



CLINICOPATHOLOGICAL CORRELATIONS OF VEGF-A AND MMP-7 GENES EXPRESSION IN DIFFERENT TYPES OF COLORECTAL ADENOMA POLYPS

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Abstract – Background: Colorectal cancer (CRC) is mostly derived from adenoma polyps. Investigation of some genes which play a critical role in angiogenesis and malignancy conversion can evaluate the risk of progression of adenoma polyps to CRC. This study aims to investigate VEGF-A and MMP-7 genes expression in different types of colorectal adenoma polyps.

Patients and Methods: In this study, 50 biopsy samples of adenoma polyp and 20 paired tissue samples were collected. VEGF-A and MMP-7 genes expression were investigated by Real-time PCR method and relative quantification. Fold change of genes expression was evaluated by ($2^{-\Delta\Delta ct}$) method.

Results: Overexpression of VEGF-A mRNA expression was found in villous type and high-grade dysplasia adenomas compared to the control group. Also, overexpression of VEGF-A and mRNA was found in polyps located in the left colon in comparison to the right colon and the male group vs. female. However, these results were not statistically significant ($p > 0.05$). MMP-7 mRNA expression was significantly higher in villous adenoma type and the grade of dysplasia compared to the control group ($p < 0.05$). However, overexpression of MMP-7 mRNA in polyps located in the left colon in comparison to polyps located in the right colon and the female group vs. the male group, was not significant ($p > 0.05$).

Conclusions: Evaluation of VEGF-A and MMP-7 genes expression can be used as a prognostic biomarker for colorectal adenoma polyps progression to malignancy. Moreover, they can be used as therapeutic targets in colorectal adenoma polyps for CRC prevention.

KEYWORDS: Adenoma polyp, Colorectal cancer, Gene expression, MMP-7, VEGF-A.

INTRODUCTION

Colorectal cancer (CRC) is the third prevalent cancer in men and women^{1,2} and the highest incidence rates were found in North America, Australia and Western Europe. The lowest CRC incidence was recorded in developing countries, although in recent years raising CRC rates have been observed³. Four abnormalities have been found in CRC including the chromosomal instability (CIN), the microsatellite

instability, the CpG island methylator phenotype (CIMP) and the TGF-beta signaling activation^{4,5}. CRC mostly derive from polyps which are resulted by genetic mutations and abnormal cell proliferation in colorectal tissue⁶⁻⁸. Colorectal polyps are divided into adenoma polyps, hyperplastic polyps, hamartomatous polyps and inflammatory polyps. Adenoma polyps are sub-classified by their histology appearance as tubular, villous and tubulovillous adenomas⁹.



Angiogenesis, the shaping of new blood vessels from preexisting vessels, plays an essential role in tumorigenesis and cancer development¹⁰⁻¹³. Vascular endothelial growth factor-A (VEGF-A), a sub-family of growth factors and VEGFRs (VEGF receptors) are the most important regulatory for angiogenesis¹³.

On the other hand MMPs (matrix metalloproteinases), especially MMP-7, which is able to degenerate extra cellular matrix and collagen, makes a suitable condition for the creation of new blood vessels, tumor growth, metastases and malignancy¹⁴.

Previous researchers show that tumor cells present a major source of VEGF and MMP-7^{14,15} but the role of these factors in different types of colorectal adenoma polyps has not been clarified yet. The aim of this study is the evaluation of VEGF-A and MMP-7 genes expression in different types of colorectal adenoma polyps including tubular, villous and tubulovillous.

PATIENTS AND METHODS

TISSUE SAMPLES

This descriptive analytical study analyzed 50 patients with adenoma polyps who were studied from 2015 and 2016. 20 paired tissue samples were selected as the control group. Samples were collected from patients who were enrolled to the Colorectal Cancer Screening Program and were undergone to colonoscopy by gastroenterologist. Pathologic features were confirmed by the pathologist in Taleghani Hospital of Teheran, Iran. The clinical information of patients were collected by a questionnaire. This study was conducted under the approval of the Ethics Committee (No. 2014/770) of the Gastroenterology and Liver Disease Research Center, Shahid Beheshti University of Medical Sciences (Teheran, Iran).

REVERSE TRANSCRIPTASE PCR (RT-PCR)

Total RNA was extracted from the samples (Yecta Tajhiz Azma kit, Cat No. YT9065, Teheran, Iran) and RNA concentration was quantified by Nanodrop. RNAs were converted to cDNA by Reverse transcriptase (RT) reaction (TaKaRa kit, Cat No. RR037A, Otsu, Shiga, Japan) according to the following: 2 µg of total RNA were picked up and denatured at 95°C for 5 min. After that, tubes were placed on ice and 5 µL of 5×primer script buffer, 0.5 µL RT enzyme, 1.24 µM oligo dt primer, 10 µM random 6 mer, 1 µM ribolock, 1 µL easy dilution, and 5 µL RNA free distilled water (dH₂O), were added. The cDNA synthesis was performed as follows: 25°C for 5 min, 42°C for 15 min, 85°C for 1 min for inactivation of the reverse transcriptase enzyme and 4°C for 10 min for hold temperature; then, cDNA products were kept at -20°C until use.

TABLE 1. VEGF-A, MMP-7 and GAPDH primers sequences.

Primer	Sequence
VEGF-A-F	5'-CCACTTCGTGATGATTCTGCC-3'
VEGF-A-R	5'-TACCTCCACCATGCCAAGT-3'
MMP-7-F	5'-GCATGAGTGAGCTACAGTGGGAAC-3'
MMP-7-R	5'-CCACATCTGGGGGTTGTGCATTA-3'
GAPDH-F	5'-TGACTTCAACAGCGACACCCA-3'
GAPDH-R	5'-CACCTGTTGCTGTAGCCAAA-3'

REAL TIME-PCR

Following cDNA synthesis, to evaluate VEGF-A and MMP-7 genes expression level, Real-time PCR and relative quantification method were performed with the SYBER Premix Ex TaqII (TaKaRa kit, Cat No RR820A, Otsu, Shiga, Japan) by using Applied Biosystems 7500 version 1 (ABI, Foster City, CA, USA). Real-time PCR was carried out by expressive primers (Table 1) under the following conditions: 95°C for 5 s, 40 cycles of 95°C for 5 s, 60°C for 34 s, 95°C for 15 s, 60°C for 1 s and 60°C for 15 s. Amplification signals for samples were normalized by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene. Fold change of genes expression was evaluated by ($2^{-\Delta\Delta Ct}$) method.

STATISTICAL ANALYSIS

Data were analyzed by Graphpad Prism version 5 software (San Diego, CA, USA). The data were non-normally distributed and the non-parametric test was used. Student *t*-test and one-way ANOVA test were performed. *p*-value < 0.05 was considered statistically significant difference.

RESULTS

Among 50 adenoma polyp samples, 14 (28%) villous adenoma polyps, 19 (38%) tubular adenoma polyps, and 17 (34%) tubulovillous adenoma polyps, were detected (Table 2).

TABLE 2. Clinicopathological parameters of patients with adenoma polyps.

Number	Type	Parameter
Types	Villous	14 (28%)
	Tubular	19 (38%)
	Tubulovillous	17 (34%)
Dysplasia	HGD	14 (28%)
	LGD	36 (72%)
Gender	Male	27 (54%)
	Female	23 (46%)
Site	Right colon	34 (68%)
	Left colon	16 (32%)
Total	50 (100%)	50

HGD indicates high-grade dysplasia and LGD refers to low-grade dysplasia.

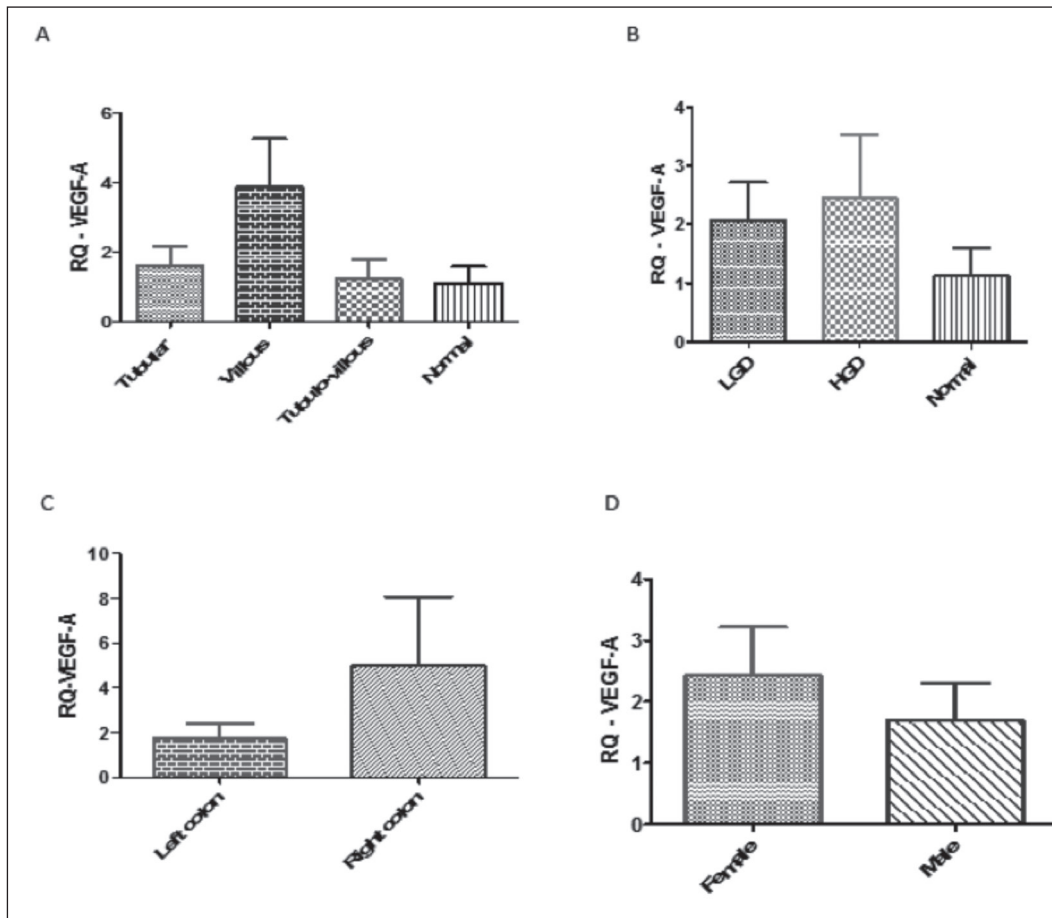


Fig. 1. Overexpression of VEGF-A mRNA in villous type (A) and high grade dysplasia (HGD) adenomas (B) compared with the control group. Upregulation of VEGF-A mRNA in polyps located in the left colon in comparison to the right colon (C) and the male group vs. female group ($p>0.05$) (D).

In this study, we observed the up-regulation of VEGF-A mRNA in villous adenoma type and high grade dysplasia (HGD) adenomas compared with the control group (Figure 1A and Figure 1B). Also, the expression of VEGF-A mRNA was increased in polyps located in the left colon in comparison to the right colon and the expression of VEGF-A mRNA in the male group was higher than the female group (Figure 1C and Figure 1D). No significant difference between clinicopathological features and VEGF-A mRNA expression level was found in adenoma polyps ($p>0.05$).

MMP-7 mRNA expression was significantly higher in villous adenoma type and the grade of dysplasia ($p<0.05$) compared with the control group (Figure 2A-B).

Comparison of MMP-7 mRNA expression level in different sites of adenoma polyps revealed overexpression of MMP-7 mRNA in polyps located in the left colon compared with polyps located in the right colon (Figure 2C). However, this difference was not significant ($p>0.05$). Expression of MMP-7 mRNA in the female group was higher than the

male group; however, no significant difference was observed ($p>0.05$) (Figure 2D).

DISCUSSION

Our results showed that VEGF-A and MMP-7 genes expression were higher in adenoma polyps compared with the control group. This may indicate the importance of these genes for polyp malignancy progressing. Previous studies suggested that VEGF-A and MMP-7 genes might have been activated in adenoma stage and contribute to the progression of CRC¹⁶⁻²¹. According to these results, overexpression of MMP-7 gene may have a positive influence on VEGF-A expression and helps the angiogenesis reinforcement in adenoma polyps.

Villous type has more malignancy potential compared with other polyp types^{8,9}. It seems that the increased expression of VEGF-A mRNA in villous type compared with tubular, tubulovillous types, and also the control group, can lead villous type to malignancy. Qasim et al¹⁷ and Kirimlioglu

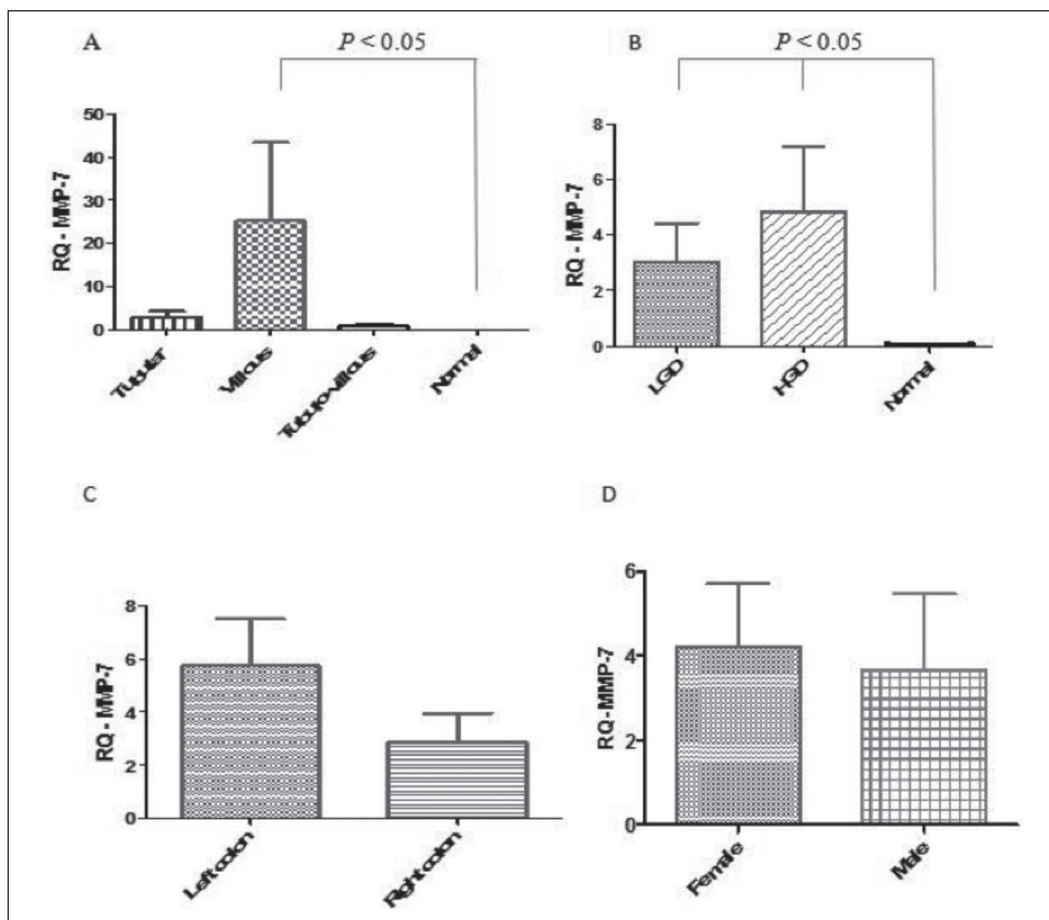


Fig. 2. Significant up-regulation of MMP-7 mRNA in villous type (A) and grade of dysplasia (HGD indicates high-grade dysplasia and LGD refers to low-grade dysplasia) (B) compared with the control group ($p < 0.05$). Over-expression of MMP-7 mRNA in polyps located in the left colon in comparison to polyps located in the right colon (C) and the female group compared with the male group ($p > 0.05$) (D).

et al²² showed that MMP-7 gene overexpression was significantly higher in villous type compared with the tubular and tubulovillous types and these data agree with our findings. In accordance with these data, increased expression of VEGF-A and MMP-7 genes together may have affected angiogenesis and speed up the malignancy transition in villous adenoma polyps.

In this study, we observed overexpression of VEGF-A mRNA in the right sided polyps compared with the left sided, as well as up-regulation of MMP-7 mRNA in the left sided adenomas, was more than the right adenomas. Ko et al²³ found that gene-regulation may have been controlled by the molecular mediator which depends on tissue-specific or site specific.

Several studies showed that VEGF-A and MMP-7 genes expression are significantly correlated with dysplasia grade of adenoma polyps^{22,24-27}. In the present study we observed over-expression of VEGF-A mRNA in HGD and low grade dysplasia (LGD) adenomas compared with the control group, but we did not find sig-

nificant difference. MMP-7 mRNA expression was significantly increased in HGD adenoma polyps compared with the control group. Accordance to results from our study and previous investigations, increased expression of VEGF-A and MMP-7 genes may have an effective role in creating dysplasia in adenoma polyps; they also can push LGD adenomas to HGD adenomas. In this study, we showed overexpression of VEGF-A mRNA and MMP-7 mRNA in the female group compared with the male group. Based on these results we assume that VEGF-A and MMP-7 genes expression in colorectal adenomas may have been influenced by sex hormone²⁸.

CONCLUSIONS

Overexpression of VEGF-A and MMP-7 genes in colorectal adenoma polyps may be essential molecular processes for induction of angiogenesis in colorectal adenoma polyps and lead them to CRC. Our results showed that VEGF-A and MMP-7 genes

may be correlated together since MMP-7 gene can digest extracellular matrix in adenoma polyp cells and provides suitable conditions for blood vessels cells placement; then, VEGF-A gene induces angiogenesis. Finally, evaluation of VEGF-A and MMP-7 genes expression can be used as a prognostic biomarker for colorectal adenoma polyps progression to malignancy. Moreover, VEGF-A and MMP-7 can be used as therapeutic targets in colorectal adenoma polyps for CRC prevention.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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