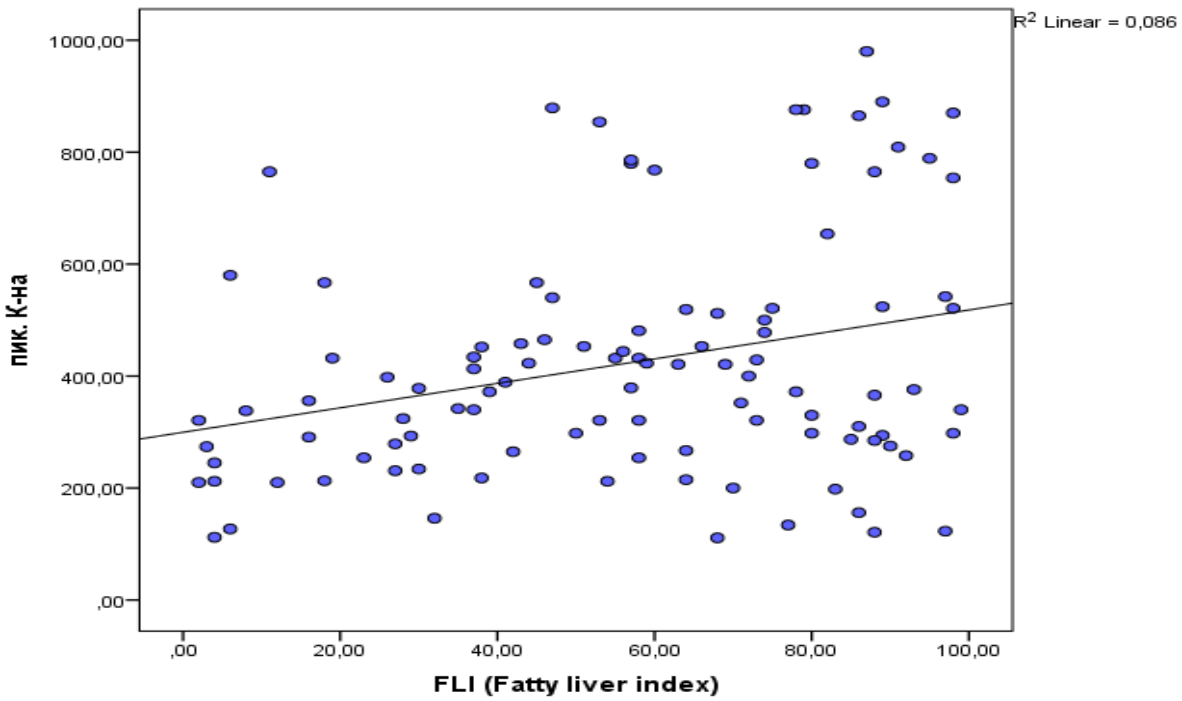


Fig. 6. Correlation between FLI and uric acid

There was a significant difference in uric acid values between men and women with hormonally inactive adrenal adenomas (p<0.001), with a significantly higher mean value in men (Fig. 7).

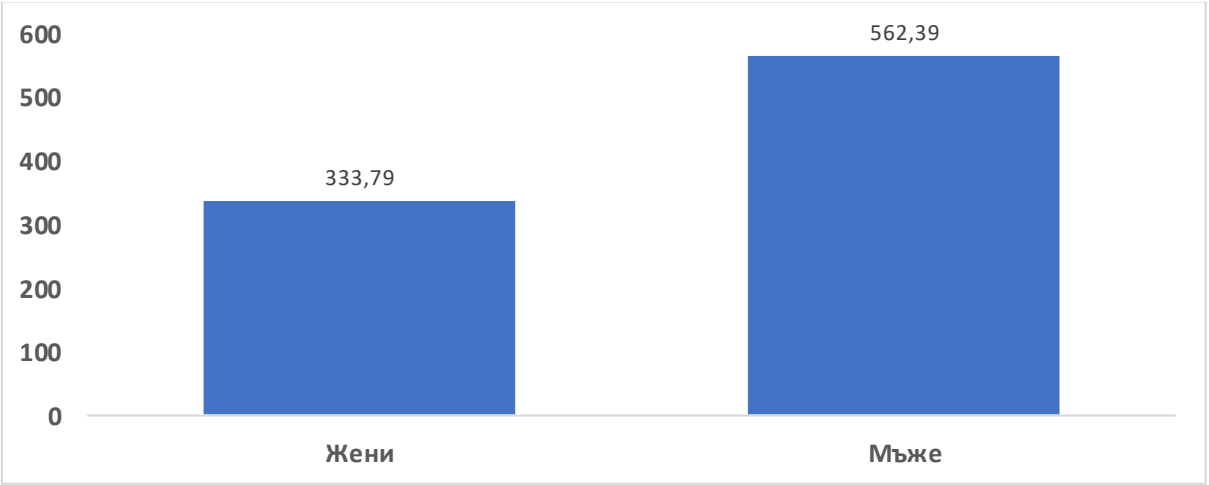


Fig. 7. Mean value of uric acid according to sex

Uric acid correlated moderately with sex (r=0.530; p<0.001), with male sex carrying a 12-fold higher risk for elevated levels above normal (OR=12.72 (4.657-34.757); p<0.001). The mean uric acid levels in patients with hormone inactive adenomas and MetSyn(405 μmol/l) were higher than those in individuals with NI without MetSyn (365 μmol/l), with no statistical significance found (p=0.213) (Fig. 8) There was also a trend for an increase in the mean value of uric acid increase in the number of c of metabolic signs,with no statistical significance .

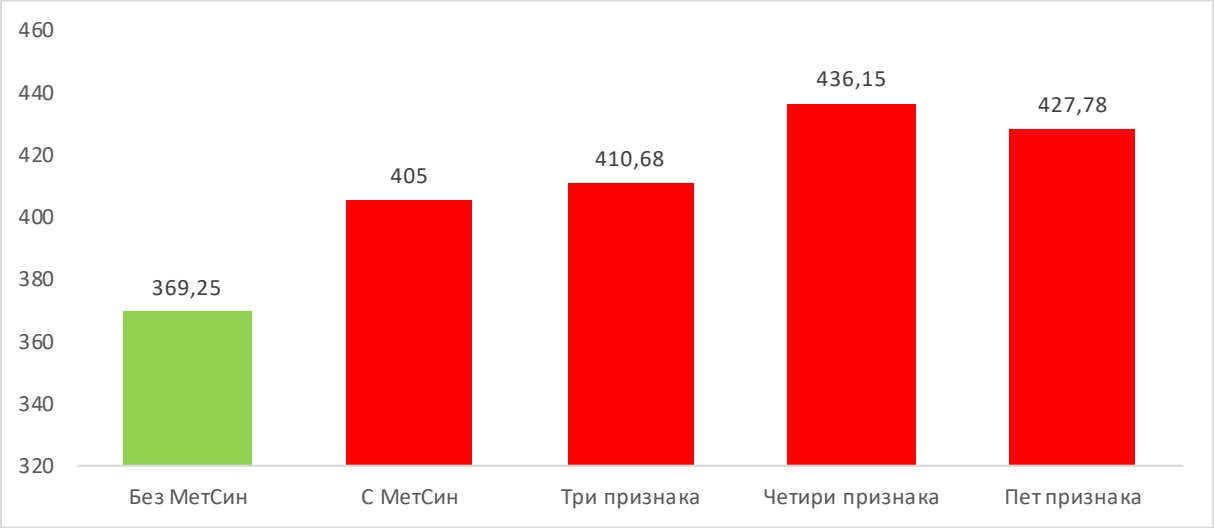


Fig. 8. Mean uric acid value according to MetSyn

The disease that is most often involved in the formation of the metabolic profile of patients with adrenal hormone-reactive adenomas is arterial hypertension - (93.3%); in second place is obesity (BMI over 25 kg/m ²84.7%, in third place in frequency are carbohydrate disorders - 76.2% (found in blood glucose above 5.6 mmol/l) .

Our results showed that obese patients (49.52%) were the most common NCD patients, followed by overweight patients (35.24%). Obesity and overweight are associated with insulin resistance, and elevated insulin could be a factor in the development of benign as well as malignant adrenal lesions. Currently, more than one-third of the world's population is overweight or obese (22, 208). Being overweight carries a threefold higher risk of developing type 2 DM and carbohydrate disorders compared with individuals of normal weight.(14) In addition, 80% of individuals those overweight or obese have already been diagnosed with type 2 DM.(162) Obesity also significantly increases overall mortality,as well as being a cause of a number of cancers. In 2007, 6% of all adenomas and carcinomas were associated with obesity (181), and in 2010, approximately 3.4 million deaths were caused by overweight or obesity (139).

The reported increase in the incidence of obesity may also coincide with the increased recording of adrenal adenomas. Because of the parallel improvement in imaging, it is difficult to prove a causal relationship between obesity and NI based on epidemiological data. (181) Obesity is more common in the female population in developed as well as developing countries. Similarly, insulin resistance is also more prevalent in women (30, 90, 147), and obesity probably increases the risk of NI in the female sex.

A number of epidemiological studies have demonstrated an association between hyperuricaemia and increased cardiovascular risk. A large number of studies have been conducted to determine whether uric acid is independently important in the pathogenetic process of MetSyn. Studies to date have come to different results. Serum uric acid has been shown to be a safe pathogenetic factor in patients with diabetes, arterial hypertension and heart failure (133). The Framingham study argued that hyperuricemia is not an independent risk factor for CVD because it is not independent of hypertension (55). From our study, it was

observed that elevated uric acid level was positively correlated with GGT and liver function index (FLI). Zheng X et al. reported similar results that an elevated uric acid level showed a positive correlation with the prevalence of non-alcoholic fatty liver disease (NAFLD) (250) Elevated serum uric acid is also a powerful predictor for the development of obesity (149) and hepatic steatosis (131, 144, 178, 196, 204, 233)

Further prospective studies are needed to clarify whether patients with NI and an altered metabolic profile have increased cardiovascular morbidity and mortality as has been demonstrated in the general population (123).

Such data will be critical in selecting optimal treatment for these patients, which currently remains largely empirical (29, 49, 53, 115, 191, 214, 245).

4.2 Results of hormonal investigations (serum cortisol rhythm, SCU, express blockade with 1 mg dexamethasone, aldosterone/renin ratio) and their relationship with age, anthropometry and tumour size in individuals with non-secreting adrenal adenomas.

The mean morning serum cortisol in our sample was 369.1±110.7 (101-613.1 nmol/L). There were no individuals with morning cortisol below 118 nmol/L nor those with levels above 618 nmol/L We found a mean evening serum cortisol of 101.1±33.1 (23.68-181.0 nmol/L). In 42 (40%) of the patients studied, an evening cortisol above 150 nmol/L was reported According to the European Society of Endocrinology clinical guidelines for the management of adrenal incidentalomas, the most informative screening test for the detection of autonomic cortisol secretion is considered to be express blockade with 1 mg of dexamethasone (76). It is recommended in all cases of incidentally detected adrenal incidentalomas, even without clinical signs of hypercortisolism and with a normal cortisol rhythm. All subjects suppressed morning cortisol after 1mg of suppression dexamethasone test below 50 nmol/L

The mean value of free cortisol in 24-h urine was 167.24±58.26 (37.70-392.00 mkg/24 h). Thus, we ruled out autonomous glucocorticoid hypersecretion in all our patients with adrenal incidentalomas by examining the 24-hour total cortisol excretion, the presence of a preserved circadian rhythm of serum cortisol, and its suppression in the course of the suppression test with 1 mg of dexamethasone.

A number of studies among such hormone-reactive adrenal adenomas have shown an increased incidence of cardiovascular and metabolic diseases such as hypertension, type 2 DM, obesity, and MetSyn (52). This increased cardiovascular risk was confirmed by the results of our study, in which patients with adrenal adenomas had significantly higher BMI and had worse metabolic parameters (higher blood glucose, triglycerides, and low HDL) compared with the general population. According to the literature, even clinically inactive tumors have the capacity for hormone production and express all the enzymes of steroidogenesis (155). These two reasons to consider the study of streoid precursors in NI patients as well, to look for early and subclinical disturbances in steroidogenesis. The levels of 11-deoxycortisol, 17-OH progesterone, DCEAC, and various 11-deoxycortisol/cortisol and 17-OH-progesterone/11-deoxycortisol ratios could be investigated for research purposes.

The mean aldosterone/renin ratio in our patients was 2.62±0.94 (1.20-4.60) and within the reference range. Plasma metanephrine and normetanephrine levels were also within normal limits, thus definitively assuming that the sample consisted of only hormone-inactive NI.

Individuals with abnormal indices, with or without clinical manifestation of hypersecretion were primarily excluded from the analysis (see exclusion criteria).

Tab. 10 presents the results of hormonal tests as well as some anthropometric indices of the patients. One of the main characteristics of adrenal adenomas is their size. Among the 105 patients with hormonally inactive adrenal adenomas who were examined at the Clinic of Endocrinology and Metabolic Diseases of St. Marina Hospital, 72.4% had adenoma size less than 40 mm, and 27.6% had adenoma size more than 40 mm. The mean adenoma size was 31.71 ±13.24 mm ranging from 10-86 mm . There were no significant differences in the two sexes with respect to localization. 48% of patients had adenoma located on the left,and 52% on the right. No difference was also found in the distribution among genders with respect to localization. Vassilev et al (6) also found no difference in terms of localization(left right NBW) in both sexes. Age wise we divided the patients into two categories above 60 years were 41.0%, below 60 years were 59.0%. Their mean age was 53.9±12.3 from 24 to 76 years. Patients of fifth,sixth decade predominated in our sample. Vassilev (6) found in a retrospective study,that NI was most commonly found in the sixth and seventh decades.

Tab. 10. Characteristics of patients by hormonal and atropometric data

Indicator		**Number /%**
Gender	Men	41/39.0 %
	Women	64/61.0 %
Age (years)	mean±SD (range)	53.9±12.3 (24-76)
	< 60 г.	62/59.0 %
	> 60 г.	43/41.0 %
Weight (kg)	mean±SD (range)	87.4±18.4 (45-161)
BMI (kg/m^2)	mean±SD (range)	30.02±5.79 (16.50-58.00)
	Underweight	2/1.9 %
	Normal weight	14/13.3 %
	Overweight	37/35.2 %
	Obesity	52/49.5 %
Waist (cm)	mean±SD (range)	94.55±12.13 (68-138)
	> 80 cm for women	58/90.6%
	> 94 cm for men	28/68.3%
Cortisol mean±SD (range)	Sutreshen (118-618 nmol/L)	369.1±110.7 (101-613.1)
	< 118	0
	> 618	0
	Evening (up to 150 nmol/L)	101.1±33.1 (23.68-181.0)
	> 150	42/40.0 %
	SCU (55-286 mkg/24 h)	167.24±58.26 (37.70-392.00)
	< 55	3/2.90%
	> 286	1/1.00%
Aldosterone/renin ratio	mean±SD (range)	2.62±0.94 (1.20-4.60)
Adenoma size (mm)	mean±SD (range)	31.71±13.24 (10-86)
	< 40 mm.	46/72.4 %
	> 40 mm.	29/27.6 %
Smoking	Yes	73/69.50 %

Smoking does not stand out as a risk factor for increasing adenoma size. No difference in age was found between men and women.

There was no difference in BMI according to gender, with the majority of patients falling into the overweight and obese group.

There was no statistically significant difference in cortisol levels according to sex, although lower cortisol values were reported in men (Fig. 9). There was no difference in the aldosterone/renin ratio according to sex (2.65 in women and 2.58 in men, respectively, p=0.457 r= 0.112). There was no difference in adenoma size according to sex (31 mm. in women and 32.8 mm. in men, respectively, p=0.310 r= 0.536).

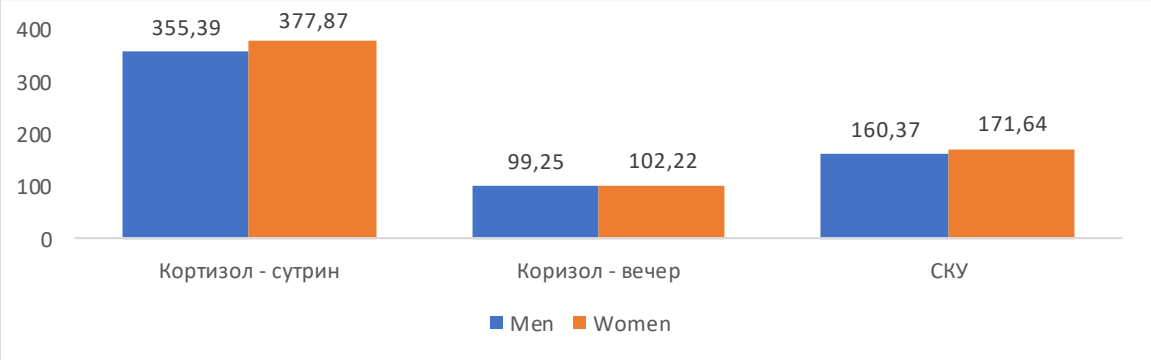


Fig. 9. Mean cortisol levels according to sex

We found no relationship between age and morning cortisol level. Regarding evening cortisol levels and age, we found a positive correlation (r=0.281; p=0.004), i.e., as age increased, evening cortisol levels increased, illustrated in Fig. 10. There is a weak positive correlation between age and WCS, without reaching statistical significance.

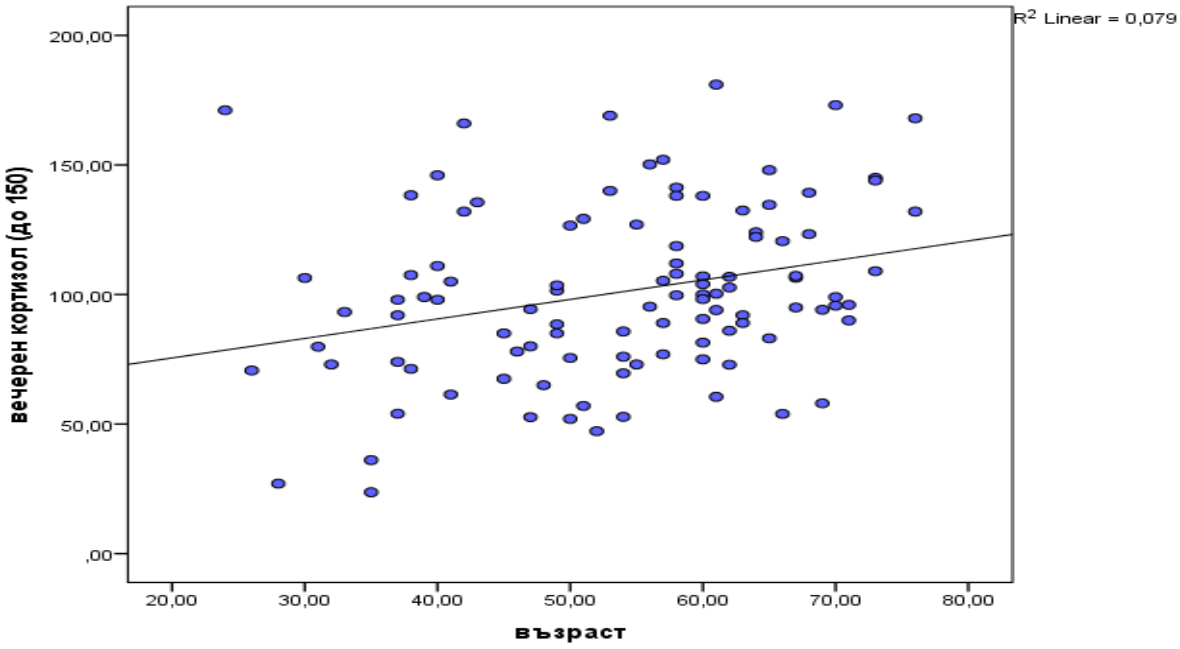


Fig. 10. Relationship between age and evening cortisol levels

The correlation analysis demonstrated a direct positive relationship between adenoma size and evening cortisol (p=0.051, r=0.088), and between adenoma size and WCS (p=0.03 r=0.014). Already in one of the first studies in this country of fat and carbohydrate metabolism in Cushing's syndrome, it was shown that upper-bound cortisol levels have subsequent impairments in glucose disposal. (13). Studies have shown that evening cortisol, even upper-limit cortisol, plays an important role in triggering metabolic disturbances in this group of patients with hormone-reactive adenomas. 40% of patients with adrenal hormone-reactive adenomas studied had evening cortisol above normal but subsequently suppressed in the course of a suppression test with 1 mg dexamethasone. Patient age correlated negatively with aldosterone/renin ratio (r=-0.281; p=0.004), suggesting that aldosterone/renin levels decrease with increasing age (Fig. 11). Despite the decrease in aldosterone/renin ratio, 93.3% of patients had arterial hypertension.

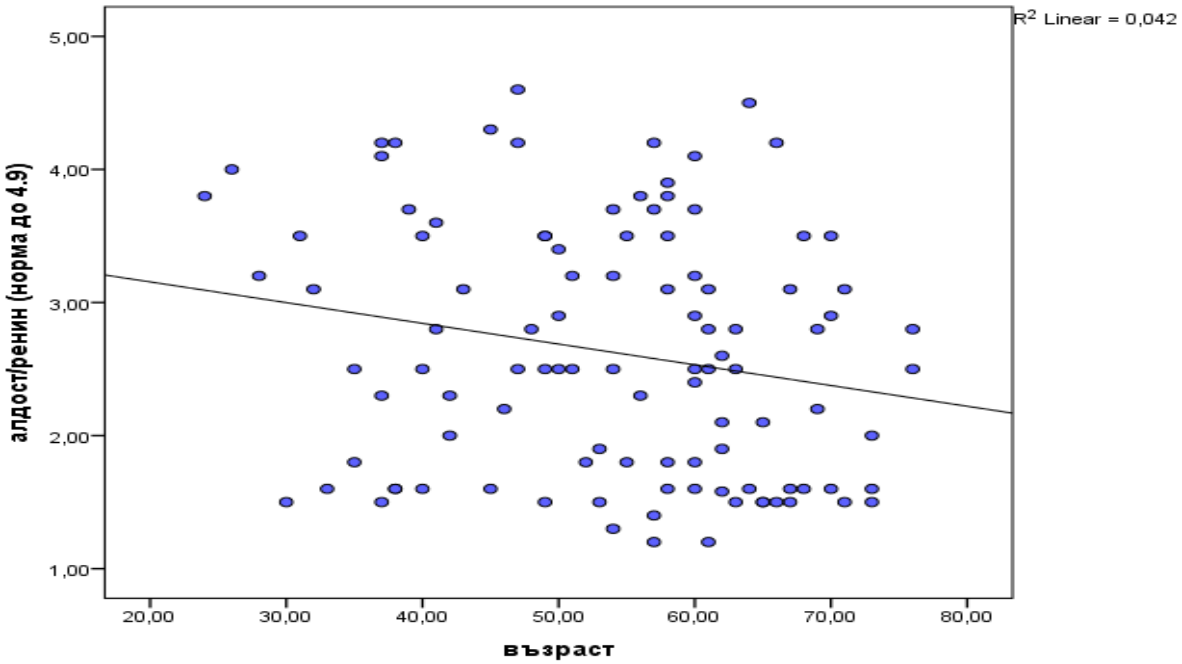


Fig. 11. Relationship between age and aldosterone/renin ratio

The age of the patients did not correlate with the size of the adenoma. Adenoma size had a positive effect on cortisol levels and no effect on the aldosterone/renin ratio. Tumor size in patients with cortisol-producing adenomas typically ranges from 2 to 5 cm. (247). The mean incidentaloma size was 41.71±14.25 mm (23-56 mm) in our group with evening cortisol above 150 nmol/L. The mean adenoma size in the SCU group was 51.71±14 mm (26-65 mm) above the mean laboratory normal. It is assumed that adrenal formations must have a certain critical mass to be able to produce a sufficient amount of cortisol to result in the manifestation of clinical symptomatology. The size of the adrenal mass is thought to correlate positively with the likelihood of upper-bound evening cortisol levels (247).

4.3. Comparison of the frequency of metabolic disorders between patients with hormone-secreting adrenal adenomas and the general Bulgarian population.

Figure 12 shows the distribution of the patients we studied according to the number of metabolic traits.

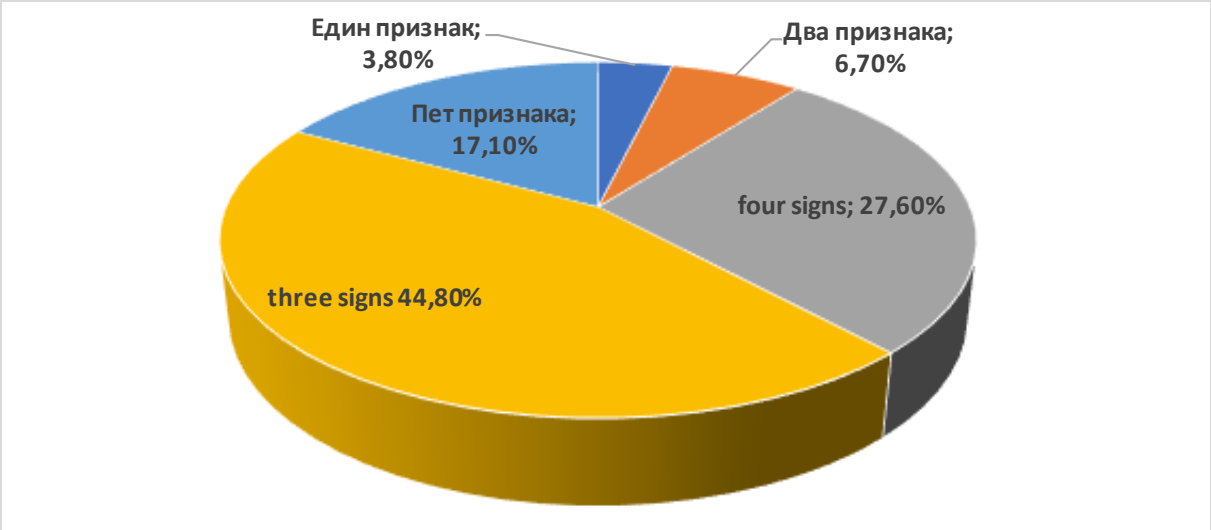


Fig. 12. Distribution according to the number of MetSyn features

No individuals were identified without any MetSyn criteria. It is noteworthy that patients in the present study were predominantly those with three features (44.8% of all subjects and 50.0% of MetSyn patients, respectively). In the national study by Borisova et al (43), those with three signs accounted for 17.7% of the total population and 49.6% of MetSyn subjects, respectively ($p<0.05$). In the cited study, the diagnosis of MetSyn by three criteria was also prevalent.

In our study, MetSyn diagnosed by three features was the most common (50.0%). The fewest individuals met all 5 criteria of MetSyn (19.1%). The cited population-based study in our country in 2012 reported the following distribution of individuals with MetSyn: covering three features- 49.6%, four features- 36.8% and with 5 features- 13.6%(1) . A Greek study in 2005 reported a similar arrangement, with 61% covering three signs, 29% covering 4 signs and 10% covering 5 signs (26).

When comparing the results according to the number of signs, some differences are found - Table 11. The relative proportion of persons with three criteria among all persons with NI we studied was higher compared to the results of Borisova et al. (44.8% to 17.7%; $p<0.05$). The relative proportion of persons with MetSyn with three features in Ana-Maria Borisova's study was similar to our results (49.6% to 50.0%, respectively; $p=0.102$).

Tab. 11. Comparative analysis of the number of signs of MS

Research	Metabolic syndrome (MS)	Three sign	Four sign	Five sign
Own research	Of all persons examined	44.8%	27.6%	17.1 %
	From persons with MS	50,0%	30,9 %	19.1%
	Of all persons surveyed (n=1967)	17.7%	13.1 %	4.9 %

Ana-Maria Borisova	Of persons with MS (n=702)	49.6%	36.8%	13.6%
	P1 of all examined persons	<0.05	<0.01	< 0.01
	P2 of persons with MS	0.102	<0.05	< 0.05

When comparing the proportion of individuals with the four traits in the self-reported (at NI) versus population-based study, there was a significant difference (27.6% versus 13.1%; p<0.01). A similar difference was found in the analysis of MetSyn patients, favoring persons with NI and MetSyn versus those with MetSyn alone from the population data (30.9% versus 36.8%; p<0.05).

In the group of persons with five features, a significant difference was observed both in the total group (17.1% to 4.9%, respectively; p<0.01) and in persons with MetSyn (17.1% to 13.6%, respectively; p<0.05).

On Tab. 12 presents the results of the comparative analysis of individuals according to BMI. A significant difference in the two studies was found only in the group of normal subjects (13.3% to 28.1%; p<0.05) and obese subjects (49.5% to 33.2%; p<0.05).

Normal body weight was found significantly more often in the general population compared to patients with established NI. It is likely that NI, although non-secreting, has an adverse effect on body weight, as confirmed by the significant difference in the proportion of obese individuals with BMI ≥ 30.0 kg/m ^{2} (49.5% in NI vs. 33.2% in the general population, p<0.05).

Tab. 12. Comparative analysis of the distribution of the examined persons according to BMI

BMI (kg/m^2)	Own study (n=105)	Borisova et al. (n=1958)	P value
< 18.5	2/1.9%	31/1.58%	>0.05
18.5 - 24.99	14/13.3%	552/28.1%	<0.05
25.0 - 29.99	37/35.2%	725/37.0%	>0.05
≥ 30.0	52/49.5%	650/33.2%	<0.05

The adverse trend that the presence of NCDs adds in terms of body weight is significant, as obesity is one of the leading preventable causes of death worldwide. (258,259). Large US and European studies have shown that the risk of death is lower at BMIs between 20 - 25 kg/m (^{2}260,261). It is clear that BMI, despite its limitations relative to waist circumference, is a rapid, inexpensive and important diagnostic tool.

There was a significant difference in BMI according to the number of MetSyn criteria (p=0.026), with the highest BMI observed in individuals meeting four criteria (31.34 kg/m ^{2}) (Fig. 13).

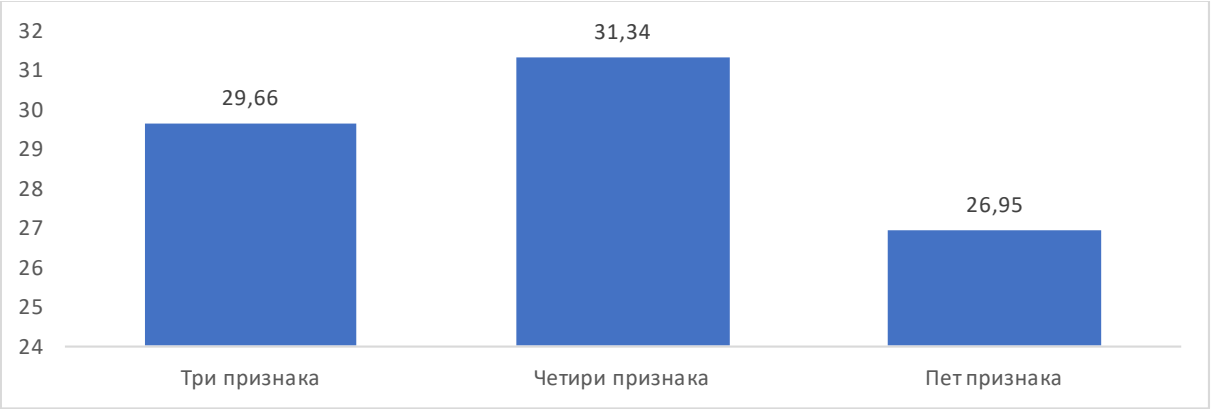


Fig. 13. Mean BMI according to the number of MetSyn criteria

On Tab. 13 a comparative analysis of dyslipidemic subjects is presented, and the results show that there is a significant difference in all indicators between the two cohorts - our NCD patients and the participants with the national study of Borisova et al. ($p<0.05$).

Tab. 13. Comparative analysis of dyslipidemia

Indicator		Own study (n=105)	Ana-Maria Borisova (n=1967)	P value
HDL - cholesterol	Abnormal level (< 1.3 women < 1.03 men)	55 (52.4%)	648 (32.9 %)	< 0.05
	Normal level (> 1.3 women > 1.03 men)	50 (47.6%)	1319 (67.1%)	< 0.05
Triglycerides	Abnormal level (> 1.7 mmol/l)	72 (68.6%)	663 (33.7%)	< 0.05
	Normal level (< 1.7 mmol/l)	33 (31.4%)	1304 (66.3%)	< 0.05

The results show that the group of subjects with abnormal HDL-cholesterol and triglyceride levels dominated the relative proportion of subjects in the self-reported study (52.4% to 32.9% for HDL-cholesterol and 68.6% to 33.7% for triglycerides, respectively), suggesting an additional adverse effect of NI on lipid profile.

The results in Figure 14 show that there was a significant difference in the relative proportion of individuals with low HDL with MetSyn (92.7% to 60.2%; $p<0.05$) and without MetSyn (7.30% to 39.8%; $p<0.05$). MetSin subjects dominated the relative proportion of patients in the self-reported study, whereas the non-MetSin group dominated those in the Borisova study. These data suggest persons with NI and low HDL-cholesterol more frequently deployed MetSyn than persons without NI but with established low HDL-cholesterol.

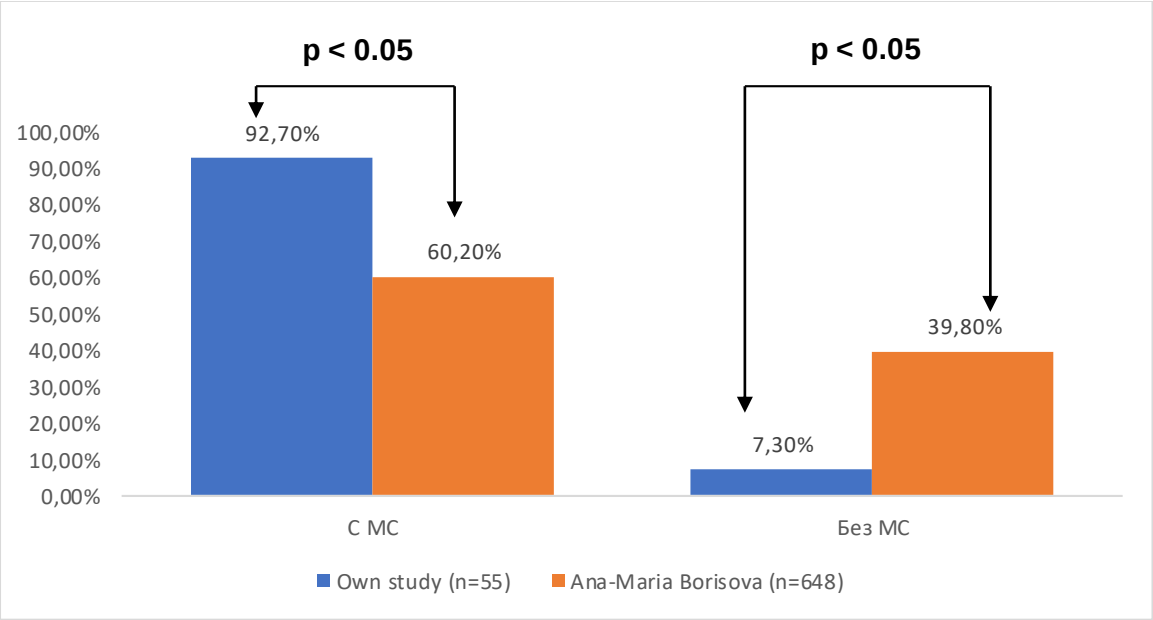


Fig. 14. Comparative analysis of the relative proportion of persons with low HDL in patients with and without MS

Figure 15 shows the distribution of patients according to sex, number of MetSyn features and low HDL. There was no significant difference between the two sexes. There was the highest relative proportion of individuals with four signs.

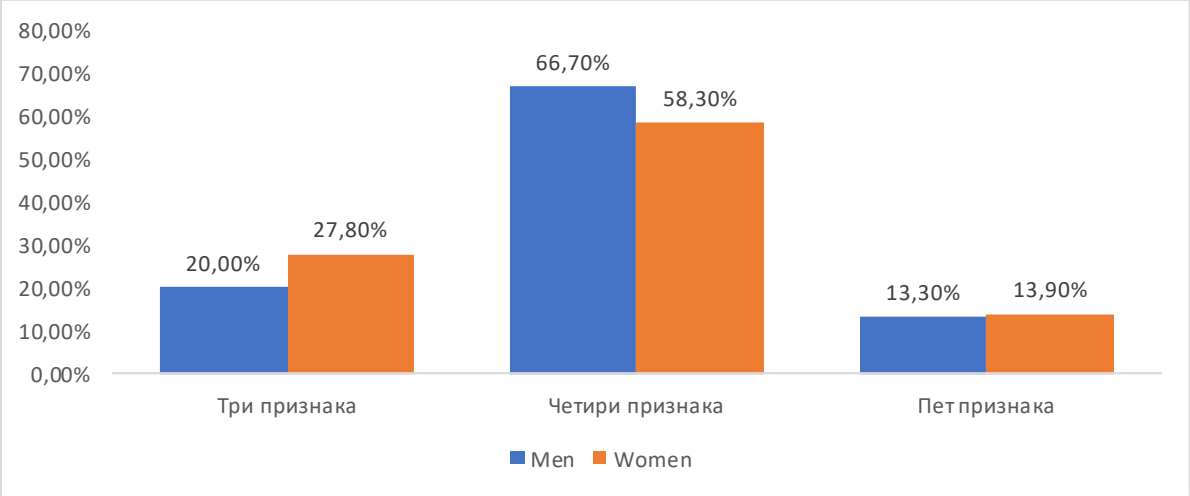


Fig. 15. Distribution of men and women with low HDL according to MetSyn

Tab. 14 presents a comparative analysis of hypertriglyceridemia, finding a difference between the two studies. The relative proportion of individuals with triglycerides >1.7mmol/l in the total group was significantly higher in the self-reported study (68.6% to 33.7%; $p<0.01$). On the other hand, in the study by Borisova et al (262), men with hypertriglyceridemia predominated (64.9% to 43.1%; $p<0.05$), whereas in the own study women had a higher relative proportion (56.9% to 35.1%; $p<0.05$).

Tab. 14. Comparative analysis of hypertriglyceridemia

Triglycerides > 1.7mmol/l	**Own research (n=105)**	**Ana-Maria Borisova (n=1967)**	**P value**
Total	72/105 (68.6 %)	663/1967 (33.7 %)	< 0.01
Proportion of men among persons with hypertriglyceridemia	31/72 (43.1 %)	430/663 (64.9 %)	< 0.05
Proportion of women among persons with hypertriglyceridemia	41/72 (56.9 %)	233/663 (35.1 %)	< 0.05

Table 14 shows that gender plays a role on triglyceride levels. In women, the incidence of hypertriglyceridemia was significantly higher in the presence of NI compared to the general population (56.9% vs 35.1% p < 0.05). Conversely, hypertriglyceridemia was significantly more common in men in the general population compared to men in our sample (64.9% vs. 43.1%; p<0.05).

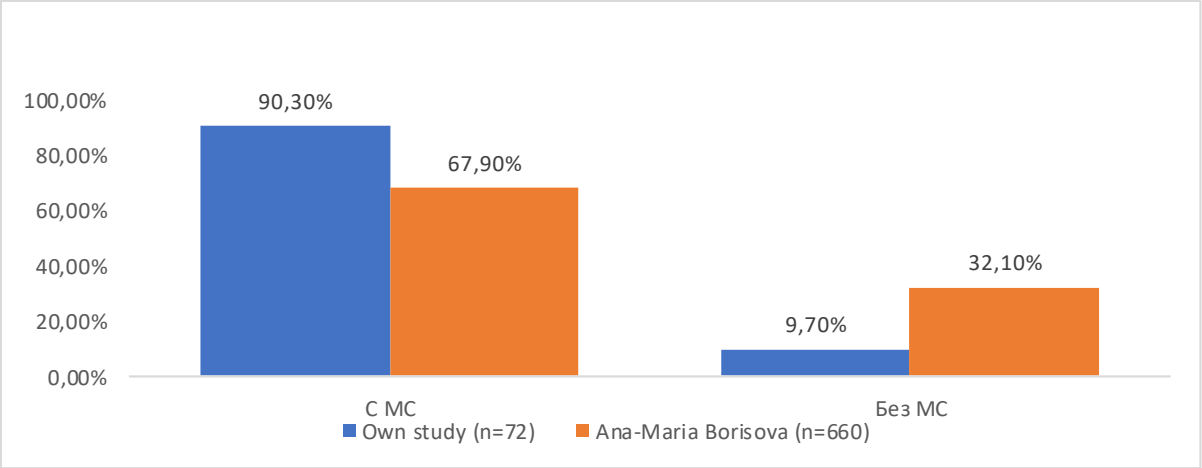


Fig. 16. Comparative analysis of the relative proportion of persons with hypertriglyceridemia in patients with and without MS

The results in Figure 16 show that there was a significant difference in the relative proportion of individuals with hypertriglyceridemia in both the non-MetSyn group (p<0.01) and the MetSyn group (p<0.05) between the two studies. In the MetSin group, the relative proportion of subjects in the own study predominated, whereas in those without MetSin, the relative proportion of subjects in the study by Borisova et al.

Figure 17 shows the distribution of NI patients according to the number of MetSyn signs and hypertriglyceridemia. Individuals with three signs had the highest relative proportion (50.8%).

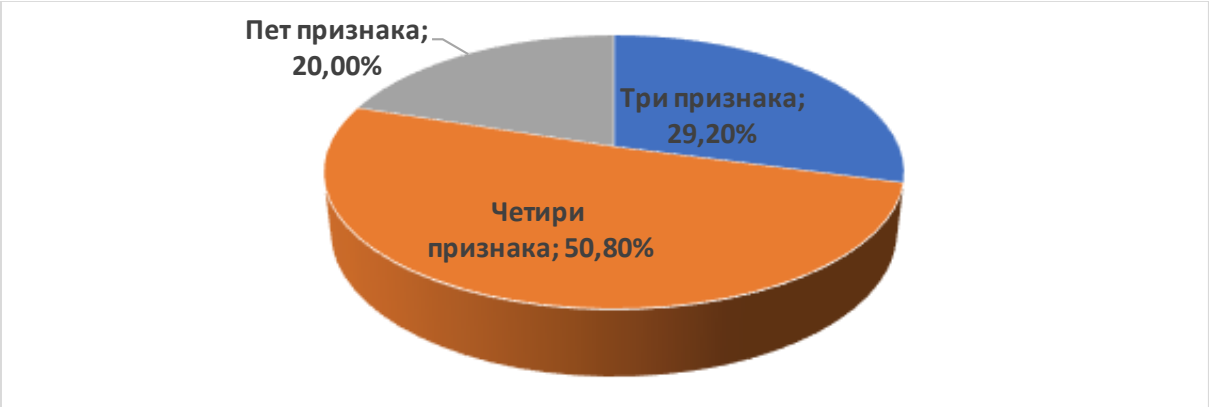


Fig. 17. Distribution of persons with hypertriglyceridemia according to the number of signs

On Tab. 15 presents a comparative analysis of the subjects according to the blood glucose level > 5.6 mmol/l in the two studies. In both the own study and the Borisova study, there was a difference in the relative proportion of subjects with MetSyn and blood glucose > 5.6 mmol/l (86.2% for the own study and 75.8% for the Borisova study, respectively), with no static significance. Similarly, among subjects with elevated glucose but without MetSyn, there was no statistical difference in the proportions in the own and population study - Tab. 15 and Fig 18.

Tab. 15. Comparative analysis of subjects according to blood glucose level > 5.6 mmol/l

Indicator	Own study (n=80)	Ana-Maria Borisova (n=495)	P value
With MetSyn	69/ 86.2 %	375/ 75.8 %	>0.05
Without MetSyn	11/ 13.8 %	120/ 24.2 %	>0.05
P value	0.042	< 0.05	

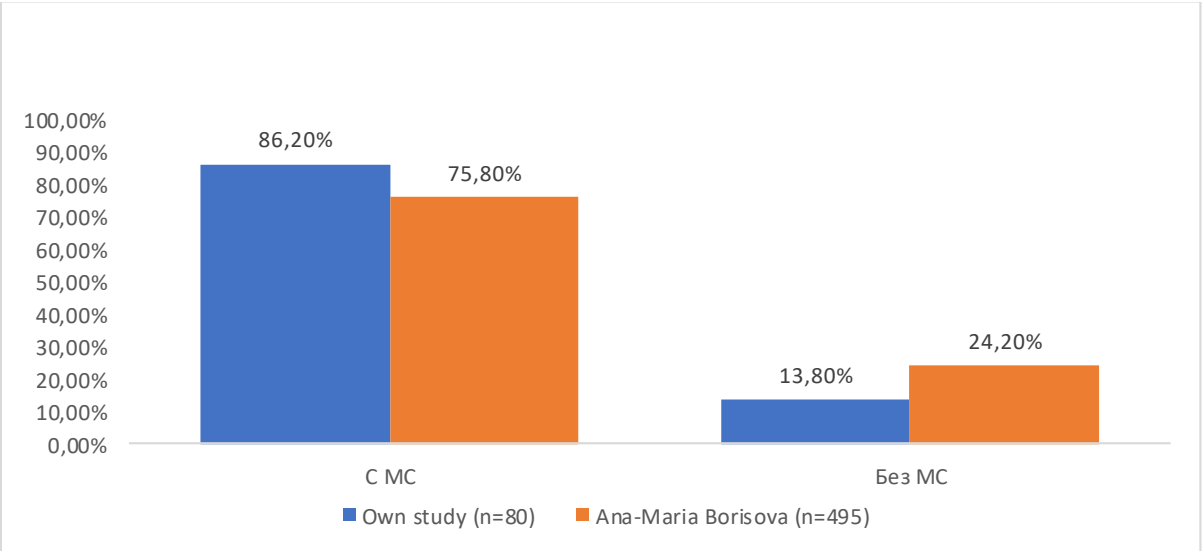


Fig. 18. Comparative analysis of the relative proportion of persons with blood glucose > 5.6 mmol/l in patients with and without MS

According to the results presented in Fig. 19, more than half of the individuals with blood glucose > 5.6 mmol/l and MetSyn available, covered it in four features (50.80%).

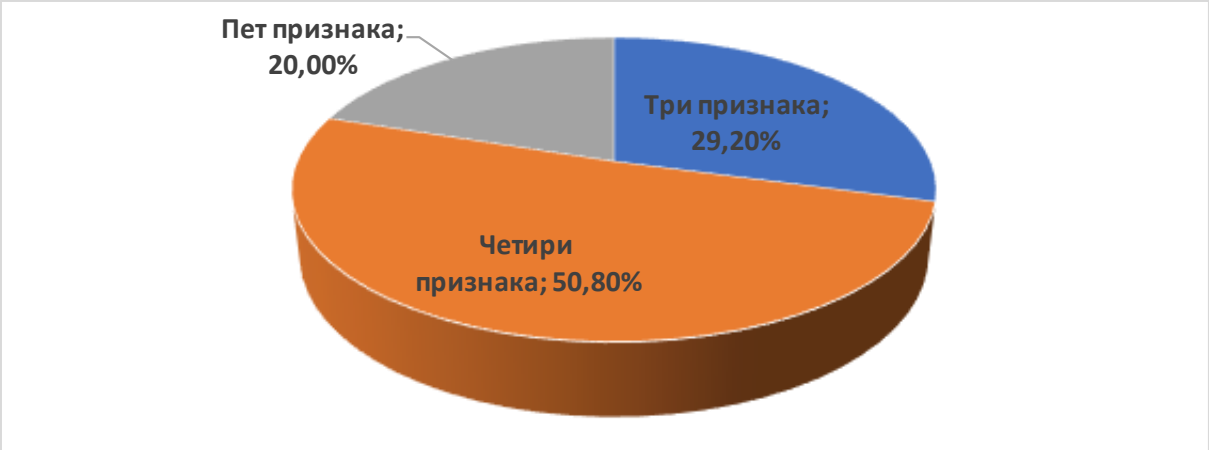


Fig. 19. Distribution of persons with blood glucose > 5.6 mmol/l and MetSyn coverage according to the number of MetSyn criteria

On Tab. 16 presents a comparative analysis of persons with arterial hypertension in the two studies. The results show that there is a significant difference among patients in the own study (p=0.024), with 91.8% of hypertensive patients covering MetSyn, whereas no significant difference was found in the Borisova study, i.e. the presence of hypertension in the general population does not necessarily imply the presence of deployed MetSyn.

Tab. 16. Comparative analysis of examined persons according to AH

Indicator	Own study (n=98)	Ana-Maria Borisova (n=1112)	P value
With MetSyn	90/91.8 %	597/53.7 %	<0.05
Without MetSyn	8/8.2 %	515/46.3 %	<0.001
P value	0.024	> 0.05	

According to the results presented in Figure 20, there was a significant difference in the relative proportion of individuals with arterial hypertension and MetSyn between the two cohorts (p<0.05). In persons with NI, the combination of hypertension and MetSyn was significantly more frequent than in the general population (91.8% to 53.7%; p<0.05). The likelihood of persons with NI and hypertension not having MetSyn was 8.2% versus 46.3% in the general population, highlighting the adverse metabolic effect of NI (8.2% versus 46.3%; p<0.001).

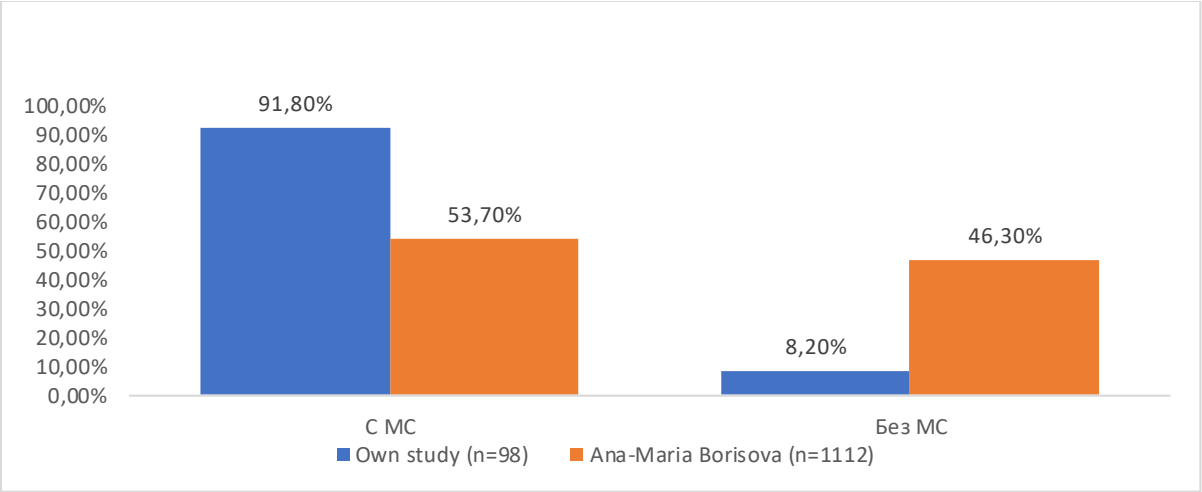


Fig. 20. Comparative analysis of the relative proportion of persons with hypertension in patients with and without MS

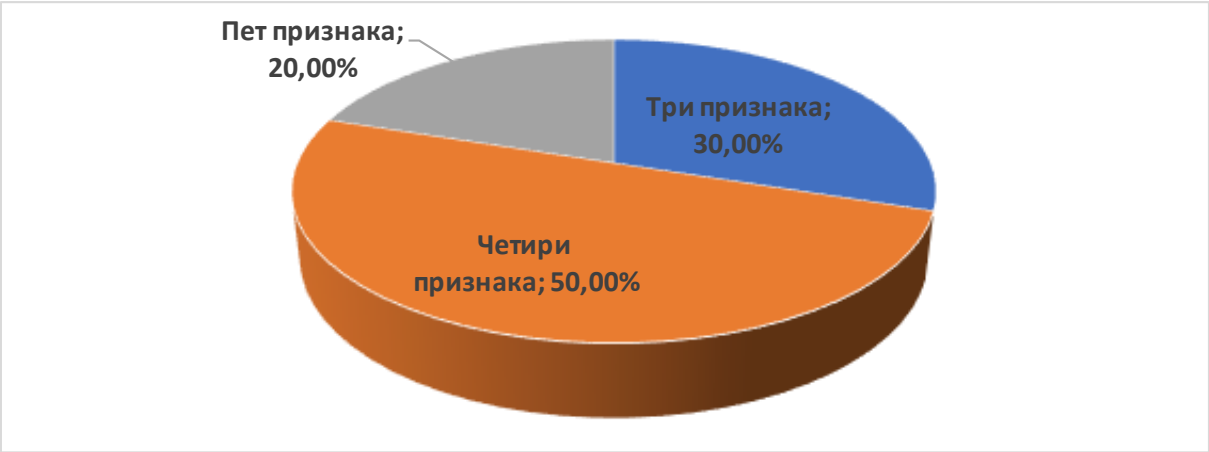


Fig. 21. Distribution of persons with hypertension according to the number of signs

Half of the patients with hypertension and MetS deployed in our sample covered it in four features (50.0%) (Fig. 21).

Arterial hypertension among patients with hormone-reactive adenomas was the most potent risk factor for MetS unlocking, and such was observed in 91.8% of individuals with NI and hypertension. Elevated triglycerides and low HDL-cholesterol levels also contribute to the development of carbohydrate disorders. Our results also highlight the role of dyslipidemia in adrenal hormone-reactive MetSyn adenomas. In our study, female sex and arterial hypertension were risk factors of high severity.

4.4. Results of the studies on carbohydrate disorders and insulin resistance in patients with adrenal non-secreting adenomas.

Using the harmonized criteria for MetSyn diagnosis, with a blood glucose level above 5.6 mmol/l, 76.2% of the subjects with NI met this criterion (Fig. 22).

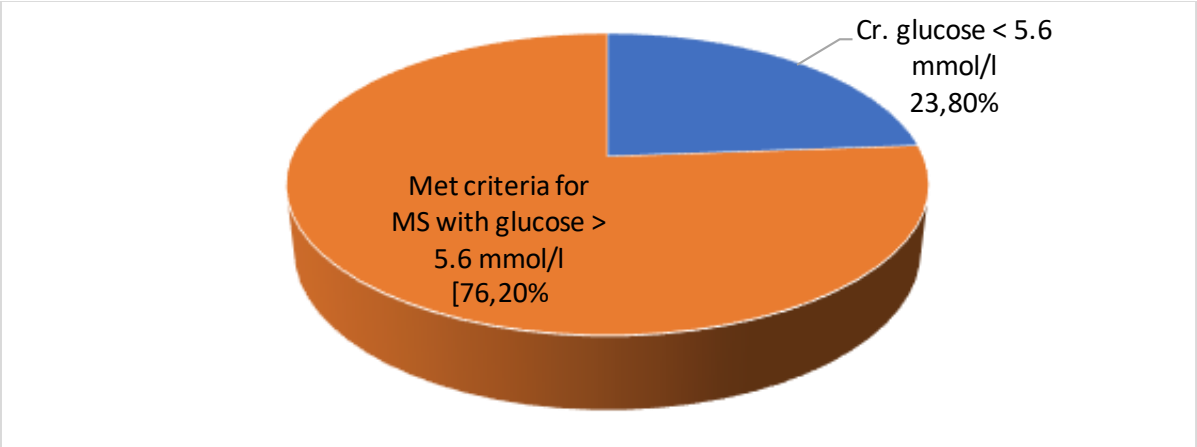
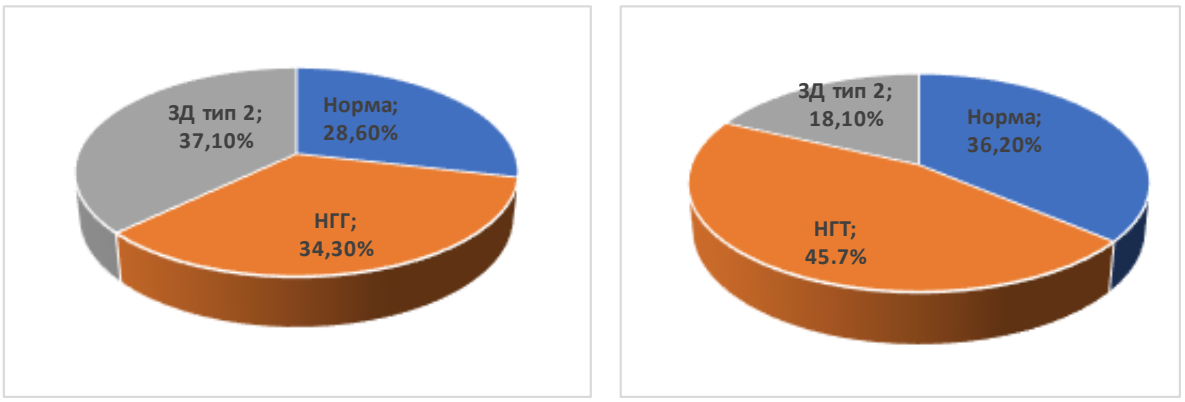


Fig. 22. Distribution of patients according to glycaemic disorders (for values above 5.6 mmol/l)

Figure 23 shows the distribution of patients with hormone-reactive adenomas according to the WHO, 2006 criteria for diagnosis of different glucose tolerance levels.



Blood glucose 0' **Blood glucose 120'**

Fig. 23. А. Distribution of patients with hormonally inactive adenomas according to fasting blood glucose. Б. Distribution of NI patients according to blood glucose at 120 min of OGTT

Of the 105 NI patients studied, 28.60% had normal fasting blood glucose values (lower than 6.1 mmol/l). With a fasting plasma glucose ≥ 7.0 mmol/l, with a compliance interval of at least 8 hours since the last meal, 37.1% of the individuals with NI were. They formed the group of newly diagnosed diabetes mellitus by first pathological point (0 minute). Abnormal fasting glycaemia (AGG) - fasting plasma glucose greater than or equal to 6.1 mmol/l to 6.9 mmol/l inclusive was recorded in 34.3% of individuals with NI. The data are presented in Figure 23 A.

In all 105 patients, OGTT was performed to search for NGT and normal blood glucose, as well as to detect the combination of both prediabetic state (NGT and NGT) and type 2 diabetes mellitus by the second pathological point (120 min >11.1mmol/l). The distribution of patients is presented in Figure 23 B.

With normal glucose tolerance and glucose at 120 minutes < 7.8 mmol/l were (36.2%). With glucose at 120 min ≥ 11.1 mmol/l, classified as diabetes mellitus were (18.1%). The

relative proportion of patients with glycemic disorders at 120 min was 63.8%, without glycemic disorders 36.2% .

Forty-eight of the 105 patients (45.70%) had a 120-minute blood glucose ≥ 7.8 and < 11.1 mmol/l. In 31 of these 48 (64.5%), the corresponding fasting blood glucose fell in the range ≥ 6.1 and < 6.9 mmol/l. They formed the prediabetic group with the presence of two glycemic disorders (NGG+NGT). 35.4% (17/48) had normal fasting blood glucose (below 6.1 mmol/l).

The use of only one pathological point may be assumed to lower the actual incidence of disorders related to glucose metabolism.

Using the 0' and 120' criteria, the following categories of NI patients were formed according to their glucose tolerance (Table 17):

Tab. 17. Distribution of NI patients according to their glucose tolerance using the two OGTT scores (0' and 120').

Category	Blood glucose at 0'	Blood glucose at 120'	Number of patients/ %
Normal glucose tolerance	< 6.1 mmol/l	<7.8 mmol/l	11/ 10,5%
NGH	≥ 6.1 and ≤ 6.9 mmol/l	< 7.8 mmol/l	6/5,7%
NGT	<7.0 mmol/l	≥ 7.8 and < 11.1 mmol/l	48 /45.7%
NGH+NGT	≥ 6.1 and ≤ 6.9 mmol/l	≥ 7.8 and < 11.1 mmol/l	31/48 /(64.5%)
Diabetes mellitus	≥ 7.0 mmol/l	≥ 11.1 mmol/l	40/38,1%

Fig. 24 shows the mean age of patients according to their carbohydrate disorders. Although it did not reach statistical significance, the mean age of patients with newly diagnosed DM (55.29 years) and with NGT (53.76 years) was found to be higher compared to the other two groups (Fig. 24).

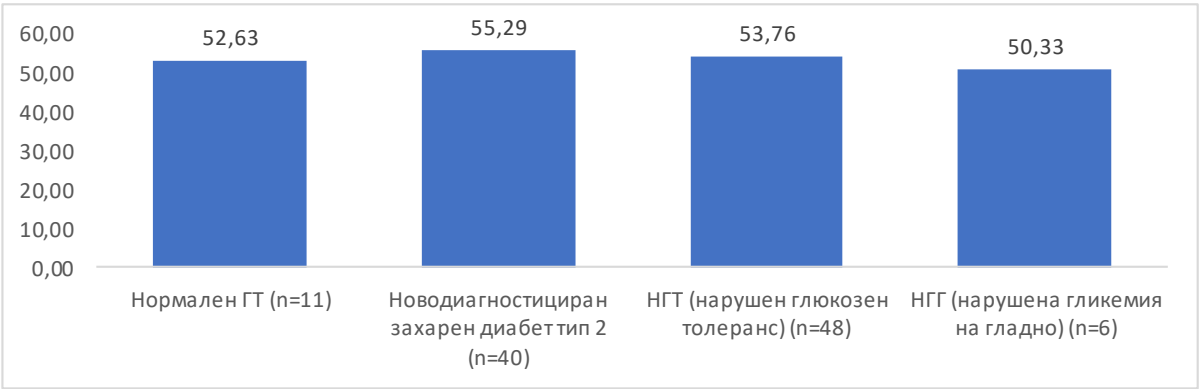


Fig. 24. Distribution of patients with hormonally inactive adenomas according to carbohydrate disorders and mean age

The results of the analysis of carbohydrate disorders according to sex showed that there was no significant difference between men and women (Fig. 25). Normal glucose tolerance was observed in 11 patients, 72.7% , of whom were women with NI and 27.3% were men with NI. In patients with newly diagnosed diabetes mellitus, 55.00% were female and 45.00% were male. In the group with NGT also female sex prevailed 54.20% to 45.80% for male sex. Among those with NGG, 52.20% were female and 47.80% were male. There was no statistically significant difference in the carbohydrate disorder groups with respect to gender. In patients with normal glucose tolerance, female gender predominated, the number of patients in the group was small.

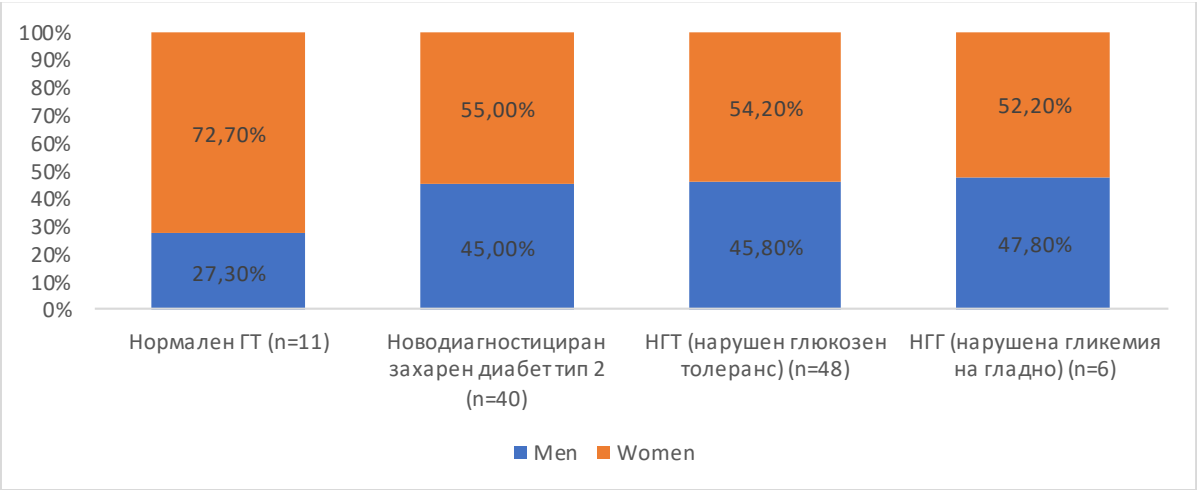


Fig. 25. Distribution of patients with hormonally inactive adenomas according to carbohydrate disorders and gender

All patients with carbohydrate disorders have elevated BMI values ≥ 30 kg/m^2 . Fig. 26 shows the distribution of NI patients according to carbohydrate disorders and the mean BMI value in the respective group. It is noteworthy that individuals with normal glucose tolerance and NI have a borderline BMI between that determining overweight and obesity. In the NGT group, the mean BMI was the highest at 32.36 kg/m ^{2}, and in the NIG group, the mean BMI was 30.17 kg/m ^{2} .

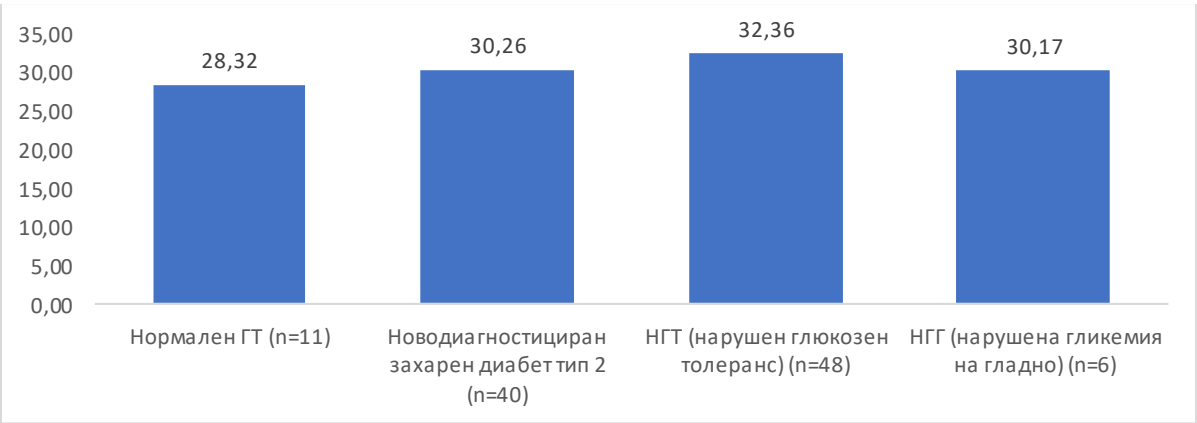


Fig. 26. BMI in different groups of patients with hormonally inactive adenomas according to their carbohydrate disorders

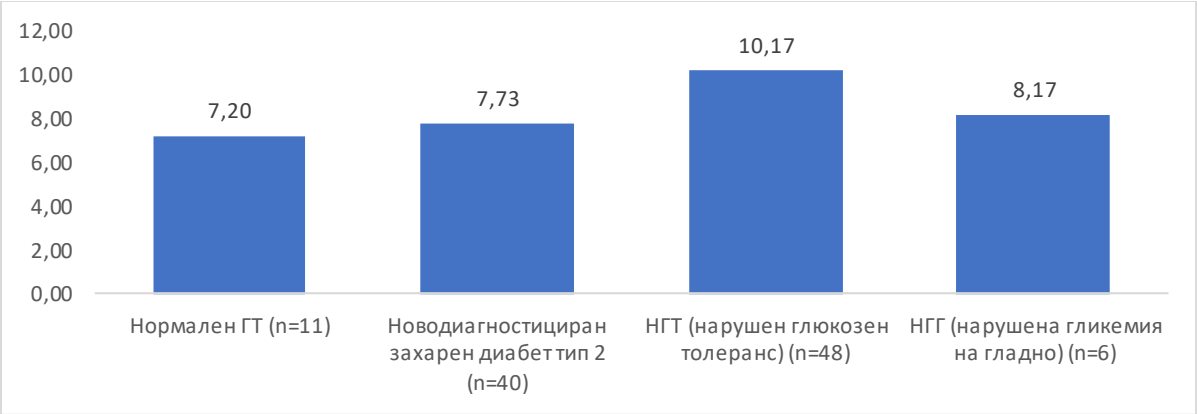


Fig. 27. Baseline insulin levels (at 0 min of OGTT) in different groups of patients with hormonally inactive adenomas according to their carbohydrate disorders

Fig. 27 shows a comparative analysis of mean insulin values at 0'min of OGTT in patients with NI and carbohydrate disorders as well as those in the normal range. The results showed that there was no significant difference in insulin levels between patients with normal GIT and those with newly diagnosed DM ($p>0.05$). An increase in insulin levels was observed in patients with NGT compared to those with normal GT, and the difference was statistically significant (10.17 IU to 7.20 IU $p<0.01$, respectively). A significant difference in insulin values was not observed in the comparative analysis of patients with normal GT and those with NGT, with values in the latter group of patients being significantly higher (7.20 IU to 8.17 IU, $p>0.05$). There was also no difference in insulin values between patients with newly diagnosed type 2 diabetes mellitus and those with NGG, reaching statistical significance (7.73 to 8.17 IU $p>0.05$). A significant difference was found with respect to the group of patients with NGT and those with NGG (10.17 to 8.17 IU, respectively, $p<0.05$)

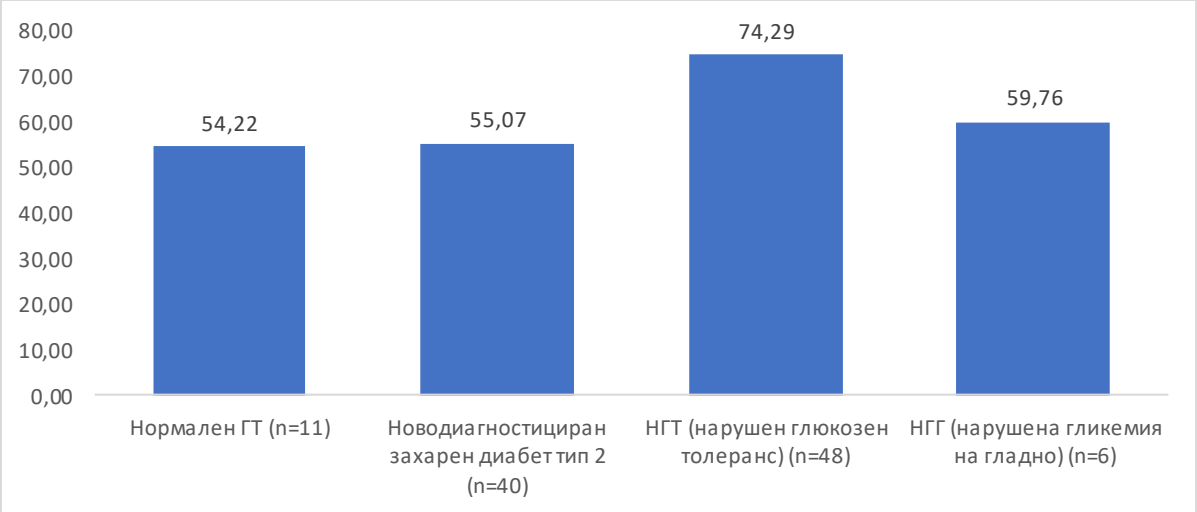


Fig. 28. Distribution of patients with hormonally inactive adenomas according to carbohydrate disturbances and insulin at 120 min of OGTT

From the data presented in Fig. 28, it can be seen that there was no significant difference in insulin levels at 120' between the normal GOT, NGT and NGG patient groups. On the other

hand, there was a significant difference in the insulin levels of patients with newly diagnosed type 2 diabetes, which were significantly lower than those with NGT ($p<0.05$).

The mean increase in insulin at 120' versus 0' in patients with hormonally inactive adenomas was 7.32±4.04 times, with a minimum increase of 1.88 times and a maximum of 28.5 times. More than two-thirds (67.6%) of the patients had a greater than 5-fold increase in insulin levels within 2 hours' (Fig. 29).

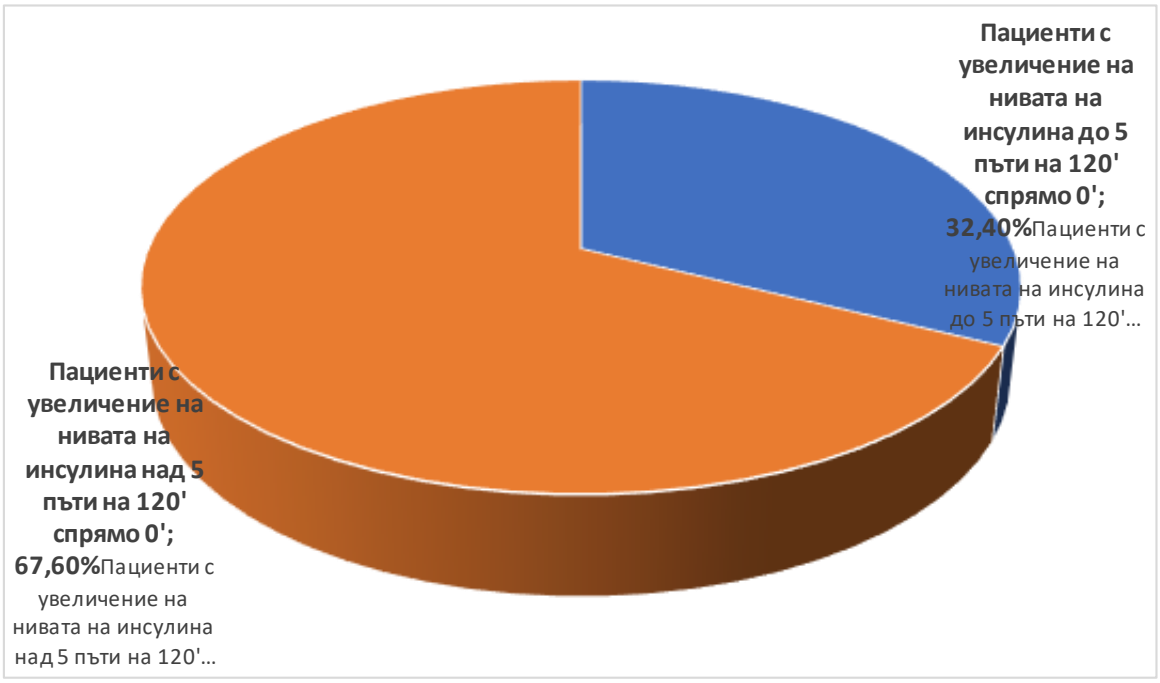


Fig. 29. Distribution of patients versus insulin levels at 0 min and rising and over 5 times at 120 min

It is possible that some of the effects of hyperinsulinemia (at 120 min, at 5-fold magnification) alter adenoma size, even if it is not the sole causative factor, insulin resistance appears to be one important element in the complex of interacting disorders that cause the development and progression of adenomas. Chronic hyperinsulinemia is a contributing factor to the onset or progression of adenomas in patients with diabetes and is primarily attributable to the mitogenic effect of insulin. Hyperinsulinaemia could induce various changes, such as those in the IGF (insulin-like growth factor 1) system, which may provoke cell proliferation and resistance to apoptosis. Factors enhancing cellular resistance may independently modify both adenoma risk and insulin resistance, including subclinical inflammation and obesity. (110). In a future prospective study, it would be interesting to unpack the role of insulin at 120 min in correlation with IGF-1 and subclinical inflammation.

The difference in insulin levels at 0' and 120' in the study groups is presented in Fig. 30, with no significant difference between the groups considered.

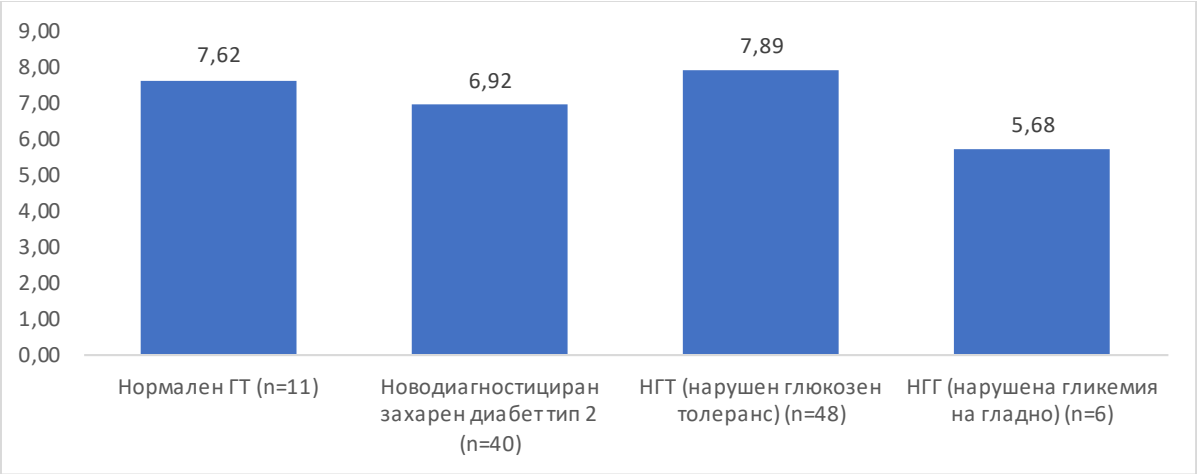


Fig. 30. Distribution of patients with hormonally inactive adenomas according to carbohydrate disturbances and the difference in insulin levels between 0' and 120' (fold)

On Tab. 18 presents a correlation analysis between anthropometric and carbohydrate indices and tumor size in patients with hormonally inactive adenomas. The results showed that tumor size did not correlate with carbohydrate indices nor with insulin resistance. Waist circumference correlated weakly orthogonally with blood glucose levels at 0', insulin at 0', and HOMA-IR. There was a moderate orthogonal relationship between waist circumference and insulin level at 120'.

Tab. 18. Correlation analysis between anthropometric and carbohydrate indices and tumor size in patients with hormonally inactive adenomas

	Size of adenoma	**Thalia**	**ITM**	**HOMA-IR**
Blood glucose 0'	r=0.125 P=0.204	r=0.065 P=0.508	r=0.090 P=0.359	**r=0.235** **P=0.016**
Blood glucose 120'	r=0.034 P=0.728	**r=0.192** **P=0.05**	r=0.137 P=0.163	**r=0.312** **P=0.001**
Insulin 0'	r=0.062 P=0.532	**r=0.289** **P=0.003**	**r=0.333** **P=0.001**	**r=0.948** **P<0.001**
Insulin 120'	r=0.112 P=0.255	**r=0.316** **P=0.001**	**r=0.423** **P<0.001**	**r=0.769** **P<0.001**
HOMA-IR	r=0.027 P=0.782	**r=0.279** **P=0.004**	**r=0.322** **P=0.001**	-
Insulin resistance - QUICKI	r=0.072 P=0.467	**r=-0.243** **P=0.012**	**r=0.255** **P=0.009**	**r=0.764** **P<0.001**

BMI was moderately correlated with insulin levels at 0' and 120' and with HOMA-IR, while insulin resistance was inversely correlated (p=0.001).

HOMA-IR was correlated with all carbohydrate indices, with only blood glucose levels at 0' showing an orthogonally weak relationship, and with glucose at 120' the relationship was

orthogonally moderate. With insulin at 0' and 120', an orthoproportional strong dependence was found.

Tab. 19. Correlation analysis between anthropometric and carbohydrate indices and tumor size in patients with hormonally inactive adenomas and newly diagnosed type 2 diabetes mellitus

	Size of adenoma	**Thalia**	**ITM**	**HOMA-IR**
Blood glucose 0'	r=-0.026 P=0.877	r=-0.015 P=0.930	r=0.053 P=0.750	r=0.255 P=0.118
Blood glucose 120'	r=-0.038 P=0.818	r=0.216 P=0.187	r=0.162 P=0.325	**r=0.315** **P=0.05**
Insulin 0'	r=-0.120 P=0.465	r=0.174 P=0.290	r=0.243 P=0.136	**r=0.983** **P<0.001**
Insulin 120'	r=-0.075 P=0.651	r=0.158 P=0.338	**r=0.307** **P=0.05**	**r=0.813** **P<0.001**
HOMA-IR	r=-0.132 P=0.422	r=0.163 P=0.320	r=0.235 P=0.150	-
Insulin resistance - QUICKI	r=0.105 P=0.523	r=0.014 P=0.932	r=-0.053 P=0.747	**r=0.733** **P<0.001**

On Tab. 19 shows correlation analysis between anthropometric and carbohydrate indices and tumor size in patients with hormonally inactive adenomas and newly diagnosed type 2 diabetes mellitus. The results of the analysis showed that adenoma size and waist circumference in this group of patients did not correlate with the carbohydrate indices considered.

BMI correlates linearly with insulin levels at 120 minutes only.

HOMA-IR correlated moderately with blood glucose levels at 120' and showed a strong correlation with insulin levels at 0' and 120'.

Table 20 shows the correlation analysis between anthropometric and carbohydrate indices and tumor size in patients with hormonally inactive adenomas and NGT. The results of the analysis showed that adenoma size did not correlate with the carbohydrate indices considered, and waist circumference and BMI correlated inversely weakly with blood glucose levels at 0'.

HOMA-IR correlates inversely with insulin levels at 0' and 120', while insulin resistance shows a strong inverse correlation.

Table 20. Correlation analysis between anthropometric and carbohydrate indices and tumor size in patients with hormonally inactive adenomas and NGT

	Size of adenoma	Thalia	ITM	HOMA-IR
Blood glucose 0'	r=0.112 P=0.457	**r=-0.281** **P=0.05**	**r=-0.291** **P=0.049**	r=0.065 P=0.667
Blood glucose 120'	r=-0.044 P=0.771	r=0.088 P=0.562	r=0.131 P=0.386	r=0.159 P=0.290
Insulin 0'	r=0.005 P=0.975	r=0.042 P=0.782	r=0.110 P=0.466	**r=0.983** **P<0.001**
Insulin 120'	r=-0.026 P=0.863	r=0.113 P=0.455	r=0.261 P=0.080	**r=0.752** **P<0.001**
HOMA-IR	r=0.005 P=0.976	r=0.015 P=0.921	r=0.097 P=0.522	-
Insulin resistance - QUICKI	r=-0.076 P=0.614	r=-0.034 P=0.821	r=-0.054 P=0.721	**r=0.793** **P<0.001**

Table 21 shows the correlation analysis between anthropometric and carbohydrate indices and tumor size in patients with hormonally inactive adenomas and NGG. The results showed that adenoma size and waist circumference did not correlate with the carbohydrate indices considered.

BMI correlates moderately orthogonally with insulin levels at 120'.

HOMA-IR correlated linearly with insulin levels at 0' and 120'.

Table 21. Correlation analysis between anthropometric and carbohydrate indices and tumor size in patients with hormonally inactive adenomas and NGG

	Size of adenoma	Thalia	ITM	HOMA-IR
Blood glucose 0'	r=0.349 P=0.103	r=0.205 P=0.348	r=0.283 P=0.191	r=-0.036 P=0.871
Blood glucose 120'	r=-0.319 P=0.137	r=0.234 P=0.283	r=0.019 P=0.932	r=0.221 P=0.310
Insulin 0'	r=0.174 P=0.426	r=0.193 P=0.378	r=0.088 P=0.690	**r=0.998** **P<0.001**
Insulin 120'	r=0.049 P=0.823	r=0.347 P=0.105	**r=0.454** **P=0.030**	**r=0.770** **P<0.001**
HOMA-IR	r=0.194 P=0.374	r=0.207 P=0.342	r=0.106 P=0.630	-
Insulin resistance - QUICKI	r=-0.003 P=0.990	r=-0.284 P=0.188	r=-0.194 P=0.374	**r=0.863** **P<0.001**

Results showed that adenoma size did not correlate with carbohydrate indices and insulin levels in patients with hormonally inactive adenomas, whereas HOMA-IR correlated linearly strongly with insulin levels at 0' and 120'.

ROC analysis was performed to determine the threshold insulin value of 0' and 120', respectively, above which there is an increased risk of increasing adenoma size (Fig. 31). The

analysis showed that for insulin at 0' the threshold value was 6.5 (AUC=0.580 (0.457-0.703); p<0.05), and for insulin at 120' the threshold value was 45.5 (AUC=0.599 (0.474-0.724); p<0.05), with a sensitivity of 54.1% and specificity of 58.1%.

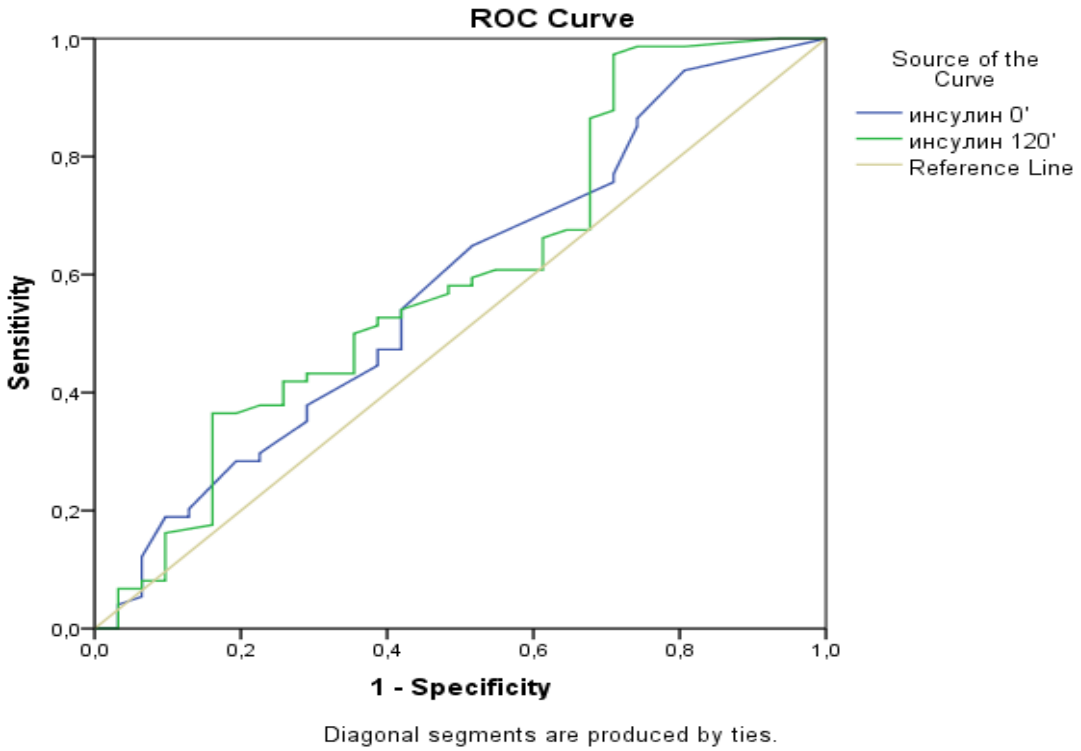


Fig. 31. ROC curve analysis to determine insulin threshold values for adenoma size development

ROC analysis was performed to determine the threshold blood glucose value at 0' and 120' above which there is an increased risk of increasing adenoma size (Fig. 32). It was found that for blood glucose at 0' the threshold value was 6.35 (AUC=0.476 (0.357-0.595); p<0.05) and for blood glucose at 120' the threshold value was 8.25 (AUC=0.547 (0.431-0.662); p<0.05). Sensitivity was 52.7% and specificity was 54.8%.

ROC analysis was performed to determine the threshold value of the HOMA index above which there is an increased risk of increasing adenoma size (Fig. 33). The results showed that the HOMA-IR threshold value was 1.85 (AUC=0.564 (0.436-0.692); p<0.05), with a sensitivity of 50.0% and specificity of 54.8%.

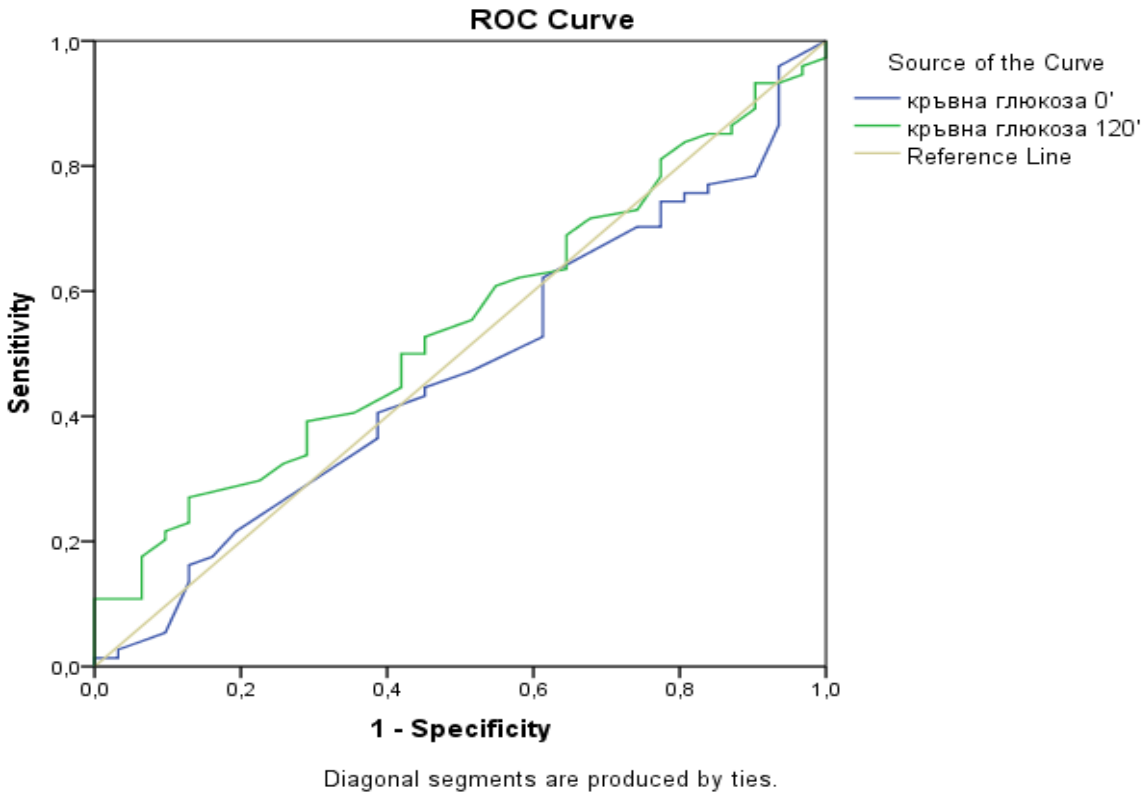


Fig. 32. ROC curve analysis to determine blood glucose threshold values for adenoma size development

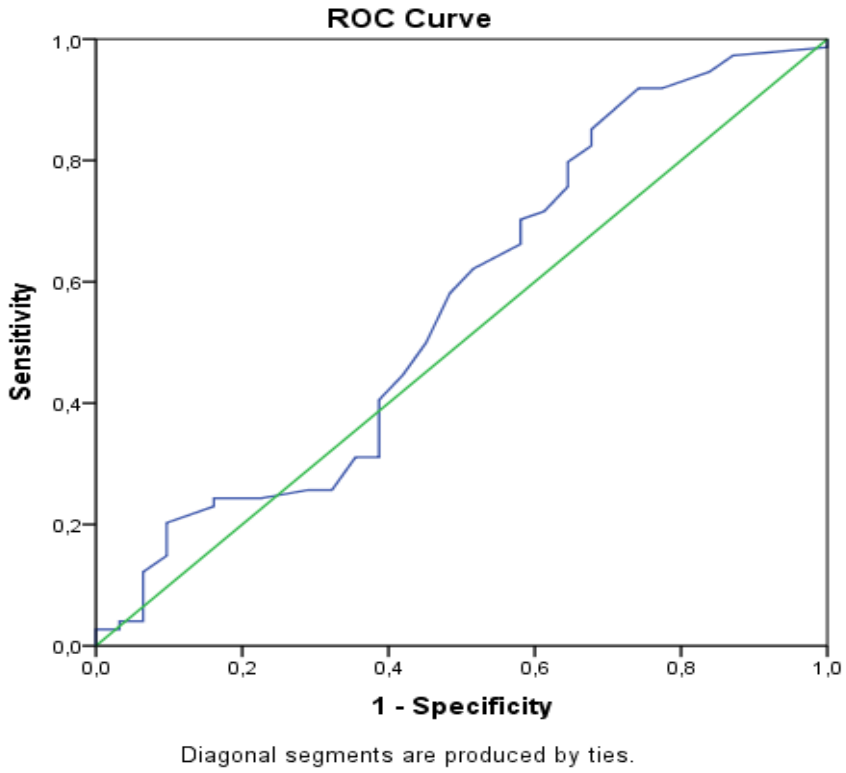


Fig. 33. ROC curve analysis to determine threshold values of HOMA index for adenoma size development

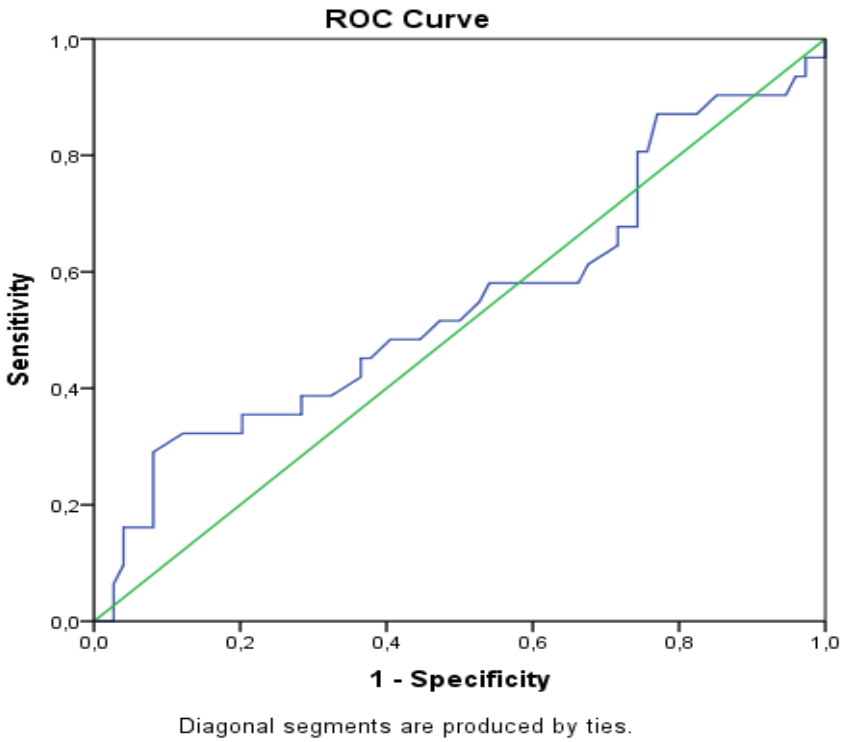


Fig. 34. ROC curve analysis to determine QUICKI threshold values for adenoma size development

ROC curve analysis was performed to determine the QUICKI threshold value above which there is an increased risk of increasing adenoma size (Fig. 34). From the results, the threshold value for QUICKI was found to be 0.348 (AUC=0.547 (0.418-0.677); $p<0.05$). Sensitivity was 51.6% and specificity was 52.7%.

Table 22. Comparative analysis of the prevalence of diabetes mellitus in the general population in the Republic of Bulgaria. Bulgaria and among patients with hormonally inactive adenoma

Indicator	Total population (Borisova et al., 2012)	Own study (persons with NI, 2021)	P value
Normal glucose tolerance	1763/ 86.7 %	11/ 10.5 %	< 0.001
Newly diagnosed diabetes	50/ 2.5 %	40/38.1 %	< 0.001
Impaired glucose tolerance	32/ 1.6 %	48/45.7 %	< 0.001
Impaired fasting glycaemia	43/ 2.1 %	6/5.7%	< 0.05

There was a statistically significant difference between the results of the population-based study by Borisova et al. (2012) and those of the present study among patients with hormone-reactive adrenal adenomas. All carbohydrate disorders were significantly more common in individuals with NI compared to the general population.

Studies on the prevalence of carbohydrate disorders are important to detect early and delay the evolution of prediabetes into diabetes. Data from the Bulgarian population survey say a prevalence of diabetes mellitus in Bulgaria of 9.6% (known and newly diagnosed at the time of the survey). Regarding a glucose level above 5.6 mmol/l as a diagnostic criterion for MetSyn, Borisova et al. found that individuals with MetSyn had fasting glucose above 5.6 mmol/l in 53.4% in the presence of MetSyn and 9.5% in the absence of MetSyn, respectively, $p<0.001$. In our study, the incidence of newly diagnosed DM in persons with NI was 37.10%, significantly higher compared to the general population ($p<0.001$). For comparison, the national prevalence of diabetes in other Eastern European countries was 6.65% for Slovakia and 10.57% for Poland (103), as well as Italian data of 4.8% and German data of 8.9% (112). Screening for carbohydrate disorders according to blood glucose values at zero minutes is not sufficient to categorize carbohydrate disorder, as it misses individuals with impaired glucose tolerance as well as those with glucose levels above 11.1 at 120'. When conducting blood glucose tracing at 120 minute along with identifying individuals with NGT, the proportion of diabetes mellitus changes due to redistribution of patients. In addition, 34.3% of the individuals with NI had glycemia at 0' between 6.1 and 6.9 mmol/l. This proportion of patients with NGT is overestimated, as glucose testing after OGTT identifies some of these patients as diabetic and some as having NGT, the latter with a combined prediabetic state (NGT+NGT). It is appropriate to perform OGTT in all patients with adrenal hormone-reactive adenomas.

In Bulgaria, the prevalence of type 2 DM is higher in men compared to women - 56.4% compared to 46.3% (Borisova 2006-2011). (3) Our results showed no difference in the prevalence of diabetes in the two sexes.

The increase in the global population and the relative proportion of the elderly population also increases the prevalence of socially significant diseases, including carbohydrate disorders. (57)

Data from the present study among individuals with hormonally inactive adrenal adenomas showed an incidence of NGT 47.5% and NGG 5.7% In comparison with the general Bulgarian population (NGT-1.6% and NGG 2.1%), individuals with NI showed significantly more frequent prediabetes ($p<0.001$). According to the Diabetes Atlas Europe, prediabetes affected ((IDF Diabetes Atlas, 2017) 7.3% of the adult population in 2017, and the prevalence is expected to increase to 8.3% by 2045, with an estimated 30% progressing to diabetes (100, 212). Therefore, early detection of individuals with prediabetes should be a strategy in the population of patients with adrenal hormonally inactive adenomas. This will allow early diagnosis, follow-up of risk factors, and treatment. Several studies have shown a potential association between adrenal hormone-inactive adenoma and prediabetic conditions and similar results to our study (3, 24, 195, 156). Androulakis et al. showed that individuals with hormone-inactive adenoma had a higher incidence of insulin resistance and prediabetic conditions compared with healthy controls without adrenal tumors (24). Adrenal hormone-inactive adenomas have been associated with a higher prevalence of impaired glucose tolerance and type 2 DM in numerous studies (25, 220, 61, 62). In a recent retrospective study of 242 patients with adrenal hormone-inactive adenomas followed for 7 years, the risk of developing diabetes in the group with adenomas was significantly higher compared with the risk of finding diabetes in controls without adrenal lesions (absolute risk of diabetes with AI = 15.6%) (25).

In our study, all patients with carbohydrate disorders had an elevated BMI. Similar studies have shown that significantly more NI patients are overweight or obese compared with age-matched controls. Similar to our results, a female sex predominance is reported among persons with NI at all ages, which the authors interpreted with a possibly higher number of imaging studies performed on women than on men. (25, 30). In our sample, there was no statistical significance between males and females in the carbohydrate abnormality groups. However, there was a slight predominance in the percentage of women relative to men.

Of the OGTTs performed with insulin at 0 min and 120 min in our study, 67.6% had at least a 5-fold increase in insulin at 120 min. Currently, there are no detailed data on insulin values during OGTT in hormone-reactive adenomas worldwide. The increased mitogenic action of insulin under conditions of hyperinsulinemia is due to a decrease in the metabolic component, with a consequent impairment of the effect and, consequently, increased mitogenic activity at the level of insulin receptor and postreceptor cascades. Hyperinsulinaemia (with an emphasis on 120 min should be the subject of clinical search and early detection to prevent the whole range of disorders with which they are associated, one of which is the increased risk of malignant transformation of a particular cell type increasing adenoma size.

Midorikawa et al. (154) demonstrated improvement in glucose tolerance and insulin resistance after adrenalectomy in patients with adrenal hormone-reactive adenomas, suggesting that insulin levels at both zero and 120 min may be related to adenoma size and part of its pathogenesis. In contrast, our correlation analysis showed no significant association of adenoma size with either blood glucose or insulin levels. A similar lack of correlation was reported by Lazurova et al. (126).

The main value of the present study is the remarkably higher prevalence of carbohydrate disorders among patients with nonfunctioning adrenal adenomas than in the general population. The present data suggest that the incidental detection of an adrenal tumor should encourage clinicians to diagnose earlier and treat possible abnormalities in glucose homeostasis.

Adrenal hormone-reactive adenomas can be a public health problem if only because of the proportion of carbohydrate abnormalities. Early diagnosis is important, not only with a single fasting blood glucose value, but also by performing an OGTT with blood glucose and insulin testing at 0 and 120 min. Only in this way will there be a complete picture of carbohydrate metabolism and patients can be properly interpreted, monitored and treated. An increase in carbohydrate metabolism testing would have a positive effect in order to look for hidden morbidity and prevent associated complications in a timely manner. (128)

4.5 Results of fatty liver index (FLI), liver enzymes in NI patients and their relationship with adenoma size.

Liver enzyme assay in subjects with adrenal hormone-reactive adenomas showed upper-bound transaminases (for ACAT 30 to 35 U/l; for ALT 35 to 40 U/l) in 34 patients (32.4% of subjects with NI), with no significant difference between men and women. Mildly to moderately elevated (value for ASAT 25 to 30 for ALT 30 to 45) serum levels of aspartate aminotransferase and alanine aminotransferase or both were found in 28.5% The aminotransferase assay is not a sufficient laboratory marker of NAFLD, and underestimates its prevalence. (20) The assessment of liver injury in NAFLD includes the examination of GGT and the calculation of the fatty liver index (FLI).

A combination of elevated ASAT, ALT and GGT was found in 40 of the NI patients (38.09%). In 60 patients (57.1%) a combination of elevated ASAT, ALT, GGT and FLI were found. The use of FLI contributed to detect an additional (almost 20%) risk of liver injury in our sample.

The mean FLI among the patients studied was 56.48±28.61 and ranged from 2.0 to 99.0 (Fig. 35).

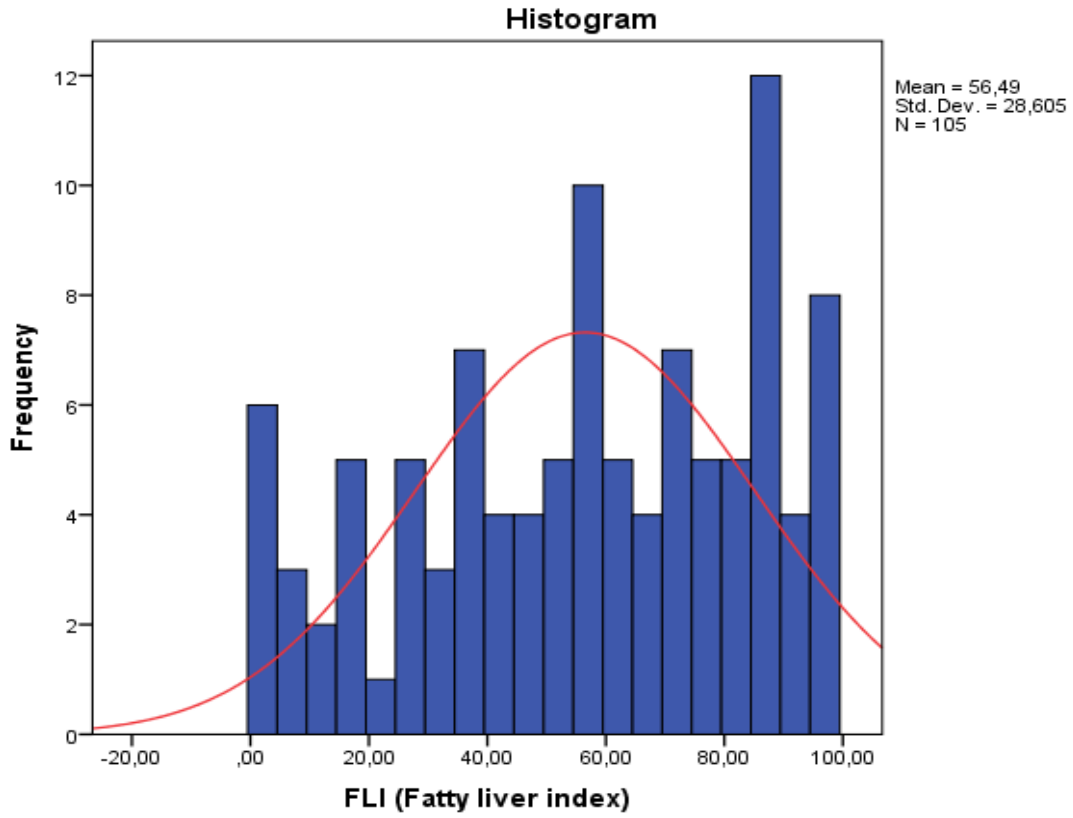


Fig. 35. Mean value of FLI

The FLI is used as a screening index. FLI scores range between 1 and 100. FLI scores below 30 indicate that hepatic steatosis should be ruled out(low risk). Values between 30 and 60 patients are at intermediate risk and further non-exclusive investigations are required.Results above 60 are used to indicate high risk.

In our sample, 49 of the NI patients (46.7%) had elevated FLI (≥60). Those at intermediate risk for developing NAFLD (FLI between 30 and 60) were 34 individuals (32.4%), and those at low risk (normal FLI <30) were 20.9% of the NI patients.

Fig. 36. Risk of developing hepatic steatosis with FLI score

The diagnosis of Non-Alcoholic Fatty Liver Disease (NAFLD) requires a combination of invasive and non-invasive tests. Worldwide, there is disagreement among researchers about the methods for diagnosing NAFLD. Including a wide range of changes, NAFLD represents an accumulation of fat (triglycerides) in at least 5% of hepatocytes - nonalcoholic steatosis (NAS) alone or in combination with hepatocellular injury and inflammation (nonalcoholic steatohepatitis - NASH), with or without fibrosis and cirrhosis. NAFLD is taken in the absence of significant alcohol consumption (<20 g absolute alcohol/day for women and <30 g/day for men) and other cause of liver disease excluded. Diagnosis of NAFLD is difficult due to the lack of specific non-invasive diagnostic tests (159).

Most patients are asymptomatic or have nonspecific complaints. Laboratory changes are also nonspecific, not correlated with histological changes. Metabolic disturbances and elevation of ALT with or without elevation of ACAT (ALT>ACAT) are common.

Serum aminotransferases typically fluctuate within reference ranges with normal values in 80% of NIs. Normal liver enzymes do not exclude significant liver injury, including cirrhosis. Conventional ultrasonography, characterized by specific imaging in steatosis with involvement of more than 30% of hepatocytes, is the main tool for the diagnosis of NAFLD - ultrasonographically based NAFLD Imaging modalities - ultrasonography, CT and MRI are insensitive in terms of inflammation, fibrosis, ballooning degeneration. There is no correlation between histological findings and results of imaging modalities. (114, 145, 170). Liver biopsy with histological examination is the gold standard for the diagnosis of NAFLD and allows the assessment of steatosis, the degree of inflammation and the stage of fibrosis, respectively the evolution of the disease (221).

A drawback of the present study is the failure to perform liver ultrasonography and elastography, which is the method of choice for the diagnosis of NAFLD. According to the literature, ultrasound has a sensitivity of 89% and 77% for detecting steatosis and fibrosis, respectively, with a specificity of 93% for steatosis and 89% for fibrosis (33).

Of interest is the analysis of the relationship between liver parameters and adenoma size in patients with hormone-reactive adrenal adenomas. When the correlation between FLI and

adenoma size was examined, no correlation was found (p=0.485). The mean FLI in the group of adenomas less than 40 mm was 58.18, and in the group of larger adenomas (≥40 mm) was 52.45, with no statically significant difference in the two groups.

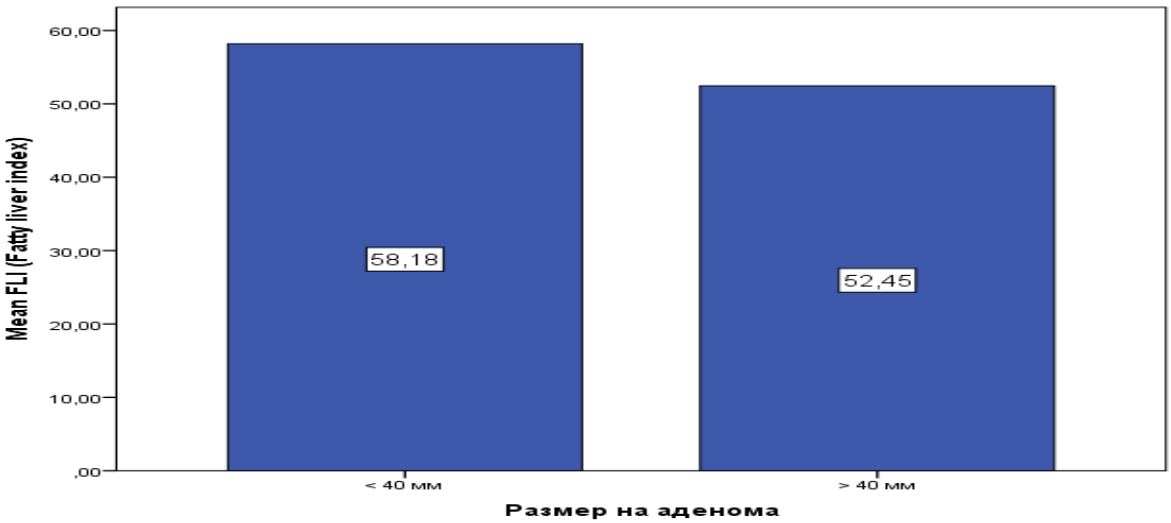


Fig. 37. Mean FLI according to adenoma size

Table 23. Distribution of patients according to FLI and adenoma size (number of patients)

		Size of adenoma		Total
		< 40 mm	> 40 mm	
FLI	Low risk <30	14	8	22
	Intermediate risk 30< 60	25	9	34
	High risk >60	35	14	49
Total		74	31	105

There was no difference in FLI and adenoma size by group.

There was no correlation between ACAT, ALT with FLI and adenoma size.

An orthogonal moderate correlation was found with respect to FLI and GGT (r=0.404; p<0.001), which is logical given that the GGT value is embedded in the formula for FLI calculation. Analysis of FLI results in patients with NI showed that 16.3% of the change in FLI was due to a change in GGT (Fig. 38). This indicates that triglycerides and waist circumference, i.e. metabolic parameters, could be interpreted as parameters of greater importance in NCD patients for the calculation of FLI.

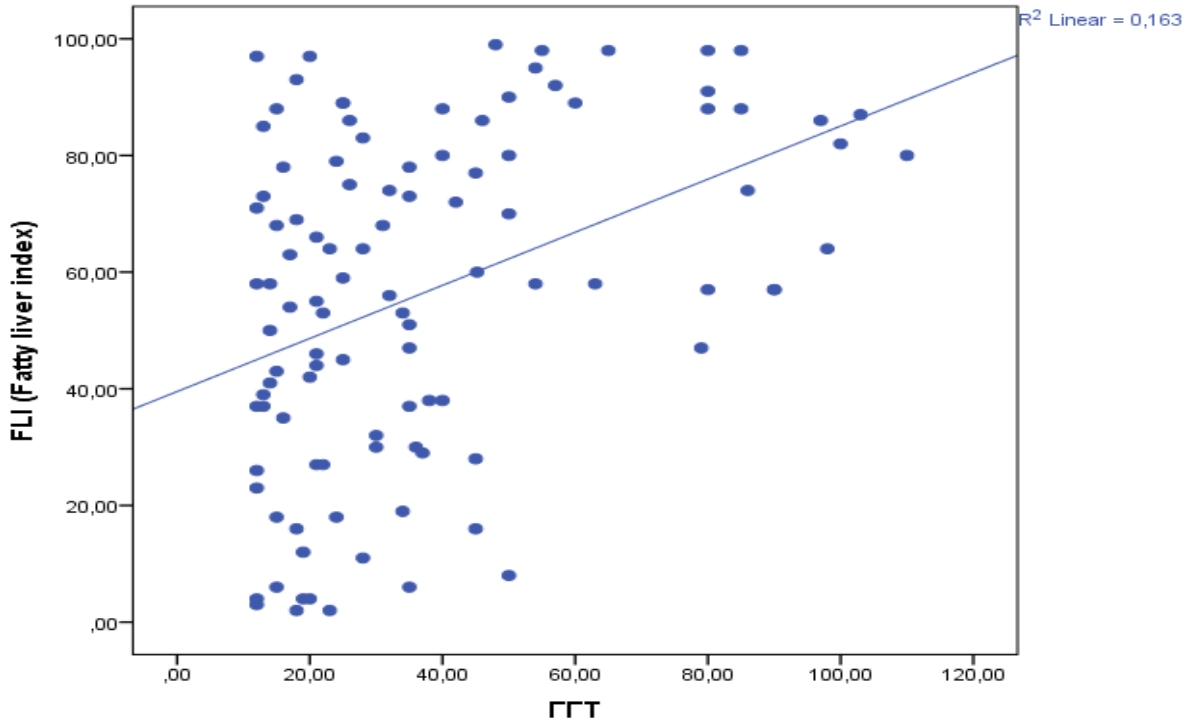


Fig. 38. Correlation analysis between FLI and GGT

A moderate orthogonal relationship was also found for FLI and TG (r=0.473; p<0.001), with 22.4% of the change in FLI attributable to a change in TG (Fig. 39).

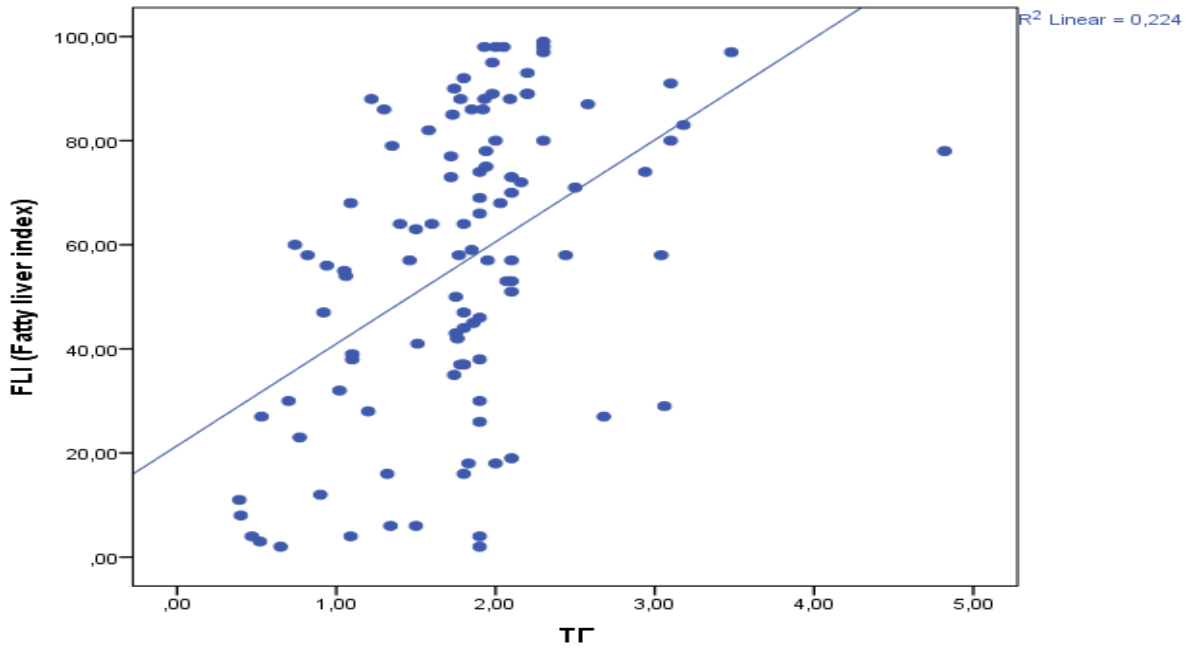


Fig. 39. Correlation analysis between FLI and TG

Adenoma size was found not to correlate with GGT and TG.

For the purposes of the present study, we determined the threshold FLI value above which an increase in adenoma size could be predicted (AUC=0.551 (0.426-0.676) (Fig. 40).

The results showed that an FLI value above 57.5 could predict an increase in adenoma size with a sensitivity of 55.4% and a specificity of 54.8%.This is important in terms of follow-up of patients with adrenal hormone-reactive adenomas.

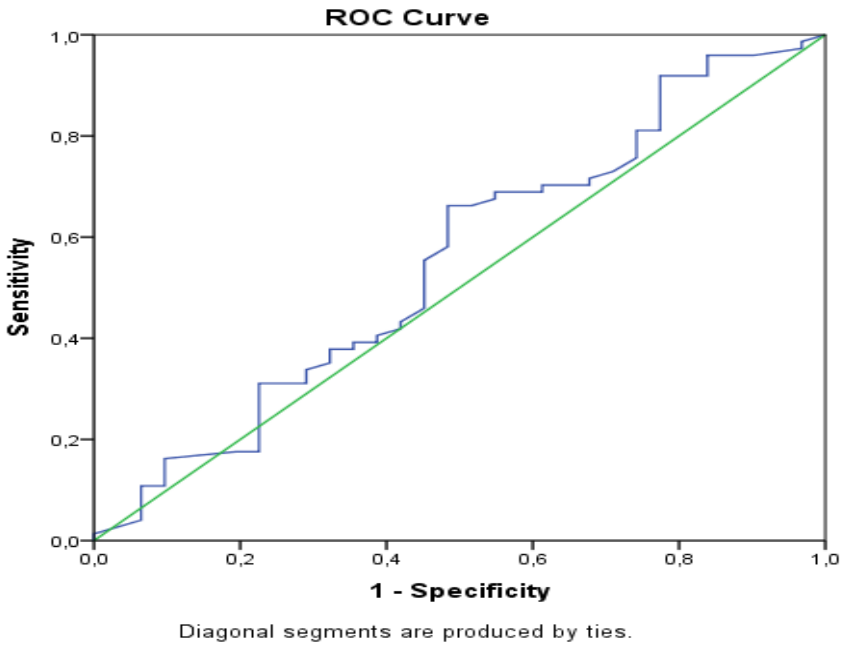


Fig. 40. ROC curve analysis to determine FLI threshold values for adenoma size development

The FLI threshold value for determining obesity in patients with adrenal adenomas was 58.5 with a sensitivity of 78.8% and specificity of 81.1% (AUC=0.867 (0.797-0.938) (Fig. 41).

FLI was first proposed by Bedogni et al. to predict liver "fatness" in a sample of Dionisos et al. (216 patients with and 280 patients without suspected liver disease) aged 18 to 75 years with a good AUC of 0.84 (95% CI: 0.81-0.87) (118)

Koehler et al (118) reported an FLI AUC of 0.807 for fatty liver and 0.813 for NAFLD with a sensitivity of 88% and specificity of 85.3% in a large patient population, similar to that reported by Bedogni et al (33)

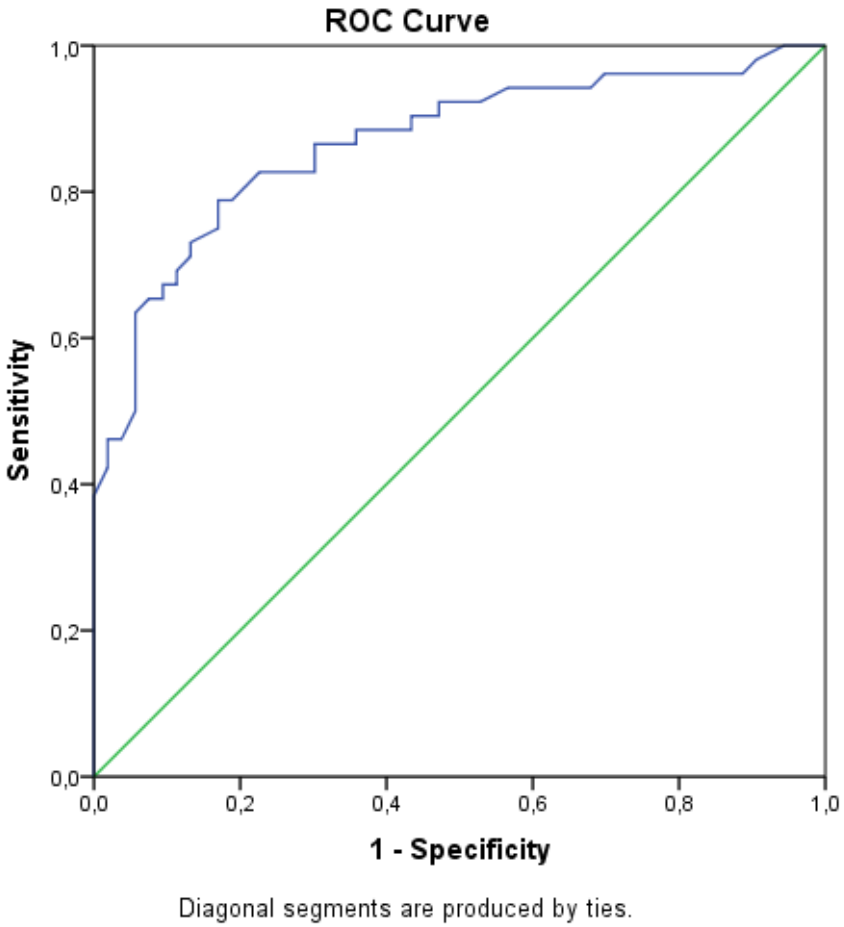


Fig. 41. ROC curve analysis to determine FLI threshold values for obesity determination

Although there are different clinical characteristics of fatty liver disease, FLI shows good AUC values for predicting NAFLD in patients with adrenal hormonally inactive adenomas and obesity. The FLI has good discriminatory ability compared with lipid profile or transaminase testing alone for predicting NAFLD in NI patients.

The use of FLI and combinations of clinical and metabolic parameters in the extended diagnostic evaluation of adrenal hormone-reactive adenomas appears to be an early predictive marker for NAFLD. Definitive diagnosis is made after liver biopsy, or with non-invasive ultrasonographic methods. The extent to which FLI correlates with ultrasonographic findings and histological outcome in individuals with NAFLD is of interest, but this is a subject for future research.

4.6. Diagnostic algorithm for searching for metabolic disorders in patients with hormone-reactive adrenal adenomas.

The clinical guidelines of various expert institutions include the development of algorithms for the management of patients with adrenal incidentalomas. Of course, their application in daily practice is recommended, not mandatory, and depends on the specifics of the region, available specialists, equipment, and the cost of diagnosis and treatment.

An algorithm for management of persons with NI based on international recommendations is presented in Fig. 42. All patients with adrenal incidentaloma should be

examined for clinical and biochemical signs of hormonal overproduction. Screening for hypercortisolism should be performed in all patients. The most readily available test for autonomic cortisol secretion in incidentalomas is express blockade with 1 mg dexamethasone. Plasma or urine metanephrines should be tested in all patients, and hyperaldosteronism should be excluded by aldosterone/renin ratio. If autonomic secretion of cortisol, aldosterone, or catecholamines is confirmed, the adrenal incidentalomas should be resected. The main purpose of imaging studies is to differentiate adrenal adenomas from carcinomas, pheochromocytomas, and metastases. Lesions with an enhancement factor of less than 10 HU on native CT represent adenomas. A subependymal appearance is present at enhancement above 10 HU. Patients with adrenal adenomas smaller than 4 cm and benign radiologic features are subject to radiographic follow-up at 6,12,24 months. Hormonal evaluation is performed at diagnosis and annually thereafter for up to 5 years. Surgical treatment is necessary if the tumor grows more than 1 cm and hormonal activity occurs.

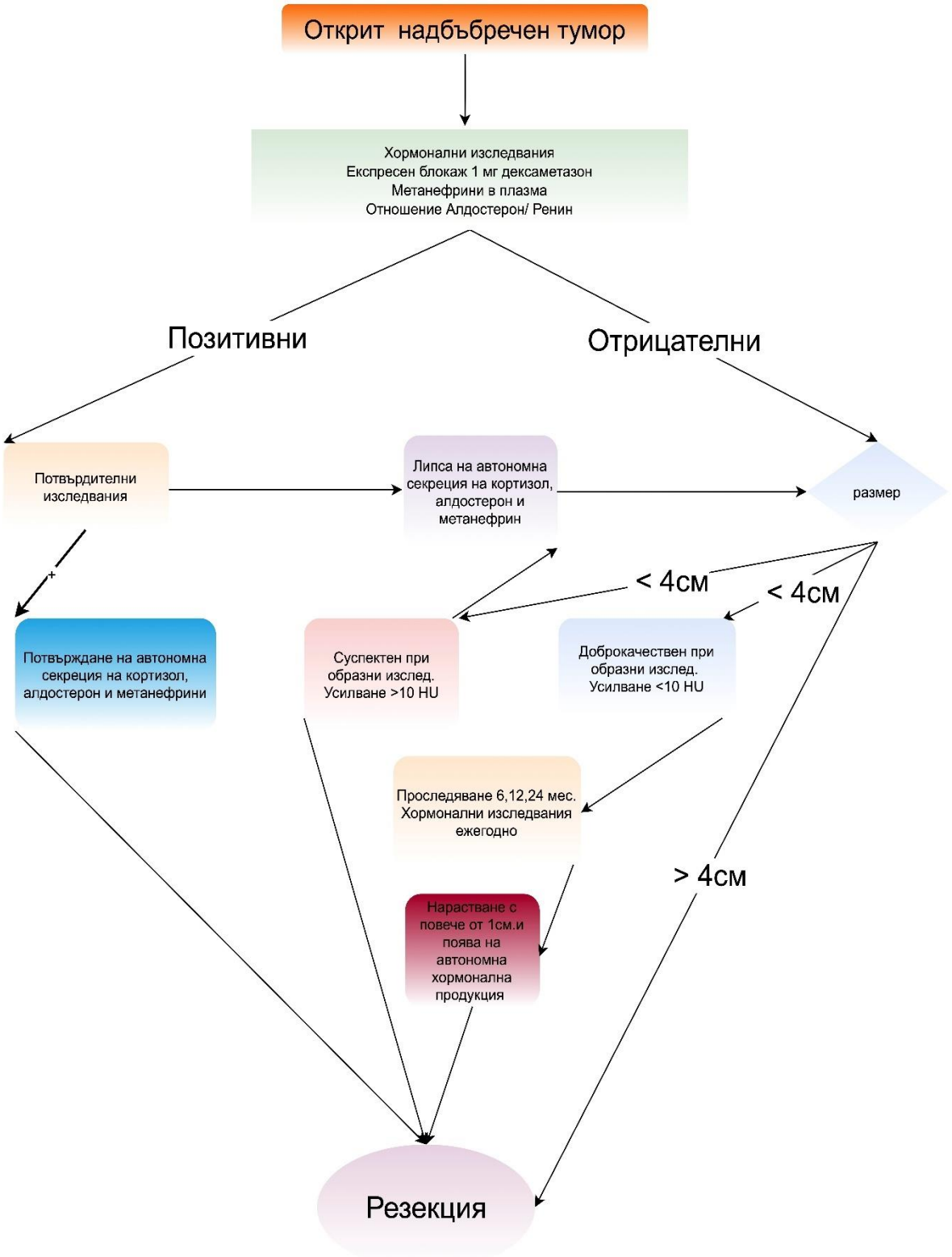


Fig. 42. Algorithm for management of persons with NI

The issue of follow-up of patients with adrenal incidentalomas is controversial. The US National Institutes of Health recommends regular hormone screening with a nocturnal

suppression test with dexamethasone 1 mg and determination of urinary catecholamine and metabolite levels - once a year, for 4 years, as the risk for hyperfunction reaches a plateau after this period. CT scanning is considered appropriate with tumor follow-up at 6-12 months and discontinuation of radiation for tumors that show no change in size (90).

Adrenalectomy is suggested when the tumour size increases (by 1 cm or more) and/or changes in its characteristics during follow-up.

Laparoscopic adrenalectomy is recommended for all patients with benign tumors where surgical treatment is indicated

The AME Task Force believes that patients in whom there is no indication for surgical intervention should be followed clinically for detection and subsequent control of cardiovascular risk factors commonly found in adrenal incidentaloma . Careful clinical follow-up of patients at high cardiovascular risk and optimal therapy of comorbidities (hypertension, diabetes, etc.) according to clinical recommendations is suggested (216).

Fig. 43 shows the metabolic parameters that form the metabolic profile of NI patients. The majority of algorithms for NI do not include the metabolic aspect in the absence of hormone secretion. It is likely that patient follow-up would be complicated if metabolic indices were included. The results of the present study suggest that patients without indications for surgical treatment should also be followed for cardiovascular risk factors that usually accompany adrenal incidentalomas. Analyses similar to the present study, accompanied by cardiovascular risk follow-up after a period of conservative therapy as well as after surgical removal of NI, suggest worsening metabolic status and cardiovascular risk profile, independent of optimal medical therapy, as an indication for reassessment of endocrine hyperfunction or assessment of the benefit of surgical treatment.

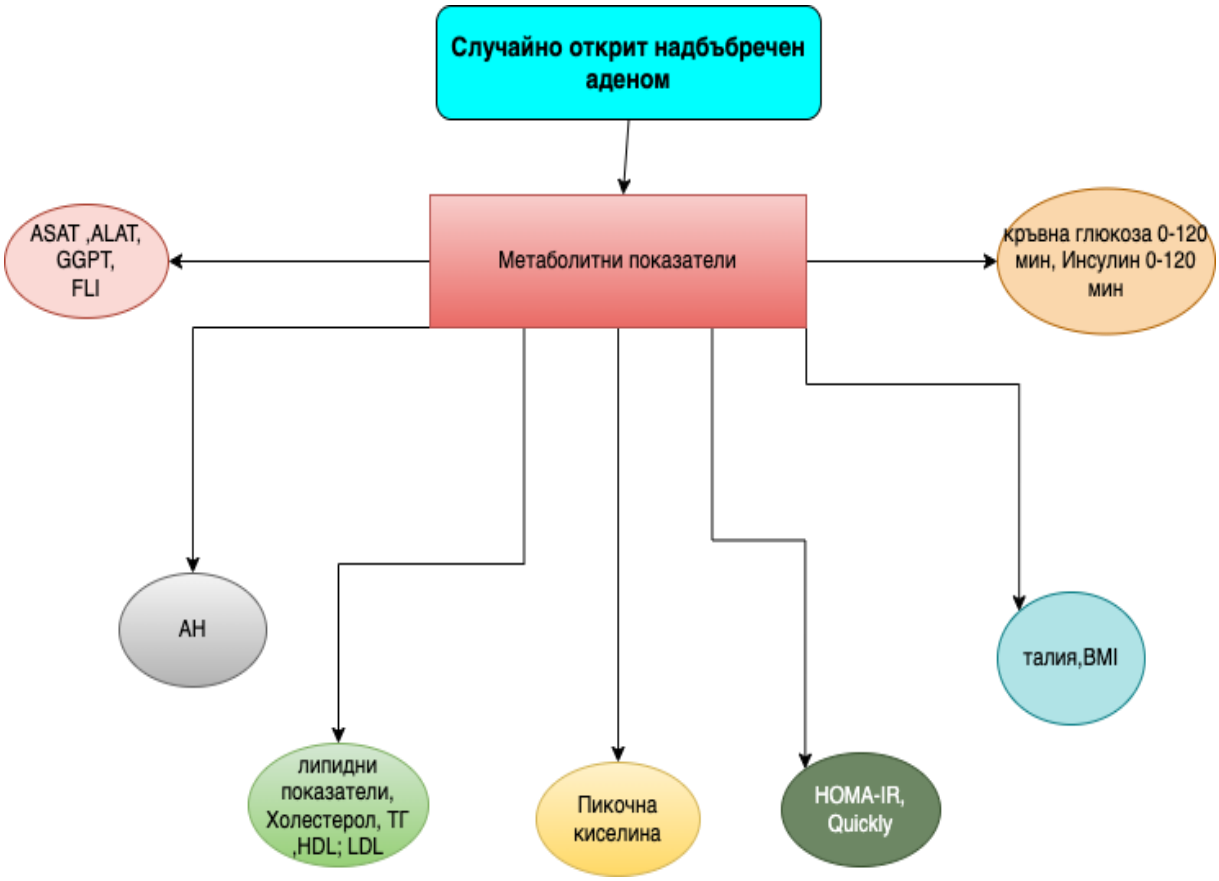


Fig. 43. Metabolic parameters that form the metabolic profile of patients with NI

The present work could become the basis for building a registry to track patients with hormone-reactive adrenal adenomas. The construction of such a database could be a powerful tool for monitoring these patients undergoing different treatment alternatives, as well as factors influencing prognosis and quality of life, patterns of care, and evaluation of the effectiveness and safety of different treatments.

FINDINGS

1. In the present study, we found a high prevalence of metabolic syndrome (89.5%) among patients with adrenal hormone-reactive adenomas - (5 signs 17.10%, four signs 27.6%, three signs 44.8%)
2. The disease that is most often involved in the formation of the metabolic profile of patients with adrenal hormone-reactive adenomas is arterial hypertension - (93.3%); the second place is overweight and obesity (BMI over 25 kg/m2) 84.7%, the third most common is carbohydrate disorders - 76.2% (found in blood glucose above 5.6 mmol/l) . Female sex is at risk for waist circumference obesity among patients with hormone-reactive adenomas
3. Arterial hypertension appears to be a risk factor among patients with hormone-reactive adenomas, in addition to elevated triglyceride levels and low HDL levels that correlate with carbohydrate disorders.
4. Uric acid, as an additional metabolic marker, correlates moderately with sex, with males carrying a 12-fold higher risk for levels above normal. There was a trend for an increase in mean uric acid with increasing criteria for metabolic syndrome in patients with hormone-reactive adenomas.There was a positive moderate correlation between serum uric acid levels with GGT and with FLI
5. In men with hormonally inactive adrenal adenomas, a waist circumference > 94 cm carries an 8-fold higher risk of lowering serum HDL levels.
6. Due to increased prevalence of CT , the incidence ofadrenal adenomas is increased , and female sex predominates. Adenoma size positively influences evening cortisol and SCU levels and does not influence the aldosterone/renin ratio.
7. The incidence of metabolic syndrome among patients with chorionically inactive adenomas is significantly higher compared to the general population.
8. Glycemic disorders were present in 89.5% of the studied patients, A high frequency of carbohydrate disorders was found : newly diagnosed DM (38.1%), NGG (5.7%), NGT(45.7%). This necessitates an active search for carbohydrate disorders among patients with hormone-reactive adenomas.
9. We found an incidence (67.60%) of NI patients who had more than a 5-fold increase in insulin levels at 120 min.
10. There was a significant difference between insulin levels in patients with normal Gt and those with newly diagnosed diabetes mellitus and NGH .We found that HOMA-IR correlated with blood glucose at 0' and 120', insulin at 0' and 120' and QUICKI.
11. Adenoma size does not correlate with carbohydrate indices. Determine the optimal value of HOMA-IR and QUICKI. at which adenoma size is expected to increase
12. Visceral obesity (evidenced by increased waist circumference) is a risk factor in patients with adrenal hormone-reactive adenoma. BMI and waist circumference correlate with insulin at 0' and 120', HOMA-IR and QUICKI.
13. 46.7% of NI patients had a high risk of developing hepatic steatosis as assessed by FLI screening. We determined a threshold FLI value of 57.5 above which adenoma growth could be expected. This value may serve as a noninvasive method for developing screening programs in this group of patients.

14. An algorithm for monitoring metabolic parameters in patients with adrenal hormone-reactive adenomas was proposed.

CONCLUSION

Adrenal hormone-reactive adenomas are defined as formations arising from the adrenal glands that are incidentally detected during imaging in conditions unrelated to suspected adrenal disease. The introduction of ultrasound, CT and MRI into clinical practice has increased the ability to detect adrenal tumours.

The main aim of our study was to analyze the diagnostic, metabolic, biochemical and hormonal aspects of patients with hormone-reactive adrenal adenomas. To make a contemporary assessment of some additional markers associated with metabolic syndrome,non-alcoholic steatohepatosis and cardiovascular risk in patients with hormone-reactive adrenal adenomas undergoing a single university-based specialized endocrinology clinic.

We found a high prevalence of metabolic syndrome as well as its individual components in patients with adrenal hormonally inactive adenomas and their association with multiple anthropometric indices. On the other hand, we compared the incidence of metabolic disorders between patients with hormone-non-secreting adrenal adenomas and the general Bulgarian population.We were able to demonstrate statistically significant differences between patients with hormone-non-secreting adrenal adenomas and the general population on the individual components of the metabolic syndrome. Arterial hypertension among patients with hormone-inactive adenomas was the most potent factor in triggering and increasing the number of MetSyn signs.We found that elevated triglycerides and low HDL-cholesterol levels also contribute to the development of carbohydrate disorders. Our results also highlight the role of dyslipidemia in adrenal hormone-reactive MetSyn adenomas. We also included the role of uric acid in the analysis. We investigated the screening marker-fatty liver index (FLI), liver enzymes in patients with hormone-inactive adenomas and found associations with adenoma size.

On the other hand, we investigated some hormonal parameters (serum cortisol rhythm, SCU, express blockade with 1 mg dexamethasone, aldosterone/renin ratio) and their relationship with age, anthropometry and size in non-secreting adenomas. We assumed that adrenal formations must have a certain critical mass in order to be able to produce a sufficient amount of cortisol to lead to the expression of clinical symptomatology. The size of the adrenal mass was thought to correlate positively with the likelihood of upper-bound evening cortisol levels. We determined the relative proportion of newly diagnosed glycemic disorders and the role of insulin resistance in patients with adrenal adenomas. The main value of this study is the remarkably higher prevalence of carbohydrate disorders among patients with hormonally inactive adrenal adenomas than in the general population. The present data suggest that the incidental detection of an adrenal tumor should encourage clinicians to diagnose earlier and treat possible abnormalities in glucose homeostasis.

Adrenal hormone-reactive adenomas can be a public health problem if only because of the proportion of carbohydrate abnormalities. Early diagnosis is important, not only with a single fasting blood glucose value, but also by performing an OGTT with blood glucose and insulin testing at 0 and 120 min. Only in this way will there be a complete picture of carbohydrate metabolism and patients can be properly interpreted, monitored and treated. An increase in carbohydrate metabolism testing would have a positive effect in order to look for hidden morbidity and prevent associated complications in a timely manner. We proposed a

diagnostic algorithm to search for metabolic disorders in patients with hormone-reactive adrenal adenomas.

Further prospective studies are needed to clarify whether patients with hormone-reactive adenomas and altered metabolic profiles have increased cardiovascular morbidity and mortality as has been demonstrated in the general population. Such data and comprehensive evaluation will be critical in selecting optimal treatment for these patients, which currently remains largely empirical.

CONTRIBUTIONS

Contributions of scientific and theoretical nature

1. For the first time in Bulgaria the relationship between hormonally inactive
2. adrenal adenomas and the individual components of the metabolic syndrome.
3. The present study confirms a higher incidence of metabolic abnormalities among patients with hormone-reactive adenomas compared to the general population.
4. For the first time in Bulgaria, the relationship between different degrees of carbohydrate tolerance and its abnormalities in patients with hormonally inactive adrenal adenomas was investigated.
5. For the first time, insulin levels at 120 min and their frequency at 5-fold rise were determined among patients with hormone-reactive adenomas.
6. For the first time in Bulgaria the risk factors for the development of metabolic syndrome in patients with hormonally inactive adrenal adenomas have been identified
7. We determined mean uric acid levels in patients with hormone-reactive adenomas and completed their metabolic profile.
8. For the first time, the use of novel non-invasive markers for screening of hepatic steatosis in patients with hormone-reactive adrenal adenomas was investigated.
9. An extension of the current algorithm with metabolic markers assessing metabolic complications in patients with hormone-reactive adrenal adenomas is proposed.

LIST OF DISSERTATION PUBLICATIONS

1. Zlatanova, E.B., Siderova, M.V., Christozov, K.K. Metabolic syndrome and adrenal incidentalomas - are they related? Journal of Endocrinology 3/2020
2. Zlatanova E., Siderova M., Hristozov K. Carbohydrate metabolism disorders in hormonally-inactive adrenal adenomas. Scientific reviews. General Medicine,2020, 22 (5): 80-84.
3. Zlatanova E., Siderova M., M.D., Khristozov K. Metabolic syndrome in adrenal hormone-reactive adenomas. MEDINFO, ISSUE 5 /2021

PARTICIPATION IN CONGRESSES RELATED TO THE THESIS

1. Zlatanova E, Hristozov K, Stoyanova D. Suprarenal incidentaloma-myelolipoma. Jubilee Congress "50 years of the Bulgarian Society of Endocrinology", 8-11 October 2015
2. Zlatanova E. Adrenal adenomas and metabolic syndrome. Varna Endocrine Days - Golden Sands, November 2016
3. Zlatanova E. Metabolic indices in adrenal hormone-inactive adenomas (own data). Varna Endocrine Days, November 2017
4. Zlatanova E, Siderova M, Kerekovska V, Khristozov K. Adrenal incidentalomas and cardiometabolic risk. XI National Congress of Endocrinology 11-13 October 2018

Acknowledgements

In conclusion, I express my most sincere and heartfelt gratitude to:
-my scientific supervisor Assoc. Ph. M. Siderova for the full support during the development of the thesis
- my colleagues from the Clinic of Endocrinology and its head Prof. Dr. K. Hristozov
- Last but not least, thank you to my parents and my husband Raiko for understanding, infinite patience and love.