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The effect of the consumed meal and of serum sample freezing/thawing cycles on the chromogranin A (CgA) blood level**

Wpływ spożytego posiłku oraz cyklów zamrażanie/rozmrażanie próbek surowicy na stężenie chromograniny A (CgA) we krwi

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Keywords

chromogranin A, CgA, neuroendocrine tumours, postprandial test

Słowa kluczowe

chromogranina A, CgA, guzy neuroendokrynne, test poposiłkowy

Summary

Introduction. Chromogranin A (CgA) is a non-specific biomarker of neuroendocrine neoplasms (NEN). One of unsettled important question concerned with this marker is the effect of a meal on the level of chromogranin A. Yet another important analytical question is associated with *in vitro* stability of a CgA molecule.

Aim. The study was aimed at assessment of the effect of a meal on the blood level of chromogranin A in a postprandial test, and assessment of stability of a CgA molecule in serum samples undergoing three cycles of freezing/thawing.

Material and methods. The study group consisted of 27 persons (7 men, 20 women) age ranging between 22 and 76 years. A postprandial test was made, using the design a sample collected on fasting (0 minutes), and a second one collected 60 or 120 minutes after a meal. The second study group consisted of 10 serum samples collected from patients with CgA levels between 28.6 and 1115.1 ng/ml. Design of the test: CgA determination in a primary sample, and then after each of three serum sample freezing/thawing cycles. The CgA level was determined using the immunoradiometric method (IRMA).

Results. No statistically significant differences in CgA levels determined in the postprandial test were found: 0'-60' ($p = 0.0580$) and 0'-120' ($p = 0.1024$), nor after each of consecutive three freezing/thawing cycles ($p = 0.0624$).

Conclusions. In majority of subjects a meal had no effect on the blood CgA level, but in some single cases the effect was significant (24-37%), and therefore the recommendation that blood should be drawn on fasting seems valid. Three cycles of freezing/thawing of serum samples had no effect on the determined CgA blood levels.

Streszczenie

Wstęp. Chromogranina A (CgA) jest niespecyficznym biomarkerem guzów neuroendokrynnych (NEN). Jednym z ważnych zagadnień przygotowania pacjenta do badania jest ocena wpływu posiłku na stężenie chromograniny A. Inny ważny aspekt analityczny dotyczy stabilności cząsteczki CgA w warunkach *in vitro*.

Cel pracy. Celami badawczymi pracy była ocena wpływu posiłku na stężenie chromograniny A we krwi w teście poposiłkowym oraz ocena stabilności cząsteczki CgA w 3 cyklach zamrażanie/rozmrażanie.

Materiał i metody. Grupa badana liczyła 27 osób (7 mężczyzn, 20 kobiet) w wieku 22-76 lat. Wykonano test poposiłkowy według schematu: na czczo (0 minut) i 60 lub 120 minut po posiłku. Drugą grupę badaną stanowiło 10 próbek surowic pozyskanych od pacjentów w zakresie stężeń CgA wynoszącym 28,6-1115,1 ng/ml. Schemat testu: oznaczenie stężenia CgA w próbce pierwotnej, a następnie w 3 cyklach zamrożenie/rozmrożenie próbki surowicy. Stężenie chromograniny A oznaczono metodą immunoradiometryczną (IRMA).

Wyniki. Nie wykazano istotnych statystycznie różnic w stężeniu CgA oznaczanej w teście poposiłkowym: 0'-60' ($p = 0,0580$) oraz 0'-120' ($p = 0,1024$) oraz w 3 kolejno następujących po sobie cyklach zamrażanie/rozmrażanie próbek surowicy ($p = 0,0624$).

Conflict of interest
Konflikt interesów

None
Brak konfliktu interesów

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Wnioski. Spożycie posiłku u większości badanych nie miało wpływu na stężenie CgA we krwi, ale w pojedynczych przypadkach wpływ był znaczący (24-37%) i dlatego uzasadnione jest zalecenie, aby krew na to badanie pobierać na czczo. Trzykrotne zamrożenie/rozmrożenie próbek surowicy nie wpłynęło na wynik oznaczeń stężenia CgA we krwi.

INTRODUCTION

Chromogranin A (CgA) belongs to the family of secretory proteins called granins/secretogranins (1). CgA is a soluble, acidic glycoprotein, with molecular mass of 48 kDa, composed of a single polypeptide chain (2). It is present in human neuroendocrine cells (3, 4). It is stored and secreted along with catecholamines, peptide hormones and cell-specific neurotransmitters (5). CgA is released to blood where it may be determined as a, so called, circulating biomarker (6) and is classified as non-specific marker of neuroendocrine neoplasms (NEN) (7). Determination of blood CgA levels is useful in diagnostics of NEN, assessment of response to treatment and in detection of progression and recurrence at early stage of the disease (8, 9).

The knowledge of various pre-analytical and analytical factors that may influence the level of a tested analyte is an important question for laboratory research. The effect of a meal on the blood level of chromogranin A is one of important issues. Another analytical problem is associated with stability of a CgA molecule in *in vitro* conditions, in which the compound will be determined for clinical purposes.

AIM

The study was aimed at assessment of the effect of a meal on the blood level of chromogranin A in a postprandial test, and of stability of a CgA molecule in a test sample of serum undergoing three cycles of freezing/thawing.

MATERIAL AND METHODS

Materials

The study group consisted of: 27 subjects (7 men, 20 women; age 22-76 years, mean age ± SD was 39 ± 17 years). They were healthy volunteers and patients in whom a postprandial test was performed. Design of the test: two blood drawings: on fasting (0 minutes) and 60 or 120 minutes after a meal. The meal consisted of: 2-3 sandwiches and water. Exclusion criteria: patients treated with proton pump inhibitors (PPI), H_2 histamine receptor blockers, corticosteroids, serotonin reuptake inhibitors, and patients with chronic renal and hepatic diseases, inflammatory conditions of the alimentary tract, prostatic cancer, rheumatoid arthritis, severe arterial hypertension and neuroendocrine neoplasms.

The second study group consisted of: 10 samples of serum collected from patients with chromogranin A levels ranging between 28.6 and 1115.1 ng/ml. The study consisted of initial determination of the CgA level in a serum sample, and repeated determination after each three consecutive cycles of freezing/thawing.

The study was approved by the Bioethics Commission of Centre of Postgraduate Medical Education.

CgA level determination

Venous blood was drawn from the ulnar vein into a tube containing a clotting activator. 30 minutes later blood was centrifuged (10 minutes at 3500 rpm, in room temperature), and the obtained serum was frozen and stored at -30°C until the test (not longer than for a month).

Chromogranin A level was determined by the immunoradiometric method (IRMA) using CIS bio International kit (CGA-RIACT, France). The reference range for serum samples, according to test producer, was 10-100.0 ng/ml. Sensitivity of the method was 1.5 ng/ml. Intra-serial variability (CV) for samples at various concentrations: 29.9, 144 and 998 ng/ml was 6.0, 3.8 and 2.2%, respectively. Inter-serial variability for samples at various concentrations: 29.9, 144 and 998 ng/ml was: 8.5, 5.7 and 5.3%, respectively.

Statistics

Results are presented as median and range of concentration and as mean ± SD. Normality of distribution was analysed using the Shapiro-Wilk test. Differences of results between groups in the postprandial test were analysed using the Wilcoxon's sign rank test, and differences of results between groups of frozen/thawed samples were analysed using the Friedman's test.

RESULTS

Table 1 presents CgA levels in the postprandial test recorded on fasting (0 minutes) and 60 minutes after a meal (n = 9), and on fasting (0 minutes) and 120 minutes after a meal (n = 18) in the group of patients and in the group of healthy volunteers. On fasting, the median CgA level was 26 ng/mL (19-46 ng/mL), and 60 minutes after a meal it was 34 ng/mL (22-44 ng/mL). The difference was 0-24% (p = 0.0580). And in the 0'-120' test, on fasting the median CgA level was 33.0 ng/mL (12-89 ng/mL), and 120 minutes after a meal it was 33.5 ng/mL (19-91 ng/mL). The difference was 0-37% (p = 0.1024) (fig. 1 and 2).

CgA was determined in serum samples within the concentration range of 28.6-1115.1 ng/ml. Median values and concentration ranges for CgA in three freezing/thawing cycles: 0 (before freezing) and after 1, 2, and 3 serum sample freezing/thawing cycles were: 215.7 ng/ml (30.6-1076.0 ng/ml), 203.2 ng/ml (28.6-1115.1 ng/ml), 209.0 ng/ml (29.3-1113.1 ng/ml), and 208.7 ng/ml (29.9-1106.7 ng/ml) (tab. 2).

Tab. 1. Differences in the CgA level in the postprandial tests 0'-60' and 0'-120'

CgA [ng/ml]		CgA [ng/ml]	
0' (fasting)-60' (after meal)		0' (fasting)-120' (after meal)	
0' (n = 9)	60' (n = 9)	0' (n = 18)	120' (n = 18)
median 26 mean ± SD – 29 ± 8.7	median 34 mean ± SD – 32 ± 7.2	median 33 mean ± SD – 37 ± 20.6	median 33.5 mean ± SD – 40 ± 21.6
Difference 0-24% (p = 0.0580)		Difference 0-37% (p = 0.1024)	

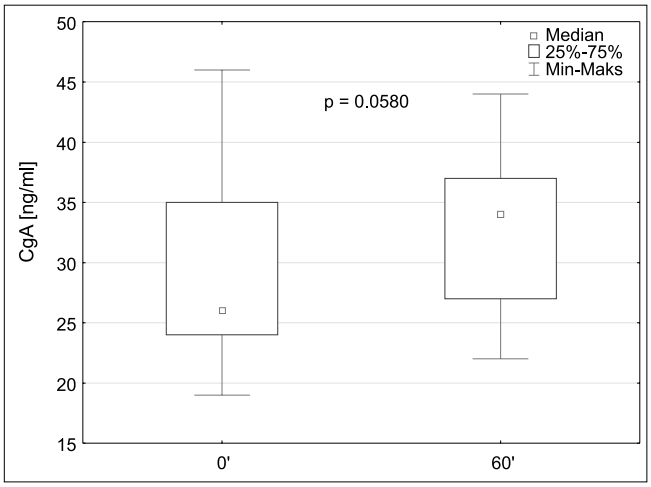

Fig. 1. Differences in CgA level in the postprandial test (on fasting and after 60 minutes)

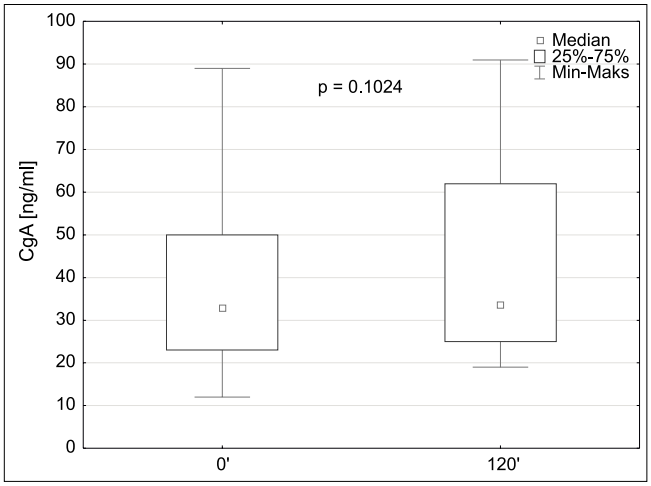

Fig. 2. Differences in the CgA level in the postprandial test (on fasting and after 120 minutes)

The difference in CgA concentration determinations was 0.1-12.5% (median 5.2%) and was not statistically significantly different (p = 0.0624) in three subsequent freezing/thawing cycles. Results of the analysis are presented in the table 3 and in the figure 3.

Tab. 3. Differences in stability of chromogranin A molecule after three freezing/thawing cycles

CgA	n	Difference (%)	p value
1x	10	median = 5.5% (0.4-10.0%)	p = 0.0624
2x	10	median = 3.7% (0.1-11.5%)	
3x	10	median = 5.5% (1.2-12.5%)	

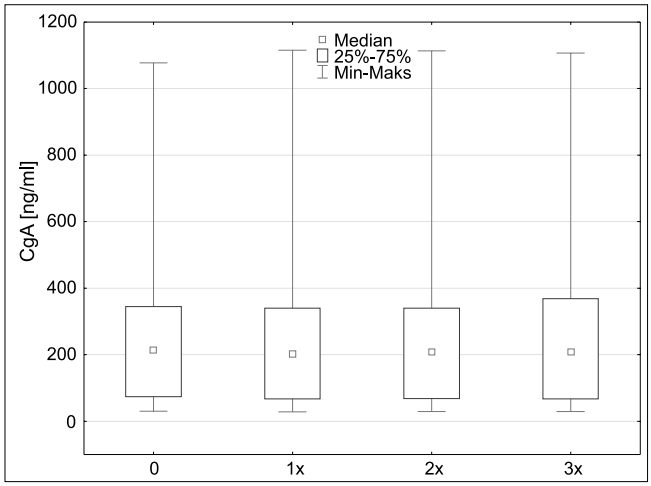

Fig. 3. Differences in stability of chromogranin A molecule after three freezing/thawing cycles

DISCUSSION

Chromogranin A molecule undergoes numerous post-translational modifications involving biochemical processes: carboxylation, glycosylation, phosphorylation, sulphatation. Those processes lead to formation of numerous peptides playing various biological functions. Determination of a whole, intact CgA molecule is particularly useful in diagnostics of neuroendocrine neoplasms, such as: hormonally active and non-active gastro-entero-pancreatic neuroendocrine neoplasms (GEP-NEN), catecholamine-secreting tumours (pheochromocytoma, paraganglioma) and some other conditions (10-12). Sensitivity of CgA determination in NEN is 60-100%, and specificity is 70-100% (13).

Among numerous pre-analytical factors important in preparation of a patient for an examination, the assessment of the effect of a consumed meal on the level of

Tab. 2. Differences in stability of chromogranin A molecule after three freezing/thawing cycles

Freeze – thaw cycle	CgA [ng/ml]				
	Median	Range (min/max)	Mean ± SD	Lower quartile	Upper quartile
0	215.7	30.6-1076.0	298.3 ± 316.5	74.10	344.80
1	203.2	28.6-1115.1	295.8 ± 328.5	67.60	340.20
2	209.0	29.3-1113.1	293.7 ± 325.5	67.90	340.20
3	208.7	29.9-1106.7	298.9 ± 325.3	67.40	368.50

a tested substance is mentioned. In case of biochemical diagnostics of NEN it is recommended that some biomarkers are determined on fasting, especially in case of insulin, proinsulin, gastrin and pancreatic polypeptide (PP). A meal, and particularly its composition, may affect the level of a tested substance. Also transient lipemia in tested serum or plasma sample collected just after a meal may affect the analytical measurement.

A meal may stimulate G and ECL (enterochromaffin-like cells) cells, belonging to the, so called Diffuse Endocrine System (DES) for the increased CgA secretion to blood. That is particularly important in case of gastric enterochromaffin cells (14). In one study, authors made a postprandial test in patients with MEN 1 syndrome (Multiple Endocrine Neoplasia type 1) and in a control group, determining blood CgA levels in the 30th and 60th minute of the test. CgA in the group of patients increased by 20-31% compared to the baseline, and in the control group by 16% (15). Another study demonstrated a 2-3-fold increase of CgA level after a meal (16). However, in yet another study authors did not observe any differences between samples collected before and after a meal – it should be noted that the study used a very small study group (17). Our observations demonstrated increased CgA levels in some patients and healthy volunteers. The difference was 24-37%, thus confirming results of our pilot study (18). It has to be considered as well, that the use of some drugs, particularly of PPI group, may lead to hyperplasia of ECL cells in the gastric wall. In that case one could expect an increased, or even greatly increased blood CgA and gastrin levels, which may be also associated with enhanced reaction to stimulation with food (19, 20).

Majority of currently used methods for determination of blood CgA levels are manual methods (RIA, IRMA, ELISA). It is advised that after blood collection serum/plasma samples should not be stored in a non-frozen state, e.g., in a refrigerator (+4°C to +8°C), or in room temperature. Therefore a biological material (serum, plasma) has to be stored frozen until the assay. Hence the question, if freezing/thawing cycles of a sample influence the CgA level. Our observations indicated that three cycles of freezing/thawing of serum samples had no significant effect on the CgA level. Also authors of a similar study focused on the assessment of the effect of freezing/thawing cycles on concentration of numerous endocrine parameters, including CgA, did not demonstrate any significant differences in relation to the initial sample (21).

CONCLUSIONS

In majority of subjects a meal had no effect on the blood CgA level, but in some single cases the effect was significant (24-37%), and therefore the recommendation that blood should be drawn on fasting seems valid.

Three freezing/thawing cycles of serum samples had no effect on determination of CgA, which indicates its high stability during the analytical process.

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