The Cyclooxygenase-2 and Nuclear Factor-*kappa* B Expressions in Colorectal Polyps

Ahmar Abyadh, Diah Rini Handjari**, Murdani Abdullah***, Pamela Abineno Damaledo**, Abdul Aziz Rani****

* Department of Internal Medicine St. Elisabeth Hospital, Bekasi
** Department of Anatomical Pathology, Faculty of Medicine University of Indonesia Dr. Cipto Mangunkusumo General National Hospital, Jakarta
***Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine University of Indonesia/Dr. Cipto Mangunkusumo General National Hospital, Jakarta

ABSTRACT

Background: *Cyclooxygenase-2 (COX-2) is the rate-limiting enzyme in prostaglandin synthesis, while nuclear factor kappa B (NF-κB) is a family of transcription factors. Both play an important role in tumorigenesis. In the present study, we examined NF-κB and COX-2 expressions pattern, and their association in neoplastic and non-neoplastic colorectal polyps (CP).*

Method: *Formalin-fixed and paraffin embedded tissue blocks from 77 patients with CP were immunostained with anti-NF-κB (p 65) and anti-COX-2. Expressions of NF-κB, and COX-2 were detected immunohistochemically. The relationship between these expressions and the two types of CP, and other clinicopathological findings were evaluated.*

Results: *The expressions of NF-κB and COX-2 in patients with neoplastic and non-neoplastic CP were high. The results of this study indicated that generally in CP, NF-κB was associated with COX-2 and the association was also seen in neoplastic and non-neoplastic polyps. There was no significant difference of NF-κB and COX-2 expressions in terms of patient's age, sex, histologic type, and location of the CP. Neoplastic CPs were more common in the distal colon, female patients and older patients (≥ 60 years) compared with non-neoplastic CPs. Neoplastic CP were located more at the distal colon, more in female, and more in older (≥ 60 years) patients as compared with the non-neoplastic CP. Further studies are needed to elaborate the role of inflammation in sporadic colorectal carcinogenesis.*

Conclusion*: The expressions of NF-κB and COX-2 in patients with CP were high, and strong correlated each other. There were no significant differences between expression of NF-κB and COX-2 in neoplastic and non-neoplastic polyps.*

Keywords: *colorectal polyps (CP), NF-κB, COX-2, inflammation*

INTRODUCTION

Colorectal polyps (CP) are classified histologically as neoplastic (adenomas) and non-neoplastic type.[1,2] Most studies support CP being considered precursors to the development of colorectal cancer (CRC).[3-5] The incidence of adenomas and carcinomas of the colon is high in western Europe and the United State in contrast to the low incidence of both among populations of Afro-Asian origin.[5-9] There are some differences between CRC in developed countries and in Indonesia. The prevalence of CRC in developed countries in patients aged 50 years or less was 3.1-8.6%,[1,3] while in Indonesia, the proportion of CRC patients aged 40 years or less was higher.[2] Another point is, the young onset CRC in western population commonly associated with a positive family history,[10,11] involving germ-line mutations of mismatch repair (MMR) genes, following microsatellite instability (MSI) pathway,[12] while young CRC Indonesian

Correspondence:
Ahmar Abyadh
Department of Internal Medicine
St. Elisabeth Hospital
Jl. Raya Siliwangi 202 Bekasi 17116 Indonesia
Phone: +62-87881408576
E-mail: ahmar.umar@yahoo.co.id

patients did not show any positive family history and there was no difference in term of expression of MMR proteins compared with older patients.[13] Abdullah concluded that colorectal carcinogenesis of young patients seems to be the same as in elder patients and might follow a sporadic pathway.[14] It is thought that chronic process gives conducive microenvironment milieu for inducing malignant changes of epithelial cells.[15] In this inflammatory-induced carcinogenesis pathway, the nuclear factor kappa B (NF-κB), which is an important mediator of the growth and development of inflammatory-induced tumorigenesis,[16] could well be an important player since it is activated in chronic inflammation, and might involved dysplastic epithelial cells rather than adenoma.[17-19]

In sporadic cancers, mutations may occur through different types of molecular alterations induced by carcinogens, tumor promoters, carcinogenic viral proteins, chemotherapeutic agents, and γ-irradiation. The alterations involved irregulation of NF-κB activation and pro-inflammatory gene products such as tumor necrosis factors (TNF) and members of its superfamily, IL-1a, IL-1b, IL-6, IL-8, chemokines, matrix metallopeptidase 9 (MMP-9), vascular endothelial growth factor (VEGF), cyclooxygenase-2 (COX-2), and 5-lipoxygenase (5-LOX), that participate in the development and progression of cancer and mediate a critical role in suppression of apoptosis, proliferation, angiogenesis, invasion, and metastasis.[20-25] COX-2 may have a crucial involvement in intestinal carcinogenesis by inducing changes in cellular adhesion, local invasion, and inhibition of apoptosis, and is upregulated in consecutive stages of the colorectal adenoma-carcinoma sequence in patients with sporadic colorectal cancer and in familial adenomatous polyposis.[16,18,26,27] However, COX-2 enzyme was not expressed in 61% of CRC patients with high MSI or negative expression of MMR proteins.[28] COX-2 is not detectable in most normal tissues, but it is induced at inflammation area by epithelial injury as part of inflammatory response to infections such as cytokines, growth factors, tumor promoters and other s timuli.[26,29-31] COX-2 expression was associated with other inflammatory markers, such as interleukin (IL)-1β, IL-6 and sub-unit p65 NF-κB both in the epithelial or stromal cells[27] suggesting that inflammatory process play roles in sporadic colorectal carcinogenesis.[14,19]

Molecular studies of inflammatory markers in CP in Indonesia are limited. So far, as our knowledge there is no study assessing the role of NF-κB and COX-2 in neoplastic and non-neoplastic cases. This study was aimed to explore the expression pattern NF-κB and COX-2 in Indonesian patients and to compare their expressions between neoplastic and non-neoplastic CP. Finding of such relation will assist in developing new modalities in the course of preventive and curative efforts for CRC, which are considered as chemoresistant tumors.[32] In addition, this study will provide data for further study about chemoprevention of colorectal cancer.

METHOD

Study Materials

The study was performed on archived human paraffin-embedded polypectomy tissue sections from medical record in the Department of Anatomical Pathology at Cipto Mangunkusumo hospital, Jakarta in the year 2007 and 2008. All of the hematoxylin and eosin-stained glass slides of these cases were reviewed by a single pathologist to confirm the histopathologic diagnosis in each case. The samples were excluded if histological findings revealed the presence of malignancy, if the available tissue sections were damaged, and if the patient medical record were missing.

Immunohistochemistry

Four-μm-thick step sections of representative formalin-fixed paraffin-embedded tissue blocks were prepared for immunohistochemical analysis. NF-κB and COX-2 expressions were evaluated using the avidin/biotin complex immunohistochemistry procedure.[31] We used rabbit polyclonal antibodies against human NF-κB p65 subunit (RelA) (Cat No.#ab7970, Abcam, UK) and human COX-2 protein (Cat No.#ab15191, Abcam, UK) as primary antibodies.

Sections were sequentially deparaffinized and rehydrated through xylene and graded alcohol solutions. Then, slides were pretreated with epitope retrieval system and preheated in a microwave for 5 minutes. Afterwards, slides were immersed in phosphate buffer saline (PBS) for 5 minutes. Endogenous peroxidase activity was blocked by immersing the sections into a solution mixture of hydrogen peroxide 3% and methanol for 10 minutes in refrigerator, and then were rinsed with PBS for 5 minutes. Slides were marked by a Pap pen and then were put again in PBS. After removal from PBS, slides were placed in a treatment chamber. Blocking solution was applied into each section and the chamber was closed for 10 minutes. Primary antibodies were diluted in distilled water with 1:100 for COX-2 and 1:150 for NF-κB. Sections were then incubated with primary in the treatment chamber for 45 minutes. After washing with PBS, antibody binding was detected by incubation with bitinylated secondary antibody for 10 minutes. Then the slides were washed again with PBS and incubated with straptavidin-horse radish

peroxydase. Staining was developed by applying the chromogen 3.3 diaminobenzidine in distilled water and then counterstain with hematoxylin. Finally, specimens were dehydrated and mounted. Positive and internal negative controls were included in each staining. Internal negative control sections run without the addition of primary antibodies. Positive control was fibroblast cells known to have positive COX-2 expression.

Evaluation of Immunostaining

The immunostaining reactions were evaluated for NF-κB and COX-2 expressions in the lesions, with light microscopy. The periluminal expression of NF-κB and COX-2 observed in polyps was scored. The scoring was accomplished according to area of staining (0-4) + intensity (0-3) in each polyp. Then, the NF-κB and COX-2 expression scores were correlated each other. The NF-κB and COX-2 expression were classified as low, if the scores were 5 or lower, and classified as high if the scores were six or higher. After classification, the expressions were correlated with histopathogical type, location of CP, and age of the patients.

Statistical Analysis

Statistical analysis was performed using SPSS 17 (SPSS Inc, Chicago, IL). Clinical characteristics in CP were analyzed using chi-square and logistic regression. Correlation between the NF-κB and COX-2 expression score was analyzed using Pearson test. Analysis of NF-κB and COX-2 expression in relation with patient's age, type, and location of CP were done using logistic regression. The difference between groups were considered significant if $p < 0.05$.

RESULTS

A total number of 77 CPs were diagnosed in the 2007-2008. There were 39 (50.6%) males, and 38 (49.4%) female patients with age range from 17 to 86 years with mean age ± SD of 54.8 ± 15.9 (table 1). There were no significant difference of frequency between non-neoplastic and neoplastic CP in terms of patient's age, and location of CP. In addition, there was no apparent correlation between non-neoplastic and neoplastic CP in expression of NF-κB and COX-2. However, there was good correlation between NF-κB and COX-2 expression scores of CP in general (table 2). There was no significant difference between expression of NF-κB in terms of patient's age, type, and location of the CP (table 3). Although there was no significant difference between expression of COX-2 in terms of type and location of CP, but there was significantly higher COX-2 expression in the group of CP patients aged < 50 years as compared with patients aged 50-59 and above 60 years (table 4).

Table 1. Characteristic of the 77 patients with CPs

Clinical characteristics	Non-neoplastic	Neoplastic	p	OR	(95% CI)
	n (%)	n (%)			
Sex					
Male	15 (38.46)	24 (61.54)			
Female	7 (18.42)	31 (81.58)	0.08		
Location					
Proximal	7 (46.67)	8 (53.33)			
Distal	15 (24.19)	47 (75.81(	0.11		
NF-κB expression					
Low	4 (19.05)	17 (80.95)			
High	18 (32.14)	38 (67.86)	0.39		
COX-2 expression					
Low	3 (18.75)	13 (81.25)			
High	19 (31.15)	42 (68.85)	0.54		
Age (years)					
<50	10 (37.04)	17 (62.96)	Reference		
50-59	4 (22.22)	14 (77.78)	0.30	2.06	(0.53-8.01)
≥ 60	8 (25.00)	24 (75.00)	0.32	1.77	(0.58-5.40)

OR: Odd Ratio, CI: Confidence Interval

Table 2. NF-κB and COX-2 expression scores

	Mean ± SD	Correlation	p
NF-kB	5.10 ± 1.40	0.63	p = 0.00
COX-2	5.80 ± 1.40		

Table 3. NF-kB expression in relation with patient's age, type, and location of CP

Characteristics		NF-kB expression		OR (CI)	p
		Low	High		
		n (%)	n (%)		
Age (years)	<50	5 (18.52)	22 (81.48)	Reference	
	50-59	6 (33.33)	12 (66.67)	0.46 (0.11-1.81)	0.26
	≥ 60	10 (15.15)	56 (84.85)	0.50 (0.15-1.70)	0.27
Type	Non-neoplastic	4 (18.18)	18 (81.82)	Reference	
	Neoplastic	17 (30.91)	38 (69.09)	0.50 (0.15-1.69)	0.26
Location	Proximal	6 (40.00)	9 (60.00)	Reference	
	Distal	15 (24.19)	47 (75.81)	2.09 (0.64-6.83)	0.22

Table 4. COX-2 expression in relation with patient's age, type, and location of CP

Characteristics		COX-2 expression		OR (CI)	p
		Low	High		
		n (%)	n (%)		
Age (years)	<50	1 (3.70)	26 (96.30)	Reference	
	50-59	6 (33.33)	12 (66.67)	0.08 (0.01-0.71)	0.02
	≥ 60	9 (28.13)	23 (71.87)	0.10 (0.01-0.84)	0.03
Type	Non-neoplastic	3 (13.64)	19 (86.36)	Reference	0.33
	Neoplastic	13 (23.64)	42 (76.36)	0.51 (0.13-2.00)	
Location	Proximal	2 (13.33)	13 (86.67)	Reference	
	Distal	14 (22.58)	48 (77.42)	0.53 (0.11-2.6)	0.43

DISCUSSION

In the present study, we found high expression of NF-κB and COX-2 in CPs. This hints at a potential role for NF-κB in the regulation of expressed COX-2 protein in CP, and clinically. This finding strongly corroborates the previous findings where there was greater NF-κB and COX-2 expression in human colorectal adenoma than in adjacent normal colonic mucosa. There are several evidence that COX-2 might be a rate-limiting step in colon carcinogenesis. Firstly, COX-2 inhibit apoptosis in colon tumor cell lines and malignant colon tissue. COX-2 inhibitors exert their antiproliferative effects in mouse models for CRC and in colon tumor cell lines most likely via induction of apoptosis. Secondly, inhibition of COX-2 in mice with a defective adenomatous polyposis coli (APC) tumor suppressor gene, protects against the development of intestinal tumors. Thirdly, epidemiological evidence shows that individuals who take non-steroidal anti inflammatory drugs (NSAIDs) have a markedly reduced risk of developing CRC and its presumed non-malignant precursor, the adenomatous polyp. Finally, expression of COX-2 was found to correlate with an unfavorable progression of the disease.[27] Expression of COX-2 parallels expression of transcription factor NF-κB. These observations suggest that immunomodulatory approaches and/or modulation of NF-κB expression and activation could be possible pharmacological strategies to interfere with the development andprogression of CP.

McLean also found significant increase in COX-2 expression in colonic polyps.[33] We found a good correlation of NF-κB and COX-2 in CP, however we found no apparent difference of NF-κB and COX-2 between neoplastic and non-neoplastic CP. This fact showed that in this study. First, NF-κB are involved in COX-2 induction in CP, and second, in non-neoplastic CP, inflammation might play an important role. Studies with an attempt to find the relationship between chronic colonic inflammation and CP are needed. Charalambous found high expression of NF-κB and COX-2 in neoplastic colorectal epithelial cells, with the expression of COX-2 and NF-κB highly correlated.[34] In our study COX-2 expression occur both in neoplastic and non-neoplastic cases. Our finding was synchronous with previous studies done in Jakarta.[14] Another thing to remind is that patients above 50 years of age expressed COX-2 significantly higher than the younger ones.

The proportion of the neoplastic type was slightly higher than the non-neoplastic type in our study. This was also the case in the west, where the neoplastic type makes around 80-85%. Brazowsky et al have shown that expression of COX-2 in familial juvenile polyps was associated with dysplasia,[35] while in our study it was not the case. The reason was that they worked on familial juvenile polyps only, while in the present study we included all types of polyps. We must put a stress, however, that our study was not designed to find the prevalence of neoplastic polyps in the entire CPs population, as we were well aware of the limitations of a retrospective study design, this study was designed primarily to explore the expression

pattern NF-κB and COX-2 in Indonesian patients and to compare their expressions between neoplastic and non-neoplastic CP. Nevertheless, the present study provides important information on this particular subset of patients.

CONCLUSION

The expression of NF-κB and COX-2 in patients with CP were high, and strong correlated each other. There were no significant differences between expression of NF-κB and COX-2 in neoplastic and non-neoplastic polyps.

SUGGESTION

The expressions of NF-κB and COX-2 in patients with CP were high, and well correlated each other. This finding led us to identify who is at risk of NF-κB and COX-2-expressing CP and for new drugs that can provide benefits for CP patients without the risks associated with the drug. The differences between CP that expresses high or low levels of NF-κB and COX-2 have to be investigated, and this understanding can form the basis for clinical implications. This study describes the expression of COX-2 in CP. COX-2 overexpression appears as a rationale for the chemoprevention in CP and immunomodulatory approaches and/or modulation of NF-κBexpression.

There were no significant differences between expression of NF-κB and COX-2 in neoplastic and non-neoplastic polyps. This finding led us to be aware of that even in non-neoplastic CP there was high percentage of NF-κB and COX-2 expression. Further, in future NF-κBand COX-2 expression are suggested to be included in routine investigations for colorectal growth and some other tumors.

REFERENCES

1. Fenoglio CM, Pascal RR. Colorectal adenomas and cancer: pathologic relationships. Cancer 1982;50:2601-8.
2. Fenoglio-Preiser CM, Hutter RV. Colorectal polyps: pathologic diagnosis and clinical significance. CA Cancer J Clin 1985;35:322-44.
3. Mayer. Gastrointestinal tract cancer. In: Harrison's Principles of Internal Medicine. Vol 1. 17 ed. New York: McGraw-Hill Inc 2008.p.573-8.
4. Morson B. The polyp-cancer sequence in the large bowel. Proc R Soc Med 1974;67:451-7.
5. Vatn MH, Stalsberg H. The prevalence of polyps of the large intestine in Oslo: An autopsy study. Cancer 1982;49:819-25.
6. Ekelund G, Lindström C. Histopathological analysis of benign polyps in patients with carcinoma of the colon and rectum. Gut 1974;15:654-63.
7. Berg JW, Downing A, Lukes RJ. Prevalence of undiagnosed cancer of the large bowel found at autopsy in different races. Cancer 1970;25:1076-80.
8. Bremner CG, Ackerman LV. Polyps and carcinoma of the large bowel in the South African Bantu. Cancer 1970;26:991-9.
9. Eichhorn RD, Bentlif PS, Kelsey JR. Ethnic differences in occurrence of rectal polyposis in over 6,000 proctoscopies. Am J Dig Dis 1971;16:433-5.
10. Ferlay J, Autier P, Boniol M, Heanue M, Colombet M, Boyle P. Estimates of the cancer incidence and mortality in Europe in 2006. Ann Oncol 2007;18:581-92.
11. Tanaka T. Colorectal carcinogenesis: review of human and experimental animal studies. J Carcinog 2009;8:5.
12. Yasui Y, Kim M, Oyama T, Tanaka T. Colorectal carcinogenesis and suppression of tumor development by inhibition of enzymes and molecular targets. Curr Enzyme Inhibitions 2009;5:1-26.
13. Sudoyo AW. Karakteristik molekular kanker kolorektal pada usia muda: ekspresi MLH1 dan MSH2 sebagai indikator instabilitas mikrosatelit. Disertasi. Fakultas Kedokteran Universitas Indonesia Jakarta 2004.
14. Abdullah M, Sudoyo AW, Pranowo BS, Rini D, Sutrisna B, Rani AA. Expression of NF-kB and COX-2 in young versus older patients with sporadic colorectal cancer. Acta Med Indones 2009;41:70-4.
15. Lu H, Ouyang W, Huang C. Inflammation, a key event in cancer development. Mol Cancer Res 2006;4:221-33.
16. Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol 2005;5:749-59.
17. Wang S, Liu Z, Wang L, Zhang X. NF-kappaB signaling pathway, inflammation and colorectal cancer. Cell Mol Immunol 2009;6:327-34.
18. Karin M, Cao Y, Greten FR, Li ZW. NF-êB in cancer: from innocent bystander to major culprit. Nat Rev Cancer 2002;2:301-10.
19. Itzkowitz SH, Yio X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. Am J Physiol Gastrointest Liver Physiol 2004;287:G7-17.
20. Baldwin AS. Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappaB. J Clin Invest 2001;107:241-6.
21. Karin M. Nuclear factor-kappaB in cancer development and progression. Nature 2006;441:431-6.
22. Dolcet X, Llobet D, Pallares J, Matias-Guiu X. NF-κB in development and progression of human cancer. Virchows Arch 2005;446:475-82.
23. Bours V, Dejardin E, Goujon-Letawe F, Merville MP, Castronovo V. The NF-κB transcription factor and cancer: high expression of NF-κB- and I κB-related proteins in tumor cell lines. Biochem Pharmacol 1994;47:145-9.
24. Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 1999;18:6853-66.
25. Pacifico F, Leonardi A. NF-κB in solid tumors. Biochem Pharmacol 2006;72:1142-52.
26. Van Hattem WA, Brosens LA, Marks SY, Milne AN, van Eeden S, Iacobuzio-Donahue CA, et al. Increased cyclooxygenase-2 expression in juvenile polyposis syndrome. Clin Gastroenterol Hepatol 2009;7:93-7.
27. Maihofner C, Charalambous MP, Bhambra U, Lightfoot T, Geisslinger G, Gooderham NJ, et al. Expression of cyclooxygenase-2 parallels expression of interleukin-1beta, interleukin-6 and NF-kappaB in human colorectal cancer. Carcinogenesis 2003;24:665-71.
28. Karnes WE, Shattuck-Brandt R, Burgart LJ, DuBois RN, Tester DJ, Cunningham JM, et al. Reduced COX-2 protein in colorectal cancer with defective mismatch repair. Cancer Res 1998;58:5473-7.
29. Mazhar D, Gillmore R, Waxman J. COX and cancer. QJM 2005;98:711-8.

30. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. Ann Rev Biochem 2000;69:145-82.
31. Subbaramaiah K, Telang N, Ramonetti JT, Araki R, DeVito B, Weksler BB, et al. Transcription of cyclooxygenase-2 is enhanced in transformed mammary epithelial cells. Cancer Res 1996;56:4424-9.
32. Yamamoto Y, Gaynor RB. Therapeutic potential of inhibition of the NF-kappaB pathway in the treatment of inflammation and cancer. J Clin Invest 2001;107:135-42.
33. McLean MH, Murray GI, Fyfe N, Hold GL, Mowat NAG, El-Omar EM. COX-2 expression in sporadic colorectal adenomatous polyps is linked to adenoma characteristics. Histopathology 2008;52:806-15.
34. Charalambous MP, Maihofner C, Bhambra U, Lightfoot T, Gooderham NJ. Upregulation of cyclooxygenase-2 is accompanied by increased expression of nuclear factor-kappa B and I kappa B kinase-alpha in human colorectal cancer epithelial cells. Br J Cancer 2003;88:1598-604.
35. Brazowski E, Rozen P, Misonzhnick-Bedny F, Gitstein G. Characteristics of familial juvenile polyps expressing cyclooxygenase-2. Am J Gastroenterol 2005;100:130-8.