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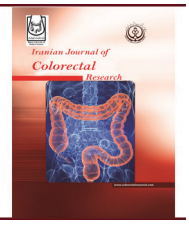

# A Cross-Sectional Study Comparing Mir-503-5p Expression in Gastric Cancer Patients Before and After Chemotherapy

***Seyedeh Azra Shamsdin[1], PhD; Sayed Ali Hijazi Hosseini[2], MD; Hassan Akrami[1]*, PhD; Nasrollah Erfani[3,4], PhD; Yousef Nikmanesh[1], PhD;Mohammad Reza Fattahi[1], MD; Mastaneh Zeraatiannejad[1], Msc***

*[1]Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
*[2]Student research committee, Shiraz university of Medical sciences, Shiraz, Iran*
*[3]Department of Immunology School of Medicine Shiraz university of Medical sciences, Shiraz, Iran*
*[4]Cancer Immunology and Immunotherapy Group, Shiraz Institute for Cancer Research, Shiraz University of Medical Sciences, Shiraz, Iran*

***Corresponding authors:***
Hassan Akrami, PhD; Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Postal code: 71935-1311, Shiraz, Iran.
**Tel/Fax:** +98 71 36281442;
**Email:** hassan_akrami@yahoo.com, h_akrami@sums.ac.ir

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## Abstract

**Background:** Gastric cancer is the third most fatal cancer among all cancer types. Early diagnosis of this cancer dramatically increases the five-year survival rate of patients. MicroRNAs (miRNAs) play a significant role in tumorigenesis, angiogenesis, invasion, metastasis, and drug resistance in gastric cancer, similar to other cancers. The expression levels of miRNAs can influence tumor progression or suppression. This study aimed to investigate the expression levels of miR-503-5p in the serum of gastric cancer patients both before and after chemotherapy.

**Methods:** This observational study was conducted from 2022 to 2024 at Namazi and Shahid Faghihi Hospitals. Thirty patients with gastric cancer with a definitive diagnosis by gastroenterologists and pathologists and had not received any prior treatment, were included in the study. A blood sample of 5 ml was drawn from each patient and sent to the laboratory for analysis. After the completion of thechemotherapy, blood samples were collected again. The expression level of miR-503-5p was measured using real-time PCR. Data analysis was performed using SPSS version 24, and differences in miR-503-5p expression and clinicopathological characteristics were assessed using ANCOVA and paired *t*tests.

**Results:** Comparing serum levels of miR-503-5p in gastric cancer patients before and after chemotherapy revealed a 2.12-fold increase in miR-503-5p expression post-treatment (P=0.001). Furthermore, expression levels of miR-503-5p in the serum of patients with metastasis were significantly lower than patients without metastasis (P<0.05).

**Conclusion:** Considering the increased expression of miR-503-5p following treatment in gastric cancer patients, these findings suggest a suppressive role for miR-503-5p in gastric cancer. miR-503-5p may potentially serve as a biomarker for evaluating gastric cancer treatment.

**Keywords:** Gastric cancer; MicroRNAs; Chemotherapy; Gene expression

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## Introduction

Gastric cancer is characterized by the abnormal and excessive proliferation of stomach cells, with various types named according to the type of proliferating cell. For instance, adenocarcinoma originates from mucosal cells. Other types of stomach tumors include gastrointestinal stromal tumors (GISTs), which originate from stromal and connective tissue cells, carcinoid tumors (neuroendocrine cells), lymphoma (lymphatic tissue), and adenocarcinoma, which accounts for over 90% of all gastric cancers (1).

Based on the anatomical location of the lesion, tumors are categorized into cardiac (located near the esophagus-stomach junction) and non-cardiac (distal stomach) subgroups, each associated with different risk factors. Over recent decades, the incidence of non-cardiac gastric cancer has decreased. Another classification of gastric cancer is based on histological and pathological findings, dividing it into intestinal and diffuse subtypes, each characterized by distinct etiologies, risk factors, and prognoses (2-4).

In 2022, over 968,000 new cases of gastric cancer were diagnosed, with approximately 70% resulting in death, making gastric cancer the third most lethal cancer that year. It is estimated that this number will increase to 1.48 million by 2040 (5).

Chronic infection with *Helicobacter pylori* is the most significant cause of gastric cancer. This bacterium contributes to the development of gastric cancer through multiple mechanisms, including direct effects on gastric epithelial cells and through the inflammatory response of gastric tissue. Additionally, *H. pylori* can disrupt the microbial balance in the stomach, driving the tissue toward carcinogenesis (6-8).

The diagnosis of gastric cancer is confirmed through endoscopy, and staging is determined by tumor invasion, the number of affected lymph nodes, and the presence or absence of metastasis (9).

Studies indicate that the five-year survival rate for patients with gastric cancer in South Korea and Japan is significantly higher than in other countries. This improvement is attributed to nationwide screening programs, which enable the early detection of the disease and substantially reduce mortality rates (10, 11).

The primary treatment modalities for gastric cancer are chemotherapy and surgery. The type of surgical intervention depends on the location of the lesion, with the standard surgical method being endoscopic mucosal resection. Laparoscopic surgery, the least invasive approach, is performed only for lesions located in the distal stomach. Although surgery removes most of the tumor, residual tumor tissue may regrow and lead to disease recurrence. To enhance the efficacy of surgery, patients undergo multiple chemotherapy sessions both before and after surgery, depending on the tumor type and level of invasion. Chemotherapy regimens are primarily based on fluorouracil (12-14).

The most critical factors indicating patient survival after treatment are tumor depth and the number of metastatic lymph nodes. The deeper the tumor invades tissue or the more lymph nodes involved, the higher the stage, which reduces the likelihood of a favorable response to treatment (15). Five-year survival rates are 50% for patients with advanced stages and 95% for those with early-stage (11).

MicroRNAs (miRNAs) are small, non-coding RNAs consisting of 19-25 nucleotides that regulate gene expression post-transcriptionally by binding to the 3' untranslated region (3' UTR) of target mRNAs (16).

Mature miRNAs bind to Argonaute (AGO) proteins through the RNA-Induced Silencing Complex (RISC), attaching to their target sequences. This process involves a 7-8 nucleotide sequence from the 5' end of the miRNA complementarily binds to its target in the 3' UTR, leading to either stimulation or inhibition of translation in the target strand (17).

The connection between miRNAs and cancer was first identified in 2002, following decreased levels of miR-15 and miR-16-1 in chronic lymphocytic leukemia (CLL). Since then, extensive molecular studies have been conducted to compare miRNA expression levels in various tumors with those in healthy individuals. Alterations in miRNA expression in different cancers arise from mechanisms such as chromosomal changes, defects in miRNA synthesis, and epigenetic modifications (18-21). Additionally, miRNAs also affect cytochrome P450 activity, which alters drug metabolism; consequently, levels of drug-metabolizing enzymes are higher in some cancers compared to healthy individuals.

In gastric cancer, as in other cancers, miRNAs play a crucial role in tumorigenesis, angiogenesis, invasion, metastasis, and drug resistance (22). miRNAs influence signaling pathways, leading to increased cellular stimulation, inhibition of apoptosis, loss of intercellular junctions, angiogenesis, and progression of tumors (23).

miR-503-5p is one of the tumor-suppressor miRNAs belonging to the miRNA-15/16 family. The biosynthesis of miR-503-5p is similar to that of other miRNAs. Initially, its precursor is synthesized from the double-stranded DNA chain on the X chromosome by RNA polymerase (24). Following partial processing, it is transported to the cytosol, where final modifications occur, resulting in the formation of its mature form.

In this study, we investigated the serum levels of miR-503-5p in gastric cancer patients both before the initiation of chemotherapy and after the completion of at least three chemotherapy sessions.

## Materials and Methods

This cross-sectional study included patients with gastric cancer with a confirmed diagnosis via endoscopy and pathology. The patients were selected

from individuals hospitalized at Namazi and Shahid Faghihi Hospitals. After obtaining informed consent and providing a comprehensive explanation of the research protocol, participants were selected.

The sample size was calculated based on a prior study (25) using MedCalc software, with 90% power using the following formula, resulting in a minimum of 16 participants. A significance level of 0.05 was considered as $n=(Z\alpha/2+Z\beta)^2\times2\times\sigma^2/d^2$. The inclusion criteria were clinical and pathological diagnosis of gastric adenocarcinoma and no history of infectious or inflammatory diseases within the past month. The exclusion criteria included patients who refused to complete treatment, gastric tumors other than adenocarcinoma (e.g., GIST, lymphoma, or neuroendocrine tumors), and patients already undergoing treatment.

In this study, blood samples were collected from the participants. Ethical considerations Patients were included in the study after providing informed consent and receiving comprehensive explanations about the research (Shiraz University of Medical Sciences granted the ethics code as: IR.SUMS.MED.REC.1402.163).

Blood samples (5 ml) were collected from these patients before starting chemotherapy and after the completion of at least three chemotherapy sessions to measure serum levels of miRNA-503-5p. The blood samples were sent to the Gastroenterology and Hepatology Research Center Laboratory of Shiraz University of Medical Sciences.

The collected blood samples were centrifuged, and the serum was stored at -70°C until analysis. RNA was extracted from all serum samples using the PsPure Total RNA Extraction Kit (Pishgaman Sanjesh Company, Iran). c-DNA specific to miR-503-5p was synthesized using reverse transcriptase (M-MLV) and stem-loop primers. The sequences of the specific primers for miR-503-5p and those used for PCR are provided in Table 1.

The mature sequence of miR-503-5p is as follows: 5'-UAGCAGCGGGAACAGUUCUGCAG -3'

Gene expression changes in patients before and after chemotherapy were analyzed using Real-Time RT-PCR. Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) was used as the internal control gene, and the expression level of miR-503-5p was calculated using the $2^{-\Delta\Delta Ct}$ method (26). According to the $2^{-\Delta\Delta Ct}$ formula, ΔCt was calculated, and the expression levels of miR-503-5p in patients who underwent chemotherapy compared to those who did not receive treatment.

Data related to age, gender, and clinicopathological characteristics of the patients, including tumor stage, Tumour, Node, Metastasis (TNM) classification, and tumor differentiation grade, were extracted from their medical records and analyzed.

### *Statistical Methods*

The obtained results were analyzed using SPSS software, version 24 (IBM Company, USA). The normality of the data distribution was assessed using the Kolmogorov-Smirnov test. To evaluate the relationship between miR-503-5p expression levels before and after chemotherapy, the paired *t* test was applied. To compare miR-503-5p expression between sexes (man and woman) and among different clinicopathological groups, including metastasis status, tumor location, and family history of cancer, *t* tests, ANOVA and ANCOVA were used. A significance level of 0.05 was considered significant for all statistical analyses.

## Results

In the present study, a total of 30 untreated patients with gastric cancer were included. Patients who did not meet the inclusion criteria were excluded. Due to the malignancy of gastric cancer, high mortality rates, and lack of patient cooperation for follow-up, only 30 patients completed the study. The mean age of the patients was 64.43±13.44 years (30-84 years). Among the patients, 20 individuals (66.67%) were men and 10 individuals (33.33%) were women.

The comparison of serum miR-503-5p levels in gastric cancer patients before and after chemotherapy revealed that the expression level of miR-503-5p was significantly higher in patients following chemotherapy compared to before treatment (fold change=2.12, P=0.001). The result of the expression levels of miR-503-5p are presented in Figure 1.

The comparison of serum levels of miR-503-5p in patients with gastric cancer with tumors located in the proximal or distal stomach revealed that the expression level of miR-503-5p was higher in patients with tumors in the proximal part of the stomach compared to those with tumors in the distal part . However, this change in the expression of miR-503-5p wasn't significant (Table 2).

In the present study, we compared the expression levels of miR-503-5p between individuals with a family history of cancer and those without such a history.

**Table 1:** The sequences of the specific primers for miR-503-5p and those used for PCR are provided

| Gene ID | Primer name | Sequence (5'to 3') | Product Length (bp) |
|---|---|---|---|
| miR-503-5p | SLRT-503 | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTGCAG | 50 |
| | mir503-F | CCAGCATAGCAGCGGGAACAG | 21 |
| | mir503-R | CCAGTGCAGGGTCCGAGGTA | 20 |
| *GAPDH* | GAPDH-F | ACTCTGGTAAAGTGGATATTGTTGC | 25 |
| | GAPDH-R | GGAAGATGGTGATGGGATTTC | 21 |

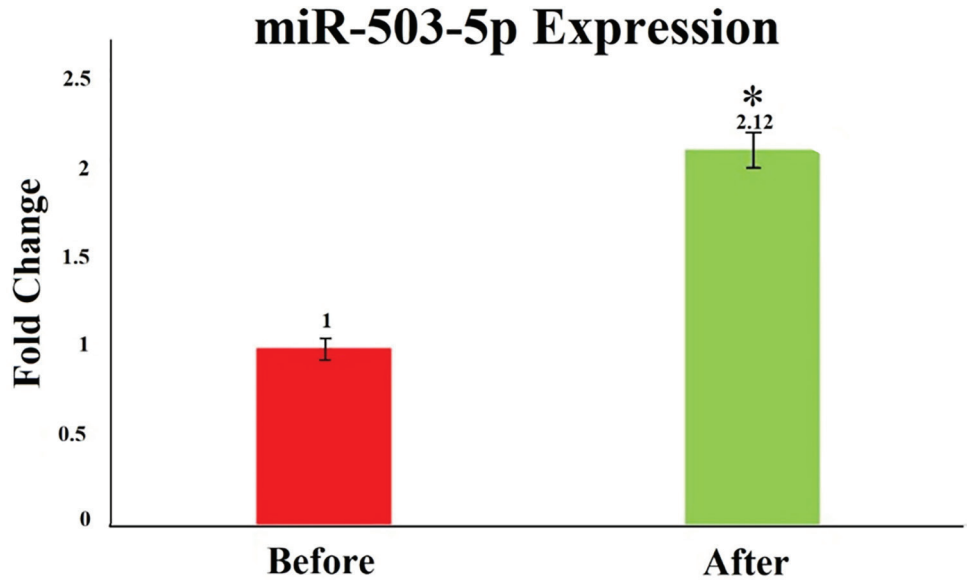


**Figure 1:** Comparison of serum miR-503-5p levels in patients before and after chemotherapy. *Indicated a Significant Difference between two groups (patients before and after chemotherapy) (Change fold=2.12, P=0.001).

**Table 2:** Comparison of serum miR-503-5p levels in patients with and without clinical characteristics

| | | N(%) | Mean±SE | P value |
|---|---|---|---|---|
| Location of tumor | Proximal | 15(50%) | 8.85±2.28 | 0.804 |
| | Distal | 15(50%) | 5.09±1.31 | |
| Family history of cancer | Yes | 12(40%) | 3.75±2.15 | 0.392 |
| | No | 18(60%) | 6.06±1.62 | |
| Gender | Men | 20(66.66%) | 4.57±1.79 | 0.542 |
| | Women | 10(33.33%) | 6.28±1.57 | |
| Metastasis | Yes | 5(16.66%) | 1.02±1.91 | 0.049 |
| | No | 25(83.33%) | 5.96±1.46 | |

**Table 3:** Analysis of serum miR-503-5p levels in patients with and without clinical characteristics with ANCOVA. A significance level of 0.05 was considered for all statistical analyses. Dependent Variable: ΔCt between after and Pre-treatment

| Parameter | Beta | P value | 95% Confidence Interval | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| Family hx of cancer=Yes | 0.038 | 0.931 | -0.855 | 0.930 |
| Family hx of cancer=No | Ref | | | |
| Gender=woman | 0.028 | 0.952 | -0.910 | 0.965 |
| Gender=man | Ref | | | |
| Metastasis=Yes | -0.044 | 0.929 | -1.041 | 0.954 |
| Metastasis=No | Ref | | | |
| Location of tumor=proximal | -0.173 | 0.634 | -0.916 | 0.570 |
| Location of tumor=distal | Ref | | | |
| Age | -0.005 | 0.753 | -0.038 | 0.028 |

The comparison revealed that the mean expression level of miR-503-5p was lower in patients with a family history of cancer than in those without. However, this change in the expression of miR-503-5p was not statistically significant. The results and the frequency of individuals are presented in Table 2.

The comparison of miR-503-5p levels between male and female patients showed that the expression level of miR-503-5p was higher in women than in men; however, this change in the expression of miR-503-5p was not statistically significant (Table 2).

The comparison of serum miR-503-5p levels in gastric cancer patients with metastasis to other tissues revealed that the levels were significantly lower than in patients without metastasis. The results of this comparison are presented in Table 2.

The serum levels of miR-503-5p in patients with and without clinical characteristics were analyzed by using ANCOVA; however, the results were not significant (P≥0.05). The results of this comparison are shown in Table 3.

## Discussion

In the present study, serum levels of miR-503-5p in gastric cancer patients who underwent treatment

were significantly higher compared to those who did not receive treatment.

Yang Peng et al. similarly demonstrated that the expression of miR-503-5p was lower in tumor in gastric compared cell lines healthy cells. In their study, the expression levels of miR-503-5p in gastric cancer cell lines were measured and compared to those in non-tumor cells. The findings indicated a reduction in miR-503-5p levels in tumor cells, supporting its role as a tumor suppressor in gastric cancer, which aligns with the results of the current study. Additionally, they found that the expression of miR-503-5p was higher in non-metastatic cells compared to metastatic gastric cancer cells, suggesting its anti-metastatic role (27). Similarly, in the present study, serum levels of miR-503-5p were significantly lower in patients with metastasis compared to those without, further confirming its anti-metastatic function in gastric cancer.

Wu et al. investigated serum miR-503-5p as a diagnostic and prognostic biomarker for gastric cancer. They found that serum levels of miR-503-5p were lower in cancer patients compared to healthy individuals. A significant correlation was found between low serum levels of miR-503-5p and higher tumor invasiveness. Patients with higher miR-503-5p expression had better long-term survival rates compared to those with lower levels. Furthermore, the sensitivity of miR-503-5p as a diagnostic factor was compared to CEA (Carcinoembryonic Antigen), with miR-503-5p demonstrating significantly higher sensitivity (28). The alignment of their findings with the current study highlights the potential of miR-503-5p as a suitable diagnostic marker for gastric cancer.

Wenjing Li et al. investigated the role of miR-503-5p in the proliferation and invasion of gastric cancer cells. In their study, the expression of miR-503-5p was measured in tumor tissues from 46 patients with gastric cancer who underwent surgery, as well as in gastric tumor cell lines (MKN-45, SGC-7901, MKN-28, AGS, and BGC-823) and normal cell lines (GES-1) using real-time RT-PCR. The results indicated a significant reduction in miR-503-5p expression in tumor tissues compared to surrounding normal tissues (24). Furthermore, a notable correlation was observed between reduced miR-503-5p levels and increased tumor size, lymph node metastasis, and decreased survival rates. Increased miR-503-5p levels were found to inhibit the proliferation and invasion of gastric cancer cells. Although their study focused on tissue expression, the findings are consistent with the present study, which examined serum expression levels.

The consistency of these studies with the present findings underscores the potential of miR-503-5p as an anti-tumor and anti-metastatic factor in gastric cancer. Its diagnostic and prognostic capabilities make it a promising marker for evaluating and managing gastric cancer. However, some limitations were observed in our study, including a lack of the large sample size and also, some patients died during the study.

## Conclusion

In this study, the serum level of miR-503-5p in patients with gastric cancer showed a significant increase following chemotherapy compared to before treatment. Additionally, the expression level of miR-503-5p in the serum of patients with metastasis was significantly lower than in those without metastasis. These findings suggest the inhibitory and anti-metastatic role of miR-503-5p in gastric cancer. More studies with larger sample size on miR-503-5p and also other miRNAs are needed to confirm our results and prepare potential diagnostic biomarkers for gastric cancer.

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## Availability of Data and Material

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

## Ethics Approval

This study was approved by the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.MED.REC.1402.163).

## Authors' Contribution

Study concept and design: Hassan Akrami. Analysis and interpretation of data: Hassan Akrami, Seyedeh Azra Shamsdin and Ali Hijazi Hosseini. Drafting of the manuscript: Hassan Akrami, Seyedeh Azra Shamsdin and Ali Hijazi Hosseini. Critical revision of the manuscript for important intellectual content: Hassan Akrami and Seyedeh Azra Shamsdin. Statistical analysis: Hassan Akrami and Seyedeh Azra Shamsdin. Experimental technical and material support: Seyedeh Azra Shamsdin, Ali Hijazi Hosseini, Hassan Akrami, Nasrollah Erfani and Mastaneh Zeraatiannejad. Sample preparation: Mohammad Reza Fattahi and Yousef Nikmanesh.

**Conflicts of interest:** None declared.

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