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Helicobacter pylori Infection and miRNA-139 Dysregulation in Hepatocellular Carcinoma: A Clinical Biochemical Case–Control Study

Tarek S. Ebrahim[1], Ahmed M. El-Hilaly[2], Heba A. Elshahawy[3], Tarek M. Okda[4]

[1] *Medical laboratory specialist Gastrointestinal Surgery Center, Faculty of Medicine, Mansoura University, Egypt.*
[2] *Gastrointestinal Surgery Center, Faculty of Medicine, Mansoura University, Egypt.*
[3] *Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Egypt.*
[4] *Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, Damanhour University, Egypt.*
Author for correspondence: Tarek M. Okda, Professor of Biochemistry and Molecular Biology, Faculty of Pharmacy, Damanhour University, Egypt.

Abstract
Background and aim*: Hepatocellular carcinoma (HCC) remains one of the leading causes of cancer-related mortality worldwide. Emerging evidence suggests that Helicobacter pylori infection may contribute to hepatocarcinogenesis through chronic inflammation, immune dysregulation, oxidative stress, and modulation of molecular signaling pathways. However, the clinical and molecular relationship between H. pylori infection and HCC progression remains incompletely understood.* ***Methods****: A prospective case–control study was conducted on 180 participants recruited from the Gastrointestinal Surgery Center, Mansoura University, Egypt, between 2023 and 2024. Participants were divided into three groups: HCC patients with H. pylori infection (n=60), HCC patients without H. pylori infection (n=60), and healthy controls (n=60). Demographic characteristics, hematological parameters, liver function tests, inflammatory markers, viral hepatitis markers, tumor biomarkers, and miRNA-139 expression were assessed. miRNA-139 expression was quantified using qRT-PCR.* ***Results:*** *HCC patients demonstrated significantly older age and marked male predominance compared with controls. miRNA-139 expression was significantly downregulated in the HCC/H. pylori group (0.373±0.0138) compared with controls (1.02±0.0118) and HCC patients without H. pylori infection (0.759±0.0273). Serum AFP levels were markedly elevated in HCC/H. pylori patients (29.5±4.41 IU/mL) compared with HCC patients without H. pylori infection (16.9±3.54 IU/mL) and controls (2.04±0.126 IU/mL). Similar trends were observed for CEA and CA19.9. Significant elevations in ALT, AST, ALP, GGT, bilirubin, CRP, creatinine, and uric acid were also observed in HCC groups, accompanied by reduced albumin, hemoglobin, RBCs, and platelet counts. HCV positivity was highly prevalent among HCC patients.* ***Conclusion:*** *H. pylori infection may aggravate liver dysfunction and tumor progression in HCC through inflammatory and molecular mechanisms involving miRNA-139 dysregulation. miRNA-139 may represent a promising biomarker for early diagnosis and therapeutic targeting in HCC patients with H. pylori co-infection.*

Keywords: *Hepatocellular carcinoma, Helicobacter pylori, miRNA-139, AFP, liver cancer, inflammation, biomarkers.*

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I. Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver and represents approximately 75–90% of all liver cancers worldwide. It remains one of the leading causes of cancer-related mortality, ranking among the top causes of cancer deaths due to its aggressive nature, late diagnosis, high recurrence rate, and limited therapeutic responsiveness in advanced stages [1,2]. Despite substantial progress in diagnostic and therapeutic approaches, the prognosis of HCC patients remains poor, particularly in developing countries where viral hepatitis and chronic liver diseases are highly prevalent [3].

The pathogenesis of HCC is a multifactorial and complex process involving chronic hepatic inflammation, oxidative stress, fibrosis, cirrhosis, genetic instability, and dysregulation of intracellular signaling pathways [4]. Chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) remains the major etiological factor associated with hepatocarcinogenesis, especially in the Middle East and North Africa,

including Egypt, where HCV infection has historically represented a major public health burden [5]. Additional risk factors include alcohol consumption, aflatoxin exposure, metabolic dysfunction-associated fatty liver disease (MAFLD), obesity, diabetes mellitus, and chronic inflammatory disorders [6].

In recent years, increasing attention has been directed toward the potential contribution of bacterial infections and gut microbiota dysbiosis in liver diseases and carcinogenesis. Among these microorganisms, Helicobacter pylori has emerged as a potentially important extra-gastric pathogen implicated in chronic liver injury and HCC progression [7]. H. pylori is a Gram-negative, spiral-shaped, microaerophilic bacterium that colonizes the gastric mucosa and infects nearly half of the world's population [8]. It is well established as the principal etiological agent of chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma, and gastric adenocarcinoma [9]. However, accumulating evidence suggests that H. pylori may exert systemic effects extending beyond the stomach, including cardiovascular, hematological, autoimmune, metabolic, and hepatobiliary disorders [10].

Several studies have demonstrated the presence of H. pylori DNA and bacterial antigens in hepatic tissues, bile ducts, gallbladder specimens, and liver biopsies obtained from patients with chronic liver diseases and HCC [11,12]. The exact mechanism by which H. pylori contributes to hepatocarcinogenesis remains incompletely understood; however, several pathogenic mechanisms have been proposed. Chronic H. pylori infection may induce persistent systemic inflammation through activation of inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and nuclear factor-κB (NF-κB) signaling pathways [13]. These inflammatory mediators may subsequently promote hepatocyte injury, fibrosis, angiogenesis, cellular proliferation, and inhibition of apoptosis, thereby facilitating malignant transformation [14].

Moreover, virulence factors produced by H. pylori, particularly cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA), have been shown to interfere with intracellular signaling pathways regulating cellular differentiation, proliferation, migration, and genomic stability [15]. CagA-positive strains are particularly associated with increased inflammatory responses and carcinogenic potential through activation of MAPK/ERK, PI3K/Akt, and Wnt/β-catenin signaling pathways [16]. Additionally, oxidative stress induced by chronic H. pylori infection may lead to excessive generation of reactive oxygen species (ROS), DNA damage, mitochondrial dysfunction, and chromosomal instability, all of which contribute to carcinogenesis [17].

Recently, molecular biomarkers have attracted significant interest in cancer research because of their potential role in early diagnosis, prognosis, and targeted therapy. Among these biomarkers, microRNAs (miRNAs) have emerged as critical regulators of gene expression and tumor biology [18]. miRNAs are small non-coding RNAs approximately 18–25 nucleotides in length that regulate post-transcriptional gene expression through mRNA degradation or translational repression [19]. Dysregulation of miRNAs has been implicated in various hallmarks of cancer including proliferation, invasion, metastasis, angiogenesis, immune escape, and resistance to apoptosis [20].

miRNA-139 is recognized as an important tumor suppressor miRNA in several human malignancies, including hepatocellular carcinoma [21]. Previous investigations have demonstrated that miRNA-139 regulates multiple oncogenic pathways involved in tumor progression, epithelial–mesenchymal transition, metastasis, and angiogenesis [22]. Downregulation of miRNA-139 has been associated with enhanced tumor aggressiveness, poor prognosis, increased metastatic potential, and reduced overall survival in HCC patients [23]. Furthermore, inflammatory and infectious conditions may influence miRNA expression profiles, suggesting a possible molecular link between chronic H. pylori infection and HCC progression [24].

In addition to molecular alterations, biochemical and hematological abnormalities play a crucial role in evaluating liver dysfunction and tumor progression in HCC patients. Elevated serum alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19.9), liver enzymes, inflammatory markers, and coagulation abnormalities are frequently associated with advanced disease stages and poor prognosis [25]. Combining conventional biomarkers with molecular indicators such as miRNA expression may improve diagnostic accuracy and provide deeper insight into disease pathogenesis [26].

Although several studies have investigated the relationship between H. pylori infection and liver diseases, the precise interaction between H. pylori, miRNA dysregulation, and hepatocellular carcinoma remains insufficiently explored, particularly among Egyptian patients. Therefore, the present study aimed to investigate the possible association between H. pylori infection and hepatocellular carcinoma through comprehensive clinical, biochemical, hematological, inflammatory, and molecular assessment, with special emphasis on the expression pattern of miRNA-139 and its potential role as a diagnostic and prognostic biomarker in HCC patients.

II. Subject and Methods

Study Design:

The present work was designed as a prospective case–control study conducted between January 2023 and March 2024 at the Mansoura University, Egypt. The study aimed to investigate the possible association between Helicobacter pylori infection and hepatocellular carcinoma (HCC), with special emphasis on biochemical alterations, inflammatory status, tumor biomarkers, and molecular expression of miRNA-139.

Ethical approval was obtained from the Ethical Committee of the Faculty of Pharmacy, Damanhour University. All participants signed written informed consent before enrollment in accordance with the Declaration of Helsinki guidelines for human research [27].

Study Population

A total of 180 individuals were enrolled in this study and classified into three groups (n = 60 for each group): Group I: Patients diagnosed with hepatocellular carcinoma associated with positive Helicobacter pylori infection. Group II: Patients diagnosed with hepatocellular carcinoma without H. pylori infection. Group III: Apparently healthy age- and sex-matched control subjects.

The diagnosis of hepatocellular carcinoma was established according to clinical examination, radiological imaging findings, elevated serum alpha-fetoprotein (AFP), and histopathological examination whenever applicable according to international diagnostic criteria [28]. Healthy control subjects had no history of chronic liver disease, malignancy, autoimmune disorders, inflammatory diseases, or active infection.

Inclusion Criteria

The following criteria were used for patient enrollment: Adult patients above 18 years of age, confirmed diagnosis of hepatocellular carcinoma, patients willing to participate in the study and availability of complete clinical and laboratory investigations.

Exclusion Criteria

Patients were excluded if they had: Recent administration of antibiotics, proton pump inhibitors, or bismuth compounds within four weeks before sampling, other malignancies, autoimmune diseases, severe renal or cardiac diseases, immunosuppressive therapy and pregnancy or lactation. These exclusion criteria were selected to avoid factors that could interfere with inflammatory biomarkers, H. pylori detection, or miRNA expression analysis [29].

Clinical Assessment

All participants underwent comprehensive clinical evaluation including detailed medical history, smoking history, medication history, physical examination, and radiological assessment.mDemographic characteristics including age and sex were recorded. Clinical manifestations related to chronic liver disease and hepatocellular carcinoma were also evaluated.

Blood Sample Collection

Approximately 10 mL of fasting venous blood samples were collected from each participant under complete aseptic conditions. The collected blood samples were divided as follows: EDTA tubes for complete blood count analysis, sodium citrate tubes for coagulation profile assessment and plain tubes for serum separation. Samples were centrifuged at 3000 rpm for 10 minutes, and serum was separated and stored at −80°C until biochemical and molecular investigations were performed.

Hematological Investigations

Complete blood count (CBC) parameters including hemoglobin concentration (Hb), red blood cell count (RBCs), white blood cell count (WBCs), and platelet count (PLTs) were analyzed using an automated hematology analyzer.

Procedure

Whole blood samples collected in EDTA tubes were gently mixed to avoid clotting and directly loaded into the automated hematology analyzer. Cellular components were measured using impedance and flow cytometric principles according to the manufacturer's instructions. CBC analysis was performed because hematological abnormalities including anemia, thrombocytopenia, and leukocytosis are commonly associated with chronic liver disease and HCC progression [30].

Liver Function Tests

Serum ALT and AST activities were measured kinetically based on enzymatic conversion reactions producing pyruvate and oxaloacetate. ALP activity was determined using p-nitrophenyl phosphate substrate hydrolysis, whereas GGT was measured through cleavage of gamma-glutamyl substrates.

Total and direct bilirubin concentrations were measured colorimetrically using diazo reaction methods. Serum albumin was determined using bromocresol green dye-binding assay, while total protein concentration was measured using the biuret method. Absorbance values were recorded spectrophotometrically according to kit instructions. These biomarkers were evaluated because elevated liver enzymes reflect hepatocellular damage and cholestasis, whereas decreased albumin indicates impaired hepatic synthetic capacity [31].

Renal Function Tests

Renal function assessment included measurement of serum creatinine, blood urea nitrogen (BUN), and serum uric acid. Serum creatinine was determined using the Jaffe kinetic method based on the reaction between creatinine and picric acid in alkaline medium. Blood urea nitrogen was measured enzymatically using urease-based assays. Uric acid was determined using uricase-peroxidase enzymatic methods. Renal assessment was important because advanced liver dysfunction may contribute to hepatorenal complications in HCC patients [32].

Assessment of Inflammatory Marker

C-Reactive Protein (CRP)

Serum CRP levels were measured as an indicator of systemic inflammatory response. CRP concentration was determined using immunoturbidimetric assay methods in which serum CRP reacts with anti-CRP antibodies forming antigen-antibody complexes. The resulting turbidity was measured spectrophotometrically. Elevated CRP levels are associated with tumor progression and poor prognosis in hepatocellular carcinoma [33].

Coagulation Profile

International Normalized Ratio (INR)

Coagulation status was assessed by measuring prothrombin time and INR values. Citrated plasma samples were incubated with thromboplastin reagent and calcium chloride. Clotting time was measured automatically and INR values were subsequently calculated. Assessment of coagulation profile is important because liver dysfunction impairs synthesis of coagulation factors [34].

Tumor Biomarker Assessment (AFP, CEA and CA19.9)

Serum AFP concentrations were determined using sandwich enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Optical density was measured using a microplate reader and concentrations were calculated from standard calibration curves. Elevated AFP levels are strongly associated with tumor burden and HCC progression [35]. CEA concentration was determined using ELISA technique based on antigen-antibody binding followed by colorimetric detection using enzyme substrate reaction. Serum CA19.9 was measured using commercially available ELISA kits. Absorbance was measured spectrophotometrically and concentrations were determined using standard calibration curves.

Detection of Viral Hepatitis Markers

HBsAg and anti-HCV antibodies were detected using ELISA kits according to the manufacturer's instructions based on antigen-antibody immunological reactions with colorimetric detection [36].

Detection of Helicobacter pylori Infection

Fresh stool samples were collected and analyzed using rapid immunochromatographic stool antigen tests according to the manufacturer's protocol. Positive samples showed visible colored bands indicating the presence of H. pylori antigens. Serum anti-H. pylori IgG and IgM antibodies were measured using ELISA kits. Serum samples were added to antigen-coated microplates followed by incubation with enzyme-conjugated antibodies. Color development was measured spectrophotometrically.

Bile Antigen Detection

Bile samples were examined for detection of H. pylori antigens using immunological assay techniques to evaluate possible hepatobiliary bacterial colonization. Combined serological and antigen detection techniques improve sensitivity and specificity for diagnosis of H. pylori infection [37].

Quantitative Real-Time PCR Analysis of miRNA-139

RNA Extraction

Total RNA enriched with small RNAs was extracted from serum samples using commercially available miRNA extraction kits. Serum samples were lysed using extraction buffer followed by phase separation and purification using silica membrane columns. Purified RNA was eluted using RNase-free water and stored at −80°C until analysis. RNA purity and concentration were assessed using NanoDrop spectrophotometry.

cDNA Synthesis

Reverse transcription of miRNA into complementary DNA (cDNA) was performed using miRNA-specific reverse transcription kits according to the manufacturer's protocol.

Quantitative Real-Time PCR (qRT-PCR)

Amplification reactions were performed using SYBR Green master mix in a real-time PCR system. Thermal cycling included initial denaturation followed by repeated denaturation, annealing, and extension cycles. Relative miRNA-139 expression was calculated using $2^{-\Delta\Delta Ct}$ after normalization against endogenous housekeeping genes according to Livak and Schmittgen method [38]. miRNA-139 was selected because previous studies demonstrated its tumor suppressor role and involvement in regulation of proliferation, invasion, migration, and apoptosis in hepatocellular carcinoma [39].

Statistical Analysis

Statistical analysis was performed using Graphpad software version 8.1.0. Quantitative data were expressed as mean ± standard deviation (SD), whereas qualitative variables were expressed as numbers and percentages. One-way analysis of variance (ANOVA) was used for comparison among groups. Post hoc tests were used for multiple comparisons whenever appropriate. A p-value < 0.05 was considered statistically significant [40].

III. Results

Demographic characteristics of the studied groups

The demographic characteristics of the enrolled participants were analyzed to evaluate potential differences among the studied groups. The mean age of the control group was 36.78 ± 1.89 years, whereas significantly higher mean ages were observed in both the HCC group (54.63 ± 1.58 years) and the HCC/H. pylori group (54.93 ± 1.68 years). The close similarity between the HCC and HCC/H. pylori groups suggests that]age distribution was comparable between the diseased groups. Moreover, confidence interval analysis demonstrated statistically significant differences between the control and patient groups, indicating a possible association between advanced age and the development of HCC, either alone or in combination with H. pylori infection. Gender distribution analysis revealed a predominance of male patients in all studied groups. In the control group, 36 males and 24 females were enrolled, corresponding to a male-to-female ratio of 3:2. In the HCC/H. pylori group, males constituted the majority, with 48 males and 12 females, yielding a ratio of 4:1. Similarly, the HCC group included 43 males and 17 females, with a male-to-female ratio of approximately 2.5:1, indicating a higher prevalence of HCC among males (Figure 1).

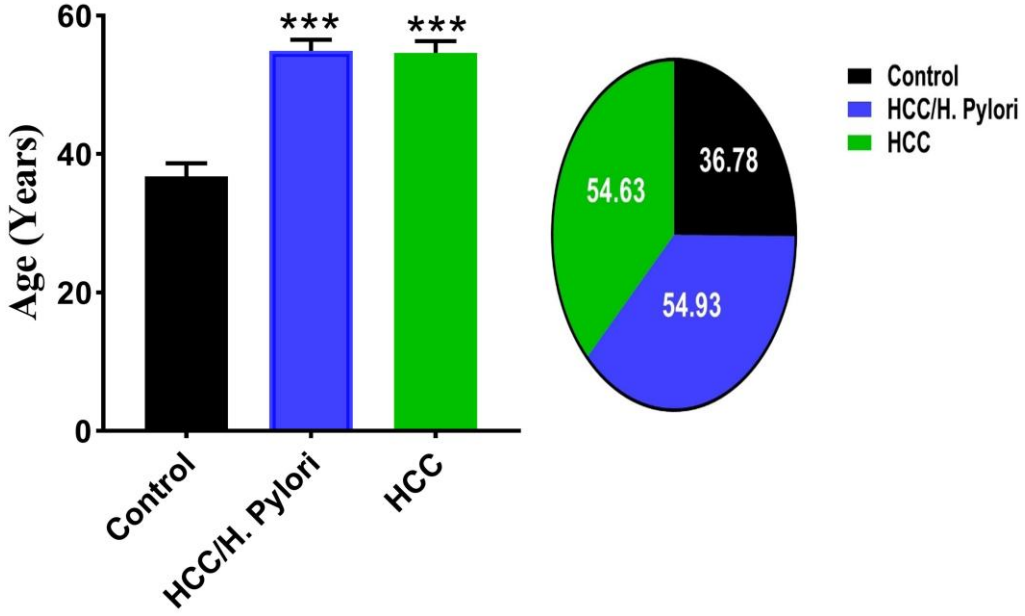

Figure 1: The mean of Age of participants

Expression of miRNA-139 assessed by qRT-PCR

The expression profile of miRNA-139 was evaluated using quantitative real-time PCR (qRT-PCR). A significant downregulation of miRNA-139 expression was observed in the serum of patients with HCC associated with H. pylori infection (0.373 ± 0.0138) compared with the control group (1.02 ± 0.0118). In contrast, patients with HCC without H. pylori infection exhibited significantly higher miRNA-139 expression levels (0.759 ± 0.0273) than those observed in the HCC/H. pylori group. Comparative analysis demonstrated

statistically significant differences among all studied groups, with the lowest expression detected in the HCC/H. pylori group, while the highest expression was observed in the HCC group without H. pylori infection. Confidence interval analysis further confirmed the statistical robustness of these findings, highlighting the differential regulation of miRNA-139 according to H. pylori infection status (Figure 2).

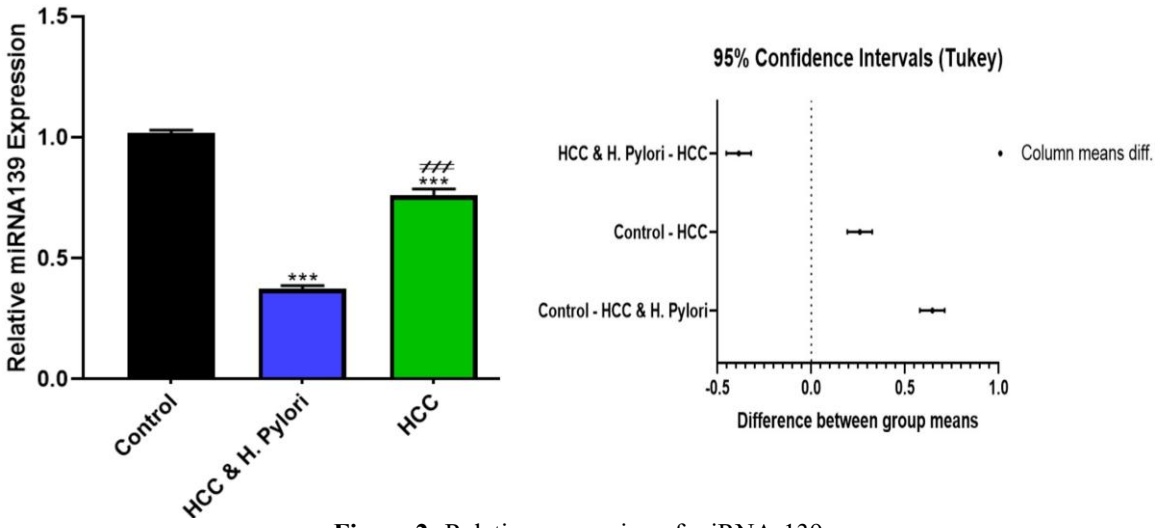

Figure 2: Relative expression of miRNA-139

Serum alpha-fetoprotein (AFP) levels

Serum AFP concentrations were significantly elevated in both patient groups compared with controls. The HCC/H. pylori group demonstrated the highest AFP levels (29.5 ± 4.41 IU/mL) compared with the control group (2.04 ± 0.126 IU/mL). Likewise, AFP levels in the HCC group without H. pylori infection were significantly increased (16.9 ± 3.54 IU/mL) relative to controls. Furthermore, AFP concentrations were significantly higher in the HCC/H. pylori group than in the HCC group, indicating a possible synergistic effect of H. pylori infection on AFP elevation. Scatter plot analysis supported these observations and revealed greater inter-individual variability among patients with HCC, particularly those co-infected with H. pylori. Confidence interval analysis demonstrated non-overlapping 95% confidence intervals between groups, confirming the statistical significance of these differences (Figures 3).

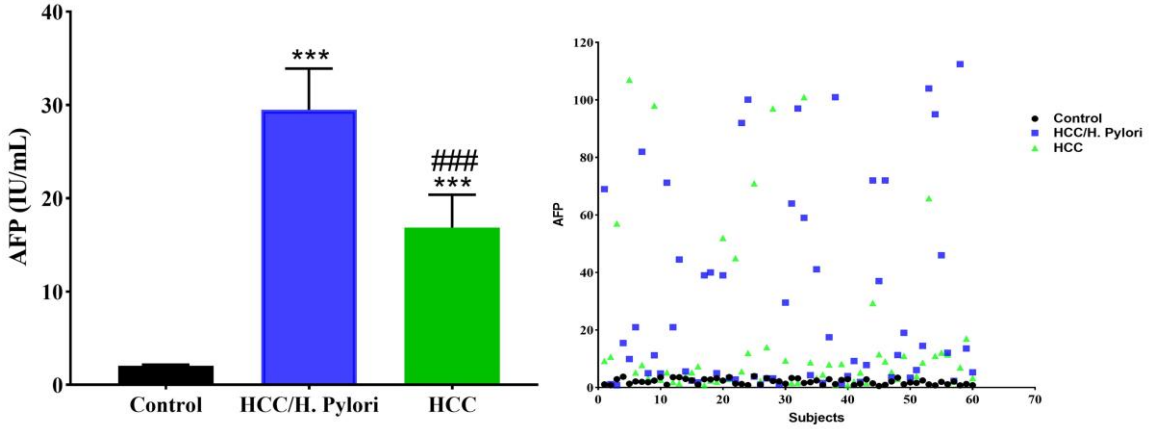

Figure 3: The mean of AFP levels

Serum carcinoembryonic antigen (CEA) levels

Serum CEA levels were evaluated among the studied groups. The control group exhibited a mean CEA concentration of 2.04 ± 0.157 ng/mL, reflecting physiological baseline levels. In contrast, significantly elevated CEA levels were observed in the HCC/H. pylori group (4.13 ± 0.647 ng/mL) and the HCC group (3.80 ± 0.309 ng/mL). Although both patient groups demonstrated increased CEA levels compared with controls, the HCC/H. pylori group showed slightly higher values and greater variability (Figure 4).

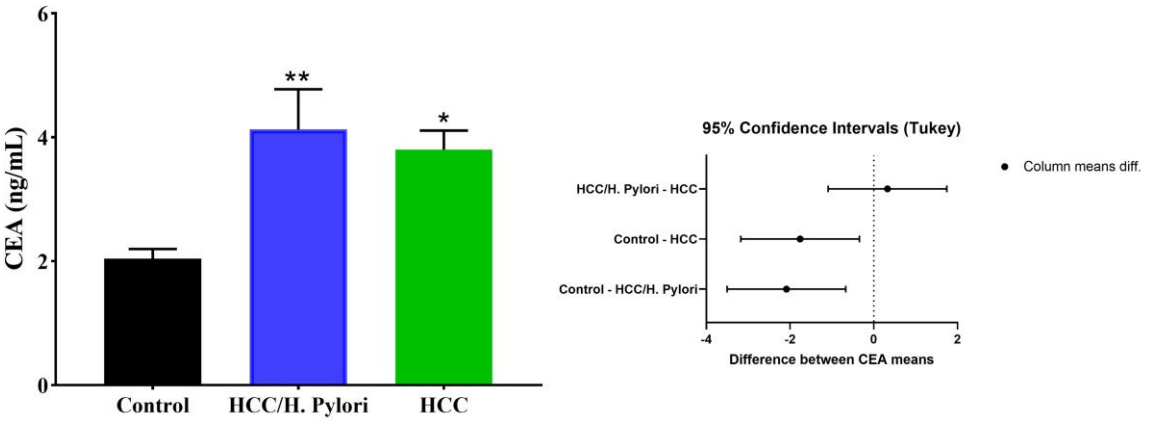

Figure 4: The mean of CEA levels

Determination of CA19.9 levels

Serum CA19.9 concentrations were significantly increased in the patient groups compared with healthy controls. The control group showed a mean CA19.9 level of 9.82 ± 0.769 U/mL, whereas the HCC/H. pylori group exhibited markedly elevated levels (27.3 ± 3.12 U/mL). Similarly, the HCC group demonstrated increased CA19.9 concentrations (15.8 ± 2.11 U/mL), although these values remained lower than those recorded in the HCC/H. pylori group. These findings suggest a potential association between H. pylori infection and enhanced tumor marker expression in HCC patients (Figure 5).

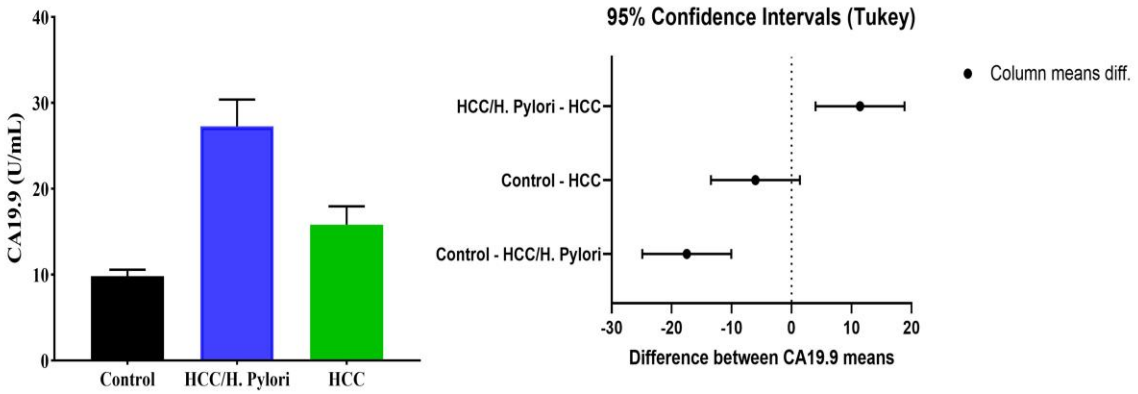

Figure 5: The mean of CA19.9 levels

Detection of HCV and HBV infection

The prevalence of HCV infection was assessed among the studied groups. No HCV-positive cases were detected in the control group. In contrast, the HCC/H. pylori group included 35 HCV-positive and 25 HCV-negative cases, while the HCC group comprised 37 HCV-positive and 23 HCV-negative cases, indicating a predominance of HCV infection among HCC patients. Regarding HBV infection, no positive cases were identified in either the control or HCC groups. However, a single HBV-positive case was detected in the HCC/H. pylori group, whereas the remaining 59 participants were HBV-negative (Figure 6).

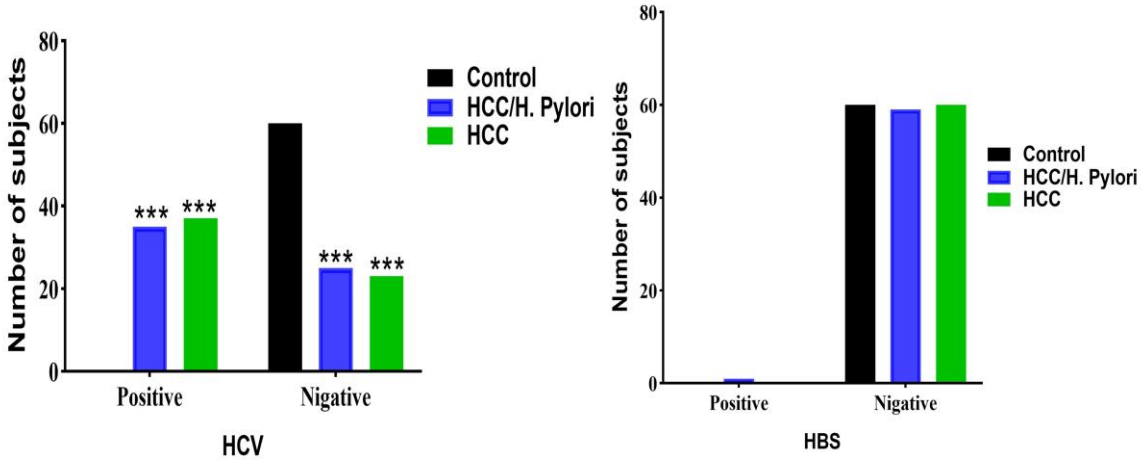

Figure 6: The prevalence of HCV and HBV infection

Liver enzyme activities (ALT, AST, ALP and GGT)

Assessment of liver enzyme activities revealed marked elevations in both patient groups. Serum ALT (GPT) levels increased significantly from 24.7 ± 0.847 U/L in controls to 76.2 ± 11.0 U/L in the HCC/H. pylori group and 91.9 ± 10.8 U/L in the HCC group. Similarly, AST (GOT) activity rose significantly from 25.4 ± 0.794 U/L in controls to 68.6 ± 8.97 U/L in the HCC/H. pylori group and 80.7 ± 9.98 U/L in the HCC group. A marked increase in GGT activity was also observed, with mean levels rising from 9.13 ± 0.630 U/L in controls to 93.2 ± 13.2 U/L in the HCC/H. pylori group and 114 ± 17.6 U/L in the HCC group. These findings collectively indicate severe hepatic and biliary dysfunction among HCC patients (Figures). Serum ALP activity was significantly elevated in the diseased groups compared with controls. The control group exhibited a mean ALP level of 8.13 ± 0.175 U/L, while increased levels were observed in both the HCC/H. pylori group (11.0 ± 0.666 U/L) and the HCC group (11.3 ± 1.09 U/L). The highest ALP activity was detected in the HCC group, reflecting substantial hepatic or biliary dysfunction (Figure 7).

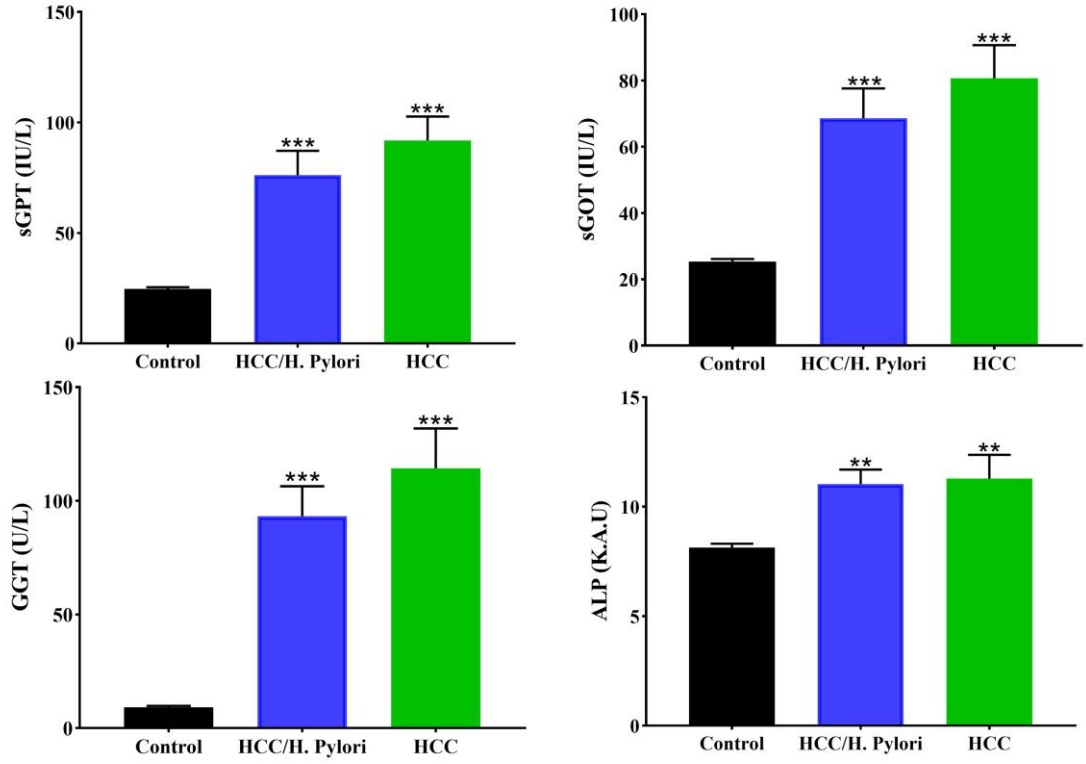

Figure 7: Liver enzyme activities (ALT, AST, ALP and GGT)

Serum albumin and Bilirubin levels

Serum albumin concentrations showed a progressive decline across the studied groups. The control group demonstrated the highest mean albumin level (4.57 ± 0.091 g/dL), whereas reduced levels were observed in the HCC/H. pylori group (3.54 ± 0.076 g/dL) and the HCC group (3.26 ± 0.071 g/dL). These findings indicate impaired hepatic synthetic function in HCC patients, particularly in those without H. pylori infection (Figure). Total bilirubin concentrations were significantly elevated in the patient groups compared with controls. The control group showed a mean total bilirubin level of 0.659 ± 0.023 mg/dL, whereas higher levels were observed in the HCC/H. pylori group (2.62 ± 0.576 mg/dL) and the HCC group (3.00 ± 0.683 mg/dL). Likewise, direct bilirubin levels increased significantly from 0.175 ± 0.007 mg/dL in controls to 1.50 ± 0.281 mg/dL in the HCC/H. pylori group and 1.64 ± 0.314 mg/dL in the HCC group, indicating progressive hepatic impairment and cholestatic dysfunction (Figures 8).

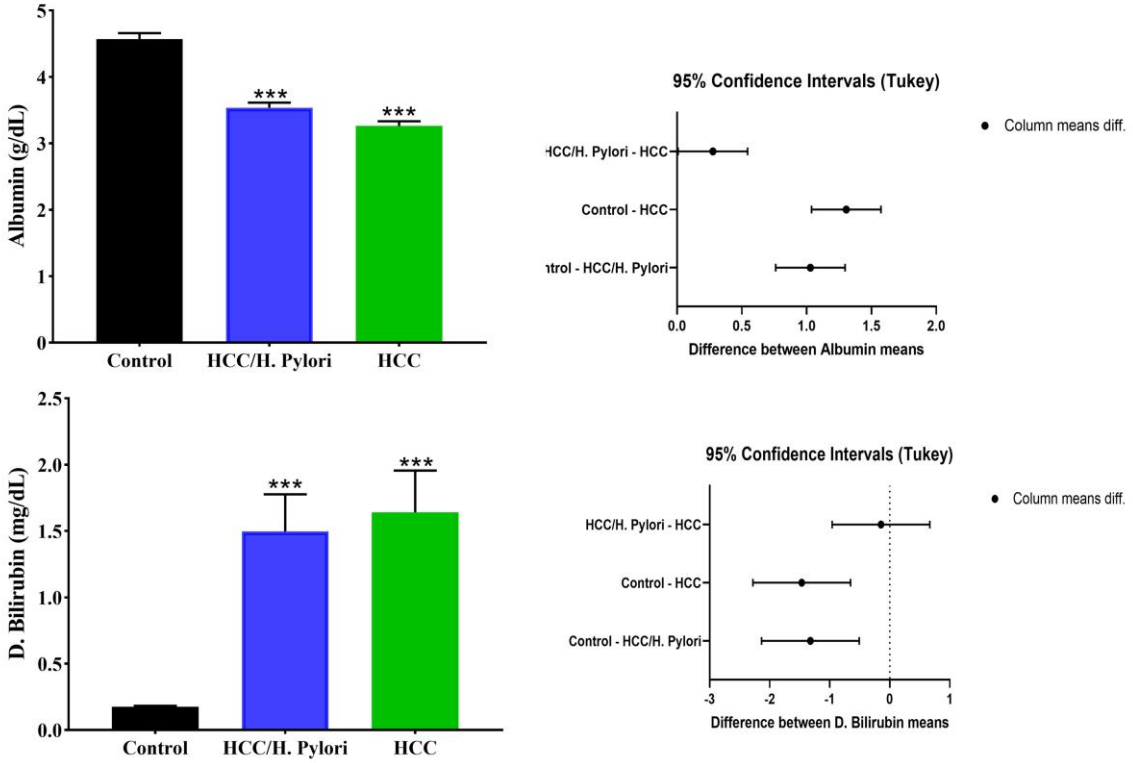

Figure 8: Estimation of Albumin and Bilirubin levels

Hematological parameters

Significant hematological alterations were observed among the studied groups. The mean RBC count declined progressively from 5.02 × 10^{12}/L ± 0.066 in controls to 3.96 × 10^{12}/L ± 0.117 in the HCC/H. pylori group and 3.53 × 10^{12}/L ± 0.114 in the HCC group. Similarly, hemoglobin levels decreased significantly from 14.0 ± 0.169 g/dL in controls to 10.7 ± 0.344 g/dL in the HCC/H. pylori group and 9.88 ± 0.305 g/dL in the HCC group, reflecting progressive anemia associated with liver disease. In contrast, WBC counts were elevated in the patient groups, increasing from 6.87 × 10^{9}/L ± 0.224 in controls to 8.35 × 10^{9}/L ± 0.659 in the HCC/H. pylori group and 7.81 × 10^{9}/L ± 0.588 in the HCC group, suggesting enhanced inflammatory and immune responses. Platelet counts showed a marked reduction in the diseased groups, decreasing from 285 × 10^{9}/L ± 7.72 in controls to 182 × 10^{9}/L ± 14.7 in the HCC/H. pylori group and 156 × 10^{9}/L ± 12.7 in the HCC group, indicative of thrombocytopenia associated with chronic liver disease (Figure 9).

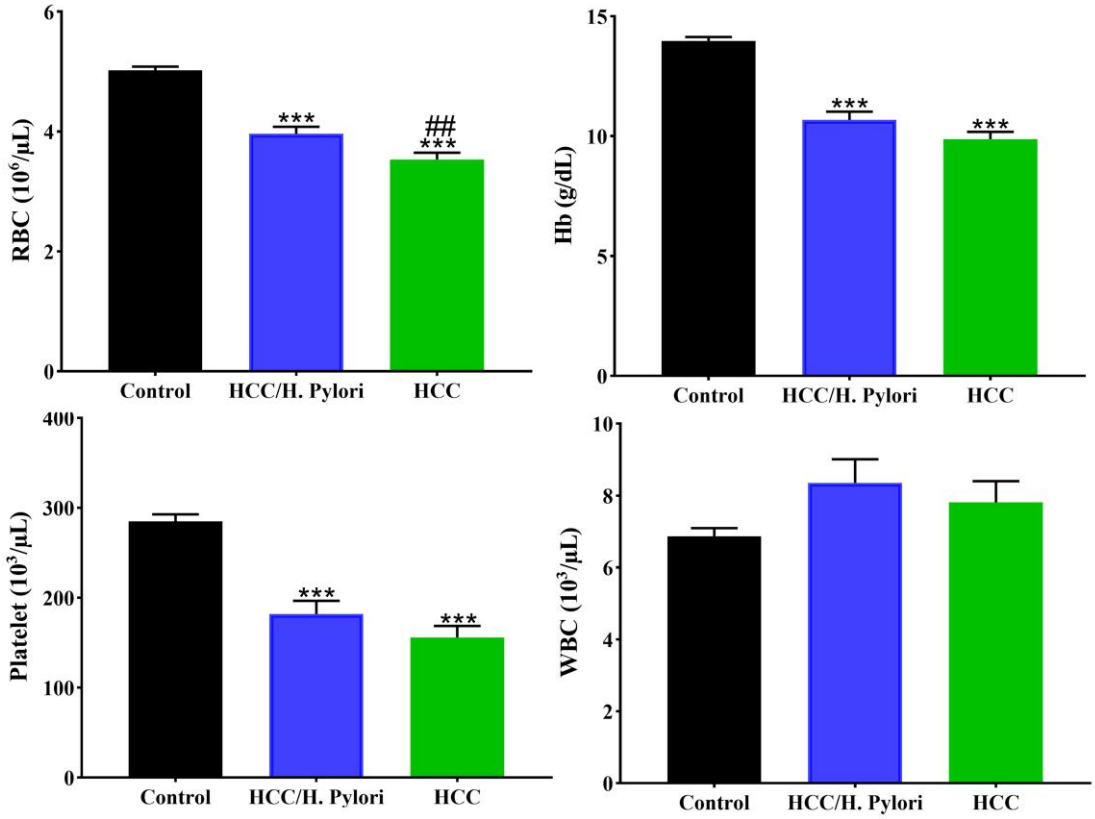

Figure 9: The mean RBC count, Hb, Platelet and WBC

The INR values demonstrated progressive coagulation impairment, increasing from 1.0 in controls to 1.15 ± 0.034 in the HCC/H. pylori group and 1.31 ± 0.056 in the HCC group. CRP levels were markedly elevated in the patient groups, rising from 3.01 ± 0.193 mg/L in controls to 27.0 ± 3.23 mg/L in the HCC/H. pylori group and 29.7 ± 2.83 mg/L in the HCC group, reflecting substantial systemic inflammation (Figure 10).

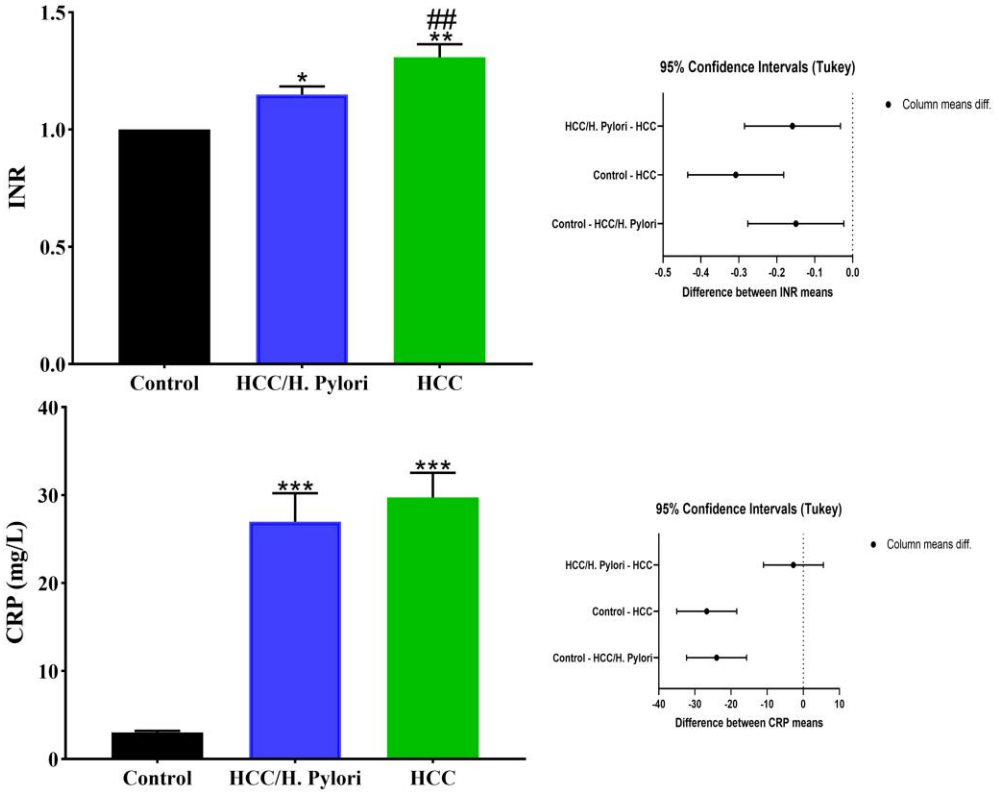

Figure 10: The INR values and CRP levels

Serum uric acid and creatinine levels

Serum uric acid levels increased progressively across the studied groups. The control group exhibited a mean level of 3.93 ± 0.147 mg/dL, while higher levels were detected in the HCC/H. pylori group (4.65 ± 0.205 mg/dL) and the HCC group (5.37 ± 0.787 mg/dL), suggesting metabolic disturbances associated with HCC progression. Similarly, serum creatinine levels were elevated in the patient groups compared with controls. The control group demonstrated a mean creatinine concentration of 0.805 ± 0.0180 mg/dL, whereas the HCC/H. pylori and HCC groups showed increased levels of 0.997 ± 0.0607 mg/dL and 1.02 ± 0.0807 mg/dL, respectively, indicating potential renal impairment secondary to liver disease progression (Figure 12).

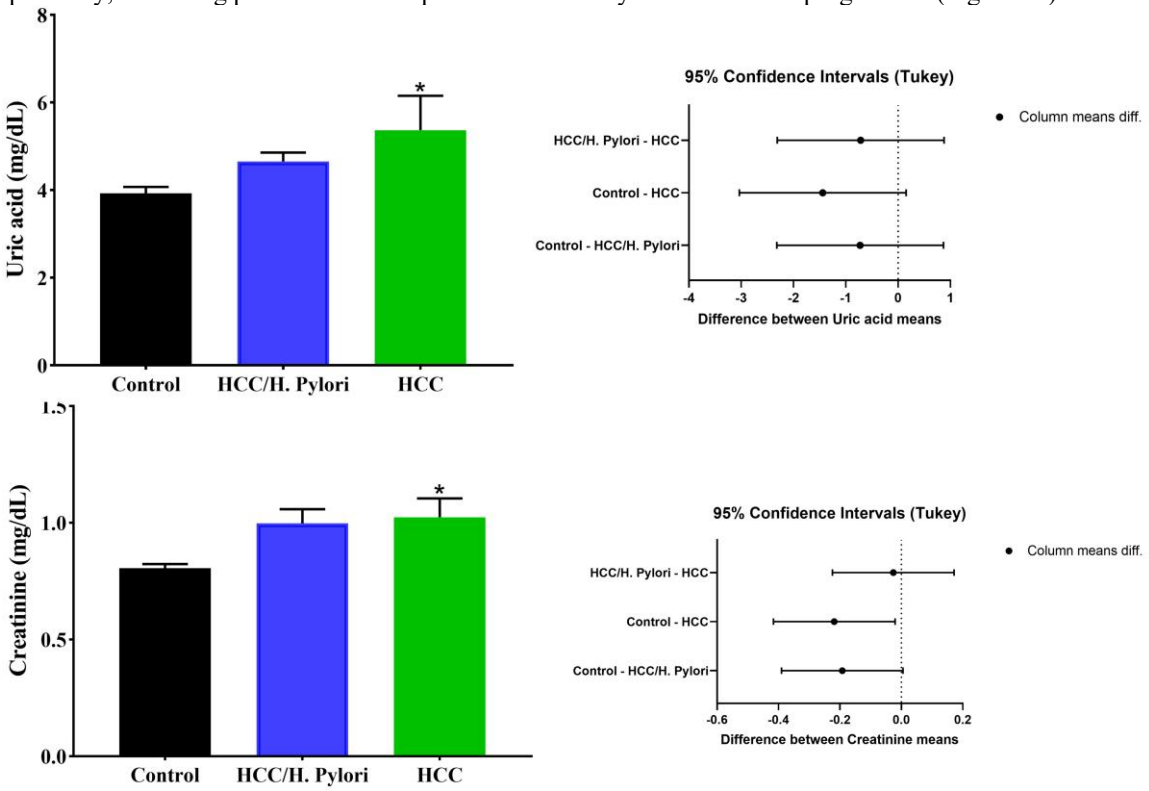

Figure 12: Levels of Serum uric acid and creatinine concentration

IV. Discussion

The present study demonstrated significant demographic, molecular, biochemical, and hematological alterations among patients with hepatocellular carcinoma (HCC), particularly those with concomitant Helicobacter pylori infection. The observed increase in mean age among HCC patients compared with healthy controls is consistent with the established role of aging as a major risk factor for hepatocarcinogenesis. Aging is associated with prolonged exposure to chronic inflammatory stimuli, oxidative stress, genomic instability, and progressive hepatic fibrosis, all of which contribute to malignant transformation of hepatocytes [41,42]. The similarity in age distribution between the HCC and HCC/H. pylori groups indicates that age-related carcinogenic processes may contribute equally in both groups.

Male predominance was evident in both diseased groups, particularly in patients with HCC associated with H. pylori infection. These findings are in agreement with previous epidemiological studies reporting a substantially higher incidence of HCC in males compared with females [43]. This gender disparity has been attributed to hormonal influences, particularly the stimulatory role of androgens on inflammatory signaling and hepatocyte proliferation, whereas estrogens exert anti-inflammatory and anti-proliferative effects [44]. Moreover, environmental and behavioral risk factors such as smoking, occupational exposure, and viral hepatitis are more prevalent among males, further increasing susceptibility to HCC development.

One of the most important findings of the present study was the significant downregulation of serum miRNA-139 expression in HCC patients, particularly in those co-infected with H. pylori. This observation strongly supports the tumor suppressor role of miRNA-139 in hepatocarcinogenesis. Several studies have demonstrated that miRNA-139 inhibits tumor proliferation, migration, invasion, and epithelial–mesenchymal transition through modulation of multiple oncogenic pathways, including PI3K/Akt, Wnt/β-catenin, ROCK2, and CXCR4 signaling pathways [45,46]. Therefore, reduced expression of miRNA-139 may promote uncontrolled cellular proliferation and tumor progression in HCC patients.

The markedly lower expression of miRNA-139 observed in the HCC/H. pylori group suggests that H. pylori infection may exacerbate epigenetic dysregulation during hepatocarcinogenesis. Chronic H. pylori infection induces sustained inflammatory responses characterized by increased production of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α, in addition to excessive reactive oxygen species generation [47].

These inflammatory mediators contribute to DNA methylation abnormalities and alterations in microRNA expression profiles. Furthermore, H. pylori virulence factors, particularly cytotoxin-associated gene A (CagA), have been implicated in activation of NF-κB and STAT3 signaling pathways, thereby promoting oncogenic transformation and suppression of tumor suppressor miRNAs [48]. Accordingly, the current findings suggest that reduced miRNA-139 expression may represent a mechanistic link between chronic bacterial infection and accelerated HCC progression.

The significant elevation of AFP levels observed in both HCC groups is consistent with the recognized role of AFP as a classical biomarker for HCC diagnosis and monitoring [49]. AFP production increases during hepatocyte dedifferentiation and malignant transformation. Interestingly, AFP levels were significantly higher in patients with concomitant H. pylori infection, suggesting enhanced tumor aggressiveness and inflammatory activity in co-infected individuals. Similar findings have been reported in studies linking chronic inflammatory conditions with increased AFP synthesis and tumor progression [50].In addition to AFP, serum CEA and CA19.9 levels were markedly elevated in the patient groups compared with controls. Although these markers are traditionally associated with gastrointestinal malignancies, accumulating evidence suggests their relevance in advanced liver disease and HCC progression [51]. Elevated CA19.9 levels may reflect biliary epithelial activation, cholestasis, or inflammatory alterations within the tumor microenvironment. The higher levels observed in the HCC/H. pylori group further support the hypothesis that chronic bacterial infection amplifies inflammatory and neoplastic activity in HCC patients.

The predominance of HCV-positive cases among HCC patients in the present study is consistent with previous reports identifying chronic HCV infection as a major etiological factor for HCC, particularly in Egypt [52]. Persistent HCV infection promotes chronic hepatic inflammation, oxidative stress, fibrosis, and cirrhosis, ultimately leading to malignant transformation. In contrast, HBV infection was nearly absent among the studied population, which aligns with regional epidemiological data indicating that HCV remains the predominant viral cause of HCC in Egyptian patients [53].

Significant elevations in liver enzymes, including ALT, AST, ALP, and GGT, were observed in both patient groups. Increased ALT and AST activities reflect hepatocellular membrane damage and leakage of intracellular enzymes into the circulation [54]. Elevated ALP and GGT levels indicate cholestasis and biliary dysfunction, which are commonly associated with advanced hepatic malignancy. Notably, GGT has recently gained considerable attention as a marker of oxidative stress and tumor progression, as excessive GGT activity contributes to glutathione metabolism and reactive oxygen species generation, thereby promoting DNA damage and carcinogenesis [55].

The observed hyperbilirubinemia in HCC patients further confirms severe hepatic dysfunction and impaired bilirubin conjugation and excretion. Similarly, the marked reduction in serum albumin levels reflects deterioration of hepatic synthetic capacity. Hypoalbuminemia is commonly associated with advanced cirrhosis, portal hypertension, systemic inflammation, and poor clinical prognosis in HCC patients [56]. Interestingly, some liver function abnormalities appeared more severe in the HCC group than in the HCC/H. pylori group, suggesting that tumor burden itself may exert profound effects on hepatic integrity independent of bacterial infection.

Hematological abnormalities were also prominent among HCC patients. Significant reductions in RBC count, hemoglobin concentration, and platelet count were observed, indicating anemia and thrombocytopenia commonly associated with chronic liver disease. Multiple mechanisms may contribute to anemia in HCC patients, including chronic inflammation, nutritional deficiencies, gastrointestinal bleeding, hypersplenism, and impaired erythropoiesis [57]. Thrombocytopenia is frequently associated with splenic sequestration and reduced thrombopoietin production secondary to cirrhosis and portal hypertension.

Conversely, elevated WBC counts and CRP levels indicate activation of systemic inflammatory responses. CRP is synthesized by hepatocytes in response to IL-6 stimulation and has been identified as an independent prognostic marker in HCC patients [58]. Chronic inflammation plays a crucial role in hepatocarcinogenesis through promotion of angiogenesis, oxidative DNA damage, immune evasion, and tumor cell proliferation. The markedly elevated inflammatory indices observed in the HCC/H. pylori group further support the role of bacterial infection in amplifying inflammatory signaling pathways associated with cancer progression.

The present study also demonstrated progressive elevations in serum creatinine and uric acid levels among HCC patients. Increased creatinine concentrations may indicate impaired renal perfusion, hepatorenal dysfunction, or systemic circulatory abnormalities commonly encountered in advanced liver disease [59]. Elevated uric acid levels may result from enhanced nucleic acid turnover, oxidative stress, and impaired renal clearance. Hyperuricemia has been increasingly recognized as a contributor to inflammatory activation and cancer progression through stimulation of oxidative and inflammatory pathways [60].

Collectively, the present findings strongly suggest that miRNA-139 may serve as a promising non-invasive biomarker for HCC diagnosis and prognosis, particularly in patients with concomitant H. pylori infection. The inverse relationship observed between miRNA-139 expression and tumor markers, inflammatory mediators, and liver dysfunction parameters supports its tumor suppressive role in hepatocarcinogenesis. Circulating miRNAs possess several advantages as biomarkers, including high stability in biological fluids, reproducibility, and non-invasive detection [61]. Therefore, incorporation of miRNA-139 into diagnostic panels alongside AFP and conventional liver function tests may significantly improve early HCC detection and disease stratification.

Furthermore, restoration of miRNA-139 expression may represent a novel therapeutic strategy for HCC management. Experimental studies have demonstrated that miRNA replacement therapy effectively suppresses tumor growth, angiogenesis, and metastasis in several malignancies, including HCC [62]. Consequently, future investigations are warranted to further explore the mechanistic interactions between H. pylori infection, miRNA dysregulation, and hepatocarcinogenesis, as well as the potential therapeutic applications of miRNA-based interventions.

V. Conclusion

The present study demonstrates that hepatocellular carcinoma is associated with profound molecular, biochemical, hematological, and inflammatory alterations, which become more pronounced in the presence of concomitant Helicobacter pylori infection. A significant downregulation of serum miRNA-139 expression was identified in HCC patients, particularly in those co-infected with H. pylori, highlighting the potential involvement of this microRNA in hepatocarcinogenesis and tumor progression. Furthermore, the inverse association between miRNA-139 expression and tumor burden markers supports the tumor suppressive role of miRNA-139 and emphasizes its potential utility as a sensitive non-invasive biomarker for early HCC detection, disease monitoring, and prognostic evaluation. The remarkable stability and accessibility of circulating miRNAs further enhance their clinical applicability in precision oncology.

Collectively, the current findings provide novel evidence supporting a potential mechanistic interaction between H. pylori infection and miRNA-139 dysregulation during HCC progression. These results may open new perspectives for the development of miRNA-based diagnostic tools and targeted therapeutic strategies aimed at improving the clinical management and prognosis of HCC patients. Nevertheless, further large-scale multicenter studies and mechanistic investigations are required to validate these findings and clarify the underlying molecular pathways linking H. pylori infection to hepatocarcinogenesis.

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