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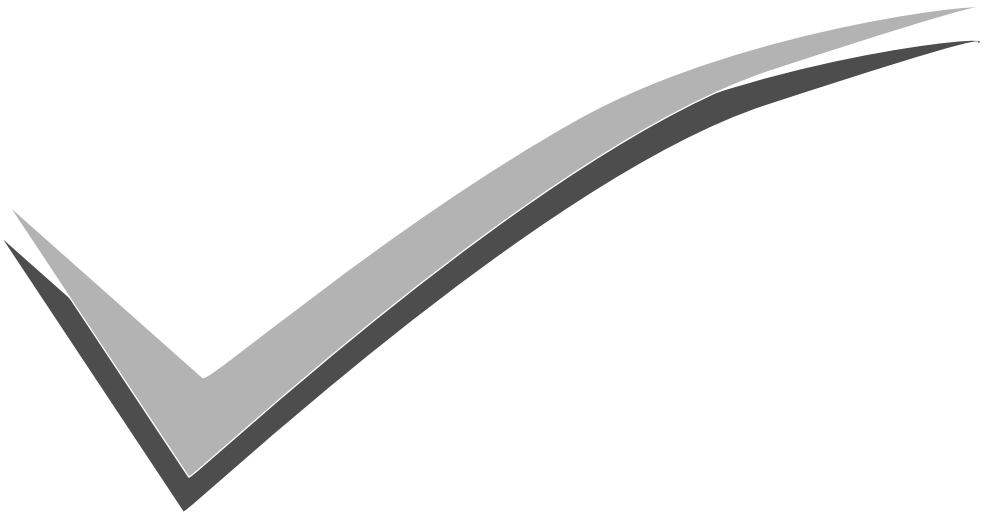

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Research

Hepatitis C virus and Human Immunodeficiency Virus coinfection among attendants of Voluntary Counseling and Testing Centre and HIV follow up clinics in Mekelle Hospital

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Key words: Hepatitis C virus, human immunodeficiency virus, co-infection, seroprevalence

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Abstract

Introduction: Hepatitis C virus remains a large health care burden to the world. HIV and HCV coinfection is major global health concern worldwide. However, there is limited information on the prevalence of HCV/HIV co-infection in Ethiopia. The aim of the study was to assess the magnitude of HIV/HCV coinfection and the potential risk factors in attendants of voluntary counseling and testing centre and HIV follow up clinics of Mekelle hospital. **Methods:** A cross sectional seroprevalence survey of HCV infection was carried out on 300 HIV negative and positive subjects attending voluntary counseling and testing (VCT) center and HIV follow up clinics of Mekelle hospital, Ethiopia from December 2010-February 2011. Serum samples were tested for anti-HCV antibodies using immunochromatographic test. **Results:** Of the 300 study participants, 126(42%) were HIV negative and 174(58%) HIV seropositive from VCT and HIV follow up clinics, respectively. The overall anti-HCV prevalence was 18(6.0%). There were no significant differences in HCV seroprevalence among the different categories of age and sex ($p > 0.05$). Of the 174 persons with HIV, 16 (9.2%) cases had antibodies to HCV, where as among 126 HIV negative subjects 2 (1.58%) were HCV seropositive ($p = 0.006$, OR= 6.28, 95% CI= 1.42-27.82). **Conclusion:** Accordingly, there was a significant difference in sero-positivity of HCV between HIV positive and HIV negative participants. No apparent risk factor that caused HCV infection was inferred from this study.

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Introduction

Hepatitis C virus (HCV) is a significant healthcare problem affecting more than 170 million people worldwide and as many as four million new infections occur annually [1] HCV may increase the rate of progression to acquired immune-deficiency syndrome (AIDS), impair immune reconstitution and the risk of hepa-toxicity. HCV infection increases the number of complications in persons who are co-infected with human immune-deficiency virus (HIV) [2]. Of those exposed to HCV, 80% become chronically infected, and at least 30% of the carriers develop chronic liver disease [3]. Risk factors associated with HCV infection include injection drug use, receipt of blood products, long term haemo-dialysis, organ transplantation, receipt of tattoo from unsanitary facilities, vertical transmission during pregnancy and sexual exposure [4]. The WHO estimated that about 3% of the world´s population is chronically infected with HCV [5]. The overall prevalence in Sub-Saharan Africa is estimated 3% [6]. Decline of mortality due to opportunistic infections in HIV-infected patients since the introduction of highly active antiretroviral therapy (HAART) has led to an increase in morbidity and mortality related to hepatitis virus infections. Co-infection of HIV and HCV is common because of their similar routes of transmissions of exposure. The sero-prevalence of HCV in Ethiopia was reported as 7.5% [7] and HIV/HCV co-infection rate was 1.4%-11.6% [8-10]. Screening of blood products is the only way to prevent transfusion associated cases. Diagnostic tests for HCV infection include serologic tests to detect HCV antibodies, molecular tests and genotyping techniques [11]. Screening based on antibody detection has markedly reduced the risk of transfusion-related infection [12]. The aim of the study was to assess the magnitude of HIV/HCV co-infection rate and potential risk factors are associated with HCV sero-positivity among consecutive attendants of voluntary counseling and testing (VCT) centre and HIV follow up clinics of Mekelle hospital.

Methods

Study population, data collection and laboratory procedures.
The prospective study was conducted during December 2010 to February 2011among attendants of VCT centre and ART clinic in Mekelle hospital, North Ethiopia. Three hundred subjects (126 HIV-negative from VCT and 174 HIV-positive subjects in ART follow up) were enrolled in the study. Socio-demographic data including risk behaviors, drug injection, dental procedure, catheterization, surgery, blood transfusion, hospitalization, history of tattooing, scarification and bloodletting were collected. Five millilitre of blood sample was collected by finger prick from consecutive attendants of VCT and ART clinics in the hospital. Serum was screened for anti-HCV antibody using rapid test kits (Flavicheck-HCV WB, Qualpro diagnostics, India) and one step HCV serum test strip (Biocare TM diagnostics, China). The reactive sera were further tested by Enzyme-Linked Immuno-sorbent assay (ELISA) for confirmation. HIV and anti-HCV positive samples were retested by 4th generation ELISA assay (HCV antibody ELISA, Human diagnostics, Germany). The presence of antibodies to HIV was determined using three different immune-chromatography rapid test kits: HIV (1+2) Rapid Test Strip (Shanghai Kehua Bioengineering co., Ltd), HIV ½STAT-PAK Assay (Chembio Diagnostic Systems, inc.) and Uni-Gold HIV (Trinity biotech plc, Ireland). Drops of blood were taken by finger-prick using pipette. About 40?l of the sample was added to the HIV (1+2) rapid test cassette and then one drop of the sample diluents was added to the same area. After 2-3 min, one band if negative or two bands if positive were observed. When the anti-HIV was positive by the HIV Rapid Test from, the sample was retested by the second HIV ½ STAT-PAK assay. HIV-seropositive subjects were counseled and negative sera were retested by the second Rapid Test and confirmed by Uni-Gold HIV test. Rapid test reactive specimens were retested using 4th generation ELISA assay.

Data analysis

Data were analyzed using SPSS 17.0 statistical software. Chi-square (X^2) test was utilized to compare variables. P-value <0.05 was considered as significant. Odds ratio (OR) and 95% confidence interval (CI) were used to measure the strength of association.

Ethical considerations

Ethical clearance was obtained from Department of Microbiology, School of Medicine of Addis Ababa University. Informed written consent was obtained from study participants.

Results

Of the 300 participants, 181(60.3%) were females (male to female ratio was 0.6:1). The mean age of the participants was 28.95±9.4 (range: 6 months to 63 years). The majority, (53.0%) were adults aged 20-29 year of age. Children 60 years age were 1.3% (**Table 1**).

Of the 300 study participants tested for anti-HCV, 135(74 females, 61 males) were from VCT centre and 165(107 females, 58 males) were HIV positive cases from ART clinic in Mekelle hospital. Among the 174 HIV positive subjects, 16(9.2%) were positive for HCV antibody. The prevalence of HCV infection among HIV negative VCT centre attendants was 2/126(1.6%). The overall prevalence of anti-HCV antibody was 18(6.0%) {95%CI, 3.6-9.3}. The age specific prevalence was higher 1/8(12.5%) for the 50-59 year age groups. Males positive for anti-HCV were 5(4.2%) while females were 13(7.2%). HCV infection was higher in females than males (OR, 0.57; 95%CI, 0.20-1.63, P=0.33) (**Table 1**).

The overall prevalence of HIV/HCV co-infection in HIV positive patients was 16(9.2%). Of the 174 persons with HIV, 5/62(8.1%) were males and 11/112(9.8%) females. Among 126 HIV-negative subjects, 2(1.6%) females were positive for HCV-antibody. The proportion of HCV infection in HIV cases was increased nearly 6 fold compared to HIV negative individuals which was 9.2% and 1.6%, respectively (OR, 6.28; 95%CI, 1.42-27.82, P=0.006) (**Table 2**). The age specific pattern of HIV and HCV co-infection shows that the frequency of HCV infection was in similar trend to the frequency of HIV infection in each age group of the study subjects.
A relatively higher proportion of HCV infection was observed in respondents with history of multiple sex partners and using tattoo, 6/59(10.2%) and 3/35(8.6%), respectively. A total of 2(8.3%) respondents had history of admission to hospital, 1/17(5.9%) had history of dental procedure (**Table 3**).

Regarding the various occupational groups, 2(11.1%) of 18 farmers had positive HCV-antibody and 5/50(10.0%) of office workers had HCV prevalence, while daily laborers had 4/43(9.3%) prevalence (**Table 4**).

Discussion

Sero-prevalence of HCV among HIV positive individuals of 16(9.2%) is higher than HIV-negative of 2(1.6%). This result is higher than the 0.7% report in HIV-positive subjects by Tessema et al [13] and 1.7% by Gelaw and Mengistu [14]. Lower HIV/HCV co-infected rates

were reported from Gambia [15], Nigeria [16], South Africa [17] and Uganda [18], in 0.6%, 1.86%, 1.9% and 3.3%, respectively. In India, prevalence of HCV-antibody in HIV-positive patients showed 1.6% [19]. In Nigeria, another study showed 2.3% HCV- antibody co-infection in HIV-positive patients [20] while in Greece 7.5% [21]. In a similar study carried out in Iran, the co-infection rate of HCV in HIV-positive patients was 74% [22]. Findings of 8.2% in Nigeria [23] and 8.6% in Cameroon [24] were reported which were comparable with the present study. In contrast, the co-infection rate obtained in the present study 9.2% is lower than 11.6% reported by an earlier study in VCT and HIV follow up clinic [8]. This was lower than co-infection rate of 30-50% reported some industrialized countries, such as in North America and Europe [25,26], where intravenous drug use (IDU) is a major risk factor for both infections. The high co-infection rate in HIV-positive persons could demonstrate that patients may be exposed to HCV infection by sexual contact and it suggests co-transmission of both viruses [9].The frequency of HCV transmission to sexual partners is significantly higher when HIV virus is also transmitted. This would suggest that HIV could be co-factor for the sexual transmission of HCV infection [27]. Therefore, investigation of HCV in HIV-positive patients is vital in order to take care of them [28]. This finding agrees with other studies in where HCV infection was significantly higher in HIV infected cases than in HIV negative individuals 11.6% vs 2.6% [8]. In addition, Tessema *et al* [13] reported the presence of a significant association between HCV and HIV infections. The possible elucidation for this variation might be due to the rapid test positive samples in the present study was not confirmed by other tests.

The overall prevalence of HCV infection among HIV negative persons was 2/126(165%). This result is lower than the 5% rate observed among VCT attendants reported by Gebre [8]. Injection drug use is uncommon in the study area. Regarding occupational groups, 2/8(11.1%) of farmers were positive for HCV-antibody and office workers had HCV prevalence of 5/50(10.0%), while day laborers had 4/43(9.3%) prevalence. In another study, HCV-antibody distribution with respect to occupation was reported uniform [8]. Seroprevalence rates of HCV antibody was higher in individuals with multiple sex partners and in sex workers suggesting that sexual transmission may be possible.

This study has several limitations. Supplemental anti-HCV testing (i.e., RIBA) for all anti-HCV positive confirms the presence of anti-HCV. The presence of screening test negative result is common in immune-suppressed individuals (HIV infection) and during window period of the disease. Hence, these assays do not exclude the possibility of exposure to HCV virus for the individuals with negative results.

Conclusion

Co-infection rate of HIV/HCV in this study is high. Thus, diagnosing HCV in HIV-positive patients is vital in order to take care of them and allot resources in health planning. Preventive measures against HCV should target primarily on HIV infected people. Therefore, providing opportunity of screening for HCV infection in all HIV-infected patients is essential. Implementation of more effective public health education and counseling are essential to reduce the dangers of HIV/HCV co-infection.

Competing interests

The authors declare no conflict of interests.

Authors' contributions

HH conceived the study. HH, SG and AM initiated and designed the study. HH conducted the laboratory work, undertook statistical analysis and drafted the manuscript. SG and AM corrected the manuscript. All authors contributed to the writing of the manuscript and approved the submitted version of the manuscript.

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Table 4: HCV prevalence by occupation in attendants of VCT centre and HIV follow up clinics, Mekelle hospital

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Table 1: HCV prevalence by age and sex in attendants of VCT centre and HIV follow up clinics, Mekelle hospital

Age group (years)	HCV-Antibody positive No (%)		Total No (%)
	Males	Females	
0-9	0(0.0)	0(0.0)	0(0.0)
10-19	0(0.0)	1(25.0)	1(9.1)
20-29	2(3.9)	7(6.5)	9(5.7)
30-39	2(6.5)	3(5.5)	5(5.8)
40-49	0(0.0)	2(25.0)	2(8.7)
50-59	1(14.3)	0(0.0)	1(12.5)
>60	0(0.0)	-	0(0.0)
Total	**5(4.2)**	**13(7.2)**	**18(6.0)**

Table 2: Coinfection of HIV and HCVin attendants of VCT centre and HIV follow up clinics, Mekelle hospital					
HIV status	**HCV antibody**		**Total No (%)**	**OR, 95%CI**	**P value**
	Positive No (%)	**Negative No (%)**			
Positive	16(9.2)	158(90.8)	174	6.28(1.42-27.82)	0.006
Negative	2(1.6)	124(98.4)	126		
Total	18(6.0)	282(94.0)	300(100)		

Table 3: HCV prevalence by risk factors in attendants of VCT centre and HIV follow up clinics, Mekelle hospital					
Risk factors	**HCV-antibody, No (%)**			**OR (95% CI)**	**p value**
	Positive	**Negative**	**Total**		
Community acquired					
Tattooing	3(8.6)	32(91.4)	35(14.0)	1.56(0.43-5.69)	0.45
Blood letting	0(0.0)	8(100.0)	8(3.2)	-	1.00
Scarification	1(3.1)	31(96.9)	32(12.8)	0.48(0.06-3.70)	0.70
Hospital acquired					
Hospitalization	2(8.3)	22(91.7)	24(9.6)	1.48(0.32-6.84)	0.65
Blood transfusion	0(0.0)	6(100.0)	6(2.4)	-	1.00
Dental procedure	1(5.9)	16(94.1)	17(6.8)	0.98(1.22-7.82)	1.00
Minor surgery	0(0.0)	16(100.0)	16(6.4)	-	0.61
Behavioral acquired					
Multiple sex partners	6(10.2)	53(89.8)	59(23.6)	2.16(0.78-6.02)	0.14
STI/STD	0(0.0)	30(100.0)	30(12.0)	-	0.23
Abortion	0(0.0)	23(100.0)	23(9.2)	-	0.38
Total	13(5.2)	237(94.8)	250(100.0)		

Table 4: HCV prevalence by occupation in attendants of VCT centre and HIV follow up clinics, Mekelle hospital			
Occupational category	**HCV- antibody (%)**		**Total**
	Positive	**Negative**	
Health workers	0(0.0)	2(100.0)	2(0.7)
Sex workers	1(6.7)	14(93.3)	15(5.3)
Day laborers	4(9.3)	39(90.7)	43(15.4)
Office workers	5(10.0)	45(90.0)	50(17.9)
Handicrafts	0(0.0)	12(100.0)	12(4.3)
Farmers	2(11.1)	16(88.9)	18(6.4)
Merchants	0(0.0)	1(100.0)	1(0.4)
Housewives	3(3.6)	80(96.4)	83(29.6)
Drivers	0(0.0)	7(100.0)	7(2.5)
No job	2(4.1)	47(95.9)	49(17.5)
Total	**17(6.1)**	**263(93.9)**	**280(100.0)**

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HBV and HCV seroprevalence and their correlation with CD4 cells and liver enzymes among HIV positive individuals at University of Gondar Teaching Hospital, Northwest Ethiopia

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Abstract

Background: The co-existence of viral hepatitis caused by HBV and HCV become common causes of severe liver complication and immunological impairment among HIV infected individuals. The aim of this study was to assess the seroprevalence of HBV and HCV and their correlation with CD4 and liver enzyme levels among HAART naïve HIV positive individuals.

Method: A Cross-sectional study was conducted from March-May, 2011 at University of Gondar Teaching Hospital, Northwest Ethiopia. HBV and HCV serological tests and liver enzymes as well as CD4 T cell level determination were assessed following the standard procedures. Socio-demographic data was collected by using structured questionnaire. The data was entered and analyzed by using SPSS version 20.0 statistical software and $p < 0.05$ was considered as statistically significant.

Result: Among 400 study participants, the overall prevalence of HIV-viral hepatitis co-infection was 42(11.7%). The prevalence of HIV-HBV, HIV-HCV and HIV-HBV-HCV co-infections were 20(5.6%), 18(5.0%) and 4(1.1%) respectively. Study participants who had HIV-HBV, HIV-HCV and HIV-HBV-HCV co-infection have relatively raised mean liver enzyme levels (ALT, AST and ALP) than HIV mono-infected once. Individuals with HIV-HBV, HIV-HCV and HIV-HBV-HCV co-infection also had a lower mean CD4 levels than HIV mono-infected study participants. The mean CD4 value in males was lower than females.

Conclusion: The prevalence of HBV and HCV was higher than reports from general population of the country. Raised levels of liver enzymes and lowered mean CD4 counts were seen in HIV-HBV, HIV-HCV and HIV-HBV-HCV co-infections. These findings underscore the importance of screening all HIV positive individuals before initiating antiretroviral treatment.

Keywords: HBV, HCV, HIV, CD4 T cells, Liver enzymes

Introduction

Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) are the most common cause of chronic liver diseases worldwide. The infection continues to have a severe and invasive impact on the health of millions of people around the world and the infection is often asymptomatic [1-4]. Due to similar routes of viral transmission [5], co-infection of HIV with HBV and/or HCV is common. The co-infection pattern of these viruses showed that 10.0% of the HIV-infected population estimated to have chronic HBV infection and around a third estimated to have chronic HCV infection worldwide [2,5]. However, studies reported [6-9] that the rates of co-infection of HIV with either HCV or HBV vary from region to region, study population and risk factors for HIV acquisition. A systematic review in 18 sub Saharan African countries also showed that the prevalence of HBV and

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HCV in HIV-infected individuals ranged from 3.9-7.3% and 6.9%, respectively [10].

Despite the effective decline of the mortality and morbidity rate from HIV/AIDS as the result of highly active antiretroviral therapy (HAART), liver diseases due to chronic HBV and HCV infections become a leading cause of death. Although the direct impact of HCV upon HIV disease progression remains controversial in many reports [8,11-15], the complex interactions between HIV-HBV/HCV co-infection and HAART are increasingly apparent in HIV disease progression [16].

In HIV-HBV co-infections, HIV infection causes increased rates of persistent HBV infection, increased cirrhosis and liver-related mortality and increased risk of hepatocellular carcinoma at lower CD4T cell counts [17]. Similarly in HIV-HCV co-infections, there is a more rapid progress to cirrhosis, end-stage liver disease and hepatocellular carcinoma [18].

The impact of HBV and HCV could not limited in causing liver hepatotoxicity but also results in failure in immunological recovery in HIV positive patients. For example, a study in Tanzania reported slow rate of immunologic recovery after initiation of HAART treatment and higher risk of hepatotoxicity among HIV/HBV and HIV/HCV co-infected patients [19]. Thus the management of HBV and HCV in HIV infection is complicated and bring high burden in particular where HIV is rampant. As the result, globally HIV, HBV and HCV become the major public health concerns [20,21]. In some countries, screening of HIV-infected individuals for HBV and HCV is highly recommended before initiation of antiviral treatment [22].

In Ethiopia, the seroprevalence of HBV and HCV among HIV positive individuals is scarce except few reports among blood donors and infection prone groups [23-25]. In addition, there is no report about liver enzyme levels and CD4 count determination among HIV-HBV and/or HIV-HCV co-infected patients. Therefore, the aim of this study was to assess the seroprevalence of HBV and HCV and CD4 cells as well as liver enzyme levels among HIV positive individuals at University of Gondar Teaching Hospital, Northwest Ethiopia.

Materials and methods

Study design, area, and period

A cross-sectional study was conducted from March to May, 2011 at University of Gondar Teaching Hospital, which is found in Gondar town, Northwest Ethiopia.

Source population and study participants

The source populations were all HIV positive individuals who had access to be served at University of Gondar Teaching Hospital. The study participants were all HAART naïve HIV positive adult individuals who had visited ART clinics at Gondar University Hospital during the study period. A total of 403 study participants were enrolled by considering 95% confidence interval, 5% margin of error, 50% proportion (since there is no previous estimation of HBV and HCV among HIV infected individuals in the area) and 5% contingency. However, 2 (0.5%) of them had refused to participate and the other 1(0.2%) were excluded due to chronic alcoholism and 400(99.3%) HIV positive individuals were used for the final analysis.

Inclusion and exclusion criteria

All ART naïve adult HIV positive individuals who visited ART clinic for CD4 and liver enzyme level determinations for their pre-ART follow up during the study period were included. But individuals who were on ART follow up and those ART naïve but who refused to give informed consent were excluded from the study. Individuals who have been vaccinated against HBV and those who visited ART clinic and requested for laboratory investigation for the second and consequent follow up at the time of the study period were also excluded from the study to avoid duplication. Patients who had TB, malaria, leishmaniasis, opportunistic infections (OIs), drug induced hepatotoxicity and chronic alcoholism were assessed and excluded as per the HIV management guidelines of Ethiopia [26].

Data collection procedures

Socio-demographic information and other relevant possible risk factors of the study participants were collected using structured and pre tested questionnaire by trained nurses and physicians. Ten milliliter (10 ml) of veinous blood was aseptically collected using plain and EDTA vacutainer tube (5 ml in each tube) for the determination of HBV and HCV seroprevalence and CD4 and liver enzyme levels from each study participants. The blood specimen in the plain tube was centrifuged at 3000 RPM for 5 minutes to separate the serum and used for determination of liver enzyme levels within one hour of separation. The remaining serum kept in deep refrigerator (−40°C) until detection of HBV and HCV. The second tube that contains whole blood was used for the CD4 levels determination.

The collected sera were checked for the presence of HBsAg using TULIP'S INSTANT (TULIP DIAGNOSTICS (P) LTD. 88/89, Phase II C, Verna Ind. Est., Verna, Goa-403 722, INDIA) which have sensitivity and specificity of 100%. Anti-HCV antibody was detected using Flavicheck-HCV WB (TULIP DIAGNOSTICS (P) LTD. 88/89, Phase II C, Verna Ind. Est., Verna, Goa-403 722, INDIA) following the manufacturer's instructions. The catalytic activities of the liver enzyme levels were analyzed by using clinical chemistry analyzer (Humastar 80)

and the CD4 count was done by BD FACS count flow cytometry machine.

Quality control

The standard operational procedures were strictly followed for the quality control issues. Both hepatitis B and C kits were checked by using known HBsAg and anti-HCV antibody positive and negative control samples. Similarly the quality of both CD4 and liver enzyme reagents were regularly monitored by running control materials in each morning before the actual work was done.

Data analysis

The data was entered and analyzed using SPSS Version 20.0 statistical software and the differences in proportions was evaluated by Pearson's Chi-square test (x^2 test) and P-value of less than 0.05 was considered as statistical significant. Mean plus standard deviation with 95% confidence interval (CI) was also used for continuous variables and the difference in means was compared with independent-sample t-test.

Ethical consideration

The study was conducted after obtaining ethical clearance from ethical committee of Department of Medical Microbiology, Immunology and Parasitology, University of Gondar (University of Gondar, College of Medicine and Health Sciences, Ref. No: V/D06/07/2011). Informed consent was also obtained from each study participants.

Result

Socio-demographic characteristics

Among 400 study participants, 122(30.5%) were males (mean age: 37 ± 9 years) and 278(69.5%) were females (mean age: 32 ±9 years) with male to female ratio of 0.4:1. The lowest and the highest age of the study participants were 18 and 70 years respectively. The median age of the study participants was 32 years. Majority 167 (41.8%) of the study participants were in the age group of 30–39 years old. Among the study groups, 151 (37.7%) were illiterate (Table 1).

Table 1 Socio-demographic characteristics of HIV positive study participants at University of Gondar Teaching Hospital, Northwest Ethiopia, 2011

Characteristics	Male N (%)	Female N (%)	Total N (%)
Age group			
18-29	21(16.4)	107(83.6)	128(32.0)
30-39	50(29.9)	117(70.1)	167(41.8)
40-49	40(51.3)	38(48.7)	78(19.5)
≥50	11(40.7)	16(59.3)	27(6.8)
Marital status			
Single	27(36.0)	48(64.0)	75(18.8)
Married	76(38.0)	124(62.0)	200(50.0)
Divorced	17(20.7)	65(79.3)	82(20.5)
Widowed	2(4.7)	41(95.3)	43(10.7)
Religion			
Christian	111(30.2)	256(69.8)	367(91.8)
Muslim	11(33.3)	22(66.7)	33(8.2)
Education			
Illiterate	34(22.5)	117(77.5)	151(37.7)
Elementary	31(32.3)	65(67.7)	96(24.0)
High school	37(33.3)	74(66.7)	111(27.8)
Certificate and above	20(47.6)	22(52.4)	42(10.5)
Residence			
Urban	103(30.7)	232(69.3)	335(83.7)
Rural	19(29.2)	46(70.8)	65(16.3)
Occupation			
Civil servant	33(56.9)	25(43.1)	58(14.5)
Merchant	18(42.9)	24(57.1)	42(10.5)
Daily laborer	21(52.5)	39(97.5)	60(10.0)
Farmer	15(60.0)	10(40.0)	25(6.3)
House wife		114(100)	114(28.5)
Student	3(42.9)	4(57.1)	7(1.8)
Driver	8(80.0)	2(20.0)	10(2.5)
Commercial sex worker		5(100.0)	5(1.3)
No work	7(14.6)	41(85.4)	48(12.0)
Other	17(54.8)	16(51.6)	31(7.7)

Seroprevalence of HBVand HCV

The overall prevalence of viral hepatitis (HBV and HCV) was 42(11.7%). The seroprevalence of HBV and HCV were 20(5.6%) and 18(5.0%), respectively. Only 4(1.1%) of HIV positive individuals showed triple (HIV-HBV-HCV) infections.

HIV-HBV and HIV-HCV co-infection and sociodemographic characteristics

Significantly higher prevalence of HIV-HBV co-infection was observed in males 11(9.4%) compared to females 9 (3.4%) ($X^2 = 5.714$, P = 0.017). Although statistically non-significant, higher 6(8.3%) ($X^2 = 3.083$, P = 0.379) prevalence of HIV-HBV co-infection was observed in the age group between 40–49 years. Individuals who were widowed 4(9.8%) ($X^2 = 3.681$, P = 0.298) and those who had better educational status (certificate, diploma and above) 4(10.3%) ($X^2 = 2.602$, P = 0.457) showed non-significantly higher HIV-HBV positive rate. The prevalence of HIV-HBV co-infection in urban and rural residences were 17(5.3%) and 3 (5.0%) respectively. Non-statistically significant higher prevalence of HIV-HCV

co-infection was also observed in females 15(5.6%), in the age group of 40–49 years 5(7.0%), in rural residence 4(6.6%), in married 12(6.3%) and in the housewives 8 (7.2%). The co-existence of both HBV and HCV in males and females were 2(1.8%) and 2(0.8%) ($X^2 = 0.786$, P = 0.375) respectively (Table 2).

Liver enzyme levels and mean CD4 count in HIV/HBV, HIV/HCV and HBV/HCV/HIV co-infection

The mean serum levels; ALT, AST, and ALP in HIV mono-infected study participants were 25 international units (IU), 27 IU and 243 IU respectively. However, in HIV-HBV co-infected study participants, the levels of

Table 2 Sociodemographic characteristics and their association with HIV-HBV and HIV-HCV co-infections at University of Gondar Teaching Hospital, Northwest Ethiopia, 2011

Variables	HIV-HBV			HIV- HCV			HIV-HBV-HCV		
	Negative N (%)	Positive N (%)	Sign	Negative N (%)	Positive N (%)	Sign	Negative N (%)	Positive N (%)	Sign
Age									
18-29	118(95.9)	5(4.1)	$X^2 = 3.083$	118(96.7)	4(3.3)	$X^2 = 1.461$	118(99.2)	1(0.8)	$X^2 = 12.169$
30-39	150(94.3)	9(5.7)	P = 0.379	150(94.9)	8(5.1)	P = 0.691	150(100.0)	0(0.0)	P = 0.007
40-49	66(91.7)	6(8.3)		66(93.0)	5(7.0)		66(98.5)	1(1.5)	
≥50	24(100)	0(0)		24(96.0)	1(4.0)		24(92.3)	2(7.7)	
Sex									
Male	106 (90.6)	11(9.4)	$X^2 = 5.714$	106(97.2)	3(2.8)	$X^2 = 1.395$	106(98.2)	2(1.8)	$X^2 = 0.786$
Female	252(96.6)	9(3.4)	P = 0.017	252(94.4)	15(5.6)	P = 0.238	252(99.2)	2(0.8)	P = 0.375
Residence									
Urban	301(94.7)	17(5.3)	$X^2 = 0.12$	301(95.6)	14(4.4)	$X^2 = 0.501$	301(99.0)	3(1.0)	$X^2 = 0.242$
Rural	57(95.0)	3(5.0)	P = 0.913	57(93.4)	4(6.6)	P = 0.479	57(98.3)	1(1.7)	P = 0.623
Marital status									
Single	72(96.0)	3(4.0)	$X^2 = 3.681$	72(100.0)	0(0.0)	$X^2 = 4.653$	72(100.0)	0(0.0)	$X^2 = 1.777$
Married	178(96.2)	7(3.8)	P = 0.298	178(93.7)	12(6.3)	P = 0.199	178(98.3)	3(1.7)	P = 0.620
Divorced	71(92.2)	6(7.8)		71(94.7)	4(5.3)		71(98.6)	1(1.4)	
Widowed	37(90.2)	4(9.8)		37(94.9)	2(5.1)		37(100.0)	0(0.0)	
Religion									
Christian	327(94.5)	19(5.5)	$X^2 = 0.327$	327(94.8)	18(5.2)	$X^2 = 1.699$	327(99.1)	3(0.9)	$X^2 = 1.311$
Muslim	31(96.9)	1(3.1)	P = 0.567	31(100.0)	0(0.0)	P = 0.192	31(96.9)	1(3.1)	P = 0.252
Education									
Illiterate	133(94.3)	8(5.7)	$X^2 = 2.602$	133(95.0)	7(5.0)	$X^2 = 0.672$	133(97.8)	3(2.2)	$X^2 = 4.553$
Elementary	89(95.7)	4(4.3)	P = 0.457	89(96.7)	3(3.3)	P = 0.880	89(100.0)	0(0.0)	P = 0.208
High school	101(96.2)	4(3.8)		101(94.4)	6(5.6)		101(100.0)	0(0.0)	
Certificate & above	35(89.7)	4(10.3)		35(94.6)	2(5.4)		35(97.2)	1(2.8)	
Occupation									
Civil servant	49(89.1)	6(10.9)	$X^2 = 12.352$	49(96.1)	2(3.9)	$X^2 = 4.427$	49(98.0)	1(2.0)	$X^2 = 5.695$
Merchant	37(90.2)	4(9.8)	P = 0.194	37(97.4)	1(2.6)	P = 0.881	37(100.0)	0(0.0)	P = 0.770
Daily laborer	54(91.5)	5(8.5)		54(98.2)	1(1.8)		54(100.0)	0(0.0)	
Farmer	22(95.7)	1(4.3)		22(95.7)	1(4.3)		22(95.7)	1(4.3)	
House wife	103(99.0)	1(1.0)		103(92.8)	8(7.2)		103(99.00)	1(1.0)	
Student	7(100.0)	0(0.0)		7(100.0)	0(0.0)		7(100.0)	0(0.0)	
Driver	9(90.0)	1(10.0)		9(100.0)	0(0.0)		9(100.0)	0(0.0)	
Com. sex worker	4(100.0)	0(0.0)		4(100.0)	0(0.0)		4(100.0)	0(0.0)	
No work	44(97.8)	1(2.2)		44(93.6)	3(6.4)		44(100.0)	0(0.0)	
Other	29(96.7)	1(3.3)		29(93.5)	2(6.5)		22(95.7)	1(4.3)	

ALT, AST and ALP were non-significantly raised (29 IU, 31 IU and 262 IU, respectively). Similarly, in HIV-HCV co-infected study participants, the mean levels of ALT, AST and ALP were 27 IU, 32 IU, and 290 IU respectively. Statistically non-significant raised mean serum ALT, AST and ALP were found in HIV-HBV-HCV triple infected study participants (Table 3).

The mean CD4 count of HIV mono-infection was 288cells/mm^3. However, in HIV-HBV, HIV-HCV and HIV-HBV-HCV co-infections, the mean CD4 count were 250 cells/mm^3, 274 cells/mm^3 and 125 cells/mm^3 respectively. However, the difference was not statistically significant (Table 3). The CD4 value in females was higher than males (299 ± 197 vs 249 ± 152). Males study participants who had both HBV and HCV have the lowest mean CD4 count. The highest and lowest mean CD4 values were observed in the age groups of 18–29 and ≥50 years respectively (Table 4).

Discussion

This study investigated the seroprevalence of HBV and HCV among HIV positive study participants and tried to assess levels of liver enzymes and CD4 count for HIV mono infected, HIV-HBV and HIV-HCV co-infected and HIV-HBV-HCV triple infected individuals. The overall prevalence (11.7%) of hepatitis (both HBV and HCV) among the study participants was very high. In this study, HIV-HBV co-infection rate was 5.6% which is more or less comparable with 7.1% prevalence [24] among blood donors in the same hospital. However, the present prevalence was lower as compared to studies reported in Nigeria (9.2%) [27], Ethiopia (10.9%) [23] and Malawi (20.4%) [28]. In the present study, the prevalence of HIV-HBV co-infection was higher in males than females (9.4%% vs 3.4%) which are in line with some other reports [29-31]. Generally, as several studies reported and anticipated in different parts of the world, such co-infection differences could be due to differences in geographic regions, types of risk groups and the means of exposures involved [26,27,32-35].

The seroprevalence rates of HIV-HCV co-infection in this study was 5.0% which is almost comparable with the studies which were done in Nigeria (5.8%), Malawi (5.0%), Burkina Faso (4.8%) and Senegal (8.0%) [27,28,36,37]. However, the epidemiological survey of HCV in Ethiopia showed variation from 2-3% in the general population in early 1990s [38-40] and recently, the co-infection rates of HIV-HCV ranges from 3.6-13.3% in different reports [24,25,41-45]. The reasons for the HCV variation both in HIV infected individuals and the general population could share the factors responsible HBV prevalence variations discussed above.

The seroprevalence of HIV-HBV-HCV triple infection in this study was 1.1%, which is more or less comparable to reports from Senegal (0.5%), Kenya (0.26%), Nigeria (1.5%) and Egypt (0.44%) [37,46,47]. However, higher prevalence of HCV-HBV-HIV triple co-infection was reported in Argentina (9.5%) and Iran (9.2%) [48,49]. For such variations, risk factors which accounts for HBV and HIV prevalence difference might work for the triple infection.

Despite absence of statistical significance difference in the mean levels of the liver enzymes between HIV-mono-infected and HIV-viral hepatitis co-infected individuals, raised ALT, AST and ALP were found in HIV-HBV, HIV-HCV and HIV-HBV-HCV co-infected individuals. However, in a study which was conducted in South Africa, 70% of HIV-HBV and HIV-HCV co-infected study participants had significantly elevated AST and ALT, 56% of them had elevated ALP [35]. Similarly, significantly raised ALT was found in 14% of HIV/HBV co-infections and 20% in HIV-HCV co-infected patients in India [50]. These liver enzyme levels difference between different studies may be due to difference in study design, duration of the viral

Table 3 Mean CD4 and liver enzyme levels and their association with HBV and HCV co-infection at University of Gondar Teaching Hospital, Northwest Ethiopia, 2011

Immunological and liver biomarkers	HIV alone	HIV/HBV	P-value	HIV alone	HIV/HCV	P-value	HIV alone	HIV/HBV/HCV	P-value
CD4 mean ± SD	288 ± 190	250	0.375	288 ± 190	274 ± 138	0.754	288 ± 190	125 ± 96	0.087
Normal value	500-1300	500-1300		500-1300	500-1300		500-1300	500-1300	
ALT mean ± SD	25 ± 21	29 ± 18	0.356	25 ± 21	27 ± 17	0.472	25 ± 21	39 ± 6	0.170
Normal value	0-37	0-37		0-37	0-37		0-37	0-37	
Abnormal High (%)	12.3%	20%		12.3%	22.2%		12.3%	50%	
AST mean ± SD	27 ± 19	31 ± 18	0.419	27 ± 19	32 ± 17	0.339	27 ± 19	33 ± 15	0.587
Normal value	0-34	0-34		0-34	0-34		0-34	0-34	
Abnormal High (%)	20.1%	25%		20.1%	27.8%		20.1%	50%	
ALP mean ± SD	243 ± 130	262 ± 118	0.515	243 ± 130	290 ± 127	0.135	243 ± 130	332 ± 228	0.176
Normal value	72-306	72-306		72-306	72-306		72-306	72-306	
Abnormal High (%)	19.6%	15%		19.6%	33.3%		19.6%	50%	

Table 4 Mean CD4 values in relation to gender and different age categories at University of Gondar Teaching Hospital, Northwest Ethiopia, 2011

Variables	Mean CD4 count				
	HIV alone	HIV-HBV	HIV-HCV	HIV-HBV-HCV	Over all
Gender					
Male	256 ±155	197 ± 113	334 ± 133	75 ± 30	249 ± 152
Female	302 ± 201	314 ± 188	262 ± 140	176 ± 129	299 ± 197
Age categories					
18-29	338 ± 223	383 ± 201	328 + 151	84 ± 0	337 ± 219
30-39	261 ± 151	169 ± 108	241 + 128		255 ± 149
40-49	292 ± 203	260 ± 121	286 + 173	53 ± 0	286 ± 196
≥50	203 ± 135		260 +0	182 ± 121	204 ± 129

hepatitis infection as well as the patient's condition like having chronic alcoholism or other drug induced hepatotoxicity. In addition, HIV can also infect the hepatic or kupffer cells [51] that may farther contributes for the development of liver fibrosis and raised liver enzyme levels. However, the magnitude of the complication of the liver may be worse if the HIV positive patients co-infected with HBV and HCV as indicated in the above.

In the present study, there is no statistically significant CD4 count mean difference between HIV mono-infected, HIV-HBV and HIV- HCV co-infected study participants. However, study participants who had HIV-HBV co-infection in this study have the mean CD4 count (250 cells/mm^3) which was incomparable with mean CD4 count of 141.6 cells/mm^3 and 121 cells/mm^3 in the studies which were conducted in South Africa and Nigeria respectively [35,46]. These controversial results may be due to the differences in the immune status of the individual who have been participated in the study or it may be due to the viral hepatitis. In individuals who have both HIV and HBV infections, there may be high HIV and HBV viral replication that may farther contribute for the impairment of the immune system of the patients.

In this study, the mean CD4 count (274 cells/mm^3) was found in HIV-HCV co-infected study participants which is comparable with a mean CD4 count of 260 cells/mm^3 and 288.6 cells/mm^3 that were reported in Nigerian and Indian studies respectively [46,50]. Such high values of mean CD4 count in HIV-HCV co-infected study participants than study participants who had HIV-HBV co-infections were unclear. However, HIV-HCV co-infected study participants have relatively lower mean CD4 values than HIV mono-infected study participants. This low CD4 count in HIV-HCV co-infected may be associated with an increased HIV and HCV replication, reflecting the immunosuppressed state.

Among HIV-HBV-HCV infected individuals, the mean CD4 count was 125cells/mm^3. This CD4 levels is comparable with the mean CD4 count of 116/mm^3 which was reported in India [50]. Similarly in a study which was done in Nigeria, HIV-HBV-HCV infected individuals had the mean CD4 count of 106 cells/mm^3 [46]. However, all of these results showed the mean CD4 count of less than 200 cells/mm^3. This could be due to the fact that the presence of both HBV and HCV in HIV positive individuals may highly contribute for the impairment of the immune system of an individual that may also further lead the person for the development of advanced HIV diseases. In addition, there is also inverse relationship between CD4 values and HIV diseases progression. However, the impact of viral hepatitis on the immune system and liver enzymes needs farther studies in both on HAART and HAART naïve HIV positive patients.

In the present study, there was also difference on the mean CD4 values in relation to gender. The mean CD4 value in the males was lower than females (249 cells/mm^3 vs 299 cells/mm^3). Similar findings were reported in studies which have been conducted in Nigeria [52] and Uganda [53]. Male study participants who had both HBV and HCV had also the lowest mean CD4 values. This lower CD4 count in males may be associated with their daily activities. In addition, males are mostly more muscular and may not be ready to accept their HIV, HBV and/or HCV results and they may develop mental stress that farther contribute for the impairment of their immune system or lower CD4 count. Farther more, males may spend most of their time with hard works for a long period of time and this may contribute for lower CD4 count. We have also analyzed the mean CD4 values in different age categories and the lowest mean CD4 values was observed in the age groups of 50 years and above. As age increases, there may be impairment of the immune system of the individuals specially HIV positive patients and older age groups may have severed HIV diseases progression than younger once.

In conclusion, the prevalence of viral hepatitis (HBV, HCV) among HIV positive individuals was higher than

the prevalence of the respective viruses in the general population. Thus screening of HBV and HCV before initiation of antiretroviral treatment is mandatory for strict monitoring and a regular evaluation of liver enzyme levels and CD4 status in order to minimize the complication of the liver and for effective HIV treatment.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
YW: Participated in conceived, designed and proposed the research idea, data collection, data entry, clearance, analysis and interpretation of the findings and drafting the manuscript and write up. MA: involved in data entry, clearance, analysis, and interpretation of the findings. FA: involved in data entry, clearance, analysis, and interpretation of the findings. YB: Participated in conceived, designed and proposed the research idea, data collection, data entry, clearance, analysis and interpretation of the findings. All authors involved in reviewing the manuscript and approval for publication.

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HIV CO-INFECTION AND VIRAL HEPATITIS

P42

Assessment of triple infections of HIV, TB and hepatitis (B and C); and associated risk factors in selected district of North Wollo Zone, Ethiopia

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Coinfection of tuberculosis and hepatitis is a major concern for the treatment of HIV/AIDS patients worldwide. In addition to tuberculosis, hepatitis is becoming a serious public problem in Ethiopia among HIV/AIDS patients. Hence, accurate information on the triple infection is being needed to tackle the problem, undertake the integrated prevention and control program and in order to base therapeutic decisions. The aim of this study was to assess the prevalence of triple infection of HIV, TB and Hepatitis (B&C) in TB patients who were following their treatment in the year 20012/13 in selected District of North Wollo Zone, Amhara Regional State, Ethiopia. A cross sectional study was conducted to assess the HIV serostatus by Rapid HIV testing kits; while the hepatitis serostatus was determined by Instant One step HBsAg kits (for HBV) and Flavicheck-HCV WB kits (for HCV). A total of 374 TB patients participated and 49 (13%) of them were positive for HIV. Among TB/HIV coinfected patients 16 (32.65%) were positive for hepatitis (11 for HBV and five for HCV). Demographic and socioeconomic factors including having multiple sexual partners, urban residents, history of STI (discharge & ulcer), intermittent condom user, partners living separated, individuals whose partner is died & divorced contributed to the highest percentage of seropositivity. The present study result confirmed the existence of triple infections in the study area. Hence, it underlines the need for integrated plan for treatment, prevention and control program of these infections.

TRANSPLANTATION AND VIRAL HEPATITIS

P43

Is recipient interleukin-28B (rs12979860) polymorphism associated with clinically recurrent HCV after living donor liver transplantation?

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BACKGROUND: Interleukin 28B (rs12979860) polymorphism (CC) is associated with better response of chronic HCV to treatment and less complication. HCV induced liver cirrhosis is one of the main indications of liver transplantation. Virological recurrence is universal, however only a group of patients may have clinical recurrence (elevated ALT, HCV RNA PCR positive, and histopathological inflammation). The aim of the work was to evaluate the relationship between IL-28B polymorphism (rs12979860) and clinical recurrence of HCV in recipients after living donor liver transplantation.

MATERIALS AND METHODS: Serum of 50 patients who underwent living donor liver transplantation due to HCV liver cirrhosis with or without HCC was tested for IL-28B (rs12979860) polymorphism. Demographic, laboratory, and histopathological data of patients were collected. Follow-up until 6 months after liver transplantation was done. Clinical recurrence of HCV was considered if there is unexplained elevated liver function tests associated with HCV RNA positive and confirmed by inflammatory activity in liver biopsy. Predictors of clinical recurrence were identified by univariate and multivariate analysis.

RESULTS: Out of the 50 patients, 10 (20%) had CC allele, 9 (18%) had TT, and 31 (62%) had CT IL-28B polymorphism. Clinical recurrence of HCV was found in 16 patients (32%). Clinical recurrence was associated with donor age ($p = 0.04$), platelet count ($p = 0.02$), HCV RNA viral load pre and post transplant ($p = 0.001$), post transplant Hb, ALT, AST ($p = 0.05$, =0.001 and =0.001 respectively) and presence of T allele ($p = 0.04$). Clinical recurrence was found in 2/10 patients with CC (20%), and 8/21 with CT (38.1%) and 6/9 with TT (66.6%). The only independent predictor for clinical HCV recurrence in logistic regression analysis is HCV RNA viral load.

CONCLUSIONS: A substantial number of LDLT due to HCV has clinical recurrence. Clinical recurrence is more significantly in patients who have T allele for IL-28B polymorphism. The independent predictor for clinical recurrence is pretransplant viral load which may suggest treatment of HCV patients on liver transplant list especially those with TT IL-28B polymorphism.

P44

Slow response to antiviral therapy in liver transplant recipients

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BACKGROUND: Despite new antiviral agents are under consideration now for hepatitis C treatment in liver transplant recipients (LTR), peginterferon (PEGIFN) remains the backbone of most antiviral regimes.

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RESEARCH ARTICLE Open Access

Impact of hepatitis C virus co-infection on HIV patients before and after highly active antiretroviral therapy: an immunological and clinical chemistry observation, Addis Ababa, Ethiopia

Solomon Taye[1,2*] and Mekuria Lakew[2]

Abstract

Background: Hepatitis C virus (HCV) is an RNA virus which has been known to cause acute and chronic necro-inflammatory disease of the liver. It is the leading cause of end-stage liver disease and hepatocellular carcinoma. HIV is known to have a negative impact on the natural disease outcome and immune response of HCV infection, whereas the reverse remains unclear. We evaluated the impact of HCV co-infection on recovery of $CD4^+$ and $CD8^+$ T-cells and liver enzyme levels before and after initiation of highly active antiretroviral therapy (HAART) in HIV/HCV co-infected patients.

Methods: A hospital-based, observational, prospective cohort study design was used for this study. Pre-antiretroviral treatment (Pre-ART) and under HAART HIV mono-infected and HCV/HIV co-infected individuals who are under regular follow-up were recruited for this study. 387 blood samples were collected from volunteer, known HIV positive Ethiopian patients and screened for HCV. Twenty five HCV/HIV co-infected patients were prospectively followed for four years. $CD4^+$ and $CD8^+$ T-cells and liver enzyme levels were determined annually for each of the participant.

Results: The prevalence of HCV/HIV co-infection in this study was 6.5%. Both HCV/HIV co-infected and HIV mono-infected under HAART groups showed $CD4^+$ recovery (343 Vs 426; $P < 0.004$, OR = 4.97, 95% CI = 2.41 to 10.27) respectively; but, the recovery rate was higher in mono-infected (80 Vs 426) than co-infected group (148 Vs 343). The recovery and/or decline pattern of $CD8^+$ T-cells was the same with that of $CD4^+$. In 75% of co-infected groups, the mean alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were above the upper limit of normal reference range. Analyses restricted to individuals who initiated HAART and pre-ART showed similar results.

Conclusion: We found that $CD4^+$ T-cell recovery was negatively affected by the presence of ongoing HCV replication in under HAART co-infected individuals and fast decline of $CD4^+$ T-cells in pre-ART patients. It was also associated with increased ALT and AST enzyme levels in both HAART initiated and treatment naïve co-infected patients.

Keywords: Immunological, HCV/HIV co-infection, Pre-ART, HAART, $CD4^+$, $CD8^+$, GOT, GPT, Alkaline phosphatase

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Background

Hepatitis C virus (HCV) is a RNA virus which has been known to cause acute and chronic necroinflamatory disease of the liver. It infects more than 170 million people worldwide. In Western countries, HCV is the leading cause of end-stage liver disease and hepatocellular carcinoma, as well as the main indication for liver transplantation [1,2]. Because of shared routes of transmission, co-infection with HCV and HIV is quite common. In the era of HAART, HCV-related liver disease has emerged as a significant cause of morbidity and mortality due to the increased risk for hepatotoxicity of HAART and likelihood of onset of an AIDS-defining illness [3].

Both innate and Cell-mediated immune responses are crucial in the early control of viral infections. Although the role of T-cell immunity during acute and chronic HCV infection and its relationship with HCV replication remains controversial, $CD4^+$ T-cell responses particularly to non-structural HCV proteins, specific $CD8^+$ T cell cytolytic action, and high level local gamma-interferon production are believed to be important [4]. It has been suggested that the deficiency of cell-mediated immune response against HIV infection actually favors the chronic development of acute HCV infection and also the progression of chronic hepatitis to cirrhosis [5]. HCV/ HIV co-infection often associated with elevated liver biochemical enzymes such as alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase [6].

Although the co-infection of HIV with HCV has been recognized worldwide in individuals exposed to blood borne diseases, limited data are available on the extent of co-infection, effect of these viruses on the immune system and liver in developing countries. Ethiopia belongs to the group of countries which are highly endemic for viral hepatitis. Few studies have been done on HIV/HCV co-infection prevalence in Ethiopia but the knowledge about the interrelationship between these viruses and their effect on the immune system remains unclear [7].

Therefore, the aim of this study was to estimate the prevalence of HCV sero-positivity in a cohort of people living with HIV in Addis Ababa and to investigate its effect on the recovery of $CD4^+$ and $CD8^+$ T-lymphocytes and liver enzymes in the era of HAART and before HAART in Ethiopia.

Methods

Study design, population and sampling technique

A hospital-based, observational, prospective cohort study design was used for this study. The study population was comprised of HIV patients on follow-up and VCT attendants at Yekatit-12 and Zenbaba General Hospitals in September 2006, Addis Ababa, Ethiopia. From September 1–30, 2006, a total of 387 HIV positive patients were screened for HCV and HBV. Hence, a convenient, non-probability sampling technique was employed and no scientific method were used to calculate the sample size, instead, we screened all eligible patients who visited the two hospitals during the month September 2006 and we followed volunteer 25 HCV/HIV co-infected patients. The control groups (HCV negatives) were also screened for HBV infection.

All those 25 HCV/HIV co-infected and 25 HIV mono-infected patients were enrolled in this hospital-based observational follow-up study. All selected patients were paired on the basis of world health organization (WHO) clinical disease stages (All were WHO stage II patients). The study groups were prospectively followed for four years from Sep 2006 to Nov 2010 in order to determine the impact of HCV/HIV co-infection on immunological and liver enzyme levels of HIV patients.

Patient grouping

Selected study cohort participants were arranged in to four groups based on their HCV and HAART (status: I) 16 under HAART patients only with HIV infection (Group 1), II) 16 HCV/HIV co-infected patients receiving HAART (Group 2), III) 9 HIV positive pre-ART patients without HCV infection (Group 3) and IV) 9 HIV/HCV co-infected pre-ART patients (Group 4). Both co-infected and HIV mono-infected under HAART patients has been taking the same combination therapy (Zidovudine (ZDV) + Lamivudine (3TC) + Efavirenz) and there were no considerable difference on the duration of treatment initiation between co-infected and HIV mono-infected groups (8 and 7 months) respectively. Annual immunological and clinical chemistry tests were done only for selected patient. The study subjects were also screened for HCV and HBV each year.

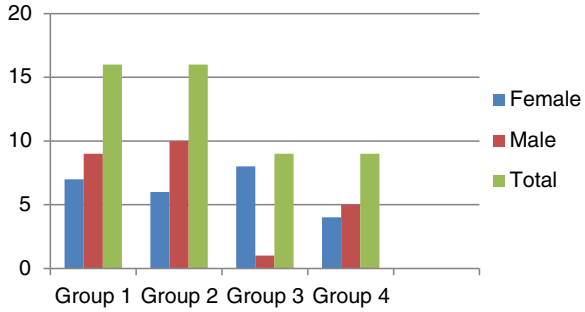

Figure 1 Sex distribution of study subjects in each group at Yekatit-12 and Zenbaba General Hospitals, Addis Ababa (Sep. 2006- Nov. 2010).

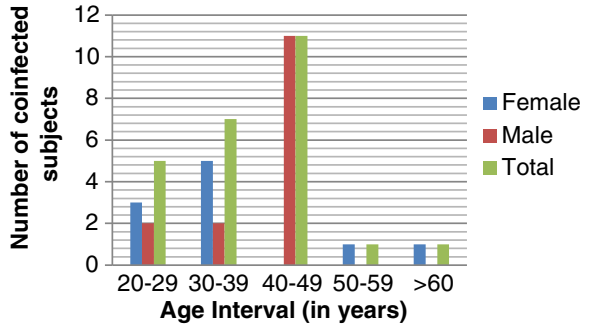

Figure 2 Sex distribution of HCV/HIV co-infected study subjects with age interval at Yekatit-12 and Zenbaba General Hospitals, Addis Ababa (Sep. 2006- Nov. 2010).

HCV and HBV screening

Flavicheck-HCV, a commercial fourth generation, rapid, qualitative, two-site sandwich immunoassay test device (Qualpro Diagnostics, 88/89, phase IIC, Verma Industrial Estate, Verna, Goa-403 722, India, 2004) was employed according to the manufacturer's instructions. It detects total antibodies specific to HCV in serum or plasma. It uses a multi-epitope recombinant peptide antigen that is broadly cross-reactive to all major HCV genotypes. Except the recombinant peptide inserted the principle, procedure and interpretation of HBV are the same with that of HCV.

$CD4^+$ and $CD8^+$ T-cells Enumeration

$CD4^+$ and $CD8^+$ T-cell counts were enumerated for each patient annually for four consecutive years starting from Sep 2006 through Nov 2010. Both tests were measured by standard 3-color flow cytometry using Fluorescent Activated Cell Sorter (FACscan) machine (Becton Dickinson Biosciences, San Jose, CA 95131–1807, USA). No viral load test was performed for all groups of patients because of scarcity of resources.

Measurement of GPT and GOT

Humastar180, chemistry analyzer (Human GmbH.65205 Wiesbaden, Germany) was used to measure the liver enzyme levels (serum GPT, GOT and ALP). Principle of operation is based on the fact that substances of clinical interest selectively absorb or emit energy (light) at different wavelengths.

Statistical analysis

Data entry and analysis was done using computer software SPSS version 16. Data was summarized and presented in a descriptive measure such as a table, figures and line graphs. Group comparisons were done using logistic regression, hazard ratio (HR) and odds ratio (OR) to determine the independent effect of the variables by calculating the strength of the association between the infection and risks. Line graph was done to show the trends of $CD4^+$ and $CD8^+$ T cells counts and liver enzyme levels along the four years follow up. Kaplan-Meier survival analysis done to predict the survival time of HCV/HIV co-infected patients from diagnosis of AIDS to termination of the study. P-value of less than 0.05 was considered statistically significant.

The study protocol was approved by Addis Ababa University, biology department research ethics committee. All participants gave informed and written consent, and HCV-positive cases were contacted with nurses and doctors for further management.

Result

Prevalence of HCV/HIV among the study subjects

The total prevalence of HCV among the 387 HIV patients (182 female and 205 male) who visited the two hospitals in the month September 2006 and were screened for the study was 6.5%. Of these, 206 (53.23%) were under HAART and 181 (46.77%) pre-ART patients. Relatively more HCV infected patients, (7.8%), were from those under HAART and (5%) from pre-ART. The mean age for the patients under investigation was 38.9 years. Even though it was not statistically significant ($p = 0.06$), females were relatively younger (34.5 years) when compared to males (42.6 years). The mean age of patients in the four groups (1–4) was comparable; 41.2, 41.3, 46.2 and 40.2 years respectively, indicating there were no a statistical age differences across the groups ($p = 0.09$). There were also no statistical difference

Table 1 Mean baseline and fourth year $CD4^+$ and $CD8^+$ cell count of under HAART subjects

Cells/μl	Year	HIV/HCV co-infected (G2) (n = 16) Mean ± SD	HIV mono-infected (G1) (n = 16) Mean ± SD	p value
$CD4^+$	1^{st} year	148 ± 30	80 ± 151	*P < 0.002*
	4^{th} year	343 ± 119	426 ± 113	*P < 0.004*
$CD8^+$	1^{st} year	1104 ± 420	950 ± 631	*P < 0.003*
	4^{th} year	1259 ± 430	1247 ± 420	*N/S*

(Number of patients in parenthesis), N/S- not significant.
Key: G = Group, HAART = highly active antiretroviral therapy, ART = Antiretroviral treatment, μl = microliter, CD = Cluster designation/differentiation, SD = standard deviation.

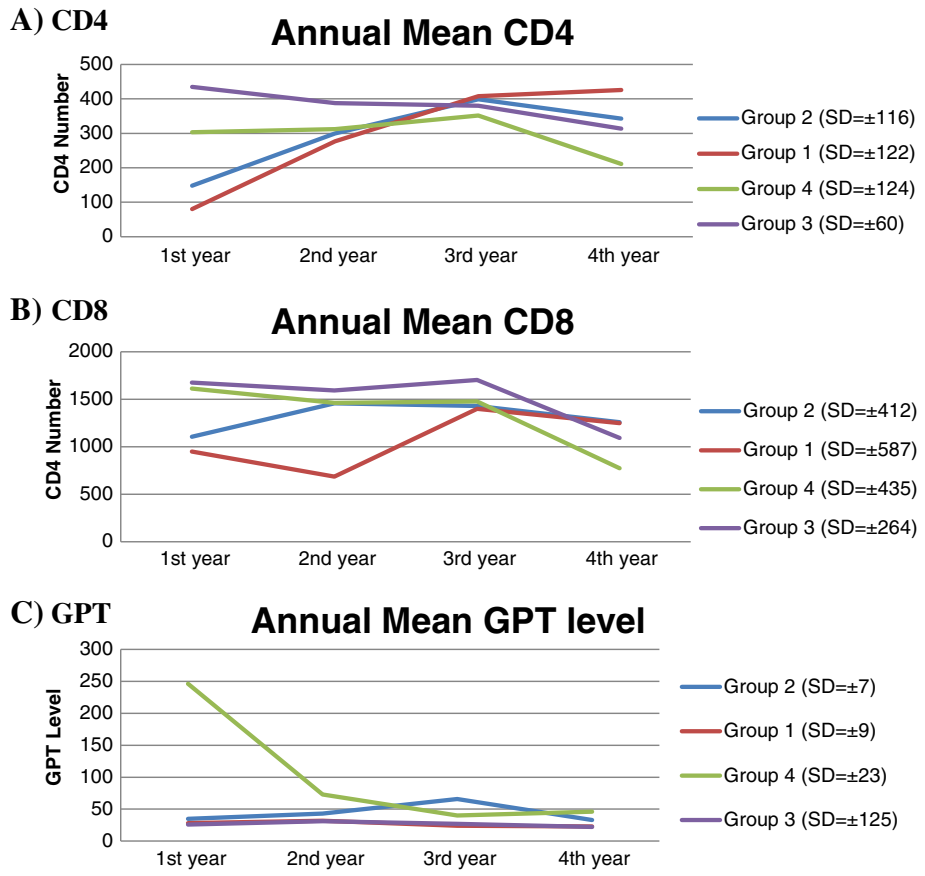

Figure 3 Line graphs showing trends of $CD4^+$ (A), $CD8^+$ (B) and GPT (C) respectively along the 4 years in all groups of patients.

($p = 0.08$) between the overall mean age and group mean ages of the study subjects (38.9 Vs 41.2, 41.3, 46.2 and 40.2). As shown in Figures 1 and 2, 23 (92%) of the total co-infected patients fall between 20–49, 1 (4%) between 50–59 and 1 (4%) above 60 years of age. In this study, HCV/HIV co-infection was higher in males than females (60% Vs 40%, $p = 0.06$). Furthermore, 11(73.33%) of the co-infected males were over the age of 40 years.

Table 1 and Figure 3A-B shows the mean baseline and fourth year $CD4^+$ and $CD8^+$ cell count of HIV/HCV co-infected and HIV mono-infected under HAART patients. The $CD4^+$ and $CD8^+$ recovery of co-infected group 2 was impaired by the co-infection of HCV despite the presence of HAART ($CD4^+$: 148 Vs 343; $P < 0.003$ and $CD8^+$: 1104 Vs 1259; $P < 0.004$) at the baseline and the last (fourth year). However, in HIV mono-infected group 1 both variables recovered significantly ($CD4^+$: 80 Vs 426; $p < 0.000$ and $CD8^+$: 950 Vs 1247; $P < 0.003$). Even though both groups showed recovery during the last $CD4^+$ count; however, the recovery rate was high in mono-infected group 1 than co-infected group 2 (426 Vs 343; $P < 0.004$, OR = 4.97, 95%CI = 2.41 to 10.27).

Table 2 and Figure 3A-B shows the mean baseline and fourth year $CD4^+$ and $CD8^+$ cell count of HIV/HCV co-infected and HIV mono-infected pre-ART individuals.

Table 2 Mean baseline and fourth year $CD4^+$ and $CD8^+$ cell count of pre-ART subjects

Cells/μl	Year	HIV/HCV co-infected (G4) (n = 9) Mean ± SD	HIV mono-infected (G3) (n = 9) Mean ± SD	p value
$CD4^+$	1st year	303 ± 73	435 ± 52	*P < 0.003*
	4th year	211 ± 163	314 ± 27	*P < 0.004*
$CD8^+$	1st year	1612 ± 380	1676 ± 343	*N/S*
	4th year	772 ± 552	1092 ± 177	*P < 0.002*

(Number of patients in parenthesis), N/S- not significant.
Key: G = Group, HAART = highly active antiretroviral therapy, ART = Antiretroviral treatment, μl = microliter, CD = Cluster designation/differentiation, SD = standard deviation.

Table 3 Mean baseline and fourth year CD4$^+$ and CD8$^+$ cell count of HIV/HCV co-infected under HAART and pre-ART patients

Cells/μl	Year	HIV/HCV co-infected (G2) under HAART (n = 16) Mean ± SD	HIV mono-infected (G4) pre-ART (n = 9) Mean ± SD	p value
CD4$^+$	1st year	148 ± 30	303 ± 73	*P < 0.001*
	4th year	343 ± 119	211 ± 163	*P < 0.004*
CD8$^+$	1st year	1104 ± 420	1612 ± 380	*P < 0.003*
	4th year	1259 ± 430	772 ± 552	*P < 0.003*

(Number of patients in parenthesis), N/S- not significant.
Key: G = Group, HAART = highly active antiretroviral therapy, ART = Antiretroviral treatment, μl = microliter, CD = Cluster designation/differentiation, SD = standard deviation.

At the baseline, there was a statistically significant CD4$^+$ difference between co-infected and HIV mono-infected groups (303Vs 435; *P < 0.003*) whereas, both groups had a comparable CD8$^+$ count (1612 Vs 1676) respectively. The last cell count (fourth year) clearly indicated that HCV co-infection was associated with fast decline of both CD4$^+$ and CD8$^+$ cells count than HIV mono-infected groups (CD4$^+$: 211 Vs 314; *P < 0.004* and CD8$^+$: 772 Vs 1092; *P < 0.002*) respectively.

Table 3 shows the mean CD4$^+$ and CD8$^+$ counts of both co-infected groups of patients at the beginning and fourth year. In the pre-ART group (G4), both CD4$^+$ and CD8$^+$ count were declined to initiate HAART (303 to 211, *P < 0.004* and 1612 to 772, *P < 0.001*) respectively, whereas, in the HAART group (G2) both cells recovered even though the rate of recovery was impaired by the presence of HCV (CD4$^+$: 148 Vs 343; *P < 0.003* and CD8$^+$: 1104 Vs 1259; *P < 0.004*) respectively.

The survival analysis curve shows that, HCV/HIV co-infected patients experienced significantly decreased durations of survival from the time of AIDS diagnosis (hazard ratio [HR), 2.85; 95% CI, 1.16-3.13) (Figure 4). The use of HAART improved survival duration (HR, 0.31; 95% CI, 0.11-0.37). HCV/HIV co-infected patients also experienced shorter durations of survival from the date of diagnosis of HIV infection to AIDS diagnosis than did HIV mono-infected patients (HR, 3.82; 95% CI, 1.32- 4.23) (Figure 5).

Table 4 shows liver enzyme levels of both co-infected under HAART and pre-ART patients at the beginning and fourth year sampling result. It shows a decline to the normal reference values from baseline to the fourth year which indicates the improvement of hepatotoxicity. The pre-ART co-infected group 4 patients improved better (246 IU/L to 46 IU/L) in GPT level than under HAART co-infected group patients (35 IU/L to 33 IU/L). The mean liver enzyme levels of group 1 and group 3 were generally fall within the normal reference range. However, the two co-infected groups had slightly greater than the normal range (Figure 3C and Table 4).

Discussion

Hepatitis C virus infection is one of the major diseases of mankind and is a serious global public health problem. The precise effect of HCV co-infection on the recovery of immune cells and liver enzymes in HIV patients before and after HAART remains controversial. However, a number of studies have suggested that the presence of HIV infection accelerates the course of HCV-related liver disease in HCV/HIV co-infected patients [5,8]. HIV is known to impair T-helper type 1 immune response which in turn alters the response of immune cells to HCV. This permits greater HCV replication and consequently, greater infection and injury to hepatocytes which leads to more rapid progression to

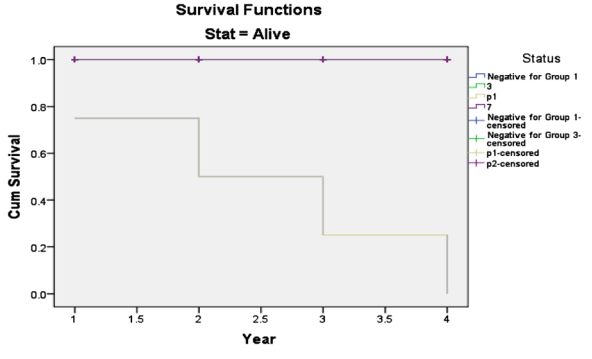

Figure 4 Survival curve for time from diagnosis of AIDS to termination of the study, stratified by HCV co-infection status, CD4$^+$ cell count and HAART use.

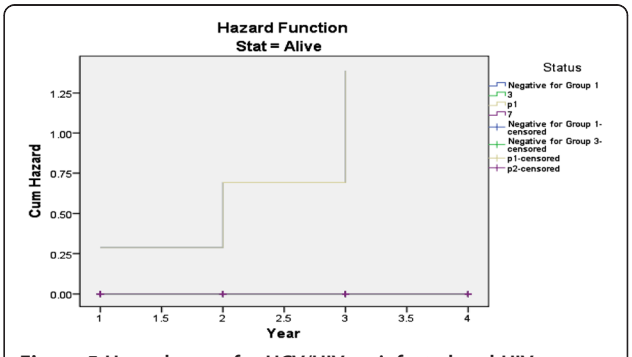

Figure 5 Hazard curve for HCV/HIV co-infected and HIV mono-infected patients, stratified by HCV co-infection status, CD4$^+$ cell count and HAART use.

Table 4 Mean baseline and fourth year liver enzyme levels of HIV/HCV co-infected under HAART and pre-ART patients

Enzyme level (IU/l)	Year	Under HAART co-infected Group (G2) (n = 16), Mean ± SD	Pre-ART Co-infected group (G4) (n = 9), Mean ± SD	p value
GPT	1st year	35 ± 5	246 ± 22	P < 0.000
	4th year	33 ± 12	46 ± 10	N/S
GOT	1st year	32 ± 5	207 ± 12	P < 0.000
	4th year	36 ± 14	35 ± 14	N/S
ALP	1st year	243 ± 50	239 ± 93	N/S
	4th year	235 ± 124	207 ± 85	N/S

(Number of patients in parenthesis), N/S- not significant.
Key: GPT = Glutamate pyruvate transaminase, GOT = Glutamate oxaloacetate transaminase, ALP = Alkaline phosphatase, IU = International unit, l = liter.

HCV-related liver diseases (fibrosis, cirrhosis and hepatocellular carcinoma) [5].

Given the above interaction of the two viruses and their implications on the proper management of the co-infected cases, the aim of this work was to describe the prevalence of HCV co-infection among HIV patients at Yekatit-12 and Zenbaba General Hospitals and assess the immunological and clinical chemistry results over a four years period. Results show that the prevalence of HCV among the 387 Addis Ababa resident-HIV patients, who visited the two hospitals during one month (September 2006), was 6.5%. This was low in accordance with the previous HIV/HCV co-infection studies in Ethiopia by Workinesh, *et al.* [7] and Addisu *et al.* [2] which was 8.6% and 10.5% respectively. Compared to Spain (33%), U.S.A (30%), France (24.3%) and Morocco (19.8%), the present prevalence was still low [5,9]. The co-infection prevalence in under HAART (7.8%) was more than Pre-ART patients (5%). This might be due to the higher numbers of under HAART (N = 206) than the pre-ART (N = 181) patients. In this study, from the 25 co-infected patients more males were co-infected than females (60% Vs 40%). This might be due to the higher number of males than females in the sample population, hence, does not justify to saying that it is gender influenced.

The distribution of HCV/HIV co-infection, in Figure 2 shows the direct link of age to HCV prevalence. It starts with 20% in age group 20–29 and grows to nearly 45% in age groups 40–49 years suggesting an association with age. A higher prevalence in older age groups could be a reflection due to the chronic nature of the disease, sexual behavior or it may be related to hormone and immunity. Our finding was in agreement with the work of Sugimoto, *et al.* [11] that found HIV/HCV co-infection is higher in males over 40 years of age. This is a serious indicator for over 50-80% of co-infected patients develop chronic infection that gives rise to liver cirrhosis (4-20%) and hepatocellular carcinoma in 1-5% [10].

The observation on immunological parameters over four years showed that an improvement of $CD4^+$ and $CD8^+$ counts in both HCV positive and negative under HAART patients. However, the $CD4^+$ increase in those not co-infected with HCV was much better when compared with those of HCV infected (426 ± 113 Vs 343 ± 119, $P < 0.004$). This suggests that although HAART does improve the immune system of HCV co-infected patients, its efficiency is relatively compromised by HCV interactions (Figure 3 and Tables 1 and 2). Our finding was in agreement with researchers who concluded that even though HAART suppresses HIV and increase $CD4^+$ count response, however, it is affected by the presence of HCV co-infection [4]. In addition, HAART adversely affect HCV outcomes by increasing HCV viral load, hepatotoxicity and increased HCV related liver disease progression in HIV/HCV co-infected people [12].

Variety of factors is incriminated in influencing these treatment outcomes. The factors include presence of high HCV RNA load, low treatment responses of genotypes 1 and 4, high rate replication (10^{12} virions/day) and its exceptionally high mutation rate producing a genetic variety [1]. Our result though does not have data on the influence of viral interaction, has shown that the presence of HCV decreases the efficiency of HAART when compare to those that were negative for HCV, hence goes well with the conclusions of Kedziora, *et al.* work [1]. The improvement of the immune status with $CD4^+$ and $CD8^+$ counts on its own improves survival of patients the minimum by slowing viral load and elimination of infected cells.

In the present study, the liver enzyme levels were much higher than the above limits of reference in co-infected than HIV mono-infected patients. In supporting the present study; DeSemone and his colleagues [13] found that increased liver enzyme level often associated with HCV co-infection. The mean GPT level of pre-ART co-infected group 4 patients during 1st year was at least fivefold greater than that of under HAART co-infected group 2 patients. From 2nd year and onwards, both group 2 and group 4 had comparatively increased levels of liver enzymes (Figure 3 and Table 4). In this study, more than 70% of the co-infected patients showed increased levels of GPT above the limits of reference which agreed with the finding of Lawson [14] in which only 30% of HCV patients have normal GPT levels.

Moreover, Sulkowski and his colleagues [6] found that HCV has been confirmed to be a risk factor associated with a 3 to 5-fold chance of developing elevated transaminases during HAART, compared to HIV patients without HCV which was compatible with the present study. However, the present work was incompatible with the study done by Gatti, *et al.* [8] that showed the synergetic mitochondrial damage by HCV and HAART (especially nucleoside analogs) were responsible for elevation of GPT in HAART groups. In addition, the higher enzyme level in the co-infected pre-ART group in this study indicates that the effect of HCV is more pronounced in pre-ART era than in the era of HAART. Furthermore, the HIV mono-infected group 1 and group 3 had normal amount enzyme levels throughout the follow up time. In supporting the normal enzyme levels of HIV mono-infected group 1 and group 3 in this study, Marina and Vincent [15] found that HAART do not associated with increased liver enzymes.

The present study has several strengths. Although viral load, HCV genotype and alcohol consumption status of patients are crucial in clinical medicine however, $CD4^+$ count is the main test routinely done to follow immune recovery. The present study clearly showed the pattern of $CD4^+$ changes considering different factors during the time of follow up. The information obtained in this study may promote our understanding of the impact of HCV infection on immunological parameters and liver enzyme levels among HIV/HCV co-infected individuals. Viral load, HCV genotype, alcohol consumption status and other clinical information of patients were not done in this study. Thus, with those limitations, we believe that this cohort may provide an accurate reflection of current clinical trends regarding this dual infection.

Conclusion

The present study has shown that HCV infection has an impact on the recovery of $CD4^+$ and $CD8^+$ cells of on under HAART patients. The improvement in $CD4^+$ cell count of under HAART HIV/HCV co-infected subjects were lower than the HIV mono-infected subjects and the mono-infected patients responded better to HAART than the co-infected patients. Moreover, HCV has a significant association with higher liver enzyme level than $CD4^+$ in HIV/HCV co-infected patients. Because of the lower prevalence rate of HIV/HCV co-infection reported from previous few local studies in Ethiopia, the disease was given less attention and seems forgotten at various levels of health delivery institutions so that the significance of the problem has been underestimated. Therefore, it is advisable to make HCV-antibody screening for every HIV infected individual prior to initiation of HAART. This will in turn influence their clinical management as well as outcome.

Competing interests
As authors, we declare that, we have no any competing interests.

Authors' contributions
The study was designed by ST. He also carried out data collection and laboratory works, performed data analysis and interpretation. ML revised the result critically and contributed to the final write up and finally, approved the manuscript.

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Seroprevalence of Hepatitis B Surface Antigen and Antibodies to Hepatitis C Virus at an Indian State Bordering Myanmar: A Hospital-Based Study

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Abstract:
In Manipur, one of the six high HIV-prevalent states of India, information is limited regarding Hepatitis B and C infections, which share a similar parenteral mode of transmission. The aims of this study are to investigate the seroprevalence of Hepatitis B surface antigen (HBsAg) and Hepatitis C antibodies (HCVAb) and to identify the seropositivity rates at different age-groups. A retrospective cross-sectional study was conducted at the Microbiology department of Jawaharlal Nehru institute of Medical Sciences, Manipur, on existing data from 2010- 13. A total of 21358 serum samples were screened for HBsAg and HCVAb using rapid immunochromatography tests and 3rd generation enzyme-linked immunosorbant assays (ELISA). 75(1.57%) and 259(5.4%) of 4790 males were positive for HBsAg and HCVAb respectively, and 132(0.8%) and 78(0.5%) of 16568 females were positive for HBsAg and HCVAb respectively. 21(0.44%) males and 5(0.03%) females were positive for both infections. For HBsAg the yearly seroprevalence ranged from 1.55 to 2.38% among the males and from 0.54 to 1% among the females. For HCVAb, it ranged from 3.91-8.17% among the males and from 0.30-0.64% among the females. Difference in seroprevalence between males and females was found to be statistically significant at P-value < 0.05. Seropositivity rates were maximum at 41-50 years for both infections among positive males, and at 21-30 years for HBsAg and 31-40 years for HCVAb among positive females. The rising seroprevalence rates of both hepatitis infections, especially those of HCV infection among the males, need urgent attention.

Keywords*: ELISA, hepatitis B, hepatitis C, immunochromatography, seroprevalence*

1. Introduction

Manipur is one of the six high prevalence states of India regarding human immunodeficiency virus (HIV) infection. HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV) all share a common route of parental transmission. Hepatitis B and C affect the liver chronically and hence is a cause of high morbidity and mortality. It was against the above backdrop that the present study was undertaken to estimate the seroprevalence of hepatitis B and hepatitis C infections among individuals attending our medical institute in the capital of this state and to identify the seropositivity rates at different age-groups of infected persons.

2. Materials and Methods

The study was carried out in the Serology and Immunology Section of the Department of Microbiology, Jawaharlal Nehru Institute of Medical Sciences, Imphal, Manipur. It was a retrospective cross-sectional data review, and the identity of the patients had been delinked from the data. Hence, Institutional Ethical Board clearance was not sought. Data evaluated included the findings of blood samples from all individuals who registered consecutively at the Out Patient Department (OPD) or were admitted to the In Patient Department (IPD), from January 2010 to December 2013 and who were advised to undergo screening for Hepatitis B surface antigen (HBsAg) and Hepatitis C Virus antibodies (HCVAb). About five ml of venous blood was received from each individual and the blood was allowed to clot for 30 minutes at room temperature. The serum sample was separated after centrifugation at low speed.

The serum was tested for HBsAg using a rapid card method (Hepacard, manufactured by Biomed Industries/ Diagnostic Enterprises, India). Reactive samples were re-confirmed by another rapid test (Hepaview, manufactured by Qualpro Diagnostics, India) or by an Enzyme Linked Immunosorbant assay (ELISA) (Hepalisa, manufactured by J. Mitra & Co. Pvt Ltd., India).

Antibodies to HCV were determined using rapid test device (Flavicheck-HCV WB, manufactured by Qualpro Diagnostics, India). Samples reactive by this test were re-confirmed using another rapid test (HCV Tridot, manufactured by Diagnostic Enterprises, India) or by a 3rd generation ELISA (SD HCV ELISA 3.0 , manufactured by Standard Diagnostics, Inc., Korea).

All the tests were performed in accordance with the manufacturer's instructions using adequate controls. A patient is considered positive for any one or both of these infections if at least two of three tests used for each type of infection were reactive.

The prevalence of both hepatitis B and C infections were analysed by using Student's t-test at probability level of 5%. A *P*- value of less than or equal to 0.05 was considered statistically significant.

3. Results

Of 21417 consecutive blood samples received over a period of four years, five failed to produce adequate amount of serum for the tests, and 54 were considered not fit for the tests as they were haemolysed. A total of 21358 serum samples were included in the study. Table 1 shows the year-wise distribution of individuals screened for HBsAg and HCVAb in terms of gender and religion. Females (77.57%) outnumbered males (22.43%). More than half of them were Hindu by religion (64.56%). Table 2 shows the year-wise seropositivity rates of HBsAg and HCVAb. Over a period of four years from 2010 and 2013, not only can we see a steady increase in the number of individuals screened, but an overall increase in the percentage of positivity rates for both sexes. Out of 4790 males screened 2% (96=14+17+28+37) were HBsAg positive and 5.8% (280= 34+43+76+127) were HCVAb positive. Out of 16568 females screened, 0.8% (137=22+23+40+52) was HBsAg positive and 0.5% (83= 10+14+26+33) were HCVAb positive. The difference in the seroprevalence of HBsAg between the male and the female patients was found to be statistically significant for all four years of the study at *P*-values of 0.0019, 0.0063, 0.0003, and 0.0024 consecutively. The corresponding *P*-values for HCVAb were all statistically significant at zero.

Year	Total	OPD	IPD	Male	Female	Hindu	Muslim	Christian	Others
2010	4084	3069	1015	767	3317	2750	482	429	423
2011	4560	3536	1024	1100	3460	2892	554	634	480
2012	5978	4556	1422	1368	4610	3686	702	978	612
2013	6736	5079	1657	1555	5181	4461	747	844	684
Total	21358	16240	5118	4790	16568	13789	2485	2885	2199

Table 1: Year-wise distribution of individuals screened for HBsAg and HCVAb.

		2010		2011		2012		2013	
		Pos / Total tested	% Pos	Pos / Total tested	% Pos	Pos / Total tested	% Pos	Pos / Total tested	% Pos
HBsAg	M	14/ 767	1.83	17/ 1100	1.55	28/ 1368	2.05	37/ 1555	2.38
	F	22/ 3317	0.66	23/ 3460	0.66	40/ 4610	0.87	52/ 5181	1.00
	T	**36/ 4084**	**0.88**	**40/ 4560**	**0.88**	**68/ 5978**	**1.14**	**89/ 6736**	**1.32**
HCVAb	M	34/ 767	4.43	43/ 1100	3.91	76/ 1368	5.56	127/ 1555	8.17
	F	10/ 3317	0.30	14/ 3460	0.40	26/ 4610	0.56	33/ 5181	0.64
	T	**44/ 4084**	**1.08**	**57/ 4560**	**1.25**	**102/ 5978**	**1.71**	**160/ 6736**	**2.38**

Table 2: Year-wise seroprevalence rates of HBsAg and HCVAb
M = Males, F = Females, T = Total, Pos = Positive

Table 3 shows the seropositivity rates of positive patients in different age groups for both infections. Among the males the highest seroprevalence for both HBsAg and HCVAb were found to occur in the 41-50 years age group. Among the females highest seroprevalence for HBsAg and HCVAb were found in the 21-30 and 31-40 years age groups respectively.

Age Group	HBsAg Positive		HCV antibody Positive		HBV-HCV Positive	
	Male N=75	Female N=132	Male N=259	Female N=78	Male N=21	Female N=5
0 - 10	0	2 (1.52%)	0	0	0	0
11 - 20	7 (9.33%)	16 (12.12%)	0	2 (2.56%)	0	0
21 - 30	9(12%)	55 (41.67%)	11 (4.25%)	13 (16.67%)	3 (14.29%)	2(40%)
31 - 40	21 (28%)	34 (25.76%)	101 (39%)	25 (32.05%)	7(33.33%)	2(40%)
41 - 50	27 (36%)	4 (3.03%)	122 (47.10%)	16 (20.51%)	9(42.86%)	1(20%)
51 - 60	8 (10.67%)	4 (3.03%)	25 (9.65%)	9 (11.54%)	2 (9.52%)	0
61-70	3 (4%)	2 (1.52%)	0	9 (11.54%)	0	0
71 & above	0	0	0	4 (5.13%)	0	0

Table 3: Seropositivity rates of HBsAg and HCVAb in different age-groups

4. Discussion

Out of 21358 serum samples studied a majority of 16240 (76.04%) were from patients registered at the OPD and the rest were from those admitted in the wards.(Table 1) A majority of 16659 (78%) samples were from asymptomatic individuals and 4699 (22%) had complaints related to viral hepatitis such as icterus with or without fever, anorexia, nausea, vomiting, right upper quadrant abdominal pain and hepatomegaly. Among the 16568 female patients, most of them 13420 (81%) were females undergoing antenatal screening for Hepatitis B and C infections. A much lesser 149 (0.9%) of them were undergoing pre-operative screening and none of them revealed a history of being HIV positive. Of the 4790 males 204 (4.3%) were known HIV positive patients and 575 (12%) were screened pre-operatively.

Out of 4790 males screened it has been found that 75 (1.57%) of them were only HBsAg positive whereas a much higher 259 (5.4%) tested positive for only HCVAb. Out of 16568 females studied, 132 (0.8 %) and 78 (0.5 %) of them were only HBsAg and only HCVAb positive respectively. 21 (0.44%) males and 5 (0.03%) females were positive for both infections and all were found to be symptomatic for viral hepatitis. Though it could not be determined whether these were co-infections or super-infections, it is certainly known that both lead to high morbidity and mortality. [1]HBsAg in serum is the first seromarker to indicate active HBV infection, either acute or chronic. Based on the prevalence of chronic hepatitis B, countries have been variably classified as high (≥8%), intermediate (2-7%), and low prevalence (≤2%) areas.[2] India has been placed in the intermediate zone of prevalence by WHO.[3] In this study, the yearly seroprevalences among the males ranged from 1.55 to 2.38% whereas those among the females ranged from 0.66 to 1%. (Table 2) Comparing the HBsAg seropositivity rates in different age groups, we can observe that they are higher among the females upto 30 years of age. Thereafter the seropositivity rates start to reverse and the percentage of positive males predominate. (Table 3)

Among the 96 HBsAg positive males 28 (29.17%) were in the 31-40 years age-group and 36 (37.5%) were in the 41-50 years age-group. Considering the fact that all of the known HIV positive males belonged to these two age-groups, and that most HIV positives in this state are or were intravenous drug users (IVDUs) it is easy to understand why more number of males were positive in these two age-groups.[4] Beyond these two age-groups, the numbers of HBsAg positive males are much lower at 12(12.5%) and 10(10.42%) in the 21-30 and 51-60 years age-groups respectively.

Comparing the HBsAg seropositivity rates in different age-groups of 137 HBsAg positive females, we observed that a maximum of 93 (67.88%) are in the 21-30 and 31-40 years age-groups put together. Taking into consideration the high percentage (81%) of pregnant females among the total number of females screened this high rate of seropositivity in the child bearing age is expected to translate into high perinatal transmission rates. Perinatal transmission is the most common mode of HBV transmission worldwide. It occurs at or near the time of birth, because neonatal vaccination prevents new born infection in about 80% -95% of cases.[5] Centers for Disease Control and Prevention (CDC) guidelines include mandatory screening of all women for hepatitis B during the first prenatal visit because this virus is highly contagious, and the risk that the newborn infant will develop hepatitis B is 10 -20% if the mother is positive for HBsAg.[6] CDC also noted that risk factor-based screening did not identify 35%-65% of all HBsAg positive mothers. Thus screening the expectant mother could go a long way in prevention of prenatal HBV transmission. In India, unlike the policy for HIV infection screening among pregnant women, there is no policy for HB infection screening for them. Perinatal transmission of HBV infection has declined steadily in the United States, consistent with the successful implementation of universal screening of pregnant women and vaccination policies.[7] If the mother is HBsAg positive, appropriate active and passive immunoprophylaxis should be given in the form of hepatitis B immunoglobulin and hepatitis B vaccine.

The presence of anti-HCV Ab indicates previous exposure to hepatitis C virus. This antibody is present in only 40% of acute infections but in more than 95% of chronic infections.[8] Hepatitis C virus (HCV) infection establishes a state of chronic infection in as many as 85% of acutely infected patients, whereas about 15% of acutely infected patients spontaneously clear the infection.[9] In India, anti-HCV Abs are present in approximately 15 million people with a prevalence rate of 1.2 – 1.8%. [10,11,12]. In this study, the yearly overall seroprevalence ranged from 1.08% to 2.38%. Those among the females continued to be under 1%. In contrast, those among the males were well above the national average and ranged from 3.91 to 8.17%. (Table 2) This high seroprevalence of HCV infection among the males could be due to the fact that this institute also runs an Anti-retroviral Therapy ART) centre under the aegis of the National AIDS Control Organisation (NACO). In Manipur, HIV-HCV co infection among people living with HIV (PLHIV) is 79.1% in 2008.[4] In India, HCV is the most infectious disease among IVDUs. A recent study conducted in another medical institute in the same city reported the seroprevalence of HCVAb as 0.40% among the voluntary blood donors and 1.11% among the replacement/relative blood donors.[13] Evidently more lives could be lost due to HCV than did HIV among people who have or had high risk behaviour such as sharing of needles and syringes, and also of other injecting-related equipment, because the cost of treatment is very high and there is no Government policy to screen these patients as in the case of HIV/AIDS.

On the other hand, universal screening for hepatitis C in pregnancy is not recommended. Firstly, the efficiency of hepatitis C virus (HCV) transmission by sexual activity remains controversial. [14] Secondly, an effective hepatitis C vaccine has not been developed and thirdly, the drugs used most commonly to treat hepatitis C in both children and adults, interferon and ribavirin are not recommended for use in pregnancy. [15,16] HCV-infected children may be born to mothers who were anti-HCVAb positive. Intrapartum infection is more common than in-utero infection. [17] Elective CS is not recommended; as even non-viremic women cannot be assured they have no chance of infecting their neonates, although the risk appears to be very low. [17,18] Yet, in this study, a large number of pregnant females were screened for HCVAb as clinicians have a tendency to test both HBV and HCV infections together.

In this study, 575 (12%) of 4790 males and 149 (4.7%) of 3148 non-pregnant females underwent pre-operative screening for these two infections. Out of 575 males only 5 (0.87%) and 1 (0.17%) were positive for HCV Ab and HBsAg respectively. Only 1 (0.67%) of the 149 females were reactive for HBsAg. It has been suggested that a minimum prevalence of 1 in 1000 (0.1%) in the general population justifies screening of an infection. [19] Universal pre-operative testing for HBV and HCV may be done if a protocol for management of infected patients, including testing of family members of confirmed cases, is available as non-sexual intra-familial modes of transmission of HBV and HCV have been reported.[20,21,22] Patient and institutional resources may be saved by elective screening dependent on a carefully worked out clinical risk assessment plan.

5. Conclusion

This study shows that the ever rising seroprevalence rates of hepatitis B and C infections, especially that of HCV infection among the males, is a cause of alarm in this state of NE India bordering Myanmar. Taking into consideration that there is no vaccine or immunoglobin prophylaxis for hepatitis C infection, and that screening is justified in terms of the seroprevalence rates of both infections for four consecutive years, a large study among the general population could be conducted to make the consideration for national policies for both hepatitis more conclusive.

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Original Article

"Hematological and Biochemical analysis of β-thalassemic Major patients"

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ABSTRACT

β-thalassemia is a single gene disorder requiring regular multi-blood transfusions. In patients with β-thalassemia major impaired biosynthesis of the beta globin leads to accumulation of unpaired alpha-globin chain. Repeated blood transfusions, ineffective erythropoiesis and increased gastrointestinal iron absorption lead to iron overload in the body. Thus shortened red cell lifespan and iron overload cause functional abnormalities in various organ systems. An attempt was made to study hematological and biochemical parameters in β-thalassemia major patients in order to assess the present status of their organ functions as well as test for transfusion transmitted infection (HBV, HCV & HIV) were performed, for this blood samples were collected just before scheduled blood transfusion from 58 β-thalassemia major children who were on regular blood transfusion and chelation therapy and 50 blood samples from healthy children belonging to same age group. Highly significant difference was noticed for CBC count including deteriorated liver and kidney functions test in patients as compared to controls. Not a single positive case of HBV, HCV & HIV were observed.

Conclusion: β-thalassemia major patients were associated with multiple renal abnormalities and deranged liver enzymes due to continuous blood transfusion and iron overload. Appropriate chelation therapy and regular monitoring of the status of iron overload is very much necessary.

Keywords:
β-thalassemia major, iron overload, LFT, KFT.

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Introduction

Thalassemia is one of the major hemoglobinopathies among the population all around the world. It is single gene hereditary disorder in humans. In thalassemia there is impaired production of alpha or beta chains of hemoglobin are. If the production of alpha chains is impaired the condition is called alpha thalassemia and if the production of beta chain is impaired the condition is called beta thalassemia. (Asha Shah, 2004).

β-thalassemia represents a group of recessively inherited hemoglobin disorders characterized by reduced synthesis of β-globin chain. Three classes of β-thalassemia have long been recognized clinically, β-thalassemia major, intermedia and minor (Thein, 2004). β- Homozygous state presents with variable degree of anemia from early childhood and are generally transfusion dependent, a condition clinically known as thalassemia major. β-heterozygous cases (thalassemic minor) are almost asymptomatic with normal or slightly reduced levels of hemoglobin. However an intermediate condition which may have either heterozygous or homozygous pattern of inheritance, requires minimal or no blood transfusion and has milder clinical course than thalassemic major but is severe enough as compared to thalassemic minor. It manifests generally after two years of age and does not require regular

transfusion therapy. (Rund, 1997, Tyagi, 2003).

Reportedly, there are about 240 million carriers of β-thalassemia worldwide and in India alone the number is approximately 30 million with a mean prevalence of 3.3%. (Yashis, 2007, Verma, 1992, Yagnik, 1997).But among certain communities and religions like Punjabi's, Sindhi's, Bengali's, Jams and Muslim's the incidence of β-thalassemia trait ranges between 8-15 %. (Marwah and Lal, 1994)

Thalassemic major child is born if both parents carry a hemoglobinopathy trait, since there is a 25% chance with each pregnancy for an affected child. Once a child is diagnosed to have thalassemia homozygous disorders, he/she has to take lifelong treatment. Management includes regular filtered packed red cell transfusion, chelation therapy for iron overload, management of complications of iron overload and transfusion, including osteoporosis, cardiac dysfunction, endocrine problem, hepatitis B and C, HIV infection etc.

Iron overload is the life limiting complication commonly found in thalassemics.(Wangruangsattit S.et.al. 1999).The progressive iron overload in β-thalassemia major patients is consequence of ineffective erythropoiesis, increased gastrointestinal absorption of iron, lack of physiologic mechanism for excreting excess iron, and above all multiple transfusions. A unit of red blood cells transfused contains approximately 250mg of iron, while the body cannot excrete more than 1mg of iron per day. The iron which exceeds the iron binding capacity of transferrin appears in the plasma as non transferrin bound iron, which is highly toxic to the tissues. (Giardina PJ and Grady RW, 2001). Iron overload- produces reactive oxygen species that damage the heart (cardiomyopathy), liver (fibrosis and cirrhosis),nervous system, can lead to diabetes mellitus, hypothyroidism, hyperparathyroidism as well as adrenal and pituitary insufficiencies.(Rund D, Rachmilewitz E.,2005). Deferroxamine, Deferiprone and Deferasirox are few commonly used iron chelator in India. Due to high cost of such chelators most of the patient cannot afford this. Regular blood transfusion is available in most of the countries, is lifesaving and improves short time quality of life. But iron chelation therapy is essential for long term survival (S Mallik, C Chaterjee et.al.2009)

Beside this, Transfusion transmitted infection (TTI) is a major challenge to the transfusion services all over the World. The problem of transfusion transmitted infection (TTI) is directly proportional to the prevalence of the infection in the blood donor's community. In India HIV, HBV, HCV, Syphilis, Malaria, Human T-lymphocyte virus (HILv-1 and HILv-2) and bacterial infection are important causes of concern (Bhasin R et.al. 2003).Blood transfusion transmitted infection (TTI) occurs in patients who are dependent on blood transfusion. Multiple blood transfusions are required mainly in patients of thalassemia, Sickle cell anemia, hemophilia, aplastic anemia, patients of chronic hemodialysis etc.

Thus the most important cause of mortality and morbidity in these patients is organ failure related with shortened red-cell life span ,rapid iron turnover and tissue deposition of excess iron. These are major factors responsible for functional and physiological abnormalities in β-thalassemic major patients as well as number of transfusion transmitted infections. An attempt was made to study hematological and biochemical parameters in β-thalassemic major patients attending the private and Muncipal hospital of Akola in order to assess the present status of their organ function as well as prevalence of blood transfusion transmitted infections among them.

Materials and Method:

58 samples were analyzed for the study which was conducted in research lab of dept. of Biochemistry at Shri Shivaji College, Akola. Sample collection was done from Hedgewar Blood Bank, Thalassemic unit of Indrani hospital and Muncipal hospital of Akola.

Study of Biochemical parameters including Serum urea, Serum creatinine, Serum bilirubin, SGPT, Serum Alkaline phosphatase, Serum iron, TIBC were done on Robonik Biochemical analyzer while hematological study was done on ERMA made Fully automatic Cell counter and Sodium, Potassium, Calcium analysis was done on Sod-Pot analyzer(Roche 9180 electrolyte analyzer). Seroprevelence of Hepatitis B, Hepatitis C and HIV were done by using Acon One step immunoassay Rapid test, Flavicheck HCV, Rapid immunochromatographic test for HCV antibodies and Aspen One step immunoassay

Rapid test respectively.

Result & Discussion:

Total 58 blood samples of β-thalassemic patients were collected from Hedgewar Blood Bank, Thalasemic Unit of Indrani hospital just before scheduled blood transfusion. The age range of patients lies between 5 to16 years. All the patients were on regular blood transfusion as well taking the oral iron chelator i.e. Kelfer. Similarly a camp was organized at Shri Shivaji High School, Akola and 50 blood samples as a control from students were collected belonging to same age range. Complete blood count and biochemical analysis were performed. The hematological data was tabulated in table no.1 while biochemical data was shown in table no.2 which was statistically analyzed using Z-score test.

Table No.1: Hematological Parameters

Sr. No.	Hematological Parameters	Control group (n=51)	β-thalassemic major patients (n=58)	Z-score	P value (level of significance)
1.	WBC X 10^3/ul	8.07±0.26	11.19±0.09	17.24	HS
2.	RBC X 10^6/ul	4.38±0.05	3.19±0.02	29.96	HS
3.	Hgb g/dl	12.09±0.13	7.58±0.11	36.69	HS
4.	HCT %	35.52±0.43	23.75±0.21	35.87	HS
5.	MCV fl	81.04±0.51	74.35±0.40	14.62	HS
6.	MCH Pg	27.77±0.37	23.84±0.42	9.85	HS
7.	MCHC g/dl	34.15±0.41	32.12±0.61	3.93	HS
8.	RDW %	14.64±0.10	18.55±0.30	19.33	HS
9.	PLT X 10^3/ul	277.80±9.19	361.74±5.41	11.49	HS

Key: HS= Highly significant (p< 0.001), S= Significant (p<0.01)

Table No. 2: Biochemical Parameters

Sr. no.	Biochemical parameters	Control group (n=51)	β-thalassemic major patients (n=58)	Z-score	P value (level of significance)
1.	Urea mg/dl	27.52±1.17	30.83±1.03	3.01	S
2.	Creatinine mg/dl	0.78±0.02	0.92±0.03	4.94	HS
3.	Sodium meq/L	135.57±0.46	150.46±0.48	31.42	HS
4.	Potassium meq/L	3.7±0.04	4.94±0.12	14.50	HS
5.	Calcium mg/dl	9.61±0.05	9.05±0.03	12.38	HS
6.	SGPT Units/ml	23.58±1.04	37.13±0.57	16.81	HS
7.	Alkaline phosphatase KA units	5.56±0.17	9.36±0.20	19.82	HS
8.	Total Bilirubin mg/dl	0.63±0.04	1.29±0.02	19.02	HS
9.	Direct Bilirubin mg/dl	0.16±0.01	0.38±0.006	20.99	HS
10.	Serum iron ug/dl	88.15±1.79	182.09±1.63	54.67	HS
11.	TIBC ug/dl	276.92±31.52	237.65±29.34	9.50	HS
12.	Transferrin saturation %	32.20±5.84	77.85±11.68	38.81	HS

Key: HS= Highly significant (p< 0.001), S= Significant (p<0.01)

β-thalassemia major is an inherited, autosomal recessive hemoglobinopathy that results in a large number of hematological, biochemical and systemic abnormalities. The β-thalassemic children included in the present work showed a significantly altered hemogram, especially in red blood cell mass and related indices (Hb, RBC,HCT and MCV,MCH).Our hematological findings in β-thalassemic patient s were found to be in similar in view as that of Rigano et.al. (2001) that showed in their study significantly altered hemograms with severe anemia, thrombocytosis and leucocytosis of β-thalassemic Sicilian patients. Yassin et.al. (2013) had studied over β-thalassemic Palestinian patients and reported severe anemia with decreased hemoglobin, HCT along with significant thrombocytosis and leucocytosis among the patients as compared to controls.

A rise in iron indices observed in β-thalassemic patients may be due to erythrocytes

hyperhemolysis and due to chronic blood transfusion.Similar results were found in the study of Asma K .*et.al.*(2003). The acute iron overload found in beta-thalassemia can lead to an iron intestinal hyperabsorption and to an abnormal molecular iron form (non-transferrin-bound: NTBI) accumulation. NTBI has hepato and cardio-cytotoxic properties. Furthermore, NTBI contributes to the formation of free radicals and increases hemolytic process (Borgna–Pignatti *et al.*, 2004). We observed highly significant iron increase leading to iron overload, in patients as compared with mean serum iron levels of 182.09 vs 88.15 ug/dl respectively. Mean value of TIBC in patients and control group was found to be 237.65 and 276.92 ug/dl whereas mean value of transferrin saturation was observed to be 77.85 and 32.20 % respectively.

Our result revealed a significant increase in serum urea level in experimental group as compared to control group.(30.83 and 27.52 mg/dl respectively).The concentration of creatinine in serum is the most widely used and commonly accepted measure of renal function in clinical medicine (Persone *et. al.* 1992).Our study showed highly significant difference in creatinine concentration in the experimental group as compared to control group.(0.92 and 0.78 mg/dl respectively).The increasing level of urea and creatinine in β-thalassemic patients possibly due to higher iron deposition in their kidneys, shortened red cell lifespan and excess iron which causes functional and physiological abnormalities in various organ systems in thalassaemia patients.

Electrolyte levels are tightly controlled by several hormones and by the kidneys, which are primarily responsible for retaining and removing electrolytes when necessary and keeping them in a constant state of balance. An electrolyte imbalance can lead to serious health issues, including eventual death if not corrected. The most common imbalances occur with sodium and potassium (Kamal *et.al.* 2013). Our findings revealed that there was highly significant increase in serum Sodium and Potassium in the patient group as compared to control group (150.46 and 135.57 meqL, 4.94 and 3.70 m eq/L respt).There was a significant decrease observed in the mean value of serum calcium in experimental group as compared to control.(9.05 and 9.61 mg/dl restp).Increase in serum Sodium and potassium and decrease in serum calcium was observed on 70 β-thalassemic patients by Hina Akram and Tabassum Mahboob (2004).

Frequent blood transfusion can also lead to iron overload in liver. Liver has a large capacity to produce proteins, which bind the iron and store it in the form of ferritin, therefore it can produce severe iron overload .Thus our study was also aimed to assess the liver status of β-thalassemic patients.Highly Significant change was observed in the SGPT level of patients as compared to control group (37.13 and 23.58 u/L respectively),similarly highly Significant difference was also observed in total and direct billirubin of experimental group as compared to control group (1.29 & 0.63 mg% and 0.38 & 0.16 mg% respectively) and highly significant increase in alkaline phosphatase was also noticed in experimental group as compared to control(9.36 & 5.56 KA units respectively)

Abnormal liver function represented by elevated levels of SGOT, SGPT and serum alkaline phosphatase, which was observed more frequently in patients with iron overload than in patients with a lower level of iron (Wanachiwanamin et.al.2003).

Regular blood transfusion improves the overall survival of patients with β-thalassemia, it carries a definite risk of infection with blood borne viruses (Amarapurkar DN *et.al.*, 1992, Mirmomen S *et.al.* 2006) and complication like secondary hemosiderosis, liver failure and renal failure. Infections are major complication and constitute the second most common cause of mortality and morbidity. In our study fortunately no single patient was found to be positive for Hbs antigen, anti HCV antibodies and anti HIV antibodies.

In case of hepatitis B, since an effective vaccine is available, immunization against this virus before transfusion management would effectively protect against transfusion transmitted hepatitis B virus. However since no such vaccine is so far available against Hepatitis C virus, the only effective protective measure is provision of HCV negative blood for transfusion. Therefore, screening of transfused blood for HCV as well as for HIV should be done mandatory, by using most sensitive screening methods with least possible false negative results. (Mohd. Y *et.al*, 2004). The decrease in seropositivity amongst multitransfused patients is because of implementation of measures such as, donor education, strict standards for donor selection criteria, improved serological screening protocols, improved blood collection and transfusion technique. (Vidja PJ, *et.al.* 2011)

Incidence of HIV positivity has decreased due to strict mandatory screening of all blood bags as well as by decreasing the window period by using improved technology. Prevalence of HCV is still there in frequent blood recipients like thalassemia major patients. This may be due

to late starting of screening for HCV antibody as well as no vaccine is available for protection against HCV. (Bhavsar H *et al.*, 2011)

Conclusion:

β-thalassemic patients present multiple renal abnormalities which may be due to iron overload. Thus appropriate chelation therapy and regular monitoring of the status of iron overload is very much necessary. Well balanced nutrition, patient education, diet counseling and supplementation therapy of calcium and vitamin-D for high risk group of β-thalassemics is strongly recommended. Ideally all patients of thalassemia major should complete vaccination for hepatitis B before starting transfusion or as soon as possible. Since increased titer of antibodies is protective against Hepatitis B viral infection. Though we didn't get any hepatitis positive case, references proved that prevalence of HCV infection is much higher as compared to HBV and HIV infections in β-thalassemic patients.

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