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Name of Journal: *World Journal of Gastrointestinal Oncology*

Manuscript NO: 75851

Manuscript Type: ORIGINAL ARTICLE

Case Control Study

Oxidative imbalance increases the risk for colonic polyp and colorectal cancer development

Tsounis D *et al.* Oxidative imbalance in CRC development

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Abstract

BACKGROUND

³ The role of oxidative stress in the pathogenesis of colorectal carcinoma (CRC) has garnered considerable interest recently. Specific oxidative factors have been implicated in the pathogenesis of adenomatous polyps and ultimately adenocarcinoma.

⁸ AIM

To evaluate the effect of oxidative imbalance, as quantified by specific serological markers, ⁸ in the development of sporadic colon adenocarcinoma.

METHODS

A total of 170 patients that underwent endoscopy of the lower gastrointestinal tract in a tertiary center within three years ¹⁷ were included in the study. They were allocated in three groups; those with sporadic colon adenocarcinoma ($n = 56$, 32.9%), those with colonic polyps ($n = 33$, 19.4%) and healthy controls ($n = 81$, 47.7%). All patients were

evaluated for oxidant activity and antioxidant capacity, with serum measurements of specific markers such as vitamins A, 25(OH) D3, E, C, B12, folic acid, glutathione, selenium (Se), zinc (Zn), free iron (Fe^{2+}), and malondialdehyde and results were compared between groups.

RESULTS

Serum levels of vitamins C, E, D, Se, Zn, vitamin B12, and total antioxidant capacity were significantly lower in the combined neoplasia/polyp group than in the control group ($P = 0.002$, $P = 0.009$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.020$ and $P < 0.001$ correspondingly). Increased levels of vitamin E ($P = 0.004$), vitamin D ($P < 0.001$), Se ($P < 0.001$), and Zn ($P < 0.001$) seem to bestow a protective effect on the development of CRC. For vitamin D ($P < 0.001$) and Zn ($P = 0.036$), this effect seems to extend to the development of colon polyps as well. On the other hand, elevated serum levels of malondialdehyde are associated with a higher risk of CRC (OR = 2.09 compared to controls, $P = 0.004$). Regarding colonic polyp development, increased concentrations of vitamin A and Fe^{2+} are associated with a higher risk, whereas lower levels of malondialdehyde with a lower risk.

CONCLUSION

Increased oxidative stress may play an important role in the pathogenesis and progression of CRC. Antioxidants' presence may exert a protective effect in the very early stages of colon carcinogenesis.

Key Words: Oxidative imbalance; Reactive oxygen species; Colorectal adenocarcinoma; Colonic polyps; Antioxidant capacity

Tsounis D, Villiotou V, Melpidou A, Pantiou C, Argyrou A, Giannopoulou C, Grigoratou A, Rontogianni D, Mantzaris GJ, Papatheodoridis G. Oxidative imbalance

increases the risk for colonic polyp and colorectal cancer development. *World J Gastrointest Oncol* 2022; In press

Core Tip: ³ The role of oxidative stress in the pathogenesis of colorectal carcinoma (CRC) has garnered considerable interest recently. Here, we evaluated oxidant activity and antioxidant capacity of the patients, with serum measurements of specific markers. Increased levels of vitamin E, vitamin D, selenium and zinc seem to bestow a protective effect regarding the development of CRC, whereas elevated serum levels of malondialdehyde are ¹ associated with a higher risk of CRC. Increased oxidative stress ³ may play an important role in the pathogenesis and progression of CRC. Antioxidants' presence may exert a protective effect in the very early stages of colon carcinogenesis.

INTRODUCTION

The term oxidative stress or oxidative imbalance refers to a series of intracellular complex metabolic processes that lead to overproduction and accumulation of oxidative products, otherwise called free radicals or reactive oxygen species (ROS), including hydrogen peroxide, hydroxyl radical, superoxide anion, and peroxynitrite^[1]. These, in turn, collaborate to overcome the protective action of existing intracellular antioxidant mechanisms. The overproduction of ROS has been shown to ultimately result in toxicity inimical practically to every cellular macromolecule, including all intracellular organelles with a special emphasis on membranes and mitochondria. The net effect of this process is local and eventually generalized impairment of human organ system function, either in the form of inflammation, or carcinogenesis^[1].

The pathogenesis of sporadic adenocarcinoma (adenoCa) of the large intestine, is a complex process, involving both genetic and epigenetic factors^[2-4]. The genetic component refers to the gradual accumulation of multiple genetic mutations in key growth regulatory genes^[5], leading to two main types of gene instability (chromosome and microsatellite) which characterize sporadic adenoCa and define its biological behavior^[4]. Furthermore, multiple other conditions have been identified as implicated in the pathogenesis of large bowel adenoCa, possibly through chemical modification of DNA bases during the replication process, especially in the stage of aberrant crypt foci (ACF) formation^[5,6]. ACF represents the earliest histological alterations in the formation of colorectal neoplasia^[6]. Risk factors associated with colorectal cancer include inflammatory bowel disease, nutrition habits (western type of diet), type II diabetes mellitus, sedentary (*e.g.* low exercise) lifestyle, professional exposure to specific irritants (*e.g.* mutagenic chemicals such as asbestos), previous medical procedures (*e.g.* cholecystectomy, radiotherapy of the pelvis, ureterocolic anastomosis), as well as smoking and obesity.

Among the aforementioned risk factors, nutritional habits and their potential influence in particular on the equilibrium between oxidative and antioxidative substances, have garnered recently a significant amount of interest. Epidemiological

studies indicate that colon adenocarcinoma is observed more frequently among individuals with a diet characterized by a low intake of fibers and calcium and higher consumption of saturated fatty acids and proteins, especially of bovine origin (red meat)^[7-10]. This dietary profile has been associated with increased production of potentially carcinogenic substances such as toxic bile acids, and free iron^[11]. Furthermore, the resulting positive energy balance (due to the high accumulation of calories when following the western type of diet) and subsequent obesity have been suggested to lead to metabolic stress, including overproduction of ROS or other organic compounds, such as malondialdehyde (MDA)^[12]. Contrariwise, antioxidant substances, including polyphenols, tocopherols, carotenoids, curcumin, vitamin A, vitamin C, and vitamin D seem to obtain a protective role against colorectal cancer^[1]. It is intriguing to ascertain the exact role of oxidative imbalance in the pathogenesis of initially precancerous lesions such as colonic polyps and ultimately colon cancer. To our knowledge, there are only a few studies, that have tried to detect a pattern of total oxidant activity and antioxidant capacity among patients with established sporadic adenocarcinoma of the large intestine, as this may be assessed through the measurement of specific serum compounds and these studies have produced conflicting results^[13,14]. Thus, we designed a prospective, case-control, single-center study aiming to evaluate the role of oxidative imbalance and its effect, as a possible primary agent in the development of sporadic adenocarcinoma of the large intestine. We tried to achieve this by determining serum levels of specific markers that reflect oxidant capacity in patients with established colorectal carcinoma in comparison to patients with colonic polyps and healthy controls.

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MATERIALS AND METHODS

Study population

A total of 6500 patients, that were over 50 years old and successfully underwent colonoscopy within three years in a major tertiary Greek hospital were screened for participation in the study. A set of specific exclusion criteria were used to curtail the

influence of confounding risk factors in data analysis (Table 1). Ultimately 170 patients were included in our study. Among them, three specific groups were defined, those with a histologically confirmed sporadic adenoCa of the colon, those with a diagnosis of colonic polyps, and a healthy control group consisting of patients with no significant findings or findings irrelevant to the development of CRC (*e.g.* diverticula).

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Study protocol

All patients gave informed consent for their participation in the study and were interviewed by a gastroenterologist not involved in their endoscopic management. Relevant demographic, epidemiological, and clinical characteristics were recorded. Full colonoscopy was performed on all patients following the established sedation, preparation, and safety protocols in our Centre, following international guidelines. All procedures were performed by the same experienced gastroenterologist (with more than 2000 colonoscopies/year and a rate of over 98% for successful completion of the procedure). When endoscopic findings, suggestive of adenocarcinoma or polyps of the large intestine were identified, single or multiple biopsy samples were obtained and sent to the Pathology department of our hospital. Biopsy specimens were assessed by two independent and experienced pathologists, unaware of the endoscopic findings, with a high inter-observer agreement (> 90%). Sporadic adenocarcinoma was evaluated for the following parameters: (1) Staging according to the Astler-Coller system of classification^[15]; (2) Grading according to a three-degree system of differentiation, based on the architectural model of development of sporadic adenoCa, defined by the presence of adenoCa blasts, as follows: poorly differentiated (0%-49% adenoCa blasts), moderately differentiated (50%-95% adenoCa blasts), well-differentiated (> 95% adenoCa blasts); (3) size of adenoCa (< 1 cm, 1 cm, > 1 cm); (4) the number of adenoCa (1, > 1-synchronous adenoCa); (5) pathologic classification as ulcerative, fungating and polyp-shaped (either with a stalk or not); and (6) location in the colon. Colonic polyps were evaluated for the following parameters: (1) histological classification of adenoma, according to World Health Organization (WHO) classification as tubular, villous,

tubulovillous^[16]; (2) grading of adenoma's dysplasia (mild, moderate, and severe according to WHO criteria); (3) size of polyp (< 0.5 cm, > 0.5 cm); (4) number of polyps (1, > 1); (5) presence of stalk or not; and (6) location in the colon.

The definite diagnosis of either sporadic adenocarcinoma or colonic polyp was made within a maximum of 2 d from endoscopy. Until definite pathological diagnosis, patients underwent fasting (nil per os), after which blood samples were obtained.

¹ The study protocol was approved by the Ethics Board of our Hospital.

Measurement of serum markers

Calculation of oxidative agents' levels took place as follows: Oxidant activity ² was measured, by using a colorimetric test system for the quantitative determination of total lipid peroxides in serum (PerOx Assay, Immundiagnostik AG, Bensheim, Germany). Malondialdehyde was quantitatively ³¹ measured, using the reverse-phase high-performance liquid chromatography method (HPLC-Analytik, Immundiagnostik AG, Bensheim, Germany) and the Vp Series HPLC System (Series LC-10 ADVp Gradient pump, Series RF-10AXL Fluorescence detector, Series SPD-10ADVp UV detector, Shimadzu, Germany). Ferrum (Fe²⁺) levels were calculated, using a colorimetric assay [FerroZine, Roche Diagnostics GmbH (COBAS), Mannheim, Germany]. Triglycerides were quantitatively calculated by using a colorimetric enzymatic test [Trinder endpoint reaction, Roche Diagnostics GmbH (COBAS), Mannheim, Germany].

Calculation of antioxidative agents' concentrations took place as follows: ² Total antioxidant capacity was measured, by using a colorimetric test system (Imanox Assay) for the quantitative determination of the residual exogenous provided hydrogen peroxide (H₂O₂) that could not be eliminated from the total antioxidants in serum, after having completed an eliminating reaction ³³ with a certain amount of exogenously provided hydrogen peroxide (Immundiagnostik AG, Bensheim, Germany). Fat-soluble vitamins A (retinol) and E (α-tocopherol) levels were determined simultaneously in human serum, using the reverse-phase HPLC method with the 22000 ClinRep Kit (Recipe Chemicals + Instruments GmbH & Co KG Labortechnik, Munich, Germany).

Retinol was monitored at 325 nm and α -tocopherol at 295 nm with the Up series HPLC System (Series LC-10ADVp Gradient pump, Series RF-10AXL Fluorescence detector, Series SPD-10ADVp UV detector, Shimadzu, Germany). Fat-soluble vitamin D (25-OH vitamin D) was measured, using a chemiluminescent immunoassay method (DiaSorin LIAISON 25 OH Vitamin D Total Assay, DiaSorin S.p.A, Italy). Water soluble vitamin C was quantitatively measured, using the reverse-phase HPLC method (Immundiagnostik AG, Bensheim, Germany). Glutathione (GSH) levels were calculated as the ratio between GSH reduced/GSH total, using the reverse-phase HPLC method (Immundiagnostik AG, Bensheim, Germany). Cobalamin/vitamin B12 and folate acid concentrations were calculated, using a radioimmunoassay method, from MP Biomedicals Inc., New York, United States, with the Packard cobra autogamma g counter from Packard, United States. Selenium (Se) and zinc (Zn) serum levels were determined, using a graphite furnace atomic absorption spectrophotometry method with the AA spectrophotometer model 2100 (Perkin Elmer, Norwalk CT, United States).

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Statistical analysis

Statistical analyses were conducted using the SPSS statistical package (IBM Statistical Package for Social Sciences v. 19.0, Chicago, Illinois, United States). At first, cases were distributed according to demographic characteristics and smoking habits, as well as levels of the studied compounds (median, 25th, and 75th percentile for each compound and each patient category). As most variables measured followed a skewed distribution (with the notable exception of triglyceride levels), non-parametric tests (Mann-Whitney and Kruskal-Wallis) were used to compare means between different groups. In order to minimize the effect of possible confounding factors in our results, we then used multiple regression to compare log-transformed serum compound levels between patients with diagnosed pathology (either adenocarcinoma or polyp) *vs* controls (using two dummy variables for pathology diagnoses correspondingly), controlling for age (as a continuous variable), sex (male *vs* female), blood triglyceride (as a continuous variable) and smoking habits (as smokers *vs* non-smokers). Finally, we applied multiple

logistic regression models to investigate the association between levels of the measured compounds and either risk of adenocarcinoma or risk of polyp development, controlling for the same variables as in the log-linear models. A two-tailed P value of < 0.05 was considered statistically significant for all comparisons.

RESULTS

The patients' main characteristics are summarized in Table 1. Briefly, our study included 56 patients with adenocarcinoma, 33 patients with colonic polyps, and 81 patients in the control group (colonoscopy negative for cancer or precancerous lesions). Patients with adenocarcinoma tended to be relatively older (71.5 ± 6.6 years, mean \pm SD), compared to those with colon polyps (66.1 ± 9.4 years) or with the control group (68.4 ± 8.5 years). Among patients with adenocarcinoma or polyps, men presented a clear majority (62.5% and 63.6% respectively) compared to women. In the adenocarcinoma subgroup, 89.3% of patients were smokers (from which 72% with a high rate of use, *e.g.* more than 20 cigarettes per day) compared to 51.5% of patients who were smokers in the polyps group, whereas smoking habits in the control subgroup bore a close resemblance to those of the adenocarcinoma subgroup (84% smokers from which 70.6% smoked more than 20 cigarettes/day).

When comparing patients with adenocarcinoma or polyp(s) with the control group there were notable differences in practically all antioxidant markers (Table 2). Thus, serum levels of vitamins C, E, D, as well as Se, Zn, and B12 and total antioxidant capacity were significantly lower in the combined neoplasia/polyp group than in the control group ($P = 0.002$, $P = 0.009$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.02$ and $P < 0.001$ correspondingly). For the antioxidant capacity, in particular, there is a clear picture of higher measurements in the control group when compared to a patient with neoplastic lesions (Figure 1). On the other hand, serum levels of oxidant activity presented the opposite pattern ($P < 0.001$ for the difference among the three groups) (Figure 2). In sum, all antioxidant substances were statistically significantly lower among patients with adenocarcinoma compared to controls, except vitamin A which did not present

any differentiation (Table 3). Vitamin D presented the greatest difference since it was lower by 56.8% (95%CI: 50.2% to 62.6%). Although no statistically significant differences regarding the levels of each measured oxidant substance in isolation were observed, total oxidant activity was statistically significantly increased among adenocarcinoma patients.

In the group of patients with colonic polyps, most anti-oxidants did not present significant differences in serum concentration, when compared to controls. Notable exceptions included, vitamin D which exhibited significantly lower levels by 46% (95%CI: 35.4% to 54.9%) (Figure 3), and total antioxidant capacity, which was reduced by 40.1% (95%CI: 38.5% to 41.6%). Interestingly, vitamin A though nominally an anti-oxidant displayed significantly higher levels compared to controls. Finally, patients with colonic polyps, presented lower levels of malondialdehyde by 31.6% (95%CI: 22.3% to 39.8%), but higher Fe²⁺ concentrations by 43.5% (95%CI: 22.2% to 68.5%) (Figure 4), as well as total oxidant activity levels (95%CI: 111.7% to 128.9%).

Next, multivariate analyses were conducted to ascertain which variables presented a true correlation with the neoplastic process. Results were similar between univariate and multivariate analyses regarding the risk of adenoCa (Table 4, which presents the risk of adenocarcinoma in comparison to controls for change in the levels of the measured compounds equal to one standard deviation of its distribution). A significant protective effect was shown especially for vitamin D (OR = 0.04, 95%CI: 0.02 to 0.12) and Zn (OR = 0.16, 95%CI: 0.09 to 0.31) (Figure 5) but also for vitamin E (OR = 0.57, 95%CI: 0.39 to 0.84) and Se (OR = 0.35, 95%CI: 0.22 to 0.55). Increase of the levels of the abovementioned substances equal to one standard deviation reduced the risk of colon adenocarcinoma to about 50%. The relation between low levels of the aforementioned antioxidants and increased risk of adenocarcinoma remained significant after mutual adjustment *e.g.* OR for Se becomes 0.34 (95%CI: 0.13 to 0.88 *P* = 0.027). As far as the oxidant substances are concerned, the solitary finding was that a doubling of malondialdehyde serum concentration is associated with an approximately twofold

increase in the risk for development of colon adenocarcinoma (OR = 2.09 95%CI: 1.27 to 3.45 $P = 0.004$).

Due to the small number of patients in the colon polyp subgroup, fewer associations retained their significance for the development of colon polyps (Table 5). Nevertheless, similarly to results from the CRC group increased levels of vitamin D (OR = 0.27, 95%CI: 0.15 to 0.48, $P < 0.001$) and Zn (OR = 0.39, 95%CI: 0.16 to 0.94, $P = 0.036$) exhibited an association with a reduced risk for colon polyp development, whereas it is worthy of note that increased levels of vitamin A were associated with almost 9 times higher risk of colon polyps compared to controls (OR = 8.84, 95%CI: 3.76 to 20.74, $P < 0.001$). Moreover, regarding the oxidant group, elevated serum levels of Fe^{2+} seem to double the risk for the development of colon polyps, (OR = 2.58 95%CI: 1.53 to 4.33, $P < 0.001$). Interestingly, in contrast to results from the CRC group higher malondialdehyde levels seem to exert a protective role (OR = 0.35 95%CI: 0.2 to 0.6 $P < 0.001$). These results remain stable after the mutual adjustment of the measured compounds.

DISCUSSION

CRC is the third cause of cancer-related mortality in both men and women worldwide^[1,2]. Current therapeutic options including surgery, chemotherapy, radiotherapy, and molecular-targeted therapy are still limited for advanced tumors. Thus, identifying new strategies, that will increase the probability of early detection of this clinical modality while keeping the public health costs reasonable (as endoscopy confers a significant financial burden on health budgets) remains an important challenge.

Comparative international epidemiological data indicate that the difference between the highest and lowest sporadic colon cancer incidence is approximately 10-fold, suggesting that environmental factors in the pathogenesis of colon cancer occupy a more prominent role than their genetic counterparts. The dominant environmental factor identified so far is the low-fiber, high-fat diet of Western industrialized countries^[17,18].

Although numerous studies dedicated to elucidating the exact role of dietary factors in CRC pathogenesis, and research conducted ¹⁴ in a variety of in-vitro and in-vivo animal models strongly hint in favor of a protective effect of antioxidants regarding CRC development, even in the stage of ACF formation, results derived from human populations, are not as clear-cut in their results and are in fact at times conflicting^[11,12,19,20].

In our study, a basic assumption was made that serum levels of oxidant/antioxidant compounds, may accurately reflect the dietary intake habits of individuals and thus, could be used to evaluate oxidative imbalance^[12]. Over fifty natural and synthetic compounds have been shown to exert a relevant chemotherapeutic effect, but since for the majority of these agents literature concerning their role is comparatively scarce, we opted to focus on a variety of compounds with a reasonably established place in the management of the oxidative/anti-oxidative equilibrium^[1].

Regarding the oxidant markers, MDA is an endogenous genotoxic end product of lipid peroxidation by ROS and has been utilized *in vivo* as a bio-marker of oxidative stress (peroxidability index)^[21]. It is thought to participate in harmful processes that lead to DNA damage and mutation, mainly through the formation of DNA adducts^[22]. MDA-induced DNA lesion, called DNA interstrand cross-link seems to be implicated in the gene-toxic effects associated with lipid peroxidation and oxidative stress^[21]. Therefore, MDA has been suggested to be strongly associated with CRC pathogenesis, a suggestion that the results of our study strongly endorse. However, the results from the polyp subgroup present a different picture as MDA levels ⁹ were significantly lower than those in the control group. This latter finding seems odd, considering the fact that colonic adenomas are established precursors of sporadic CRC, but firstly the small number of cases in this subgroup may skew the results in an unexpected direction, and secondly, this finding may account for a more prominent role of MDA in the second part of the neoplastic process that leads to the evolution of precancerous lesions such as polyps to adenoCa.

On the other hand, elevated levels of free iron (labile iron) another recognized strong oxidant, actually doubled the risk of colon polyp development in our cohort, whereas no similar correlation was observed for the adenocarcinoma group of patients. Dietary iron can be consumed in the form of heme iron (Fe^{2+}) and nonheme iron (Fe^{3+})^[23]. Heme iron contributes to colorectal cancer *via* the Fenton reaction. Hydroxyl radicals produced by the Fenton reaction can alter DNA, leading to oxidative base damage^[24]. Furthermore, heme iron contributes to cancer development by inducing colonic hyperproliferation through modulation of the intestinal microbiota and inducing mutations through DNA adducts or by increasing the formation of lipid peroxyl radicals (ferroptosis), such as malondialdehyde and 4-hydroxynonenal, which are potent carcinogens^[24-26]. These findings provide a strong association between excessive intestinal heme iron and colorectal cancer. However, no sufficient evidence is available, to our knowledge, to link a mechanism of nonheme iron and colorectal cancer^[25]. However, emerging evidence suggests that reduced iron intake and low systemic iron levels are also associated with the pathogenesis of colorectal cancer^[25]. This is important because patients with colorectal cancer often present with iron deficiency. The mechanism supporting iron deficiency and colorectal cancer development is not fully understood; it may involve cellular functions' requirement for iron, which, when deficient, may hinder immune cells' ability to protect against cancer, providing the potential for a suppressed immunosurveillance response, affecting growth and differentiation of immune cells, as well as influencing cell-mediated immune response and cytokines activities, which may contribute to tumor immune-cell evasion and inadequate tumor cell destruction^[27]. On the contrary, the association between high iron concentration and the risk of formation of adenomatous (colonic) polyps, is ambiguous^[28]. Several studies suggest that the presence of high levels of Fe^{2+} may induce the formation of colonic polyps, suggesting a potent involvement in the early rather than later steps of colorectal carcinogenesis^[1,28,29].

Regarding the antioxidant markers, Vitamin A (retinol) did not exhibit any protective role in our study population. Elevated vitamin A concentrations were associated with

almost 9 times higher risk of colon polyps compared to controls, with no significant effect observed in the adenoCa subgroup. This is not as confusing as it may seem as results regarding the role of vitamin A in the prevention or recurrence of adenomas have been conflicting so far. Several studies^[30-32] suggested a protective effect of vitamin A and its derivatives (retinoids) against CRC, whereas Andersen *et al*^[33] failed to establish a beneficial role for vitamin A in CRC. On the contrary, recent studies on the metabolism of vitamin A in CRC imply that despite the presence of high concentrations of retinol or all-trans-retinoic acid (ATRA), CRC is being promoted instead of obtaining decreasing cancer cell proliferation^[34-36]. The growth and differentiation of the colonic epithelial cells are strongly controlled by retinoid-activated genes, which contain retinoic acid receptors (RARs) in their promoter regions. RARs bind to ATRA to induce the transcription of these genes. In many epithelial-derived adenomas and carcinomas, the expression of one or more RAR is lost and the cell loses its ability to regulate normal growth, a phenomenon called "ATRA-resistance". In addition, as CRC progresses, colorectal tumor cells lose the ability to produce ATRA^[34]. Kropotova *et al*^[37] claimed that these dysregulated pathways were more observed in adenomas rather than in more advanced carcinomas^[37]. Consequently, the high levels of vitamin A in the polyp group in our study might reveal the inadequate protective mechanism of retinol, possibly due to decreased ATRA production and loss of RAR in the colonic epithelial cells.

On the other hand, Vitamin D was by far the compound with the most significant decrease in concentration, among patients with adenocarcinoma or colorectal polyps when compared to controls in our analysis. It should be noted, that we assessed vitamin D levels by measuring circulating 25(OH)D3 (calcidiol) levels, thus providing an overall estimate of vitamin D status, as described elsewhere^[38]. Since 1980, a large number of epidemiological and experimental studies support the association of vitamin D deficiency with a large variety of human diseases, including an increased incidence of colorectal cancer^[39]. The most active metabolite of vitamin D, which is 1,25-dihydroxy vitamin D3 [1,25(OH)2D3, (calcitriol)], is synthesized in a highly regulated multi-step process, by mitochondrial 25(OH)D3-1 α -hydroxylase^[38]. Several cell types, including

colon cells, have been described to contain vitamin D receptors (VDRs)^[38]. When these receptors are activated by calcitriol, they are thought to induce differentiation, regulate detoxification metabolism, sensitize cells to apoptosis and inhibit proliferation, invasiveness, angiogenesis, and metastatic potential^[39]. In general, according to epidemiological studies, vitamin D deficiency may be linked to a higher risk for neoplasia. A recent meta-analysis of case-control and cohort demonstrated a consistent inverse relationship between serum 25(OH)D3 levels and CRC risk^[40]. Another systematic review of studies evaluating the association of vitamin D intake or serum levels of 25(OH)D3 and the risk of CRC suggests as well an inverse correlation between CRC risk and both serum 25(OH)D3 and vitamin D intake^[41]. This mostly positive observational data have failed to be confirmed by human intervention studies in which supplemental vitamin D administration was found to be ineffective in reducing colon cancer risk in contrast with dietary sources of vitamin D. These disappointing results may be explained by the timing of administration, indicating that colon lesions may progress to a stage where they become unresponsive to vitamin D, bearing, therefore, the hallmarks of an epigenetic change^[42]. Moreover, gene expression and activity controlled by VDRs have been described as up-regulated at the early stages of colorectal tumorigenesis with a subsequent sharp decline in advanced CRC^[43]. Further investigations of VDR expression at different stages of colon cancer development have come to a consensus that VDR expression is frequently increased at the pre-neoplastic ACF and the early stages before being lost in more advanced lesions, suggesting a possible role for vitamin D supplementation in early stages, with no benefit conferred in advanced cases of this neoplasia^[44,45].

Vitamin E is a generic term that describes a group of lipid-soluble chain-breaking antioxidants that exist in nature as eight structurally related forms with α -tocopherol as the isomer found in the highest concentrations in serum and dietary supplements^[1]. The results of our study show a potent protective effect for vitamin E in the adenocarcinoma group of patients compared to controls, although studies focusing on vitamin E have produced conflicting results so far^[46,47]. Non-significant trends toward reduced blood

concentrations of α -tocopherol have been observed in subjects subsequently developing colorectal cancer when compared with controls^[47]. Conversely, intakes of other forms of vitamin E (γ -tocopherol, δ -tocopherol, γ -tocotrienol, and δ -tocotrienol) suggest a highly significant inverse trend between serum concentration of vitamin E and cancer risk ($P < 0.001$)^[30,47]. In a recent intervention study, the administration of a combination of resveratrol and vitamin E to prevent the development of colonic adenomas exhibited clear benefits^[48]. Therefore, our findings though interesting must be further evaluated within larger sample size studies.

Se, an essential trace element, is one of the most extensively studied anti-oxidant compounds^[8]. A protective effect of Se for the prevention of colorectal adenomas development has been convincingly described^[49-52]. Data from the European Prospective Investigation into Cancer and Nutrition cohort that evaluated the effect of Se supplementation according to the dose supplied demonstrated a statistically significant decrease in the incidence of CRC, although only for a subgroup of subjects with baseline Se concentration $< 100 \mu\text{g/L}$ ^[52]. These reports are in agreement with our results of a protective effect of higher Se levels regarding CRC risk. In summary, it can be concluded that an inverse dose-response correlation between the level of Se in serum and the risk of colorectal cancer may exist, albeit this association may be stronger in particular subgroups of patients.

Zn is another potent compound that has been found to play a crucial role mainly in antioxidant defense systems, as a specific activator of many enzymatic reactions (*e.g.* CuZn Superoxide dismutases), in DNA synthesis, as well as in immune functions^[53]. Zn has also been shown to inhibit chemically induced neoplastic progression in the colon and to promote the cell cycle arrest of colon cancer cells in animal models^[53,54]. Reports regarding Zn levels in biological fluids from CRC patients have been limited but encouraging^[54]. In a large Mendelian randomization study, the analysis suggested that increased dietary Zn intake may be associated with a decreased risk of both proximal and distal colon cancer^[55]. Similar findings were reported by a recent meta-analysis of nineteen studies, that suggested a statistically significant inverse dose-response

association of Zn intake with CRC risk^[54]. The aforementioned findings are in agreement with our results that hint at a protective role for elevated serum levels of Zn in regards to CRC pathogenesis both in the early and later steps of this process.

There are several limitations to this study. From the antioxidant compounds analyzed in our study, we observed that vitamin D provided the strongest argument in favor of a protective role in the prevention of CRC. An important issue though, that should be taken into account concerning vitamin D assessment is the age of the participants, as a potential confounder since ¹¹ it is known that vitamin D insufficiency ^{is} strongly associated with increasing age^[56-59]. Of particular interest is also the finding that ²⁹ elevated serum levels of Fe²⁺ ^{were associated with a} twofold ^{increase in the} risk of colon polyp development, suggesting a possible role in the formation of colonic polyps and more specifically involvement in early rather than late stages of colorectal carcinogenesis. To our knowledge, there are not many studies in the literature in favor of this association, probably because in most of them, bound -and not free- iron was under scrutiny^[29]. In our analysis, we also noticed a trend for certain antioxidant substances ³ to be associated with a lower risk of colonic polyp rather than CRC subgroups of patients. This could be possibly explained by a more prominent role in the protective effect of these antioxidants in the early stages of CRC pathogenesis *i.e.* before the formation of precancerous cells. This effect, when overcome by the sum of tumorigenic factors, will be then attenuated when the adenoma stage is reached, rendering interventions such as nutritional antioxidant supplementation incapable of stabilizing or reversing the neoplastic phenotype. This is an attractive theory, especially considering the often inconsistent and even negative results from intervention trials with antioxidant supplementation^[1,60,61]. It is known, that selecting the exact timing and duration of the intervention (*e.g.* the age of the patient at enrolment and the supplementation period) is challenging^[62,63]. It remains unclear if interventions given for a relatively short period, as in most of the trials due to practical reasons, have the potential to interrupt the tumorigenic sequence. Furthermore, it is difficult to ascertain ⁷ the optimal follow-up duration for such a trial ^{to detect an effect on} the ^{incidence of} a

disease such as CRC with a time-extensive pathogenetic process. Apart from that, clinical trials cannot provide evidence concerning the exact point at which chemoprevention begins to take effect concerning the start of treatment or concerning the precise nature of this effect (whether this is gradual or constant)^[61,63-65]. In most studies, the relative risk predicted for the incidence of colonic polyp formation or CRC is assumed to be constant because of a lack of data to the contrary, thus suggesting that chemoprevention does not offer any cumulative protection^[64,65]. Our study followed the "top-down" approach to studying the exosomal risk factors for CRC onset^[2,66]. Thus, it suffers from the known limitations of this approach, which we mentioned earlier, mainly that the time-points for specific marker measurements were limited and that the crucial pathophysiological effects regarding CRC pathogenesis may have already taken place. On the other hand, it presents a clear and unbiased approach to the biochemical serum profile of several factors, important to the oxidative balance, in a sizeable CRC cohort. Thus, while a causal effect can by no means be proven for these compounds, intriguing correlations emerge from our analysis that may be the trigger for further research and new insights.

CONCLUSION

In summary, we describe a possible protective effect for Se, Zn, vitamin E, and vitamin D regarding CRC pathogenesis, while elevated levels of MDA were associated with a two-fold increase in the risk for CRC. Regarding the development of colonic polyps, higher serum levels of vitamin D and Zn correlated with a decreased risk of adenoma, whereas elevated levels of vitamin A and Fe²⁺ bestowed a higher risk. Interestingly, lower levels of MDA were found in patients with polyps when compared to controls. Our findings indicate that increased oxidative stress and a reduced antioxidant defense mechanism as assessed by a variety of serum compounds may participate in CRC pathogenesis and progression. Moreover, the possible protective effect of antioxidants may be more important in the very early stages of colon carcinogenesis, probably through an interactive mechanism in the early stages of ACF formation^[1,6]. Total

antioxidant intake may represent a better predictor of colorectal cancer risk as opposed to specific foods and nutrients^[12]. Further trials are needed that should focus on the effect of total antioxidant intake in high-risk for CRC populations, but prevention of CRC through manipulation of the oxidative balance in the human body *via* nutritional supplementation may represent a worthwhile future research target.

ARTICLE HIGHLIGHTS

Research background

The role of oxidative stress in the pathogenesis of colorectal cancer (CRC) has recently attracted considerable interest. Specific oxidative factors have been implicated in the pathogenesis of adenomatous polyps and ultimately adenocarcinoma.

Research motivation

Several studies have evaluated the association between oxidative imbalance and the development of colorectal adenocarcinoma, although the results are conflicting. Thus, the study was designed to assess the correlation between the dietary intake habits of individuals with either colonic polyps or CRC, through measurements of oxidant/antioxidant serological markers, aiming to introduce novel serum indicators of colonic cancer, even in the stage of aberrant crypt foci.

Research objectives

The main objective of the study was to evaluate the effect of total oxidant activity and antioxidant capacity in the development of sporadic colon adenocarcinoma.

Research methods

A total of 170 patients that underwent endoscopy of the lower gastrointestinal tract in a tertiary center within three years were included in the study. They were allocated in three groups; those with sporadic colon adenocarcinoma ($n = 56$, 32.9%), those with colonic polyps ($n = 33$, 19.4%) and healthy controls ($n = 81$, 47.7%). All patients were

evaluated for oxidant activity and antioxidant capacity, with serum measurements of specific markers such as vitamins A, 25(OH) D3, E, C, B12, folic acid, glutathione, selenium (Se), zinc (Zn), free iron (Fe^{2+}), and malondialdehyde and results were compared between groups.

Research results

Serum levels of vitamins C, E, D, Se, Zn, vitamin B12, and total antioxidant capacity were significantly lower in the combined neoplasia/polyp group than in the control group ($P = 0.002$, $P = 0.009$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.020$ and $P < 0.001$ correspondingly). Increased levels of vitamin E ($P = 0.004$), vitamin D ($P < 0.001$), Se ($P < 0.001$), and Zn ($P < 0.001$) seem to bestow a protective effect on the development of CRC. For vitamin D ($P < 0.001$) and Zn ($P = 0.036$), this effect seems to extend to the development of colon polyps as well. On the other hand, elevated serum levels of malondialdehyde are associated with a higher risk of CRC (OR = 2.09 compared to controls, $P = 0.004$). Regarding colonic polyp development, increased concentrations of vitamin A and Fe^{2+} are associated with a higher risk, whereas lower levels of malondialdehyde with a lower risk.

Research conclusions

In conclusion, increased oxidative stress may play an essential role in the pathogenesis and progression of CRC. Antioxidants' presence may exert a protective effect in the early stages of colon carcinogenesis.

Research perspectives

Further research in high-risk CRC populations is needed in order to assess the role of oxidative imbalance in the development of CRC and the potential for colonic cancer by dietary modifications regarding specific oxidative serum markers.

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