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## Association of hypercalcemia with biochemical parameters in patients with endocrine disorders

**Abstract. Background.** Although today primary hyperparathyroidism and cancers are considered the main factors of hypercalcemia (HC), the latter is quite often detected in various endocrinopathies. According to modern data, ionized calcium ( $Ca^{2+}$ ) is an intracellular mediator in the functioning of enzymes and hormones. All this determines the importance of finding out the features of the associations between HC and biochemical indicators in various endocrine disorders, which was the purpose of this study. **Materials and methods.** Retrospectively, according to the medical histories of 537 patients with detected HC, who were treated at the endocrinology clinic of the V. Danilevsky Institute for the Endocrine Pathology Problems in 2020–2021, we have analyzed the features of correlations between HC (by the content of  $Ca^{2+}$ ) and the levels of phosphorus (P), total protein (TP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in various endocrine pathologies. **Results.** A significant difference between the spectrum and degree of probability of associations was found even within the limits of one type of pathological conditions. In the group with hypothyroidism, there was an inverse correlation of HC with P ( $p < 0.01$ ) and a highly probable correlation with AST ( $p < 0.001$ ). At the same time, a direct association with P ( $p < 0.01$ ) and an inverse correlation with TP ( $p < 0.001$ ), AST ( $p < 0.001$ ) and ALT ( $p < 0.01$ ) was revealed in case of Graves' disease. The following data were obtained for tumors of endocrine organs: nontoxic multinodular goiter — direct with P ( $p < 0.001$ ) and inverse with TP ( $p < 0.001$ ) and ALT ( $p < 0.01$ ); thyroid cancer — direct correlation with P ( $p < 0.01$ ) and inverse highly probable with TP ( $p < 0.001$ ) and ALT ( $p < 0.001$ ); nodular pathology of the adrenal glands — direct correlation with TP ( $p < 0.01$ ) and inverse with AST ( $p < 0.01$ ) and ALT ( $p < 0.001$ ). In diabetes mellitus, only an inverse correlation with P was found ( $p < 0.01$ ). There was an inverse correlation with P ( $p < 0.01$ ) and TP ( $p < 0.001$ ), and a direct one with ALT ( $p < 0.001$ ) in primary hyperparathyroidism. **Conclusions.** Metabolic disorders in the presence of hypercalcemia in patients with various endocrine pathologies have their own characteristics. The nature of revealed correlations between ionized calcium and biochemical parameters in terms of various endocrine diseases must be taken into account when developing a therapeutic strategy for patients with hypercalcemia.

**Keywords:** hypercalcemia; hypothyroidism; Graves' disease; thyroid cancer; nodular thyroid pathology; primary hyperparathyroidism; aspartate aminotransferase; alanine aminotransferase

### Introduction

The pathogenesis of the disorders of calcium metabolism is not fully understood [1]. Calcium is the most abundant cation found in the human body and plays an integral role in neural transmission, enzyme activity, myocardial function, coagulation, and other cellular functions [2]. Most of the calcium is found in the bones as calcium phosphate while a small percentage is found in the cells and extracellular fluids.

In the serum, about 45 % of calcium is bound to proteins, 45 % exists as free or ionized calcium that is the active form, while 10 % is bound to anions [3].

Systemic acidosis decreases calcium binding to albumin increasing serum levels while alkalosis causes the opposite effect. Serum calcium concentrations are highest in neonates and infants, decrease during childhood and adolescence, and stabilize at adult values by 17 years of age [4, 5].

The main cause of hypercalcemia is an excess parathyroid hormone (PTH). PTH-mediated causes include adenoma/hyperplasia of the gland, familial hypocalciuric hypercalcemia, and multiple endocrine neoplasia syndromes (type 1, 2A). Familial hypocalciuric hypercalcemia is an autosomal dominant condition due to an inactivating mutation in the calcium-sensing receptor gene [6].

At the same time, endocrine diseases are also associated with hypercalcemia [7]. Based on the fact that hyperparathyroidism (HPT) is an endocrine disease, and oncopathology can also be localized in the endocrine glands, it can be assumed that it is endocrine pathology that is the main cause of hypercalcemia.

Taking into account the peculiarities of the pathogenesis of various endocrine disorders, the question arises whether there are differences in the associative relationships between hypercalcemia and biochemical parameters in patients with endocrine disorders. To date, it is known that ionized calcium ( $\text{Ca}^{2+}$ ) serves as an intracellular mediator in the action of enzymes and hormones, which is of great importance. There is an opinion that  $\text{Ca}^{2+}$  rather than total calcium is the preferred indicator of its status [8, 9]. A significant role of  $\text{Ca}^{2+}$  as a mediator of the action of such hormones as vasopressin, angiotensin II, aldosterone, adrenaline, glucagon, cholecystokinin, serotonin has been established [3, 10, 11]. There are works on correlations of hypo- or hypercalcemia with alkalosis/acidosis, levels of phosphorus, and total protein [4, 12].

Establishment of the specific features of the profile of associations between hypercalcemia and biochemical markers in various endocrine disorders can correct the therapeutic strategy for monitoring such patients.

The **purpose** of this study was to establish the features of biochemical parameters in the development of hypercalcemia in patients with endocrine disorders.

## Materials and methods

We identified hypercalcemia in 537 patients with various endocrine disorders in the endocrinological clinic of the V. Danilevsky Institute for Endocrine Pathology Problems during 2020–2021. The associative relationships of hypercalcemia with the levels of phosphorus (P), total protein (TP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were studied.

Serum levels of total Ca and P were studied to assess the calcium-phosphorus metabolism by colorimetric method using kits from the company Spainlab (Ukraine) (for Ca) and Filisit diagnostics (for P). The level of ALT and AST was assessed by the kinetic method on a LabLine biochemical analyzer using Spainlab kits (Ukraine). TP was determined by the colorimetric method with a biochemical semi-automatic analyzer on a Solar PM 2111 photometer using a kit for determining total protein NVL Spainlab (Ukraine).

The features of associative links in various endocrine pathologies are analyzed.

Statistical analysis included an assessment of the normality of the distribution of the variables under study using the Kolmogorov-Smirnov test, the calculation of the main statistical parameters (mean value and its error). To study the relationship between the indicators, we used the method of correlation analysis with the determination of the correlation coefficient ( $r$ ) and the establishment of its significance by the  $t$ -test with a 95% level of reliability ( $p < 0.05$ ).

**Table 1. Correlation of serum calcium levels and biochemical parameters in case of thyroid dysfunction**

Diagnosis	n	Ca, mmol/l	$\text{Ca}^{2+}$ , mmol/l	P, mmol/l	TP, g/l	AST, IU/l	ALT, IU/l	
Hypothyroidism	57	M $\pm$ m	2.66 $\pm$ 0.02	1.15 $\pm$ 0.01	1.14 $\pm$ 0.05	72.30 $\pm$ 0.75	27.81 $\pm$ 2.38	25.66 $\pm$ 1.97
		r	0.880687	–	–0.33709	–	–0.44173	–
		p	< 0.001	–	< 0.01	–	< 0.001	–
Graves' disease	42	M $\pm$ m	2.68 $\pm$ 0.03	1.15 $\pm$ 0.02	1.16 $\pm$ 0.06	73.09 $\pm$ 2.04	25.48 $\pm$ 9.67	28.96 $\pm$ 11.57
		r	0.445118	–	0.329618	–0.75731	–0.59594	–0.38995
		p	< 0.001	–	< 0.01	< 0.001	< 0.001	< 0.01

**Table 2. Correlation of blood calcium level and some biochemical parameters in tumor processes in endocrine organs**

Diagnosis	n	Ca, mmol/l	$\text{Ca}^{2+}$ , mmol/l	P, mmol/l	TP, g/l	AST, IU/l	ALT, IU/l	
NMG	158	M $\pm$ m	2.68 $\pm$ 0.01	1.15 $\pm$ 0.01	1.09 $\pm$ 0.04	74.00 $\pm$ 0.75	35.95 $\pm$ 6.05	46.05 $\pm$ 8.27
		r	0.365033	–	0.339	–0.43243	0.218999	–0.78322
		p	< 0.01	–	< 0.01	< 0.001		< 0.001
Thyroid cancer	68	M $\pm$ m	2.70 $\pm$ 0.02	1.15 $\pm$ 0.01	1.13 $\pm$ 0.07	73.00 $\pm$ 1.59	27.15 $\pm$ 9.65	30.79 $\pm$ 16.41
		r	0.888011	–	–0.50305	–	–1.0	–1.0
		p	< 0.001	–	< 0.001	–	< 0.001	< 0.001
Formations in the adrenal glands	15	M $\pm$ m	2.67 $\pm$ 0.04	1.21 $\pm$ 0.01	1.14 $\pm$ 0.09	68.71 $\pm$ 0.96	46.49 $\pm$ 18.92	40.97 $\pm$ 10.34
		r	0.940809	–	–	0.536311	–0.35116	–0.61483
		p	< 0.001	–	–	< 0.001	< 0.01	< 0.001

The Ethics Commission of V. Danilevsky Institute for Endocrine Pathology Problems of the National Academy of Medical Sciences of Ukraine considered and approved the design of the study, information for the patient, and the form of the informed consent to participate in the study (protocol 4/2020).

## Results

Depending on pathology of the endocrine glands, the patients were divided into subgroups: with thyroid dysfunction (hypothyroidism and Graves' disease); tumor processes in the endocrine organs (nontoxic multinodular goiter (NMG), thyroid cancer (TC), adrenal adenomas); primary hyperparathyroidism and diabetes mellitus.

Analyzing the correlations of hypercalcemia in terms of the  $\text{Ca}^{2+}$  level with the studied set of biochemical parameters, one should note significant differences between the spectra and the degree of significance of associations even within the same class of diseases. The results are presented in Tables 1–3.

Thus, in groups of patients with thyroid dysfunction in hypothyroidism there was a negative correlation of  $\text{Ca}^{2+}$  with P ( $p < 0.01$ ) and a highly significant correlation with AST ( $p < 0.001$ ). At the same time, in Graves' disease there was a direct correlation with P ( $p < 0.01$ ) and an inverse correlation with TP ( $p < 0.001$ ), AST ( $p < 0.001$ ) and ALT ( $p < 0.01$ ).

In groups of patients with tumor lesions of the endocrine organs, each of the considered pathologies had its own characteristics. Nodular pathology of the thyroid gland was characterized by the presence of a direct correlation with P ( $p < 0.01$ ), an inverse highly significant correlation of HC with TP ( $p < 0.001$ ) and ALT ( $p < 0.001$ ). At the same time, a highly significant negative correlation with P ( $p < 0.001$ ), AST ( $p < 0.001$ ) and ALT ( $p < 0.001$ ) was determined in thyroid cancer. In case of nodular pathology of the adrenal glands, there was a direct correlation with TP ( $p < 0.001$ ), an inverse correlation with AST ( $p < 0.01$ ) and a closer correlation with ALT.

In DM, only an inverse correlation with P ( $p < 0.01$ ) was revealed, while in PHPT, it was inverse with TP ( $p < 0.01$ ) and direct with ALT ( $p < 0.01$ ).

## Discussion

Today, parathormone-dependent hypercalcemia, which is the most common variant, and parathormone-independent hypercalcemia, mainly due to malignant tumors, are distinguished [7]. At the same time, endocrine pathology is also often accompanied by HC, which is characterized by certain features of correlations with biochemical parameters.

The data obtained by us regarding the spectrum of associative relationships between HC and a number of biochemical markers in various pathologies of the endocrine organs provide an opportunity to obtain additional information regarding the pathogenesis of the considered pathologies. Its importance is associated with the significant role of  $\text{Ca}^{2+}$  as a mediator of hormone action. It is known that a complex cascade of reactions involving  $\text{Ca}^{2+}$  and enzymes controlled by it mediates the physiological action of hormones on cells [13, 14]. It has been established that an increased concentration of  $\text{Ca}^{2+}$  is accompanied by a different spectrum and direction of associative relationships with the studied biochemical parameters against the background of hyper- and hypothyroidism. In groups of patients with tumor lesions of the endocrine organs, each of the considered pathologies had its own characteristics. Nodular pathology of the thyroid gland was characterized by the presence of an inverse highly significant correlation of HC with TP and with ALT ( $p < 0.001$ ). At the same time, in thyroid cancer, a highly significant inverse correlation with P, AST and ALT was determined ( $p < 0.001$ ). In nodular pathology of the adrenal glands, there was a direct correlation with TP ( $p < 0.001$ ) and an inverse correlation with AST ( $p < 0.01$ ) and a closer correlation with ALT ( $p < 0.001$ ).

The diagnostic value of increasing the levels of AST and ALT in various pathologies is today an urgent problem in terms of interpreting the results. Far from all patients, it is associated with liver pathology, and is often detected with obstruction and cardiac pathology [15, 16]. There is evidence that hyperthyroidism and insulin resistance may be independent causes of an increase in the activity of serum liver enzymes or the development of hepatocyte fibrosis [17, 18]. AST and ALT are two important metabolic links between carbohydrate and protein metabolism [18, 19]. Their activity is relevant for cells with high metabolic activity. It is known that an increase in AST is largely associated with the risk of cardiovascular disease, and ALT tends to more accurately characterize the state of the liver. In the studies conducted, HC significantly correlated with AST in all the considered pathologies, except for NMG, and ALT, except for hypothyroidism. A number of indicators identified during the study deserve special attention: — the absence of significant correlations between HC and the level of P in tumor processes in the adrenal glands; the absence of significant correlations in patients with diabetes between an increase in  $\text{Ca}^{2+}$  and TP, as well as AST and ALT; the predominance of ALT levels compared with AST in NMG and thyroid cancer; a more pronounced increase in AST and ALT in benign thyroid nodular pathology and adrenal glands.

**Table 3. Correlation of blood calcium levels and some biochemical parameters in DM and PHPT**

Diagnosis	n	Ca, mmol/l	$\text{Ca}^{2+}$ , mmol/l	P, mmol/l	TP, g/l	AST, IU/l	ALT, IU/l
Diabetes mellitus	M ± m	2.67 ± 0.01	1.19 ± 0.04	1.12 ± 0.02	72.85 ± 0.51	29.39 ± 1.48	29.78 ± 1.91
	r	–	–	–0.33565	–	–	–
	p	–	–	< 0.01	–	–	–
PHPT	M ± m	2.97 ± 0.06	1.27 ± 0.03	0.94 ± 0.04	72.96 ± 0.79	26.89 ± 3.84	20.68 ± 3.46
	r	0.812618	–	–0.442	–0.47193	–	0.537392
	p	< 0.001	–	< 0.01	< 0.001	–	< 0.001

The results obtained make it possible to specify the features of the pathology of each of the considered pathologies of the endocrine glands, as well as to provide a differentiated approach to monitoring patients with HC against the background of various endocrine pathologies.

## Conclusions

1. Disorders of metabolic processes in the presence of hypercalcemia in patients with various endocrine disorders have some features with a different nature of endocrinopathies.
2. The nature of the established features of the association of hypercalcemia with biochemical parameters should be taken into account when choosing a therapeutic strategy.

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