ejpmr, 2018,5(2), 39-43

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

THE PREVALENCE OF CO-INFECTION WITH HIV, HEPATITIS C AND TB AMONG PATIENTS INFECTED BY HIV/AIDS IN SENEGAL

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Article Received on 21/11/2017 **Article Revised on 12/12/2017** **Article Accepted on 04/01/2018**

ABSTRACT

Background: Viral hepatitis constitutes a major public health problem world wide. The consequences of chronic liver diseases put a considerable economic burden upon the communities afflicted with the diseases. Viral hepatitis is the predominant risk factor associated with hepatocellular carcinoma. **Objective:** The aim of the present research is to carry out epidemiological studies concerning the laboratory out-come management of Hepatitis C co – infection with HIV and TB in a well defined hospital based population. **Materials and Methods:** Epidemiological studies were carried out among 1320 patients admitted at Bio-Amarie Laboratory of biology/Kaolack come management of hepatitis C (HCV) and Co – infections with HIV and TB over a period of four years (2012 – 2016). The grand diagnostic kits were used to analyze blood samples for HBV, HCV and HIV while sputum samples were analyzed for TB using the Zel Nielsen (ZN) staining technique. **Results:** A CD4 lymphocyte count of 0-200 cells/mml was found in 625 (47.3%) individuals. Out of these, 393 were found to be in WHO stage l clinical status. A further 409 with CD4 count of 201-400 cell/mml were grouped as stage lll (254) and stage IV (155).These together was determined as being eligible for anti-viral therapy. Of those eligible for anti-viral 52 and 80 were infected with HCV and HIV respectively. With respect to subjects management outcome in the hepatitis C infected subjects, follow-up was lost in 44(71.0%) while 11(17.7%) subjects survived and 7 (11. 3%) died. Similarly, subjects managements out-come amongst HIV and TB co-infected with HCV infections were fallow-up was lost in 34 (77.3%), 7 (15.9%) survived and 3 (6.8%) died. With respect to TB smear positive co-infected with HCV, subjects management out-come in 17 (58.6%) fallow-up was lost, 6 (20.6%) survived while 6 (20.6%) died. **Conclusion:** In respect to CD4 lymphocyte count and WHO clinical staging subjects were selected for Antiviral therapy management out-come showed that there was loss of fallow-up for majority of the subjects. However, in those that were successfully monitored, survival rate was consistently higher than mortality rate for all categories of patients except for cases of co-infections by HCV/TB where survival and mortality rates was equal.

INTRODUCTION

Hepatitis C therapy has steadily advanced since the hepatitis C virus (HCV) was isolated in 1989. From the introduction of interferon (IFN) immunotherapy to the current standard of care, combination therapy with pegylated (peg) interferon- plus ribavirin, the efficacy of achieving a sustained virologic response (SVR), consisting of HCV RNA undetectable 24 weeks after treatment completion, has improved (McHutchison 1998; Manns 2001; Fried 2002). However, still almost half of all patients with chronic hepatitis C do not achieve a sustained virologic response. The success of the current standard treatment strongly depends on the HCV genotype with SVR rates of only 40-50% in patients infected with genotype1, contrasted with SVR rates of approximately 80% in those infected with genotypes 2 or 3 (Manns 2001; Fried 2002; Hadziyannis 2004; McHutchison 2004).

In addition, treatment with interferon-and ribavirin is long (up to 72 weeks) and associated with numerous side effects that lead to early discontinuation in up to 20% of patients. Further more, a significant proportion of patients contraindications to IFN-based therapy due to concomitant diseases and circumstances (Fried2002). The most important reason to treat hepatitis C in HIV-co-infected individuals is the unfavorable course of hepatitis C in the setting of HIV co-infection particularly with the increased life expectancy gained by successful HAART. An increased risk of hepatotoxicity after HAART initiation in HIV/HCV-co-infected patients, possibly limiting the long-term benefit of HAART in this particular patient group, further underlines the need for successful treatment of hepatitis C (Sulkowski 2000). Most recently, several studies have demonstrated that successful treatment of hepatitis C dramatically reduces subsequent complications of preexisting liver disease. This implies that once viral clearance is achieved with

hepatitis C combination therapy the prognosis of liver disease dramatically improves (even in the presence of already developed liver cirrhosis) and once HCV infection is eradicated further liver complications are very unlikely. If chronic hepatitis C is detected early in the course of HIV infection (before the initiation of HAART) treatment for chronic HCV is advised. However, if a co-infected patient has severe immune deficiency (CD4 count <200 cells/l), the CD4 count should be improved using HAART prior to commencing anti-HCV treatment. Patients with a CD4 relative percentage >25 % are more likely to achieve SVR than those with lower CD4 percentages (Opravil 2007).

HIV and HCV share transmission pathways, which explains the high rate of co-infection with both viruses. Of the 33 million HIV-infected persons worldwide in 2016 it is estimated that 4-5 million of them have concomitant hepatitis C virus infection. Whereas both viruses are transmitted with high efficacy via direct blood to- blood contact, HCV is less easily transmitted via the sexual route. Thus, the prevalence of hepatitis C co-infection within different countries, regions and populations is closely related to the prevalence of blood-borne (mainly intravenous drug use) HIV infection. Among all HIV-infected patients in Europe, Australia and the US, at least one out of four is also infected with hepatitis C (Rockstroh 2004). Particularly high hepatitis C co-infection rates are observed in Eastern European countries like Byelorussia and the Ukraine where intravenous drug use is the main route of HIV transmission, with hepatitis C co-infection rates as high as 70%. This highlights the importance of preventing further spread of hepatitis C infection as one of the major co-morbidities in HIV infected individuals. The average estimated risk of transmission for hepatitis C in as clear as HIV's influence on the accelerated disease progression for HCV associated liver disease is, HCV' influence on the course of HIV disease is conflicting. The Swiss Cohort first revealed a blunted CD4 cell response associated with a faster progression to AIDS after initiation of HAART in HIV/HCV co-infected patients (Greub 2000). Interestingly, four-year follow-up data from the same cohort study did not see any significant differences with regard to CD4 cell count recovery between HCV-positive and HCV-negative HIV patients (Kaufmann,2003).

MATERIALS AND METHODS

Study area

Bio-Amarie Laboratory of biology/Kaolack was selected for the present study, is one of the reference laboratory in Kaolack where people from various parts of Senegal.

Study population

The population studied was a heterogeneous population of different age groups, ethnicities and educational status. Biodata and other information were collected via the counselors after obtaining written informed consent from each patient with the assurance that all information obtained would be treated with utmost confidentiality

Study design

General descriptive epidemiological study was used to design out the survey. Cross-sectional (Prevalence) survey was carried out according to the ethical standards for human experimentation.

Sample size

A total of 1320 patients were recruited for the study. 3ml of blood was collected from each patient by vein-puncture using sterile Syringes and needles into a sterile container with a screw cap. The blood samples collected were centrifuged at 2000rpm for 5 minutes in order to separate the sera. After centrifugation, the sera were labeled and stored in plastic tubes at 2^0C – 8^0C for analysis within 24 hours.

Serological examination

The grand diagnostic rapid test kits were used to analyze the samples for HCV antibodies. This is a rapid chromatographic immunoassay for the quantitative detection.

All procedures for the test were based on recommendation of the manufacturers. The steps for the procedure are as follows: Test strips and serum samples were allowed to equilibrate to room temperature 25-30c prior to testing. Test strip was removed from the sealed pouch and used as soon as possible. With arrows pointing towards the serum the test strip was immersed vertically in to the serum for at least 10-15 seconds. The strip was placed on a non-absorbent flat surface with the timer on and was observed for the red line (s) to appear. The results were read after 15 minutes. No result was interpreted after 30 minutes. Two distinct red lines indicated positive, one line in the control region (C) and another line in the test region (T). One red line in the control region (C) and no apparent red line or pink line in the test region (T) indicated negative. Results were considered invalid when Control line failed to appear. Insufficient specimen volume or incorrect procedural techniques were the most likely reasons for control line failure.

Retroviral screening (RVS) procedure

To detected cases of co-infections of HCV with HIV, retroviral screening was conducted on all blood samples. The Chembio HIV1/2 start-park test device and rapid strip test were used for the screening process. The test pack was removed from the pouch and placed on a flat surface, 5µL of the patient's blood sample was transferred into the sample pad at the center of the sample well and 3 drops of the buffer was slowly dropped in to the sample well. The result was read within 10 minutes after addition of the running buffer (in most cases the test line appeared in less than 10 minutes, however, 10 minutes). When the determinant was used (i.e. rapid strip test) the same procedure was adopted

except that there was no running buffer in the determinant test device.

Tuberculosis screening procedure

In order to detect cases of TB Co-infections with HCV, three Sputum samples were collected from each subjects and examined for the presence of Acid fast bacilli using the Ziehl's Neelsen staining technique. Sputum collection was done in the open air with the patients facing away from the wind and also away from others, during expectoration (Fujiki, 1998).Two samples were collected for follow-up. Sputum was smeared using applicator stick in biosafety cabinets. The smears were air dried in the biosafety cabinet. The slides were then fixed by passing them through flame 2-3 times. The slides were stained by pouring strong carbol-fuschin solution to cover the whole surface and heated to steam without boiling and allowed to stand for 5 minutes. The slides were tilted, washed with distilled water and decolorized with 3% acid alcohol for 3-5 minutes. The slides were washed with distilled water and tilted to drain excess water. Counter staining was done with 0.1% methylene blue for 1 minute and then washed with distilled water. The slides were then placed on the slide rack and allowed to dry. The smears were examined under x100 objective. Positive showed pink rod shape bacteria while the other substances appeared blue.

CD4 lymphocyte count determination: At first visit all subjects were examined clinically by physician and CD4 count was tested. Staging of all participants based on clinical findings was carried out to guide the diagnoses, evaluation and management of the subjects as well as determine eligibility for antiviral therapy. Clinical criteria for anti-viral were CD4 count below $200x10^6$ cells/l or advance WHO stage III or stage IV.

5ml blood sample was obtained from each subject and analyzed for CD4 count. For each Patient sample, two 12x75mm test tubes were labeled as follows: Tube #1 was labeled with specimen identification number and "A+B" while test tube #2 was labeled with specimen identification number and "C" Reagent C is then prepared by putting 100µl of the reagent into test tube labeled "C". 100µl of whole blood (collected in EDTA container (anti coagulated) was placed at the bottom of the test tube labeled "A + B". Blood droplets around the top of any test tube was avoided in order not to result in nonlysis of red blood cells which can lead to erroneous $CD4^+$ lymphocytes count. 10µl of reagent A is added to the blood in the test tube labeled "A + B" (test tube #1). Holding the test tube vertically by hand the mixture of the whole blood sample and reagent A in the test tube #1 is gently mixed up by shaking for about 2 minutes. To the same test tube #1 labeled "A + B" 10µl of reagent B was added. 10µl of the Blood-latex spheres mixture in the test tube "A + B" was added to test tube #2 labeled as "C" and mixed gently for 10 – 15 second, just to allow the red blood cells to lyses. Both chambers of the 0.1mm deep (or one chamber of the 0.2mm deep) hemacytometer were loaded with the sample from the above step. The hemacytometer was then placed in a moisture chamber and the cells were allowed to settle for 2-3 minute. The prepared samples were allowed to become stable in a moisture chamber for 15 minutes. The cells that have three or more large latex spheres attached to them as $CD4^+$ lymphocytes were counted under the microscope. The $CD4^+$ lymphocytes in all 9 squares of one chamber of the 0.2mm deep (total of $9mm^2$), hemacytometer were counted $CD4^{+.}$ Lymphocytes that touch or were intersected by the upper and left hand boundary lines were not counted. To calculate the absolute number of $CD4^+$ lymphocytes per 1µl using a 0.1 mm deep hemacytometer, when $18mm^3$ (both sides of the hemacytometer) are counted. The number of $CD4^+$ lymphocytes counted was multiplied by 7.3. For example, if the number of $CD4^+$ lymphocytes counted in a 0.1mm deep hemacytometer is 75.The value of the absolute number of $CD4^+$ lymphocytes can be calculated as follows: 74 x 7.3 = 547.3 (or 547) $CD4^+$ lymphocytes. The same factor of 7.3 was used to multiply the count when 0.2mm deep hemacytometer was used.

Determination of Clinical Stage: Subject clinical stage was determined based on clinical findings of the physician. WHO staging system (2004) was used as follows.

Clinical stage i: Asymptomatic, persistent generalized lymphadenopathy.

Clinical stage ii: Unexplained moderate weight loss (<10% of presumed or measured body weight) Recurrent respiratory track infection (sinusitis, tonsillitis, otitis media and pharyngitis), herpes zoster, angular chelits. Recurrent oral ulceration, Papular pruritic eruption seborrhoeic dermatitis, fungal nail infections.

Clinical stage iii: Unexplained severe weight loss (>10% of presumed or measured body weight) Unexplained chronic diarrhea for longer than one month, Unexplained persistent fever (above 37.5^0C intermittent or constant for longer than one month), Persistent oral Candidiasis, oral hairy Leukoplakia, Pulmonary tuberculosis severe bacteria infection eg Pneumonia, bone or joint infection, Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis, Unexplained aneamia (<8.0g/dl, neutropaenia ($<0.5x10^9$ per litre) or chronic thrombocytopaenia ($<50x10^9$ per liter)

Clinical stage iv: HIV wasting syndrome, Pneumoncytis pneumonia, Recurrent sever bacterial pneumonia Oesophageal Candidiasis (or Candidiasis of trachea, bronchi or lungs), Cytomegalovirus infection (retinitis or infection of other organs). Chronic cryptosporidiosis, chronic isosporiasis, recurrent septicemia (including non-typhoidal salmonella, Disseminated mycosis (extra pulmonary histoplasmosis or coccidiomycosis), Symptomatic HIV associated nephropathy or symptomatic HIV associated cardiomyopath.

RESULTS

Results of the studies on the Management out-come of hepatitis C and HIV and TB Co-infections with HBV and HCV are summarized in table 1- 3.The results show, 62 of the total subjects screened as sero-positive for HVC 44(71.0%) subjects there was loss of follow-up, 11(17.7%) were alive while 7(11.3%)died. Mortality rate was higher among males 5(8.1%) than females 2(3.2%) as in table 1.Results of the management out-come on HCV/HIV co-infection showed that, follow-up was lost in 34(77.3%), 7(15.9%) survived while 3(6.8%) died. Mortality rate was slightly higher in females (4.5%) than males (2.3%) (Table2).7 subjects were co-infected with HBV/HCV/ HIV; however, all were lost in the follow-up. Results of the management out-come on HCV/TB co-infection showed that, follow-up was lost in 17(58.6%), 6(20.6%) survived while 6(20.6%) died. Mortality rate was higher in males (13.7%) than females (6.9%) (Table 3).5 subjects were co-infected with HBV/HCV/ TB; however, 3 were lost in the fallow-up, 1 survived while 1 died.

Table. 1: Sex related management out-come among HCV positive Subjects.

HCV	MANAGEMENT OUT- COMES.				
		Loss of follow up	Alive	Dead	Total
Sex	Males	27 (43.5)	5 (8.1)	5 (8.1)	37(59.7)
	Females	17 (27.4)	6 (9.7)	2 (3.2)	25 (40.3)
	Total	44 (71.0)	11 (17.7)	7 (11.3)	62 (100)

Table. 2: Sex related management out –come among HCV/HIV Co-infected Subjects.

HCV/HIV	MANAGEMENT OUT- COMES.				
		Loss of follow up	Alive	Dead	Total
Sex	Males	20(45.5)	4(9.1)	1(2.3)	25(56.8)
	Females	14((31.8)	3(6.8)	2((4.5)	19(43.2)
	Total	34(77.3)	7(15.9)	3(6.8)	44(100)

Table. 3: Sex related management out-come among HCV/ TB Co-infected Subjects.

HCV/TB	MANAGEMENT OUT- COMES.				
		Loss of follow up	Alive	Dead	Total
Sex	Males	13(44.8)	2(6.9)	4(13.7)	19(65.5)
	Females	4(13.7)	4(13.7)	2(6.9)	10(34.5)
	Total	17(58.6)	6(20.6)	6(20.6)	29(100)

DISCUSSION

With respect to subjects management out-come, before the introduction of hepatitis B vaccines, numerous effective hepatitis B prevention measures had been employed to some degree, including screening of blood donors, preparation of plasma-derived products in a way that inactivates the virus, implementation of infection control measures, and administration of hepatitis B immune globulin following suspected exposure, especially for infants born to HBsAg-positive women. Although all of these activities can reduce the risk of HBV transmission, none have been as effective as active immunization with hepatitis B vaccine, which remains the single most important hepatitis B prevention measure (MMWR, 2003).Early antiviral treatment may only be required in less than 1% of patients, whose infection takes a very aggressive course (fulminant hepatitis) or who are immunocompromised. On the other hand, treatment of chronic infection may be necessary to reduce the risk of cirrhosis and liver cancer. Chronically infected individuals with persistently elevated serum alanine aminotransferase, a marker of liver damage and candidates for therapy (Lai and Yuen, 2007).

Although none of the available drugs can clear the infection, they can stop the virus from replicating, thus minimizing liver damage. Currently, there are seven medications licensed for treatment of hepatitis infection in the United States. These include antiviral drugs lamivudine (Epivir), adefovir (Hepsera), tenofovir (Viread), telbivudine (Tyzeka) and entecavir (Baraclude) and the two immune system modulators interferon alpha-2a and PEGylated interferon alpha-2a (Pegasys). The use of interferon, which requires injections daily or thrice weekly, has been supplanted by long-acting PEGylated interferon, which is injected only once weekly (Diensan, 2008). The response rate and safety of HCV therapy in HIV/HCV-co infected individuals are now well established in several clinical trials, which show improved response rates using pegylated formulations of interferon plus ribavirin when compared to standard interferon plus ribavirin (Carrat *et al.*, 2004). However, the long-term benefits of HCV therapy in the HIV/HCV-co infected patient including improvements in survival and quality of life have yet to be demonstrated. (www.hiv.va.gov 2008). One study found that only one-third of HIV/HCV co-infected patients are eligible for HCV treatment. (Fleming *et al.*, 2003) This proportion was not different from patients with HCV infection only. The major barriers to HCV treatment in HIV/HCV co-infected patients identified in this study included non adherence to medical visits, ongoing drug or alcohol use

in the preceding 6 months, decompensated liver disease, advanced HIV disease, and medical co morbidities (Fleming *et a.l*, 2003).

However, some individuals are much more likely to respond than others and this might be because of the genotype of the infecting virus or the patient's heredity. The treatment reduces viral replication in the liver, thereby reducing the viral load (the amount of virus particles measured in the blood) (Pramoolsinsup, 2002).Results of the subjects management out-come of HCV were successfully treated by Directly Observed Therapy (DOT) in the study, treatment success rate for subjects that survived were recorded as 40.0%,17.0% respectively while that of TB and HIV Co-infection with HCV were 35.3%,20..6%,15.4% and15.9% respectively. These rate was lower than estimated success rate of 80% from the analysis of World wide treatment out come (WHO, 1994).It is also lower than estimated success rate of 79% in Nigeria (Dorsum, 2001).The success rate from this study and many previous studies are less than the global targets of 85%.This may be attributed to the facts that, in a significant number of subjects follow up was lost and some subjects included in the study were treated before the Direct Observed Treatment Short Course (DOTS) were fully established. High death rate recorded during the study among the population studied may be due to the co-infections with TB and HIV which probably reflecting their immunity level.(Harries *et al*.,2001, Mukadi,2001).Treatment endpoints for HCV are the same for HCV mono infected patients and HIV/HCV co-infected patients. An undetectable HCV RNA level or a 2-log reduction in HCV RNA levels at 12 wk is referred to as an EVR. An undetectable HCV RNA at the end of therapy is referred to as an end-of-treatment response (ETR) and undetectable HCV RNA 6 months after therapy is referred to as SVR. Because failure to achieve an EVR has been shown to predict strongly an inability to achieve an SVR in both HCV mono infected and HIV/HCV co-infected patients, discontinuation of therapy should be considered at 12 wk in the absence of an EVR. (Perronne *et al*, 2004).Currently, there is no vaccine for hepatitis C, but research is in progress. Like HIV, HCV can mutate easily, which makes vaccine development complicated. As no vaccine exists, all measures should be taken to prevent HCV transmission. (James, 2008).

CONCLUSION

CD4 lymphocyte count and WHO clinical staging subjects were selected for Antiviral therapy management out-come showed that there was loss of fallow-up for majority of the subjects. However, in those that were successfully monitored, survival rate was consistently higher than mortality rate for all categories of patients except for cases of co-infections by HCV/TB where survival and mortality rates was equal.

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