**Abstract.** – **OBJECTIVE:** This study aimed to investigate the CYP24A1, AHR, CPEB4, TRIP13, and PIK3CA mRNA expression in the blood of colorectal cancer patients in Egypt. This was performed to elucidate if there's a link between this gene expression and other clinicopathological characteristics of the tumor.

**PATIENTS AND METHODS:** A case-control study including 50 colorectal cancer patients and 50 healthy controls was conducted. Real-time polymerase chain reaction (rt-PCR) was utilized to assess the expression of CYP24A1, AHR, CPEB4, TRIP13, and PIK3CA mRNA in blood samples.

**RESULTS:** Patients with colorectal cancer had significantly higher levels of mRNA for the genes CYP24A1, AHR, CPEB4, TRIP13, and PIK3CA (p<0.001, p=0.021, p<0.001, and p<0.001, respectively) compared to controls. Remarkably, the gene expression of AHR, TRIP13, and PIK3CA genes did not exhibit a significant correlation with the tumor stages (p=0.379, p=0.095, and p=0.526, respectively). However, there was a strong correlation between CYP24A1 and CPEB4 gene expression and tumor stages (p<0.001 and p=0.002, respectively).

**CONCLUSIONS:** Therefore, we can conclude that increased mRNA levels of CYP24A1, AHR, CPEB4, TRIP13, and PIK3CA in blood samples withdrawn from colorectal cancer patients could be a biomarker for the disease.

**Key Words:** Biomarker, Carcinogenesis, Colorectal cancer, Gene expression profiling, Peripheral blood.

**Introduction**

Colorectal cancers start in the colon or rectum of the human body. Colorectal cancer is one of the most frequent reasons for cancer-related fatalities and the third most prevalent cancer type globally. Early diagnosis of such cancer is connected to the discovery of gene biomarkers and the advancement of diagnosis techniques, all of which could improve the disease prognosis. In research on carcinogenesis, gene expression profiling is used to discover specific changes in gene expression associated with the emergence of tumors and identify and categorize tumors according to their molecular characteristics.

There are startling differences between the gene expression patterns of adenoma and normal mucosa. According to numerous investiga-


**Patients and Methods**

**Subjects**

Mansoura Faculty of Medicine’s Ethical Committee approved this study with code: R.21.05.1339.R1.R2. Before participating in this study, all subjects signed a written consent form. We obtained clinical information and biochemical findings from patient medical records. The current case-control study, which had 100 participants (50 CRC patients and 50 controls), was conducted at the Faculty of Medicine, Mansoura University, Egypt, in the medical biochemistry, general surgery, and tropical medicine departments. Between June 2019 and May 2021, one hundred individuals were separated into two groups: fifty CRC patients were recruited from Mansoura University Hospitals for the first group and fifty normal individuals for the second group.

CRC was diagnosed according to American College of Gastroenterology (ACG) guidelines. This study excluded patients with malignancies other than colorectal cancer, patients with chronic inflammatory disorders, patients with septicemia, patients with liver cirrhosis, patients with pancreatitis, smokers, patients with inflammatory bowel diseases, and patients who received any chemotherapeutic agents. Every patient and member of the control group underwent a thorough physical examination as well as standard laboratory tests. These tests included a complete blood count, virology markers (hepatitis B virus antibody, hepatitis B surface antigen, and human immune deficiency virus antibody), liver function tests (serum
albumin, serum bilirubin, serum glutamic pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), and prothrombin time), serum creatinine, and tumor markers [carcinoembryonic antigen or (CEA) and carbohydrate antigen 19-9 (CA 19-9)]. Also, according to the tumor, node, and metastasis (TNM) staging, colorectal cancer staging was carried out.  

**RNA Extraction**

RNA extraction was performed from blood samples in all the study groups. We gathered (5 mL blood samples in EDTA). In an adequately sized tube, one volume of whole human blood was mixed with five volumes of erythrocyte lysis (EL Buffer). The tube was swiftly vortexed twice while incubating on ice for 10 to 15 minutes. Following incubation, the tube was separated from the supernatant and centrifuged. A pellet of leukocytes will form after centrifugation, and RNA was extracted using the Mini Kit miRNeasy described by Qiagen manufacturer (Valencia, CA, USA). Total RNA from the nucleated cells was taken out.

**RT-PCR**

Supplementary Table I illustrates the B-actin and selected genes’ primer sequences. Primer 3 software (https://primer3.ut.ee/) was used to create primer sets for the CYP24A1 gene, AHR gene, CPEB4 gene, TRIP13 gene, PIK3CA gene, and B-actin gene. Primers were designed to span exon-exon junction to preclude amplification of genomic DNA (Primers were chosen and designed so that one half hybridizes to the 3 ends of one exon and the other half to the 5 ends the adjacent exon of the target genes). CYP24A1, AHR, CPEB4, and PIK3CA genes were verified in The Cancer Genome Atlas Program (TCGA) data, while there was no data about TRIP13 gene in TCGA data. Two Micrograms (ug) of RNA were added to reverse transcription with random primers using Gene-specific PCR primers purchased from Vivantis Technologies Sdn Bhd (Selangor, Malaysia) in proportion to the manufacturer’s directions. As a control gene, we checked the integrity of cDNA using the amplification of B-actin gene simultaneously. The PCR amplifications were performed using a thermocycler (Applied Biosystem 7500, CA, USA) Supplementary Table II.

**Statistical Analysis**

The data was entered into and evaluated using Microsoft Excel. The Statistical Package for the Social Sciences (SPSS) 27.0 (IBM Corp., Armonk, NY, USA) was used to import and analyze the data. In categorical data, the baseline characteristics of the study population were presented as frequencies and percentages (%). On the other hand, the non-parametric quantitative data were exhibited as median and interquartile ranges, while mean values and standard deviations (SD) were utilized for the quantitative parametric data.

The Chi-Square and Fisher’s exact tests were used to compare two or more distinct groups of qualitative data. Also, an independent t-test was performed to compare two quantitative parametric data sets. For continuous data, a Mann-Whitney test was performed to look for a significant difference between two groups that didn’t have a normal distribution. The Registrars of Companies (ROC) curve assessed a test’s diagnostic performance or precision in separating diseased and non-diseased cases. p-values < 0.05 and 0.01 were regarded as very significant and significant, respectively.

**Results**

Demographic Data of Both Groups and Tumor Characterization in the Colorectal Cancer Group

The two primary groups in this study were the 50 colorectal cancer patients (first group), who had an average age of 55.86±11.87 years and were made up of 22 men and 28 women, and the 50 healthy control subjects (second group), who had an average age of 54.04±15.45 years. In the colorectal group, 49 patients had the colon as their primary site of tumor growth, including 7 in the ascending colon, 5 in the caecum, 7 in the descending colon, 8 in the hepatic flexure, 12 in the sigmoid colon, 6 in the splenic flexure, and 4 in the transverse colon. There were 7, 16, 26, and 1 patients in stages I, II, III, and IV, respectively. Regarding the histopathology of colorectal carcinoma, 42 individuals had adenocarcinomas with moderate differentiation, 4 had adenocarcinomas with poor differentiation, 3 had villous carcinoma, and 1 had signet ring cell carcinoma (Figure 1).

Gene Expression Analysis

CYP24A1, AHR, CPEB4, TRIP13, and PIK3CA had significantly higher levels of mRNA in the blood of colorectal cancer patients when com-
CYP24A1, AHR, CPEB4, TRIP13, and PIK3CA genes expression in colorectal cancer patients

Compared to the controls (p<0.001, p=0.021, p<0.001, p<0.001, and p=0.006 respectively) (Supplementary Table III and Figure 2).

**ROC Curve Analysis for Gene Expression to Identify Colorectal Cancer**

The CYP24A1 gene’s cut-off value was 0.89, with a sensitivity of 80%, a specificity of 6%, and an area under the curve (AUC) of 0.813. AUC was 0.634, sensitivity 82%, specificity 46%, and 0.795 was the cut-off value for the AHR gene. The CPEB4 gene’s cut-off value was 0.875, with a sensitivity of 72%, a specificity of 58%, and an AUC of 0.718. The TRIP13 gene’s cut-off value was 0.895, with a sensitivity of 74%, a specificity of 64%, and an AUC of 0.725. The PIK3CA
The gene’s cut-off value was 0.795, with a sensitivity of 86%, a specificity of 40%, and an AUC of 0.660 (Supplementary Table IV and Supplementary Figure 1).

**Association of Gene Expression Levels and Tumor Staging**

There was a significant association between CYP24A1 and CPEB4 gene expression and tumor staging.
staging ($p<0.001$ and $p=0.002$, respectively). At the same time, we observed no significant association between $AHR$, $TRIP13$, and $PIK3CA$ gene expression and tumor staging ($p=0.379$, $p=0.095$, and $p=0.526$, respectively) (Supplementary Table V and Figure 3).

Association of Gene Expression Levels and Tumor Histopathology

There was no significant association between $CYP24A1$, $AHR$, $CPEB4$, $TRIP13$, and $PIK3CA$ gene expression levels and histopathological characteristics of the tumor ($p=0.123$, $p=0.561$, $p=0.499$, $p=0.807$, and $p=0.543$, respectively) (Table I).

Discussion

The different patterns of expression of the biomarkers could be used as a tool to support already-present histological elements in patient follow-up and treatment strategies in colorectal cancer.

Colon cancer is typically found at an advanced tumor stage, with a very low survival rate, despite the growing importance of screening procedures. Different gene expression patterns can be observed as colon cancer develops, as is well known. Based on all genome expression, previous research aims to identify, create, and use therapeutically useful biomarkers for routine tumor classification diagnoses. To the best of our knowledge, this study is the first to elucidate the influence of different gene expressions in patients with colorectal cancer in Egypt. Our study outcomes could have a significant consequence for early diagnosis. In addition, it is planned to create and use useful biomarkers in routine tumor categorization diagnosis based on all genome expression investigations.

In this current study, $CYP24A1$ mRNA level was upregulated in colorectal cancer patients compared to controls. According to Androutsopoulos et al$^{23}$, active CYP1 enzymes were overexpressed in 35% of colon cancer cases. In contrast, $CYP1A1$ was overexpressed in 80% of colon cancer cases, whereas the mRNA of $CYP1B1$ was overexpressed in 60% of cases. Additionally, Androutsopoulos et al$^{23}$ discovered that bladder and colon cancers had increased mean $CYP1B1$ and $CYP1A1$ mRNA levels and mean CYP1 activity when compared to normal tissues ($p<0.05$).

This study showed a significant link between the expression of $CYP24A1$ and the tumor stage. This contrasts with Androutsopoulos et al$^{23}$, who found that CYP1 expression levels were independent of TNM status. Aldrghi et al$^{24}$ found a significant relationship between the gene expression of $CYP24A1$ and colorectal cancer. Still, it differs from our study that the samples were colon cancer tissue and control healthy tissue samples. These findings were in agreement with Sun et al$^{25}$, who found that the expression of $CYP24A1$ is strongly correlated with the development of CRC and may represent a unique predictive biomarker for CRC and can be used as a prognostic marker as it is correlated with tumor depth and tumor progression stages$^{25}$.

In the present investigation, colorectal cancer patients had higher $AHR$ mRNA levels than healthy controls. Colon cancer cells exhibit moderate levels of $AHR$ expression, according to the mRNA expression of $AHR$ of 967 human cancer cell lines. However, it is unclear how these levels relate to those of normal intestinal epithelial cells$^{26}$. Aldrghi et al$^{24}$ found a highly significant relationship between $AHR$ gene expression and colorectal cancer. Still, it differs from our study that the samples were colon cancer tissue and control healthy tissue samples. The different expression rates of the $AHR$ gene may be due to racial distribution, different sample sizes, and different technical methods for detection.

A link between aberrant $CPEB4$ expression and some cancer types suggests that $CPEB4$ may

<table>
<thead>
<tr>
<th>Stage</th>
<th>Number</th>
<th>$CYP24A$</th>
<th>$AHR$</th>
<th>$CPEB4$</th>
<th>$TRIP13$</th>
<th>$PIK3CA$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderately differentiated</td>
<td>42</td>
<td>2.75 (0.7-4.5)</td>
<td>0.9 (0.7-4.5)</td>
<td>2.25 (0.7-4.5)</td>
<td>2.5 (0.7-4.5)</td>
<td>0.9 (0.6-4.5)</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>4</td>
<td>1.5 (0.8-4)</td>
<td>0.85 (0.7-1.5)</td>
<td>1.65 (0.8-4)</td>
<td>0.9 (0.7-4.5)</td>
<td>1.7 (0.9-3)</td>
</tr>
<tr>
<td>Signet ring cell carcinoma</td>
<td>1</td>
<td>3.5 (0.7-0.9)</td>
<td>0.9 (0.7-1.5)</td>
<td>3 (0.8-0.9)</td>
<td>2.5 (0.9-2)</td>
<td>0.8 (0.8-0.9)</td>
</tr>
<tr>
<td>Villous carcinoma</td>
<td>3</td>
<td>0.8 (0.7-0.9)</td>
<td>0.8 (0.7-1.5)</td>
<td>0.9 (0.8-0.9)</td>
<td>0.9 (0.9-2)</td>
<td>0.8 (0.8-0.9)</td>
</tr>
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$p$-value: 0.123, 0.561, 0.499, 0.807, 0.543
considerably control cancer growth and metastasis. It is particularly recommended that CPEB4 is a target for cancer treatment since it is thought to be crucial for cancer cell migration and invasion. Furthermore, it is crucial to comprehend the biology of colorectal cancer, identify RNA-binding proteins associated with the disease, and perhaps establish new objectives for the prognostic biomarkers. According to research, high mRNA levels have been associated with advanced tumor stages, metastasis, as well as reduced prognosis in individuals with colorectal cancer. CPEB4 is expressed at high levels in colorectal cancer tissues. According to the current investigation, patients with colorectal cancer had higher levels of CPEB4 mRNA than healthy controls, which was consistent with Söylemez et al. In contrast to our research, Xu and Liu discovered that prostate cancer and the surrounding tissues had lower levels of CPEB4 mRNA than the control. According to the investigations, CPEB4 has been found in various types of tumor tissues. Given that CPEB4 has been identified as highly expressed in different types of tumors, it is believed to exert preoncogenic effects on tumor development, invasion, and vascularization. Another study discovered that while pancreatic ductal cancer increased CPEB4 gene expression, hepatocellular carcinoma displayed a decrease. It is thought that CPEB4 is overexpressed in several malignancies, such as kidney, skin, and colorectal cancers, and that this overexpression may be advantageous for tumor development. High expression levels of CPEB4 are linked to a low prognosis in colorectal cancer. In addition, its potential involvement in tumor invasion and metastasis has been hypothesized. Similar to this study, it has been noted that cases of colorectal cancer have significant levels of CPEB4 expression in their peripheral blood.

It has been established that TRIP13 is a protein localized in the kinetochore and facilitates proper cell division. Many kinetochore-localized proteins are abundantly generated in various malignancies. TRIP13 has been associated with elevated expression or amplification in several types of human cancer. In our study, the amount of TRIP13 mRNA significantly increased, in agreement with Söylemez et al. Similar research was conducted by Kurita et al. on the variation in TRIP13 mRNA levels between cancer and normal tissues. They stated that TRIP13 is involved in developing and invading colorectal cancer cells and might be a marker for the illness. The advanced TNM stage was significantly linked with higher TRIP13 expression, according to Sheng et al. It is important to emphasize that accurate chromosomal segregation in this scenario depends on the expression and function of the TRIP13 gene. Thus, TRIP13 overexpression may be a general characteristic of colorectal cancer and a potential biomarker or signal for the disease’s detection.

The proto-oncogene PIK3CA, which codes for phosphatidylinositol-3-kinases, is in the EGFR tyrosine-kinase domain (PI3K). As a result, the protein kinase B enzyme AKT is phosphorylated, activating the AKTmTOR signaling pathway. The phosphoinositol-3-kinase (PI3K) pathway has been recognized as an enzyme activity connected to a viral oncoprotein in human malignancies. According to Söylemez et al., it was shown in our investigation that peripheral blood PIK3CA mRNA levels rose in comparison to the control group. Numerous cancers, including colorectal cancer, have mutations in the catalytic subunit of PI3K, known as PIK3CA. Exon 9 and exon 20 at two hot locations, which account for 10% to 20% of colorectal cancers, have been reported to include about 80% of PIK3CA mutations. Yan et al. explored the possible role of the PIK3CA mutation in colorectal cancer treatment. We looked at the relationship between PIK3CA mutation and first-line treatment response in 440 colorectal cancer patients’ medical data.

PIK3CA gene mutations have been discovered to occur 9.55 percent of the time in colorectal cancer patients, and they have been linked to late TNM staging and low histological grade. Primary chemotherapy has been shown to have a worse response in colorectal cancer patients with the PIK3CA mutation than in people without the mutation. Both in vitro and in vivo testing revealed low sensitivity of PIK3CA mutant tumor cells to first-line chemotherapy. The findings showed that the PIK3CA mutation activates PI3K/Akt, promoting chemotherapy-resistant colorectal cancer stem cells’ survival and growth. Multiple meta-analyses found that PIK3CA exon 20 mutations may be a sign of resistance to anti-EGFR therapy. Combining mRNA analysis with mutation analyses is necessary to accurately identify the PIK3CA effect in patients with colorectal cancer.

Limitations

The current study had potential limitations as it is a small sample study, only one patient was in stage IV of the tumor. It is a single-center study, so a larger-scale validation study is required to prove these results.
Conclusions

In conclusion, according to the study's findings, a spike in the mRNA levels of CYP24A1, AHR, CPEB4, TRIP13, and PIK3CA in the blood of persons with colorectal cancer may be a potential biomarker for the diagnosis of this disease. The study’s findings suggest that the increase in the mRNA levels of CYP24A1, AHR, CPEB4, TRIP13, and PIK3CA could contribute to the development of colorectal cancer and can be used as an indication of disease occurrence. Our data contain genetic information that might help accurately detect and diagnose colorectal cancer.

Authors' Contributions
Conceptualization, Ahmed Mohamed El Nakib, Mohamed Elsaeed, Ramy A. Abdelsalam, and Sally Abdallah Mostafa; Data curation, Ramy A. Abdelsalam, Khalil Wafi, and Engy Elekhnawy; Formal analysis, Mohamed Elsaeed, Ramy A. Abdelsalam, and Sally Abdallah Mostafa; Investigation, Ahmed Mohamed El Nakib, Mohamed Elsaeed, Khalil Wafi, Walaa A Negm, and Sally Abdallah Mostafa; Methodology, Ahmed Mohamed El Nakib, Mohamed Elsaeed, Ramy A. Abdelsalam, Khalil Wafi, and Sally Abdallah Mostafa; Resources, Mohammed Alrouji, Mansour Alsaleem; Software, Mohammed Alrouji, Mansour Alsaleem; Validation, Engy Elekhnawy, Gaber El-Saber Battha, and Wala A Negm; Writing – original draft, Ahmed Mohamed El Nakib, Mohamed Elsaeed, Ramy A. Abdelsalam, Engy Elekhnawy, Wala A Negm, and Sally Abdallah Mostafa; Writing – review and editing, Ahmed Mohamed El Nakib, Khalil Wafi, Mohamed Elsaeed, Ramy A. Abdelsalam, Engy Elekhnawy, Nada H. Aljarba, Walaa A Negm, and Sally Abdallah Mostafa. All authors have read and agreed to the published version of the manuscript.

Ethics Approval
Mansoura Faculty of Medicine's Ethical Committee approved this study with code: R.21.05.1339.R1.R2.

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Conflicts of Interest
The authors declare no conflict of interest.

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