Published in final edited form as:

Dig Dis Sci. 2017 August ; 62(8): 2159–2165. doi:10.1007/s10620-017-4626-7.

Clinical and Pathological Risk Factors Associated with Liver Fibrosis and Steatosis in African Americans with Chronic Hepatitis C

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Abstract

Background & Aim—Several factors involved in the development of liver fibrosis in African American patients with chronic hepatitis C (CHC) have not been well-studied. We aimed to evaluate some of these risk factors.

Methods—We reviewed pathology and medical records of 603 African Americans with chronic hepatitis C virus (HCV) infection at Howard University Hospital from Jan. 2004 to Dec. 2013. Among the clinical and pathological data collected were HIV (human immunodeficiency virus), HCV genotype, Hepatitis B virus (HBV), diabetes mellitus (DM), hypertension (HTN), body mass index (BMI), and hepatic steatosis.

Results—The frequency of DM, HTN, HIV and HBV were 22%, 16%, 11%, and 4%, respectively. Median BMI was 27.3 kg/m². The frequency of fibrosis stages 0, 1, 2, 3 and 4 were 2%, 48%, 28%, 11% and 11%, respectively. In multivariate logistic regression, we found a significant association between liver fibrosis stage (3–4 vs. 0–2) and HIV infection (OR=2.4, P = 0.026), hypertension (OR=3.0, P = 0.001), age (OR=2.6 for every 10 years, P<0.001), weight (OR = 1.1 for every 10 pound increase, P = 0.002) and steatosis grade (OR = 1.6, P = 0.002). The frequency of liver steatosis was 73%. In an ordinal logistic regression, significant risk factors for steatosis were female gender (OR = 1.5, P = 0.034) and inflammation grade (P=0.001).

Conclusion—This study shows that steatosis is independently associated with fibrosis in African American patients with HCV infection. Female patients were at higher risk for steatosis.

Keywords

Hepatitis; fibrosis; HCV; African Americans

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Introduction

Hepatitis C Virus (HCV) infection is a major cause of liver disease and remains an important public health problem worldwide. According to the World Health Organization, nearly 3% of the world's population (about 150 million people) is infected with HCV and about 700,000 die from hepatitis C-related liver disease ¹. Although chronic HCV infection begins as an asymptomatic condition with few short term effects, it can progress to fibrosis, cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC) ^{2,3}. Three to 4% of chronically infected individuals can also develop fatal hepatocellular carcinoma (HCC) ⁴. In the United States, people born between 1945 and 1965 (Baby-boomers) are at higher risk of harboring chronic HCV and therefore are prone to developing complications from the infection ⁵. A chronic liver injury leads to fibrosis, a structural modification of the liver that promotes accumulation of extracellular matrix proteins such as collagen in response to wound healing. Fibrosis staging is critical for the clinical management and prognosis of chronic hepatitis C because as a prognostic factor, it is correlated with the risk of developing cirrhosis and end-stage liver disease.

Progression of fibrosis can be accelerated by factors such as age, duration of HCV infection, sex, coinfection with HBV or HIV and alcohol intake. Also, Diabetes and insulin resistance not only increase fibrosis but are also predictors of liver fibrosis particularly in patients harboring genotype-1 ⁴⁹⁻⁵¹.

In patients with HCV infection alone, steatosis was associated with accelerated progression of liver disease and HCV genotype 3, BMI, visceral adiposity, insulin resistance and hyperlipidemia ⁵²⁻⁵⁵. Furthermore, insulin resistance can cause hepatic steatosis and is associated with progression of fibrosis and inflammation⁶. HCV infection is more prevalent in African-Americans (AA) as compared to most other racial and ethnic groups, although fibrosis rates may be slow, hepatocellular carcinoma and mortality rates appear to be higher among African Americans ⁷.

Steatosis is defined as the accumulation of fat in hepatocytes. Physiologically, hepatocytes contain small amounts of fat necessary for their metabolism. When liver fat exceeds 5–10% of its weight, we can suspect a diagnosis of steatosis ⁸. Accumulation of fat in the cytoplasm of hepatocytes is a complex pathological process in which many factors are involved leading to an alteration of fat metabolism ⁹. There are two discrete forms of steatosis that may be found in patients infected with HCV. Metabolic steatosis can coexist with HCV, regardless of genotype, in patients with risk factors such as obesity, hyperlipidemia, and insulin resistance. The second form of hepatic steatosis in HCV patients is a result of the direct cytopathic effect of genotype 3 viral infections ¹⁰. Steatosis occurs frequently in hepatitis C patients. However, the mechanisms leading to this lesion are still unknown, and the role of steatosis in the progression of liver fibrosis remains controversial ¹¹. Insulin resistance (IR) occurs when normal amounts of insulin do not stimulate insulin activity in cells. Both steatosis and insulin resistance have been described in hepatitis C virus infected patients and are thought to be biologically related ¹². Steatosis and steatohepatitis liver inflammation caused by fat deposits, are present in 23% and 30%, respectively, in HIV/HCV co-infected patients, and both are associated with an increased risk of advanced stage fibrosis ¹³. Hepatic

steatosis is common in chronic hepatitis C patients and has been linked to concurrent obesity, insulin resistance, diabetes, and disease severity¹⁴. Diabetes is not an independent predictor of the degree of fibrosis. In contrast, BMI is an independent predictor of both fibrosis and steatosis in HCV patients¹⁵. Both host and viral factors may be important contributors to the natural history of HCV. Male gender¹⁶, acquisition of HCV at an older age¹⁷ and BMI^{18, 19} are associated with faster progression of liver disease. Alcohol intake has been shown to promote the progression of HCV, even in patients with relatively low alcohol intake¹⁸.

Although non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome, the clinical association between non-alcoholic steatohepatitis (NASH), the severe version of NAFLD, and lifestyle-related diseases such as obesity, type 2 diabetes mellitus (T2DM), hypertension (HTN) and dyslipidemia has not been clarified²⁰. Ethnicity and BMI were associated with the progression of fibrosis and the presence of cirrhosis. Hispanics had the highest fibrotic index and prevalence of cirrhosis, whereas African-Americans had the lowest¹². Our study was prompted by published reports that suggested that the rate of liver fibrosis progression was slower in African-Americans than European-Americans with chronic hepatitis C virus (HCV) infection³⁷. We wished to determine the clinical and pathologic factors associated with the course of the disease.

In this study, we analyzed risk factors associated with fibrosis and steatosis in African American patients with HCV.

Methods

Patients

We extracted data from 603 AA patients diagnosed with chronic hepatitis C and from whom a liver biopsy was taken between 2004 and 2013 at Howard University Hospital in Washington D.C. Initial diagnosis for CHC infection were made through serological assays for antibodies and molecular techniques for detecting virus particles. Hepatitis C quantitation or viral titer was accomplished by HCV Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Patients were included if they had at least one liver biopsy in the last 10 years. Biopsy evaluation was performed using Batts-Ludwig fibrosis staging score. All fibrosis diagnosis met the minimum requirement (>1.5 CM and >6 portal track). Logistic regression analysis was used to assess the predictors of steatosis and fibrosis. Liver biopsies were performed percutaneously with ultrasound guidance or through transjugular needle biopsy. Hematoxylin & Eosin, and Reticulin & Trichrome staining were used to visualize liver tissues and for fibrosis and steatosis staging. Two pathologist (B.S & E.L) graded and staged steatosis. Brunt scale was used for grading steatosis. Data for fibrosis, steatosis, inflammation were extracted from pathology reports while the demography and clinical data such as age, BMI, DM, HTN, HIV, HBV were obtained from Electronic Medical Records. Iron stain was also used to assess iron deposition within the hepatocytes.

Statistical analysis

Continuous variables were presented by median (interquartile range). We tested for trend the bivariate association between the clinical and demographic factors with steatosis grade and stage of fibrosis using a nonparametric test for trend. Variables with $P \leq 0.2$ from bivariate analysis were entered into logistic regression model (for fibrosis Stage of 3–4 vs. 0–2). We also used an ordinal logistic regression model for the predictors of different steatosis stages. We used a backward stepwise approach to select the final significant predictors of each outcome ($p < 0.05$). All analyses were performed in STATA 14.0 (StataCorp., College Station, TX).

Results

General characteristics of the study population

The majority of the study population were male ($n=388$, 64%). Median (range) of age and BMI were 54 years (19–83) and 27.3 (24.4–32.2) kg/m^2 , respectively. The frequency of fibrosis, all stages combined, was 98% ($n=589$). Fibrosis stages 1–4 was 48%, 28%, 11% and 11%, respectively. Among 593 patients with reported steatosis data, 434 (73%) had steatosis, including 48% grade 1, 14% grade 2 and 11% with grade 3 steatosis. The HBV and HIV co-infections were 4% and 11%, respectively. Other major comorbidities were diabetes mellitus (22%) and hypertension (16%). In univariate analysis; age, hypertension and steatosis were significantly associated with higher stages of fibrosis. In higher stages of fibrosis, the inflammation grade was also significantly higher (Table 1).

Additionally, the Odds Ratios (OR) of significant clinical factors associated with fibrosis are displayed in Table 2. As shown, in a logistic regression model of predictors of fibrosis (stage 3–4 vs. 0–2), HIV (OR = 2.4), older age (OR = 2.6 for each 10 years), hypertension (OR = 3.0), weight (OR = 1.1 for each 10 pound increase) and steatosis grade (OR = 1.6) significantly predicted higher fibrosis stages.

Among 593 patients with reported steatosis data, 159 had grade 0 and 434 (73%) had some grade of steatosis, including 38% ($n=228$) grade 1, 13% ($n=76$) grade 2, 12% grade 3 ($n=69$). In a logistic regression analysis (comparing absence of steatosis vs. any steatosis), female gender (OR = 1.5, 95% CI = 1.01–2.3, $P = 0.044$) and inflammation grade (OR = 1.4, 95% CI = 1.1–1.9, $P = 0.005$) were associated with higher risk of steatosis. There seems to be a significant relationship between hepatocyte Iron levels and steatosis stages ($p=0.022$) although a clear explanation may not be evident. In an ordinal logistic regression (comparing different stages to no steatosis), both female gender (OR = 1.4, $P = 0.034$) and inflammation grade (OR = 1.4, $P = 0.001$) remained significant predictors of steatosis stage.

Discussion

Our retrospective study showed that there are risk factors, such as HIV co-infection for progression of liver fibrosis in the African American population with chronic hepatitis C. For HIV-HCV co-infected individuals, the burden of disease is largely related to their HCV disease, including a faster progression to liver fibrosis, cirrhosis and liver-related deaths²¹. This may be due to a wide number of molecular and pathogenetic mechanisms that could

accelerate liver fibrosis and its progression in HCV/HIV co-infected individuals. Although it is beyond the scope of our study, it has been demonstrated *in vitro* that HIV augments the replication of HCV in hepatocytes possibly by way of transforming growth factor (TGF)- β 1-mediated pathway³⁶. TGF- β 1 is the leading fibrogenic cytokine in the liver, even though HIV can also enhance fibrogenesis through the induced production of reactive oxygen species by hepatic stellate cells in the presence of HCV³⁸.

This study showed that hypertension, HIV, higher age and obesity were found to be risk factors for liver fibrosis in the study population. Also, steatosis was clearly shown to be an independent risk factor for fibrosis. Steatosis in general could be due to alcohol, metabolic syndrome or viral hepatitis. Several studies have reported that liver steatosis is a common histological feature of chronic hepatitis C infection. The prevalence of steatosis ranges from 40% to 86%^{22, 23} in these patients. It is difficult to assess the impact of steatosis on necroinflammatory activity and the process of liver fibrosis in patients with chronic hepatitis C, since these processes are multifactorial⁸. However, several clinical and experimental studies agree with our results that that hepatic steatosis accelerates the development and progression of fibrosis in chronic hepatitis C patients^{24, 25}.

A number of previous studies have investigated the role of iron stores in NAFLD by assessing the presence of stainable hepatic iron deposits, the biochemical hepatic iron content, or both^{26–28}. However, the findings thus far have been conflicting, with some studies finding hepatic iron deposition to be associated with increased disease severity^{29, 30}. In our study, we found that the higher the grade of deposition of iron, the more pronounced steatosis was in the biopsies.

Chronic viral hepatitis infection increases liver fibrosis and stiffness and is an important cause of liver cirrhosis³¹. Chronic hepatitis C virus infection is typically characterized by slowly progressive hepatic fibrosis. However, it is recognized that some patients do not progress while others rapidly develop significant fibrosis³². Nonalcoholic fatty liver disease is associated with major cardiovascular risk factors, such as type 2 diabetes mellitus, and has been recently considered to be a new component of metabolic syndrome and it serves as a criterion for the hepatic manifestation of the latter³².

We conducted a study in African Americans with chronic hepatitis C to define clinical factors, such as DM, HTN, obesity, age, and viral co-infections that can be predictive of fibrosis. In our results, age was also a risk factor in the progression of fibrosis. Similarly in agreement with other published data involving older groups of Japanese³⁹, age is strongly associated with the development and progression of NASH³³. However, this agreement does not preclude the observation that in children with NAFLD, steatosis is associated with advanced fibrosis as indicated in the latest published report⁴⁰. Other reports also indicate that DM is associated with NASH in older male NAFLD group whereas a higher frequency of advanced fibrosis was observed in an older female NASH group. This increasing prevalence of NASH and progression to fibrosis in both sexes may be related to lifestyle changes and the presence of other comorbidities in older populations.

We also found in our study that steatosis is more prevalent in African American female patients. This is surprising because following the initial infection, hepatitis C is a disease that disproportionately affects men because women are more likely to clear the virus spontaneously³³. Women also have slower rates of liver disease progression than men if they become chronically infected. However, this rate of disease progression changes over time in women as postmenopausal women have increased rates of fibrosis compared with women of reproductive age because they have lost the protective effects of estrogen. Another study, which is consistent with our results, showed that steatosis associated with chronic hepatitis C was disproportionally more frequent in females³⁴.

Steatosis is a pathological feature that was also highly associated with fibrosis. In a cross sectional study of nondiabetic HCV-infected individuals, steatosis' severity was correlated with severity of fibrosis, independent of age³⁵.

We also noticed that inflammation was highly correlated with both fibrosis and steatosis. Hepatic fibrosis is commonly preceded by chronic inflammation, and persistence of this inflammation has been associated with progressive hepatic fibrosis and the development of cirrhosis³⁰.

However, we were not able to find significant relationship between DM, end-stage renal disease (ESRD), and hepatitis B co-infection in our population. We were not able to find correlation between male gender with the progression of liver fibrosis. In contrast, in a study by Poynard et al¹⁷, male gender has a stronger association with progression of fibrosis than virological factors such as HCV infection. The reason for this difference may be due to genetic factors related to African American ethnicity or environmental factors which may need more studies. More importantly, liver fibrosis (i.e. mild or moderate) once considered irreversible is now reversible with early detection and intervention mainly due to recent progress in understanding its molecular pathogenesis. This has been demonstrated by observations of haemochromatotic patients and patients with hepatitis B and C following successful interferon-based therapy and other antiviral therapies^{41–48}. These observations are highly reassuring and advance the notion that the liver has an ability to rebuild a scar tissue that would prevent progression to cirrhosis, which is far more difficult to reverse. This is significant because in African-Americans the natural history of HCV and the progression to fibrosis is less accelerated whereas development of cirrhosis and hepatocellular carcinoma are more evident.

A clear limitation of our retrospective study is the lack of inclusion of advanced fibrosis with a staging score of 3 and above (i.e. cirrhosis) and the associated clinical and pathologic factors in its progression.

In summary, our results show that steatosis is independently associated with fibrosis in African American patients infected with HCV. Female patients were at higher risk for steatosis. Also, HIV, HTN and age were found to be risk factors for fibrosis in African-Americans. Future clinical guidelines may include the early detection of fibrosis through routine measurement of steatosis for HCV and HIV patients.

Acknowledgments

This project was supported (in part) by the National Institute on Minority Health and Health Disparities of the National Institutes of Health under Award Number G12MD007597. We would like to thank Dr. El-Serag for his critical review of this manuscript.

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Table 1

Distribution of clinical and pathologic variables by fibrosis stage. Results are in median (interquartile range) unless otherwise specified.

N = 603	Stage 0 N=14	Stage 1 N=287	Stage 2 N=166	Stage 3 N=68	Stage 4 N=68	P value for trend
Age, year	47 (45–55)	54 (49–58)	54 (51–59)	55 (51–60)	56 (52–61)	<0.001
Female, no (%)	2 (14%)	100 (35%)	59 (36%)	27 (40%)	27 (40%)	0.2
Weight, pound	168 (156–194)	175 (160–200)	179 (155–205)	200 (156–217)	199 (163–230)	0.2
BMI, kg/m ²	28 (24–19)	27 (24–31)	27 (25–33)	28 (24–34)	28 (25–36)	0.1
Inflammation grade	1 (1–1)	1 (1–1)	2 (2–2)	3 (2–3)	3 (2–3)	<0.001
Iron grade	6 (4–8)	2 (1–3)	1 (1–2)	1 (1–4)	1 (1–6)	0.1
Iron in hepatocyte, no (%)	5 (38%)	143 (53%)	74 (47%)	31 (51%)	31 (51%)	0.1
Steatosis, no (%)	8 (62%)	187 (67%)	131 (79%)	54 (81%)	52 (80%)	0.002
HBV, no (%)	0	8 (3%)	7 (4%)	6 (9%)	1 (1%)	0.4
HIV, no (%)	1 (7%)	23 (8%)	23 (14%)	12 (18%)	8 (12%)	0.003
CHF (congestive heart failure), no (%)	1 (7%)	9 (3%)	6 (4%)	0	0	0.1
HTN (hypertension), no (%)	1 (7%)	33 (12%)	27 (16%)	18 (26%)	15 (22%)	0.02
ESRD (end stage renal dis.), no (%)	0	11 (4%)	5 (3%)	1 (1%)	0	0.1
DM (diabetes mellitus), no (%)	6 (43%)	53 (18%)	44 (27%)	15 (22%)	14 (21%)	0.1

Table 2

Multivariate logistic regression of predictors of fibrosis stage.

	OR (95% CI)	P value
Age (each 10 years)	2.6 (1.6–4.0)	<0.001
HIV	2.4 (1.1–5.1)	0.026
Hypertension	3.0 (1.6–5.6)	0.001
Weight (each 10 pound)	1.1 (1.06–1.2)	<0.001
Steatosis grade	1.6 (1.2–2.2)	0.002

Variables entered into model: age, weight, gender, HIV, HTN and DM, steatosis. ESRD and CHF were removed from model due to frequency of events.

Area under curve for model = 0.78

P for goodness of fit >0.9

Table 3

Distribution of clinical and pathologic variables by grade of steatosis. Results are in median (interquartile range) unless otherwise specified.

N = 593	Grade 0 N=159	Grade 1 N=283	Grade 2 N=82	Grade 3 N=69	P value for trend
Age, year	53 (49–58)	55 (51–59)	54 (50–58)	53 (50–56)	0.6
Female, no (%)	46 (29%)	103 (36%)	35 (43%)	28 (41%)	0.032
Weight, pound	180 (160–205)	180 (160–203)	171 (150–205)	170 (150–213)	0.3
BMI, kg/m ²	28 (25–32)	27 (25–32)	27 (23–31)	27 (23–32)	0.1
Inflammation grade	2 (1–2)	2 (1–2)	2 (2–3)	2 (1–3)	<0.001
Iron grade	2 (1–3)	2 (1–3)	2 (1–4)	1 (1–4)	0.7
Iron in hepatocyte, no (%)	79 (54%)	140 (53%)	31 (39%)	28 (42%)	0.022
HBV, no (%)	5 (3%)	12 (4%)	3 (4%)	3 (4%)	0.7
HIV, no (%)	21 (13%)	26 (9%)	11 (13%)	8 (12%)	0.9
CHF, no (%)	4 (3%)	9 (3%)	3 (4%)	0	0.5
HTN, no (%)	22 (14%)	42 (15%)	16 (20%)	12 (17%)	0.3
ESRD, no (%)	7 (4%)	9 (3%)	1 (1%)	1 (1%)	0.1
DM, no (%)	40 (25%)	56 (20%)	18 (22%)	14 (20%)	0.4