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Influence of Hepatitis B and Hepatitis C Virus Infections and Human Immunodeficiency Virus Therapy on Liver Status of Patients with Human Immunodeficiency Virus

Tary Abdel-Hamid Salman[1,2], Khaled Mohammad Attallah[1,2*], Omkolsom Mohamed EL-Haddad[2], Azza Mohamed Abdel-Latif[3] and Mohamed Abdou Awid[4]

[1]Tropical Medicine, National Liver Institute, Menoufiya University, Egypt.
[2]Gastroenterology and Hepatology, National Liver Institute, Menoufiya University, Egypt.
[3]Microbiology and Immunology, National Liver Institute, Menoufiya University, Egypt.
[4]Alexandria Fever Hospital, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author TAHS designed the study and revised the draft of the manuscript. Author KMA wrote the manuscript, managed the literature searches and analyses of the data of the study. Author OMEH revised the manuscript. Author AMAL managed the experimental process. Author MAA performed data collection. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Hepatitis B virus (HBV) and hepatitis C virus (HCV) are emerging among Human Immunodeficiency Virus (HIV) patients.
Aim: To determine the impact of the presence of hepatitis B and hepatitis C viral infections as well HIV treatment on the liver of patients with HBV, HCV and HIV co-infections.
Methods: A descriptive, cross-sectional study on 115 HIV patients that were subdivided into two

**Corresponding author: Email: kmatalah@yahoo.com;*

groups; group A included 67 patients who had HIV mono-infection and group B included 48 patients who had HIV plus HBV, HIV plus HCV or HIV plus both.
Results: 48 HIV patients (41.7%) had HBV and/or HCV infections. 4 patients (3.5%) were positive for HBV Surface Antigen (HBsAg), 37 patients (32.2%) were positive for HCV Anti-body (Anti-HCV Ab) and 7 patients (6.1%) were positive for both. HIV treatment caused significant impairment of liver functions especially in group B patients. CD4+ T Lymphocytes significantly increased after HIV therapy in group A versus group B.
Conclusion: HIV may accelerate liver damage caused by HBV and HCV. HIV therapy carries a potential risk for hepatotoxicity.

Keywords: AIDS; HAART; HBV; HCV; HIV; CD4+ T lymphocytes.

1. INTRODUCTION

Human immunodeficiency virus infection / acquired immunodeficiency syndrome (HIV / AIDS) has become one of the world's most serious health challenges with approximately 35 million people currently living with HIV and tens of millions of people have died from AIDS related disease since the beginning of the epidemic [1]. HIV infects primarily vital human immune system cells such as CD4+ T lymphocyte cells (mature helper T cells), macrophages and dendritic cells. When CD4+ T cell lymphocytes numbers decline below a critical level; cell-mediated immunity is lost and the body becomes progressively more susceptible to opportunistic infections and malignancies [2]. The majority of HIV infections are acquired through un-protected sexual relations, infected blood specially in intravenous drug abusers, haemophiliacs and recipients of blood transfusions or vertical transmission (mother-to-child) which can occur during the birth process or during breast feeding. HIV has been found at low concentrations in the saliva, tears and urine of infected individuals, but there are no recorded cases of infection by these secretions [3]. HCV and HIV share similar modes of transmission but their transmission efficiencies differ [4]. HCV is most efficiently spread by exposure to contaminated blood or blood products particularly by injection drug use. Rates of vertical and perinatal transmission are relatively low (3-6%) although this rate increases approximately 5-fold when the mother is HIV infected [5]. Introduction of effective combined anti-retroviral therapy has made HIV infection a chronic illness. Substantial reductions in the number of AIDS-related deaths have been accompanied by an increase in liver-related morbidity and mortality due to co-infection with chronic HBV and HCV, non-alcoholic fatty liver disease (NAFLD), HIV drug-induced hepatotoxicity and hepatocellular carcinoma (HCC) [6]. HIV-infected patients with advanced liver disease bear a high risk for life-threatening complications of cirrhosis such as liver failure, variceal bleeding and hepatocellular carcinoma [7]. Chronic infections with HBV and HCV represent the most significant cause of liver disease in HIV-infected persons. The shared routes of transmission of HBV, HCV and HIV help to explain this phenomenon [8]. Worldwide chronic HBV infection defined as persistently detectable hepatitis B surface antigen (HBsAg) more than 6 months after infection affects approximately 7-15% of HIV-infected persons [9]. HIV infection modifies the natural history of HBV infection which can result in higher rates of HBV persistence and relapse [10]. Among patients with persistent HBV infection the severity of liver disease and risk of liver-related mortality is substantially increased in patients with HIV co-infection [11]. This study aimed to determine the impact of the presence of hepatitis B and hepatitis C viral infections as well HIV treatment on the liver of patients with HBV, HCV and HIV co-infections.

2. MATERIALS AND METHODS

2.1 Study Design

A descriptive, cross-sectional study was conducted on 115 HIV-positive patients that were enrolled from HIV centre of Alexandria Fever Hospital and co-studied at National Liver Institute Hospital in the centre of the River Nile Delta in North Egypt. Study design was approved by the National Liver Institute, Menoufia University Institutional Research Board (IRB) and Alexandria Fever Hospital ethical committee and ethical considerations from both social and cultural aspects were taken into consideration.

Patients were eligible for the study if they have HIV infection diagnosed by Enzyme Linked Immunosorbent Assay (ELISA) for Detection of anti-HIV antibodies using Bio-Rad Genscreen

Plus HIV version 2 Kit (Steenvoorde, France) which is a microplate double-sandwich enzyme-linked immunosorbent assay. In the first step, the sample (75 µl) is incubated with biotinylated anti-p24 polyclonal antibodies (conjugate 1) in a microtiter plate coated with anti-p24 monoclonal antibodies and purified HIV-1 and HIV-2 antigens (recombinant gp160, artificial functional consensus group O gp41 polypeptide, and synthetic gp36 polypeptide). After a first washing step, avidin and HIV-1 and HIV-2 antigens (HIV-1 group M, gp41 synthetic polypeptides, artificial functional consensus group O gp41 polypeptide, and synthetic gp36 polypeptide) labelled to horseradish peroxidase (conjugate 2) are added. After a second washing step, a TMB substrate solution is added. The presence of HIV antigen or antibody is proportional to the binding of the conjugate and peroxidase activity.

Results were confirmed by Western blot HIV Blot 2.2 assay (MP Biomedicals Asia Pacific Pte Ltd., Singapore).

Steps:

1. 2 ml of diluted wash buffer were added to each well.
2. Using forceps, carefully remove required number of strips from the tube and place numbered side up into each well. Include strips for Strong Reactive, Weak Reactive and Non-Reactive controls.
3. Incubate the strips for 1 to 2 minutes at room temperature (25±3ºC) on a rocking platform (speed of 12 to 16 cycles per minute). Remove buffer by aspiration. (Note: Do not allow the strips to dry. Failure may result in watery marks on developed strips for some specimens).
4. Add 2 ml of blotting buffer to each well.
5. Add 20 µl each of patients' sera or controls to appropriate wells. Care should be taken to ensure specimens are not added directly on the strips.
6. Cover the tray with the provided cover and incubate for 1 hour at room temperature (25±3ºC) on the rocking platform.
7. Carefully un-cover the tray to avoid splashing or mixing of samples. Tilt the tray to aspirate the mixture from the wells. Change aspirator tips between samples to avoid cross-contamination.
8. Wash each strip 3 times with 2 ml of diluted wash buffer allowing 5 minutes soak on the rocking platform between each wash.
9. Add 2 ml of working conjugate solution to each well.
10. Cover tray and incubate for 1 hour at room temperature (25±3ºC) on the rocking platform.
11. Aspirate conjugate from the wells. Wash as in step 8.
12. Add 2 ml of substrate solution to each well.
13. Cover tray and incubate for 15 minutes on the rocking platform.
14. Aspirate the substrate and rinse the strips at least 3 times with reagent grade water to stop the reaction (A dark background can result if washing is insufficient at this step).
15. Using forceps, gently remove strips onto paper towels. Cover with paper towels and dry. Alternatively, allow strips to dry in the wells of the tray.
16. Mount strips on worksheet (non-absorbent white paper). Do not apply adhesive tape over the developed bands. Observe the bands and grade the results. For storage, keep the strips in the dark.

Patients were divided into 2 groups:

Group A: 67 patients had HIV mono-infection.
Group B: 48 patients were co-infected with HIV and HBV, HIV and HCV or HIV and both.

2.2 Medical Data

- Age, gender, risk factors, mode of transmission of HIV as well as history of liver disease and other infections.
- Clinical examination.

2.3 Laboratory Investigations

- Complete blood count (CBC).
- CD4+ T lymphocytes count before and after 6 months of treatment.
- Biochemical liver tests before and after 6 months of treatment: serum Alanine Aminotransferase (ALT) and serum Aspartate Aminotransferase (AST), serum total bilirubin (T. Bil.), serum alkaline phosphatase (ALP), serum albumin (Alb.), Prothrombin time (P.T.), prothrombin concentration (P.C.) and international normalized ratio (INR).
- Urine analysis, Urea and creatinine.
- Hepatitis B Virus Surface Antigen (HBsAg) was detected by a sandwich Enzyme Immuno Assay (EIA) using DiaSorin S.r.l kit (13040 Saluggia, Vercelli, Italy).

Principle of the procedure:

The HBsAg test is an in vitro neutralization assay for detection of the presence of HBsAg in human serum and plasma samples. The HBsAg test is based on the principle of neutralization of binding activity. A neutralizing reagent containing human antibodies to HBsAg is added to one aliquot of each specimen (neutralized aliquot). As a control procedure, anti-HBs negative human serum is added to the other aliquot (non-neutralized aliquot). If the neutralizing reagent has been added to a sample containing HBsAg forming an antigen-antibody complex. If the neutralizing reagent has been added to a sample containing an interfering substance, the antibodies in the neutralizing reagent will not bind to the interfering substance. In the confirmation procedure, each sample is incubated in a solid matrix coated with mouse monoclonal antibodies to HBsAg. Next the antibody conjugate from the screening kit, which contains sheep antibodies to HBsAg, is added. If an antibody-antigen-antibody complex is present, the antibody conjugate will bind to the complex only partially. If an antibody-interfering substance complex is present, the antibody conjugate will bind non-specifically to the interfering substance. If the signal of the neutralized aliquot is significantly lower than the signal of the non-neutralized aliquot, the presence of HBsAg in the sample is confirmed.

- Hepatitis C Virus Anti-body (Anti-HCV Ab) was detected by EIA, using 3rd generation enzyme-linked immunosorbent assay (ELISA) (INNOTEST) HCV antibody III kit, Innogenetics, Ghent, Belgium).

Principle:

The test samples are incubated in the wells coated with a mixture of HCV antigens Core, NS3, NS4, and NS5. Virus specific antibodies to HCV, if present in the sample, will bind to the solid phase antigens. Subsequently, an affinity purified rabbit anti-human IgG (H chain specific) labelled with the enzyme horseradish peroxidase (HRP) is added. In case of a positive reaction, this labelled antibody binds to any solid phase antigen/antibody complex previously formed. Incubation with enzyme substrate produces a blue colour in the test well, which turns yellow when the reaction is stopped with sulfuric acid.

Procedure:

First, all reagents and samples were allowed to reach the room temperature (15-30ºC) before use.

1. 100 µl of the sample diluent was added to each test well.
2. 10 µl of the negative control was added to each well B1 and C1 and 10 µl of the positive control was added to each well D1 and E1.
3. The wells were covered with an adhesive sheath and incubated in an incubator for 60 minutes at 37ºC.
4. Just before the end of the incubation period, the conjugate solution was prepared by diluting the concentrated conjugate 1:100 with conjugate diluent.
5. At the end of the incubation, the plate was washed 5 times with wash solution (prepared by diluting concentrated wash solution 1:25 with distilled water), adding 400 µl to each well, waiting for at least 30 seconds and rewashing.
6. 200 µl of the prepared conjugate solution was added to each well.
7. The wells were again covered with an adhesive sealer, and incubated at 37ºC for another 60 minutes.
8. The wells were again washed 5 times.
9. Prepared substrate solution (20 µl) were added to each well (substrate solution was prepared by diluting concentrated TMB 100 with substrate buffer e.g. 200 µl to 20 ml).
10. The wells were incubated at room temperature (20-25ºC) for 30 minutes.
11. The reaction was stopped by adding 50 µl of the stop solution (sulphuric acid) to each well, in the same sequence and time interval as the substrate solution.
12. A sample is reactive if the absorbance at 450nm is equal to or more than the cut off value.

2.4 Radiological Investigations

- Real-Time abdominal ultrasound using Philips ATL HDI 5000 with SONOCT equipment.
- Plain X-Ray Chest.
- Computerized Tomography (CT) if applicable.

2.5 HIV Therapeutic Strategies

Patients with CD4+ T lymphocytes count <350 cells/mm3 were given HAART (Highly Active Anti-Retroviral Therapy); consisting of a combination of at least 3 drugs, including protease inhibitors (PIs) such as Ritonavir, non-nucleoside (or nucleotide) reverse transcriptase

inhibitors (NNRTIs) such as Efavirenz and nucleoside (or nucleotide) reverse transcriptase inhibitors (NRTIs) such as Lamivudine, Emtricitabine, Tenofovir Disoproxil Fumarate and Zidovudine. Combinations were selected according to the drugs availability and the international recommendations at the time of prescription. 30 patients from each group received HAART; 20 of them received first line {Efavirenz + Lamizidine (Lamivudine 150 mg + Zidovudine 300 mg)} and 10 received second line {Efavirenz + Truvada (Emtricitabine 200 mg + Tenofovir Disoproxil Fumarate 300 mg)}.

2.6 Statistical Analysis

Data were analysed using SPSS software package version 20. Qualitative data were described using number and percent. Comparison between different groups regarding categorical variables was tested using Chi-square test. When >20% of the cells have expected count <5; correction for chi-square was conducted using Fisher's exact test or Monte Carlo correction.

Distributions of quantitative variables were tested for normality using Shapiro-Wilk test and D'Agstino test, also Histogram and QQ plot were used for vision test. If it reveals normal data distribution, parametric tests were applied. If the data were abnormally distributed, non-parametric tests were used.

Quantitative data were described using mean, standard deviation, median, minimum and maximum. For normally distributed data, comparison between two independent populations were done using independent t-test while if more than two populations were analysed; F-test (ANOVA) and Post Hoc test were used, also paired t-test was used to analyse two paired data. For abnormally distributed data, Mann-Whitney Test (for data distribution that was significantly deviated from normal) was used to analyse two independent populations. If more than 2 populations were analysed Kruskal Wallis test was used. Wilcoxon signed ranks test was used to compare between the different periods. Significance test results were quoted as two-tailed probabilities. P (probability) value ≤0.05 was considered significant.

3. RESULTS

Male gender was predominant in both groups with statistically significant difference (p=0.004). Male patients were 101 (87.8%) while female patients were 14 (12.2%). Males were 54 (80.6%) in group A and 47 (97.9%) in group B, while females were 13 (19.4%) in group A and 1 (2.1%) in group B. Mean age of patients was 35.9±9.68 years, ranging between 3-63 years (p=0.693).

Their residence was variable; 77 patients (67%) were from Alexandria, 13 patients (11.3%) from Beheira, 9 patients (7.8%) from Kafr Elsheikh, 7 (6.1%) from Matrouh and 9 (7.8%) from the neighbour country of Libya.

85 patients (73.9%) had a positive history of IV drug abuse, 18 patients (15.7%) had a history of sexual transmission and un-protected sex, 9 patients (7.8%) had a history of both IV drug abuse and sexual transmission, 1 patient (0.9%) had a history of multiple blood transfusions and lastly 2 patients (1.7%) had a history of peri-partum transmission. There were predominant history of IV drug abuse in both groups; 42 patients (62.7%) in group A and 43 patients (89.6%) in the group B with no statistically significant difference (p=0.935). History of sexual transmission and un-protected sex was found in 17 patients (25.4%) in group A and 1 patient (2.1%) in group B, still with no statistically significant difference (p=0.336). Only 1 patient (1.5%) and 2 patients (3%) in group A had a history of multiple blood transfusion and peri-partum transmission respectively (Fig. 1).

There were no concomitant HBV or HCV infections in group A. However in group B; 4 patients (3.5%) were positive for HBsAg, 37 patients (32.2%) were positive for anti-HCV antibody and 7 patients (6.1%) were positive for both HBsAg and anti-HCV antibody (Fig. 2).

Among patients of group B; 11 patients (22.9%) were suffering from ascites, compared to none in group A with statistically significant difference (p<0.001).

There was a significant increase in ALT, AST, total bilirubin and Alkaline phosphatase in group B patients with mean±SD: (65.04±27.03 U/l), (65.35±27 U/l), (1.78±0.77 mg/dl) and (193.58±48.26 U/l) respectively, compared to group A with mean±SD: (38.04±19.36 U/l), (36.87±18.72 U/l), (1.01±0.37 mg/dl) and (165.09±34.89 U/l) respectively (p≤0.001). There was a significant decrease in serum albumin and prothrombin concentration in group B patients

with mean±SD of (3.13±0.54 g/dl) and (92.65±8.24%) respectively, compared to group A (3.56±0.51 g/dl) and (96.21±5.59%) (p<0.001, p=0.011 respectively). There was an increase in INR for group B (1.11±0.13) compared to group A (1.07±0.10) but such changes did not reach the level of statistical significance (p=0.074) (Table 1).

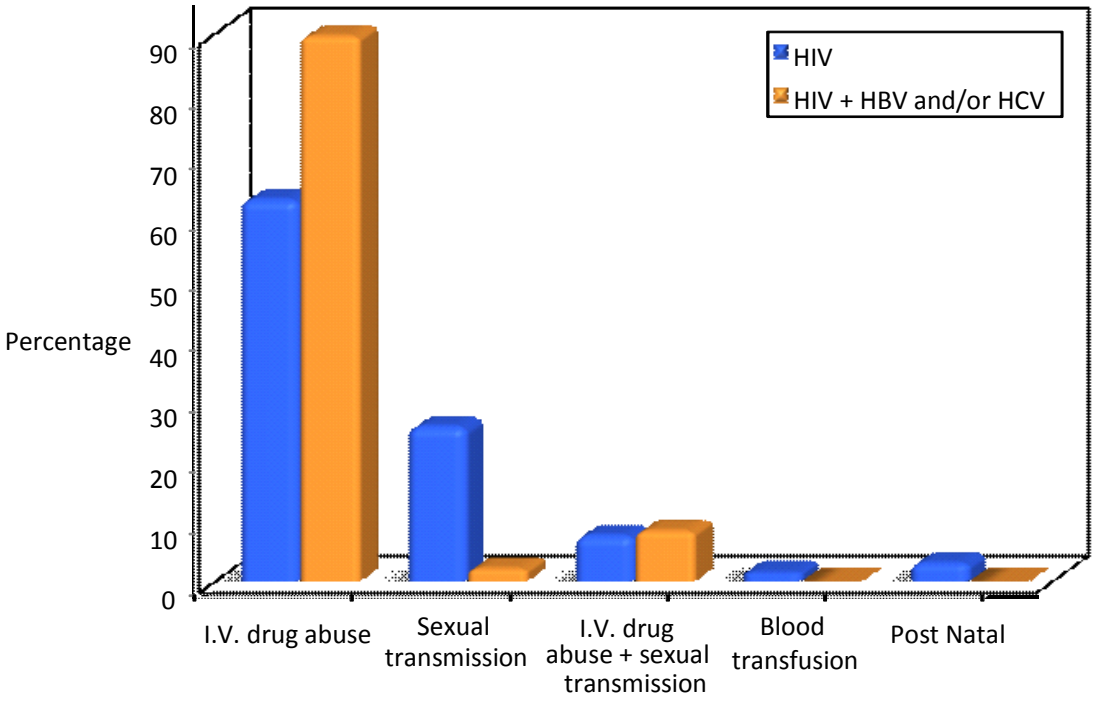

Fig. 1. Mode of transmission of HIV among the studied patients

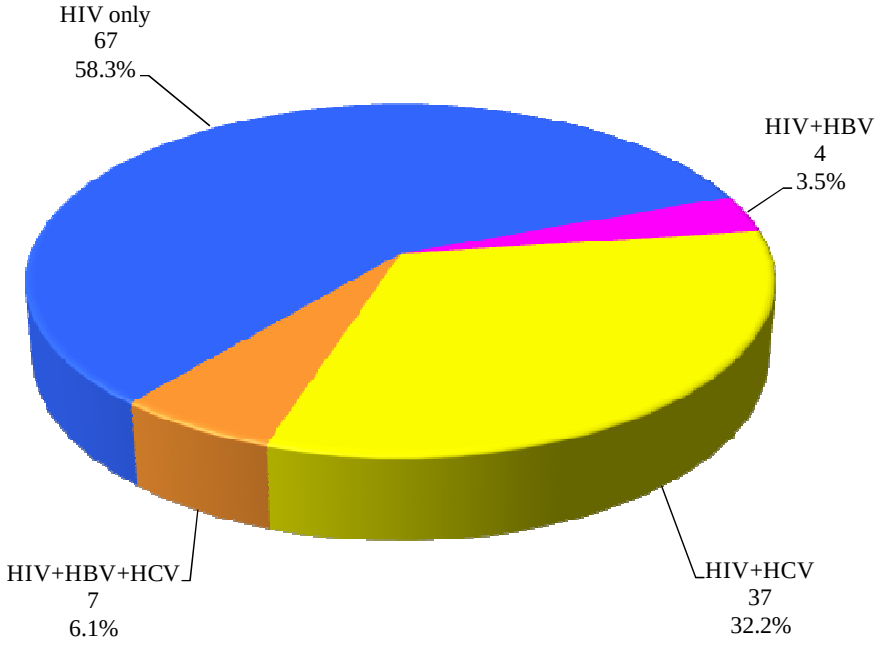

Fig. 2. Prevalence of concomitant infection (HBV and/or HCV) in the studied patients

Table 1. Laboratory Investigations of the studied groups before HIV treatment (HAART)

Variable	Group A (n=67)			Group B (n=48)			P
	Range	Mean±SD	Median	Range	Mean±SD	Median	
CD4+ (500-1200 cells/mm3)	19-915	346.48±209.812	306	28-1087	298.48±195.553	261.5	<0.0216*
ALT (10-44 U/l)	15-88	38.04±19.36	33	30-120	65.04±27.03	59	<0.001*
AST (10- 34 U/l)	16-85	36.87±18.72	36	23-123	65.35±27	56	<0.001*
T. Bil. (0.2-1.5 mg/dl)	0.5-1.8	1.01±0.37	1	0.7-3.5	1.78±0.77	1.8	<0.001*
Alb. (3.7-4.9 g/dl)	2.8-4.6	3.56±0.51	3.7	1.8-4	3.13±0.54	3	<0.001*
ALP (45-122 U/l)	130-234	165.09±34.89	145	133-299	193.58±48.26	183	=0.001*
P.C. (70-100%)	80-100	96.21±5.59	100	67-100	92.65±8.24	95	=0.011*
INR (<1.4)	1-1.3	1.07±0.1	1	1-1.5	1.11±0.13	1.1	=0.074

**: Statistically significant at p ≤0.05*

In treated group A, there was a significant increase in level of CD4+ T Lymphocytes count in response to first and second lines of HIV treatment (p<0.001). In treated group A with Efavirenz+Lamizidine (first line), there was a significant increase in liver tests (ALT, AST and ALP) 6 months after treatment when compared to the same group before treatment (p=0.013, p=0.011, p=0.001 respectively).

Similarly there was a significant increase in serum total bilirubin (p=0.008) and a significant decrease in serum albumin (p=0.031). There were no significant changes in prothrombin concentration and INR (Table 2).

Similarly in treated group A with Evafirenz + Truvada (second line), there was a significant increase in liver tests (ALT, AST, total bilirubin and Alkaline phosphatase) during treatment when compared to same group before treatment (p=0.006, p=0.011, p=0.013, p=0.015 respectively). But there was a significant decrease in serum albumin and prothrombin concentration (p=0.009, p=0.001 respectively) (Table 3).

In treated group B; there was a significant increase in CD4+ T Lymphocytes count in response to first line and second line treatments (p<0.001). In treated group B with Efavirenz+Lamizidine (first line), there was a significant increase in ALT and INR (p<0.001, p=0.039 respectively) after 6 months of treatment, while a significant decrease was recorded in prothrombin concentration (p=0.031) when compared to the same group before treatment.

Also, after 6 months of therapy there were higher levels of AST and Alkaline phosphatase as well as lower serum albumin, but such changes did not reach the level of statistical significance (p=0.068, p=0.326, p=0.091 respectively) (Table 4).

Table 2. Impact of HIV treatment (first line HAART) on CD4+ and biochemical liver tests in Group A

Variable	Treated group A with Efavirenz+Lamizidine						P
	Before treatment (n=20)			After 6 Months of treatment (n=20)			
	Range	Mean ± SD	Median	Range	Mean ± SD	Median	
CD4+ (500-1200 cells/mm3)	58-342	182.97±86.23	168	190-900	415.41±160.38	389	<0.001#*
ALT (10-44 U/l)	15-119	46.52±23.26	45	30-120	63.83±26.61	59	=0.013#*
AST (10-34 U/l)	16-123	45.55±23.94	48	23-123	64.10±26.43	56	=0.011#*
T. Bil. (0.2-1.5 mg/dl)	0.5-3	1.26±0.36	1	0.7-3.5	1.73±0.75	1.8	=0.008#*
Alb. (3.7-4.9 g/dl)	2.7-4.6	3.49±0.56	3.5	2.3-4	3.22±0.48	3	=0.031t*
ALP (45-122 U/l)	130-266	168±39.92	146	133-299	195.72±48.37	199	=0.001t*
P.C. (70-100%)	80-100	96.41±5.31	100	80-100	94.28±5.84	95	=0.172t
INR (<1.4)	1-1.3	1.06±0.09	1	1-1.3	1.09±0.09	1.1	=0.326t

#: Wilcoxon signed ranks test
t: Paired t-test
**: Statistically significant at p ≤0.05*

Table 3. Impact of HIV treatment (second line HAART) on CD4+ and biochemical liver tests in Group A

Variable	Treated group A with Efavirenz+Truvada						P
	Before treatment (n=10)			After 6 Months of treatment (n=10)			
	Range	Mean±SD	Median	Range	Mean±SD	Median	
CD4+ (500-1200 cells/mm3)	100-342	226.14±87.91	226.5	356-1000	703.57±214.04	732.5	<0.001#*
ALT (10-44 U/l)	22-125	46.92±26.46	35	22-270	86.06±53.87	69	=0.006#*
AST (10-34 U/l)	20-132	41.92±24.94	33	23-135	64.1±26.43	56	=0.011#*
T. Bil. (0.2-1.5 mg/dl)	0.7-6	1.05±0.85	0.9	0.7-9.2	1.93±1.67	1.6	=0.013#*
Alb. (3.7-4.9 g/dl)	3-4.8	3.90±0.36	3.9	2-3.9	3.04±0.54	3	=0.009t*
ALP (45-122 U/l)	150-320	175.19±37.56	155	151-315	208.77±46.61	197	=0.015t*
P.C. (70-100%)	44-100	95.32±9.82	100	50-100	80.1±14.77	84	=0.001t*
INR (<1.4)	1-2.1	1.07±0.18	1	1-1.7	1.23±0.2	1.2	=0.538t

#: Wilcoxon signed ranks test
t: Paired t-test
**: Statistically significant at p ≤0.05*

Table 4. Impact of HIV treatment (first line HAART) on CD4+ and biochemical liver tests in Group B

Variable	Treated group B with Efavirenz+Lamizidine						P
	Before treatment (n=10)			After 6 Months of treatment (n=10)			
	Range	Mean±SD	Median	Range	Mean±SD	Median	
CD4+ (500-1200 cells/mm3)	78-343	176.57±97.15	134	234-658	403.57±120.14	394.5	<0.001#*
ALT (10-44 U/l)	30-124	72.9±28.87	69	30-229	90.41±51.15	89	<0.001#*
AST (10-34 U/l)	28-123	73.07±28.33	66	28-240	89.9±51.57	78	=0.068#
T. Bil. (0.2-1.5 mg/dl)	0.7-3.5	1.91±0.8	2	0.7-4	2.2±0.9	2.3	=0.066#
Alb. (3.7-4.9 g/dl)	1.8-4	3.06±0.52	3	1.6-4	2.92±0.62	3	=0.091t
ALP (45-122 U/l)	135-299	196.76±47.39	200	135-299	201.34±48.62	200	=0.326t
P.C. (70-100%)	67-100	88.38±9.49	89	38-100	82.69±15.23	87	=0.031t*
INR (<1.4)	1-1.5	1.17±0.14	1.2	1-1.8	1.25±0.2	1.2	=0.039t*

#: Wilcoxon signed ranks test
t: Paired t-test
**: Statistically significant at p ≤0.05*

On the other hand in treated group B with Efavirenz+Truvada (second line); there was a significant increase in ALT, AST and total bilirubin 6 months after treatment when compared to the same group before treatment (p<0.043, p<0.001, p=0.014 respectively). There was a significant decrease in serum albumin and prothrombin concentration (p=0.039, p=0.011 respectively). After 6 months of treatment there was higher level of Alkaline Phosphatase and INR but with no statistical significance difference (p=0.640, p=0.074) respectively (Table 5).

When comparing group A to group B after 6 months of treatment, group A had a significant high increase in CD4+ T Lymphocytes count with mean±SD of 703.57±214.04 cells/mm3 compared to CD4+ T Lymphocytes count in patients of group B with mean±SD 403±120.14 cells/mm3 (p<0.001).

When comparing group A to group B after 6 months of treatment; group B had a significant high increase in liver enzymes (ALT and AST) compared to group A (p=0.040, p=0.043). Similarly there was a significant increase in serum bilirubin (p=0.048), but a significant decrease in serum albumin (p=0.044). Group B had a significant increase in INR (p<0.001) but a significant decrease in prothrombin concentration (p=0.001) (Table 6).

4. DISCUSSION

In the present study HBV prevalence in HIV patients was determined to be 9.6% as compared to 3.3% in patients from Buenos Aires, Argentina [12], 4.47% from a large cohort in New York City, USA [13], 5.3% from Gabon and Botswana [14], 6.3% from Nigeria [15] and

Table 5. Impact of HIV treatment (second line HAART) on CD4+ and biochemical liver tests in Group B

Variable	Treated group B with Efavirenz+Truvada						P
	Before treatment (n=10)			After 6 Months of treatment (n=10)			
	Range	Mean±SD	Median	Range	Mean±SD	Median	
CD4+ (500-1200 cells/mm3)	106-345	226.72±74.02	234	190-739	394±132.39	370	<0.001#*
ALT (10-44 U/l)	23-123	64.1±26.43	56	28-240	89.9±51.57	78	<0.043#*
AST (10-34 U/l)	30-124	72.9±28.87	69	30-229	90.41±51.15	89	<0.001#*
T. Bil. (0.2-1.5 mg/dl)	0.5-1.9	1.01±0.3	0.9	0.7-5	1.93±1.9	1.9	=0.014#*
Alb. (3.7-4.9 g/dl)	2.5-4.2	3.51±0.47	3.6	2-3.9	3.04±0.62	3	=0.039t*
AlP (45-122 U/l)	150-320	175.16±37.56	155	153-299	188.54±36.62	177	=0.640t
P.C. (70-100%)	80-100	96.21±5.59	100	67-100	92.65±8.24	95	=0.011t*
INR (<1.4)	1-1.3	1.07± 0.1	1	1-1.5	1.11±0.13	1.1	=0.074t

#: Wilcoxon signed ranks test
t: Paired t-test
**: Statistically significant p ≤0.05*

Table 6. CD4+ and biochemical liver tests in HAART treated HIV patients

Variable	After 6 months treatment with Efavirenz+Lamizidine						P
	Group A (n=20)			Group B (n=20)			
	Range	Mean±SD	Median	Range	Mean±SD	Median	
CD4+ (500-1200 cells/mm3)	356-1000	703.57±214.04	732	234-658	403±120.14	394	<0.001 MW *
ALT (10-44 U/l)	30-120	63.83±26.61	59	30-229	90.41±51.15	89	=0.040 MW*
AST (10-34 U/l)	23-123	64.10±26.43	56	28-240	89.9±51.57	78	=0.043 MW *
T. Bil. (0.2-1.5 mg/dl)	0.7-3.5	1.73±0.75	1.8	0.7-4	2.20±0.9	2.3	=0.048 MW *
Alb. (3.7-4.9 g/dl)	2.3-4	3.22±0.48	3	1.6-4	2.92±0.62	3	= 0.044t*
ALP (45-122 U/l)	133-299	195.72±48.37	199	135-299	201.34±48.62	200	=0.661t
P.C. (70-100%)	80-100	94.28±5.84	95	38-100	82.69±15.23	87	=0.001t*
INR (<1.4)	1-1.3	1.09±0.09	1.1	1-1.8	1.25±0.2	1.2	<0.001t*

MW: Mann Whitney test
t: Student t-test
**: Statistically significant p ≤0.05*

17.8% from a large cohort in Africa [16]. The discrepancy found among former studies can be related to the different HBV levels of prevalence, endemicity and genotype profiles worldwide.

In the current study; the prevalence of HCV in HIV patients was determined to be 32.2% as compared to 33.8% among HIV positive patients in a province in Iran [17]. On the contrary, HCV prevalence rate was 77% in a study on HIV patients from Asfahan, Iran [18]. Among 822 French HIV infected patients; 29% of them were infected by HCV [19].

In contrast; the prevalence of HCV co-infection in the present study was higher than reported by a study on 569 Nigerian HIV infected patients; where 3 patients (0.5%) were infected by HCV [20]. Similarly, in a study on 260 HIV positive Nigerian persons; 6 patients (2.3%) had positive anti-HCV antibodies [21]. It was reported that chronic viral hepatitis among hospitalized HIV patients was caused by HCV in 72% of patients [22]. In accordance; very high HCV prevalence was recorded in a population of high risk HIV infected people as intravenous drug users (96.6%) and former paid blood donors (92.9%) in some Chinese provinces [23].

This wide range of HIV/HCV co-infection prevalence and variability in different studies could be explained by the virtue of changing HCV prevalence among different countries as well as the discrepancy in the studied groups of patients and research designs regarding epidemiological transmission risk factors.

This study revealed that the prevalence rates of dual co-infection of both HBV and HCV among HIV positive patients were 7/115 cases (6.1%).

Another study reported that among 5639 HIV patients the prevalence of both HBV and HCV were 89/5639 cases (1.58%) [13].

Similarly among 822 HIV infected patients; 4% of patients were co-infected by both HBV and HCV [19]. In 102 HIV patients dual presence of HBV and HCV was observed in 4 patients (3.9%) [24]. All previous controversial data could be attributed to sample size and different epidemiological patterns of prevalence.

In contrast; it was found that among 80 HIV positive patients; 20 patients (25%) were co-infected with HBV and HCV [17]. Another study that included 443 HIV positive individuals found that 3 patients (0.7%) were infected with both hepatitis B and C viruses [25]. Among 593 people living with HIV; both HBV and HCV were prevalent in 2 patients (0.3%) [12].

The previously mentioned data support the fact that both HBV and HCV are common co-infections in people with HIV probably through sharing similar modes of transmission. The risk factors of any population under study directly influence the prevalence in that particular population.

In the present study; male cases were higher than females. This is similar to two cohorts that showed male preponderance (98.5% and 82.3% respectively) [18, 22]. HIV/HBV and HIV/HCV co-infection was more likely associated with male gender (80.6% and 71.2% respectively) [13].

On the contrary, a study reported that the majority of their 260 patients (71.2%) were females [21]. Also, in another study 64% of patients were females [26].

In the present study, the mean age was 35.9±9.68 years. Young people (15-24 years) are at the centre in terms of newly acquired infections [27]. A report by the Information and Decision Support Centre which is the research arm of the Egyptian Cabinet; declared that around 75% of Egyptians living with HIV were aged 25-49, the most productive segment of society [28].

In a meta-analysis comprising published studies in medical literature, involving cohorts of HIV-positive patients co-infected with HCV, the mean age was 21-50 years [29].

The results of the present study are consistent with previous studies that emphasize intravenous drug abuse (IDU) as one of the major routes of HCV transmission. 73.9% of the patients had a positive history of IDU, 15.7% had a history of sexual transmission and un-protected sex. These results highlight the need to intensify risk reduction education, such as needle disposal awareness, safe sex practices and optimal models of integrated care, particularly for populations with IDU, to reduce the risk of transmission of HIV and hepatotropic viruses [30,31].

This is in agreement with a study that stated that among 130 HIV patients; IDU and high-risk sexual activity were 83.5% and 48% of the patients respectively [18]. IDU was emphasized as one of the major routes of transmission in HIV patients with and without HCV co-infection [32]. Another study reported that the majority of their patients were IDUs (82.2%) [22]. Moreover, IDU was reported in more than 22% of those with HIV/HCV [33].

On other hand; a study revealed that among 260 Nigerian HIV positive persons; there was no reported IV drug abuse, but major risk factors for co-infection with all three viruses were in the form of multiple un-protected sexual exposure (50%), multiple blood transfusions (25%) and scarification marks to express ethnic identity (50%) [21].

In the current study; ALT and AST were significantly higher in HIV patients co-infected with HBV and HCV (mean ALT: 65.04±27.03 U/l and mean AST: 65.35±27 U/l respectively) ($p<0.001$) when compared to HIV mono-infection (mean ALT: 38.04±19.36 U/l and mean AST: 36.87±18.72 U/l respectively) ($p<0.001$). Similarly; another study reported elevation of liver enzymes among HIV patients co-infected with HBV and HCV with mean ALT of 72.6±23.7 U/l and 85±12 U/l respectively and mean AST of 64.8±33.7 U/l and 34±7 U/l respectively. On the other hand; mean ALT was 50.7±45.2 U/l and mean AST was 30.3±21.2 U/l in HIV mono-infected patients [21].

HIV patients co-infected with chronic HBV or HCV were significantly more likely to have elevated liver tests than those without co-infection [34,35].

In the present study; there was a significant impairment of both HIV mono-infected and HIV/HBV, HIV/HCV co-infected patients throughout HAART treatment duration (whether

Evavirenz + Lamizidine or Truvada regimens). However, the impairment was severe in co-infected patients after six months of treatment when compared to pre-treatment values.

A similar finding to the present study revealed that ALT level of HIV/HCV co-infected patients under HAART, during the first year of treatment, was at least 5 fold greater than that of pre-HAART [36]. HCV has been confirmed to be a risk factor associated with a 3 fold opportunity of increased transaminases during HAART in relation to HIV patients without HCV [37]. The incidence of anti-retroviral therapy associated hepatoxicity is approximately 3 folds greater in HIV/HCV-co-infected persons than in persons without hepatitis [38].

In contrast; it was reported that ALT elevations in HIV/HBV co-infected patients on HAART over a median period of more than 5 years was low and did not increase with duration of HAART [39]. Similarly, it was concluded that no significant changes were observed in ALT serum levels over time in patients co-infected with HIV and HCV who were treated with HAART [40]. This discrepancy could be attributed to the type, dose and duration of HAART used as well as liver functional status before starting treatment.

In the present study; CD4+ T Lymphocytes counts increased significantly in both HIV mono-infection and HIV/HBV, HIV/HCV co-infection patients after HAART. However, CD4+ T Lymphocytes increase in HIV mono-infected patients was higher when compared with those of HBV or HCV co-infected with the mean CD4+ T Lymphocytes count of (703.57±214.04 cells/mm3 versus 403.57±120.14 cells/mm3, $p<0.001$). This suggests that although HAART does improve the immune system of co-infected patients, but its efficiency is relatively compromised by HBV and HCV interactions.

The former finding was in agreement with a study that showed that the presence of HIV/HCV co-infection significantly decreases the CD4+ T Lymphocytes response after HAART when compared to mono-infected patients [36]. Even though HAART suppresses HIV and increases CD4+ T Lymphocytes count response; however adverse drug reactions were worsened by the presence of HCV co-infection [41].

5. CONCLUSION

Liver disease is common among HIV-infected patients. Prevalence of viral hepatitis (either HBV or HCV) among HIV positive individuals was high. HIV infection may accelerate progression of liver damage caused by HCV or HBV infection. Thus screening of HBV and HCV is mandatory through strict monitoring and regular evaluation of liver enzyme levels and CD4+ status in order to maintain effective HIV treatment with low incidence of liver complications.

Although, HAART itself are associated with a potential risk for hepatotoxicity, this risk is even enhanced in patients with hepatitis co-infections. Consequently, these patients need to be managed in an expert multidisciplinary environment to ensure early diagnosis, effective management, HAART adjustment and the possible treatment of viral hepatitis whether HBV or HCV and meticulous screening for hepatocellular carcinoma.

Future research should focus on more detailed analysis of the effects of HAART on the progression of fibrosis in hepatitis-co-infected patients as this may open new doors toward drugs slowing down the progression of liver fibrosis, as well as studying the impact of hepatitis co-infection on antiretroviral therapy choice, toxicity and regimen change.

CONSENT

All authors declare that written informed consent was obtained from the patients (or other approved parties) for performing this research.

COMPETING INTERESTS

The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

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