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THE IMPORTANCE OF URATE PATHWAY ENZYMES ACTIVITY AND ITS RELATION WITH OXIDATIVE STRESS IN PROGRESSION AND INVASION OF HUMAN COLORECTAL CANCER

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Colorectal cancer (CRC) is one of the main reasons for the mortality connected with tumor diseases. There is still a shortage of examination including the influence of urate pathway enzymes in the progressiveness and invasion of CRC, so the present study investigated the role of xanthine oxidase (XO), adenosine deaminase (ADA) and 5'-nucleotidase (5'-NT) activity, concerning TBA-reactive substances (TBARS) as an oxidative stress (OS) marker in progression, also an invasion of human colorectal cancer.

We took tissue specimens from 50 patients with colon cancer, in all four TNM clinical stages of the disease. They were divided into 3 groups: cancer tissue, tissue surrounding the tumor and healthy control tissue group. We made 10% homogenates in which we conducted the study with proper methods.

The activity of ADA and XO in tumor tissue and tissue adjacent to the tumor is statistically higher in comparison to healthy colon tissue. The 5'-NT is not significantly higher in carcinoma tissue. The highest activity of ADA and XO is in T2 and T3 tumor stages. TBARS has the highest concentration in T3 and T4 stages of the tumor.

Presented results suggest that the possible cause of OS in colon carcinoma is high XO and ADA activity. It may include those enzymes in the transformation of the colon tissue, as well as in the progression of CRC. So, the ADA and XO activity might be helpful in determing the margins of colon resection. They can have significance in diagnosis, but in the prognosis of the disease as well.

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Key words: *colorectal cancer, adenosine deaminase, 5'-nucleotidase, xanthine oxidase, oxidative stress*

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Introduction

Colorectal cancer (CRC) is the third most frequent tumour in the human population. It is one of the main reasons for mortality connected with tumor diseases (1). Despite the fast development of diagnostic and treatment strategies, the five-year survival rate of CRC remains poor, mostly because of recurrence and metastasis. However, the precise mechanisms resulting in the initiation and progression of CRC remain unclear, and it has focused considerable scientific interest on the molecular pathogenesis of CRC. Emerging evidence has shown that enzymes of purine nucleotides are included in the pathogenesis and progression of CRC.

Adenosine deaminase enzyme (ADA) catalyses the conversion of adenosine to inosine which is in the end transformed into uric acid (2). Adenosine is a vital signalling molecule that exerts main anti-inflammatory movements in tumorous conditions including inhibition of tumour infiltration in lymphoid cells (3). Higher ADA activity can also additionally have an impact on scavenging of a vital molecule, adenosine.

Adenine nucleotide catabolism that embodies vital pathways of the intermediary metabolism maintained the regulatory effector (adenosine) and molecular energy compound, adenosine triphosphate (ATP). In a lot of tissues, it gives an excellent

adenine nucleotide pool through a specialised mechanism that correlates with adenosine 5' monophosphate (AMP) metabolism (4). Two essential enzyme sequences typically take a component withinside the catalysis of the original AMP metabolism pathway. The first is AMP deaminase, which catalyses the deamination of AMP to provide inosine monophosphate (IMP). The 2nd is 5'-nucleotidase (5'-NU), which catalyses the dephosphorylation of AMP to provide adenosine. In addition, the catabolism method consists of the conversion of adenosine to inosine through adenosine deaminase (ADA) catalytic activity (5).

Human adenosine deaminase-ADA (E.C. 3.5.4.4.) exists in lots of molecular forms, with specific molecular weights. It performs an important function as a key enzyme involved in the salvage of purine nucleosides and the utilization of purines (6). Many researchers have found out the best activity and significant molecular heterogeneousness of intestinal adenosine deaminase. Besides soluble adenosine deaminase shape, a particulate-membrane bound shape turned into isolated additionally from the normal gut, however, a cancer-unique shape became isolated in some colorectal tumours (7, 8).

The second is 5'-ribonucleotide phosphohydrolase-5'-nucleotidase (5'-NT E.C.3.1.3.5.), which catalyses the dephosphorylation of AMP to produce adenosine. This was first described by Reis in 1934. It is a phosphomonoesterase because it catalyses the hydrolytic degradation of monophosphate nucleotides (AMP, GMP, CMP, UMP, IMP) and their deoxy analogues (9). Although the activity of this enzyme has been shown to be reduced in tissues and neoplasm cells in some studies (10), some researchers have additionally found high 5'-NT activity in cancer tissue relative to surrounding normal tissue (11).

Xanthine oxidase (XO) catalyses the final degradation of purine bases which generate uric acid, that's the final product of purine catabolism (5). It is widely recognized that xanthine oxidase (XO) is an enzyme found in interconvertible forms, dehydrogenase and oxidase. Results of our previous examinations have proven that during most cancers the tissue through oxidation of sulfhydryl groups or restricted proteolysis, dehydrogenase XO activity is transformed to oxidase form that produces hydrogen peroxide and superoxide. (12). Simultaneously with the production of uric acid, XO activity liberates hydrogen peroxide and superoxide anion, which are one of the major ROS and oxidative stress-inducers. DNA damage caused through ROS performs a vital function in the carcinogenic transformation of the cell (13). There are lots of pathological conditions at some stage in which elevated plasma XO exists, like cholecystitis, shock, ischaemia-reperfusion injury, acute virus infection, adult respiration distress syndrome, carcinogenesis (14). It has not been clarified but whether or not the activity of XO will increase or decline in human cancers. Since there is still a shortage of examination including influence of these enzymes in progressiveness and invasion of CRC, the present study investigated the role of urate pathway enzyme activity, such as ADA, 5'-NT and XO, in relation to oxidative stress in progression and invasion of human colorectal cancer.

Materials and methods

Patients and tissue samples

The investigation was conducted in 50 patients with CRC at the Clinical Centre Niš, Serbia. All patients gave their knowledgeable consent for inclusion prior to their participation in the study. The investigation was carried out in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Faculty of Medicine in Niš (Decision No. 01-1591/8).

We took tissues from 50 patients with primary colorectal cancers. Patients were at different stages of the disease according to TNM classification. Tissue specimens used for this study were obtained during the surgery as soon as possible after resection of the carcinoma. In all patients, ph confirmed adenocarcinoma, and we excluded patients with other types of tumours from the study. We also excluded patients with: gravidity, co-malignancies, inoperable tumoirs, preceding chemotherapy or radiotherapy. Tumour stages are shown in Table 1.

Table 1. Tumour Stages (TNM Classification)

Tumor stage	Gender		Age (Mean)
	m	w	
T1	4	2	50.5
T2	8	3	52.5
T3	16	9	63
T4	5	3	61.5
In total	33	17	56.5

Stage I tumours (n = 6) are tumours limited to the bowel wall. Stage II (n = 11) denotes tumours that have penetrated the muscularis propria. Stage III (n = 25), the tumours have spread to involve the regional nodes. Stage IV (8), the tumours which have a faraway metastasis. Also, as a control, we gathered the identical quantity of samples from macroscopically unchanged colon areas farthest from cancer, and tissue immediately surrounding the tumour without a macroscopic or pathological manifestations.

Preparation of tissue samples

We removed tissues quickly during the process of surgery. All samples were placed in iced 0.15 mol/L NaCl solution, perfused with an isotonic solution to get rid of blood cells and other tissue residues. Further on, after removal of fat, connective tissue, and major vessels, the tissue was cut into small pieces and washed with de-mineralized water to remove RBC as much as possible and subsequently with 0.15 M phosphate-buffered (30 HIM) saline (pH 7.5). We homogenized the tissue with a homogenizer with a teflon pestle; made 10% homogenates and centrifuged them at 3,000 x g for 15 min, and the supernatant was frozen at -80 ^{O}C and kept until assayed.

Biochemical assays

The ADA activity

We measured activity of ADA according to the slightly modified method of Pederson and Berry (15, 16). We expressed enzyme activity in U/g protein.

The 5'-NT activity

The activities of the 5'-nucleotidase were determined by Wood and Wiliams method (17). Substrate was AMP at an optimal pH of 7.5 using a barbiturate-HCL buffer. 5'-nucleotidase activity is expressed as IJ/mg protein.

Xanthine oxidase activity

XO activity was evaluated with spectrophotometric method, by using xanthine as a substrate where the uric acid formation was measured. Enzyme activity was expressed in IJ/mg of protein (18).

TBARS concentration

TBA concentration of reactive substances in the homogenate was measured using the slightly modified Nabavi et al. method (19). MDA-reactive lipid peroxidation products were measured at 532 nm. We expressed concentration in nmol/mg of protein.

Protein content

The amount of protein was determined by Lowry et al. method, where by bovine serum albumin was a standard (20).

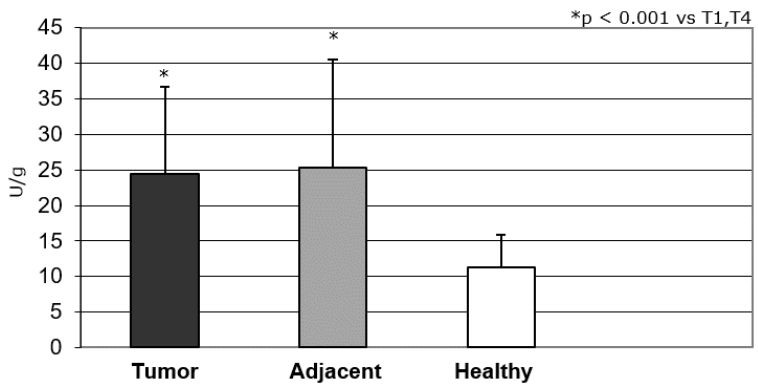
Statistical analysis

The values of all parameters were expressed as X ± SD (mean value ± standard deviation). The examination of received data was assessed via way of means of the t-test evaluating the enzyme activity of mucosa with pathological manifestations or mucosa next to tumour tissue with the activity of corresponding further healthy tissue and with the activity of acquired tissue from patients without pathological manifestations.

The statistical significance of differences between TNM stages of tumour was calculated using the ANOVA test. The limit value of $p < 0.05$ was considered to be statistically significant.

Results

The activity of ADA in tumour tissue was significantly higher in comparison to healthy control tissue ($p < 0.001$). Tissue surrounding the tumour likewise had higher activity of ADA in relation to control tissue ($p < 0.001$) (Graph 1).



Graph 1. The ADA activity in tumour, adjacent and healthy colon tissue

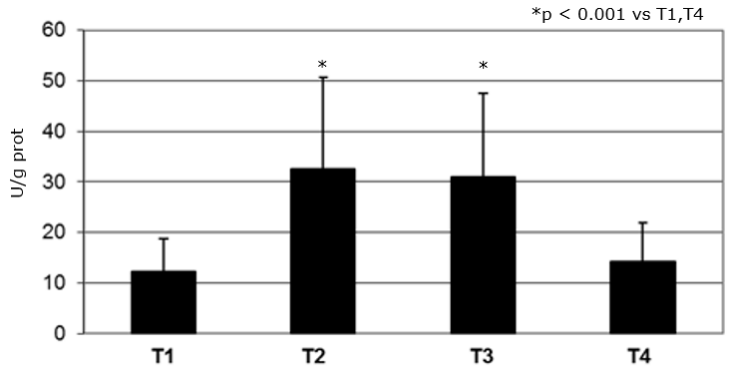
T2 and T3 tumour stages had a significantly higher activity of ADA when compared to T1, and T4 tumour stages ($p < 0.001$). Highest activity was in T2 stage without statistical significance compared to T3 stage (Graph 2).

The activity of 5'-NT was the highest in adjacent tissue, but without statistical significance (Graph 3).

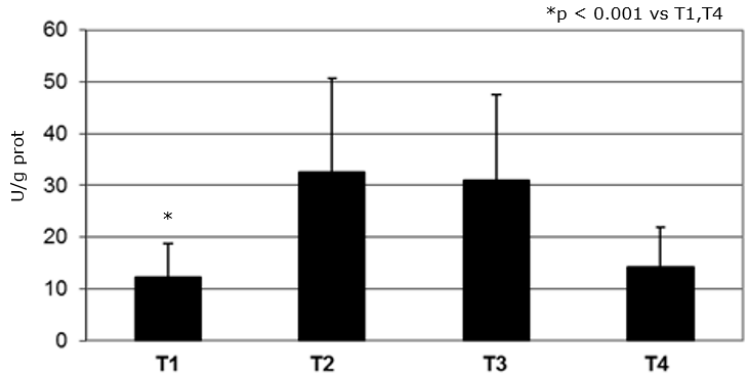
The activity of XO in tumour tissue was notably higher in comparison to control colon tissue ($p < 0.001$). Also, the tissue of tumour had significantly higher XO activity when compared to adjacent tissue ($p < 0.001$) (Graph 4).

T3 and T4 tumour stages had a significantly higher XO activity when compared to T1, T2 tumour stages ($p < 0.001$) (Graph 5).

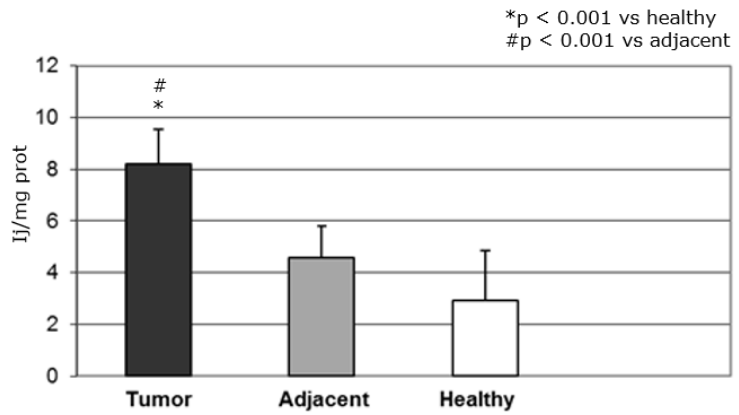
The T4 tumour stage had a significantly higher concentration of TBARS when compared to T1, T2, and T3 tumour stages ($p < 0.001$) (Graph 6).



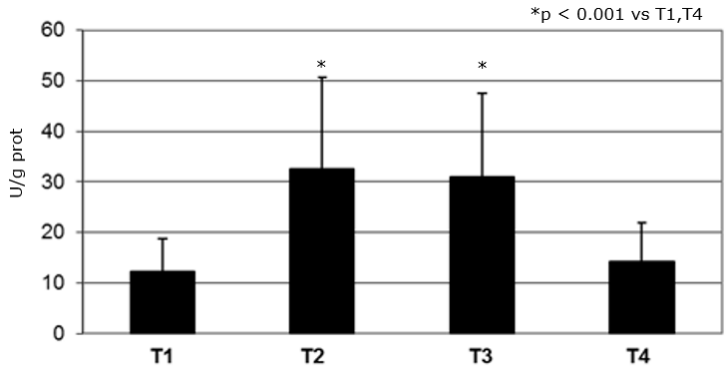
Graph 2. The ADA activity in tumour tissue, patients with T1, T2, T3 and T4 stage



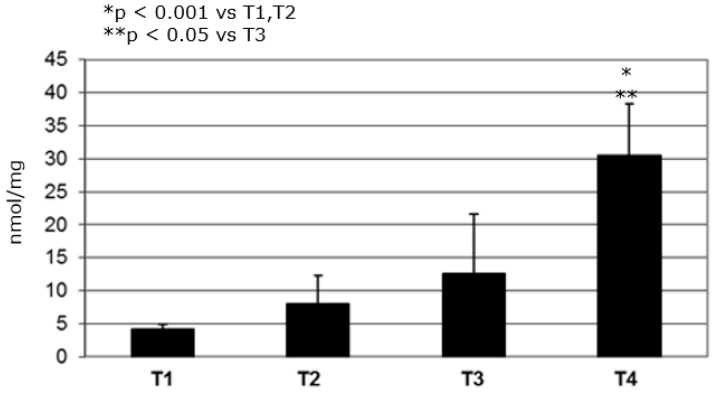
Graph 3. The 5'-NT Activity in tumour, adjacent and healthy colon tissue



Graph 4. The XO activity in tumour, adjacent and healthy colon tissue



Graph 5. The XO activity in tumour tissue of patients with T1, T2, T3, and T4 stage



Graph 6. The concentration of TBARS in tumour tissue of patients with T1, T2, T3, and T4 stage

Discussion

Alterations in the enzymology of the human colorectal tumour absolutely distinguished it from the regular colorectal mucosa. To get a higher knowledge of purine enzymology in colorectal carcinoma, we have paid a great deal interest to investigating the interrelations among the carcinogenic process and the activities of some urate pathway enzymes. CRC is a complex disease, multifactorial, related to accumulated oxidative stress and inflammation followed by fast tissue proliferation. In this study, the role of ADA, 5'-NT, and XO has been investigated concerning oxidative stress in CRC patients.

Adenosine deaminase (ADA) an enzyme of the purine metabolism is widespread in tissues and relatively high levels have been found in the villi intestinal of the large intestine epithelial cells.

Several researches have proven changes of ADA activity withinside the tumour tissue and blood serum in patients with lung, head and neck, breast, and ovarian cancer (21, 22). Additionally, a group of authors founded elevated ADA activity in the cancerous large intestine tissue (23).

ADA is particularly susceptible to stimulation by growth factors and cytokines during rapid tissue proliferation (24). Various researches have documented the rise of ADA in unexpectedly developing malignancies, where it has been documented as a tumour marker, whereas slow-developing well-differentiated tumours do not show prominent enzyme activity (25). Results of our observation additionally display that there may be a higher activity of an enzyme in cancer tissue in comparison to the control healthy tissue of colon in our study. But, the tissue surrounding the cancer has even higher activity of this enzyme. So ADA could be included in tumour proliferation and invasion of large intestine tissue where the tumour is located, directly to adjacent tissue. One of the possible reasons could be lower effect of adenosine.

Indeed, improved ADA activity in patients suggests decreased availability of adenosine, which may be a defensive molecule just in case of the tumorous condition. Adenosine is an endogenous purine nucleoside generated from ATP (26). This is an important signalling molecule that exerts important anti-inflammatory action. Tumours have an excessive concentration of adenosine that could inhibit the characteristic of tumour-infiltrating lymphoid cells (2). We may take this as a compensative mechanism against the tumorous conditions. ADA enzyme scavenges adenosine through degrading it into inosine, which eventually gets regenerated into uric acid (3). There are numerous proofs that adenosine acts as a crucial regulative autocrine and paracrine component accumulating in the cellular micro-environment (27). The concentration of adenosine, which is typically low in physiological conditions, will grow in reaction to certain conditions, including

inflammation, hypoxia, ischaemia, or trauma (28). The fast accumulation of extracellular adenosine has a protecting effect, since it prevents immoderate inflammatory reaction of the cells in order to help the tissues return to their physiological state (29). However, in the tumour environment, low adenosine concentration may be associated with immunosuppression leading to neoplasia (30), which is related to our results, which show the highest ADA activity in the tissue surrounding the tumour.

Hypoxia is one of the reasons malignant tumours no longer perform the functions necessary for cellular homeostases, such as the proper production of ATP, resulting in the decomposition of nucleotides and the discharge of adenosine. Some authors, therefore, proposed that adenosine is the main component in promoting tumour increase (31). The direct consequences of adenosine on molecular growth *in vitro* are controversial (32, 33). There is a significant evidence that low levels of extracellular adenosine, both in a paracrine or autocrine manner, can promote tumour increase in numerous ways (31). Next, the available data strongly support the low adenosine concentration as a stimulator of angiogenesis (34). Third, adenosine has a role in lowering the inflammatory and cellular immunity responses and contributes to the formation of specific, tumour immune barriers (35). It can also take part in signal transduction through specific adenosine receptors, which result in the alterations in the adenyl cyclase system and PLC activity (36).

A crucial aspect of most cancers cells is their spreading from the primary tumour that consequently produces diverse clinical symptoms. There are generally four clinical levels of CRC which are highlighted in the literature.

Results of our investigation show the highest activity of ADA in the T2 and T3 stages of the disease (Graph 2). Those are the stages where the tissue invasion and proliferation are the highest. So, ADA might be included in tumour progression.

Other authors additionally found that inflammation progresses with the development of the disorder in patients with clinical degree four compared to the ones having stages 1, 2, or 3 of breast cancer. Mahajan (37) confirmed most activity of ADA at stage three of breast cancer, but at some point of their observation probably they did not come across any affected person with stage 4 breast cancer. High levels of ADA activity can additionally be interpreted as a compensatory mechanism for tumours towards highly toxic adenosine, deoxyadenosine and its derivatives, ADP, and dATP, which are effective inhibitors of ribonucleotide reduction, a restricting enzyme in nucleic acid biosynthesis (38), which can be higher in tumour than in normal colon tissue (39).

The proof of excessive ADA activity in fast and stimulated normal tissue increase is crucial for the existence of useful purine metabolism because of the viable inactivation of adenosine and 2'-deoxyadenosine, toxic metabolites for the cells increase (40). The enzyme is mainly sensitive to stimulation by growth factors and cytokines in the course of rapid tissue proliferation (24). Therefore, a few data show that ADA isn't always directly involved in carcinogenesis, however has a metabolic function in assisting a fast increase state of relevant tissues, through the re-utilisation of nucleosides, related as RNA and associated precursors. When CRC cells are treated with deoxycoformicine, an ADA inhibitor, the cell growth is inhibited (41). The highest ADA activity in T2 and T3 tumour stages may support its influence in the progression and invasiveness of the colon carcinoma.

Some authors additionally related accumulated ADA activity to lower or deficient ADA complexing macromolecule (ADBP), a specific glycoprotein localized in the healthy colon membranes. The monoclonal antibodies against tumour represent also ADBP (42).

We have also investigated the activity of 5'-NT to test the process of enzymes resolving mononucleotide to nucleosides. Although, Sanfilippo et al. (43) have shown that there was no important difference in different activity in tumour and healthy large intestine tissue, within the study by Eroglu et al. (23) activity of 5'-NT in tumour tissue was above in tumour-free tissue. They even found that its level was related to the development of the carcinoma.

Our results do not show a statistically significant higher value of 5'-NT activity in the tumour compared to the control colon tissue and tissue adjacent to the tumour. Low activity of 5'-NT, also the accumulated ADA activity results in reduced levels of adenosine. These conditions result in enhanced OS and generate some complications as a result of the amount of remaining adenosine does not perform its physiological functions. Additionally, adenosine is a generally anti-inflammatory agent, that suppresses neoplasm necrosis factor-alpha (TNF-α) production in monocytes and macrophages, inhibiting the liberation of arachidonic acid and leukotriene production in neutrophils (44). Adenosine can also be an activator in antioxidant enzyme signalling pathways. In our preceding report, we suggested that colon cancer tissue had an extensively higher concentration of oxidative stress parameters which included TBARS and advanced oxidation protein products as compared to healthy colon tissue that represented control. The tissue adjacent to the tumour additionally had a higher concentration of those oxidative products as compared to the control, and it could consist of oxidative stress in the process of tumour development and nearby invasion. Also there may be a decreased activity of anti-oxidative enzymes (45).

Erkiliç et al. (46) reported that ADA would increase the production of ROS, like H2O2, O2-, NO, and 1O2. The high concentration of ROS causes OS, which leads to inflammation by enhancing lipid peroxidation next to the membrane.

The lower 5'-NT activity is considered to be a consequence of destruction of the membrane and its structure because of the high levels of ROS (47). Extracellular AMP is hydrolysed by the action of 5'-NT on free phosphate and adenosine. The most commonly suggested reason for depletion of activity is precisely the oxidative modification of the 5'-NT sulfhydryl (-SH) groups and the interaction with LPO also, the final product of oxidative stress.

This conclusion is derived from a previous study which shown the inhibition of 5'-NT activity by impaired sulfhydryl groups compared with many different enzymes (48).

But our results show the highest concentration of TBARS in T3 and especially T4 stadium of the disease (Graph 6). The highest ADA activity is in T2 and T3 stages, so ADA is not likely the source of ROS.

Further, the enhanced ADA activity which raises the xanthine concentration might lead to higher XO activity. Therefore, the high activity of XO may be linked to the high xanthine levels present in the cancer tissue, because it is the substrate of XO. Therefore, higher levels of xanthine raise XO activity, which might be the source of high oxidative stress. It is the main enzyme that links the metabolism of purines and free radicals along with oxidative stress.

The results of this study showed a high rise in XO activity in cancer tissues when compared with the healthy tissue and also the adjacent tissue. Tissue surrounding the tumour had lower activity when compared to tumour tissue, so XO is not the main reason of tumour proliferation.

Most of the previous studies reported lower XO activity in cancers, and it is suggested that the lower purine catabolism and higher activity of salvage pathway enzymes could favour tumour cell growth (49). Some of the previous researches have refuted the thesis that XO activity is the source of the oxidative stress (50). The lower XOR activity in more aggressive cancer cells has unexplained effects on tumour development and leads to cancer growth and metastasis.

We could relate increased XO activity to the change of the dehydrogenase form of XO into the oxidase one, by process of the oxidation of thiol groups or by proteolytic degradation caused by higher level of peroxynitrite. Furthermore, higher activity of ADA that raises the xanthine pool could lead to increased XO activity. Therefore, the high XO activity could be explained by the higher levels of xanthine.

Our results showed the highest XO activity in T3 and T4 stage (Graph 5) and it is in correlation with the TBARS concentration in the higher stage of the disease.

ROS induced by the activity of XO can influence the higher hypoxia-inducible factor 1a expression and activate NF-kB, and thus contribute to cancer-associated inflammatory signalling and to tumour progression (51, 52). One recent article has shown that xanthine oxidase inhibition could suppress migration of the cell and metastastatic potential of breast cancer (53).

High level of OS in the last cancer stage in our investigation shows the advancement of the tumour since the higher OS gives rise to inflammatory processes. ROS can also act as a secondary messenger by activating intracellular signalling pathways, particularly NF-kB, one of the major modulators of carcinogenesis. Oxidative activation, stimulates the expression of many pro-inflammatory cytokines in the epithelium of the gut, such as TNF-α, IL-8, and COX-2, and leads to inflammation and tumorigenesis (54).

Increased XO activity in the tissue of patients with cancer in our study suggests that OS may be increased in cancerous changes and processes, and could affect the course of the disease. We may relate higher XO activity in our study to increased levels of TBARS in tumour tissue representing markers of oxidative damage.

Conclusion

We can conclude that colon cancerogenesis could require a higher concentration of many metabolic changes responsible for tumour development in a co-operative fashion. Presented results could suggest that the possible cause of OS in colon carcinoma is high XO and ADA activity. It may include those enzymes in the transformation of the colon tissue, as well as in the progression of CRC. So, the ADA and XO activity might be helpful in determining the margins of colon resection. They can have significance in diagnosis, but in the prognosis of the disease as well.

The simplicity of measuring activity asserts the usefulness of these enzymes in some patients where cytopathological findings cannot lead to a clear conclusion, this simple test, together with all clinical findings can have significance in diagnosis, but in the prognosis of the disease as well.

Acknowledgments

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca A Cancer J Clin 2018; 68:394-424. [CrossRef] [PubMed]
2. Lokshin A, Tatiana R, Huang X, Zacharia LC, Jackson ED, Gorelik E. Adenosine-mediated inhibition of the cytotoxic activity and production by activated natural killer cells. Cancer Res 2006;66:7758-65. [CrossRef] [PubMed]
3. Eltzschig HK, Weissmuller T, Mager A, Eckle T. Nucleotide metabolism and cell cell interactions. Methods Mol Biol 2006;341:73-87. [CrossRef] [PubMed]
4. Banihani SA. Role of uric acid in semen. Biomolecules 2018;8(3):E65. [CrossRef] [PubMed]
5. Kimoloi S. Modulation of the de novo purine nucleotide pathway as a therapeutic strategy in mitochondrial myopathy. Pharmacol Res 2018;138:37-42. [CrossRef] [PubMed]
6. Daddona PE, Kelley WN. Analysis of normal and mutant forms of human adenosine deaminase - a review. Mol Cell Biochem 1980;29(2):91-101. [CrossRef] [PubMed]
7. Piggot CO, Brady TG. Purification of multiple forms of adenosine deaminase from rabbit inetstine. Bioch Biophys Acta 1976;429:600-7. [CrossRef] [PubMed]
8. Pedley AM, Benkovic SJ. A new view into the regulation of purine metabolism: The purinosome. Trends Biochem Sci 2017;42:141-54. [CrossRef] [PubMed]
9. Kate J, Wijnen JT, Herbschleb-Voogt E, Griffionen G, Bosman FT, Khan PM. Adenosine deaminase (ADA, E.C. no 3.5.4.4.) In colorectal adenocarcinoma in man. Adv Exp Biol Med 1984;165:299-303. [CrossRef] [PubMed]
10. Durak I, Isik AU, Canbolat O, Akyol B, Kavutcu M. Adenosine deaminase, 5'-nucleotidase, xanthine oxidase, superoxide dismutase and catalase activities in cancerous and noncancerous human laryngeal tissues. Free Radic Biol Med 1993;15:681-4. [CrossRef] [PubMed]
11. Canbolat O, Durak I, Cetin R, Kavutcu M, Demirci S, Oztürk S. Activities of adenosine deaminase, 5'-nucleotidase, guanase, and cytidine deaminase enzymes in cancerous and non-cancerous human breast tissues. Breast Cancer Res Treat 1996;37:189-93. [CrossRef] [PubMed]
12. Veljković A, Hadži-Dokić J, Sokolović D, Bašić D, Veličković-Janković L, Stojanović M, et al. Xanthine Oxidase/Dehydrogenase Activity as a Source of Oxidative Stress in Prostate Cancer Tissue. Diagnostics 2020;10(9):668. [CrossRef] [PubMed]
13. Solomon H, Brosh R, Buganim Y, Rotter V. Inactivation of the p53 tumor suppressor gene and activation of the Ras oncogene: cooperative events in tumorigenesis. Discovery Medicine 2010;9(48):448-54. [PubMed]
14. Nath K, Fischerder M, Hostetter HT. The role of oxidants in progressive renal injury. Kidney Int 1994;45: S-111—S-115. [PubMed]
15. Pederson RC, Berry AJ. Sensitive, optimised assay for serum AMP deaminase. Clin Chem 1997;23:1726-33. [CrossRef] [PubMed]
16. Lauber K. Photometric determination of nitrogen: Wet incineration followed by formation of indophenol blue with salicylate/hypochlorite. Clin Chem 1976;67:107-10. [CrossRef] [PubMed]
17. Wood RJ, Williams DG. Colorimetric determination of serum 5'-nucleotidase without deproteinization. Clin Chem 1981;27(3):464-5. [CrossRef] [PubMed]
18. Smelcerovic Z, Veljkovic A, Kocic G, Yancheva D, Petronijevic Z, Anderluh M, et al. Xanthine oxidase inhibitory properties and anti-inflammatory activity of 2-amino-5-alkylidene-thiazol-4-ones. Chemico-Biological Interactions 2015;229:73-81. [CrossRef] [PubMed]
19. Nabavi SM, Nabavi SF, Eslami S, Moghaddam AH. In vivo protective effects of quercetin against sodium fluoride-induced oxidative stress in the hepatic tissue. Food Chem 2012;132:931-5. [CrossRef]
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent, The Journal of Biological Chemistry 1951;193(1):265-75. [CrossRef] [PubMed]
21. Tayyar Kalcioglu M, Kizilay A, Ramazan Yilmaz H, Uz E, Gulec M, Ozturan O, et al. Adenosine deaminase, xanthine oxidase, superoxide dismutase, glutathione peroxidase activities and malondialdehyde levels in the sera of patients with head and neck carcinoma. Kulak Burun Bogaz Ihtis Derg 2004;12(1-2):16-22. [PubMed]
22. Aghaei M, Karami-Tehrani F, Salami S, Atri M. Adenosine deaminase activity in the serum and malignant tumors of breast cancer; the assessment of isoenzyme ADAI and ADA2 activities. Clin Biochem 2005; 38(10):887-91. [CrossRef] [PubMed]
23. Eroglu A, Canbolat O, Demirci S, Kocaoglu H, Eryavuz Y, Akgul H. Activities of adenosine deaminase and 5'-nucleotidase in cancerous and noncancerous human colorectal tissues. Med Oncol 2000;17(4):319-24. [CrossRef] [PubMed]
24. Kocic G, Vlahovic P, Djordjevic V, Bjelakovic G, Koracevic D, Savic V. Effect of growth factors on the enzymes of purine metabolism in culture of regenerating rat liver cells. Arch Physiol Biochem 1995;103: 715-19. [CrossRef] [PubMed]
25. Balis EM. Adenosine deaminase and malignant cells. Ann NY Acad Sci 1985;451:142-9. [CrossRef] [PubMed]
26. Hirata BKB, Oda JMM, Guembarovski RL, Ariza CB, de Oliveira CEC, Watanabe MAE. Molecular markers for breast cancer: prediction on tumor behavior. Disease Markers 2014;2014:513158. [CrossRef] [PubMed]
27. Kazemi MH, Mohseni SR, Hojjat-Farsangi M, Anvari E, Ghalamfarsa G, Mohammadi H, et al. Adenosine and adenosine receptors in the immunopathogenesis and treatment of cancer. J Cell Physiol 2018;233(3): 2032-57. [CrossRef] [PubMed]
28. Di Virgilio F. Purines, purinergic receptors, and cancer. Cancer research 2012;72:5441-7. [CrossRef] [PubMed]
29. Longhi MS, Robson SC, Bernstein SH, Serra S, Deaglio S. Biological functions of ecto-enzymes in regulating extracellular adenosine levels in neoplastic and inflammatory disease states. Journal of Molecular Medicine 2013;91:165-72. [CrossRef] [PubMed]
30. Sitkovsky MV, Kjaergaard J, Lukashev D, Ohta A. Hypoxia-adenosinergic immunosuppression: tumor protection by T regulatory cells and cancerous tissue hypoxia. Clinical Cancer Research 2008;14:5947-52. [CrossRef] [PubMed]
31. Spychala J. Tumour-promoting function of adenosine. Pharmacol Therapy 2000;87:161-73. [CrossRef] [PubMed]

32. Weisman GA, Lustig KD, Lane E, Huang NN, Belzer I, Friedberg I. Growth inhibition of transformed mouse fibroblasts by adenine Nucleotides occurs via generation of extracellular adenosine. J Biol Chem 1988; 263:12367-72. [CrossRef] [PubMed]
33. Fishman P, Bar-Yehuda S, Vagman L. Adenosine and other low molecular weight factors released by muscle cells inhibit tumor cell activities of adenosine deaminase, 5V-nucleotidase, guanase, and cy-growth. Cancer Res 1998;58:3181-7. [PubMed]
34. Pueyo M, Chen Y, Dangelo G. Regulation of vascular endothelial qrowth factor expression by cAMP in rat aortic smooth muscle cells. Exp Cell Res 1998; 238: 354-8. [CrossRef] [PubMed]
35. Cronstein BN, Kubersky SM, Weissmann G, Hirschhorn R. Engagement of adenosine receptors inhibits hydrogenperoxide release by activated human neutrophils. Clin Immunol Immunopathol 1987;42:4276-85. [CrossRef] [PubMed]
36. Schulte G, Fredholm BB. Signalling from adenosine receptor to mitogen activated protein kinases. Cell Signalling 2003;15:15813-27. [CrossRef] [PubMed]
37. Mahajan M, Tiwari N, Sharma R, Kaur S, Singh N. Oxidative Stress and Its Relationship With Adenosine Deaminase Activity in Various Stages of Breast Cancer. Indian Journal of Clinical Biochemistry 2013; 28(1):51-4. [CrossRef] [PubMed]
38. Zeleznikar RJ, Heyman RA, Graeff RM, Walseth TF, Dawis SM, Butz EA, et al. Evidence for compartmentalized adenylate kinase catalysis serving a high energy phosphoryl transfer function in rat skeletal muscle. J Biol Chem 1990;265:300-11. [CrossRef] [PubMed]
39. Natsumeda Y, Prajda N, Donohue JP, Glover JL, Weber G. Enzymic capacities of purine *de novo* and salvage pathways for nucleotide synthesis in normal and neoplastic tissues. Cancer Res 1984;44:2475-9. [PubMed]
40. Hofbrand AV, Janossy G. Enzyme and membrane markers in leukemia: recent developments. J Clin Pathol 1981;34:254-62. [CrossRef] [PubMed]
41. Bemi V, Tazzini N, Banditelli S, Giorgelli F, Pesi R, Turchi G, et al. Deoxyadenosine metabolism in a human colon-carcinoma cell line (LoVo) in relation to its cytotoxic effect in combination with deoxycoformycin. In J Cancer 1998;75:713-20. [CrossRef] [PubMed]
42. Kate J, Ingh HF, Khan PM, Bosman FT. Adenosine deaminase complexing protein (ADCP) immunoreactivity in colorectal adenocarcinoma. Int J Cancer 1986;15:479-85. [CrossRef] [PubMed]
43. Sanfilippo O, Camici M, Tozzi MG, Turriani M, Faranda A, Ipata L, et al. Relationship between the levels of purine salvage pathway enzymes and clinical/biological aggressiveness of human colon carcinoma. Cancer Biochemistry Biophysics 1994;14(1):57-66. [PubMed]
44. Flamand N, Boudreault S, Picard S, Austin M, Surette ME, Plante H, et al. Adenosine, a potent natural suppressor of arachidonic acid release and leukotriene biosynthesis in human neutrophils. Am J Respir Crit Care Med 2000;161(2):S88-S94. [CrossRef] [PubMed]
45. Veljković A, Stanojević G, Branković B, Pavlović D, Stojanović I, Cvetković T, et al. Parameters of oxidative stress in colon cancer tissue. Acta Medica Medianae 2016;55:32-7. [CrossRef]
46. Erkiliç K, Evereklioglu C. Çekmen M, Özkiris A, Duygulu F, Dogan H. Adenosine deaminase enzyme activity is increased and negatively correlates with catalase, superoxide dismutase and glutathione peroxidase in patients with Behçet's disease: original contributions/clinical and laboratory investigations. Mediators Inflamm 2003;12(2):107-16. [CrossRef] [PubMed]
47. Viviani LG, Piccirillo E, Cheffer A, de Rezende L, Ulrich H, Carmona-Ribeiro AM, et al. Be aware of aggregators in the search for potential human ecto-5'-nucleotidase inhibitors. Molecules 2018;23(8):E1876. [CrossRef] [PubMed]
48. Al-Haj L, Khabar KSA. The intracellular pyrimidine 5'-nucleotidase NT5C3A is a negative epigenetic factor in interferon and cytokine signaling. Sci Signal 2018; 11(518):l2434. [CrossRef] [PubMed]
49. Ikegami T, Natsumeda Y, Weber G. Decreased concentration of xanthine dehydrogenase (EC 1.1.1.204) in rat hepatomas. Cancer Res 1986;46(8):3838-41. [PubMed]
50. Veljkovic A, Hadzi-Djokic J, Sokolovic D, Cukuranovic R, Cukuranovic-Kokoris J, Basic D, et al. Local and Systemic Oxidative Stress in Balkan Endemic Nephropathy Is Not Associated with Xanthine Oxidase Activity. Oxid Med Cell Longev 2020;8209727. [CrossRef] [PubMed]
51. Romagnoli M, Gomez-Cabrera MC, Perrelli MG, Biasi F, Pallard´o FV, Sastre J, et al. Xanthine oxidase-induced oxidative stress causes activation of NF-kappaB and inflammation in the liver of type I diabetic rats. Free Radic Biol Med 2010;49:171-7. [CrossRef] [PubMed]
52. Balamurugan K. HIF-1 at the crossroads of hypoxia, inflammation, and cancer. Int J Cancer 2016;138: 1058-66. [CrossRef] [PubMed]
53. Se-Hyun O, Soon-Youn C, Hyo-Jung C, Hye-Myung R, You-Jin K, Hee-Yeon J, et al. The emerging role of xanthine oxidase inhibition for suppression of breast cancer cell migration and metastasis associated with hypercholesterolemia. FASEB Journal 2019;33(6): 7301-14. [CrossRef] [PubMed]
54. Cummins E, Doherty G, Taylor C. Hydroxylases as therapeutic targets in inflammatory bowel disease. Laboratory Investigation 2013;93(4):378-83. [CrossRef] [PubMed]

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ZNAČAJ AKTIVNOSTI ENZIMA KOJI UČESTVUJU U NASTANKU URATA I NJIHOVA VEZA SA OKSIDATIVNIM STRESOM U PROGRESIJI I INVAZIJI KOLOREKTALNOG KARCINOMA

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Kolorektalni karcinom jedan je od najčešće dijagnostikovanih karcinoma i najčešći uzrok smrtnosti od svih malignih bolesti. I dalje postoji nedostatak ispitivanja patogeneze ove bolesti, uključujući uticaj enzima uratnih puteva na progresivnost i invaziju kolorektalnog karcinoma, te je cilj ovog rada da istraži ulogu aktivnosti adenozin deaminaze (ADA), 5'-nukleotidaze (5'-NT) i ksantin oksidaze (KSO) u odnosu na TBA reaktivne supstance (TBARS), kao markere oksidativnog stresa u progresiji i invaziji humanog karcinoma debelog creva.

Uzeli smo uzorke tkiva karcinoma – zdravo kontrolno tkivo i tkivo koje okružuje tumor od 50 bolesnika sa primarnim karcinomom debelog creva u sva četiri klinička stadijuma bolesti. U 10% homogenatima sprovedeno je istraživanje odgovarajućim metodama.

Aktivnost ADA i KSO u tkivu tumora i tkivu uz tumor bila je značajno veća u poređenju aktivnosti ADA i KSO u zdravom tkivu debelog creva. Aktivnost 5'-NT nije značajno veća u tkivu karcinoma. Najveća aktivnost ADA i KSO je u stadijumima tumora T2 i T3. Najveća koncentracija TBARS je u stadijumima T3 i T4 tumora.

Dobijeni rezultati sugerišu da bi jedan od mogućih uzroka oksidativnog stresa u karcinomu debelog creva mogla biti visoka aktivnost KSO i ADA. To može dovesti u vezu te enzime sa malignom transformacijom epitela debelog creva, kao i sa napredovanjem i metastaziranjem karcinoma debelog creva. Na ovaj način, procena aktivnosti ADA i KSO mogla bi da pomogne u proceni margina prilikom odstranjivanja karcinoma, kako bi se utvrdila opsežnost resekcije debelog creva. Takođe, ovi enzimi mogu imati značaj u dijagnozi, ali i u prognozi bolesti.

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Ključne reči: *kolorektalni karcinom, adenozin dezaminaza, 5'-nukleotidaza, ksantin oksidaza, oksidativni stres*