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Comparative study of the frequency of occurrence of HBeAg, anti-HBe, anti-HIV and anti-HCV in pre-icteric, icteric and post-icteric HBsAg seropositive patients

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Abstract
Jaundice is associated with hepatitis B virus infection due to the destruction of the infected hepatocytes and intrahepatic-cholestasis caused by the scars of the healed hepatocytes. Hepatitis B patients that have never had jaundice are referred to as preicteric patients, while those that have recovered from jaundice are referred to as posticteric hepatitis patients. Those ones suffering from jaundice are referred to as icteric patients. This research work was designed to determine and compare the serological profiles of HBsAg seropositive patients. It will also be used to determine the relationship between jaundice and hepatitis B virus co-infection with HIV. The frequency of hepatitis B virus co-infection with HIV was found to be higher in hepatitis B patients that have had or are having jaundice compared to those that have never had jaundice. Frequency of Hepatitis B virus co-infection with HIV or HCV was found to be more in rural patients than in the urban patients and in rural female patients than the males. The frequency of hepatitis B patients co-infected with HIV was higher than those that were co-infected with HCV. Frequency of anti-HBe was higher in rural females than the males and urban males than the female patients; higher frequency of this antibody was also found in urban patients than the rural patients. Rural patients that expressed serum HBeAg were more than the urban patients were. However, more of the urban patients expressed anti-HBe compared with those in the rural area. Higher frequency of occurrence of HBeAg was found in icteric and preicteric patients than the posticteric patients and higher incidence of anti-HBe in posticteric patients than the pre and icteric patients were reversed during the second bleeding of the patients. Furthermore, the anti-HCV was found to be more in posticteric than the icteric patients were and none of the preicteric patients expressed anti-HCV. More anti- HIV was found in post icteric than the icteric patients were. Higher frequency of occurrence was also found in icteric than the preicteric patients.

Keywords: Jaundice; HBeAg; anti-HBe; anti-HIV; anti-HCV; HBsAg.

Introduction

The causative agent of hepatitis B is a viral particle known as hepatitis B virus (HBV). Hepatitis B virus (HBV) is a small double stranded DNA virus composed of an outer envelope containing hepatitis B surface antigen (HBsAg) and an inner nucleocapsid consisting of hepatitis B envelope antigen (HBeAg) and hepatitis B core antigen (HBcAg). Hepatitis B virus is a hepatotropic virus that replicates in the liver and causes hepatic dysfunction (Loguercio et al., 1997; Lai et al., 2003). Jaundice is associated with the pathophysiology of hepatitis B, because of the intra-hepatic cholestasis caused by the scars from damaged hepatocytes due to immune response to the presence of hepatitis B virus in liver cells, though the organism itself is not cytophatic (Ryan and Ray, 2004).

During hepatitis B infection, many virus particles are released from infected liver cells, resulting in large amount of viral antigen entering the blood. Hepatitis B surface antigen (HBsAg) is present in about 2 weeks before the onset of symptoms and persists throughout the course of the disease (Cheesbrough, 2002). At the recovery, it declines and is no longer detectable after 4 – 5 months. Persistence of HBsAg beyond six months indicates chronic infection or carrier state (Lok and McMahon, 2001: Cheesbrough, 2002). Detectable antigens of hepatitis B virus also include; hepatitis B envelope antigen (HBeAg) a secreted product of the nucleocapsid gene of hepatitis B virus during chronic hepatitis B virus infection and its presence indicates that the virus is replicating and the infected individual has a high level of hepatitis B virus. The other type of the antigen is hepatitis B core antigen (HBcAg) which is secreted by inner nucleocapsid core that encases the genome (Lai et al., 2003). Structural proteins such as pre-S1 and pre-S2 are regions involved in hepatitis B virus binding and entry into the hepatocytes. Hepatitis B envelope antibody (anti-HBe) is produced by immune system temporarily during acute

hepatitis B virus infection or consistently during or after a burst of viral replication. It may be found in the convalescence stage and often in chronic hepatitis and the carrier state (Lai et al., 2003). It is believed that the antibody response to viral envelope antigens contributes to clearance of the virus and that cytotoxic T cells mediate viral clearance by killing the infected cell (Francis, 1999).

Alao et al. (2009) analyzed the results of HBsAg screening among blood donors in General Hospital, Otukpo, an urban area of Benue State, over a three-year period (2006 – 2008), with a view to establishing the prevalence rate in this region of the Middle Belt of Nigeria. 2,500 samples were screened for HBsAg over the three-year period. The seropositivity rate among donors tested was found to be 20%. Hepatitis B virus has the same routes of transmission as Human Immunodeficiency Virus (HIV) and Hepatitis C Virus therefore the co-infection of one or both of the agents with HBV is possible. It has been demonstrated in clinical studies that HIV infection causes a more rapid progression of chronic hepatitis C / hepatitis B to cirrhosis and liver failure (Ryan and Ray, 2004; Olusoji et al., 2006; CROI, 2008). This research work was therefore designed to compare the frequency of occurrence of HBeAg, anti-HBe, anti-HIV and anti-HCV in the preicteric, icteric and posticteric HBsAg seropositive patients.

Materials and Methods

Study Area / Volunteers

Two hundred and eleven critically ill HBsAg seropositive patients aged 5 – 80 years were initially recruited but only one hundred and fifty critically ill HBsAg seropositive patients aged 5 – 79 years were successfully monitored. The one hundred and fifty [150] critically ill HBsAg seropositive patients include 50 Preicteric HBsAg seropositive test participants (patients without any episode of jaundice) aged 5 – 78 years (25 females; 25 males), 50 Icteric HBsAg seropositive patients (patients manifesting the first episode of jaundice on recruitment) aged 7 – 79 years (25 females; 25 males) and 50-Posticteric HBsAg seropositive patients/test aged 5 – 79 years (that is patients that have had at least one previous episode of jaundice but were not jaundice on recruitment). Forty three critically ill HBsAg seropositive patients (Female = 21; Male = 22) aged 6 – 76 years living in Ibadan for at least five years classified into preicteric (n=13;aged 6 - 72 years), icteric (n = 16; aged 6 - 76 years) and posticteric (n = 14; aged 6 - 76 years) patients were also investigated and the results obtained were used as urban reference data.

Rural subjects were recruited from Saki-West/Saki-East/ATISBO federal constituency of Oyo North Senatorial District, Nigeria Volunteers that have been living in this area for not less than 5 years were studied. The major complaints of the critically ill Preicteric and Posticteric patients volunteered to the clinician and the researcher were malaise, abdominal tenderness/pain, prolong headache, nausea/vomiting and loss of appetite. About 58% of the critically ill icteric patients gave these complaints in addition to the development of jaundice. However, these complaints persist in about 65% of the patients investigated. Saki-West/Saki-East/ATISBO federal constituency is a rural community. It comprises three local government areas. The local governments include Saki–West, Saki- East and ATISBO local government areas. It is located in the Northern part of Oyo–State, Nigeria and shares borders with Kwara-State, Nigeria and the Republic of Benin. It consists of 319 settlements.

Recruitment of Hepatitis B Patients

Hepatitis B surface antigen seropositive patients were recruited from 20 hospitals/clinics in Saki-West/Saki-East/ATISBO federal constituency after the ethical approval of the hospitals and the consent of the parents of the children parent aged 5 – 15 years and that of the adult patients have been obtained.

Sample collection, separation and preservation

Ten milliliters of blood was collected from each of the volunteers (tests and the controls) into a plain specimen bottles .The samples were allowed to clot and the serum was extracted. Serum extracted from each of the volunteers was used for determination of HBsAg, anti-HCV, anti-HIV, HBeAg and anti-HBe. Volunteers were all pre and post-test counseled. Blood samples were collected from each of the patients initially on recruitment and at least six months after the initial bleeding and investigations.

Ethical Issue

The sample collection was carried out after the approval of research/ethical committee of the hospital and due consent of the patients in the

hospitals / clinics where there is no research and ethical committee.

Methods

a. Patients were pre and post - test counseled to be able to meet the psychological needs of the test and control volunteers before and after the test.

b. Hepatitis B surface antigen (HBsAg) test was carried out to recruit the test and normal control volunteers by using a one step enzyme immunoassay technique of the sandwich type for the detection of HBsAg in human serum or plasma using the reagent kit of BIO–RAD Raymond Poincare, Marnes La Coquette.
Principle: MONOLISA AgHBs PLUS is a one-enzyme technique of the sandwich type using three monoclonal antibodies selected for their ability to bind themselves to the various subtypes of HBsAg now recognized by World Health Organization. The solid phase is made up of 12 strips of 8 polystyrene wells coated with the first monoclonal antibody. The two other monoclonal antibodies are bound to the peroxidase.

c. HBeAg and anti-HBe tests were carried out on the test and control volunteers by enzyme immunoassay for the determination of Hepatitis B e antigen and antibody in human plasma and sera using the reagent kit of:

DIA.PRO
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Via Columella, Milano – Italy

Principle of the test

i. HBeAg; HBeAg if present in the sample, is captured by a specific monoclonal antibody, in the 1st incubation. In the 2nd incubation, after washing, a tracer, composed of a mixture of two specific anti-HBeAg monoclonal antibodies, labeled with peroxidase, is added to the microplate and binds to the captured HBeAg. The concentration of the bound enzyme on the solid phase is proportional to the amount of HBeAg in the sample and its activity is detected by adding the chromogen / substrate in the 3rd incubation. The presence of HBeAg in the sample is determined by means of a cut – off value that allows for the semi-quantitative detection of antigen.

ii. Anti – HBeAg; anti – HBe if present in the sample compete with recombinant HBeAg preparation for a fixed amount of an anti- HBeAg antibody, coated on the microplate wells. The competitive assay is carried out in two incubations, the first with the sample and recombinant HBeAg, and the second with a tracer, composed of two anti-HBeAg monoclonal antibodies, labeled with peroxidase. The concentration of HBeAg specific antibodies in the sample is determined by means of a cut – off value that allows for the semi-quantitative detection of anti – HBe antibodies.

d. HIV screening was carried out on the test and control volunteers after pre-test counseling by using the reagent kit of Abbot Laboratories Co. Ltd., Japan. The Abott Determine HIV-1/2 is an in-vitro, visually read qualitative immunoassay for the detection of antibodies to HIV-1 and HIV-2 in human serum plasma or whole blood. The test is intended as an aid to detect antibodies to HIV-1/HIV-2 from infected individuals.

Biological Principle of the Procedure
Determine HIV – 1/2 is an Immuno-chromatographic test for the detection of antibodies to HIV-1/HIV-2. Sample is added to the sample pad. As the sample migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid – antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient window site. If antibodies to HIV-1 and or HIV-2 are present in the sample, the antibodies bind to the antigen – selenium colloid and to the antigen at the patient window forming a red line at the patient window site. If antibodies to HIV-1 and HIV-2 are absent, the antigen – selenium colloid flows past the patient window and no red line is formed at the patient window site. To ensure assay validity a procedural control bar is incorporated in the assay device.

e. The HIV confirmatory test was carried out on all the volunteers by Western blot assay, using reagent kit of Immunoetics, Inc., 27 Dryclock Avenue, Boston, USA. http//www.immunoetics.com

Principle: The QualicodeHIV1/2 kit is a

qualitative immunoblot assay based on the Western Blot principle. The assay is performed on immunoblot membrane containing HIV-1 viral lysate protein (HLTVIII B stain) and a recombinant HIV-2 protein. To produce the membrane HIV-1 viral protein are fractionated according to molecular weight on a Polyacrylamide slab gel (PAGE) in the presence of Sodium Dodecyl Sulphate (SDS). The separated HIV-1 is then transferred through electrophoretic blotting from the gel to a nitrocellulose membrane two bands are directly striped on the membrane.

1) A control band containing staphylococci protein A.
2) A recombinant HIV-2 specific envelope antigen.

The membrane is then cut into strips for individual sample testing. During the procedure, the strips containing HIV1/2 are reacted with the serum specimen and washed to remove unbound antibodies. Visualization of human globulin specifically bound to HIV-1 or HIV-2 proteins is performed by sequential reaction with goat anti-human immunoglobulin-alkaline phosphatase conjugate and BCIP/NBT substrate. Band positions are compared to those on the Reference Card developed using the HIV -1/2 Positive Control Serum. The intensity of the bands is monitored by comparison to the HIV-1/2 Weakly Reactive Control.

f. Anti – HCV test on all the volunteers was carried out by a third generation enzyme immunoassay for the determination of antibodies to hepatitis C virus in serum and plasma using the reagent kit of

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Principle of the test: Microplates are coated with HCV specific antigens derived from core and 'ns' regions encoding for conservative and immunodominant antigenic determinants (Core, NS3, NS4 and NS5). The solid phase is first treated with the diluted sample and HCV antibody are captured, if present, by the antigens. After washing out all other components of the sample, in the 2nd incubation bound anti-HCV are detected by the addition of anti – human immunologic G and M antibody, labeled with peroxidase. The enzyme captured on the solid phase, acting on the substrate / chromogen mixture, generates an optical signal that is proportional to the amount of anti – HCV antibodies present in the sample.

g. *Data Analyses*

It was carried out using simple percentage, and mean as described by Norman (1994).

Results

The serologic pattern observed in the overall patients during the 1st bleeding include; 150[100%] HBsAg; 145[96.7%] HBeAg; 22[14.7%] anti-HIV; 17[11.3%] anti-HCV and 5[3.3%] anti-HBe (Table 3). The frequencies of occurrence of the serologic markers during 2nd bleeding include; 139[92.7%] HBsAg, 94[62.7%] anti-HBe, 56[37.3%] HBeAg, 26[17.3%] anti-HIV, and 17[11.3%] anti-HCV (Table 4). During 2nd bleeding, 11 [7.3%] of the previously HBsAg seropositive patients were found to be HBsAg seronegative. Antibody to 'e' antigen was found in their respective serum. None of them was found to have any of HBeAg, anti-HCV and anti-HIV in the serum (Tables 1, 2, 3 and 4).

The serologic characteristics of the patients with respect to jaundice include; 49 (98%) HBeAg; 1 (2%) Anti-HBe; 0 (0 %) Anti-HCV; 5 (10%) Anti-HIV found in preicteric patients; 49 (98%) HBeAg; 1 (2%) Anti-HBe; 6 (12%) Anti-HCV; 8 (16%) Anti-HIV found in icteric patients and 47 (94%) HBeAg; 3 (6%) Anti-HBe; 11 (22%) Anti-HCV; 9 (18%) Anti-HIV were found in posticteric patients during the 1st bleeding (Table 1).

The serologic characteristics of the patient with respect to jaundice include; 13 (26%) HBeAg; 37 (74%) Anti-HBe; 0 (0 %) Anti-HCV; 7 (14%) Anti-HIV found in preicteric patients; 21 (42%) HBeAg; 29 (58%) Anti-HBe; 6 (12%) Anti-HCV; 8 (16%) Anti-HIV found in icteric patients and 22 (44%) HBeAg; 28 (56%) Anti-HBe; 11 (22%) Anti-HCV; 11 (22%) Anti-HIV were found in posticteric patients during the 2nd bleeding (Table 2). The frequencies of occurrence during the 1st bleeding of anti-HCV [58.8% Vs 41.2%] and anti-HBe [60% Vs 40%] were higher in HBsAg seropositive female patients than their male counterparts. The frequency of occurrence of anti-HBe in urban patients was higher than that of the rural HBsAg seropositive patients [23.3% Vs 3.3%] whereas the frequencies of occurrence of serum anti-HCV and-HIV and HBeAg in rural patients were found to be higher than the results obtained from the urban HBsAg seropositive patients [12 Vs 7%; 14.7% Vs 2.3% and 85.3 Vs76.8% respectively] (Table 5).

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and anti-HBe [60% Vs 40%] were higher in HBsAg seropositive female patients than their male counterparts. During 2nd bleeding, 11[7.3%] of the previously HBsAg seropositive patients were found to be HBsAg seronegative. Antibody to 'e' antigen was found in their respective serum. None of them was found to have any of HBeAg, anti-HCV and anti-HIV in the serum. The frequency of hepatitis B virus co-infection with HIV was found to be higher in hepatitis B patients that have had or are having jaundice compared to those that have never had jaundice. Frequency of Hepatitis B virus co-infection with HIV or HCV was found to be more in rural patients than in the urban patients and in rural female patients than the males. The frequency of hepatitis B patients co-infected with HIV was higher than those that were co-infected with HCV. Frequency of anti-HBe was higher in rural females than the males and urban males than the female patients; higher frequency of this antibody was also found in urban patients than the rural patients. Rural patients that expressed serum HBeAg were more than the urban patients were. However, more of the urban patients expressed anti-HBe compared with those in the rural area.

Higher frequency of occurrence of HBeAg was found in icteric and preicteric patients than the posticteric patients and higher incidence of anti-HBe in posticteric patients than the pre and icteric patients were reversed during the second bleeding of the patients. Furthermore, the anti-HCV was found to be more in posticteric than the icteric patients were and none of the preicteric patients expressed anti-HCV. More anti- HIV was found in post icteric than the icteric patients were. Higher frequency of occurrence was also found in icteric than the preicteric patients.

Discussion

The higher frequency of occurrence of anti-HCV found in females than male patients (Table 3) agrees with the report of Mahboob et al (2003) that of the 43 (23.4%) with anti-HCV, females were predominantly affected with female to male ratio of 5.1 : 1. Higher frequency of anti-HBe in the females than the male patients is also consistent with the report of Mario et al (1982) that males showed a greater prevalence of anti-HBs and anti-HBc, while anti-HBe was more common in females; however, higher prevalence of anti-HBs; anti-HBc and anti-HBe was found in the older age groups.

The serologic pattern observed in the overall patients during the 1st bleeding include: 150(100%) HBsAg, 145(96.7%) HBeAg, 22(14.7%); anti-HIV, 17(11.3%); anti-HCV, 5(3.3%); anti-HBe, (Table 3). According to Anna and Michael (2007) of approximately 360 million people in the world chronically infected with hepatitis B virus (HBV), 65 million reside in Africa. Thus, Africa, with 12% of the world's population, carries approximately 18% of the global burden of HBV infection, with hepatocellular carcinoma and cirrhosis accounting for 2% of the continent's annual deaths (Anna and Michael, 2007). The report of Anna and Michael (2007) could not be associated with the frequency of occurrence of HBsAg as this is one of the criteria for recruiting the patients for the study, though serum HBsAg has been reported to persist in patients with an impaired immune response (Kumar and Clark, 2002).

Kobayashi et al (2002) reported that out of the 637 patients, 323 (50.7%), 51 (8.0%) and 97 (15.2%) were positive for HIV Ab, HBsAg and HCV Ab, respectively. While prevalence of HBV was significantly higher in HIV-positive patients (10.5%; 34/323) than in HIV-negative ones (5.4%; 17/314) (p=0.026), prevalence of HCV was significantly lower in HIV-positive patients (12.1%; 39/323) than in HIV-negative ones (18.5%). It was also reported that two patients were triply infected with HIV, HBV and HCV, and one patient was dually infected with HBV and HCV. They suggested that HIV infection could be a co-factor for HBV infection, but that HCV infection may occur independently of HIV infection. This study also revealed evidence of HBsAg seropositive patients co-infected with HIV or HCV (Table 4).

The 11 (7.3%) of the previously HBsAg seropositive patients that were found to be HBsAg seronegative during the 2nd bleeding (Tables 1,2,3 and 4) . This is attributable to the fact that HBsAg could be cleared after at least 6 months (Ryan and Ray, 2004). Higher frequency of occurrence of HBeAg was found in icteric and preicteric patients than the posticteric patients and higher incidence of anti-HBe in posticteric patients than the pre and icteric patients. This could be associated with the severity of hepatitis B in pre and icteric patients as HBeAg has been associated with high level of Hepatitis B virus and severity of hepatitis B (Ryan and Ray, 2004). Moreover, during the second bleeding of the patients, the results obtained were the reverse of the above. These could be a pathological condition, altered immunity, co-infection, clinical

interventions and body normal reaction to infections (Kumar and Clark, 2002).

Furthermore, the anti-HCV was found to be more in posticteric than the icteric patients were and none of the preicteric patients expressed anti-HCV. More anti- HIV was found in post icteric than the icteric patients were. Higher frequency of occurrence was also found in icteric than the preicteric patients. These findings could be attributed to the fact that co-infection of Hepatitis B virus with HCV or HIV could be found in hepatitis B patients as they share common routes of infection (Ryan and Ray, 2004; CROI, 2008). The co-infection is also consistent with the reports of Christy et al (2004), Mustapha and Jubrin (2004) and Uneke et al (2005).

Higher frequency of anti-HBe in the males than the female patients is not in agreement with the report of Mario et al (1982) that males showed a greater prevalence of anti-HBs and anti-HBc, while anti-HBe was more common in females. It is however in agreement with the report of Uneke et al (2005) that found a slightly higher HBsAg seroprevalence in the males (14.6%) than females (12.9%) of the blood donors. Among the HIV-infected patients, the males had considerably higher HBsAg seroprevalence than the females (31.8 vs 22.1%) with the highest prevalence of HBsAg occurring in the 51-60 years age group (44%), followed by those of 31-40 years (28.2%). The HIV co-infection obtained in this study is compatible with the report of Uneke et al (2005) that confirmed the high endemicity of HBV infection in Jos, Nigeria and the significantly greater prevalence of HBV infection among HIV -infected patients than among blood donors (Uneke et al, 2005) and that HBsAg seropositivity was greater among HIV-infected patients than among blood donors. Uneke et al (2005) also reported that this is consistent with findings from Florianopolis-Brazil where 18.9% of HIV-infected persons and 0.7% of blood donors. Jesse et al (2008) also reported gender stratification showed that males had a higher prevalence of HBV (17.9% vs 10.7%). The HBsAg seropositivity of 14.3% among blood donors and 25.9% among HIV-infected patients confirmed that Jos, Nigeria is still endemic for HBV-infection. Their results were in conformity with earlier reports from community and hospital based studies in some parts of Nigeria, which showed high prevalence HBsAg ranging from 7.4-26% (Ekpo et al, 1995). Frequencies of occurrence of HCV - HBV (11.3 %) and HBV – HIV (14.7%-1st bleeding and 17.3%-2nd bleeding) co-infections found in this study is higher than a relatively high prevalence of HBV - HIV (11.9%) and HCV – HIV (4.8%) co-infected patients reported by Jesse et al (2008).

The frequency of occurrence of anti-HBe in urban patients was higher than that of the rural HBsAg seropositive patients (23.3% Vs 3.3%) whereas the frequencies of occurrence of serum anti-HCV and -HIV and HBeAg in rural patients were found to be higher than the results obtained from the urban HBsAg seropositive patients (12 Vs 7%;14.7% Vs 2.3% and 85.3 Vs76.8% respectively) (Table 5).This could also be attributed to access difficulties to quality health facilities .This report is not in agreement with the report of Özdemir et al (2007) that the presence of HBsAg and Anti-HCV was similar in patients living in rural and urban areas (Ozdemir et al, 2007). The major factors that cause non-attendance of the available services in the rural area included the high costs of drugs (29%) and service charges (19%), easy access to traditional healers (39%) and difficulty in getting transport to a health facility (30%). There were also varying degrees of socio-economic and geographic inequalities in treatment expenditures, providers seen and payment modalities that were used. the poorest SES [socio-economic status] group and rural dwellers are the major sufferers of inequality and this could be mitigated through improved provision of primary healthcare services in rural areas and initiation of exemptions, vouchers and other pro-poor payment strategies for the poorest SES groups (Obinna et al., 2005). The above results in rural patients compared with the urban patients could also be attributed to the report of Salim et al (1986) that attributed higher incidence of HBsAg seropositive patients in rural than the urban inhabitants to rural cultural practices like ear piercing, scarification and poor control of mosquito as it could transmit hepatitis B virus.

Conclusion

This recent study has therefore been used to compare the frequency of occurrence of HBeAg, anti-HCV,anti-HBe and anti-HIV in preicteric, icteric and posticteric HBsAg seropositive patients. The result obtained in this study showed variations in the frequency of occurrence of HBeAg, anti HCV, anti-HBe, and anti-HIV in preicteric, icteric and post icteric HBsAg seropositive patients due to the evidence of co-infection and pathological changes.

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Tables follow……

Table 1: Serologic characteristics of preicteric, icteric and posticteric patients (1st bleeding).

Serology	Preicteric N =50	Icteric N =50	Posticteric N =50
Anti-HCV	0 (0 %)	6 (12%)	11 (22%)
Anti-HIV	5 (10%)	8 (16%)	9 (18%)
Anti-HBe	1 (2%)	1 (2%)	3 (6%)
HBeAg	49 (98%)	49 (98%)	47 (94%)

Table 2: Serologic characteristics of preicteric, icteric and posticteric patients (2nd bleeding).

Serology	Preicteric n =50	Icteric n =50	Posticteric n =50
Anti-HCV	0 (0 %)	6 (12%)	11 (22%)
Anti-HIV	7 (14%)	8 (16%)	11 (22%)
Anti-HBe	37 (74%)	29 (58%)	28 (56%)
HBeAg	13 (26%)	21 (42%)	22 (44%)

Table 3: Frequency of occurrence of anti –HCV, anti- HIV, anti-HBe, HBsAg and HBeAg in the patients during the 1st bleeding.

Serology	Male +VE	Female +VE
Anti HCV [n= 17]	7(41.2%)	10(58.8%)
Anti HIV [n = 22]	11(50%)	11(50%)
Anti HBe [n = 5]	2(40%)	3(60%)
HBsAg [n = 150]	75(50%)	75(50%)
HBeAg [n = 145]	72(49.7%)	73(50.3%)
	Male –VE	**Female –VE**
Anti HCV[n =133]	68(51.1%)	65(48.9%)
Anti HIV[n= 128]	64(50%)	64(50%)
Anti HBe [n = 145]	73(50.4%)	72(49.6%)
HBsAg [n = 150]	0(0%)	0(0%)
HBeAg [n = 5]	2(40%)	3(60%)
	Total +VE	**Total –VE**
Anti HCV [n = 150]	17(11.3%)	133(88.7%)
Anti HIV [n = 150]	22(14.7%)	128(85.3%)
Anti HBe [n = 150]	5(3.3%)	145(96.7%)
HBsAg [n = 150]	150(100%)	0(0%)
HBeAg [n = 150]	145(96.7%)	5(3.3%)

NB:- + VE – Positive; -VE – Negative

Table 4: Frequency of occurrence of anti –HCV, anti- HIV, anti – HBe, HBsAg and HBeAg in the patients during the 2nd bleeding.

Serology	Male +VE	Female +VE
Anti-HCV [n = 17]	8(47.1%)	9(52.9%)
Anti-HIV [n = 26]	11(42.3%)	15(57.7%)
Anti-HBe [n = 94]	45(47.9%)	49(52.1%)
HBeAg [n = 56]	29(51.8%)	27(48.2%)
HBsAg [n =139]	66(47.5%)	73(52.5%)
	Male -VE	**Female –VE**
Anti-HCV [n =133]	65(48.9%)	68(51.1%)
Anti-HIV [n =124]	62(50%)	62(50%)
Anti-HBe [n = 56]	28(49.1%)	29(50.9%)
HbeAg [n =94]	49(52.1%)	45(47.9%)
HbsAg [n =11]	7(63.6%)	4(36.4%)
	Total +VE	**Total -VE**
Anti-HCV [n = 150]	17(11.3%)	133(88.7%)
Anti-HIV [n =150]	26(17.3%)	24(82.7%)
Anti-HBe [n =150]	94(2.7%)	6(37.3%)
HBeAg [n =150]	56(7.3%)	4(62.7%)
HBsAg [n =150]	139(2.7%)	1(7.3%)

NB:- + VE – Positive; -VE – Negative

Table 5: Results of the comparative study of the serologic pattern of urban and rural patients.

Serology	Total +VE	Male +VE	Female +VE
Urban patients Anti-HCV	3(7.0%)	2(66.7%)	1(33.3%)
Rural patients	18(12%)	10(55.6%)	8(44.4%)
Urban patients Anti-HIV	1(2.3%)	1(100%)	0(0%)
Rural patients	22(14.7%)	11(40%)	11(58%)
Urban patients Anti-HBe	10(23.3%)	4(40%)	6(60%)
Rural patients	5(3.3%)	3(60%)	2(40%)
Urban patients HBeAg	33(76.8%)	18(54.6%)	16(48.5%)
Rural patients	128(85.3%)	64(50%)	64(50%)
Urban patients HBsAg	43(100%)	21(48.8%)	22(51.2%)
Rural patients	150(100%)	75(50%)	75(50%)

NB:- + VE – Positive; -VE – Negative