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Expression of APC, β-catenin and E-cadherin genes in pathological types of colorectal polyps: A comparative study

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Abstract

Background: The onset of colorectal cancer requires the transformation of normal epithelial cells into neoplastic cells and eventually the growth of polyps and the progression to malignancy. The purpose of this study was to compare the expression of E-cadherin, β-catenin, and APC genes at the mRNA level and their relationship with the pathological features of colorectal polyps.

methods: The present descriptive-analytical study was conducted on 40 biopsy specimens collected from patients with colorectal polyps and 10 healthy tissue samples from the Department of Gastroenterology at Ayatollah Taleghani Educational Hospital in Tehran (Iran), subsequently recording their demographic and clinical characteristics. After RNA extraction and cDNA synthesis, the expression of E-cadherin, β-catenin, and APC genes was evaluated by real-time PCR.

Results: According to the present findings, the APC gene expression level indicated a significant decrease in the types of adenomatous polyps, but no change was shown in hyperplastic polyps. In addition, the β-catenin gene expression level did not change in adenomatous and hyperplastic polyps. The E-cadherin gene expression level was decreased in the group of villous polyps.

Conclusion: Studies have shown that a decrease in the expression level of the APC gene is one of the primary events in the formation of colorectal polyps, as well as a decrease in the expression level of the E-cadherin gene is considered a late event of polyp malignancy. These genes can be applied as biomarkers for early detection of colorectal cancer.

Keywords: colorectal cancer, Real-time PCR, E-cadherin, β-catenin, APC

Background

A colorectal polyp is a fleshy growth of clumps on the inner lining of the colon or rectum. Failure to treat some of these polyps can lead to colorectal cancer. In colorectal cancer, the polyps are small and benign at first. Gradually, the polyps grow and progress into malignant tumors, resulting in cancer. Sometimes it can take up to 10 years from start to complete progress. This period would be a suitable opportunity for screening, prevention, and early diagnosis of the disease (1, 2).

In a classification, polyps are pathologically divided into two categories, non-neoplastic colon polyps, and neoplastic colon polyps. The neoplastic type has the potential to become cancerous, while the non-neoplastic type cannot. Non-neoplastic colon polyps in turn include hyperplastic polyps, inflammatory pseudopolyps or (pseudopolyps), and hamartomatous polyps or juvenile polyps. Neoplastic polyps are divided into three categories: adenomatous polyps, carcinomatous or malignant polyps, and serrated polyps. Adenomatous polyps are progressively invasive and metastatic to carcinogenesis and are divided into three types based on their appearance: tubular, villous, and tubulovillous adenomas. The most common types of polyps are adenomatous polyps (adenomas) or tubular adenomas, accounting for 70% of colonic polyps (3, 4).

Various genetic changes can contribute to colorectal cancer, some of which involve mutations in the TGF-β receptor gene and genes including SMAD2 and SMAD4 as part of the TGF-β pathway (5).

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The DCC gene (deleted in colorectal cancer) has also been mutated in studies on colorectal cancer (6). Mutations in the RAS and TP53 genes, as well as LOH at 5q and 18q, appear to accumulate during the progression from colorectal adenoma to carcinoma (7). The accumulation of changes is more important than the order in which they occur in the progression of carcinoma. More than one of these four changes is seen in only 7% of small primary adenomas. As the adenoma progresses in size and the histological features of the malignancy develop, two or more changes occur with increased frequency. More than 90% of carcinomas show two or more changes and there are three of these changes in almost 40% of cases (8). In addition, the same mutation in some inherited cancer syndromes can cause cancer in different parts of the body in different people, possibly due to the interaction of inherited polymorphic changes in several genes or by a variety of environmental factors (9).

The Wnt family of molecules are secretory glycoproteins with several conserved cysteine-rich domains that are implicated in various processes such as embryonic induction, cell polarization, and cell fate determination (10). Receiving and sending Wnt signals as a result of Wnt protein binding to intramembrane receptors of Frizzled receptor (Fzd) family is performed by exploiting members of the LDL receptor-related protein (LRP) family. In the Wnt/β-catenin pathway, binding of Wnt to fzd causes hyperphosphorylation of a cytoplasmic protein called Dishevelled (Dvl/Dsh) and leads to inhibition of the cytoplasmic activity of glycogen synthase kinase-3 beta (GSK-3β), a member of serine/threonine family of protein kinases through phosphorylation in serine residue at position 9. This eventually leads to the release and stabilization of β-catenin and, consequently, its increase in the cell cytoplasm. β-catenin enters the nucleus and forms a heterodimer with Tcf/Lef, a family of transcription factors, and regulates the expression of target genes including cyclin-D1 and c-myc. In the absence of Wnt molecules, GSK3-β binds to the β-catenin and APC complexes through a molecule called Axin, leading to its rapid degradation by β-catenin phosphorylation through the ubiquitination process (11, 12). Given that activation of the Wnt pathway can lead to the progression of primary polyps to malignancy, examining the expression pattern of key genes related to the Wnt pathway such as β-catenin, E-cadherin, and APC in pathological types of colonic polyps and comparing this expression pattern in different types of polyps, in addition to increasing our knowledge of the molecular changes in the progression of polyps, can also be useful in determining a molecular marker for prognosis.

2. Materials and Methods

2.1 Study participants

The presentation was conducted on 40 colorectal polyp tissue samples collected from patients referred to Ayatollah Taleghani Hospital in Tehran (Iran), whose colonoscopic results were confirmed by a pathologist. Additionally, 10 samples of healthy intestinal tissue were used as controls. Polyp type classifications were done according to polyp histology; hyperplasic (HP), tubular adenoma (TA), and tubulovillous polyp (TVP), where dysplasia grades and polyp sizes were characterized by a pathologist. In this study, all patients signed written informed consent after explaining the methodology and objectives of the research and about participating voluntarily in the project. Demographic, clinical, and history information for patients were obtained and recorded in the relevant information forms. The study was approved by the Clinical Research Ethics Committee of Shahid Beheshti University of Medical Sciences and the Ethics Committee of Taleghani Hospital, Tehran, Iran with No.2014/770.

2.2 RNA extraction and cDNA synthesis

RNA samples were extracted using an RNA extraction Kit (Yekta Tajhiz Azma Co., Tehran, Iran) based on the manufacturer's protocol. For the purity assessment of RNA, the ratio of absorbance at 260 and 280 nm (A260/280) was determined using NanoDrop® ND-1000 (BioTek, USA). Total RNA integrity was assessed by gel electrophoresis on 1.0% agarose gel. RNA samples were reverse transcribed using the PrimeScript™ RT reagent Kit (Perfect Real Time) (Takara), as per the manufacturer's protocol. cDNA synthesis was performed using the rimeScript RT Enzyme, Oligo (dT) primers, and random 6 mers in a final volume of 10 µL according to the appropriate thermal profile (42 °C for 15 min and 85 °C for 30 s). All cDNA samples were stored at -20 °C. 0.5 µL of template cDNA (corresponding to 10 ng of RNA) was assessed in Real-time quantitative PCR (RT-qPCR).

2.3 Real-time PCR method

To amplify CTNNB1 (β-catenin), CDH1 (E-cadherin), and APC genes, Real-time PCR primers were designed using Primer Express version 3.0 (Applied Biosystems, USA). Finally, the designed primers were blasted by NCBI and Gene Runner software to analyze accuracy and specificity (Table 1).

Table 1. Sequence of primers designed for real-time PCR process

Gene	Primer Sequence	Product length (bp)
CTNNB1	F(GTGCTATCTGTCTGCTCTAGTA) R(CTTCCTGTTTAGTTGCAGCATC)	154
CDH1	F(TACACTGCCCAGGAGCCAGA) R(TGGCACCAGTGTCCGGATTA)	103
APC	F(CCTCATCCAGCTTTTACATGGC) R(GCCCGAGCCTCTTTACTGC)	78
β2m	TGCTGTCTCCATGTTTGATGTATCT TCTCTGCTCCCCACCTCTAAG	86

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Real-time PCR was performed using an ABI 7500 system (Applied Biosystem, USA).
Amplification reactions were performed using SYBR green master mix obtained from Kapa Biosystems. PCR protocol was as follows: 95 °C for 5 min, followed by 40 cycles of 95 °C for 60 s, 60 °C for 45 s. No Template Control (NTC) reactions were included in each experiment to control the DNA contamination in the reagents. Each amplification was carried out in duplicates in a final reaction volume of 20 µL using 10 pmol of each primer, 7.5 µL of PCR Master Mix, 3 µL of cDNA (corresponding to the cDNA reverse transcribed from approximately 10 ng RNA), and 3.5 µL of RNase/DNase-free distilled water. and β2m was used as the reference gene to normalize target gene expression. The relative gene expression values were calculated using the comparative cycle threshold (Ct) method.

2.4 Statistical analysis

Statistical analyses were performed using GraphPad Prism software version 5 (San Diego, CA, USA). All data were reported as mean ± standard deviation. The data collected were analyzed using one-way ANOVA or t-test as appropriate. P-value < 0.05 was considered statistically significant.

3. Results

Among 40 polyps, 11 (27.5%) were identified as hyperplastic, 13 (32.5 %) as tubular,10 (25%) as tubulovillous, and 6 (15%) as villous. About 10% of these 40 had gastrointestinal symptoms as well as blood in the stool and abdominal pain, 25% were smokers and 10% were alcoholics. They were also screened for IBD; none of them had the disease. Clinicopathological parameters of polyps are presented in Table2.

Table 2. Clinicopathological Parameters of Patients

Parameter	Type	Number
Polyp types	Hyperplastic	11 (27.5%)
	Tubular	13 (32.5%)
	Tubullovillous	10 (25%)
	Villous	6 (15%)
Dysplasia	HGD	38 (95%)
	LGD	2 (5%)
site	Rectum	6 (15%)
	AC	7 (17.5)
	AD	13 (32.5%)
	TC	14 (35%)
size	< 5mm	38 (95%)
	> 5mm	2 (5%)

Based on these results, there was a significant relationship between the lower expression level of APC in the polyp cases compared with normal samples (P-value< 0.05). On the other hand, β-catenin gene expression was significantly up-regulated in the patients' group (P-value< 0.05) (Figure 1).

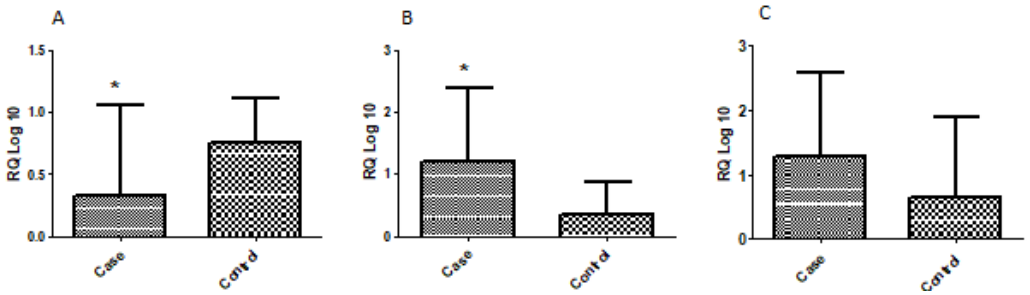


Figure 1. Rates of expression changes in APC (A), β-catenin (B) and E-cadherin (C) genes between polyps compared to normal tissue.

Also, Figure 1 shows the expression level of the studied genes in different types of polyps. The APC mRNA level (Figure 2-A) was significantly decreased in adenoma polyps (tubular, villous, and tubulovillous) compared to Hyperplastic samples (P-value< 0.001). Although the level of β-catenin expression was lower in all types of adenoma polyps compared with Hyperplastic that was not significant. (Figure 2-B), while there were significant differences in E-cadherin expression between adenoma polyp types as well as in Hyperplastic tissue (P-value< 0.01) (Figure 2C).

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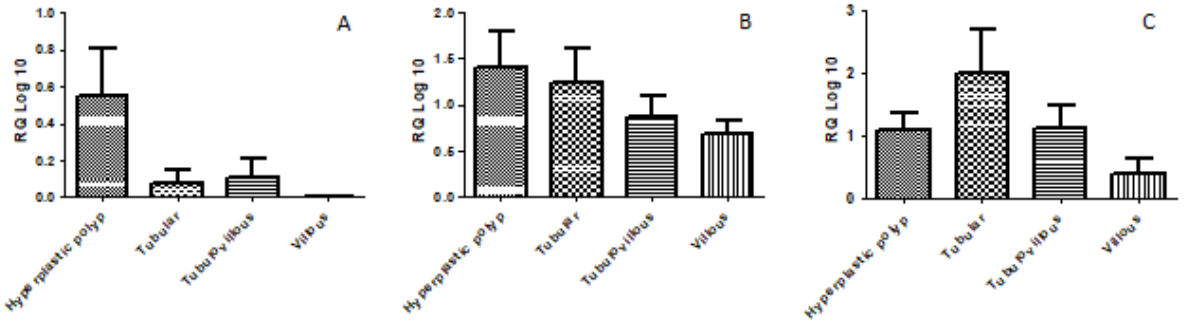


Figure 2. The gene expression of APC (A), β-catenin (B) and E-cadherin (C) between different types of polyps

Consequently, the gene expression was evaluated in both high-grade dysplasia (HGD) and low-grade dysplasia (LGD) in contrast to the control group, where no significant difference levels were achieved.

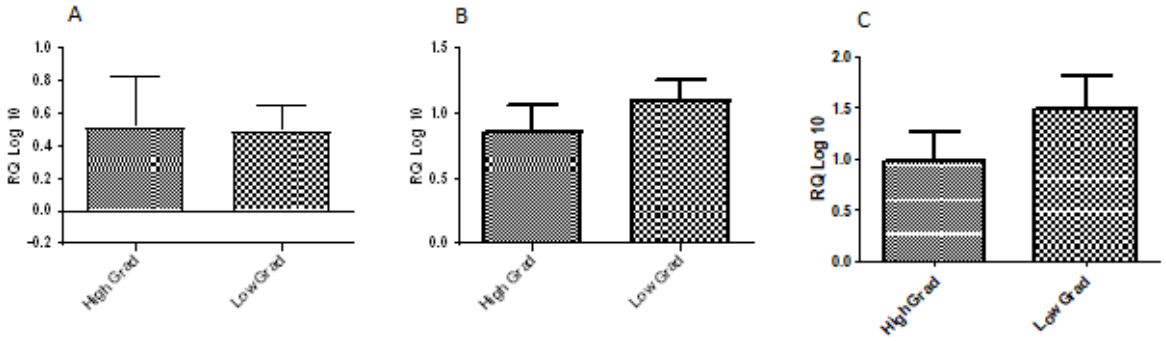


Figure 3. The mRNA level of APC (A), β-catenin (B) and E-cadherin (C) in HGD and low-grade dysplasia (LGD) of studied polyps

4. Discussion

The main reason for the occurrence and spread of colorectal cancer is the presence of polyps in the colon or rectum and the progression of adenomatous to carcinoma (13). Colorectal polyps occur as a result of the overgrowth of intestinal epithelial cells and the accumulation of these cells (2). Early diagnosis of colorectal cancer, prognosis of patient survival rate and polyp examination for malignancy are of great importance. Finding markers capable of detecting malignancy in colon polyps earlier than cell changes is a research prerequisite in the field of cancer (14). Accordingly, the present study investigates the expression level of molecules involved in the Wnt signaling pathway as an important pathway in regulating cell fate, cell polarity, differentiation, and apoptosis and today as a promoter of colon carcinogenesis. Key genes in this pathway, including β-catenin and APC, have been shown to play a very important role in colon carcinogenesis. The E-cadherin gene, which is linked to α-catenin and β-catenin as a cell adhesion molecule, may also be involved in the metastatic stage of colon cancer (15). In this study, the polyps collected from patients who were referred to Ayatollah Taleghani Hospital for screening and underwent colonoscopy were evaluated at the RNA level. According to the available results, decreased level of APC gene expression was observed in tubular, villous, and tubulovillous adenomas. Meanwhile, villous polyps showed a more severe decrease in gene expression levels. On the other hand, no difference in the level of gene expression was observed in hyperplastic polyps. Therefore, since the expression of this gene has decreased in tubular, villous, and tubulovillous adenomas, a defect in this gene may have occurred from the beginning of the process of change in colon tissue and therefore it may be used as an early diagnostic marker.

Various studies have been conducted on the pathological types of polyps and their causative pathways. For example, Nguyen et al. examined the distinctive expression pattern of Wnt pathway genes in tubular adenomas and villous adenomas and concluded that although mutations in the APC gene are a priority for cancer, villous adenomas have a higher potential for cancer and malignancy than tubular adenomas. They suggested that Wnt pathway activation is distinct in two types of adenomas (16). Silva et al. worked on the polyps of 91 patients with colorectal cancer and examined the expression of APC and β-catenin proteins in adenocarcinoma and their polyps using immunohistochemistry. This study confirmed the association of changes in the expression levels of APC and β-catenin proteins between colon adenocarcinoma and polyps (17). In experiments with immunohistochemistry on APC protein expression, Bourroul et al. observed a significant decrease in protein expression in adenomas and adenocarcinomas and reported this gene involvement in the mechanism of action of carcinogenesis (18). According to Roper et al., the inactivation of APC and inhibition of β-catenin degradation induces the accumulation of β-catenin as well as the transcription of target genes including cyclin-D and c-myc. Late adenoma occurs when k-RAS is activated and the 18q portion of the chromosome is lost. Finally, carcinoma

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develops due to P53 mutations as the cell guard (19).

According to the results of the present study, β-catenin gene expression did not change in tubular, villous, and tubulovillous adenomas. This gene may be involved in the process of deformity and malignancy of colon tissue toward late-stage carcinoma. Although Scholer-Dahirel et al. revealed that nuclear levels of β-catenin in human adenocarcinoma are associated with progressive tumors (20). This difference in results indicates that the control of β-catenin activity is more at the level of degradation than gene expression.

Based on the data of the present study, there was a decrease in the expression level of the E-cadherin gene in villous polyps. On the other hand, no change in the expression level of this gene was observed in tubulovillous and hyperplastic polyps. The tubular polyps also experienced increased gene expression. Given that the results of the present study did not report a change in the expression level of the E-cadherin gene in hyperplastic and tubulovillous polyps, it can be concluded that this gene is ineffective in the early stages of change in colon tissue, and often play a role in the late-stage carcinogenesis, metastasis and spread of cancer. Ogun et al. measured the expression levels of E-cadherin, β-catenin, EGFR, and CYLIN D1 genes and reported that the rapid progression of primary metastasis and the risk of local recurrence of the disease after radiotherapy and chemotherapy were related to the expression level of these genes (21).

Our results also showed that the expression level of the APC gene could be different in various colon polyps and different grades of polyps, while β-catenin and E-cadherin genes did not show much difference in expression level. Decreased levels of APC gene expression in adenomas polyps in the study suggest that a decrease in the expression level of this gene is one of the earliest events of polyp formation in susceptible individuals. Studying the reasons for this decrease in expression levels may offer valuable insights into molecular methods of prevention. Measuring the level of this marker along with pathology information can help to distinguish cancerous polyps.

5. Conclusion

In general, we concluded that different expression levels of APC, β-catenin and E-cadherin genes in polyps may play a significant role in the progression of polyps to CRC and malignancy.

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