



Determining the Role of miR-30a as a Diagnostic Biomarker for Gastric Disorders

¹Bashir A. Ahmed, ²Nuha J. Kandala

^{1,2} Biotechnology Department, College of Science, University of Baghdad

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Abstract: Gastric cancer (GC) arises from the interplay of various risk factors, including both environmental and genetic influences. Peripheral blood samples represent a straightforward method for collection and analysis, owing to their accessibility and ease of acquisition. MicroRNA is a short non-coding RNA comprising 18–24 nucleotides, which plays a significant role in regulating gene expression and influencing the progression of various cancer types. The study examined the relationship between miR-30a gene expression in 100 Iraqi participants, comprising sixty patients with various gastric diseases and forty healthy controls. The models were analyzed using qRT-PCR technology. The mean $\pm$ SD relative expression of miR-30a in patients was 1.661 ± 1.4113 , significantly exceeding that of healthy controls (mean $\pm$ SD 1 ± 0) ($p = 0.004$). The expression levels of miR-30a were evaluated in healthy controls and patients diagnosed with different gastric conditions, including gastric ulcers, gastritis, gastric cancer, and duodenal ulcers. The mean $\pm$ SD of relative expression of miR-30a was significantly elevated in patients with duodenal ulcers (2.142 ± 2.256), gastritis (1.693 ± 1.415), gastric cancer (1.537 ± 1.05), and gastric ulcers (1.284 ± 2.257) compared to healthy controls (1 ± 0) (all $p < 0.001$, Mann-Whitney U test). The findings suggest that the upregulation of miR-30a is associated with gastrointestinal disorders and may serve as a biomarker for differentiating healthy individuals from patients. MicroRNA serves as a biomarker for the early identification of diseases and facilitates straightforward treatment.

Keywords: Gastric cancer, Peripheral blood, MicroRNA, Gene expression.

Corresponding author: (Email: basheer.aed1106a@sc.uobaghdad.edu.iq).

Introduction

MicroRNAs, which have 18–24 nucleotides and are tiny non-coding RNAs, regulate several biological processes, including the onset of cancer (1-3). Numerous activities of tumor cells, such as invasion, migration, metastasis, and proliferation, are mediated by microRNAs. This is because they are regulators of signaling pathways. MicroRNAs have the ability to either stimulate or inhibit the development of an oncological process through their interactions with certain genes in signaling pathways (4). RNA

polymerase II (Pol II) transcriptionally generates the majority of miRNA genes and, on occasion, RNA polymerase III (Pol III). The hairpin structure of the long primary transcripts, or prim-miRNAs, is what forms the miRNA sequence (5, 6). The two essential elements of the microprocessor complex involved in the production of miRNA are as follows: DiGeorge syndrome Critical Region 8 (DGCR8) is a cofactor for the double-stranded RNase III enzyme (DROSHA). These play a special role in the nucleus cleavage procedure that yields around

70 nt of (stem-loop- precursor miRNAs) or premiRNAs(7, 8). Therefore, it is believed that the mirtron pathway is independent of DROSHA but dependent on DICER. For processing, premiRNAs have been remapped to exportin-5's nucleus (9). These double-stranded miRNAs are converted by helicases into single-stranded miRNAs, which then connect with proteins called Argonaute (AGO) to create a complex known as RNA-induced silencing. In this complex, miRNAs inhibit or degrade a specific mRNA's ability to translate (10, 11). miRNAs split an mRNA chain into two segments after binding to a specific mRNA, which reduces the stability of the mRNA and prevents it from being translated into a protein (12). According to Ross and Davis (13), miRNAs regulate the expression of genes in many biological mechanisms, such as cell differentiation and combining, cell death, and cell cycle progression. Multiple targets can be concurrently anchored by a single miRNA. This occurrence underscores the crucial function of miRNAs in both beneficial and detrimental cellular processes and illustrates the bidirectional nature of their influence (14-18). Potential GC biomarkers have been proposed in a number of published publications that examine the association between microRNAs and tumor chemoresistance and metastasis (19, 20). According to a number of studies, an intronic transcriptional unit produces miR-30a, which is located on chromosome 6q.13 (21). Compared to endoscopy or tissue biopsy, blood samples provide a number of benefits, not the least of which being their ease of acquisition, less invasive nature, and widespread usage in clinical practice. Furthermore, the majority of people find it acceptable, and its cost is manageable (22, 23). Different

phenotypes of gastric cancer stem cells (GCSCs) are caused by aberrant miRNA expression (24). They are known as tumor suppressors or oncogenes and have the ability to target many genes simultaneously. Based on the studies of Azimi, Totonchi, and Ebrahimi, six miRNAs were identified: miR-30a, miR-34a, miR-23a, miR-100, miR-27a, and miR-19b. These miRNAs were shown to play a role in the regulation of treatment resistance, stemness, and metastasis in gastric cancer (GC). These miRNAs may be utilized to make GC cells more susceptible to chemotherapy (25). According to Otmani, and Lewalle miR-30a targeted *BCL9* and *COX-2* in *H. pylori*+ GC cell lines, which controlled metastasis and promoted cell proliferation. They discovered that changes in the level of miR-30a cause abnormal expression of the miR-30a target mRNAs, which is associated with the advancement of GC (26). Moreover, gastritis, dysplasia, and gastric cancer were demonstrated in miR-30a-knockout mice infected with *H. pylori*(27). The blood test is direct and easy. It is also ideal for properly diagnosing all cancers, including stomach cancer, and controlling and resisting it before progression to more severe phases. On this basis, we decided to target in this study the role of microR-30a as a diagnostic marker and accurate warning of the possibility of stomach cancer.

Materials and Methods

Clinical specimens

The 100 specimens (blood sample) used in this study were split into 40 specimens for the control group, which consists of roughly healthy individuals who have not been diagnosed with any digestive system diseases. Both males and females, aged 18 to 76, were represented. Additionally, 60 specimens

of male and female patients with a range of ages (19–71) who displayed digestive system symptoms and signs (diarrhea, vomiting, weight loss, and indigestion) were included in this investigation. Through histological investigation, a doctor correctly diagnosed the patients as having a gastrointestinal disease. Face-to-face procedures were used for the patient interviews. Several questions were utilized in the questionnaire to collect data on each patient's demographics, clinical picture, disease history, prior treatment regimens, and family history. Blood samples were taken for this descriptive research from 100 participants at Fallujah Hospital in Anbar, Iraq between March 14 and September 15, 2023. Following whole blood collection, the blood was allowed to coagulate for around half an hour at room temperature. After that, a centrifuge with a 10-minute setting of 2,000 x g was used to extract the clot. Serum is the term for the leftover supernatant. The liquid component (serum) was rapidly transferred into a sterile Eppendorf tube using a Pasteur pipette. The University of Baghdad's College of Science Research Ethics Committee gave its approval to this work (CSEC/0723/0055).

The Gene expression for miR-30a using q RT-PCR

Using the TRIzol Reagent procedure from ELK Biotechnology in China, the total RNA was extracted from the 100 serum samples that were collected. The isolated RNA was then submitted to Two Step RT-PCR (cDNA synthesis, Real Time PCR) quantification. Two primers were designed (Reverse transcript primer miR-30a 5` GTTGGCTCTGGTGCAGGGTCCGA GGTATTCGCACCAGAGCCAACCT TCCA -3` and Forward primer miR-30a: 5`-

GGTTTTTTTTGTAAACATCCTCGA C -3`) Macrogen / Korea and an additional universal reverse primer for real-time PCR: 5`- GTGCAGGGTCCGAGGT -3` Macrogen / Korea, as well as, ( Reverse transcript primer miR-16-1-primer: 5`GTTGGCTCTGGTGCAGGG TCCGAGGTATTCGCACCAGAGCC AACCGCCAAT -3` and Forward primer miR-16-1: 5`- GGTTTTTTTTAGCAGCACGTAAAT -3` Macrogen / Korea) serve as a housekeeping gene in gene expression analysis.

Statistical analysis

Using the housekeeping gene (miR-16-1) as an example, the following equations were utilized to determine the target gene's gene expression. The difference in CT value and fixed levels of fluorescence are represented mathematically in the equation, which is followed by the determination of the ΔCT of two genes (miR-30a and miR-16-1) for both patient and control clinical specimens. The following mathematical formulas were used to determine the gene's relative gene expression:

- $\text{Folding} = 2^{-\Delta\Delta CT}$
- $\Delta\Delta CT = \Delta CT_{\text{patient}} - \Delta CT_{\text{Control}}$
- $\Delta CT = CT_{\text{gene}} - CT_{\text{House Keeping gene}}$

Results and Discussion

Demographic and clinical characteristics

Sixty individuals with different gastric illnesses were among the 100 participants in the current research (Table 1). There was no discernible difference in the groups' median ages ($p=0.813$), with the control group's median age was 42 years (interquartile range, IQR=25.3) and the patient group's median age was 38 years (IQR=21.8). Furthermore, there was a comparable gender distribution in both groups: in the control group, there were

37.5% men and 41.7% females, while in the patients group, there were 46.7% males and 53.3% females ($p=0.414$). In both groups, the percentage of smokers was likewise very equal, with 30% of individuals smoking and 70% not smoking ($p=0.999$). The proportion of patients with comorbidities (chronic disease include: diabetes and blood pressure) was greater in the patient group (40%) than in the control group (22.5%), although the difference was not statistically significant ($p=0.084$). The groups' residential distributions were also comparable, with 55% of the patients group and 57.5% of the control group living in urban regions and 42.5% and 45% in rural areas, respectively ($p=0.840$). Figure 1A shows the frequency and proportion of patients with different gastrointestinal problems within the patient group. With 51.7% of patients suffering from gastritis, it was the most common ailment, followed by stomach cancer (23.3%), duodenal ulcers (13.3%), and gastric ulcers (11.7%). Moreover, Figure 1B shows the distribution of these circumstances

by sex. It's noteworthy that the prevalence of gastritis was the same in men (16 patients) and females (15 patients), but there were no appreciable variations in the prevalence of duodenal ulcers in females (6 patients) and males (2 patients). Male and female patients with stomach cancer were equally represented at seven each, whereas patients with gastric ulcers were distributed similarly, consisting of three male and four female patients. Furthermore, Figure 1C shows the age distribution of various gastrointestinal disorders. It's interesting to note that patients under 40 years old had a higher prevalence of gastritis (21 individuals) than patients over 40 years old (10 patients). However, patients that are older than 40 years of age had a higher incidence of stomach cancer (10 patients) compared to 4 patients who were younger than 40 years old. In both age groups, the distribution of gastric and duodenal ulcers was comparable, with 3 and 4 patients in the age group under 40 and 4 patients in the age group over 40, respectively.

Table (1): The study participants' demographic and clinical features.

	Controls (n= 40)	Patients (n= 60)	P
Age, years	42 (25.3)	38 (21.8)	0.813
Sex			
Male	15 (37.5)	28 (46.7)	0.414
Female	25 (41.7)	32 (53.3)	
Smoking status			
Smoker	12 (30)	18 (30)	0.999
Non-smoker	28 (70)	42 (70)	
Comorbidities			
Present	9 (22.5)	24 (40)	0.084
Absent	31 (77.5)	36 (60)	
Residence			
Urban	23 (57.5)	33 (55)	0.840
Rural	17 (42.5)	27 (45)	
<i>H. pylori</i> infection			
Positive	0 (0)	53 (88.3)	0.001
Negative	40 (100)	7 (11.7)	
Medical treatment			
Yes	24 (10)	38 (63.3)	0.834
No	16 (90)	22 (36.7)	

The interquartile range (IQR) and median for age are displayed, while the frequencies (and percentages) of the categorical data—sex, residence, comorbidities, and smoking status—are given. *P*-values have been computed using Mann Whitney's *U* test for age and Fisher's exact test for categorical data.

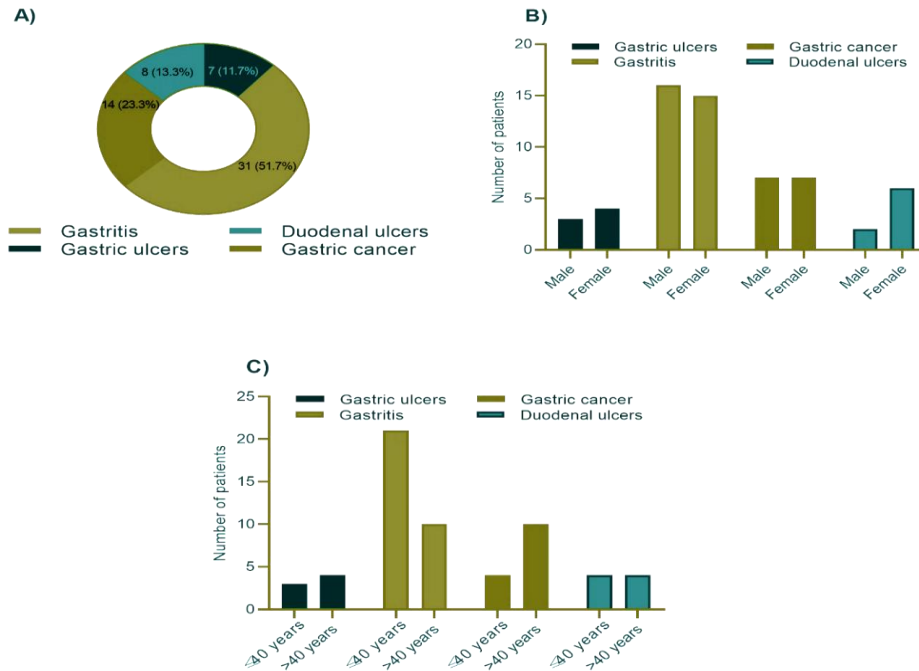
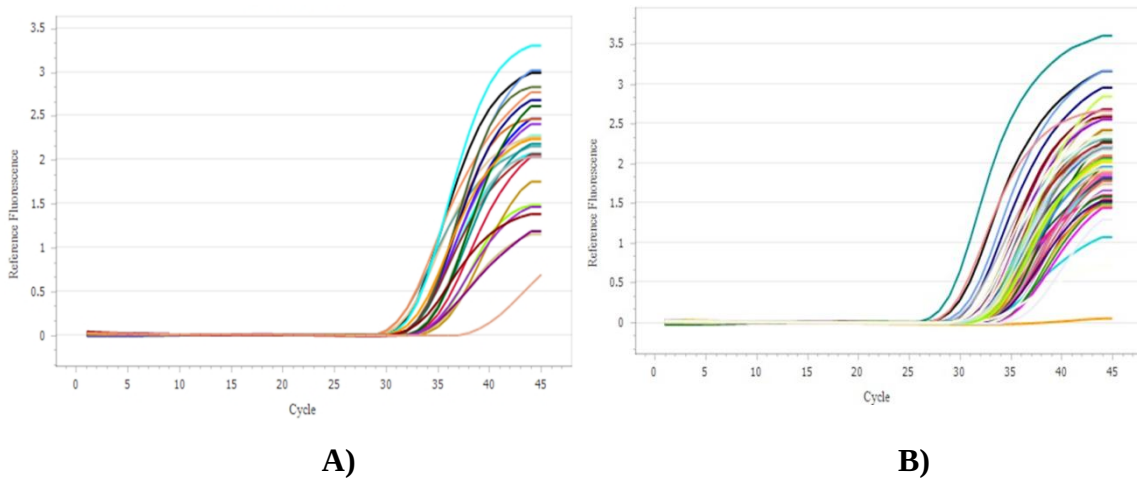


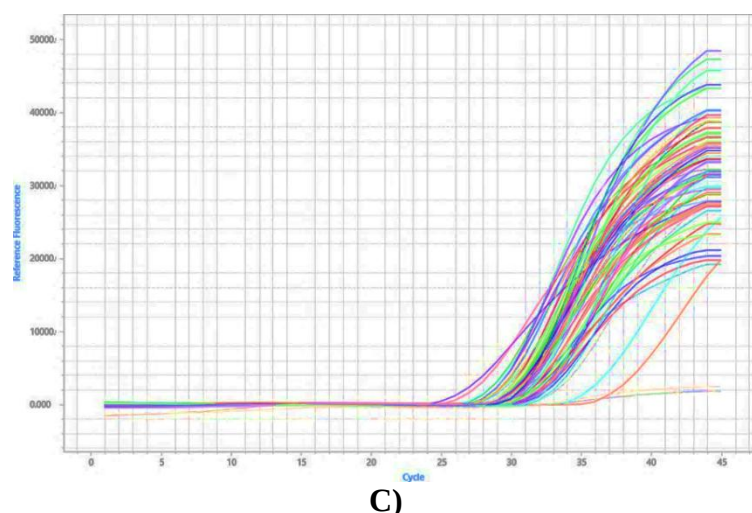
Figure (1): Patients' distribution of stomach conditions: A) Frequency and proportion of patients with duodenal ulcers, gastritis, stomach cancer, and gastric ulcers; B) Condition distribution by sex; and C) Condition distribution by age, with patients divided into those over 40 and those under 40.

Relative expression of *miR-30a*

Comparing the different cycle identified by threshold values (*Ct*) at a constant fluorescence level is the basis

for quantitative- real-time Polymerase - Chain Reaction, [qRT-PCR], result calculations, as shown in Figures 2 [A, B, and C].





C)
Figure (2): Typical Real-Time amplification curve, showing the stages of the reaction when any fluorescence over a threshold is noticed. A) Amplification curve for miR-30a control (40) samples. B) Amplification curve for miR-30a patients. C) Amplification curve for miR-16-1 (housekeeping gene) patients and control (100) samples.

The amplification curve for the forty miR-30a control samples is displayed in Figure 2 A. The Ct value ranged from (30.06 – 38.11). The amplification curve for the 60 samples from miR-30a patients is shown in (Figure 2 B), with a Ct value ranging from (27.45 – 35.54). The amplification curve for miR-16-1 (housekeeping gene) patients and control samples (100) is displayed in Figure 2 C. The Ct value ranged from 15.02 and 36.59. When comparing patients with stomach disorders to healthy controls, the relative expression of microR-30a was considerably higher, (Table 2) and (Figure 3). The Mean $\pm$ SD relative expression of miR-30a in patients was 1.661 ± 1.4113 , which was significantly higher than that in healthy controls (Mean $\pm$ SD 1 ± 0) ($p=0.004$). The maximum relative expression in patients (3.8637) was higher than that in healthy controls (1). There was a statistically significant difference between the two groups based on the 95% confidence intervals for the mean relative expression of microR-30a in patients and controls. These results imply that the upregulation of miR-30a is linked to the emergence of gastric diseases, and that the relative

expression of this gene may be used as a biomarker to differentiate between gastric illness patients and healthy persons. Abbasi *et al.*, (28) found that upregulating miR-30a through all-trans retinoic acid (ATRA) treatment suppressed autophagy by targeting the Beclin-1 gene. This suppression enhanced the sensitivity of GC cells to cisplatin (CDDP), a common chemotherapeutic agent, promoting apoptosis and inducing G2/M cell cycle arrest. Overexpression of miR-30a-3p that revealed by Wang *et al.*, (29) led to the downregulation of MAD2L1, a gene involved in cell cycle regulation, thereby inhibiting the proliferation of GC cells and causing cell cycle arrest at the G0/G1 phase. Some studies have reported that miR-30a can function as an oncomiR, promoting tumor growth. For instance, research by Hu *et al.*, (30) indicated that miR-30 expression was significantly increased in GC tissues and cell lines. Upregulation of miR-30 enhanced cell proliferation and inhibited apoptosis in GC cells, suggesting a complex role of miR-30a in gastric cancer.

Table (2): Relative expression of miR-30a in patients and control

Folding	Patients (N=60)	Control (N=40)	<i>p</i> -value (<i>p</i> <0.05)
	Mean $\pm$ SD	Mean $\pm$ SD	
Relative expression of miR-30a	1.661 $\pm$ 1.4113	1 $\pm$ 0	<0.004*

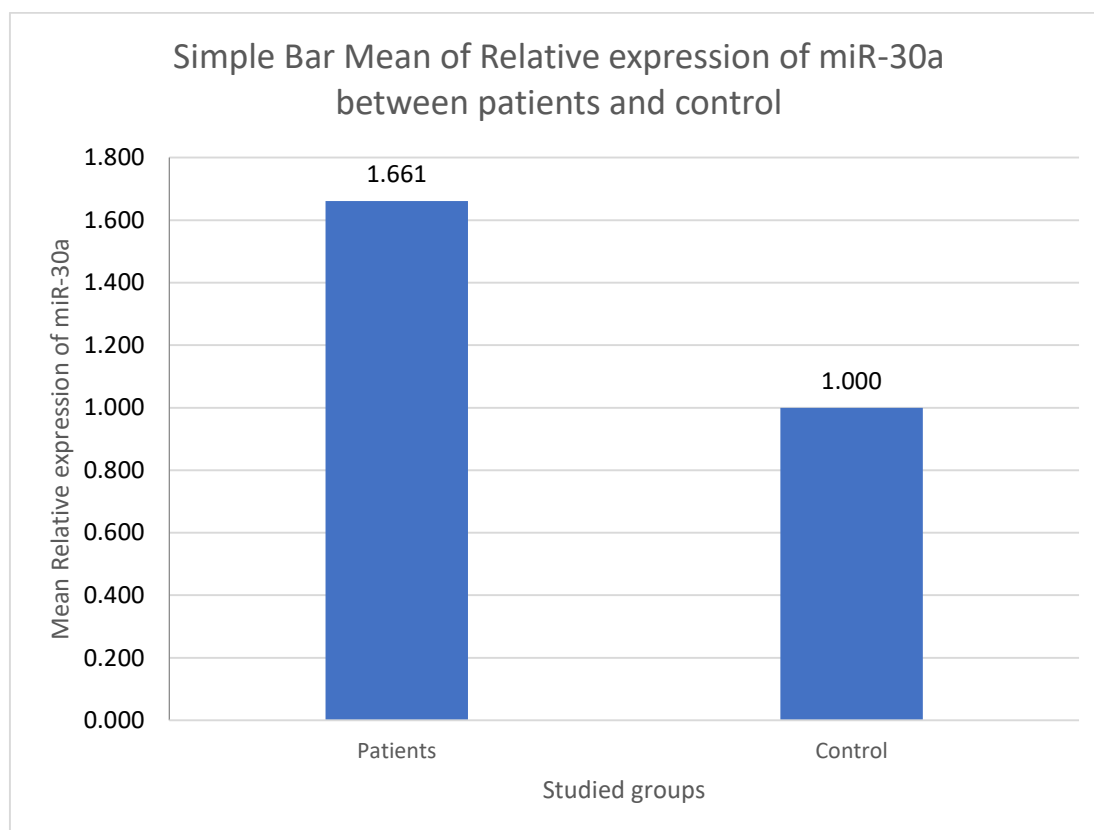


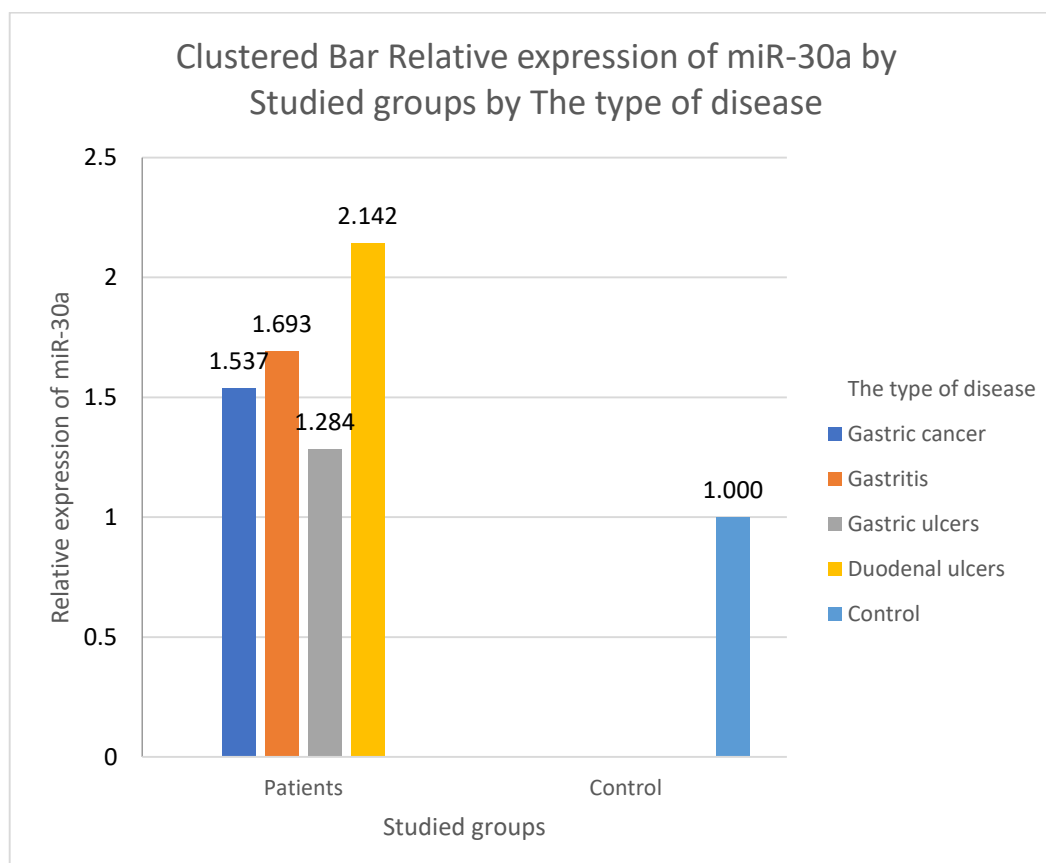
Figure (3): Simple Bar Mean of Relative expression of microR-30a between healthy controls and patients with stomach-related conditions *:*p*<0.05**

The relative expression of miR-30a was also assessed in healthy controls and patients with various gastric diseases (gastric ulcers, gastritis, gastric cancer, and duodenal ulcers) (Figure 4) and (Table 3). The type of disease, Mean $\pm$ SD of Relative expression of miR-30a was significantly higher in patients with Duodenal ulcers (Mean $\pm$ SD, 2.142 $\pm$ 2.256), gastritis (Mean $\pm$ SD, 1.693 $\pm$ 1.415), and gastric cancer (Mean $\pm$ SD, 1.537 $\pm$ 1.05) and Gastric ulcers (Mean $\pm$ SD, 1.284 $\pm$ 2.257) compared to healthy controls (Mean

$\pm$ SD, 1 $\pm$ 0) (all *p*<0.001, Mann-Whitney U test). However, the relative expression of miR-30a in patients with duodenal ulcers (median: 1.100, IQR: 0.6475 - 1.758) did not substantially vary from that of the healthy controls (Mann-Whitney U test, *p*>0.05). The minimum relative expression of miR-30a was lowest in the gastritis group (0.01000), followed by gastric ulcers (0.2100), gastric cancer (0.2500), and duodenal ulcers (0.4000), all of which were lower than the minimum value observed in healthy controls (0.8300).

Table (3): Relative expression of miR-30a in subgroup patients and control.

Groups	Patient (N=60) Mean $\pm$ SD	N	Control (40N) Mean $\pm$ SD	N	p-value ($p < 0.05$)
Gastric cancer	1.537 $\pm$ 1.055	17	1 $\pm$ 0	40	< 0.029*
Gastritis	1.693 $\pm$ 1.415	28			
Gastric ulcers	1.284 $\pm$ 2.257	7			
Duodenal ulcers	2.142 $\pm$ 2.256	8			

**Figure (4): miR-30a expression in relation to a variety of gastric disorders and healthy controls.**

The maximum relative expression of miR-30a was highest in healthy controls (7.380), followed by gastritis (3.560), duodenal ulcers (2.230), gastric cancer (2.180), and gastric ulcers (1.880). These findings suggest that the upregulation of miR-30a is associated with the development of gastric ulcers, gastritis, and gastric cancer, and duodenal ulcers, and its relative expression could potentially serve as a biomarker for distinguishing patients with these specific gastric diseases from healthy individuals. It confirms its role as oncoMiR, which plays an effective

role in the development of cancer. This study on miR-30a confirms what has been reported in many studies, as researchers in this field have shown that miR-30a is up-regulated, which leads to the development of the disease (31, 32). This study contradicts with the studies of Min *et al*, (33) found down-regulation of miR-30a in pre-neoplastic lesions and its tumor suppressor functions by targeting ITGA2 in GC. Similarly, several investigations have shown that the RNA under investigation functions as an oncogene inhibitor and is adversely regulated in a number of

stomach cancer and illness instances (34, 35) Chan *et al*, (36) developed the miR-30a deletion mouse model to investigate the function of microR-30a *in vivo*. Among the several genome-editing technologies that have developed in recent years are zinc-finger- nucleases, ZFNs, transcription activator-like effector- nucleases (TALENs), and the sequence-specific (CRISPR/Cas9) nuclease system (36).

The association between relative expression of miR-30a with risk factors

The relative expression of microR-30a was further analyzed in subgroups based on sex, age, residence, smoking status, comorbidities, and treatment status to identify potential factors influencing its expression in healthy controls and patients with gastric diseases (Table 4). Age-based analysis revealed significant differences in miR-30a expression between (≤ 40 and > 40) within the control (Mean $\pm$ SD: 1 ± 0) or patient groups (Mean $\pm$ SD: 1.579 ± 1.504) Vs 1.762 ± 1.310 respectively; $p < 0.034$, Figure 5).

Likewise, when comparing sex groups (males vs. females), significant

differences were observed between the control (Mean $\pm$ SD: 1 ± 0) and patient groups (Mean $\pm$ SD: 1.739 ± 1.587 vs 1.572 ± 1.202) respectively $p < 0.035$.

As well, smoking status significantly influence miR-30a expression within the control (Mean $\pm$ SD: 1 ± 0) and patient groups (Mean $\pm$ SD: (1.605 ± 1.439) smoker vs (1.715 ± 1.835) nonsmoker; $p < 0.035$).

The presence of comorbidities significantly affects miR-30a expression within the control (Mean $\pm$ SD: 1 ± 0) and patient groups (Mean $\pm$ SD: (1.625 ± 1.068) , for those with comorbidities vs. (1.715 ± 1.835) for those without; $p < 0.039$). Residence-based analysis showed significant differences in miR-30a expression between urban and rural residents within the control (Mean $\pm$ SD: 1 ± 0) or patient groups (Mean $\pm$ SD: (1.880 ± 1.473) vs. (1.482 ± 1.355) , respectively; $p < 0.0001$). Finally, treatment status significantly influence miR-30a expression within the control (Mean $\pm$ SD: 1 ± 0) and patient groups (Mean $\pm$ SD: (1.564 ± 1.1564) for treated vs. (1.829 ± 1.7868) for untreated; $p < 0.028$).

Table (4): Subgroups relative expression of miR-30a and clinical characteristics of the study participants.

Subgroups	Patient (N=60) Mean $\pm$ SD	Control (N=40) Mean $\pm$ SD	p-value ($p < 0.05$)
Age, years			
≤ 40	33(1.579 ± 1.504)	18 (1 ± 0)	$< 0.034^*$
> 40	27(1.762 ± 1.310)	20 (1 ± 0)	
Sex			
Male	32(1.739 ± 1.587)	25(1 ± 0)	$< 0.035^*$
Female	28(1.572 ± 1.202)	15(1 ± 0)	
Smoking			
Smoker	43(1.605 ± 1.439)	28 (1 ± 0)	$< 0.035^*$
Non-smoker	14 (1.802 ± 1.37)	12 (1 ± 0)	
Comorbidities			
Present	36 (1.625 ± 1.068)	31 (1 ± 0)	$< 0.039^*$
Absent	24(1.715 ± 1.835)	9 (1 ± 0)	
Residence			
Urban	27 (1.880 ± 1.473)	17 (1 ± 0)	$< 0.0001^*$
Rural	33 (1.482 ± 1.355)	23 (1 ± 0)	
Treatment			
Yes	38 (1.564 ± 1.1564)	4 (1 ± 0)	$< 0.028^*$
No	22 (1.829 ± 1.7868)	36 (1 ± 0)	

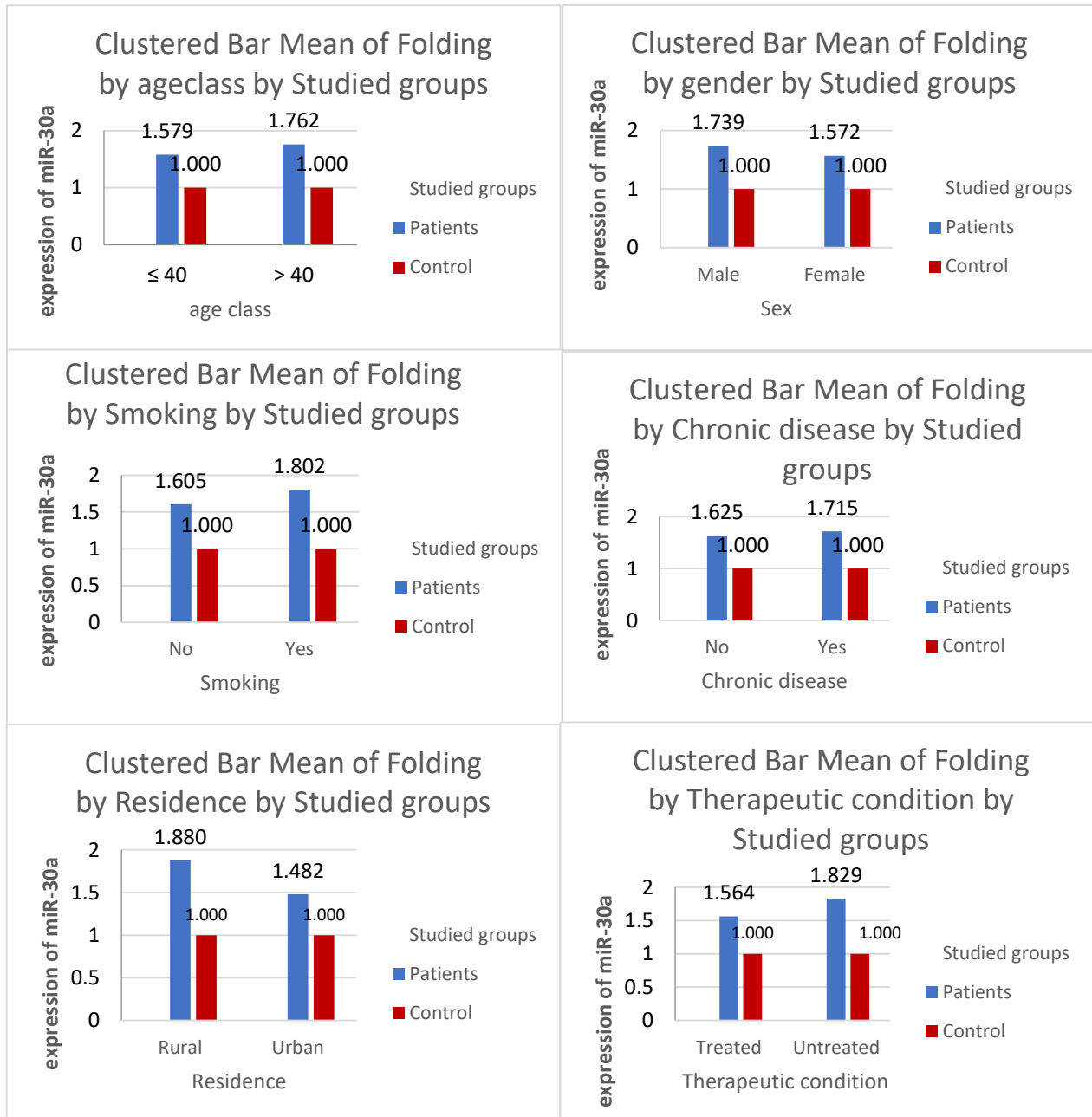


Figure (5): Subgroup analyses of miR-30a relative expression in healthy controls and patients with gastric diseases based on sex, age, residence, smoking status, chronic disease, and treatment status.

The regulation of miRNAs, including miR-30a, may be affected by various biological processes, including changes in gene expression, epigenetic modifications, and cellular senescence. Studies suggest that certain miRNAs are upregulated or downregulated with age, influencing aging-related diseases, including cancer. The relationship between aging and miRNA expression is complex, as older individuals may

experience changes in immune function, inflammation, and gastric microbiota, which can influence miRNA expression profiles (37).

The impact of sex on miR-30a gene expression in the context of gastric diseases, including gastric cancer, is a subject that has not been extensively studied, but some research suggests that gender-related biological differences, such as hormone levels, genetic factors,

and susceptibility to infections, may influence miRNA expression, including miR-30a. In this regard, gender-specific variations in the expression of miRNAs could play a role in the development and progression of gastric diseases, including gastric cancer (38).

There is evidence suggesting that these factors can influence the expression of certain microRNAs, including miR-30a, and thus potentially impact gastric cancer development and progression. While direct studies on residence-specific effects on miR-30a are limited, there are insights into how environmental factors, dietary habits, and genetic predispositions related to geographical regions could influence miR-30a regulation in gastric diseases (39).

Overexpression of miR-30a can enhance gastric cancer cell survival, proliferation, and migration by suppressing the expression of tumor-suppressive factors. For example, miR-30a can target genes like MMP-9 (Matrix Metalloproteinase-9), which is involved in the degradation of the

extracellular matrix and facilitates metastasis (40).

Receiver operating characteristic analysis

We investigated the differential expression of microR-30a in gastric patients compared to healthy controls and performed subgroup analyses to assess its diagnostic efficiency in various gastric diseases (gastric ulcers (GU), gastritis, gastric cancers (GC), and duodenal ulcers (DU)). The receiver-operating characteristic (ROC) curve analysis was used to evaluate the sensitivity, specificity, and overall diagnostic accuracy of miR-30a expression in differentiating between these groups. At a cut-off value of ≤ 1.67 , miR-30a showed a sensitivity of 88.3% (95% CI: 77.4 - 95.2) and a specificity of 72.5% (95% CI: 56.1 - 85.4) when compared to healthy controls. With an area-under-curve (AUC) of 0.865 ($p < 0.001$), the diagnostic performance was high. According to Figure (6), the positive likelihood ratio (+LR) was 3.21, whereas the negative likelihood ratio (-LR) was 0.16.

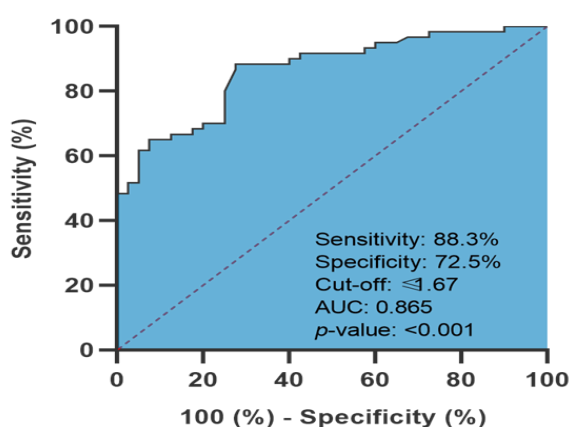


Figure (6): Examining the receiver -operating characteristic (ROC) curve for the expression of microR-30a to distinguish gastric patients from healthy controls. AUC: area under the curve.

In the subgroup analysis, miR-30a exhibited robust diagnostic accuracy in recognizing specific gastric diseases from healthy individuals. The results for

stomach ulcers showed a cut-off value of ≤ 1.56 (AUC = 0.886, $p < 0.001$, Figure 7 A), with sensitivity and specificity of 85.7% (95% CI: 42.1 -

99.6) and 75.0% (95% CI: 58.8 - 87.3), respectively. Likewise, in the gastritis subgroup, at a cut-off value of ≤ 1.64 (AUC = 0.857, $p < 0.001$, Figure 7 B), miR-30a produced a sensitivity of 90.3% (95% CI: 74.2 - 98.0) and a specificity of 72.5% (95% CI: 56.1 - 85.4). Moreover, in the gastric cancer subgroup, miR-30a demonstrated a sensitivity of 92.9% (95% CI: 92.86) and a specificity of 72.5% (95% CI: 56.1 - 85.4) at a cut-off value of ≤ 1.67 (AUC = 0.890, $p < 0.001$, Figure 7 C). The +LR and -LR for these subgroups ranged from 3.28 to 3.43 and 0.10 to 0.19, respectively, which confirm the ability of miR-30a to accurately identify specific gastric diseases. Furthermore, in the duodenal ulcer subgroup, miR-30a showed a sensitivity of 62.5% (95%

CI: 83.1 - 99.4) and a specificity of 95.0% (95% CI: 24.5 - 91.5) at a cut-off value of ≤ 1.11 (AUC = 0.831, $p = 0.003$, Figure 7 D). The +LR and -LR were both 3.38 and 0.1, respectively, indicating that miR-30a expression could effectively distinguish duodenal ulcers from healthy controls. According to a several studies, miRNAs have a significant and high role in regulating gene expression and have unique expression patterns in different malignancies, which highlights their significance for a correct diagnosis (41). Therefore, the identification of miRNA biomarkers may hold great potential to make tumor cells more receptive to specific treatment drugs and halting the spread of cancer (42- 44).

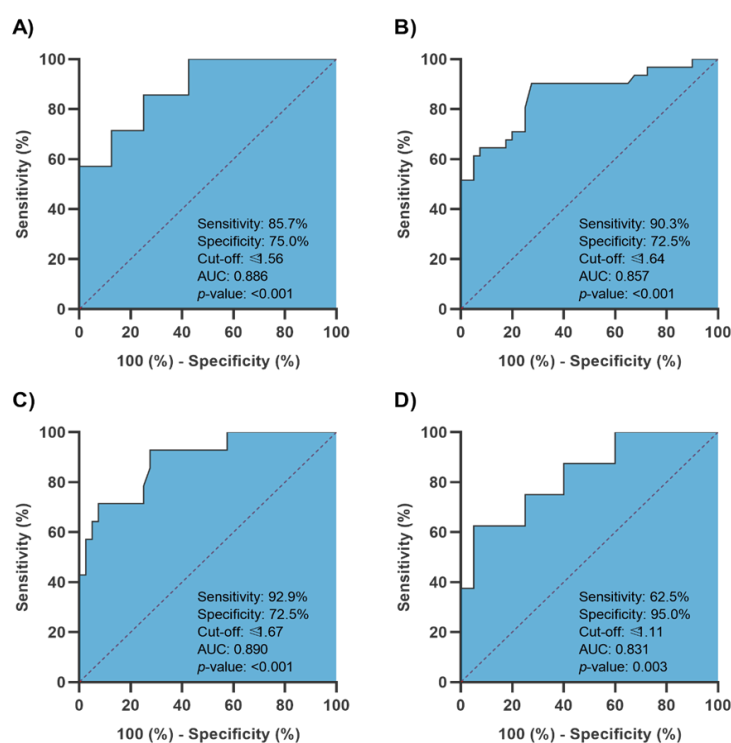


Figure (9): Receiver operating characteristic (ROC) curve analysis of miR-30a expression in distinguishing patients with specific gastric diseases from healthy controls: A) gastric ulcers, B) gastritis, C) gastric cancers, and D) duodenal ulcers. AUC: area under the curve.

Conclusion

Our study on miR-30a showed diagnostic power and also discriminatory ability among

individuals suffering from various gastric diseases and gastric cancer. It has been proven that miR-30a has high sensitivity and specificity for diagnosis,

providing its advantage as a biomarker for early prediction of gastric cancer.

Ethics approval

The University of Baghdad's College of Science Research Ethics Committee granted its authorization for this study's conduct (CSEC/0723/0055).

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