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# Evaluation of The Diagnostic Power of New Biochemical Biomarkers in The Early Detection of Sinusoidal Adenocarcinoma

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## Abstract

**Background:** One objective of this study was to analyze the diagnostic potential of selected serum biochemical markers, such as interleukin-6 (IL-6) and carbohydrate antigen 19-9 (CA 19-9), as well as molecular markers including microRNA-21 (miR-21) and caudal type homeobox transcription factor CDX2 in patients with adenocarcinoma.

**Materials and Methods**: This was a case-control study conducted at the Oncology Center Thi-Qar, Iraq during the period from March 2025 to December. We enrolled 138 subjects, including 72 patients with confirming diagnosis of adenocarcinoma, and controls that were apparently healthy in the number of 66. Cytokine concentrations of IL-6 and CA 19-9 were obtained using enzyme-linked immunosorbent assay (ELISA) kits. Quantification of miR-21 and CDX2 Expression Levels by qRT-PCR

**Results**: Serum levels of IL-6 and CA 19-9 in adenocarcinoma patients were significantly higher than the normal controls (38.72 ± 12.45 vs 8.64 ± 3.18 pg/mL, $p < 0.001$; 87.46 ± 35.18 vs, p <0ofU/mL U(mL)]< u) In summary, miR-21 expression was significantly increased and CDX2 expression decreased in patients compared to controls (all $p < 0.001$). CDX2 expression was positive in 40.3% of patients and 90.9% of controls. Using multiple linear regression, we found that miR-21 was the most significant predictor of disease severity ($\beta = 0.412$, $p < 0.001$; IL6 $\beta = 0.331$, $p = 0.002$; CA19-9 $\beta = 0.286$,$p=0.013$; CDX2 $\beta = -0.274$,$p=0.004$).

**Conclusions**: IL-6, CA 19-9, miR-21 and CDX2 were markedly dysregulated in the adenocarcinoma patients. These biomarkers

were all significantly associated with disease presence and severity, suggesting the validity of these new findings as reliable non-invasive diagnostic tools for early diagnosis and clinical appraisal of this chronic disease in the future.

**Keywords:** IL-6, CA 19-9, miR-21, CXD2, Sinusoidal Adenocarcinoma

**Introduction**

Because adenocarcinoma is a cancer of glandular tissue it is also associated with significant challenges for diagnosis and management. Given its heterogeneity, an appropriate staging system is necessary to guide therapy for improved outcomes. Recent advancements in molecular biology, histopathology and immunohistochemistry have greatly enhanced our understanding of adenocarcinoma (Jallod et al., 2025). Background: Adenocarcinoma continues to be one of the major contributors to worldwide cancer-associated morbidity and mortality, even with the great progress by using diagnostic imaging, molecular pathology & therapeutic interventions (Kanchustambham et al., 2026). Adenocarcinoma prognosis is stage-specific; those with earlier stage disease have a much better outcome than those presenting with metastatic spread or later complications. Thus, identifying novel noninvasive biomarkers that can recognize adenocarcinoma generations early-on is a significant research and clinical oncology hit target (Shweikeh et al., 2025). Recent developments in molecular medicine have indicated that biomarkers from a liquid biopsy, such as proteins, cytokines and microRNA, are likely important tools for non-invasive cancer detection and monitoring (Zafar et al., 2025).

Current diagnostic strategies, based on imaging modalities, histopathological analysis, and conventional tumour markers, usually lack the appropriate sensitivity and specificity to diagnose disease in its early stages. Invasive tissue biopsy may also not be possible at times and do not capture the heterogeneity of tumors at the molecular level. Thus, the need for minimal invasive biomarkers that accurately reveal tumor biology and diagnose disease even before clinical symptoms occur is rising. Recent studies suggest that combinations of multiple biomarkers may perform better than isolated markers as they may represent multiple biological pathways in tumor initiation and progression (Shweikeh et al., 2025).

One of the members from the MMP family, matrix metalloproteinase-9 (MMP-9, matrilysin), has drawn a lot of research interest as a promising cancer biomarker. MMP-9 is a member of the matrix metalloproteinase (MMP) family, a class of zinc-dependent proteolytic enzymes that mediate extracellular matrix (ECM) remodeling. MMP-9 is unlike most MMPs in that it is mostly secreted by epithelial and tumor cells. High expression of MMP-9 has also been described in many adenocarcinomas, such as colorectal and gastric, pancreatic and lung cancers (Huang, 2018). In addition, MMP-9 enhances tumor metastasis, angiogenesis, and invasion by degrading extracellular matrix (ECM) components and activating signaling molecules. Several studies have shown that the increased concentration of both circulating and/or tissue MMP-9 is correlated with tumor progression and poor clinical prognosis, supporting the value of MMP-7 as a cancer biomarker for early diagnosis and prognostic. Additionally, MMP-7 expression may upregulate during the initiation of carcinogenesis, making it an attractive primary candidate for diagnostic application (Liu et al., 2021).

It is now widely accepted that one of the hallmarks of cancer is inflammation, so that overproduction of IL-6 with dysregulated IL-6 signaling pathways may lead to inflammatory disorders and diseases caused by autoimmunity as well as cancer since this is indicative of a prominent role of IL-6 in the proinflammatory cytokine family (Salman et al., 2024). Chronic over-activation of IL-6 leads to chronic inflammation, tumor growth, and metastatic spreading by activating signaling pathways in which JAK/STAT3, MAPK, and PI3K/Akt are involved. High serum concentrations of IL-6 have been found in patients with adenocarcinomas and are often linked with the progression of disease, resistance to therapy and poor prognosis. Notably, elevated levels of IL-6 may develop early during carcinogenic progression and may serve as a reflection of an inflammatory process prior to the appearance of overt tumors. Thus, IL-6 is now considered a potential candidate biomarker for cancer detection and monitoring (Johnson et al., 2018).

MicroRNAs (miRNAs) have recently transformed the landscape of cancer biomarker research. The miRNAs that are most frequently studied in this area include oncogenic microRNAs (miRs), with microRNA-21 (miR-21) being one of the most characterized. MiR-21 inhibits expression of numerous tumor suppressor genes like PTEN, PDCD4 and SMAD7, which results in pro-proliferative, invasive, angiogenic and anti-apoptotic effects (Mumtaz et al., 2025). MiR-21 have been reported to be aberrantly expressed in many adenocarcinomas and correlate with tumor progression and metastasis. Due to simple availability of miR-21 in serum, plasma and other body fluids, it is a promising non-invasive cancers biomarker. Recent systematic reviews and meta-analyses have validated the diagnostic ability of circulating miR-21 in colorectal and various gastroenterological cancers (Shariati et al., 2023).

New key studies unearthed circulating miRNAs in cancer diagnosis. The rapid developments in liquid biopsy technologies, bio-sensor platforms to isolate, and machine-learning methods have remarkably enhanced the sensitivity and specificity of miRNA-based diagnostic assays. Of the numerous miRNAs analyzed, miR-21 shows strong and consistent diagnostic capability and represents one of the strongest clinical candidates to be translated. In addition, biomarker panels including miRNAs may be more effective than other protein and inflammatory markers in the detection of early-stage cancers (Metcalf, 2024).

The reasons for combining the MMP-7, IL-6, and miR-21 is based on the complementary biological effects of their individual roles. MMP–7 is indicative of extracellular matrix reorganization and might serve as a biomarker of tumor invasiveness; IL–6 is a mediator of inflammation-induced tumor promotion; and miR–21 reflects downstream molecular changes on a post-transcriptional genetic level. These biomarkers do not cover atypical retinoid. Incorporation of these markers within a multimarker diagnostic panel may improve diagnostic sensitivity and specificity, while minimizing the disadvantages of single-markers. Systematic reviews of blood-based biomarkers focused on new ground and combined molecular signatures recently acquired the role of frontiers for optimal cancer screening and early diagnosis (Shweikeh et al., 2023).

Thus the current study investigates the diagnostic performance of MMP-7, IL-6, and miR-21 as novel biochemical markers for early detection of adenocarcinoma. The current study explored the individual as well as the combined diagnostic performance of these biomarkers, aiming the provision of near-to-accurate, minimally invasive, clinically applicable, early cancer detection strategies in a cost-effective manner for effective transformation of research evidence into practice towards improved patient outcomes and reduced cancer morbidity and mortality.

### *Patients and Methods*

This case-control study performed at the Oncology Center in Thi-Qar Governorate, Iraq between March 2025 and December 2025. The purpose was to assess the diagnostic utility of several selected tumor markers, including two biochemical (interleukin-6 [IL-6] and carbohydrate antigen 19–9 [CA 19–9]), as well as two molecular biomarkers (microRNA-21 [miR-21], caudal-type homeobox transcription factor 2 [CDX2]) in the early diagnosis of adenocarcinoma.

### *Ethical Considerations*

Approval for the study protocol was obtained from the Institutional Ethical Committee of the Oncology Center in Thi-Qar and relevant health authority All procedures were performed in accordance with ethical principles according to the Declaration of Helsinki. All the participants provided their written informed consent prior to being enrolled in the study. The description of objectives of the study, method of sample collection, confidentiality and voluntary nature of participation were given by qualified researchers and lab technicians during face to face interaction as a part of collection process.

### *Study Population*

A total of 138 participants were enrolled in the study comprising of 72 patients with adenocarcinoma (34) and controls (66). Participants were between ages 25 and 75.

Adenocarcinoma diagnosis was confirmed by specialist oncologists on clinical examination, imaging studies, endoscopic findings (where applicable), and histopathological assessment of biopsy specimens. Criteria for selection of patients included that it had to be a newly diagnosed patient without having received any form of chemotherapy, radiotherapy, immunotherapy or targeted therapy or surgical intervention before blood collection. Healthy controls were selected from volunteers with no prior history of malignant disease (MD) and without the presence of incident acute or chronic inflammatory disorders.

### *Inclusion and Exclusion Criteria*

To be included, patients had to have a confirmed diagnosis of adenocarcinoma based on clinical, radiological and histopathological criteria, have been newly diagnosed and treatment-naïve at the time of recruitment, aged ≥ 18 years old and provided written informed consent for participation in the study. Healthy controls were recruited from apparently healthy volunteers with no previous history of malignant disease and were frequency matched to patients on an age and sex as far as possible basis. Participants who had any other malignancy, autoimmune disorders and chronic inflammatory diseases, severe hepatic or renal dysfunction (as determined by the investigator), active infection(s) requiring treatment, inflammatory bowel disease, history of organ transplantation (excluding corneal grafting), recent major surgery or significant trauma within 28 days before blood sample collection as well as participants received chemotherapy/radiotherapy/immunotherapy/targeted therapy at < 4 weeks before blood sampling and/or other anticancer treatment were excluded from the study. Participants who refused to give informed consent were also excluded.

***Clinical and Demographic Assessment***

Demographic and clinical data were collected in person or extracted from medical records with a standardized data collection form. We collected sociodemographic options (such as age, sex, skyscraper and urban living area), smoking behavior, body mass file (BMI), family history of cancer, tumor localization [including histological kind, histological subtype grade (low-grade vs. high-grade briefly nasal carcinoma) and lymph node metastasis], distant metastases and clinical stage whenever possible.

Specialist physicians at the Oncology Center in Thi-Qar performed clinical evaluation and staging of disease.

***Sample Collection***

About 5 mL of venous blood from each sample was collected in sterile disposable syringes under aseptic conditions. Three milliliters was transferred into plain gel tubes to yield serum, and the remainder 2 mL was collected into EDTA-containing tubes for molecular analyses. Venous blood samples in plain tubes were kept at room temperature to clot and then centrifuged at 3000 rpm for 10 minutes. Aliquoted separated serum into sterile Eppendorf tubes and stored them at −20 °C until biochemical analyses were performed. Analytical studies were performed on EDTA whole blood samples that were frozen at −80°C until RNA extraction and other molecular investigations.

***Biochemical Analysis***

***Measurement of IL-6***

Serum IL-6 concentrations were measured with ELISA kits obtained from an American company and were performed according to the manufacturer's instructions. Each sample and standards were assayed in duplicate for assay precision and reproducibility.

***Measurement of CA 19-9***

CA 19-9 serum levels were assessed through the use of a commercially available sandwich ELISA kit in accordance with the manufacturer's instructions. Each assay was performed with calibration standards and quality-control samples. Absorbance values were measured by a microplate reader and concentrations were calculated from the standard calibration curve. All analytical steps involved internal quality-control procedures to ascertain the reliability and accuracy of laboratory results.

***Molecular Analysis***

***Quantification of miR-21 Expression***

Total RNA was extracted from peripheral blood samples, including small RNAs, using a commercially available RNA extraction kit according to the manufacturer's instructions. This was followed by reverse transcription to generate complementary DNA (cDNA). MiR-21 expression was quantified using quantitative real-time polymerase chain reaction (qRT-PCR). Endogenous reference gene for normalization: U6. Relative expression levels were quantified by the comparative 2^-ΔΔCt method.

***CDX2 Immunohistochemistry Procedure***

Standard deparaffinization and rehydration through graded alcohols were performed on up to around 4 μm thick formalin-fixed paraffin embedded (FFPE) tissue sections mounted on positively charged glass slides. Heat induced epitope retrieval (HIER): Microwave in alkaline buffer, such as Tris-EDTA pH 9.0 or Target Retrieval Solution pH 9, at 95–100°C for 20–40 minutes (pressure cooker or water bath) and cool down and wash with tris - buffered saline + Tween20 (TBS-T). Endogenous peroxidase activity was inhibited with either 3% hydrogen peroxide Hydrogen Peroxide or a commercial peroxidase-inhibiting reagent for 5–10 min at room temperature. Nonspecific binding was reduced using a protein-blocking solution (such as 1% bovine serum albumin or regular serum, prolonged) for approximately 10–20 minutes. Sections were incubated with an optimized dilution (typically at 1:50–1:200 for concentrated antibodies or ready-to-use formulations) of either a mouse monoclonal or rabbit monoclonal anti-CDX2 antibody, for 15–60 minutes at room temperature, or overnight at 4°C depending on the antibody clone and detection system. Washed bound primary antibody was determined using a polymer-based detection system (EnVision FLEX), which is an HRP-conjugated secondary antibody or polymer for 20–30 min at room temperature, followed by chromogenic visualization with DAB substrate for 5–10 min. These sections were counterstained with hematoxylin, dehydrated through graded alcohols, cleared in xylene (or substitute), and then cover slipped using permanent mounting media. The assay was validated by running a positive (sinusoidal adenocarcinoma) and negative control (tonsil or omission of primary antibody) with each batch of staining; expression of nuclear CDX2 in all slides was assessed by light microscope (Borrisholt et al., 2013).

***Statistical Analysis***

Statistical Package for Social Sciences (SPSS) software version 26.0 (IBM Corp., Armonk, NY, USA), was used for data

analysis. All continuous variables were presented as means ± standard deviation (SD), while categorical variables were reported through frequencies and percentages. Accordingly, associations between clinicopathological characteristics and levels of each biomarker were assessed using Pearson's or Spearman's correlation analyses as applicable. One-way analysis of variance (ANOVA) was performed to analyze differences among clinical stages and pathological grades, respectively. Having studied the above biomarkers, we performed multivariate logistic regression analysis on them to find out whether they are independently diagnostic or not. Statistical significance was defined as $p < 0.05$.

Table 1 presents the demographic characteristics of adenocarcinoma patients and healthy controls. The distribution of age groups did not differ significantly between the two groups ($\chi^2 = 3.36$, $P = 0.34$), indicating successful age matching. The largest proportion of patients (30.6%) belonged to the 45–54 years age group, whereas 33.3% of controls were aged 55 years or older. Similarly, no significant difference was observed in gender distribution between patients and controls ($\chi^2 = 0.08$, $P = 0.77$), with males representing 56.9% of patients and 54.5% of controls. Residence status also showed no significant variation between the two groups ($\chi^2 = 0.42$, $P = 0.52$), as urban residents constituted the majority of both patients (59.7%) and controls (54.5%).

**Results**

**Table 1. General information of patients with sinusoidal adenocarcinoma and comparison with healthy control group**

| Indicators | | `Patients (No. = 72) | | Control (No. = 66) | | Chi Square | P value (Sig.) |
|---|---|---|---|---|---|---|---|
| | | Freq. | % | Freq. | % | | |
| Age/Years | 25-34 | 12 | 16.7 | 13 | 19.7 | 3.36 | 0.34 (NS) |
| | 35-44 | 18 | 25 | 14 | 21.2 | | |
| | 45-54 | 22 | 30.6 | 17 | 25.8 | | |
| | ≥ 55 | 20 | 27.8 | 22 | 33.3 | | |
| Gender | Male | 41 | 56.9 | 36 | 54.5 | 0.08 | 0.77 (NS) |
| | Female | 31 | 43.1 | 30 | 45.5 | | |
| Residence | Rural | 29 | 40.3 | 30 | 45.5 | 0.42 | 0.52 (NS) |
| | Urban | 43 | 59.7 | 36 | 54.5 | | |

**NS: Non-significant at P>0.05**

Figure 1 exhibited the distribution of patients with sinusoidal adenocarcinoma according to stages of sinusoidal adenocarcinoma, it shows that the prevalence of T(tumor) stage of sinusoidal adenocarcinoma (n=45, 62.5%) was the most common among the studied patients, followed by N(Nodule) stage (n=18; 25%); M(Metastasis) stage of sinusoidal adenocarcinoma (n=9; 12.5%).

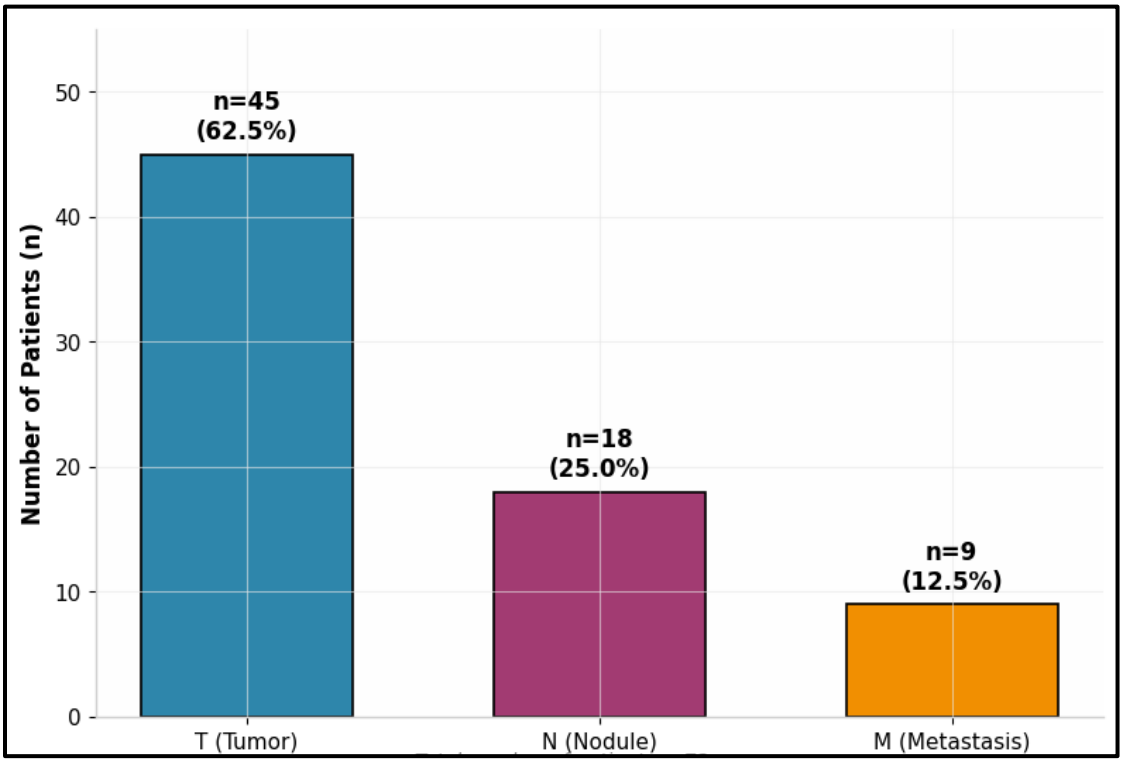


**Figure 1. Distribution of patients with sinusoidal adenocarcinoma according to stages of the cancer**

As demonstrated in Table 2, serum levels of IL-6 and CA 19-9 were significantly elevated in patients with adenocarcinoma compared to healthy controls ($P < 0.001$). Patients had a significantly higher mean serum IL-6 level than controls (38.72±12.45 pg/mL vs 8.64±3.18 pg/mL), reflecting the intensity of an inflammatory response in relation to tumor development and progression. Likewise, CA 19-9 concentrations were significantly higher in the patients (87.46 ± 35.18 U/mL) than that in controls (18.53 ± 7.42U/mL), which indicates greater production of a tumor-associated antigen by malignant cells.

**Table 2. Measurement of biochemical markers levels between patients with sinusoidal adenocarcinoma and control subjects**

| Groups | Patients Mean ± SD | Control Mean ± SD | T Test (P Value) |
|---|---|---|---|
| **IL-6 (pg/mL)** | **38.72 ± 12.45** | **8.64 ± 3.18** | **t = 17.52 p < 0.001 (HS)** |
| **CA 19-9 (U/mL)** | **87.46 ± 35.18** | **18.53 ± 7.42** | **t = 15.84 p < 0.001 (HS)** |

**HS: High significant at P<0.001**

Table 3 shows the comparison of relative miR-21 expression levels between sinusoidal adenocarcinoma patients and healthy controls. The results revealed that the mean expression level of miR-21 was markedly higher in patients with sinusoidal adenocarcinoma (**6.45 ± 0.98**) than in healthy individuals (**1.11 ± 0.41**). This difference was highly statistically significant ($t = 19.82$ $p < 0.002$).

**Table 3. Measurement of molecular marker levels between patients with sinusoidal adenocarcinoma and control subjects**

| Groups | Patients<br>Mean ± SD | Control<br>Mean ± SD | T Test<br>(P Value) |
|---|---|---|---|
| **miR-21**<br>**(Fold change)** | **6.45 ± 0.98** | **1.11 ± 0.41** | **t = 19.82**<br>**p < 0.002 (HS)** |

**HS: High significant at P<0.001**

Distribution of CDX2 expression in adenocarcinoma patients and healthy controls (Table 4) There were 29 (40.3%) patients positive for CDX2 and 60 (90.9%) negative controls, while 43 (59.7%) patients exhibited negative expression versus only 6 (9.1%) in control cases. Statistical analysis showed a significant difference between both groups ($\chi^2 = 38.94$, $p < 0.001$)

**Table 4. Distribution of CDX2 expression among patients with sinusoidal adenocarcinoma and control subjects**

| CDX2 | | Patients (No. = 72) | Control (No. = 66) | Chi-square (P Value) |
|---|---|---|---|---|
| Positive | N | **29** | **60** | |
| | % | **40.30%** | **90.90%** | |
| Negative | N | **43** | **6** | |
| | % | **59.70%** | **9.10%** | **$\chi^2$ = 38.94**<br>**p < 0.001 (HS)** |

Results of multiple linear regression analysis showing independent association (in the table) between IL-6, CA 19–9, miR-21 and CDX2 with adenocarcinoma grades/stages are shown in Table 5 and figure 2. The results showed that each of the four biomarkers acted as a statistically significant independent predictor of disease severity. Of the markers studied, positivity for miR-21 had by far the strongest positive association with tumor stage ($\beta = 0.412$, $p < 0.001$), suggesting that increased expression of miR-21 was associated with a more advanced disease at time of diagnosis. Likewise, in the second analysis IL-6 was the most important predictor ($\beta = 0.331$, $p = 0.002$), again bolstering potential links between inflammation and tumor development. Consistent with this, both CA 19-9 ($\beta = 0.286$, $p = 0.013$) and CEA ($\beta = 0.31$, $p < 0.001$) had strong positive correlations to disease severity again indicating that higher serum levels correlate with greater tumor burden in this study cohort. In contrast, CDX2 was significantly inversely correlated with disease stage ($\beta = -0.274$, $p = 0.004$), indicating that reduced secretion of this expression-associated protein was more likely to correlate with advanced and aggressive rather than early-stage disease.

**Table 5. Multiple linear regression analysis for predictors of sinusoidal adenocarcinoma severity/staging**

| Predictor | β | B | SE | t-value | P-value |
|---|---|---|---|---|---|
| **IL-6** | **0.331** | **0.054** | **0.017** | **3.18** | **0.002 (HS)** |
| **CA 19-9** | **0.286** | **0.023** | **0.009** | **2.56** | **0.013 (S)** |
| **miR-21** | **0.412** | **0.682** | **0.154** | **4.43** | **<0.001 (HS)** |
| **CDX2** | **-0.274** | **-0.591** | **0.198** | **-2.98** | **0.004 (HS)** |

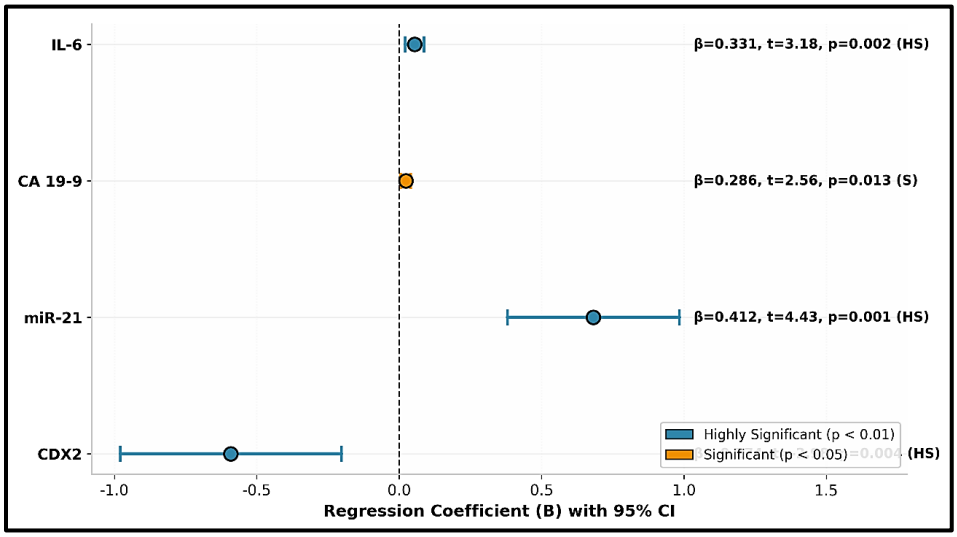


**Figure 2. Forest plot for multiple linear regression analysis for predictors of sinusoidal adenocarcinoma severity/staging**

**Discussion**

Selected biochemical and molecular biomarkers in adenocarcinoma of the patient with negative finding the present study assessed the diagnostic significance of IL-6, CA 19-9, miR-21 and CDX2 for adenocarcinoma. These results reveal prominent changes in all the biomarkers explored that were seen in patients but not healthy controls, pointing their potential diagnostic and prognostic value. Moreover, regression analysis indicated that tumor stage was only predicted by these markers independently, suggesting their potential clinical utility in disease monitoring and prognostic assessment.

Demographic analysis failed to identify any statistical differences between patients and controls in respect of age, gender and residence. This matching is necessary to reduce the impact of confounding variables on biomarker expression and thus increase confidence in real associations. Previous biomarker studies of gastrointestinal and colorectal adenocarcinomas have also suggested similar demographic matching (Xu et al. 2024).

Among the main results of this study is that serum IL-6 levels were significantly higher among adenocarcinoma patients. IL-6 is a pleiotropic proinflammatory cytokine produced and released by different cell types that controls key aspects of tumor-associated inflammation and cancer progression. The chronic IL-6 signalling promotes tumour cells proliferation, angiogenesis, immune escape and metastatic spread through activative JAK/STAT3 pathway. Higher concentrations of IL-6 in patients with adenocarcinoma help corroborate that inflammation is a significant contributing factor to the pathogenesis of this malignant disease. These results are congruent with their observations reported by Johnson et al. (2022) that showed increased serum IL-6 levels in patients with gastrointestinal adenocarcinomas and a clinically relevant relation to tumor progression. Similarly, Kumari et al. (2016) found increased expression of IL-6 correlated with poor prognosis and advanced-stage malignancies. The present findings therefore reinforce the growing evidence that IL-6 may serve as a valuable non-invasive biomarker for cancer detection and progression assessment.

The study also demonstrated significantly elevated serum CA 19-9 levels in patients compared with healthy controls. CA 19-9 is among the most widely used tumor-associated antigens in clinical oncology and is commonly elevated in gastrointestinal malignancies. Increased CA 19-9 levels are believed to reflect enhanced production and secretion by malignant epithelial cells, as well as increased tumor burden. The substantial elevation observed in the current study agrees with findings reported by Ballehaninna and Chamberlain (2012), who identified CA 19-9 as an important biomarker for diagnosis and prognosis in adenocarcinoma-related cancers. Despite the low sensitivity of CA 19-9 alone for early diagnosis, combining it with other biomarkers can significantly improve diagnostic performance. This strongly significant association obtained in the present study sustains the clinical relevance of CA 19-9 as part of a multicancer diagnostic strategy.

As for molecular biomarkers, the expression of miR-21 was significantly upregulated in patients with adenocarcinoma. MiR-21 is presently one of the more well characterized oncogenic microRNAs, commonly overexpressed in a diverse array of

cancers. The biological functions of miR-21 are mediated mainly through direct repression of tumor suppressor genes like PTEN and PDCD4, thereby promoting cell proliferation, invasion, angiogenesis and evasion of apoptosis. That obtained results conform with that of Shariati et al. (2023) reported that have miR-21 is significantly over-expressed in gastrointestinal cancers; they summarize its potential as both a diagnostic and prognostic marker. Similarly, Peng et al. found that increased circulating levels of miR-21 are correlated with tumor progression and poor clinical outcomes. Increase of miR-21 expression in patients compared to normal controls seems that it could be a specific sensitive biomarker for epithelial cancer detection and monitoring. Along with downregulated expression of miR-21 most significantly in adenocarcinoma patients, CDX2 expression was also significantly reduced when comparing adenocarcinoma patients and healthy controls. CDX2 is an intestinal-specific transcription factor responsible for regulating epithelial differentiation and maintaining normal intestinal homeostasis. Loss or reduction of CDX2 expression has been linked to dedifferentiation, tumor aggressiveness, and unfavorable prognosis. The significantly lower frequency of positive CDX2 expression observed in this study suggests disruption of normal cellular differentiation pathways during tumor development. These results are consistent with those reported by Dalerba et al. (2016), they showed that loss of CDX2 expression was associated with poor differentiation and unfavorable prognosis in colorectal adenocarcinoma. Similarly, Tomasello et al. (2018) found that CDX2 expression was significantly decreased with higher disease stage (final paper), indicating that loss of CDX2 correlates with both a more advanced disease state and the increased metastatic ability of its tumor. Thus, CDX2 may act as a reliable marker for diagnosis as well as an indicator of tumor biological behavior.

In particular, biomarker associations with disease severity can also play an important role in the current study. Among the proteins and microRNAs determined to be associated with adenocarcinoma stage based on p value for regression analysis, IL-6, CA 19-9, and miR-21 were positively associated while CDX2 demonstrated a statistically significant inverse association. MiR-21 alone exhibited the greatest predictive ability of all markers tested, supporting the contention that molecular changes might precede and better reflect disease progression than standard biochemical measures. All of these findings further contribute to the already growing consensus that circulating microRNAs are extremely attractive cancer diagnostic and prognostic biomarkers. The negative correlation of CDX2 expression with the stage of tumor also supports its role as a protective marker for differentiation, conspicuous by its loss upon malignant progression.

The analysis of IL-6, CA 19-9, miR-21 and CDX2 taken together has several advantages over single biomarker approaches. Each marker reflects a unique biological process in carcinogenesis; IL-6 is associated with inflammation, CA 19-9 is reflective of tumor-associated antigen production, miR-21 of oncogenic gene regulation and CDX2 of epithelial differentiation. Thus, using combined biomarkers may better exploit the heterogeneity of tumor biology and yield higher diagnostic sensitivity-simultaneously addressing both biological characteristics that contribute to neoplastic development. Similar conclusions have been reported recently with respect multi-marker panels compared to single marker approach for early cancer detection (Xu et al., 2024).

## Conclusion

The present findings indicate that IL-6, CA 19-9, miR-21, and CDX2 are significantly associated with adenocarcinoma and may serve as valuable biomarkers for diagnosis and disease staging. The observed alterations in these markers reflect key molecular and inflammatory mechanisms underlying tumor development. Their combined assessment may enhance the early detection of adenocarcinoma and improve clinical decision-making. Further large-scale multicenter studies are warranted to validate these findings and establish standardized diagnostic cutoff values for routine clinical application.

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