

## Original Research Article

### **Clinico-Pathological Characteristics of HCV coinfection in Chronic HBV Liver Diseases Patients in North-central Nigeria**

#### **Abstract**

**Introduction:** Viral hepatitis (HBV, HCV) infections remain a global public health problem, with high burden in sub-Saharan Africa and Asia. Coinfection ~~compounds and disease~~ worsen patient diagnosis and management approach. Local clinical and pathological data to provide basic diagnostic information necessary in patient management. Hence, this study evaluated the clinico-pathological ~~characteristics of characteristics of~~ HCV coinfection in chronic HBV liver disease patients.

**Methodology:** A total of 100 chronic HBV patients were enrolled into the study, with detailed demographic and clinical information documented into study database. Serodetection of HBsAg was determined by rapid immunochromatographic kit and Anti-HCV by Enzyme linked immunosorbent assay (ELISA) method. Hematological indices was analysed using Sysmex hematological analyser and biochemical profile, liver function tests by standard methods. Appropriate statistical method applied in analysis of study data

**Results:** The 100 study patients comprised of 65% males and 35% females, male to female ratio of 1.869:1. The mean age of coinfecting patient was  $42.0 \pm 8.8$  years, with majority within age-group of 41-50 years. Anti-HCV seroprevalence of 10%, 7 (10.8%) males and 3 (8.6%) females, M:F 1:2.3. Histological diagnosis of liver biopsies, 92% cases identified as chronic liver

disease and 4% each as cirrhosis and hepatocellular carcinoma respectively. Anti-HCV detection in coinfecting liver biopsies, 75% as hepatocellular carcinoma(HCC) cases, 25% as cirrhosis and 6.6% as chronic hepatitis. Significant association was observed between severity of liver disease and degree of infection( $p=0.01$ ). Histological activity index score was significantly low in coinfecting patients( $5.2\pm 4.2$ ) compared with mono-infection( $8.2\pm 4.2$ )( $p=0.01$ ). Significantly high mean value of aminoalanine transferase(ALT), aminotransferase(AST), alkaline phosphatase(ALP) and total protein and reduced mean of albumin were recorded among coinfecting patients(0.05). Similarly, significant mean value in white blood cell count(WBC) and ESR in coinfecting patients, while packed cell volume(PCV), lymphocyte and platelets count in mono-infected patients

**Conclusion:** The coinfection prevalence of 10% is lower than the level reported in studies conducted in Nigeria, while the clinic-pathological picture is at variance with other studies. The main findings revealed concomitant contribution of both hepatic viruses to development of chronic liver disease

**Keywords,** HCV, HBV, coinfection, chronic liver disease, north central Nigeria

## Introduction

Globally, Hepatitis B and C remains a public health problem, with high prevalence ~~and burden~~ and burden in sub-Saharan Africa and Asia responsible for chronic liver diseases, cirrhosis and hepatocellular carcinoma ~~with high~~ with high morbidity and mortality rate[1]. Characteristically, both hepatotropic viruses shared same mode of transmission ~~although~~ although ~~HBV~~ HBV infection ~~predominantly~~ predominantly ~~acquired early~~ acquired early in life via vertical and perinatal transmission ~~while,~~ while, ~~HCV~~ HCV infection by contact with

contaminated body fluid. According to the World Health Organisation, an ~~estimated, 350~~estimated, 350 million and 170 million are infected by HBV and ~~HCV worldwide~~HCV worldwide respectively, ~~while~~while approximately 1 million died annually due to liver related cases including cancer[2,3]. Clinical implication and outlook of coinfection of HBV and ~~HCV is~~HCV is complex, but common in viral hepatitis endemic region like sub-Saharan Africa, particularly West African region and Asia due to the shared mode of transmission[4]. The exact prevalence is not known, it is projected to be 1-15% globally[5], 2-10% among HCV antibody positive patient and 3-20% among hepatitis B surface antigen carriers[6,7].

In Nigeria, national surveillance study of HBV infection of 12.2% was reported [8] and Anti-HCV prevalence of pooled studies ranged between 0.4 and 5.5%[9], although these data varied with geographical location and predisposing risk factors. As epidemiological data of coinfection continued to evolve, few studies had reported coinfection prevalence in chronic liver disease subtypes, 20% in Maiduguri[10], 12.2% in Lagos[11], 11.8% in Jos[12]. Coinfection is common among IDU, haemodialysis patients, organ transplantation patient, HIV patients - positive patient, and beta-thalassemia patient 10%[7]. Risk factors are more likely to occur among individuals aged over 40 years, geographical variation, history of blood transfusion, intravenous drug user, unsafe sex, history of genetic hematological disease[7].

Serological screening for HbsAg and Anti-HCV are routinely used for screening and early diagnosis while PCR assay of HBV-DNA and HCV-RNA are used as the precise marker of active HBV replication, possible variant infection and reliable marker of infectivity. Histological diagnosis of liver biopsy is used as the investigation of choice for assessment of inflammation and fibrosis that provides

insight into assessment of liver damage, necro inflammatory changes and antiviral therapy. In addition, the Histological active Index(HAI) is used to grades/and score of hepatic disease development and progression of liver diseases. Of the histological findings, documented studies had reported conflicting changes in the liver diseases of coinfection and mono-infection based on the severity of liver diseases, while other studies reported no difference in the histological changes [13,14]. However, some studies have reported necro inflammatory changes and fibrosis in coinfection [15]. The biochemical and hematological tests are clinically used to complement the histological diagnosis in the evaluation of disease progression. Based on this observation, ~~the study~~ the study was conducted to evaluate the clinico-pathological profile of HCV coinfection of chronic liver disease cases.

### **Patients and Methods**

~~The descriptive~~ The descriptive, cross-sectional study was conducted at the Gastroenterology Unit, Jos University Teaching Hospital between March and December 2005. The 600 beds capacity hospital, provides multimedical specialties to Nigerians and foreigners. The study protocol was approved by Jos University Teaching Hospital Institution review board. A well-structured study questionnaire and informed consent was administered to the study participants, which entails the following information, demographic, clinical data of past medical history, previous history of jaundice, blood transfusion, use of intravenous drugs and traditional surgery such as scarification, uvulectomy, ~~tattoo~~ tattoo and alcohol intake.

The inclusion criteria, patients who were positive HBsAg for over six months and the following tests, full blood count, liver function test, HIV screening,

prothrombin, abdominal ultrasound scan and liver biopsy. Exclusion criteria includes, HIV infection, contraindication to liver biopsy, pregnancy.

Using the formula and HCV prevalence of 5%[16]

$$n = \frac{(Z^2 pq)}{d^2}$$

n = sample size

z = Standard Normal deviation corresponding to 5% level of significance

$$= 1.96.$$

p = Prevalence of hepatitis C virus co-infection among patients with chronic liver disease (Chronic Hepatitis B) from review of literature<sup>64</sup>

$$= 5\%. (0.05)$$

q = complementary probability to p.

$$1-p$$

$$= 1-0.05$$

$$= 0.95.$$

$d^2$  = precision = 0.05

$$n = \frac{(1.96)^2 0.05 (1-0.05)}{(0.05)^2}$$

The minimum sample size required for this study was 73. However, for the purpose of this study a sample size of 117 was taken to account for loss of questionnaire and loss to follow up.

### **Sample collection**

10 ~~millilitre~~ mL of venous blood was drawn aseptically into properly labelled sample bottle, allowed to clot, retract and centrifuged at 1500rpm for 15 minutes. The sera separated into sample bottle and kept at  $-20^{\circ}\text{C}$  for analysis. Serum transaminases, total protein and bilirubin were analysed using standard routine methods. Serodetection of HbsAg and Anti-HCV was carried out using Biotech diagnostic limited and clinotech diagnostic (ELISA-3), according to the manufacturer's instruction. Percutaneous liver biopsy was carried out using the trucut soft tissue biopsy with graduate cannula 14G(2.1mmx152mm) was ~~performed~~ performed on the 100 subjects, Minimum safety requirement of ~~heamoglobin~~ hemoglobin concentration of at least 10g/100ml, platelets count of 80,000/cm<sup>3</sup>, prolonged prothrombin time not prolonged than 3 seconds of the control was observed.

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### **Histological analysis**

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The liver tissues were fixed in 10% buffered normal-saline for 24 hours and embedded in paraffin wax. The liver tissue sections were cut at 5 $\mu\text{m}$  thickness with rotary microtome and stained with haematoxylin and eosin. Haematoxylin stains the nuclei while eosin stains the cytoplasm and other extra cellular materials. Special stains such as Reticulin to stain the reticular fibres, Masson's trichrome to demonstrate fibrous connective tissues, a Periodic Acid-Schiff (PAS) to demonstrate glycogen, and Orcein to demonstrate HBsAg were also employed. The histologic activity index of chronic hepatitis were reported according to Knodell and Ishak grading and scoring method.

Data analysis

Using SPSS version 20.0 demographic and clinical data were expressed in descriptive frequency, mean, standard deviation. ~~Categerigial~~Categorical comparison of data was done by  $\chi^2$  (chi-square) test at significance differences of  $p < 0.05$ . Fisher exact test and multiple logistic regression analysis was performed to assess the independent effects of risk factors for acquisition of HCV. The effects of the risk factors using odds ratio (OR) with 95<sup>th</sup> CI (confidence interval) were estimated.

## Results

The 100 chronic HBV related liver disease study participants (Table 1) comprised 65% males and 35% females giving male to female ratio of 1.9:1, and ratio of 1:1 of study participants aged above >40years. The mean age of study participant was  $32.7 \pm 9.3$  years, with majority within the age-group 41-60years(42%). The mean age of co-infected patients was significantly older than monoinfected subjects,  $41.2 \pm 8.8$  vs  $31.8 \pm 8.9$  years. ( $P=0.0019$ ). Significant difference was observed between the viral infection and age-group of the study participants( $p=0.001$ )

The seroprevalence of Anti-HCV was 10%, with gender distribution of 10.8%(7/65) males and 8.6%(3/35) females, giving male to females ratio 1:2.3. Of the married patients(48%), 18.8%( $n=9$ ) had coinfection and 81.2%( $n=39$ ) monoinfection, while single participants, 98.0%( $n=50$ ) had monoinfection and 20%( $n=1$ ) coinfection( $p=0.118$ ). There was no significant difference between the risk factors and viral infection( $p=0.84$ ).

Histological diagnosis of liver biopsies(table 2), chronic hepatitis recorded in 92% cases, 4% in cirrhosis and 4% in hepatocellular carcinoma respectively. There was significant association between Anti-HCV and histological diagnosis with 75% cases identified as HCC, 25% as liver cirrhosis and 6.6% as chronic

hepatitis( $p=0.01$ ). Using the histological activity index scores graded as  $<6$ ,  $6-10$  and  $>10$  of the severity of liver diseases between coinfection and mono-infection, mild necroinflammatory changes of 12.8% vs 87.2%, moderate changes of 12.0% vs 88.0% and severe necroinflammatory 3.6% vs 96.4%. Signification association was observed between the severity of liver diseases and coinfection( $p=0.0519$ ), HCC(75% vs 25%) liver cirrhosis(25% vs 75%) and chronic hepatitis(6.6% vs 93.4%) respectively. The mean histological activity index score of coinfecting patients was significantly lower than mono-infection  $5.2 \pm 4.8$  to  $8.0 \pm 4.2$ ( $p=0.05$ ) Table 3 depicts the biochemical and hematological indices. Biochemical parameters showed significantly high mean serum of AST, ALT, ALP and Total Protein among coinfecting patients compared to mono-infected patients( $p=0.05$ ), with low serum albumin level. Hematological indices showed significant difference in the mean PCV, absolute white blood cell count(WBC), platelets count and ESR levels( $p=0.05$ ). In coinfecting patients, high WBC and ESR was recorded compared to the mean PCV, lymphocyte neutrophil and PLT in mono-infected patients.

## Discussion

In this study, the mean age of coinfecting patients was higher than mono-infected patients  $41.2 \pm 8.8$  years vs  $32.12 \pm 12.5$  years, but the mean age( $41.2 \pm 8.8$  years) is comparable to the mean age reported in similar studies,  $42.9 \pm 12.3$  years and  $47.44 \pm 14.56$  years in India[17,18], and  $42.8 \pm 11.5$  years in China[19]. We observed that majority of coinfecting patients were within the age-group of 41-60 years, similar age-group was reported in studies conducted in India, 41-60 years [17], 41-50 years[20] and 30-60 years[21]. However, significant association was observed

between coinfection and age-group of older patients compared to mono-infection ( $p < 0.0019$ ), this observation is consistent with some documented studies [19,22]. This association between coinfection and older age-group alluded to the manifestation of liver diseases due to coinfection is common in the fourth decade of life [7,17,19,20].

As observed in most viral hepatitis studies, male gender predominates similar to the picture seen in our study of 65% males compared to 35% females, giving a male to female ratio of 1.9:1 [18,20,23]. The major driver risk factors of viral factors in SSA are early unprotected sexual activities involving multiple partners, and exposure to risk factors of viral hepatitis infection like transfusion of improperly screened blood units/product, and use of unsterile instrument for invasive procedure like circumcision and uvulectomy [8]. In contrary, we observed no significant difference between the risk factors of viral hepatitis infection and coinfection, which differs from findings of similar studies conducted in Indian continent that reported significant association between risk factors- blood transfusion, alcohol ingestion and intravenous drug ingestion and coinfection [18,24,25].

The coinfection prevalence of 10% is comparable to 11.0% and 12.8% reported to studies conducted in Spain [26,27], but lower than 18% in China [19], 16% in India [28], 14.4% in Maiduguri Northeast Nigeria [10], and 12.2% in Southwest Nigeria [29]. While other studies had reported lower prevalence, 4% in India [17], 5.9% in India [30] and 7.7% in Mongolia [31]. This observed variation may be due to several factors, such as method employed in the study, criteria of inclusion of patients selection and chronicity of liver diseases.

The clinical usefulness of histological diagnosis of liver biopsies in investigation and assessment of liver diseases has been well documented[10,11,15,29,32]. However, the unique characteristic is the difference in the histological picture and severity/CLD subtypes [15]. In our study, histological finding of liver biopsies revealed 92% cases were identified as chronic hepatitis and 4% each as liver cirrhosis and HCC cases respectively. Further evaluation of coinfecting liver biopsies, revealed that 60% cases were confirmed as chronic hepatitis, 30% liver cirrhosis and 10% HCC respectively. Comparing our histological findings with other similar studies revealed variation in the chronic liver disease subtypes as reported in other studies. In northeastern Nigeria, Laraba *et al* reported, 20.5% prevalence of HCC, 12.1% cirrhosis and 5.6% chronic hepatitis. While in India, Hossaniet *al* reported cirrhosis(30%), HCC(26%) and chronic hepatitis(8%)[20], cirrhosis(20%), and 30% chronic hepatitis[33]. In China, Dai *et al* reported 67% cases of chronic active hepatitis followed by 22% chronic persistent and 11% liver cirrhosis. A multicenter study of 9,997 cases in Italy, Sagnelliet *al*[15] reported 62.1% chronic hepatitis, 19.4% liver cirrhosis and 3.4% HCC.

The Anti-HCV prevalence of coinfecting biopsies revealed 75% in HCC, 25% in liver cirrhosis and 6.6% in chronic hepatitis. Studies with similar high Anti-HCV prevalence includes, 75% in Spain[34], 69.6% in Spain[35] and 65 % in Italy[36]. Some studies has also reported low Anti-HCV prevalence in HCC cases , 18% in China[19], 20% in Maiduguri[10], 12.2% in Lagos[11], 14% in Southwest Nigeria[37], 18.7% in Ibadan, and 19% in The Gambia[32]. The non-significant difference observed in histological diagnosis between coinfecting(75% in HCC) and monoinfected (chronic hepatitis 93.4%, cirrhosis 75.0%) in liver biopsies, alluded to the fact that coinfection may not necessarily aggravate liver damage[38]. Though, some studies have associated high prevalence of cirrhosis, chronic liver

disease with HCC cases[39,40] The reason for the histological picture recorded in our study may be due to HBV endemicity that could be responsible for possible superinfection, and criteria of patient selection.

Several studies have reported high prevalence of CLD subtypes, liver cirrhosis and chronic liver disease in coinfection than mono-infection[41,42], with high prevalence of Anti-HCV and severity of chronic HBV infection[ 43]. However, severity of liver disease in coinfection and mono-infection is debatable . In our study, we observed significant decrease in the mean HAI score of coinfections(52.4±4.8) compared to mono-infection( 8.2±4.2 ), comparable to other studies[15,44], this observed activity may be due to severity of liver disease which is a reflection of progression from mild to severe necroinflammatory chronic hepatitis, to cirrhosis and HCC. Using the Knodell classification, we observed mild presentation of 12.8% in coinfection compared to mono infection of 87.2%, moderate 12.0% vs 88.0% and severe case 3.6% vs 96.4%. However, the high necroinflammatory changes observed in mono-infection cases may be due to relatively low HCV prevalence in the study area, or possible HBV superinfection, capable of development and progression from cirrhotic to fibrosis stages.

Biochemical parameters are used as clinically predictors of assessing viral hepatitis disease and antiviral therapy, and values varies with severity of liver disease. However, ALT is one of the important biochemical tests in assessment of CLD but is a poor marker when considering antiviral therapy, as it shows poor correlation in patients with significant liver injury in chronic HBV infection. In this study, significantly high mean values of ALT, AST, ALP, total Protein and reduced albumin level were recorded, which is in conformity with the findings of other studies[15,19, 20,21,45-47]. The increased liver enzyme activities recorded

in coinfection is associated with liver disorder, which is associated with decreased serum albumin[47,49].

Haematological parameters assessed the haemopoetic system response to viral infection[50]. In this study, significantly high mean value of WBC and ESR was recorded in coinfecting patients, in contrast to significant mean PCV, lymphocyte and PLT counts in monoinfection. The mean hematocrit value of 39.0 vs 39.26 in coinfection and monoinfection, showed that 13%(<33%) coinfecting patients were anaemic, which is lower than 24% reported in acute viral hepatitis infection[50]. In this study, we observed increased mean absolute WBC( $7983 \times 10^9$ ), and 5% leucocytosis in coinfection, these values are lower than 12% reported in acute hepatitis study in Southwest Nigeria[50]. The mean lymphocyte count of 44.18% in monoinfection is an indication of viral infection[50,51]. Thrombocytopenia is a common haematological picture in chronic liver disease, which the count could vary with the severity of infection, from mild to severe cases[52]. In our study, increased mean PLT (403,516) was observed in monoinfection compared to coinfection of (394,757), this observed PLT count in both coinfection and monoinfection may be due to chronic infection, malignancy, chronic inflammatory disease or iron deficiency [52]. The significant difference observed in the mean PCV and ESR, is consistent to the report of Ajugwo and Ukaji [53] study which reported low PCV and ESR, indicative of inflammatory liver disease. The main findings of our study revealed concomitant contribution of both aetiological viruses in development and progression of chronic liver diseases and changes in laboratory parameters necessary for patient assessment and management approach

Despite the fact that the study findings had presented clinical, pathological and epidemiological information, necessary for better understanding and viral hepatitis infection in a low-resource setting.. There are limitations, (i) the non-serodetection

of HBeAg, an indicator of infectivity and (ii) molecular assay of HBV-DNA and HCV-RNA not included in the study had limited the vital immunological information necessary in evaluating the stage of infection and possible seroconversion.

### **Conclusion**

The coinfection prevalence of 10% is relatively lower than reported in other studies, which depict disparity and variation due to factors influencing infection outcome. The chronic liver disease subtypes prevalence was similar to those reported in other studies. However, the histological diagnosis of liver biopsies showed no significant difference in degree of severity which highlight that coinfection might not necessarily impact on liver damage.

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Table 1; Demographic variable of study patients

Variables	Coinfection	Monoinfection	p-value
Mean age(SD)(yrs)	42.0+8.8	31.8+8.9	
Age-group			
<20	1(14.3)	7(85.7)	
21-30		42(100)	
31-40	2(6.9)	27(93.1)	0.0019
41-50	6(37.5)	10(62.5)	
>51	1(20.0)	4(80.0)	
Sex			
Male	7(10.8)	58(89.2)	0.001
Female	3(8.6)	32(91.4)	
Marital status			
Divorce/widowed	0(0)	1(100)	
Married	9(18.8)	39(81.25)	0.116
Single	1(1.95)	50(98.03)	
<b>Risk factors</b>			
Blood transfusion	1(9.1)	10(90.9)	
Multiple sex partners	2(15.4)	11(84.6)	0.84

Traditional incision	1(6.3)	15(93.7)	
Uvulectomy	2(14.3)	12(15.7)	
Histopathological diagnosis			
Chronic hepatitis	6(6.6)	86(93.4)	
Liver cirrhosis	1(25.0)	3(75.0)	0.01
Hepatocellular carcinoma	3(75.0)	1(25.0)	

**Table 2; Histological diagnosis and Knodell scores of Liver biopsies**

Chronic disease	Liver HCV(+)	HCV (-)	P=	value
Chronic hepatitis	6(6.6%)	86(93.4)		
Liver Cirrhosis	1(25.0%)	3(75.0)	0.01	
HCC	3(75.0%)	1(25.0%)		
Knodell Scores				
<6	6(12.8%)	41(87.2)		
6-10	3(12.0)	22(88.0)	0.0519	
>10	1(3.6)	27(96.4)		

**Table 3 Biochemical and Heamatological parameters of dual and monoinfected patients**

Parameters	HBV +HCV	HCV(-)	p-value
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ALP(IU/L)	110.9	72.0	0.045
ALT(IU/L)	26.7	9.8	0.015
AST(IU/L)	43.3	12.0	0.045
Total Protein(g/L)	81.7	72.3	0.012
Total	13.0	13.6	0.84
Bilirubin(umol/L)			
Albumin(g/L)	40.8	39.1	0.52
PCV	39.26	39.00	0.047
WBC	7983	7212	0.013
Neutrophil	50.38	49.90	0.08
Lymphocyte	43.87	44.18	0.012
PLT	394,757	403,516	0.001
ESR	10.96	11.03	0.011

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UNDER PEER REVIEW