JCRMHS

Journal of Clinical Case Reports, Medical Images and Health Sciences

Volume 10 Issue 2, 2025

Article Information

Received date: 01/04/2025
Published date: 24/04/2025

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*Key Words:

Colon Polyps, Colorectal Cancer, NO, TGF- β1

Research article

The Clinical Significance of Serum no and TGF-β1 in patients with Colon Polyps and Colorectal Cancer

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Abstract

Objective: The levels of TGF-β1 and NO in patients with adenomatous polyps and colorectal cancer, which constitute a high risk for colorectal cancer development, was analysed and their correlations with CEA and CA19-9 levels were evaluated.

Material and Method: The study included 28 patients with colorectal cancer, 30 patients with colon polyps and 30 control subjects consisting of healthy individuals who were admitted to the hospital and diagnosed colonoscopically and histopathologically. CEA, CA 19-9, NO and TGF-β1 levels were measured in patients and controls before treatment.

Results : In our study, CEA, CA 19-9 and TGF-β1 levels were significantly higher in colorectal cancer compared to colon polyp and control groups. No significant difference was found between the three groups in terms of NO levels.

Conclusion: Our results suggest that NO and TGF- β1 are effective in the development of colorectal cancer and that the use of TGF- β1 as a tumour marker in the follow-up of colorectal cancer patients with normal CEA and CA 19-9 levels may be clinically useful, while the clinical use of NO is not appropriate.

Introduction

Colorectal cancers are common in both sexes. It is the third most common cancer in men and the second most common cancer in women worldwide. The frequency of colorectal cancer increases in direct proportion to age and this increase, which starts in the 40s, reaches its peak between the ages of 60 and 751. It is thought that 95% of colon cancers are caused by polyps. The incidence increases in direct proportion to age. The malignant potential of these polyps are related to polyp type, size and degree of dysplasia, as well as the number of polyps, age of the patient and follow-up period2,3.In colorectal cancer, a wide range of reactive oxygen radicals and increased cytokine levels have been found. Many studies have shown that these reactive oxygen radicals play a role in carcinogenesis. It was observed that antioxidant levels were lower in colorectal cancer cell lines compared to intact epithelial cells4. In some studies, a higher level of NO secretion was observed in colon adenoma and cancer tissue compared to normal colon tissue, whereas opposite findings were obtained in some studies5-8. These different effects of NO are thought to be related to the secreted level9. TGF-β has been reported to be expressed in many tumors including colorectal cancer and is believed to play a role in tumor metastasis as well as tumor progression. TGF- beta plays a role in inflammation and tumor formation, especially by modulating cell growth, differentiation apoptosis and homeostasis10,11.Screening patients with adenomatous polyps, which constitute a high risk for colorectal cancer development, for TGF-B and NO, and the use of selective inhibitors will increase knowledge about the immunological mechanisms underlying colon tumor progression, provide a better understanding of molecular pathways, and open new horizons in the diagnosis and treatment of some precancerous diseases and cancer.

Materials And Methods

The study included 30 patients with colon polyps, 28 patients with colorectal cancer and 30 control subjects consisting of healthy individuals. Patients with colon polyps who were between 30-85 years of age, who gave written informed consent before participating in the study, and who were colonoscopically and histopathologically diagnosed with colorectal cancer and tubular, villous or tubulovillous adenoma were included in the study. As the control group; 30 healthy people who had no gastrointestinal complaints and pathological physical examination findings, no infection or systemic disease was detected in clinical and laboratory tests, were not in the malignancy risk group, did not smoke, drink alcohol, use drugs, etc. were included. After signing the informed consent form, a detailed history was taken. A complete physical examination was performed. Routine biochemical tests were performed in all patients. In addition, CEA and CA 19-9 levels were measured. 4cc of venous blood was collected from all patients in the morning, fasting, sitting position, from the forearm with a disposable syringe, in order to determine NO and TGF-beta levels in serum. After the blood was separated for biochemical and haematological tests, the samples were kept at room temperature for 20 minutes. Then centrifuged at 5000 rpm for 5 minutes. The separated sera were placed in a tube, sealed with parafilm and kept in a deep freezer at - 70°C until the study day. On the study day, NO and TGF-β were analysed in batches from blood samples.

Measurement of TGF-beta l level in serum: Biosource International, Nivelles, Belgium TGF-beta l kit was used. ELISA (solid phase sandwich Enzyme Linked Immunosorbent Assay method was used. By using specific antibodies against TGF-β 1, the antigens in the sample were combined with them and then colored. A standard curve is plotted according to the concentration and optical density of the standard. TGF-B 1 concentrations in the samples are calculated using the standard curve. The minimum detectable level according to this method is 15.6 pg/ml. Expected values are 19-71 ng/ml. Tecan Sunrise ELISA reader was used for absorbance reading and Tecan Columbus ELISA washer was used for washing.

Measurement of total NO level (nitrate + nitrite) in plasma: Since serum nitrate concentrations are affected by dietary nitrite/nitrate intake, blood samples were taken after a 14-hour fasting period. To determine serum nitrite/nitrate concentrations, Cayman Chemical (Ann Arbor, USA) nitrite/nitrate kit was used. It was measured photometrically using an ELISA plate. NO3- (nitrate) is converted to NO2- (nitrite) with the help of the enzyme Aspergillus nitrate reductase and the subsequent determination of NO2- is based on the principle of conversion to pink azo compound by Griess reaction. This colored compound is directly proportional to the nitrite concentration. Nitrate concentrations are then found by subtracting NO2- from total (NO3- + NO2-) concentrations. The sensitivity of the test is 2.5Mm for nitrite/nitrate. Tecan Sunrise ELISA reader was used to read the absorbances.

'SPSS for windows' package program was used for statistical evaluation of the data. The results obtained were expressed as mean ± sd. Differences between groups were compared by Mann-whitney-U, Kruskall-wallis, Student t tests. Pearson correlation analysis was used for correlation analysis. $P<0.05$ was taken as significance level.

Results

Of the 88 patients, 28 had colorectal cancer (31.8%) and 30 had colon polyps (34%). In the colorectal cancer group (n:28), 14 were male (50%) and 14 were female (50%), in the colon polyp group (n:30), 19 were male (63.4%) and 11 were female (36.6%), and in the control group (n:30), 14 were male (46.7%) and 16 were female (53.3%). There was no statistically significant difference between the three groups in terms of gender distribution ($p> 0.05$). The ages of the patients in the colorectal cancer group ranged between 42-83 (mean: 58.8 ± 11.8), the ages of the patients in the colon polyp group ranged between 39-84 (mean: 55.5 ± 11.6), and the ages of the patients in the control group ranged between 31-85 (mean: 52.06 ± 13.4). There was no statistically significant difference between the three groups in terms of age ($p>0.05$). 6 (21.4%) of the patients with colorectal cancer (n:28) and 6 (20%) of the patients with colon polyps (n:30) were smokers. There was no statistically significant difference between the two groups in terms of smoking ($p>0.05$). When colorectal cancer, colon polyps and control group were compared in terms of CEA level; the mean CEA level in the colorectal cancer group was 64.44 ±195.09 ng/ml in the colorectal cancer group and 3.25 + 3.2 ng/ml in the colon polyp group: 3.25 + 3.2 ng/ml, in the control group: 2.27 ± 1.2 ng/ml.

The elevated CEA levels In the colorectal cancer group were found to be statistically significant compared to the colon polyp and control groups ($p< 0.05$). There was no statistically significant difference between the colon polyp group and the control group in terms of CEA levels ($p>0.05$). When colorectal cancer, colon polyps and control group were compared in terms of CA 19-9 levels; the mean CA 19-9 level in the colorectal cancer group was 149.09 ± 272.48 ng/ml in the colorectal cancer group and 14.93 ± 8.55 ng/ml in the colon polyp group: 14.93 ± 8.55 ng/ml, in the control group: 14.27 ± 12.2 ng/ml. The elevated CA19-9 levels in the colorectal cancer group were found to be statistically significant compared to the colon polyp and control groups ($p< 0.05$). There was no statistically significant difference between the group with colon polyps and the control group in terms of CA 19-9 levels ($p>0.05$).

When colorectal cancer, colon polyps and control group were compared in terms of NO level; mean NO level in the colon cancer group: 18.24 ± 4.87 µM in the colon cancer group, and in the colon polyp group: 19.10 ±8.38 µM in the control group: 19.12± 8.39 µM. There was no statistically significant difference between NO levels in colorectal cancer, colon polyp and control groups (p> 0.05).

When colorectal cancer, colon polyps and control group were compared in terms of TGF-β level; mean TGF-β level in colorectal cancer group: 3.58 ± 1.43 ng/ml, in the group with colorectal cancer: 2.91 ± 1.76 ng/ml, in the control group: 2.81 ± 1.40 ng/ml. The increase in TGF- β levels in the colorectal cancer group was statistically significant compared to the control group (p<0.05). However, no statistically significant difference was found between the cancer group and the group with colon polyps and between the control group and the group with colon polyps in terms of TGF-β levels (p>0.05).

Discussion And Conclusions:

Colorectal polyps are quite common in western countries and have been found in more than 30% of autopsied people over 60 years of age12,13. The most important factor determining the prevalence of colon adenomas is age and the incidence of adenomas increases over the age of 50. The prevalence is higher in the male population (M/F= 1.4/1) than in females (14). In our study, the mean age of 30 patients with colorectal polyps was 55.5 ± 11 years (39-84). Nineteen (63.4%) of the patients were male and 11 (36.6%) were female (M/F=1.7/1).

Similar to colon polyps, the frequency of colorectal cancers increases in direct proportion to age. Colorectal cancers are more common in men and mostly over 50 years of age. The peak incidence is in the 6th and 7th decades. Hereditary colon cancers such as familial adenomatous polyposis (FAP) can also be seen at earlier ages14. In our study, the mean age of 28 patients with colorectal cancer was 58.9 ± 11.8 years (42-83). Fourteen (50%) of the patients were male and 14 (50%) were female (M/F- 1/1). Although colorectal cancers are more common in males in previous studies, we found the rates of males and females to be equal in our study. Since colorectal cancers can be observed below the age of 50 years, screening tests should be performed at an earlier age in the presence of predisposing factors such as family history of colon cancer, inflammatory bowel disease or FAP14.

In our study, 8 (28.57%) of the patients with colorectal cancer were under the age of 50 years and 5 (62.5%) of them were female patients. However, none of the patients with colorectal cancer under the age of 50 years in our study had a family history or genetic predisposition such as FAP. The fact that colon cancers can be seen in sporadic cases under the age of 50 years suggests that screening tests should be performed more commonly and at earlier ages.

Carcinogens activated by cooking methods of foods, increased mutagenic activity in feces, excessive alcohol consumption and heavy smoking are risk factors that increase the development of colon polyps and cancer15. In our study, 6 (21.4%) of patients with colorectal cancer and 6 (20%) of patients with colon polyps were smokers.

Of neoplastic polyps, 80-86% are tubular, 8-16% tubulovillous, and 3-16% villous adenomas16. In our study, 17 (56.6%) of the polyps were tubular, 7 (23.3%) were tubulovillous and 6 (20%) were villous. Tubular adenomas were less and villous adenomas were more in our study group compared to the literature.

Simple small (<1 cm) tubular adenomas are quite common and have a low risk of malignancy. In our study, 13 of 30 cases (43.3%) with polyps were smaller than 1 cm. Advanced adenomas are polyps >1 cm and contain a high rate of villous tissue or high grade dysplasia15. In our study, 17 (56.6%) of 30 patients with polyps were larger than 1 cm.

Adenomas are mostly localized distally. This region is also the region where colorectal cancer is localized most frequently. Especially in individuals aged 60 years and older, adenoma localization shifts proximally17. In our study, 17 (56.6%) of the polyps were localized in the rectum, 5 (16.7%) in the sigmoid colon, 5 (16.7%) in the ascending colon and 3 (10%) in the transverse colon. In cancerous cases, 21 (75%) were localized in the rectum, 3 (10.7%) in the sigmoid colon, 2 (7.1%) in the transverse colon, 1 (3.57%) in the ascending colon and 1 (3.57%) in the cecum. In accordance with previous studies, we observed a parallelism in the localization of cancers and polyps.

CEA and CA 19-9 are the most commonly used tumor markers in the diagnosis and follow-up of colorectal cancers. In general, CEA is used in the evaluation of response to treatment and in the detection of postoperative recurrences and metastases in colorectal cancers1.

In our study, when colon cancer, colon polyps and control group were compared in terms of CEA levels, serum CEA levels were found to be significantly higher in patients with colorectal cancer compared to polyps and healthy control group (p<0.05). Although CEA levels were higher in patients with colon polyps compared to the control group, group, it was not statistically significant.

CA 19-9 is a high molecular weight antigen detected by monoclonal antibodies that plays a role in cancer invasion and metastasis. It is most frequently elevated in pancreatic and biliary tumors, as well as in patients with colorectal cancer. On the other hand, high serum CA 19-9 levels can also be observed in many nonneoplastic benign diseases18.

When colorectal cancer, colon polyp and control groups were compared in terms of CA 19-9 levels, the elevated CA 19-9 levels in the colon cancer group were found to be statistically significant compared to the colon polyp and control groups. There was no statistically significant difference between the colon polyp group and the control group in terms of CA 19- 9 levels. Since CA 19-9 is an antigen that plays a role in invasion and metastasis, its sensitivity is low in early stage colorectal cancers and adenomas. In our study, we found elevated serum CA 19-9 levels in colorectal cancer patients in accordance with the literature. However, we found no significant difference between patients with colorectal polyps and normal healthy group.

Nitric oxide (NO) is a mediator involved in many biological events such as vasodilation neurotransmission, angiogenesis, apoptosis and tumor progression. Recent studies have investigated NO expression and activity in human cancers19. However, NO expression in colorectal cancers is still controversial. In many studies, NO expression and activity were found to be increased in tumor tissue compared with normal mucosa, while some studies have reported the opposite results. While low levels of NO concentration accelerate tumor growth and angiogenesis, high concentrations of NO have been found to show antitumor activity by inducing cytotoxicity and apoptosis20.

Many in vitro and in vivo studies have shown that NO accelerates tumor angiogenesis. NO has been shown to induce endothelial cell growth and regulate tumor blood flow. Ziche et al. have shown that NO controls angiogenesis by modulating some angiogenic factors such as vascular endothelial growth factor released from tumor cells 21. In another study, when NO expression or NO enzyme activity was examined in tissue samples, NO expression was found at very low levels in normal colonic epithelium, while NO expression was found at high levels in 60% of adenomas and 20-25% of cancers. While NO activity was found at very low levels in normal tissue adjacent to neoplastic lesions, the highest levels were found in adenomas22.

Lagares et al. showed that tumor metastasis was higher and long-term survival was significantly lower in the group with higher NO activity in colon cancers 23.

In our study, although serum NO levels were found to be lower in patients with colorectal cancer compared to patients with polyps and control group, no statistically significant difference was found between the three groups in terms of serum NO levels. This finding suggests that low NO expression may be a negative prognostic factor in tumor progression.

In our study, although not statistically significant, NO levels in the control group and patients with colon polyps were higher than in patients with colorectal cancer. Further studies are needed to clarify the controversial results regarding NO expression in colorectal tumors and to determine the relationship between NO levels in adenomas and tumor progression.

TGF-β is a family of growth factors with many functions. TGF- β1 is released by platelets and synthesized by a variety of normal cells including activated lymphocytes, macrophages and neutrophils, as well as many transformed cells. TGF- β is thought to be involved in epithelial carcinogenesis. Many tumor cells including colorectal cancer express TGF-β and have been reported to play a role in tumor progression and metastasis development24.

It was found that TGF- βl expression was closely related to disease progression and survival in colorectal cancer patients, the 3-year survival rate was 80% in TGF- β negative tumors and 40% in TGF- β positive tumors, and it was reported that serum TGF- β1 levels were <5ng/ml in normal healthy individuals, <7.5 ng/ml in benign colonic lesions and >7.5 ng/ml in colorectal cancers. They also showed that high preoperative TGF- β1 levels may be a determining factor for liver metastasis after curative resection24,25.

Narai et al. found that plasma TGF- β1 concentrations were significantly higher in patients with colorectal cancer compared to healthy volunteers. It was also reported that serum TGF- β1 levels were closely related with the depth of tumor invasion, lymph node metastasis distant metastasis and serum CEA levels and decreased significantly after surgical resection of the tumor26.

In our study study, the difference in serum TGF- β1 levels between colorectal cancer patients and healthy control group was statistically significant (p-<0.05). However, no statistically significant difference was found between colorectal cancer and colon polyps. In addition, although serum TGF- β levels were lower in tubular adenomas, no statistically significant difference was found between villous, tubulovillous and tubular adenomas (p>0.05).

The finding that serum TGF- β1 levels are higher in patients with colon cancer compared to normal healthy group suggests that TGF- β may play a role in colorectal carcinogenesis. In screening of patients with colorectal cancer, serum CEA levels can be found normal in approximately 30% of patients. Therefore, the use of TGF-β1 as a tumor marker in the follow-up of colorectal cancer patients with normal serum CEA levels may be clinically useful.

CEA, CA 19-9 and TGF-β1 levels were found to be significantly higher in colorectal cancer compared to colon polyp and control groups. There was no significant difference between the three groups in terms of NO levels. Again, CEA, CA 19-9, NO and TGF-β1 levels were not significantly higher

in colon polyps.

In conclusion, all these data suggest that NO and TGF-β1 are effective in the development of colorectal cancer and that the use of TGF- β1 as a tumor marker in the follow- up of colorectal cancer patients with normal CEA and CA 19-9 levels may be clinically useful. It suggests that NO may play a critical role in molecular pathways in the progression of colon adenoma to colon cancer and that the use of CEA, CA 19-9, NO and TGF-β1 in the monitoring of adenomatous polyps, a risk factor for colorectal cancer development, may not be appropriate.

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Citation: Zuhal koksal. The Clinical Significance of Serum no and TGF-β1 in patients with Colon Polyps and Colorectal Cancer. Jour of Clin Cas Rep, Med Imag and Heal Sci 10(2)-2025.