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• COLORECTAL CANCER •

Relationship between serum calcium and CA 19-9 levels in colorectal cancer

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Abstract

AIM: To examine the calcium metabolism of colorectal cancer (CRC) in patients with colorectal cancer and control patients.

METHODS: Seventy newly diagnosed CRC patients were included. The healthy control group was age and gender matched (n=32). Particular attention was devoted to the relationship between serum calcium of patients, and levels of AFP, CEA, carbohydrate antigen 19-9 (CA 19-9) (that could be considered as prognostic factors). Furthermore, the Ca-sensing receptor (CaSR) gene A986S polymorphism was investigated in these patients, as well as the relationship between different CaSR genotypes and the data stated above.

RESULTS: A lower level of ionized calcium (also corrected for albumin) was found in the serum of CRC patients with normal 25(OH) vitamin D levels. The ionized calcium concentration was inversely correlated with the serum level of CA 19-9. There was no difference in the distribution of CaSR genotypes, between CRC patients and general population. The genotypes did not correlate with other data examined.

CONCLUSION: Based on these results, lower levels of serum calcium might be a pathogenic and prognostic factor in colorectal cancer.

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INTRODUCTION

Mortality statistics of the developed countries show that colorectal cancer (CRC) is the second leading cause of death. Even though tumorigenesis is a complex process, epidemiological and experimental data indicate that beside the genetic factors, eating habits (and thus the calcium intake) also play a key role in the development of CRC[1]. In the past few years, we have learned more and more about the process, but not every possible factor has been uncovered yet.

Several *in vitro* and *in vivo* studies have confirmed the chemopreventive role of calcium in CRC[1-5]. The experimental data showed that there was a definite connection between low calcium and vitamin D intake and the prevalence of CRC[6,7]. Some researchers described a protective effect against the development of CRC, when the calcium intake was 1 200 mg daily[8]. Alimentary calcium (among others) together with bile acids creating insoluble calcium-soaps inhibite the cytotoxic effect of fatty acids in the bowel, thus protecting the mucus membrane[9-12].

According to twin studies, serum calcium level was mostly determined by genetic factors[13]. One of the key factors of this determination is the calcium-sensing receptor (CaSR), which by sensing the concentration of calcium in target organs could respond to the changes of calcium level, thus regulating calcium homeostasis[13]. Also, a connection has been found between the CaSR gene A986S genotypes (986 Ala/Ser) and serum calcium concentrations within the normal range in healthy adult population[14].

In our work, we examined the calcium metabolism of 70 recently diagnosed CRC patients and analyzed its possible role in the pathogenesis of CRC, and also the relationship between serum levels of AFP, CEA, CA 19-9 (that could be considered as prognostic factors) and parameters of calcium metabolism. Furthermore, we examined the genotype frequencies (CASR A986S) of our patients and the relationship between genotypes and laboratory parameters of calcium homeostasis and tumor markers.

MATERIALS AND METHODS

Patients

Seventy newly diagnosed CRC patients were examined. All the subjects were in good general conditions. Colonoscopy was performed partly for screening purposes (n=10), partly due to symptoms (n=60), such as abdominal pain, discomfort and flatulence, anemia, hematochezia, changes in bowel habits, and partly to search for primary tumor (for example in cases of hepatic metastasis). In each selected case, the histological diagnosis was adenocarcinoma. Clinical stage was Dukes A: 20, B: 32, C: 18. Five patients had liver metastasis at presentation. The clinical data of the patients are presented in Table 1. An age and gender adjusted healthy control group (n=32) was selected for comparison of the laboratory data. The CASR genotype frequency was compared to the genotype frequency of our previously determined control group, consisting of 201 healthy adults. Written informed consent was obtained from all subjects. The study was approved by the Semmelweis University Regional and Institutional Committee of Science and Research Ethics (141/2003).

Laboratory parameters

To analyze the calcium metabolism of the subjects, serum calcium, phosphate and albumin levels were determined by photometric analysis (Roche, Mannheim, Germany). Ionized calcium levels were also measured in a similar way (Easy-Lite, Bedford, USA). The calcium levels were also corrected to the serum albumin levels (corrected calcium). HPLC (Biorad,

Hercules, USA) was used to measure the serum 25(OH) vitamin D levels, while serum levels of parathyroid hormone (PTH) were determined by chemiluminescence assay (Elecsys/Roche, Basel, Switzerland). To measure AFP, CEA and CA 19-9, immunoassays (Axxym/Abbott, North Chicago, USA) were utilized.

Sampling and histology

In each case, the diagnosis was established by colonoscopy (Fujinon, Japan), and tissue samples were taken for histological analysis.

Genotyping

The polymorphic region of *CaSR* gene was amplified by allele specific PCR technique. The following primers were used: primer M: 5' ACG GTC ACC TTC TCA CTG ACG TTT GAT GAG CCT CAG AAG TAC T 3' 43-mer; primer W: 5' GCT TTG ATG AGC CTC AGA AGA TCG ' 24-mer; and primer R: 5' CTC TTC AGG GTC CTC CAC CTC T 3' 22-mer (10 μmol/L final concentration). PCR reaction was carried out in 20 μL final volume containing: 2 μL 10× Mg free reaction buffer, 4 μL dNTP (1 mmol/L), 1.2 μL 25 mmol/L $MgCl_2$, 1 μL DNA (25 ng/mL), 3-2-1 μL (primer R, -W, -M), 0.1 μL (0.5 U/μL) Taq (Promega, Madison, USA) and 5.7 μL 2D PCR water. The PCR conditions were at 94 ℃ for 12 min, 35 cycles at 94 ℃ for 20 min, at 55 ℃ for 20 min, at 72 ℃ for 30 min and at 72 ℃ for 5 min. There were two types of CaSR allels, the allele A and allele S, so the genotypes were AA, AS, SS. Electrophoretic separation was carried out in a 70 g/L Spreadex/acrilamide-bis (29:1) gel (Elchrom, Cham, Switzerland). For the PCR reaction; Hybaid express thermocycler (Teddington, Middlesex, UK) was used.

Statistics

As the first step, the distribution of continuous parameters was analyzed (Kolmogorov-Smirnov test). Logarithmic transformation was performed as needed. However, results were presented using the original units for easier understanding. The average values for CRC and the control population as well as difference between patients with different genotypes were compared using Student *t* test with separate variance estimates. χ^2 test was used to describe the relation between the allele frequency and tumor localization. Finally, we used parametric correlation (Pearson) to describe the relationship between tumor markers and the calcium homeostasis. A P value <0.05 was considered statistically significant. All the statistical analyses were performed using SPSS 9.0 for Windows.

RESULTS

By examining the calcium metabolism of CRC patients, we found that the serum calcium, ionized and corrected calcium levels of our patients were significantly lower, than those of controls. These calcium levels were at the lower limits of the normal range. Serum phosphate and albumin levels were also determined, which were in the lower end of the normal range (Table 1).

There was no difference in serum 25(OH)-vitamin D and PTH values between patients and controls (Table 1). When examining the correlation between calcium metabolism and CEA, AFP, CA 19-9 tumor markers, we found that the ionized calcium levels of our patients were inversely correlated with the serum level of CA 19-9 tumor marker (Table 2).

There was no significant difference in the CaSR A986S genotype frequency between healthy population and CRC patients [(CRC: AA=51/70(73%), AS=19/70(27%), SS=0/70 (0%); control group: AA=145/201(72%), AS=54/201(27%), SS=2/201(1%)].

In case of the different CaSR A986S genotypes of CRC patients, no difference was observed in the examined biochemical parameters and tumor markers (Table 3). Different alleles or genotypes did not show any correlation with the localization of tumor.

Table 1 Laboratory data of CRC patients (mean±SE)

	CRC	Control	*P*	Normal value
Calcium (Ca)	2.26±0.17	2.45±0.09	0.0001	2.25-2.61 mmol/L
Corrected Ca	2.26±0.14	2.37±0.11	0.0001	2.25-2.61 mmol/L
Ionized calcium	1.07±0.07	1.2±0.05	0.0001	1.05-1.25 mmol/L
Phosphate	1.07±0.13	1.2±0.22	0.006	0.85-1.45 mmol/L
Albumin	39.5±4.61	43.87±4.37	0.0001	35-50 g/L
AFP	4.19±2.85	3.34±1.58	NS	0-15 ng/L
CEA	18.84±69	1.79±1.86	0.043	0-10 ng/L
CA 19-9	86.49±406	10.20±9.95	NS	0-37 ng/L
PTH	38.2±16.8	41.5±14.6	NS	10-65 pg/mL
25(OH) vit. D	44.7±73.8	46.84±13.7	NS	20-60 ng/L
Age (yr)	65.6±10.9	63.6±8.2	NS	-

NS=no significant difference.

Table 2 Correlation between tumor marker levels and parameters of calcium metabolism of CRC patients

	Ca	Corrected Ca	*P*	Ca^{2+}	Albumin	PTH	25 (OH) vit. D
AFP	0.67	0.33	0.53	0.40	0.80	0.87	0.30
CEA	0.21	0.79	0.80	0.23	0.15	0.94	0.95
Ca19-9	0.57	0.57	0.63	0.007	0.10	0.74	0.94

Table 3 Laboratory parameters associated with different CaSR A986S genotypes (mean±SE)

	AA	AS	*P*
Calcium	2.23±0.16	2.31±0.18	NS
Corrected Ca	2.24±0.12	2.30±0.14	NS
Phosphate	1.07±0.13	1.08±0.11	NS
Ionized calcium	1.07±0.08	1.09±0.07	NS
Albumin	39.61±4.40	39.20±5.30	NS
AFP	4.18±2.96	4.29±2.68	NS
CEA	9.2±23.8	45±125	NS
CA 19-9	68±379	143±492	NS
PTH	37.3±14.2	41.2±23.0	NS
25(OH) vit. D	51±85	27.3±11.4	NS
Age (yr)	66.18±10.96	64.8±11.2	NS

NS=no significant difference.

DISCUSSION

The results of our study support the role of low serum calcium in the pathogenesis of CRC. Contrasting to previous data of others, we found that beside normal vitamin D values the level of calcium and the concentration of serum phosphate were both lower than that of controls[7], suggesting that in CRC the function of vitamin D receptor (VDR) might be damaged. Our previous study corroborates this hypothesis since we showed that oncogene (HER-2) expression correlated with VDR genotypes in CRC patients[15].

Western style diet (low in calcium and vitamin D and high in fat) has been shown to induce hyperproliferation in colonic epithelial cells. This cell proliferation could be inhibited by calcium and vitamin D supplementation[10,16,17]. By increasing calcium intake, the number of apoptotic cells in colon epithelium increased,

as well[18]. Based on several human studies, high calcium and vitamin D intake might prevent CRC development[1,3,5,6]. A prospective study claimed that the frequency of CRC was three times less in those patients whose serum vitamin D level was above 20 ng/mL[7]. More than 3.75 μg daily vitamin D intake appeared to reduce 50% incidence of CRC, while 1 200 mg daily calcium intake decreased 75% of its incidence[7,8].

Low calcium level may influence CRC pathogenesis in several ways. Calcium ion (Ca^{2+}) is needed for cell proliferation and differentiation. On the other hand, Ca^{2+} could play an important role in intercellular connections and signal-transduction cascades[19-24]. Furthermore, it has an influence on the cell-cycle regulatory genes (p53, K-ras, epidermal growth factor) that had a well-documented role in CRC pathogenesis[25,26]. The functions mentioned above are mediated partly through CaSR. Similarly to other tissues, CaSR could be detected in normal colon epithelial cells as well. Besides, stimulation of the CaSR could increase the expression of E-cadherin and decrease that of beta-catenin. E-cadherin is the inductor of cell differentiation while beta-catenin is responsible for the genesis of malignant phenotype. The fundamental question is whether the low serum calcium level influencing CRC development is present during a life-long period or emerges only in a phase of the patient' s life right before the appearance of cancer. Answering this question is crucial in deciding when to start chemoprevention.

A986S polymorphism of the CaSR gene has been suggested to have a role in the development of parathyroid adenoma. Others have demonstrated that healthy women with CaSR 986 AA homozygote had lower levels of serum calcium compared to women having AS heterozygote and SS homozygote CaSR 986 alleles[14]. Earlier, we demonstrated that CaSR A986S allele frequencies in CRC patients were not different from those in healthy subjects. However, patients with homozygote AA genotype had a significantly higher UICC stage at the time of discovery compared to the heterozygote AS patients. We could not find an association between serum calcium concentrations and CaSR A986S genotypes in our CRC subjects. We also could not detect a correlation between CaSR polymorphism and CRC. The role of CaSR A986S polymorphism in CRC development requires further investigation.

A large array of evidence indicates that CA 19-9 tumor marker had a prognostic role in CRC. Elevated serum levels were associated with the recurrence of CRC or the presence of metastasis. In our patients, serum calcium levels inversely correlated with CA 19-9 concentration, which could support the significance of serum calcium not only as a pathogenic factor but also as a prognostic factor.

In conclusion, our results further strengthen the possibility that serum calcium might be a pathogenic and prognostic factor in the development of colorectal cancer. Our data draw attention to the possibility that by increasing calcium intake, the multi-leveled pathogenic process leading to tumorigenesis might be influenced. In order to prove this, further studies are necessary.

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