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5 Lipoxygenase Overexpression in Polyp Tissue and Normal Tissue in Patients with Colon Polyps

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Abstract

Aim: Arachidonic acid metabolism plays a role in the development and progression of many neoplasms through Cyclo Oxygenase (COX) and Lip Oxygenase (LOX) enzyme pathways. Over-expression of 5- Lipoxygenase (5-LOX) enzyme has been demonstrated in different human cancers. A few studies have investigated the relationship between colon neoplasms and 5-LOX enzymes. The aim of this study was to examine and compare 5-LOX expression in normal colonic tissue and polyp tissue in patients with colonic polyps.

Methods: The study included a total of 20 patients (10 female, 10 male). Normal colonic tissue and polyps after polypectmy were collected from all patients. Polyp and normal colonic tissues were sent to the pathology and genetic laboratories for examination of 5-LOX gene expression.

Results: All patients had 5-LOX overexpression in polyp tissue and 11 patients also had 5-LOX overexpression in normal colonic tissue. There were no statistically significant differences in respect of polyp localization and polyp histological evaluation between the 5-LOX over-expression group and the 5-LOX normal expression group in normal colonic tissue.

Conclusion: There was seen to be 5-LOX overexpression in the tissue of colonic polyps in all the patients an in normal colonic tissue in a high percentage of patients. 5-LOX overexpression may have a role in polyp formation and tumorogenesis.

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Introduction

Colorectal cancers are one of the most common types of cancers, and are considered to be the third most prevelant cancer type and the second most common cause of all cancer mortalities [1]. Currently, there are advanced methods and screening tools for the early detection of colorectal cancers. However, the current approaches are not sufficent for early detection, hence the mortality rate of colorectal cancers remains high. Early detection of colorectal cancer is a challenge for gastroenterologists and oncologists [2]. The risk factors for colon cancer include older age, obesity, consumption of red meat, smoking, alcohol, insulin resistance/diabetes mellitus, inflammatory bowel disease, and family history for colon cancer [3]. Excessive dietary fat is known to be associated with an increased risk of colorectal cancer [4,5]. Arachidonic Acid (AA) is a polyunsaturated fatty acid and the metabolism of AA is associated with human carcinogenesis. There are two major metabolic routes to control AA metabolism; first is the Cyclo Oxygenase (COX) enzyme pathway that converts to thromboxanes, prostoglandins and prostacyclins; second is the Lip Oxygenase (LOX) enzyme pathway that converts to Leuko Trienes (LTs) and Hydroxy Eicosate Tra Enoic acids (HETEs). These proinflammatory substances are known to modulate some important processes such as cell proliferation, apoptosis and angiogenesis, which are related with development of neoplasia [6-8]. Two COX enymes have been detected; the first one is COX-1, which is expressed by most tissues and maintains the homeostatic system and the second one is COX-2, which is induced by a variety of stimuli such as cytokines, growth factors, proinflammatory stimuli and tumour promoters. There are 3 major LOX enzymes in the human body, namely 5-LOX, 12-LOX and 15-LOX. 5-LOX enzyme is mainly present in leukocytes [6,8-11].

Many studies have clearly demonstrated that COX-1 ve COX-2 enzymes via cytokines, apoptosis and angiogenic factors have an important effect in the devolepment and progression of many neoplasms [12-15]. However, the contribution of 5-LOX in the development of human neoplasms has not been comprehensively investigated [7,11,16]. Over-expression of 5-LOX enzyme has been demonstrated in different human cancers, including prostate, pancreas, testicular, breast, and colon [16-20]. 5-LOX enzyme and metabolites may play an important role in tumor cells via supression of apoptosis, enhancing proliferation of cancer cells and promoting the angiogenic process similar to the effects of COX enzymes. To date, only a few studies have investigated the relationship between colon neoplasms and 5-LOX enzymes [8-16]. The aim of this study was to examine 5-LOX expression in normal colonic tissue and polyp tissue in patients with colonic polyps.

Method

The study was conducted in the Gastroenterology Department of Turgut Ozal University. The inclusion criteria were as follows; age 18-65 years, to have undergone colonoscopy and have colon polyps with ≥1 cm in diameter, voluntary participation in the study. Patients with a history of malignancy, inflammatory bowel diseases, a family history of gastrointestinal malignancy or family history of gastrointestinal polyposis were excluded. According to the inclusion and exclusion criteria, a total of 20 patients were evaluated in the study. The clinical and demographic characteristics including age, gender, treatment status of Non-Steroidal Anti-Inflammatory Drugs (NSAID), calcium combined hormones and acetyl salycilic acid, usage of tobacco and alcohol, body mass index, phsysical activity level and eating habits were all recorded. Informed consent was obtained from all patients. All the study procedures were in compliance with the principles of the Helsinki Declaration. Approval for the study was granted by the Local Ethics Committee.

Tissue material

Polyps and normal tissue were obtained from all patients. All polyps were fixed with formalin, embedded in paraffin blocks and then sent to the Department of Pathology for examination and classification.

Another sample of polyps and normal tissue that were extracted from the patients were sent to the Genetic Laboratory for examination of 5-LOX gene expression. A quantitative Real-Time RT-PCR analysis was performed using a Rotor-Gene Q real time PCR cycler (Qiagen, Hilden, Germany). The PCR mixture consisted of 2X QuantiTect SYBR Green PCR Master Mix(Qiagen, Hilden, Germany), which contains DNA polymerase, SYBR Green I Dye, dNTP mix including UTP, 5 mM $MgCl_2$, PCR buffer, 20 pmol forward and reverse primers, RNase-FreeWater and cDNA of samples in a total volume of 25 µl/mL. The amplification of a housekeeping gene, β-actin, was used for normalizing. PCR was performed with initial denaturation at 95ºC for 5 min, followed by amplification for 40 cycles, each cycle consisting of denaturation at 95ºC for 30 s, annealing at 58ºC for 30 s, polymerization at 72ºC for 1 min and, the last stage, polymerization at 72ºC for 5 min. Data were analyzed using the Rotor-Gene Q Series Software 2.1.0. Sequencing of B-Actin and 5-LOX are demonstrated in Table 1. The demonstration of agorose-gel electrophoresis is shown in Figure 1.

Statistical method

All Statistical Analyses were Performed using SPSS version 17.0 (Chicago, IL, USA). Data were expressed as mean ± Standard Deviation (SD). Continuous variables were compared using the Mann-Whitney U-test. Categorical variables were compared using the Chi-Square test. Logistic regression analysis was performed to assess the relationship between the presence of high or low grade dysplasia and 5-LOX overexpression in polyps and normal tissue samples. A value of $p<0.05$ was considered statistically significant.

Results

Evaluation was made of 20 patients with colonic polyps, comprising 10 (50%) females and 10 (50%) males with a mean age of 54.60 + 16.66 years. On admission, 5 (25%) patients had rectal bleeding, 3 (15%) had abdominal pain, 8 (40%) had anemia and 4 (20%) had non-specific complaints. The polyps of 14 patients were classified as low grade dysplasia (70%) and 6 patients were classified a high grade dysplasia (30%). 5-LOX gene over expression was demonstrated in all polyp tissue (100%). In normal colonic tissue, 5-LOX gene overexpression was seen in 11 (55%) patients. 5-LOX gene overexpression in normal colonic tissue was detected in 3 (75%) of 4 patients who had malignant polyps (Table 2).

The 5-LOX gene overexpression group and 5-LOX gene normal expression group were similar in respect of gender, age, and BMI (p=0, 20. P=0.50, p=0.55, respectively). There was no statistically significant difference between the groups in respect of polyp localization and polyp histological evaluation (p=0,46. p=0.88, respectively). The groups were similar in terms of the use of NSAID, acetylsalicylic acid, calcium and hormone replacement therapy (p=0,63. p=0.63, p=0.71, p=0.45, respectively).

No statistically significant difference was observed between the groups in respect of tobacco, alcohol consumption, dietary habits and frequency of physical activity (Table 2).

In regression analysis, the presence of 5-LOX expression in normal tissue was selected as the dependent variable and type of dysplasia was entered as the covariant. There was no significant association between 5-LOX overexpression in normal tissue and type of dysplasia in the binary logistic regression analysis (OR: 0.81, p= 0.65).

Table 2: Demographic and Clinical Characteristics of the Patients.

	5 LOX overexpression group (n=11)	5 LOX normal expression group (n=9)	P
Age, years	54.11 ± 11.22	44.48 ± 12.33	P=0.20
Gender Male Female	 6 (54.5%) 5 (45.4%)	 4 (44.4%) 5 (55.6%)	 P= 0.50
BMI, kg/m^2	28.32 ± 2.72	27.90 ± 3.06	P=0.55
Histology of polyps Tubular Tubulovillous Villous Malignant polyp Serrated adenoma	 3 (27.7%) 1 (9.1%) 2 (18.2%) 3 (27.7%) 2 (18.2%)	 4 (44.4%) - 1 (11.1%) 1 (11.1%) 3 (33.3%)	 P= 0.88
Type of Dysplasia Low Grade High Grade Dysplasia	 8 (57%) 3 (50%)	 6 (43%) 3 (50%)	 P= 0.57
Localization of polyps Rectum Sigmoid colon Descending colon Transverse colon Cecum	 3 (27.7%) 6 (54.5%) - 1 (9.1%) 1 (9.1%)	 1 (11.1%) 4 (44.4%) 2 (22.2%) 2 (22.2%) -	 P= 0.46
Calcium replacement Yes / No	 1 (9.1%)/10 (91.9%)	 1 (11.1%)/8 (88.9%)	 P= 0.71
Multivamin replacement Yes / No	 -	 1(11.1%)/8(88.9%)	 P= 0.45
Use of NSAID Yes / No	 2 (18.2%)/9 (81.8%)	 2(22.2%)/7(77.8%)	 P= 0.63
Use of acetylsalicylic acid Yes / No	 2 (18.2%)/9 (81.8%)	 2 (22.2%)/7 (77.8%)	 P= 0.63
Hormone replacement Yes / No	 -	 1 (11.1%)/8 (88.9%)	 P= 0.45
Meat Yes / No	 7 (63.6%)/4 (36.4%)	 6 (66.7%)/3 (33.3%)	 P= 0.89
Chicken meat Yes / No	 7 (63.6%)/4 (36.4%)	 4 (44.4%)/5 (55.6%)	 P= 0.25
Nut Yes / No	 10(91.9%)/1(9.1%)	 8 (88.9%)/1 (11.1%)	 P= 0.70
Milk Yes / No	 8 (72.7%)/3 (27.3%)	 5 (55.6%)/4 (44.4%)	 P= 0.42
Vegetables Yes / No	 7 (63.6%)/4 (36.4%)	 9 (100%)/-	 P= 0.09
Alcohol Yes / No	 2 (18.2%)/9 (81.8%)	 1 (11.1%)/8 (88.9%)	 P= 0.66
Tobacco Yes / No	 6 (54.5%)/5 (45.5%)	 4 (44.4%)/5 (55.6%)	 P= 0.65

Abbreviations: 5 -LOX: 5 Lipoxygenase; BMI: Body Mass Index; NSAID: Non-Steroidal Anti-Inflammatory Drugs.

Table 1: Demonstration of agorose-gel electrophoresis.

B – Actin	FORWARD	5'-TTCCTGGGCATGGAGTCCT-3'
	REVERSE	5'-AGGAGGAGCAATGATCTTGATC-3'
5-LOX	FORWARD	5'-CCAGACCATCACCCACCTTC-3'
	REVERSE	5'-GAATCTCACGTGTGCCACCA-3'

Abbreviations: 5-LOX: 5 Lipoxygenase

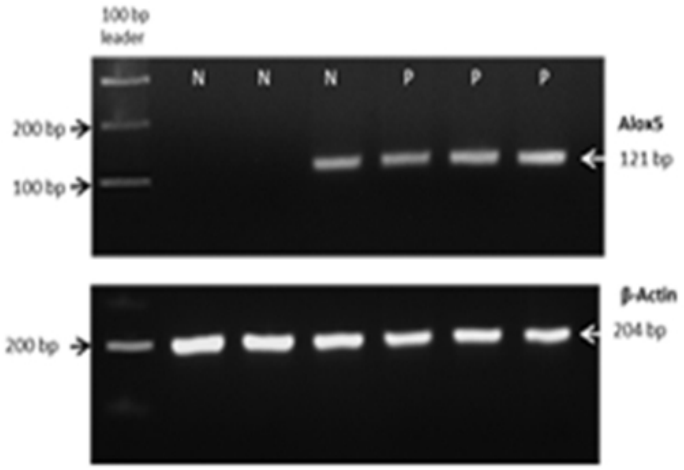

Figure 1: Demonstration of agorose-gel electrophoresis.

Discussion

The results of the present study demonstrated that 5-LOX overexpression was present in the tissue of colonic polyps in all patients and in normal colonic tissue in some patients. 5-LOX overexpression in both normal tissue and polyp tissue was not seen to be related to location and histological type of polyps.

AA, a long-chain polyunsaturated fatty acid, is transformed into prostoglandins, prostacyclins and thromboxanes by the COX enzyme pathway and into leukotrienes, and hydroxyeicosatetraenoic acids by the LOX enzyme pathway. These products play an important role in the development, progression, and growth of malignant tumors through increased cell proliferation, supression of apoptosis and increased angiogenesis [6,21,22]. Overexpression of 5-LOX, an enzyme involved in AA metabolism, has been shown in human cancers including prostate, testicular, pancreas, and esophagus [16,17,19,20,23]. However, there are few studies in literature that have reported 5-LOX overexpression in colonic neoplasms [8,16].

Soumaoro et al reported that 5-LOX expression was markedly increased in colon tumors compared to the adjacent normal tissue. The results of that study also showed that there was a positive corelation of 5-LOX level with the size and depth of tumor and invasion of vessels, but no corelation with tumor site and histological type [16]. Similarly, in the current study, no relationship was determined between 5-LOX overexpression and location and histological type of polyps.

In another study, Wasilewicz et al. also reported a high percentage (67.5%) of 5-LOX overexpression in colon polyps. It was also suggested that 5-LOX overexpression was related to histological type, degree of neoplasia, size, location of polyps and patient age [24].

Melstrom et al reported 5-LOX overexpression in polyp tissue and colon cancer, but 5-LOX expression was not detected in normal colonic epithelium [25]. In contrast, the results of the current study showed 5-LOX overexpression in normal colonic epithelium in more than half of the patients. This finding may suggest that 5-LOX overexpression in normal colonic epithelium is a trigger step for the emergence of polyps in the colon. This hypothesis may be a speculative interpretation when it is taken into account that these are preliminary data. It should be kept in mind that the sample size of this study was too small to be able to make general conclusions.

In conclusion, 5-LOX overexpression contributes to polyp formation in the colon and 5-LOX overexpression in normal colonic tissue may be an early sign of the capability to develop polyp or cancer. However, there is a need for further studies of larger samples to clarify this association.

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