Relationship of the content of vitamin D and melatonin in blood serum and pineal gland calcifications in patients with malignant bone tumors

Oleg Drobotun[a*], Mykola Safonov[a], Mykola Kolotilov[b]

[a] *R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine Vasylkivska St., 45, Kyiv, 03022*

[b] *Institute of Nuclear Medicine and Diagnostic Radiology of the National Academy of Medical Sciences of Ukraine, Platon Mayborodi St., 32, Kyiv, 04050*

olegdrobotun@gmail.com

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The aim of the study was to investigate the relationship between the vitamin D content, melatonin and the characteristics of pineal gland calcifications in patients with malignant tumors of the bones of the lower extremities. Vitamin D deficiency and pineal gland calcifications are observed in almost 100 % of patients with malignant tumors of the lower extremities' bones. The high heterogeneity of calcifications and its dynamics during the treatment of patients may indicate the processes of their litholysis and dissolution.

Introduction

The pineal gland, located in the geometric center of the human brain, is an organ of the diffuse endocrine system. Melatonin (5-methoxy-N-acetyltryptamine) is the main hormone of the gland (80 % of the total systemic melatonin), an endogenous integrator, chrono modulator and regulator of the body's circadian rhythms. The maximum secretion of melatonin is observed at night, the minimum – during the day. Melatonin has, to varying degrees, a dose-dependent antistressor, sedative, neuroprotective, geroprotective, antidepressant, antioxidant, antitumor, antiapoptotic (in normal cells), proapoptotic (in cancer cells), oncostatic, immunostimulating, hepatoprotective, geroprotective, antihypertensive, anti-inflammatory, moderate contraceptive (for women) action [1,2]. Melatonin regulates neuroendocrine functions, respiratory rate, reproductive function; osteogenic differentiation of mesenchymal stem cells, formation and protection of bones; modulates the activity of bone-forming osteoblasts and bone-resorbing osteoclasts; reduces pain sensitivity; influences intracellular calcium content [2,3]. However, we should note that most of these properties of melatonin are debatable [1].

Calcium plays a central role in initiating a cellular response, as it participates in all known cell effector systems. The difference is only in the share contribution of Ca^{+2} – the effector

system and in the source of calcium mobilization. Absolutely essential for any cell, calcium becomes a cellular toxin at its high concentration. An excessive increase in the concentration of calcium in the cytoplasm leads to dysfunction and cell death [4].

Cemeteries (possibly used sometimes as a depot) of calcium are observed in almost all organs and tissues [5]: gallbladder, kidneys, prostate, salivary glands stones, calcifications of the brain, heart valves, carotid and coronary arteries, lungs, heterotopic idiopathic ossification of muscles, tendons, ligaments, joint capsules, enterolitis, coprolitis, rhinolitis.

Deposition of mineral salts (ectopic calcification – the process is certainly pathological) in the form of solid calcifications is observed in almost all organs and tissues [4,5], but the pineal gland has the highest level of calcification among all organs and tissues of the human body. The incidence of pineal calcifications increases with age: from 2 % at age 0-9, 32 % at age 10-19, 53 % in age groups of 20-29, 8 3% in age groups over 30 and 99 % in age groups over 70 years old [5]. Pineal calcification is an active process and resembles bone formation by a number of signs [6]. This process involves mesenchymal stem cells and melatonin. Pineal calcification is a marker of aging in the body and is associated with evidence so far only with Alzheimer's disease and schizophrenia [7], although more recently calcifications were called physiological or non-pathogenic pineal gland calcifications [6]. The relationship of pineal calcification with melatonin synthesis violation and development of diseases of the central nervous system and other organs and systems of the body is being studied in the researches [7].

A typical participant in the normal formation of bones [6] and, possibly, calcifications of many localizations [8] is vitamin D, which has antiproliferative, antibacterial, anti-inflammatory (anticytokine), immunomodulatory, normoglycemic (insulin), antidepressant, analgesic, anabolic, lipolytic, organo protective, hypotensive action; regulates apoptosis, angioneogenesis. Epidemiological and experimental data have shown that low levels of vitamin (hormone) D are closely associated with overall mortality, the development of cardiovascular and oncological diseases (mainly breast, prostate, colon cancer), arterial hypertension, sarcopenia, metabolic syndrome, and insulin resistance and diabetes mellitus types 1 and 2 in adults [9,10].

The aim of the study was to investigate the relationship between the vitamin D content, melatonin and the characteristics of pineal gland calcifications in patients with malignant tumors of the bones of the lower extremities.

Material and research methods

The study included the following groups of patients: 1 – control group, 22 practically healthy male patients without chronic diseases at the age from 36 to 55 years; 2A group – 19 male patients with benign tumors (aneurysmal bone

cyst, enchondroma, chondroblastoma, osteoblastoma, giant cell bone tumor) at the age from 41 to 59 years before treatment; 2B group – 19 male patients with benign tumors of the proximal/distal femur and simultaneous arthroplasty at the age from 39 to 52 years after treatment; 3A group – 28 male patients with malignant tumors (malignant giant cell bone tumor, osteosarcoma, chondrosarcoma, fibrosarcoma, Ewing's sarcoma, periosteal and paraostal sarcomas, bone lymphoma) of the lower extremity bones at the age from 38 to 60 years before treatment; 3B group – 21 male patients with malignant tumors after radical removal of a malignant tumor of the proximal/distal femur and simultaneous endoprosthetics at the age from 44 to 60 years during stable remission; 3C group – 16 male patients at the age from 37 to 56 years with recurrence and/or metastasis.

Patient selection criteria for the study: age from 36 to 60 years; absence of osteoporosis; no pain syndrome, normal renal function (the permissible level of exceeding the upper limit of the reference values of creatinine is not more than 2.5) and liver (biochemical parameters of bilirubin, alanine aminotransferase, aspartate aminotransferase not exceeding the upper limit of the norm by more than 2.5 times); general somatic status on the Karnofsky scale is not less than 90 %; body mass index – 18.5-25 kg/m²; polymorbidity.

Criteria for excluding patients from the study: severe condition of the patient (Karnovsky index below 50 %); a history of mental illness; signs of renal or hepatic failure (ALT/AST level more than 100 U/L; creatinine more than 220 μmol/L); low hematological parameters (hemoglobin below 90 g/l; the initial number of leukocytes less than 3.0 thousand and platelets less than 50.0 thousand in the peripheral blood); violation of the dim light regime in the evening (twilight lighting), night wakefulness; the presence of an active infectious process; clinical signs of circulatory failure (peripheral edema, severe shortness of breath due to decompensation of chronic diseases of the cardiovascular system), cardiac arrhythmias; use of narcotic analgesics for pain relief.

Biochemistry. The blood serum melatonin content was measured by the enzyme immunoassay with reagent kits (№ RE54021) from IBL-Hamburg GmbH (Hamburg, Germany). The detection limit for melatonin is 1.6 pg/ml, the intra-assay coefficient of variation is 4.4 %, and the inter-assay coefficient of variation is 7.1 %.

The content of vitamin 25(OH)D (25-hydroxycalciferol) in blood serum was determined by the immunochemiluminescence method on an automatic analyzer ARCHITECT 25-OH Vitamin D Controls. The detection limit is 2.1 ng/ml, the coefficient of variation within the assay is 5.2 %, the coefficient of variation between assays is less than 7 %.

Patient stratification (in addition to the study design) was carried out according to the classification of vitamin D content [10]: deficiency – <20 ng/ml (50 nmol/l); suboptimal level – 20-30 ng/ml (50-75 nmol/l); optimal level (target status) – 30-50 ng/ml (75-125 nmol/l); high content – 50-100 ng/ml (125-250 nmol/l); dangerous level – > 100 ng/ml (250 nmol/l).

Blood sampling was carried out on an empty stomach at 7 am, in winter, before diagnostic procedures and taking medications. Blood samples were taken at 12 noon, 1 and 2 a.m. (when using a bedside lamp with illumination of 10 lux in lying position, 8 a.m. (December 2019 – January 2020), from the median cubital vein through a pre-installed catheter. Compliance with all known requirements [11, 12] for blood sampling for melatonin is limited by bioethics and compliance.

X-ray computed tomography. The patients were investigated at multi detector X-ray tomography. Calcification was determined as such by its typical location and in the presence of at least 1 pixel with an X-ray density of ≥130 units (**Figure 1**). Heterogeneity (heterogeneity describes changes in physical properties between 2 or more points) of calcifications was determined by measuring the maximum Dmax and minimum Dmin of X-ray density, the coefficient of heterogeneity was calculated by the formula Cg = Dmax/Dmin. The scale "homogeneity → heterogeneity" Cg corresponds to 1.0 → 100.0.

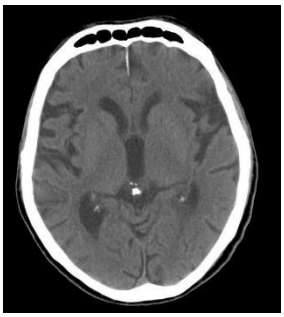

Figure 1. Patient – 59 years. Computed tomography image. Pineal calcification.

The investigations were carried out in accordance with the rules and principles of bioethics. Patients were familiarized with the content of diagnostic and treatment procedures and signed an “Informed Consent” form.

Statistical processing of the material was carried out by methods of variation statistics. The values of the arithmetic mean, mean square (standard) error of the arithmetic mean were calculated. For significant differences in the comparison of mean values in paired comparisons, the Student's t-test was taken at $p < 0.01$.

Results and discussion

The results of measurements and calculation of correlation coefficients are presented in **Tables 1, 2**.

Table 1. Content of vitamin D, melatonin, frequency and characteristics of calcifications

Group	n	Vitamin D, ng/ml	Melatonin, pg/ml	Calcifications		
				abs/%	volume, mm^3	Cg
		1	2		3	4
1	10	38,5±1,2 optimal	45,7±2,1	3/30,0	99±8	1,2±0,
	12	26,9±0,9 suboptimal	34,1±1,9	4/33,3	125±11	1,4±0,1
2A	3	31,9±1,0 optimal	28,5±1,7	1/33,3	138±11	1,5±0,1
	16	24,7±0,9 suboptimal	22,9±1,6	7/43,8	159±12	1,9±0,1
2B	6	32,4±1,1 optimal	27,4±1,7	3/50,0	141±13	1,4±0,1
	13	25,1±0,9 suboptimal	24,6±1,7	6/46,2	170±12	1,7±0,1
3A	28	14,9±0,7 deficit	17,2±1,5	28/100,0	483±18	5,8±0,2
3B	4	22,4±0,8 suboptimal	19,8±1,6	3/75,0	397±16	4,1±0,2
	17	17,4±0,7 deficit	19,1±1,4	16/94,1	435±15	6,2±0,2
3C	16	10,3±0,6 deficit	12,9±1,2	16/100,0	754±19	7,6±0,2

Table 2. Correlation coefficients

Indicators	Vitamin D	Melatonin	Volume	Kg
Vitamin D	X	0,89	- 0,90	- 0,90
Melatonin	0,89	X	-0,79	-0,79
Volume	- 0,90	-0,79	X	0,98
Kg	- 0,90	-0,79	0,98	X

In the study [10], 83 % of patients with bone tumors had a vitamin D deficiency – 19.82 ng/ml. Patients with malignant tumors had significantly lower levels of vitamin D compared to patients with benign lesions ($p = 0.0008$).

A study [9,10] showed that patients with bone metastases of breast cancer had 25-OH-D serum levels of 15.3 ng/ml (± 4.7 ng/ml), with metastatic prostate cancer – 14.7 ng/ml (± 8.3 ng/ml), with multiple myeloma – 14.8 ng/ml (± 6.3 ng/ml).

The correlation analysis between the vitamin D content and the melatonin content revealed a strong direct relationship ($t = 13.5$; $p > 0.99$), between the vitamin D content and the volume, between the vitamin D content and the coefficient of heterogeneity of calcifications - a strong inverse relationship ($t = 14.1$; $p > 0.98$).

The volume and heterogeneity of calcifications increases, and the content of vitamin D and melatonin decreases in the examined healthy and patients in the following order: norm – benign tumors – malignant tumors – relapses/metastases. The increase in heterogeneity with a simultaneous increase in the volume of calcification may reflect the processes

of mineralization/demineralization in the calcium depot. The experiment [14] showed the ability of melatonin to reduce the calcification of the aortic valve, as evidenced by a decrease in thickness and calcium deposition in the valve leaflets, an improvement in echocardiographic parameters (a decrease in the maximum transvalvular flow velocity and an increase in the area of the aortic valve). Another experiment [15] demonstrated the reduction of vascular calcification and aging with the help of melatonin.

The melatonin content in blood plasma was studied in patients with common cancers of the breast, prostate, rectum, gastrointestinal tract, melanoma: the hormone content in all untreated patients was significantly lower than in healthy people [3,16]. The studies have practically not covered rare patients with malignant bone tumors. At the same time, high levels of melatonin receptors expression in human osteosarcomas have been established, which indicates an important role of melatonin in oncogenesis [16, 17]. The antitumor activity of melatonin has been studied in osteosarcomas [18].

It is quite reasonably believed [6], that the calcification of the pineal gland is a marker of the inevitable aging process of the organism and its natural structure (but this is not yet generally accepted) is a chronicle of the specificity of lithogenesis. It is obvious (by analogy with cholelithiasis, urolithiasis, calcifying aortic valve disease) that slowing down this lithogenesis can slow down the aging process.

Conclusion

Vitamin D deficiency and pineal gland calcifications are observed in almost 100 % of patients with malignant tumors of the lower extremity bones.

The high heterogeneity of calcifications and its dynamics during the treatment of patients may indicate the processes of their litholysis and dissolution.

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