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Clinical Characteristics and Cross Analysis of HIV and HCV Co-Infection in Faisalabad Region

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Abstract

About 150 and about 35 million human beings are infected with HCV (hepatitis C virus) as well as HIV (human immunodeficiency virus) respectively. The increasing stress of HIV/HCV coinfection is supposed to infect five to seven million individuals globally due to their coinciding approaches of procurement. Co-infection of HCV/HIV in patients caused them to suffer from more liver-associated mortality and anguish. This paper aims to investigate the spread rate of the HIV and HCV coinfection in District Faisalabad. In this paper, blood samples of patients are evaluated and tested for anti-HIV/HCV antibodies employing the ICT (immune chromatography technique) for HIV/HCV confections. The six out 30 confirmed patients (real-time PCR) for HIV infection who also have HCV infection were analyzed for additional provisional clinical examination. In these patients, the hemoglobin (17.38±0.159 per dL), ALT (77 uL^{-1}) and Hematocrit (50.60±0.255%) levels increased significantly than standard reference values. On the body, these facts could be developed due to HIV/HCV co-infection burden. An inverse relation has been demonstrated by these coinfected patients in the levels of hemoglobin as well as in platelets. With the usages of better supplements/nutrients, the above differential values can be improved and ultimately beneficial for the survival of the infected individuals. The Current paper could be convenient for appropriate perpetuation of HIV/HCV co-infected patients under related treatment.

Keyword: Hepatitis C; HIV; Co-infection; Clinical Analysis; Viral coinfection; Antioxidant.

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1. Introduction

HIV (human immunodeficiency virus), a virus that convulses the human body's defense. The HIV genome is composed of two similar single-stranded molecules of RNA that are encased inside the core of the virus fragments [1]. The main target of HIV is to strive and dismantle CD4 cells of the defense system which endeavor against inflammation. The drop of CD4 cells makes it impossible for the body to compete against diseases and some cancers. Over time, if HIV is left untreated it can slowly ruin the defense system and assists AIDS. Hepatitis C is an epidemic viral infection that attacks the liver. The HCV, being a positive-sense (5'-to-3') genome is a mono-stranded RNA with exceedingly organized elements, that is about 9.6 kb in length [2]. This genomic RNA of HCV consists of a distinct open reading frame (ORF) which encodes a polyprotein that can be treated into 10 viral proteins subsequently the translation process [3]. Hepatitis C is an infectious disease that affects about 185 million people each year and can cause the death of more than 35,000 people each year [4]. Vaccine for hepatitis C is currently unavailable and liver cirrhosis may also be developed due to infection caused by the hepatitis C virus in many people. Hepatitis is an epidemic pathogen worldwide and HCV is the main reason for liver diseases and liver damage. By the worldwide survey, it is stated, that about 71 million people are affected with HCV and 400,000 patients die each year because of liver damage and hepatocellular carcinoma. Hepatitis C is the main reason for liver diseases like hepatocellular carcinoma and liver cirrhosis and causes liver failure [5]. As HIV affects the defense system of the human body, therefore people suffering from HIV have higher chances of HCV. Infection including both HIV and HCV simultaneously is known as HIV/HCV coinfection. Globally, the serious stress of coinfection (HIV/HCV) is more identified especially within the Asia/pacific areas. The chances of hepatocellular carcinoma and liver failure which results in cirrhosis are increased in the patients who are HCV and HIV coinfected. Also, changed immunological reactions to HAART (highly active antiretroviral therapy) may have seen in these coinfected patients. All these coinfected patients should be subjected to assessment of the HCV treatment, and patients with HCV infection are treated with ribavirin therapy including standard pegylated interferon in HIV-infected people [6]. Globally, it is supposed that people living with HIV are affected by 2 to 15 percent of HCV. The general estimate of stress of HIV and HCV coinfection is 2.75 million. There is an increased possibility that the HIV confirmed individuals who became infected with HCV may develop chronic hepatitis [7]. Medicines are carefully advised to HIV as well as HCV infected patients by health professionals to evade the drug-drug interaction and carefully observe those patients for any kind of side reaction [8]. HCV, being a bloodborne virus that can be transmitted via direct contact (blood plasma) of an infected individual, HIV and HCV coinfection is 62 to 80 percent common between users (drug-injection) who have HIV [9–11]. However, transmission via sexual acts (unprotected) is an essential mode of receiving between MSM with HIV [12]. In HIV- HCV- coinfected individuals, early diagnosis plays an essential role to manage the coinfection. Advanced therapies are developing including polymerase inhibitors as well as HCV protease which may expand the treatment preferences for HIV and HCV coinfected patients in the coming days. It is not an easy task to analyze and to detect such viral diseases in patients which relies on skilled people for analysis. Few research institutes are performing the study of HIV and HCV coinfected samples in Pakistan. Precise diagnosis accords an advance response to manage the treatment of these patients in a greater way. In this paper, samples of blood serum of patients (HCV-infected) are gathered from the Faisalabad region who came for the checkup at Allied Hospital

Faisalabad, and their samples are examined with RT-PCR for the potentiality of HCV and HIV coinfection. The sera of HIV including HCV-infected sufferers are administered for definite clinical analysis such as CBC (complete blood count) analysis. The evaluation of these sera-based specifications might be beneficial in the administration including the HIV/HCV from adults to teens. Even variations in these aspects of plasma (serum) are based on the method of their nutrition which may cause the biosynthesis of unbalanced hormones. Due to this, the serum is subjected to evaluate the intensive hematological values of HIV/HCV coinfected individuals against normal reference values.

2. Methodology and Materials

2.1. Specimens collection

Selected patients of HIV infection were subjected to give their fresh blood serum samples in definite vials (with anti-coagulated characteristic due to EDTA-K2) from the ostensibly preferred normal persons who have paid visit the Allied Hospital Faisalabad. From this stated collection, six patient specimens were elected, which are contaminated with chronic HCV. The blood specimens were centrifuged, and the serum was reserved at -20°C before its later use for the HIV investigation.

2.2. Immunochromatographic tests (ICT) for both HIV and HCV

The collected samples were screened by using an ICT test through a test device (Acucheck USA). 10µL serum was applied to the sample well and two drops of sample diluents were added immediately. The mixture could migrate along the test strip. Two specific lines appeared after 15 minutes, a line on the C area (Control) and the other on the T area (Test) indicated the results as positive. A line on the C area with none of the line in the T area was noted result as a negative, although the line C that decline to emerge was deliberated an inoperative outcome, so negative samples did not produce a test line. Anti-HIV/HCV antibodies positive blood samples were used for further analysis.

2.3. Diagnostic of HIV patients

The samples of HIV patients were confirmed via COBAS TaqMan (TaqMan 96) HIV-1 test by the ensuing procedure announced [13] as these patients were infected with HIV-1. The nucleic acids were extorted with a computerized Cobas AmpliPrep apparatus i.e. Roche Diagnostics, Germany with the help of the isolation kit for nucleic acid (TINAI). It is a nucleic acid elaboration test (in vitro) to calibrate the HIV-1 RNA in human serum that points to two eminently preserved areas of the genome (HIV-1), not apt to drug pressure. While doing so, it recoups for the feasibility of mutations and escalates the anticipation of disclosure [14].

2.4. Hematological analysis of the co-infected patients

Several hematological variables were investigated in the blood serum of proved patients having HIV and HCV coinfection in analogy to normal persons. The parameters including hematocrit (HCT), concentration of MCH (MCHC), Hemoglobin (Hb) level, complete blood count (CBC) mean of cell hemoglobin (MCH), mean cell

volume MCV , and RBC's classification range were driven with Hematological Analyzer (Adevia, 2120/Model , USA) and the liver marker i.e. alanine aminotransferase (ALT) determined with Beckman Coulter Automatic Biochemical Analyzer by following their procedure given in the manuals [15].

2.5. Estimation of Hb

The Hb (hemoglobin) was predicted by ensuing the procedure as previously recorded [16, 17]. Shortly, exact 20µl blood was assorted in Drabkin's reagent with the ratio (1:200). The mixture was blended assiduously for 10 minutes at ambient temperature. At 530 across blank Drabkin's reagent, the absorbance was calculated. It is also employed in the preparation of a standard solution of hemochromogens (12g per dL).

2.6. Determination of the antioxidant action

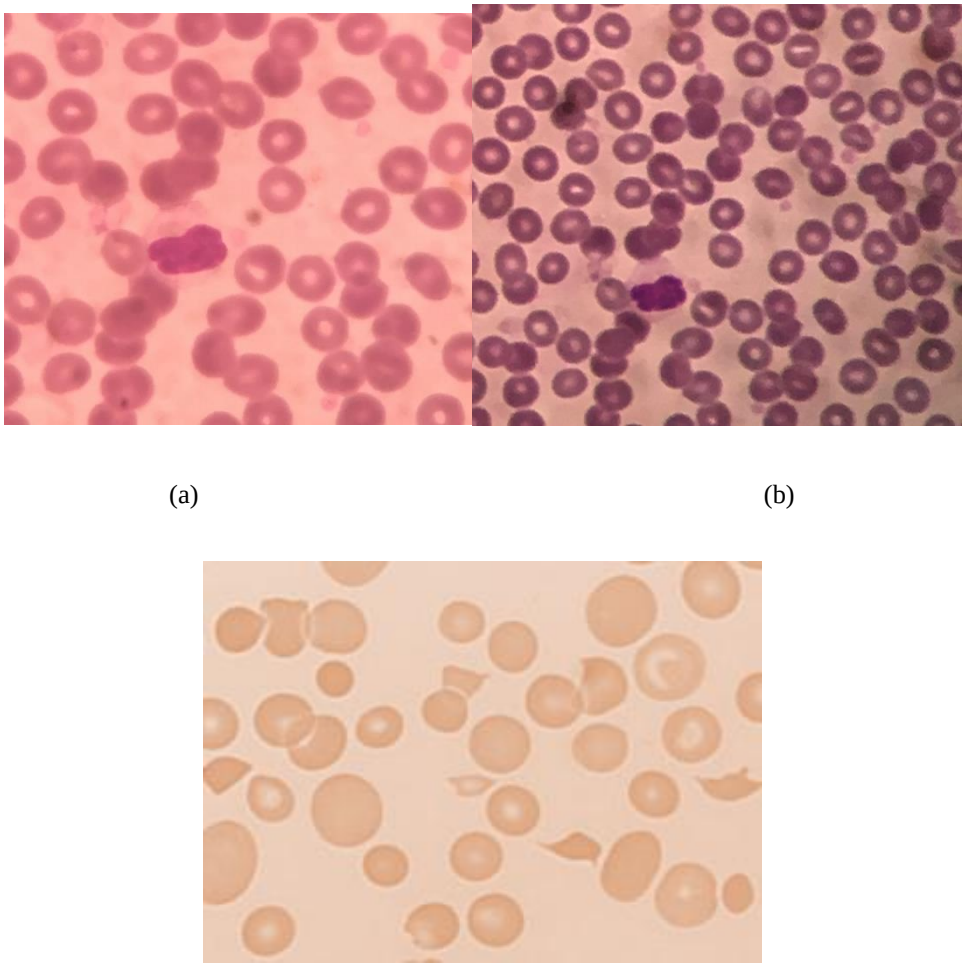
TAA (total antioxidant activity) was determined in a mixture, that was processed by fusing the 2mL 80-Tween, ascorbic acid 0.2mL (10 mM ascorbic acid), and 0.2mL solution of $FeSO_4$ [1 mM $Fe2(SO4)^3$ with hemolysate 0.1mL/0.2mL serum]. The mixture was fused completely and hatched for two days at 40°C. It's 2mL disordered with 1mL of 20% trichloroacetic acid (TCA) correctly than 1mL of its buoyant was poured with 2mL thiobarbituric acid (0.8% TBA). The mixture was steamed for 10 minutes, after that chilling to ambient temperature, OD-532 of the uppermost liquid state was captured [18–20].

2.7. Determination of glycine betaine

For the perseverance of glycine betaine, in the test tube we diluted the sample with dilute H_2O. Then, the sample was blended with specific 1mL in 1M of KI and incubated at 65^0C for 15 minutes [21].

3. Results and Discussion

The replication of the hepatitis C virus is precisely increased due to the HIV infection, amplifies the induction of HCV hepatic infection, and because of the pro-fibrinogenic cytokines programmed cell death in hepatocyte increases, increases translocation of microbes from the stomach and advances to a deterioration of immune responses specific to HCV. The increased hepatic fibrosis and inflammation seen in HIV and HCV coinfection might be explained by these synergistic effects. The coinfection of viruses (HIV and HCV) induces a contentious virulent epidemic. Recently, the interest to detect and to advance HIV therapy have been increased due to the several diagnostic tools. As the HIV infection develops in a concurrent and efficient coinfection with HCV having abnormal Alanine transaminase (ALT/SGPT). The primary HIV serological diagnostic test HIV/RNA reported in predominantly lymphoid tissues and gastrointestinal tract. Microscopic blood smear showed the manifestation of coinfection [*Figure 1: Microscopic Blood Smear of co-infection*].



(a) (b)

Figure 1: Microscopic Blood Smear of co-infection

In this paper, several HIV patients were subjected to screening while six confirmed patients with hepatitis C (HCV infection positive). These patients were then elected for the hematological analysis. The plasma of each of the patients had collected four times (on alternate days) in eight days. The samples collected each day were deliberated as a replicate and thus, a total of four replicates of each patient were organized in this way. The reflective study of the samples was based on measuring the approximate hematological values. Besides, it approves the severity of HIV sero-pervasiveness between the HCV patients (chronic active). The HCT values show blood diseases because of their unusual levels (Table 1) between the HIV along with hepatitis C virus coinfected patients than normal patients. The complete blood count (CBC) of elected confirmed patients (five male and one female) has indicated specific abnormalities for hemoglobin, hematocrit, RBC count, platelet count, and AMC (absolute monocyte count) (Table 1). The parameters including Hb, RBCs, and hematocrit were lower while greater for the PLT count. In complete blood count, a negative interrelationship has been detected between the hemoglobin levels and PLT count. The CBC variables indicate that the patient-5 have more HB (17.18±0.149), along with another patient-1 and patient-2 (Table 1). As the Hb values are essentially distinct from the healthy persons, it indicates that HIV/HCV coinfected patients are anemic [22]. The HIV/HCV co-infected patients show abnormal values CBC. The anomalous parameters of MO, hemoglobin, HCT along with MCV in patients displayed liver failure (acute) along with several complexities of kidneys well [23]. These abnormalities in the serological values may be interconnected with cell fibrosis in the other body organs along

with the liver, kidney, etc. The high parameters of ALT of HIV and HCV coinfected patients shows the strong connections for the appropriate discovery of the levels of hepatic fibrosis and infection rate (Table 1). The HIV/HCV coinfected individuals have increased ALT values than the fibrosis normal or reference (up to 36 uL^{-1} for female and up to 42 uL^{-1}) to abnormal mild-moderate (higher than normal) and abnormal severe ranges observed in patient three and four as shown in (Table 1). The results from CBC (complete blood count) to serological conclusions have indicated that HIV and HCV coinfection is more related with increased liver fibrosis advancement and greater liver decompensation rates and mortality related to HCV mono-infection, and hepatic disorder is a dominant motive of non-AIDS associated death between infected patients of HIV [24]. In the liver as well as in the heart, higher levels of cell fibrosis are induced because of the delay in the detection and the cure of both viral coinfection in the patients. The HIV along with the HCV induction is much more difficult to treat. As the level of fibrosis in hepatic cells raised to an abnormal stage in coinfection that's why it is fatal for the infected person[25].

Table 1: Comparative analysis of hematological (complete blood count) parameters of the confirmed HIV/HCV co-infected patients in comparison to standard normal reference values for healthy individuals.

No.	Parameters	Patient-1	Patient-2	Patient-3	Patient-4	Patient-5	Patient-6
01	**RBCs (10^{12}/L) (3.46-5.07)**	6.045±0.072	4.963±0.066	5.083±0.069	5.487±0.055	6.983±0.068	5.374±0.059
02	**HB (g/dL) (13.20-16.3)**	15.63±0.279	15.66±0.367	11.89±0.264	12.78±0.581	17.38±0.159	10.45±0.475
03	**NEU (%) (4.0-10.0)**	42.38±0.289	47.35±0.164	49.50±0.236	51.70±0.217	47.83±0.354	43.20±0.397
04	**MO (%) (4.40-12.13)**	7.95±0.134	13.23±0.125	5.473±0.121	8.254±0.254	9.155±0.195	5.398±0186
05	**EOS (%) (1-6)**	02.65±0.063	04.80±0.210	01.60±0.133	02.85±0.144	02.43±0.254	03.34±0.187
06	**LY (%) (20.27-55.48)**	39.60±0.432	41.80±0.228	42.73±0.252	39.88±0.232	39.88±0.212	39.58±0.272
07	**BAS (%) (0-1)**	0.630±0.046	0.245±0.045	0.165±0.047	0.450±0.042	0.300±0.048	0.470±0.041
08	**WBCs (10^9/L) (3.80-11.20)**	7.350±0.143	5.325±0.175	4.550±0.154	8.575±0.175	4.175±0.135	7.145±0.135
09	**PLT (10^8/µL) (150-400)**	153.0±2.846	86.00±2.345	126.0±2.140	173.5±2.734	139.5±2.724	93.5±2.744
10	**MCV (fl) (66.06-95.60)**	83.65±0.639	87.95±0.454	78.23±0.283	84.80±0.611	82.26±0.534	89.26±0.288
11	**HCT (%) (41.9-48.7)**	50.60±0.255	43.48±0.813	41.25±0.310	45.43±0.263	49.56±0.245	44.76±0.215
12	**MCH (pg) (21.10-31.23)**	28.65±0.232	26.00±0.395	25.68±0.264	28.95±0.266	25.35±0.233	29.75±0.253
13	**MCHC (g/dl) (28.70-34-60)**	34.25±0.260	34.25±0.340	31.33±0.121	34.65±0.230	34.35±0.210	34.65±0.260
14	**ALT/SGPT (UL^{-1}) (F= ^36 M=^42 UL^{-1})**	57 M	24 M	63 M	77 M	61 M	61 F

4. Conclusion

This paper indicates, HCV and HIV co-infection rate is escalating constantly in persons belonging to poor socioeconomic society. Likewise, pervasiveness also interacts with the literacy rate. The main goal of this paper is to locate the high predominant region of the country and to capture the consideration of the scientists and health-related companies to the trending issue. The HCV/HIV coinfection escalates hepatic cell fibrosis rate. The HCV and HIV co-infection reasonably changes the levels of Hb, RBC, lymphocytes, HCT, specifically ALT between the patients significantly. The HIV/HCV co-infected patients must survive with bloodlessness (anemia), bacteremia, and other infections, etc. Considerable awareness plans and other precautionary measures should be taken against this alarming co-infection in the study region.

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