HIF-1 ALPHA GENE EXPRESSION IS NOT A SUITABLE BIOMARKER FOR EVALUATING MALIGNANCY RISK IN COLORECTAL POLyps

S. Khatibi1, E. Nazemalhosseini Mojarad2, F. Forouzesh1, Z. Pezeshkian3, H. Asadzadeh Aghdaei2, M. Reza Zali2

1Department of Genetics, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran
2Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract – Objective: Most cases of colorectal cancer originate from adenoma polyps. The identification of the expression pattern of cancer related genes such as Hypoxia-inducible factor – 1 (HIF-1α), can be useful in detection of the malignancy in polyps. The aim of this study is the evaluation of HIF-1alpha mRNA expression and risk of malignancy transitions in colorectal polyps.

Patients and Methods: In this study, 54 samples of fresh polyps and 20 samples of normal mucosa were collected as the control group from patients. The demographic and clinical data of the patients were collected. The RNA extraction and cDNA synthesis were performed. HIF-1alpha gene expression was investigated by Real-time PCR method and relative quantification. Fold change of gene expression was evaluated by \(2^{-\Delta\Delta ct}\) method. The statistical analysis was performed by the Prism software (version 5).

Results: There was no significant difference in the expression of HIF-1α mRNA between adenoma and hyperplastic polyps compared to the control group (\(p>0.05\)), and also we did not found significant difference in the expression of HIF-1α mRNA between polyps group (\(p>0.05\)). No significant correlation between clinicopathological features and HIF-1 alpha expression was observed in colorectal polyps (\(p>0.05\)).

Conclusions: While HIF-1alpha gene has a critical role in angiogenesis and tumor malignancy, but in this study we did not found significant expression of HIF-1alpha mRNA in colorectal polyps. Finally, we conclude that, HIF-1alpha gene is not a suitable prognostic biomarker for indicating malignancy progression to CRC.

KEYWORDS: Colorectal cancer, Polyp, HIF-1 alpha.

INTRODUCTION

Colorectal cancer (CRC) is the third leading cause of death in the world4 and the highest incidence rates were observed in developed countries5. There are several abnormalities in CRC including the methylator phenotype (CIMP), the microsatellite instability, the CpG island and the chromosomal instability (CIN)3,4.

Previous investigations5,6 have shown that colorectal tumors may originate from transformed polyps within several years. Histologically, colorectal polyps are found in different shapes including adenoma, hyperplastic, hamartomatous and inflammatory polyps5. Neoplastic or adenoma polyps are more important because they are suspected of being malignant and cancerous7; however, recent investigations re-
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(dH2O), were added. The cDNA synthesis was performed as follows: 25°C for 5 minutes, 42°C for 15 minutes, 85°C for 1 minute for inactivation of the reverse transcriptase enzyme and 4°C for 10 minutes for hold temperature, then cDNA products were kept at -20°C until use.

**Real-time PCR**

Following cDNA synthesis, to assess HIF-1alpha gene expression level Real time PCR and relative quantification method were performed with the SYBR Premix Ex TaqII (TaKaRa kit, Cat. No. RR820A, Otsu, Shiga, Japan) using Applied Biosystems (ABI) 7500 version 1.3 (Foster City, CA, USA) and 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 gene expression was evaluated by \( (2^{-\Delta\Delta CT}) \) method.

**Statistical Analysis**

The data were analyzed by Graphpad Prism version5 software (La Jolla, CA, USA). The data were non-normally distributed and 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 54 adenoma polyp samples, 26 (48%) adenoma polyps, 28 (52%) hyperplastic polyps were detected. Clinicopathological features of CRC patients have been showed in Table 2. In this study, we observed that, there was no significant difference in HIF1-alpha mRNA expression in polyps compared with the control group (\( p>0.05 \)) (Figure 1). Comparison of HIF1-alpha mRNA expression level in adenoma and hyperplastic polyps did not show significant difference (\( p>0.05 \)) (Figure 2) as well as revealed that hyperplastic polyps have the malignancy potential in some cases.

Various factors, including Hypoxia-inducible factor 1 (HIF-1), participate in controlling of hypoxia during pathologic conditions like cancer. HIF-1 consists of two subunits called HIF-1alpha and HIF-1beta, which are expressed continuously in the cells. HIF-1 alpha regulates the expression process of many genes involved in cell adaptation to the condition of the hypoxia. Previous investigations showed that HIF-1alpha regulates the expression of genes involved in growth, malignancy and metastasis and angiogenesis like VEGF, in tumor cells. Several studies showed that up-regulation of HIF-1alpha was detected in CRC and strongly associated with lymph node metastasis and malignancy, but the expression of HIF-1alpha mRNA in colorectal polyps has not been investigated yet. The aim of this study is to evaluate the HIF-1alpha level in colorectal adenoma and hyperplastic polyps and prediction of malignancy transitions.

**PATIENTS AND METHODS**

**Tissue samples**

In this descriptive – analytical study which took place from 2016 to 2017, 54 patients with adenoma and hyperplastic polyps and 20 paired tissue samples as the control group were investigated. Samples were collected from participations of colorectal cancer screening program that underwent colonoscopy by gastroenterologist and pathologic features were confirmed by the pathologist in Taleghani Hospital (Tehran, Iran). The clinical information of patients was 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 (Tehran, Iran).

**Reverse cRIPTase PCR (RT-PCR)**

Total RNA was extracted from the samples (Yecta Tajhiz Azma kit, Cat. No. YT9065, Tehran, Iran) and RNA concentration was quantified by Nanodrop. RNAs were converted to cDNA by Retrotranscriptase (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 minutes. After that, the 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 (Ribonuclease inhibitor), 1 μL easy dilution, 5 μL RNA free distilled Water (dH2O), were added. The cDNA synthesis was performed as follows: 25°C for 5 minutes, 42°C for 15 minutes, 85°C for 1 minute for inactivation of the reverse transcriptase enzyme and 4°C for 10 minutes for hold temperature, then cDNA products were kept at -20°C until use.

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**TABLE 1. HIF-1 alpha and GAPDH primers sequences.**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIF-1alpha-F</td>
<td>5’-GATGTGGTTGTATTCTGTG-3’</td>
</tr>
<tr>
<td>HIF-1alpha-R</td>
<td>5’-ATCTCCTGCTTCTTTTAGTC-3’</td>
</tr>
<tr>
<td>GAPDH-F</td>
<td>5’-TGACTTCAACACCGCACACCCA-3’</td>
</tr>
<tr>
<td>GAPDH-R</td>
<td>5’-CACCTGTTGCTGTAGCCAAA-3’</td>
</tr>
</tbody>
</table>
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with the control group 17. In contrast, Simiantonaki et al16 showed that HIF1-alpha protein was not expressed in hyperplastic polyps and normal mucosa. According to this study and previous data, we assume that HIF1-alpha gene has not a prognostic role for malignancy formation in hyperplastic and adenoma polyps. Also, researchers mentioned that HIF1-alpha expression did not observed in benign tissue and up-regulation of HIF1-alpha in carcinoma may have arisen from genetic alteration and genome instability16,18.

Jiang et al19 found that there is a significant correlation between the expression of HIF1-alpha and VEGF-A genes in colorectal adenoma polyps and carcinoma. So, they suggested that up-regulation of HIF1-alpha gene may happen in the early stage of tumorigenesis and then can start angiogenesis and malignant formation in cells 19 and HIF1-alpha gene overexpression can regulate hypoxia and VEGF expression in colorectal carcinoma20. Moreover, Pezeshkian et al21 demonstrated that VEGF-A gene expression plays an important role in colorectal adenoma polyps angiogenesis and malignancy incidence. In accordance to this study, maybe some mediators, molecular pathways and epigenetic factors are involved in HIF1-alpha gene expression and activation during adenoma-carcinoma sequence 22.

CONCLUSIONS

In summary, HIF1-alpha gene has an important role in CRC pathological pathways such as angiogenesis. According to this study, HIF1-alpha gene is not a suitable prognostic biomarker for detecting the risk of malignancy incidence in colorectal polyps and

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**TABLE 2.** Clinicopathological parameters of patients with adenoma polyps.

<table>
<thead>
<tr>
<th>Number</th>
<th>Type</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Types</td>
<td>Adenoma</td>
<td>26 (48%)</td>
</tr>
<tr>
<td></td>
<td>Hyperplastic</td>
<td>28 (52%)</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>High grad</td>
<td>27 (50%)</td>
</tr>
<tr>
<td></td>
<td>Low grad</td>
<td>27 (50%)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>28 (52%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>26 (48%)</td>
</tr>
<tr>
<td>Site</td>
<td>Transverse Colon</td>
<td>16 (29.6%)</td>
</tr>
<tr>
<td>Size</td>
<td>&lt;5</td>
<td>44 (81.5%)</td>
</tr>
<tr>
<td></td>
<td>&gt;5</td>
<td>10 (18.5%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>54 (100%)</td>
</tr>
</tbody>
</table>

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![Fig. 1.](image1.png) There was no significant difference in HIF1-alpha mRNA expression in hyperplastic and adenoma polyps compared with the control group ($p>0.05$).

![Fig. 2.](image2.png) No significant difference in HIF-1 alpha expression between polyp groups was found ($p>0.05$).
their progression to CRC; also, uncovering the role of HIF1-alpha gene in colorectal polyps malignancy needs more investigations.

ACKNOWLEDGMENTS: This paper has been resulted from MSc thesis of Shirin Khatibi student at Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran. Also, this work was supported financially by a grant from the Iranian National Science Foundation (Project No. 89001357). The authors would like to thank the Research Institute for Gastroenterology and Liver Diseases of the Shahid Beheshti University of Medical Sciences for its support of this study.

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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