The serological and molecular detection of Hepatitis B and C Viruses coinfection among intravenous drug users in Egyptian patients

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ABSTRACT

Due to parenteral exposure, injection drug users are very susceptible to infection with HIV, the hepatitis B virus, and the hepatitis C virus. It is commonly known that injecting drug users have a high risk of viral infections such as hepatitis B virus (HBV), and hepatitis C virus (HCV), due to parenteral exposure. The Present study aims to evaluate the incidence of Co-infection of hepatitis B and C viruses among injection drug users. In the present study, we investigated one hundred persons who obtain drug substances aiming to evaluate the epidemiology of Co-infection with HCV and HBV among infection drug users. The present study includes serological, molecular, Hematological, and biochemical techniques. for the patients and control samples. The results indicated that the virus titer was high in the patients who used Heroin and Tramadol drugs substances more than the other drug substances and the control. We concluded from this that the other drugs substances (THC and Synthetic Cannabinoids') had an antiviral effect on the virus's replication, in addition to the host immunity. In the test group the proportions of people who suffer from positive co-infections with HCV and HBV were 33 %, 28 % of them males and 5 % of them females according to serological test (ELISA) for HCV and HBV Co-infection as compared to control group. For Regarding Multi Drugs Screening, in the test group, the distribution of drug users infected with HCV and HBV (33%), the percentages differ according to the Narcotic substance, reaching the highest percentage in the case of Tramadol 11 %, followed by THC by about 9.0 %, then Heroin by 6.0 % and these were considered among the most dangerous and widespread types, 1% synthetic cannabinoids and 1% tramadol and synthetic cannabinoids. The study concludes that the risk of liver and kidney damage is due to infectious hepatitis in injecting drug users. Measures should be implemented to combat the spread of drug abuse.

Keywords: outcome of co-infection; acute viral hepatitis; HAV; hepatitis B Virus (HBV); hepatitis C Virus (HCV); injection drug use.

INTRODUCTION:

It is well recognized that people who inject drug are at critical hazard of contracting viral diseases such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV), due to parenteral exposure. Studies have shown that people who inject drugs are exposed to HCV through the sharing of needles, poor hygiene, and lack of hand washing, leading to contamination of drug mixing containers and sexual contact people infected with hepatitis. with (Logemann and Blinkhorn, 2023).

Co-infection with HIV, HBV, and/or HCV in people who inject drugs has been reported (Logemann and Blinkhorn, 2023). Hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) are the three most common viral and blood-borne infections worldwide (Javadi et al., 2014). These are considered important public health concerns due to the associated health, social, and economic impacts (Javadi et al., 2014). In addition, considering the similar route of transmission in these infections, their coinfections would be more challenging for healthcare professionals. Injection drug users have a potential risk of acquiring mentioned infections. It is considered as an important reservoir for infections. In addition, there is evidence that HIV, HBV, and HCV coinfections are more prevalent among IDUs (Javadi et al., 2014).

However, acute co-infection with multiple hepatitis viruses is not well documented, and thus the risk of acute liver failure secondary to co-infection is unknown. We report a case of acute co-infection of HCV and HBV in a drug user with no other known risk factors for developing acute hepatitis (Logemann and Blinkhorn 2023). Determining co-infection rates may help to better understand the epidemiological specifications of the mentioned infections, especially in high-risk populations such as injection drug users.

MATERIALS AND METHODS:

The study included an interview blood and urine testing. They were patients from Abou El Azayem Psychiatric Hospital, Egypt, Cairo. A full Medical examination was carried out in the study groups. The interview was done by an expert and trained social worker who had been working with IDUs, using a structured checklist. Demographic information and risk factors for HBV and HCV infections. The demographic and risk factors of studied bloodborne infections include gender, age, marital status, job, education, surgical history, dentistry history, sex behaviors, history of sharing needles, history of imprisonment, multiple partners, tattooing, cupping, piercing, and transfusion.

One hundred patients suffering from drug dependence and one hundred healthy persons as controls were included in the present study. They were patients from Abou El Azayem Psychiatric Hospital, Egypt, Cairo. The studied groups were subjected to full clinical examinations, Laboratory investigation including the following parameters:

Complete Drug Screening qualitative. 2-Virology Panel for HCV and HBV detection (ELISA & PCR) in patient samples and control healthy samples .3- Complete blood count (HB, RBCs, WBCs, PLT) .4- Liver function (Total Bilirubin, Direct Bilirubin, Levels of AST, and ALT enzymes) 5- Kidney function tests (serum creatinine, blood urea, and uric acid).

Random blood and urine samples were collected from the patients and control groups admitted to Abou El Azayem Hospital. No fasting was recommended. 5 ml venous blood was collected in Vacutainer plain tubes, 2 ml venous blood was collected in EDTA tubes, and 10 ml Urine was used for drug analysis and collected in sterilized plastic urine containers.

Urine samples (minimum 10 ml) were collected and subjected to analysis of abused substances using rapid Chromatographic immunoassay for qualitative detection of multiple drugs and drug metabolism in urine (Hangzhous AllTest Biotech Co., China).

Detection of HCV.Ab by ELIZA "sandwich" technique 96 well plate by kit RecombiLISA (CTK Biotech, united state). the amount of color intensity can be measured, and it is proportional to the amount of antigen captured in the wells by the Microplate Reader DR_200B (LED light) (DIATEK, China).

HBsAg was detected by ELISA "sandwich" technique 96 well plate by kit RecombiLISA (CTK Biotech, united state).in which, the amount of color intensity can be measured, and it is proportional to the amount of antigen captured in the wells by the Microplate Reader DR_200B (LED light) (DIATEK, China).

In cases, where antibody assays have limited use, gene amplification is the assay of choice, RT-PCR-based technology to detect HCV genome equivalents. An important additional application for HCV RT-PCR. HCV-RNA is reverse transcribed and specific fragments of RNA are amplified with specific primers in a one-step RT-PCR. The products are detected by using specific Taqman-MGB probes. These probes are labeled at the 5-end with a fluorescent dye and at the 3-end by a non-fluorescent quencher. An internal control is supplied. Using commercial Bioer DNA technology.

Blood specimens were gently mixed by gentle inversion for 1 min. then applied to the apparatus. Full Blood Analysis (HB, RBCs, WBCs, and PLT) Auto Hematology Analyzer Device (Celltac Alpha, made in Jaban)

Liver Function Tests: Alanine aminotransferase (ALT/GPT), Aspartate aminotransferase (AST/GOT). These tests have been monitored kinetically at 340 nm by the Photometer 5010 (RIELE, Germany) at 30-37ºC. Bilirubin is measured kinetically bv 5010(RIELE, Germany) Photometer (wavelength 540 nm).

Kidney Functions Tests: Urea, Creatinine, and Uric acid are measured kinetically by using Photometer 5010(RIELE, Germany) capable of measuring absorbances at 600(+, -)10 nm at a constant temperature incubator set at 37 °C.

SPSS version 22 was used to analyze the data. The forms of frequency, means and SD were used to express descriptive statistics. Person Chi-square independent sample T-test, Mann-Whitney U test, and Kruskal-Wallis H test were used for analytic statistics. A significant p-value was considered when less than 0.05 and highly significant when less than 0.01.

RESULTS:

In the present study, we investigated one hundred persons who obtain drug substances aiming to evaluate the epidemiology of Coinfection with HCV and HBV among infection drug users. In the study, one hundred healthy persons (not obtaining drug substances) were used as controls. The two groups used in the study were inpatients in Abou El Azayem Psychiatric Hospital Cairo, Egypt. The test group, their age ranged from 18 – 70 Years and 53 persons were male and 47 females.

In the control group age ranging from 18 – 70 years, the males persons (54 %) were more than females (46 %).

Regarding the Virology Tests

In the test group the proportions of people who suffer from positive co-infections with HCV and HBV were 33 %, 28 % of them males and 5 % of them females according to serological test (ELISA) for HCV and HBV Co-infection.

In the control group, the proportions of people who suffer from positive HCV Ab were 8%, 4.0 % of them males and 4.0 % of them females,

In the control group, the proportions of people who suffered from positive only with HBs Ag were 3%, 2.0 % of them males and 1.0 % of them females according to serological tests (ELISA).

According to molecular assay (PCR) HCV RNA,

In the test group, the present study confirmed that the test group included only 13 patients (12 males + 1 female) who were positive for HCV RNA by PCR from the 33 patients who contained HCV Ab and HBs Ag co-infection.

In the control group there were 5 patients positive (2 males and 3 females) for HCV RNA from 8 control patients positive for HCV Ab.

According to molecular assay (PCR) HBV DNA

In the test group, the results confirmed that there were 4 Patients Positive (3 males + 1 female) for HBV DNA from positive 33 cases of HCV and HBV co-infection

In the control group there were 3 patients positive (2 males+1 female) for HBV DNA in PCR molecular assay in control cases which screened positive to HBs Ag by ELISA test.

Regarding Multi Drugs Screening

In the test group, the distribution of drug users infected with HCV and HBV (33%), the percentages differ according to the Narcotic substance, reaching the highest percentage in the case of Tramadol 11 %, followed by THC by about 9.0 %, then Heroin by 6.0 % and these were considered among the most dangerous and widespread types, 1% synthetic cannabinoids and 1% tramadol and synthetic cannabinoids.

All cases were males except 1 case of female (1.0 %) who used Tramadol, THC, and Synthetic Cannabinoids, 3 cases of females (3.0 %) who used Tramadol and THC, and one case of female who used Tramadol, THC, and Heroin

The results indicated that the virus titer was high in the patients who used Heroin and Tramadol drugs substances more than the other drug substances and the control according to molecular assay (PCR) for HCV RNA titer.

The results recorded that the virus titer was very high in the male patients samples whose used Heroin substance than the patients used the other drug substances, but in the control samples the titer also was high, even that, still less than in the patient obtain heroine substances, according to HBV DNA titer.

Regarding biochemical results, hematological disturbances in Test and Control groups for HB, PLT, WBCs, and RBCs.

Regarding hemoglobin concentration

In the test group the proportion of people who suffer from hemoglobin deficiency (Anemia) 13%,11% of them Males (5% abuse tramadol, 5 % THC, and 1 % Synthetic Cannabinoids), and 2 % of them are females used Tramadol + THC. While the percentage of people suffering from a high concentration of HB represents 1% of males who used THC.

In control groups, the proportion of people who suffer from hemoglobin deficiency (Anemia) is 20%, 7% of them males and 13% of them females, While the percentage of people suffering from a high concentration of HB represents 1% male.

Regarding platelets numbers

In the test group the proportion of people who suffer from Low PLT (Thrombocytopenia) 5%, 3 % of Males used Tramadol, and 2 % of them were females who used Tramadol + THC.

In Control groups the proportion of people who suffer from Low PLT (Thrombocytopenia) 1% females, While the proportion of people who suffer from High PLT (Thrombocytosis) is 1% females.

Regarding WBCs numbers

In the test group the proportion of people who suffered from low WBCs (Leucopenia) 2%

of males (abused Tramadol), While the proportion of people who suffered from High WBCs (Leukocytosis) 1% of males used THC.

In the control groups the proportion of people who suffer from Low WBCs (Leucopenia) is 3%, 1% of them male, and 2 % of them are females, While the proportion of people who suffer from High WBCs (Leukocytosis) 5%,3% of them males, 2 % of them are females

Regarding RBCs number

In the test group the proportion of people who suffer from Low RBCs is 13%, 12 % of them are males (6% Tramadol, 5 % THC, and 1 % Synthetic Cannabinoids), and 1 % of them are females who used Tramadol + THC.

In control groups, the proportions of people who suffer from Low RBCs are 39%, 30% of them males, and 9% of them are Females.

Regarding the Liver Function

In the test group the proportion of people who suffer from elevated liver function GPT 8%, 7 % of them are males (3% Tramadol, 2.0 % THC and 2.0 % Heroin) and 1 % of them are female (Tramadol + THC).

In GOT 8%,7 % of them are males (3.0 % Tramadol, 3.0 % THC and 1.0 % Heroin) and 1.0 % of them are female (Tramadol + THC).

In the control group, the proportion of people who suffer from elevated liver function (GPT and GOT) is about 8%, 2 % of them males and 6 % of them are females.

Regarding the Kidney Function

In the test group the proportion of people who suffer from, urea is low in 1 male (THC), low U.A. 2 in males (1 THC and 1 Heroin), and High U.A. in 1 male (Heroin).

In the control group, the proportions of people who suffer from low urea in 3 males, High urea in 1 male and 2 females. Low creat in 3 males, High creat in 1 female.

Generally, In the test group according to molecular assay (PCR) for HCV RNA titer, our Hematological and Biochemical analysis results show that the proportions of people who suffer from hemoglobin deficiency (Anemia) in one, (Tramadol abuse) in one male (Synthetic Cannabinoids abuse) and two males (THC abuse), low PLT (Thrombocytopenia) in one female (Tramadol +THC abuse), Low RBCs in two males (THC abuse), in one male (Tramadol abuse) and one male (Synthetic Cannabinoids abuse). While high RBCs in one male (Heroin abuse), High GPT and GOT in one male (THC abuse) Low U.A. in 7 males, and high U.A. in two males and 5 females.

Our Hematological and Biochemical analysis in the test group according to molecular assay (PCR) for HBV. DNA titer., shows that High GPT and High U.A in one male (Heroin abuse), High GPT and GOT in one male (Heroin abuse), and low U.A.

Our Hematological and Biochemical analysis in the control group according to molecular assay (PCR) for HCV.RNA titer, shows low RBCs in one male and high Gpt, Got, T. Bili, and D. Bili in one female.

DISCUSSION:

Intravenous drug users may be susceptible to acute hepatitis caused by multiple hepatitis viruses, but acute co-infections are rare. Patients should be checked for all hepatitis viruses when developing acute hepatitis. (Logemann and Blinkhorn, 2023).

In this section, one hundred drug users were included in this study to evaluate the epidemiology of HCV and HBV co-infection. This result suggests that older people tend to be exposed to new drugs less frequently than younger people (UNODC, 2018).

Early onset of substance abuse increases the risk of psychosocial problems in many areas of life, including behavioral patterns, mental disorders, family systems, and work adjustment (Poudel and Gautam, 2017).

Most of the patients in this study were male. The results showed that men were more likely than women to use almost all types of illegal drugs (SAMHA, 2016).

Our results find that 33 patients from 100 drug users were suspected of having HCV & HBV infection.

Most of the studied group (33 %) were between 24 - 67 years in age, most of them were males, representing 28 %.

The results find that tramadol was the highest substance (11.0 %) used through injections, followed by THC (9.0%) by smoking, and heroin represented 6.0 % by snorting. In a similar study by (Wafaa et al., 2020), tramadol was the most commonly abused substance in 32.5% of cases followed by cannabis representing 21.25% of cases.

This high prevalence of tramadol abuse is consistent with the study by (Fawzy, 2010), who reported the high prevalence of tramadol use among children and adolescents who presented it to the Emergency Unit of the Poison Control Center of Ain Shams University Hospitals. The results indicated that 13.0 % of the studied group suffered from anemia, most of them were male patients between 24- 45 years of age, 5.0% used Tramadol, 5.0 % used THC, 1 patient used Synthetic Cannabinoids (1.0 %) and 2.0 % females were used Tramadol with THC. While in control groups 19.0 % of healthy people suffered from anemia, the females represented 13.0 %. Drug abuse affects other hematological present parameters. In the study, erythrocytosis showed 2 % (1.0 % THC and 1.0 % used Heroin) compared to 10.0 % among control males group. 15% of the studies group had leukocytosis used THC compared to 3.0 % in the control group. In a similar Egyptian study, 10% of the addicts suffered from leukocytosis (Wafaa et al., 2022).

Compared Hb concentration, RBCs count, WBCs count, and platelet count between cannabis smokers and healthy controls (Mukhtar and Elbagir 2011). They found significant increase in the leucocyte count of compared cannabis smokers as to controls. Although most of the studied groups were injection tramadol addicts, nearly all addicts were cannabinoid smokers, which causes interference in haematological parameters.

Analysis of liver and kidney functions of abusers in the present study revealed a highly significant increase in means of ALT, AST in the patients who used Tramadol, THC and Heroin compared to values of no abusers subjects. A study conducted in Gaza to assess the tramadol addiction effects on both liver and kidney functions among tramadol.

abusers showed that serum ALT and AST levels were significantly higher in tramadol abusers compared to the control group (Elmanama *et al.,* 2015).

Egypt which is profoundly burdened with HCV infection had a ratio of 14.75% of HBV/HCV coinfection, while it was about 10-15 % worldwide in non addicts (Shiha *et al.*, 2009). The rate of HCV infection among patients was 13% after excluding positive cases for HCV Ab (18%) which showed negative PCR results, compared to 5% of control group. This is in agreement with the results of Wafaa et al, 2020 who stated that Hepatitis C virus (HCV) was positive in 15 (18.75%) of all cases.

The rate of HBV infection was 3%, which showed positive HBsAg test, while the

confirmed cases by PCR were 1%, the same percentage was evident in the study by (Wafaa et al., 2020). The results in the present study indicated also that HCV.RNA titre was high in the male patients used heroin and Tramadol drugs substances more than the other drug substances and the control group. The results with HBV.DNA titre had observed that 2 males patients from three used heroin substance and virus titre with high and very high. We concluded that the other drug substances may be had an antiviral effect on the two viruses replication. As regard to hematological analysis in test group according to molecular assay (PCR) for HCV.RNA titre, the results indicated that a mostly decrease effects in HB concentration and RBCs count in male patients used THC, Tramadol drugs and RBCs increased in one male patient used heroin substance with also low virus titre. While liver enzymes ALT, AST were highly increased in one male used THC with low virus titre. The results also indicated that not effect on kidney functions except one male state effect on uric acid content with low virus and used THC drug. From the above results with HCV-RNA titre that the drug substances.

Heroin, THC and Tramadol effected on virus replication (Low titre) and on hematological and biochemical analysis and the virus titre was mostly low and now high virus titre. while in effects increase in the control virus group in liver function tests with moderate virus titre.

While in the test group with HBV.DNA patients, the heroin drug substance had highly increase effect on GPT liver enzyme and in uric acid as a kidney function with high virus titre in one male (age 20 years) of HBV.DNA patient and very high in GPT liver enzyme and high level in GOT liver enzyme in 1 male (67 years age) with very high virus titre.

This indicates the heroin drug substance increases the HBV.DNA virus titre replication and some biochemical effects. The effects in male patients were more than in the females.

CONCLUSION:

Injectable drug use has become widespread in Egypt in recent years. According to our study, the rate of HCV infection among patients was 13% after excluding positive cases for HCV Ab (18%) which showed negative PCR results, compared to 5% of control group. While the rate of HBV infection was 3%, which showed positive HBsAg test, while the confirmed cases by PCR were 1%. Drug users

should thoroughly discuss the health risks of injecting drug use with patients, including potential HBV, and HCV. In addition, they should regularly screen asymptomatic patients for all types of hepatitis viruses, biochemical tests, hematological tests and multi drug screening. Many organizations recommend vaccination against HBV for patients with chronic HCV.; However, patients who do not have HCV and are taking injectable drugs should also consider these vaccinations as a preventative measure. In acute hepatitis, it is especially important to keep all hepatitis viruses under control, if a case is diagnosed with acute co-infection with multiple hepatitis viruses, early discussion with a liver specialist is crucial. It is necessary to consider antiviral therapy. Because there is no established treatment for acute HAV, it may be prudent to consider early initiation of antiviral treatment for HBV even in the absence of acute liver failure.

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Figure 2: Comparison between the test group according to molecular assay (PCR) HCV. RNA.

Figure 3: Distribution of the test group according to molecular assay (PCR) HBV.DNA.





Figure 4: Shows the distribution of studied cases according to the type of drug used.

Figure 5: Hematological, Biochemical analysis in Test group according to molecular assay (PCR) for HCV. RNA titer.



Figure 6: Hematological, Biochemical analysis in Test group according to molecular assay (PCR) for HBV. DNA Titer.

الكشف المصلي والكشف الجزيئي لفيروسات التهاب الكبد الوبائي (ب) و (ج) بين مرضى متعاطي المخدرات عن طريق الحقن الوريدي في مصر محمد حسن شحاته 1،. محمد عبدالفتاح سليم ^{1,*} ، عادل عبدالباسط موسي 1،. ايمان مختار محود² ¹قسم النبات والميكروبيولوجي, كلية العلوم، جامعة الأزهر، القاهرة، مصر. ²معمل مستشفى ابو العزائم, القاهرة, مصر.

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الملخص العربي

بسبب التعرض بالحقن ، فإن متعاطي المخدرات بالحقن معرضون جدا للإصابة بفيروس نقص المناعة البشرية وفيروس التهاب الكبد الوبائي ب وفيروس التهاب الكبد الوبائي . سي. ومن المسلم به جيدا أن متعاطي المخدرات بالحقن معرضون بشكل كبير لخطر الإصابة بعدوى فيروسية مثل فيروس التهاب الكبد الوبائي (ب) وفيروس التهاب الكبد الوبائي (ج) ، بسبب التعرض للحقن. تهدف هذه الدراسة إلى تقيم حدوث العدوى المشتركة لفيروسات التهاب الكبد الوبائي (ب) و(ج) بين متعاطي المخدرات بالحقن معرضون بشكل كبير لخطر الإصابة بعدوى فيروسية مثل فيروس التهاب الكبد الوبائي (ب) و(ج) بين متعاطي المخدرات بالحقن. في هذه الدراسة ، (ج) ، بسبب التعرض للحقن. تهدف هذه الدراسة إلى تقيم حدوث العدوى المشتركة لفيروسات التهاب الكبد الوبائي (ب) و(ج) بين متعاطي المخدرات بالحقن. في هذه الدراسة ، قمنا بالتحقيق في مائة شخص حصلوا على مواد دوائية تهدف إلى تقيم وبائيات العدوى المشتركة بفيروس التهاب الكبد الوبائي والتهاب الكبد الوبائ بالعدوى. تشمل الدراسة الحالية تقنيات مصلية وجزيئية ودموية وكميائية حيوية. للمرضى وعينات التحكم. وأشارت النتائج إلى أن عيار الفيروس كان مرتفعا في المرضى الذين استخدموا أدوية الهيروين والترامادول مواد أكثر من مادة الخدرات الأخرى والسيطرة عليها. استنتجنا من هذا أن مواد الأدوية الأخرى (التتراهيدروكانابينول والقنب الاصطناعي) كان لها تأثير مضاد للفيروسات على تكاثر الفيروس ، بالإضافة إلى مناعة المضيف. وخلصت الدراسة إلى أن خطر تلف الكب والكلى يرجع إلى التهاب الكبد المعدي لدى متعاطي المخدرات عن طريق الحقن. وينبغى تنفيذ تدابير لمكافة الى مناعة المضيف. وخلصت الدراسة إلى أن خطر تلف الكبد والكلى يرجع إلى التهاب الكبد المعالي الخدرات .

الكليات الاسترشادية: نتائج العدوى المشتركة ، التهاب الكبد الفيروسي الحاد ، فيروس التهاب الكبد ب ، فيروس التهاب الكبد سي ، تعاطي المخدرات بالحقن.