Virological Response to Dual Hepatitis B and C viral co-infection in Saudi patients

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As there is conflicting data regarding the impact of dual infection. Many previous studies reported that dual infection led to more severe histological changes with rapid progression to cirrhosis. While other studies did not confirm these findings .Therefore, this study aimed to compare the virological and histological changes between HBV/HCV co-infection and mono-infection of HBV and HCV in Saudi patients. Three hundred Saudi patients were enrolledin this retrospective descriptive study. Mono-infection of HCV was observed in 50 patients, considered as subgroup I, while another 25 had mono-infection of HBV, and considered as subgroup II. Also, dual infection was observed in18 patients who had dual positivity for HBs-Ag and hepatitis C virus antibody (HCV-Ab) considered as Subgroup III. Quantitative HBV-DNA polymerase chain reaction (HBV-DNA-PCR) and HCV-RNA-PCR assays, serum alanine aminotransferase (ALT) and liver biopsy (METAVIR scoring system) results were studied. All patients were naive for antiviral treatment. Regards the HBe-Ag status, it was found to be more in the subgroup II alone than in the subgroup III (p = 0.017). In addition, the raised ALT level was found to be more in the subgroup III than in the subgroup I and subgroup II with statistically significant difference between patients' subgroups (p = 0.016). When comparing the HBV viral load between the subgroup III and subgroup II, the HBV DNA levels showed a statistical significant difference (p= 0.021).Also, When comparing the HCV viral load between the subgroup III and the subgroup I, the HCV RNA levels showed a statistical significant difference (p= 0.034), histological characteristics revealed significantly higher values of necroinflammatory, fibrosis and steatosis scores in subgroup III compared to subgroup I and subgroup II. Virological and histological properties of dual infection showed significant differences compared to the single infections among Saudi patients.

Key words: Hepatitis C virus, Hepatitis B virus, Dual hepatitis B and C co-infection, Real time PCR.

Chronic infection with the hepatitis B (HBV) and C virus (HCV) represents a major health problem worldwide, roughly 400 and 200 million people respectively are infected[1].These people are at increased risk of developing cirrhosis, hepatocellular carcinoma (HCC), liver decompensation and esophageal varices bleeding, that ultimately explains why HBV and HCV infection is the current leading cause of liver-related death and the main indication for liver transplantation in developed countries[2].

Hepatitis B and hepatitis C viruses' co-infection may occur because of the common modes of viral transmission, particularly in areas where the two viruses are endemic[3].In addition, dual chronic infection with hepatitis C virus (HCV) and hepatitis B virus (HBV) is common in areas endemic for either virus[4].HBV/HCV co-infection is common

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in endemic countries and in subjects at high-risk of parenteral transmission. It is important to identify this group of patients because of the more severe liver injury and its different evolution compared to mono-infected patients[5].

Liver fibrosis is a common complication of chronic viral hepatitis leading to the progressive destruction of normal tissue architecture or the replacement of hepatic tissue with fibrous tissue. The outcome of this process is liver cirrhosis, which is the major cause of morbidity and mortality in chronic viral hepatitis[6].Several studies have reported elevated risks of cirrhosis, hepatic failure and hepatocellular carcinoma among patients with hepatitis C virus (HCV) and hepatitis B virus (HBV) co-infection[7]. HCV-HBV co-infection accounts about 5% of liver transplants performed for virus-related cirrhosis, making a significant indication for LT in Europe[8].

Although some authors have reported milder histologic disease activity in HBV-HCV–co-infected patients compared with HCV mono-infected[9, 10].Others have shown more severe liver damage among HBV-HCV–co-infected individuals[11, 12].The beneficial influence of HBV on the course of HCV infection is controversial, and the virologic patterns in co-infection are widely divergent in immunocompetent patients[13, 14].

As there is a conflicting data regarding the impact of dual infection. Many previous studies reported that dual infection leads to more severe histological changes with rapid progression to cirrhosis[11, 15, 16].While other studies did not confirm these findings[17, 18].Therefore, this study aimed to compare the virological and histological changes between HBV/HCV co-infection and mono-infection of HBV and HCV in Saudi patients.

Patients and methods
Subjects

The ethical committee of KingAbdul-AzizUniversity approved this study to be conducted at King Abdul-AzizUniversity, Saudi Arabia.Three hundred patients were enrolledin this retrospective descriptive study. Mono-infection of HCV was observed in 50 patients, considered as subgroup I, while another 25 had mono-infection of HBV, and considered as subgroup II. In addition, dual infection was observed in18 patients who had dual positivity for HBs-Ag and hepatitis C virus antibody (HCV-Ab) was considered chronic hepatitis co-infected (Subgroup III). Data on hepatitis B e antigen (HBe-Ag) status, anti-hepatitis delta virus (anti-HDV), anti-HBV core (HBc-Ab), quantitative HBV-DNA polymerase chain reaction (HBV-DNA-PCR) and HCV-RNA-PCR assays, serum alanine aminotransferase (ALT) and liver biopsy (METAVIR scoring system) results were studied. All patients were naive for antiviral treatment.

Signed written informed consents were obtained from the participants. Patients with autoimmune hepatitis, hemochromatosis, Wilson's disease, α1-antitrypsin deficiency, alcoholic liver disease, and drug induced liver disease, active Schistosomiasis, or hepatic tumors, poorly controlled diabetes mellitus, hypertension, immunodeficiency virus or under care of a psychiatrist were excluded.

Serodiagnosis of HBV and HCV infection

Virological studies included a third-generation screening ELISA test for the detection of antibody to hepatitis C virus (anti-HCV) (Abbott HCV EIA 3.0/Ortho HCV ELISA 3.0). Testing for hepatitis B markers included Anti-HBs and total anti-HBC antibodies were detected by ELISA technique according to the manufacturers' instructions (DiaSorin diagnostic kits,Italy).

HBV DNA examination

Real time quantitative PCR technology HBV Test (Roche Diagnostics, Switzerland) was evaluated (IU ml_1) using specimen processing carried out by COBAS AmpliPrep analyzer, specimens were collected by professional staff with sticking to quality standards to avoid any potential contamination, blood was collected in EDTA containing tubes, transfer of the specimen within the laboratory was maintained at 2-8°C, most samples were analyzed on the same day, while when stored for no more than 3 days, they were kept at room temperature (25-30°C).

HCV RNA examination

Real time quantitative PCR technology HCV Test (HCV RNA Super Quant TM, National Genetics Institute, Los Angeles, CA, USA) using polymerase chain reaction (PCR) amplification with a linear range of 40 international equivalent units per milliliter (IU/mL) to 2 million IU/mL.

Liver biopsy

Pathological examination performed at liver histopathology laboratory, King Abdul-Aziz

University Hospital. More than one pathologist revised the slides to minimize inter-observer variability that commonly affects explanation of liver biopsy. Liver biopsies were paraffin-embedded and stained with haematoxylin-eosin and Masson trichrome stains. The biopsies were reviewed blindly without knowledge of any parameter. The degree of hepatic fibrosis and portal inflammation was evaluated according to the METAVIR scoring system. The stage of fibrosis varied from 0 to 4 (F0 = no fibrosis; F1 = portal fibrosis without septa; F2 = portal fibrosis with few septa; F3 = septal fibrosis, without cirrhosis; F4 = cirrhosis). The grade of inflammatory activity classified into; none, mild, moderate and severe[19].

Statistical analysis

Statistical analysis of data was performed using SPSS (Chicago, IL, USA) version 15. Quantitative variables were described as mean ± SD. qualitative variables were described as number and percentage. The Fisher's exact test used in the compression of non-parametric qualitative data and Mann–Whitney test was used to compare nonparametric qualitative data. While the one way ANOVA (analysis of variance) was used to compare more than two groups as regard a quantitative variable .P<0.05 was considered significant.

RESULTS

According to serological results, patients were divided into 3 subgroups; subgroup I included 50 chronic HCV mono-infected patients, subgroup II included 25 chronic HBV mono-infected patients and subgroup III included 18 chronic HCV with HBV co-infected patients. Comparison between Patients' subgroups regarding baseline variables showed that: there was no statistically significant difference between patients' subgroups as regards sex and age the study showed significant difference (respectively; P = 0.34 and P = 0.79). Regards the HBe-Ag status, it was found to be more in the subgroup II alone than in the subgroup III (p = 0.017). In addition, the raised ALT level was found to be more in the subgroup III than in the subgroup I and subgroup II with statistically significant difference between patients' subgroups (p = 0.016). When comparing the HBV viral load between the subgroup III and subgroup II, the HBV DNA levels showed a statistical significant

Table 1. Comparison between Patients' subgroups regarding baseline and virological variables

Variable	subgroup I (n = 50)	subgroup II (n = 25)	subgroup III (n = 18)	p Value
Age	38.32 ± 7.41	37.51 ± 8.62	39.14 ± 8.53	0.79
Sex (Male: Female)	44(88%):6(12%)	21(84%):4(16%)	15 (83.3%):3(16.7%)	0.34
HBe-Ag status (Positive: Negative)	–	7(28%):0	3 (16.6%):0	0.017*
ALT level (Normal: Elevated)	28(56%):22(44%)	15(60%):10(40%)	5 (27.7%):13 (72.3%)	0.016*
HCV RNA viral load	4.81E5 ± 1463611	–	8.24E3 ± 1519274	0.034*
HBV DNA viral load	–	1.23E7 ±4264217	4.85E6 ± 178423	0.021*

* Significant level.

difference (p= 0.021).Also, When comparing the HCV viral load between the subgroup III and the subgroup I, the HCV RNA levels showed a statistical significant difference (p= 0.034), (Table 1).

Concerning the comparison between Patients' subgroups regarding histological characteristics revealed significantly higher values of necroinflammatory, fibrosis and steatosis scores in subgroup III compared to subgroup I and subgroup II. While there was significant difference between subgroups (Table 2).

Table 2. Comparison between Patients' subgroups regarding histological characteristics

Variable	subgroup I (n = 50)	subgroup II (n = 25)	subgroup III (n = 18)	p Value
Score of fibrosis stage	2.89±1.35	1.76±1.23	3.92±1.15*	0.02*
Score of necroinflammatory activity	5.21± 2.86	3.54±2.23	7.52±3.14*	0.03*
Score of steatosis stage	5.31±4.41	4.86± 2.67	9.12±5.25*	0.01*

* Significant level.

DISCUSSION

The global spread of hepatitis B virus (HBV) and hepatitis C virus (HCV), their high chronicity rates and their progression to cirrhosis and hepatocellular carcinoma, are major public health problems. To our knowledge, in Saudi Arabia there is previous no studies have evaluated the impact of dual combined HCV and HBV infection and which virus superimposes and has the upper hand.

Our study defined age and sex as an independent risk factor for HCV-HBV co-infection as the groups of patients were age and sex-matched. In the current study, we reported only three patients (16.6%) with HBe-Ag positivity and all of our patients were positive for HCV RNA these results compatible with a study conducted in Tunisia in 2010 reported that HBsAg was detected in 5% of HCV positive patients and in 9% of HCV negative controls. These results indicate that the HCV positive patients do not have an increased risk of exposure to HBV infection as compared to HCV-negative individuals[20].

Also, ALT was measured to determine the impact of this dual infection on the liver structure, which may refer to the necro-inflammatory process, and the histological changes between dual and mono-infection. In this context, a significant higher in the ALT level was found in the dual infection group versus mono-infected groups (p = 0.016) that agreed with previous studies indicated that dual infection may suffer from a more severe disease pattern than patients with mono-infection[11, 21, 22].A possible factor influencing responsiveness of HCV-positive patients to antiviral therapy may be co-infection with HBV[23].Concurrent infection with the two viruses may worsen the prognosis and result in a less favorable outcome to antiviral treatment[24-26].

In the present study, our results showed that the histological lesions were more severe in dual infection than in mono- infection, including necroinflammatory, fibrosis and steatosis scores. These results are in consistence with several previous studies have compared the histological findings between HBV/HCV co-infection in case of viral co-infection with single viral infection. Zarski et al. found that liver injury was more severe in dual infection than in HCV viral infection; criteria included Knodell score, piecemeal necrosis and incidence of liver cirrhosis[11].Also, based on controlled studies, the HBV/HCV co-infection is associated with a high-risk of developing HCC[27-29].While the cumulative rate of incidence of hepatocellular carcinoma in patients with compensated viral cirrhosis at 10 years was 45% in case of HBV/HCV co-infection compared with 16% and 28% in HBV and HCV mono-infected disease controls[30].However, In contrast to our study results Lee *et al.*, showed no difference in histological lesions between Taiwanese co-infected HCV/HBV chronic hepatitis and chronic HCV only[12].

CONCLUSION

Virological and histological properties of dual infection showed significant differences compared to the single infections among Saudi patients.

ACKNOWLEDGMENTS

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