Gut Microbiota Characterization in Patients with HCC Post Chronic HCV Infection

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Abstract

Aim: Dysbiosis of gut microbiota favors chronic hepatic inflammation with subsequent hepatic carcinogenesis. The current study aimed to evaluate the role of gut dysbiosis in the development of hepatocellular carcinoma in patients with chronic HCV infection.

Methods: This descriptive cross-sectional cohort study carried out on 400 subjects recruited from the Internal Medicine Department and Tropical Medicine and Gastroenterology Department of Helwan and South Valley University Hospitals in Egypt. The study period was from January 2017 till January 2020. The subjects were divided clinically into three groups. Group I: One hundred patients with HCC, evaluated by **Child Pugh**, **TNM** and **BCLC** scoring systems. Group II: 200 chronic hepatitis C virus-infected patients. All patients infected with hepatitis C virus genotype 4. Group III: One hundred healthy control subjects with negative hepatitis marker and normal abdominal ultrasound. PCR of stool Microbiota, complete blood counts, complete liver function tests, INR, HCV antibodies and HBsAg were done for all included subjects. HCV PCR assessment and alpha-fetoprotein (AFP) were done for all patients.

Results: No statistically significant difference was detected between HCC patients and control (p-value > 0.05) as regard Bacteroides fragilis & Akkermansia muciniphila. Faecalibacterium prausnitzii was less detected in HCC patients (51%), opposite to 70% of healthy control. With Statistically significant difference (p-value < 0.05). Bifidobacterium was less detected in HCC patients (43%), opposite to (76%) of healthy control. With highly statistically significant difference (p-value < 0.001). Lactobacillus & Enterobacteriaceae was more detected in HCC patients (80%) and (81%), in. Opposite to (36%) and (58%) in healthy control, respectively. With highly statistically significant difference (p-value < 0.001). no significant difference was detected between gut-microbiota and HCC progression with respect to Child or TNM systems. However, a significant difference was detected between number of positive stool isolate of Bacteroid Fragilis and BCLC staging system; where it was isolated from 66.7% of patients with **BCLC stage IV** opposite to 10.7% of patients with **BCLC stage I**.

Conclusion: A characteristic pattern of Bifidobacterium, Lactobacillus and Enterobacteriaceae species in patients with chronic HCV and HCC was detected. Alteration of gut microbiota may be accused as a predisposing factor for liver disease progression.

Introduction:

World Health Organization considers hepatocellular carcinoma (HCC) is the 5th most common cancer worldwide and the 2nd most predominant cause of death related to cancer. [1]

HCC is the most common liver cancer; follows chronic hepatitis B virus infection, post HCV liver cirrhosis, smoking, alcohol, and aflatoxin intake. [2]

Viral hepatitis is considered the 7th cause of death worldwide. [3] Approximately 50% of these deaths related to chronic hepatitis C virus (HCV) infection which led to liver cirrhosis that can progress to HCC

development. [4–5]

HCV infection is a public health problem in Egypt, it has the highest prevalence according to WHO. [6]

It's known that post-viral hepatitis HCC mainly related to hepatocellular inflammation, oxidative stress, abnormal signaling pathways with activation of oncogenic pathways and integration of virus DNA in host DNA. [7–8]

Intact intestinal mucosal barrier, mucus layer, secretion of IgA and associated lymphoid tissues are the intestinal barrier that prevents the passage of microbes and its metabolites across the mucosa. [9]

A close relationship exists between the gut microbiome and liver; mainly due to its anatomical position and its nutritional and blood supply from the gut via the portal vein, at the same time liver is the 1st organ vulnerable to gut-derived toxins, dangerous metabolites, bacteria, and its metabolites. [10–11]

Growth and Alteration in gut microbiota cause damage to the intestinal wall that promotes the transmission of bacteria and its metabolites that elicit systemic endotoxemia and distant organ disease including the liver. [12–13]

Dysbiosis is a loss of beneficial microorganisms with overgrowth of harmful microbes, and/or a loss of overall microbial profile. [14]

Dysbiosis of gut microbiota favors chronic hepatic inflammation with subsequent carcinogenesis; elicited by an interaction between the liver, abnormal gut microbiota, and its metabolites together with the immune system via macrophages and Kupffer cells that secrete IL-8, TNF-α, and IL1β inflammatory cytokines. [15–17]

Logically, dysbiosis of gut microbiota augments the pathophysiology of viral hepatitis by inducing chronic inflammation of the liver with subsequent hepatic HCC. [18]

Mechanisms by which viral hepatitis induce disturbance of gut microbiota are unknown; anyway, maintaining gut homeostasis can prevent viral hepatitis induced hepatic disease progression and HCC up-growth. Therefore, we herein in this study to find out any change in gut microbiota between healthy volunteers, patients with chronic HCV infection and patients with HCV induced hepatocellular carcinoma.

Aim Of The Work:

This study aims to detect the role of dysbiosis in the development of hepatocellular carcinoma in patients with chronic HCV infection.

Patients And Methods:

This cross-sectional cohort study was carried out on 400 subjects with average body volume recruited from the Internal Medicine Department and Tropical Medicine and Gastroenterology Department of two dedicated centers (Helwan University Hospital and South Valley University Hospital) in Egypt. The study period was from January 2017 till January 2020. The subjects were divided clinically into three groups

Group I: One hundred patients with HCC on top of liver cirrhosis; caused by chronic HCV infection. The diagnosis of HCC was done according to AASLD guidelines; based on clinical, laboratory, imaging data and liver biopsy when needed. (19)

Group II: 200 chronic hepatitis C virus-infected patients. All patients infected with hepatitis C virus genotype 4.

Group III: One hundred healthy control subjects with negative hepatitis marker and normal abdominal ultrasound.

The exclusion criteria include age below 18 years, previous antibiotics therapy within the last two weeks, autoimmune hepatitis, HIV or HBV co-infection, alcohol intake, and patients with other etiologies of hepatic affection.

Clinical examination

Complete history taking and full clinical examination, laboratory and radiological investigations were done for each included subject. Serum alpha-fetoprotein protein and multislice abdominal CT (MSCT) were done for patients with suspected HCC. For Cirrhotic patients with hepatic focal lesion of > 1 cm on abdominal ultrasound and AFP > 20 ng/mL a multislice CT imaging is done, if the typical features of HCC not seen but HCC still suspected; liver biopsy was done to confirm the diagnosis (according to AASLD guidelines).

All Patients with HCC were evaluated by Child-Pugh score [22]. Tumor staging was assessed by following scoring systems:

1) TNM [20]

2) Barcelona Clinic Liver Cancer (BCLC) [21]

Laboratory Investigation

Blood samples

Ten-milliliter blood samples obtained from each subject under sterile conditions and transported to the laboratory for routine laboratory investigations. The investigations include complete blood counts (CBC) by Sysmex, serum creatinine, complete liver function tests including alanine aminotransferase (ALT),

aspartate aminotransferase (AST), albumin, total bilirubin by the auto-analyzer (Dialab 450 system), prothrombin time, prothrombin concentration and INR. HCV genotype estimation and HCV PCR assessment using quantitative PCR (Roche COBAS Taq Man HCV assay version 2.0 with a lower limit of detection 15 IU/mL). HBsAg estimation using and HCV antibodies detection using the automated MiniVidas immunoassay system; Biomerieux, France. AFP (alpha-fetoprotein) assessment for patients with suspected HCC.

Stool Sample

Stool samples collected from each participant in a clean container and transported to the laboratory rapidly. At the laboratory, an aliquot was taken from stool to 1.5 ml Eppendorf and stored at -80^{O}C.

Polymerase Chain Amplification of stool Microbiota

DNA isolated from the stored sample after thawing. Extraction performed by QIAamp. DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer`s instructions.

Positive Control Strains for Polymerase Chain Reaction Analysis. The study used positive control strains: *Akkermansia muciniphila* ATCC BAA-835, *Faecalibacterium prausnitzii* ATCC 27766, *Bifidobacterium breve* ATCC 15700, *Lactobacillus acidophilus* ATCC 4356, *Bacteroides fragilis* ATCC 25285, *Escherichia coli* ATCC 25922. *Lactobacillus acidophilus* and *Bifidobacterium breve.* The control strains cultured according to the specific microbiological recommendations and DNA of these strains suspended in 1-milliliter phosphate buffer and DNA extracted from the Qiagen extraction kit.

Polymerase Chain Reaction Analysis

The amplification of different bacterial species of microbiota performed by the use of the primers summarized in Table 1.

Table 1
Demographic data of HCC infected patients.

Variables	HCC group (N = 100)
Age (years) (Mean ± SD)	50.1 ± 5.5
Sex (N%)	76 (76%)
Male	24 (24%)
Female	
Smoking (N%)	44 (44%)
Diabetes mellitus (N%)	9 (9%)
Random blood glucose (mg/dl) (Mean ± SD)	137.2 ± 32.7
AFP (ng/ml) (Mean ± SD)	211.34 ± 151.9
Complete blood count (Mean ± SD)	
Total leucocyte count (x10^3/cmm) (Mean ± SD)	6.9 ± 3.9
Hemoglobin (g/dl)	11.02 ± 1.0
Platelets (x10^3/cmm)	113.9 ± 37.6
Liver function tests (Mean ± SD)	
Albumin (g/dl) (Mean ± SD)	3.1 ± 0.5
Total bilirubin (mg/dl) (Mean ± SD)	1.98 ± 1.34
ALT (U/L) (Mean ± SD)	89.9 ± 91.8
AST (U/L) (Mean ± SD)	70.7 ± 95.7
INR (Mean ± SD)	1.5 ± 0.6
Renal function tests (Mean ± SD)	
Creatinine (mg/dl) (Mean ± SD)	1.09 ± 0.4
scoring data of patients	
Child-Pugh class	18 (18%)
A	38 (38%)
B	44 (44%)
C	

Variables	HCC group (N = 100)
TNM stage	24 (24%)
I	40 (40%)
II	28 (28%)
III (A-B)	8 (8%)
IV	
BCLC staging	28 (28%)
A	26 (26%)
B	34 (34%)
C	12 (12%)
D	

The amplification reaction was carried out in a total volume of 20 μL and consisted of 4 mM MgCl2, 0.25 μM of each primer and 2 μL of DNA template. Amplification involved an initial denaturation at 95 °C for 10 min following by 45 cycles of denaturation at 95 °C for 10 s, annealing at the specific annealing temperature (Table 1) for 5 s, and extension at 72 °C for 10 s. The products of the amplified PCR were analyzed by electrophoresis. For the primers determined in the study are shown on 7**Table I**. Figure 1.

Table (I): Bacterial species, primers sequences, annealing and melting temperatures of PCR and the result size of bp

Bacterial species	Primers sequences	bp	annealing	melting
Bifidobacterium spp.	CTCCTGGAAACGGGTGG GGTGTTCTTCCCGATATCTACA	550	56	90
Bacteroides fragilis group	ATAGCCTTTCGAAAGRAAGAT CCAGTATCAACTGCAATTTTA	495	50	86
Lactobacillus spp	AGCAGTAGGGAATCTTCCA CACCGCTACACATGGAG	341	55	86
Akkermansia muciniphila	CAGCACGTGAAGGTGGGGAC AM-1 CCTTGCGGTTGGCTTCAGAT AM-2	327	60	90
Faecalibacterium prausnitzii	GATGGCCTCGCGTCCGATTAG Fprau223F CCGAAGACCTTCTTCCTCC	199	60	88
Enterobacteriaceae	CATTGACGTTACCCGCAGAAGAAGC CTCTACGAGACTCAAGCTTGC	195	63	87

Abdominal *Muttislice CT (HCC radiological hallmark)*

The typical feature for diagnosis of HCC is arterial phase hyperintensity (wash-in) followed portal venous washout to iso- or hypodensity (delayed phases). [23]

Statistical analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 24. Qualitative data were expressed as frequency and percentage. Quantitative data were expressed as mean ± standard deviation (SD). **Mean (average)**: the central value of a discrete set of numbers, specifically the sum of values divided by the number of values. **Standard deviation (SD)**: is the measure of dispersion of a set of values. A low SD indicates that the values tend to be close to the mean of the set, while a high SD indicate that the values are spread out over a wider range. **Chi-square test**: was used when comparing between studied groups as regard gut micro-biota. **Probability (P-value)**: P-value < 0.05 was considered significant, P-value < 0.001 was considered as highly significant & P-value > 0.05 was considered insignificant.

Results:

This descriptive cross-sectional cohort study included 300 hundred patients and 100 healthy control. The first group included 100 patients with hepatocellular carcinoma (HCC); Their basic demographic and laboratory data are shown in Table 1.

The second group included 200 Patients with chronic hepatitis C virus infection. Their basic demographic data are shown in Table 2.

Table 2
Demographic data of chronic HCV infected patients.

Variables	Chronic HCV group (N = 200)
Age (years) (Mean ± SD)	53.1 ± 9.9
Sex (N%)	137 (68.5%)
Male	63 (31.5%)
Female	
Smoking (N%)	55 (27.5%)
Diabetes mellitus (N%)	35 (17.5%)
Random blood glucose (mg/dl) (Mean ± SD)	120.1 ± 46.2
Complete blood count (Mean ± SD)	
Total leucocyte count (x10^3/cmm) (Mean ± SD)	6.5 ± 4.01
Hemoglobin (g/dl)	12.7 ± 1.9
Platelets (x10^3/cmm)	201.8 ± 73.8
Liver function tests (Mean ± SD)	
Albumin (g/dl) (Mean ± SD)	3.4 ± 0.4
Total bilirubin (mg/dl) (Mean ± SD)	1.57 ± 1.3
ALT (U/L) (Mean ± SD)	39.1 ± 27.2
AST (U/L) (Mean ± SD)	38.1 ± 1.2
INR (Mean ± SD)	1.43 ± 0.4
Renal function tests (Mean ± SD)	
Creatinine (mg/dl) (Mean ± SD)	0.89 ± 0.2

The third group included one hundred healthy volunteers; their gut microbiota is evaluated to be compared with both HCC and HCV groups of patients. Their demographic data are shown in Table 3.

Table 3
Demographic data of healthy control subjects.

Variables	Control group (N = 100)
Age (years) (Mean ± SD)	49.9 ± 6.8
Sex (N%)	49 (49%)
Male	51 (51%)
Female	
Smoking (N%)	70 (70%)
Complete blood count (Mean ± SD)	
Total leucocyte count ($x10^3$/cmm) (Mean ± SD)	7.6 ± 2.3
Hemoglobin (g/dl)	13. 2 ± 1.7
Platelets ($x10^3$/cmm)	242.9 ± 92.2
Liver function tests (Mean ± SD)	
Albumin (g/dl) (Mean ± SD)	4.1 ± 0.5
Total bilirubin (mg/dl) (Mean ± SD)	0.82 ± 0.1
ALT (U/L) (Mean ± SD)	31.09 ± 4.1
AST (U/L) (Mean ± SD)	29.2 ± 4.4
INR (Mean ± SD)	1.1 ± 0.1
Renal function tests (Mean ± SD)	
Creatinine (mg/dl) (Mean ± SD)	0.81 ± 0.2

Regarding studied gut-microbiota in different groups:

1. Patients with HCC:

- High statistically significant difference was detected between HCC patients and controls **(p-value < 0.001)** as regard **Bifidobacterium** which was less detected in HCC patients (43%), compared to (76%) of healthy controls. However, in concern to **Lactobacillus & Enterobacteriaceae** they were more detected in HCC patients (80%) and (81%) compared to (36%) and (58%) in healthy controls, respectively.
- Statistically significant difference was detected between HCC patients and controls **(p-value < 0.05)** as regard **Faecalibacterium prausnitzii** which was less detected in HCC patients (51%), compared to (70%) of healthy controls.

- No statistically significant difference was detected between HCC patients and controls **(p-value > 0.05)** as regard **Bacteroides fragilis** & **Akkermansia muciniphila**. Table 4

1. **Patients with Chronic HCV infection**:

- High statistically significant difference was detected between HCV patients and controls **(p-value < 0.001)** as regard **Bifidobacterium & Faecalibacterium prausnitzii** which were less detected in HCV group of patients (44% and 38%), compared to 76% and 70% of healthy controls, respectively. On the other hand, **Lactobacillus** was more detected in HCV group of patients (62%). Compared to (36%) of healthy controls.
- Statistically significant difference was detected between HCV patients and controls **(p-value < 0.05)** as regard **Enterobacteriaceae** which was more detected in HCV group of patients (70%) compared to (58%) of healthy controls.
- No statistically significant difference was detected between HCV patients and healthy volunteers **(p-value > 0.05)** as regard **Bacteroides fragilis** & **Akkermansia muciniphila**. Table 5

Table 4
comparison between comparison between HCC patients and Control as regard studied gut micro-biota.

Gut micro-biota	**HCC patients (N = 100)**	**Control (N = 100)**	**X2**	**P-value**
Bifidobacterium (N%)	43 (43%)	76 (76%)	22.6	< 0.001 (HS)
Bacteroides fragilis (N%)	41(41%)	34 (34%)	1.04	0.307 (NS)
Lactobacillus (N%)	80 (80%)	36 (36%)	39.7	< 0.001 (HS)
Akkermansia muciniphila (N%)	46 (46%)	49 (49%)	0.18	0.671 (NS)
Faecalibacterium prausnitzii (N%)	51 (51%)	70 (70%)	7.6	0.006 (S)
Enterobacteriaceae (N%)	81(81%)	58 (58%)	12.5	< 0.001 (HS)
X^2: Chi-square test. S: p-value < 0.05 is considered significant.				
HS: p-value < 0.001 is considered highly significant.				
NS: p-value > 0.05 is considered non-significant.				

Table 5
comparison between chronic HCV infected patients and Control as regard studied gut micro-biota.

Gut micro-biota	HCV patients (N = 200)	Control (N = 100)	X^2	P-value
Bifidobacterium (N%)	88 (44%)	76 (76%)	27.5	< 0.001 (HS)
Bacteroides fragilis (N%)	82 (41%)	34 (34%)	1.37	0.241 (NS)
Lactobacillus (N%)	124 (62%)	36 (36%)	18.1	< 0.001 (HS)
Akkermansia muciniphila (N%)	83 (42%)	49 (49%)	1.52	0.217 (NS)
Faecalibacterium prausnitzii (N%)	75 (38%)	70 (70%)	28.2	< 0.001 (HS)
Enterobacteriaceae (N%)	140 (70%)	58 (58%)	4.27	0.339 (S)
X^2: **Chi-square test. S: p-value < 0.05 is considered significant.**				
HS: p-value < 0.001 is considered highly significant.				
NS: p-value > 0.05 is considered non-significant.				

By comparing between patients with HCC and chronic HCV infected patients

- High statistically significant difference was detected between HCC and HCV groups of patients **(p-value < 0.001)** as regard **Faecalibacterium prausnitzii** which was more detected in HCC group of patients (51%) compared to (38%) of HCV group. However, **Lactobacillus and Enterobacteriaceae** were more detected in HCC group of patients (80%) and (81%) compared to (62%) and (70%) of HCV group, respectively.
- No statistically significant difference was detected between HCC and HCV groups of patients (**p-value > 0.05)** as regard **Bifidobacterium, Bacteroides fragilis** and **Akkermansia muciniphila**. Table 6

Table 6
comparison between HCC patients and HCV patients as regard gut micro-biota.

Gut micro-biota	HCC patients (N = 100)	HCV (N = 200)	X^2	P-value
Bifidobacterium (N%)	43 (43%)	88 (44%)	0.027	0.869 (NS)
Bacteroides fragilis (N%)	41(41%)	82 (41%)	0.0	1.0 (NS)
Lactobacillus (N%)	80 (80%)	124 (62%)	9.9	0.002 (S)
Akkermansia muciniphila (N%)	46 (46%)	83 (42%)	0.55	0.458 (NS)
Faecalibacterium prausnitzii (N%)	51 (51%)	75 (38%)	4.9	0.026 (S)
Enterobacteriaceae (N%)	81(81%)	140 (70%)	4.2	0.041 (S)
X^2: **Chi-square test. S: p-value < 0.05 is considered significant.**				
NS: p-value > 0.05 is considered non-significant.				

Figure 2 showed the characteristic pattern of gut micro-biota; that showed a statistically significant difference between all studied groups.

In HCC group, no correlation could be detected between gut micro-biota and **Child-Pugh** grade or **TNM** stage.

A correlation was detected between the presence of **Bacteroid fragilis** microbiota and the **BCLC** staging with the highest detection in stage **IV** (66.7%). Table 7

Table 7
Correlation between studied gut- microbiota and different scores in the HCC group.

Variables	Child staging				P-value
	Child A (N = 18)	Child B (N = 38)	Child C (N = 44)		
Bifidobacterium (N%)	7 (38.9%)	13 (34.2%)	23 (52.3%)		0.239 (NS)
Bacteroides fragilis (N%)	6 (33.3%)	12 (31.6%)	23 (52.3%)		0.126 (NS)
Lactobacillus (N%)	12 (66.7%)	29 (76.3%)	39 (88.6%)		0.112 (NS)
Akkermansia muciniphila (N%)	10 (55.6%)	18 (47.4%)	18 (40.9%)		0.563 (NS)
Faecalibacterium prausnitzii (N%)	7 (38.9%)	20 (52.6%)	24 (54.5%)		0.517 (NS)
Enterobacteriaceae (N%)	14 (77.8%)	31 (81.6%)	36 (81.8%)		0.928 (NS)
TNM staging					
	TNM I (n = 24)	**TNM II (n = 40)**	**TNM III (n = 28)**	**TNM IV (n = 8)**	
Bifidobacterium (N%)	9 (37.5%)	18 (45%)	12 (42.9%)	4 (50%)	0.914 (NS)
Bacteroides fragilis (N%)	9 (37.5%)	16 (40%)	12 (42.9%)	4 (50%)	0.931 (NS)
Lactobacillus (N%)	18 (75%)	34 (85%)	21 (75%)	7 (87.5%)	0.633 (NS)
Akkermansia muciniphila (N%)	14 (58.3%)	15 (37.5%)	15 (53.6%)	2 (25%)	0.195 (NS)
Faecalibacterium prausnitzii (N%)	12 (50%)	21 (52.5%)	13 (46.4%)	5 (62.5%)	0.872 (NS)
Enterobacteriaceae (N%)	21 (87.5%)	34 (85%)	20 (71.4%)	6 (75%)	0.403 (NS)
BCLC staging					

X^2: Chi-square test. HS: p-value < 0.001 is considered highly significant.

NS: p-value > 0.05 is considered non-significant.

Variables	Child staging				P-value
	Child A (N = 18)	Child B (N = 38)	Child C (N = 44)		
	BCLC I (n = 28)	BCLC II (n = 26)	BCLC III (n = 34)	BCLC IV (n = 12)	
Bifidobacterium (N%)	9 (32.1%)	9 (34.6%)	19 (55.9%)	6 (50%)	0.201
Bacteroides fragilis (N%)	3 (10.7%)	13 (50%)	17 (50%)	8 (66.7%)	0.001**
Lactobacillus	21(75%)	22 (84.6%)	27 (79.4%)	10 (83.3%)	0.832
Akkermansia muciniphila (N%)	21 (75%)	22 (84.6%)	27 (79.4%)	10 (83.3%)	0.832
Faecalibacterium prausnitzii (N%)	12 (42.9%)	15 (57.7%)	20 (58.8%)	4 (33.3%)	0.316
Enterobacteriaceae	24 (85.7%)	20 (76.9%)	29 (85.3%)	8 (66.7%)	0.441
X^2: Chi-square test. HS: p-value < 0.001 is considered highly significant.					
NS: p-value > 0.05 is considered non-significant.					

Discussion:

Gut and liver are physiologically and anatomically connected; which known as the gut–liver axis. Gut microbiota component and their metabolites affect both hepatocytes and stromal cells (hepatic stellate cells and Kupffer cells). [24]

Intestinal dysbiosis is observed in different chronic hepatic diseases; which increased concern about its role in the development and progression of hepatic diseases with aggravation of liver disease-related complications. [25–26]

Few studies evaluated the role of gut dysbiosis in patients with viral hepatitis. So, in this study we try to compare changes that occur in gut microbiota in patients with chronic hepatitis C, HCC with normal healthy control.

The different species of Bifidobacterium has beneficial health effects, including the regulation of homeostasis of intestinal microbes, the suppression of pathogens and harmful bacteria that colonize the gut mucosa, the modification of local and systemic immune responses, the inhibition of procarcinogen enzymatic activities of the gut- microbiota and the mend of the gut mucosal barrier by lowering the level of lipopolysaccharides. [27–28]

In our study less than half of patients with HCC and chronic HCV infection have detected Bifidobacterium in their stool sample compared to 76% of healthy volunteers. This come in agreement with **Yu et al.** who found that decrease Bifidobacterium species, led to accumulation of lipopolysaccharides; which act as pathological mediator of inflammation-associated HCC. [29]

In our opinion also impairment of gut wall integrity due to absence of Bifidobacterium, may allow translocation of pathogenic bacteria and endotoxins which, may produce chronic hepatic inflammation with subsequent risk of development of HCC.

Although, its known that lactobacillus species is important for human health, by decreasing gastrointestinal PH, that protect the host against invasion by pathogens. [30] on the other hand, lactobacilli may be pathogenic in susceptible patients related to several mechanisms including, the ability of some strains to bind to intestinal wall with translocation to bloodstream leading to bacteremia, also they have ability to adhere to collagen in extracellular matrix with production of glycosidase enzyme leading to damage of affected tissues. [31–33]

In this study against usual, most patients with HCC and chronic HCV infection have lactobacillus in their stool compared with healthy controls, with more detected in HCC patients than chronic HCV infected patients.

This come with Sherid et al. Who found in their case study, an involvement of lactobacillus bacteria in the development of bacteremia and liver abscess. [34]

Against our results, Zhang et al. reported that disturbance of gut microbiota homeostasis with decrease lactobacilli, led to damage of mucosa with the development of endotoxemia, systemic inflammation and tumor formation. [35]

In healthy individuals, Faecalibacterium prausnitzii represent (> 5%) of the gut flora, it plays an important role in improvement of the immune system; it is an anti-inflammatory commensal activate IL-10 secretion and inhibiting IL-12 and interferon-γ expression. [36–37]

In this study, Faecalibacterium prausnitzii has been detected in about half of patients with HCC and (38%) of patients with chronic HCV infection, opposite to (70%) of healthy control.

This come in agreement with Munukka et al. who found that treatment of mice with Faecalibacterium prausnitzii improved hepatic ALT, AST, and decreases adipose tissue inflammation.[38]

Also, Liu et al. found in his study on patients with different causes of HCC, decreased level of Faecalibacterium resulting in reduction of the level of anti-inflammatory short-chain fatty acids.[39]

The family **Enterobacteriaceae** includes a medically important species such as Salmonella, Escherichia coli, Yersinia pestis, Klebsiella, Shigella, Proteus, Enterobacter, Serratia, and Citrobacter. Many members of this family are normally present in human as a gut microbiota. Some enterobacteria are pathogens,

because they produce endotoxins; when released into the bloodstream, cause a systemic inflammatory and vasodilatory response. [40–41]

In this study, Enterobacteriaceae was found in stool samples of about 80% of patients with HCC and 70% of chronic HCV infected patients.

This come in agreement with, Sanduzzi, Chen, Lax and Bajaj et al. who found in their studies that, increased Enterobacteriaceae was linked to progression of liver cirrhosis and development of cirrhosis related complications. [42–45]

Bacteroides is the most predominant bacteria in the colon, with Bacteroides fragilis group is the most abundant one among Bacteroides species. [46] Bacteroides together with other gut commensal bacteria provide the human body with energy through Carbohydrate fermentation producing a pool of volatile fatty acids that are absorbed in the colon. [47] Akkermansia muciniphila is one of gut micro-biota known to have an anti-inflammatory effect in humans, improves hepatic inflammation and protect against liver cell damage through an immune-mediated mechanism. Also, it's believed to have a role in cancer response to immunotherapy. [48–50]

In our study, we found no significant difference between HCC patients and chronic HCV infected patients, in comparison with control group, regarding the stool isolate of **Bacteroides fragilis.**

However, **Chen et al.** found in his study, a significant decreased in **Bacteroid** level in patients with liver cirrhosis related complications. [51] On the other hand, **Guoxiang et al.** found a marked increase in **Bacteroid** species level in mice model with HCC development post-NASH. [52]

Also, in this study, no significant difference has been detected between HCC patients and chronic HCV infected patients, compared with control group, as regard the stool isolate of Akkermansia muciniphila. Akkermansia muciniphila, was detected in the stool of less than half of patients with HCC and HCV groups.

To some extent different experimental studies on animal models; come in accordance with our results. They demonstrated that, the presence gut Akkermansia muciniphila can enhance the anticancer effect of T-cell-based immunotherapies. [53–56]

Though many staging systems for HCC were used worldwide, there is no system is considered the best in evaluating the suitable treatment and patient's prognosis. [58] **Child-Pugh** and **TNM** had a better predictive ability for overall survival than **BCLC**. [59] However, TNM fails to evaluate patient's prognosis accurately, because it only evaluates tumor extension and **BCLC** has a better prognostic ability than **TNM** staging system. [60] So, in this study we try to use different systems for evaluation of patients with HCC

In this study, no significant difference was detected between gut-microbiota and HCC progression with respect to **Child** or **TNM** systems. However, a significant difference was detected between number of

positive stool isolate of Bacteroid Fragilis and BCLC staging system; where it was isolated from 66.7% of patients with **BCLC stage IV** opposite to 10.7% of patients with **BCLC stage I**.

This come in agreement with Guoxiang et al. who found in his experimental study, marked increased bacteroid species together with lipopolysaccharides levels; with the progression of liver disease from steatosis till HCC. [61]

Conclusion

A characteristic pattern of Bifidobacterium, Lactobacillus and Enterobacteriaceae species in patients with chronic HCV and HCC was detected. So, dysbiosis may be associated with the progression of liver disease and hepatic carcinogenesis. It also may act as a clue for a possible role probiotic to prevent the progression of liver disease. However, many debates are present regarding the role of dysbiosis in the development of liver disease and hepatic carcinogenesis. further studies are needed on a large number of patients.

Declarations

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None.

Authors' contributions

Data collection and Abdominal Ultrasound for included subjects in Internal Medicine and Tropical Medicine& Gastroenterology Department; **AMMS, HAO.** Investigation and Methodology: **KM, HA**. Data analysis: **KM, HAO, HA, AMMS.** Provisional writing and final reviewing of the manuscript: **KM, HAO, HA, AMMS.**

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Consent for publication

Not required.

Conflict of interests

All Authors declared that no conflict of interest.

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Figures

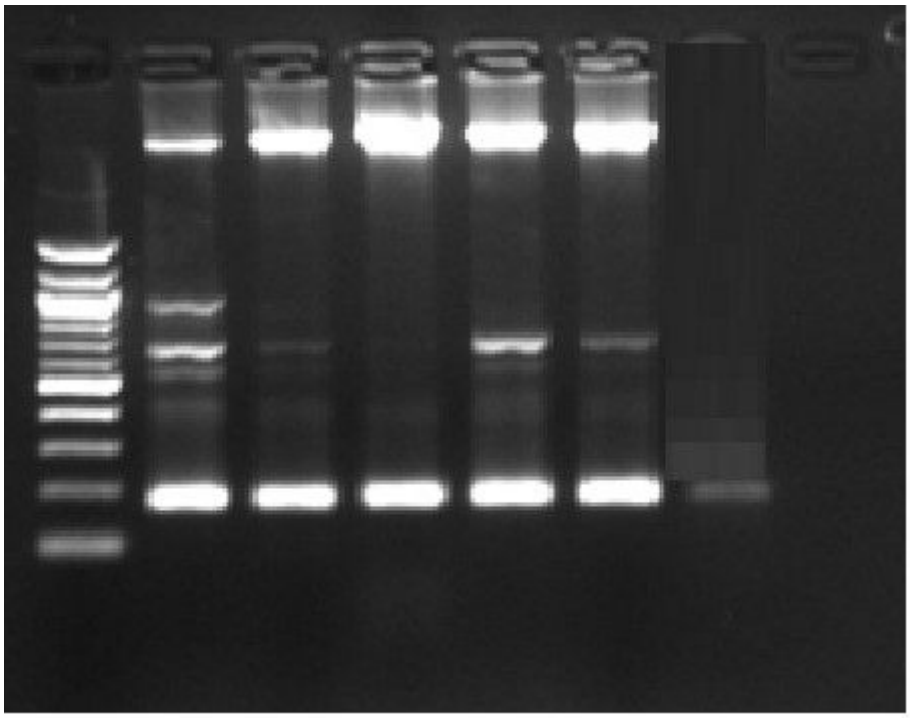

Figure 1

Positive stool sample for Faecalibacterium prausnitzii

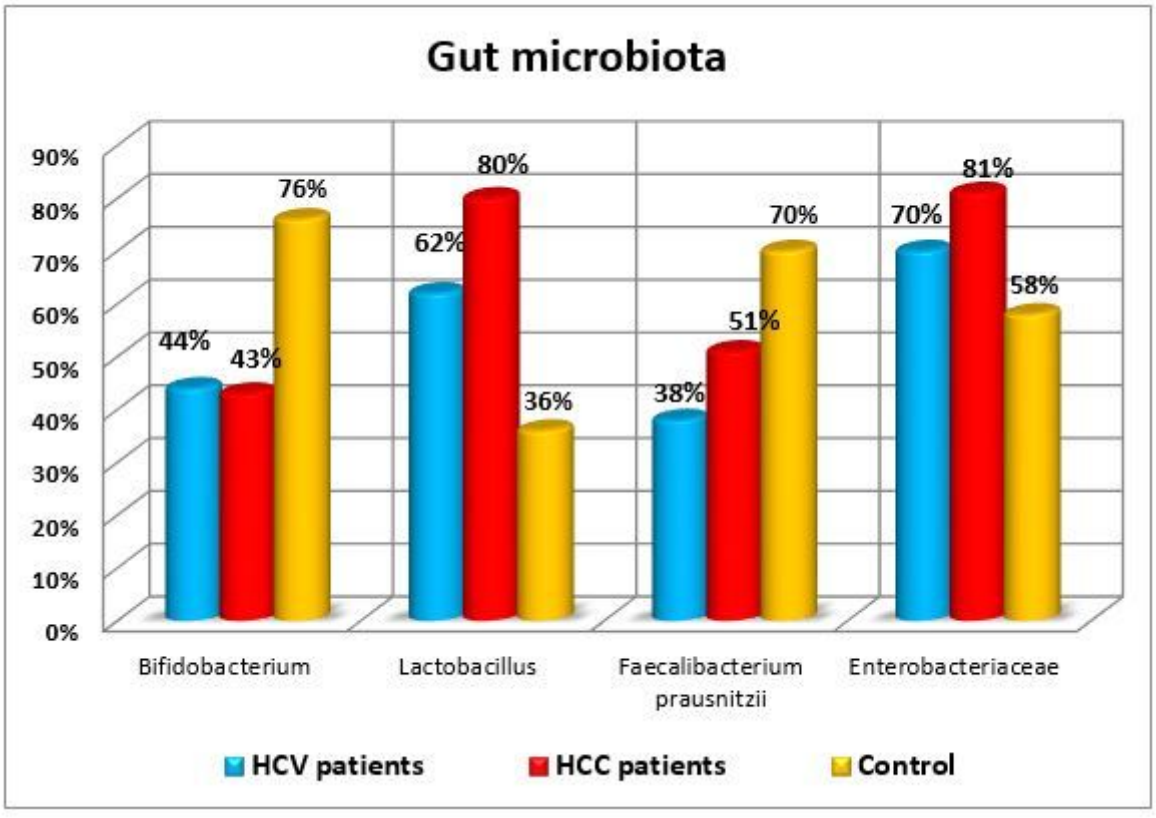

Figure 2

The characteristic pattern of gut micro-biota; that showed a statistically significant difference between all studied groups.