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Research Article

Epidemiological survey for the identification and diagnosis of viral hepatitis B and C, using Enzyme Linked Immunosorbent Assay (ELISA) technique at Al-Kut city/Iraq, and the surrounding area

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ABSTRACT

Viral hepatitis diseases, especially hepatitis B virus (HBV) and hepatitis c virus (HCV) are public health problem worldwide. Both types HBV and HCV are common causes of acute and chronic liver diseases. HBV and HCV infections are the most important infections transmitted by the parenteral route in patients. This study aimed to known the epidemiology, prevalence rate of infection with HBV, and HCV, and relationship with sex, and age in Al-Kutcity/Iraq between 2014 and 2015 years. A field survey had been carried out to investigate of infection in patients of Al-Karameh Teaching Hospital Laboratory (KTHL),held from January-2014 to January 2015. Blood sample were examined 10744, (5570) from males and (5174) from females of different years of age (1-87 years). Each specimen was examined and identified by serological test. They were screened for Hepatitis B surface antigen (HBs Ag) and anti-Hepatitis C (Anti HCV) by serological test e.g. Enzyme Linked Immunosorbent Assay (ELISA). In conclusion, the prevalence of HBsAg and anti-HcAg in patients is very high. It has been recommended that properly screened blood using a reliable technique,such as ELISA.

Keywords: Hepatitis B, Hepatitis C, ELISA technique.

INTRODUCTION

Viral hepatitis cause dangerous health problems in terms of their predominant and affecting several hundreds of millions of people[1]. There are several types of hepatitis (A-G), and the important among of them; hepatitis B and C. Lee *et al.* [2], and Sagnelli *et al.* [3] demonstrated that a sub-group of patients may have both hepatitis; B and C infections concomitantly. The rate of occurrence of hepatitis B and C occurring at the same time has been recorded from various countries and varies from a range of 1-15 %. Furthermore, one to seven It has been proposed that the real predominant is much higher in the regions where hepatitis B is mild to highly endemic, and that the existence of hepatitis B-DNA can be detected in blood serum. The jail community is at huge risk for acquiring infectious diseases, such as hepatitis B and hepatitis C. Furthermore, some investigations have identified these viruses as

important causes of deaths related to chronic liver diseases in prisoners [4, 5]. Numerous features of confined people, involving illicit drug use, low socioeconomic status, and multiple sexual partners, are predictors of these disorders. Subsequently, most are already infected at the time of imprisonment, becoming a source of propagation and maintenance of these viruses in the jail setting [6].Kramvis and Kew [7], and Simmonds[8] mentioned that HBV and HCV have been classified into 8 genotypes (A-H), and 6 HCV genotypes (1-6) respectively, and multiple subtypes have previously been described. Viral hepatitis is a cause of considerable morbidity and mortality in the human population, both from acute and chronic infection complications which include, in the case of hepatitis B, C and D, chronic active hepatitis and cirrhosis[9]. Bhaumik*et al*. [10]; Modi and Feld [11] observed that these viruses are the three most recurrent chronic hepatitis infections all worldwide, and they share same track of transmission, with main three route of transmission; parenteral, sexual, and perinatal being the most common modes of acquiring these diseases, and therefore human immunodeficiency virus infection - hepatitis B and C viruses (HIV-HBV and HIV-HCV) coinfection and/or both are widespread. Locarnini, and Zoulim[12]; Ghani *et al*. [13]and Albert *et al*[14] reported that the genotypic groups have variable geographic distribution and have been used to trace transmission routes. Moreover, the genetic diversity of these viruses appears to intervention in the activity of anti-viral therapy.

However, both of HIV and HCV are consider RNA viruses, and HBV is consider a DNA virus; but they are all similar in terms of how high they replicate in the host body. On the other hand, exposure to these viruses is followed by an immune respond which differs noteworthy in its capability to clear the infection. Moreover, among the HIV patients, there are 24 million guessed to have chronic HBV coinfection, whilst 10-12 million are confected patients with HCV [15].Both hepatitis B and C virus infections are estimated that there are 350 and 130 million chronic carriers of causative agents, respectively, which are also at risk of developing chronic hepatitis, cirrhosis, and hepatocellular carcinoma [16, 17]. In addition, hepatitis C coinfection with human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS) is associated with accelerated progression to cirrhosis and thus a higher mortality rate [18].

In addition, a predestined one-third of the deaths in HIV patients are directly or indirectly related to hepatic diseases associated with HBV or HCV infections, which makes HBV and HCV a large problem in the HIV patients. The increase rate of coinfection with HBV and HCV in HIV patients have been variable in all over the world depending on the, risk groups, geographic regions and the type of exposure involved which may be various not only from country to country, but also in various regions of the same country[19,20]. Coinfections of both HBV and HCV with HIV have been associated with decreased survival, with a high risk of progression to severe hepatic diseases and an increased risk of hepatotoxicity associated with antiretroviral therapy. Coinfection with hepatitis may also complicate the management of HIV infection (CDC).

The purpose of this study to known the epidemiology, prevalence rate of infection with HBV, and HCV, and relationship with other parameters e.g. sex, and age.

MATERIALS AND METHODS

A total number of 3985 individuals were chosen randomly from Al-Karameh Teaching Hospital Laboratory (KTHL) in Al-Kut city. This study was conducted for the period fromJanuary-2014 to December-2015. A detailed questionnaire was filled With all necessary information blood sample was drawn by vein puncture using disposable 5 ml syringe, then the blood transferred into plain plastic test tube and left to clot at room temperature (20-25C°), then spun at 3500 rpm using ordinary centrifuge, finally the sera were collect and labeled and stored at freeze temperature(-20C°) for next test, for the assessment of presence of Hepatitis B surface antigen (HBsAg) and anti-Hepatitis B core antigen (anti-HBcAg). Enzyme Linked Immunosorbent Assay (ELISA) used for the detection of HBsAg and anti-HBCAg.

All samples were examined using ELISA; For HBs-Ag, kits were purchased from Omega diagnostics limited, pathozyme HbsAg, UK. For HCV Ab detection; Kits used were either from Ortho-Diagnostics, UK or Innogenetics, Innotest, Belgium according to the purchasing order. Five ml venous blood was collected from each patient. Clear serum was obtained by centrifugation. The serum was transferred to a disposable container for HBVAg and anti HCV antibodies determination using ELISA technique. Third generation enzyme immuosorbent assay EIA for screening for anti HCV using the commercially available kits. Third generation micro particle Enzyme Immunoassay (EIA) was used for HBsAg assessment Sera from all these groups of population were examined for HBsAg and HCV antibody. For HBsAg markers, ELISA techniques (Bio test) were used as a screening test which is later confirmed by RANDOX ELISA and it's a

confirmatory test. For detection of anti HCV Ab marker, (UBI 4.0) test was used as a screening test, which is, then validated by LiaTek ELISA test.

RESULTS

Generally, a total of (685) sample was positive from (3985) samples were chosen randomly from Al-Karameh hospital in Al-Kut city from January 2014 to December 2015.

The frequency of HBsAg was 86 and that of anti-HCV were599, the result was indicative of much higher probable prevalence of HCV amongst population than HBsAg. The results were high in 2015 in HBV infections 54 (62.8%), and were 324 (54.1%) in HCV in 2014. Moreover, the infections of HCV in both years were very highly (324 and 275 in 2014 and 2015 years respectively) compared with HBV (32 and 54 in 2014 and 2015 years respectively), as outlined in Tables 1 and 2.

The highest prevalence of HBV was detected in health care workers group 31 (36%), followed by the dialysis group 15 (17.4%) followed by the renal transplant 11 (12.8%) and dentistry groups 10 (11.6%). Furthermore, the highest prevalence rate of HCV was detected in the health care workers group 141 (23.5%), followed by the renal transplant 129 (21.6%), dialysis 97 (16.2%), and laboratory groups 92 (15.4%), as summarized in Table 3.

These variations in the prevalence of both; HBV and HCV among patients groups were not statistical significant.

Frequency of HBsAg was detected in both sexes; male and female. Male being more affected than female (80.2- 19.8%) in patient with hepatitis B. However, HCV was prevalence very high positivity and affected in male compare with female (62.1-37.9%), as clarified in Table 4

In addition, anti-HCV and HBV Ag sero-positivity was no significantly differences observed between males and females. The age range of all patients was 0-84 years. The highest prevalence of HBV was in the age of 24-35years 34 (39.5%) followed by age 12-23 years and36-47 years 18 (20.9%) for each; while the highest prevalence of HCV was in the age of 12-23 years 345 (57.6%) followed by age 0-11years 111 (18.5%), and 24-35 years 88 (14.7%). The statistical analysis show no significantly differences between the age patients and the types of all hepatitis (Tables 5 and 6).

DISCUSSION

Risk of infection by HBV and HCV remains a constant problem, not only for health care workers but also for patients. Infections with hepatitis disease; HBV and HCV pose dangerous healthcare disorder, particularly in developing countries. Recently, some of the developing countries started ambitious projects to combat these diseases [21]. Hussein [22] demonstrated that HBV vaccine in Iraq was added to the expanded program of vaccination in 2000.

In the current study, all patients were tested for HBsAg and anti HCV Abs by ELISA technique. In this technique, the prevalence of HBV and HCV was prevalent. This result of present study is comparable with Saeed*et al.* [23] and Soul*yet al.* [24] who found that prevalence of HBV and HCV in health care personnel inNorth West Frontier Province and IbnSina hospital, Rabat, Morocco respectively.

Moreover, Bakhshipour *et al.* [25] and Messina *et al.* [26] demonstrated that genotyping is significant due to its supply information as to strain difference and possibility association with infections severity. Furthermore, it is of epidemiologic value due to it sheds light on whether prevalent HCV strains are similar to that endemic in a specific area. The type 4 is most common genotype in some Asia's countries e.g. Iraq, Kuwait, Yemen, and Kingdom of Saudi Arabia.

On the other hand, Mostafa *et al.* [27] observed that HBV and HCV disease are still predominant in some North Africa developing countries, such as Egypt. The overall predominant of antibodies to HCV in the general population is nearly 15-20%, this apparently high spread of HCV disease in Egypt population is of significance, due to the possibility adverse effect of HCV on the public health of Egyptian communities. As well as, there is another study conducted by Mehmet *et al.* [28], who observed that the predomonent of HBV infections in the North Asia continent e.g. South Eastern region of Turkey is at an intermediate level. The ratio of HBs-Ag positivity that might indicate chronically HBV carriers was around 7% for this southeastern area, and this ratio was higher than in civilian communities.

Furthermore, there are some disorders in the urinary tract, such as chronic renal failure is by itself a risk factor for HBV and HCV diseases even if these patients are not on dialysis; hemodialysis on the other hand was associated with highest predominance for HCV antibody and for HBsAg. Various researches on predominance of HCV in these patients observed various results in the Land of the Arabian Peninsula (Kingdom Saudi Arabia) 50-90% [29], Europe (Romania) 91.7% and Iran 28.1%[30]. In the current study, the highest predominance HBV and HCV were showed in the health care workers followed by the dialysis group and those of renal transplant. Moreover, Mehmet *et al.*[28] mentioned that the education level, higher age, and male sex positive family history of liver diseases, such as jaundice

disease may be considered as a significant danger agents.

In fact, the main problem of different types of viral hepatitis in nosocomial infections around the world has not been enough studied. Although hospitalized patients overall and especially certain high risk groups among them, represent a possible source for viral hepatitis infections of medical, nursing and auxiliary personnel caring for them [31, 32]. Ayatollahi *et al.* [33] clarified that the predominance of hepatitis infection markers in nosocomial infections has been published, in isolated groups of high danger patients and/or in hospital workers in everywhere.

In this present study, with regard to sex; males and females, this comparable with the studied of Baha *et al.* [34] and Mutamuliza*et al.* [35], who reported that the results are also indicative of much higher possible spread of HCV amongst normal population than HBV. Forbi*et al.* [36] and Mutiat*et al.* [37] observed that these results indicated that male dominates female. It may be due to gender preference, commonly seen in access to health care facilities. The high predominant of HBV in age 24-35 years, anti-HCV predominance rises with age reaching the higher predominant in the age group of 12-23 years. As well as, the low level of predominance in children (0-11) years of age may be because of the effect of mother's immunity**.**

CONCLUSION

In summary, liver diseases associated with HCV and HBV is a growing problem in HIV positive individuals. In addition to more rapid liver disease complications seen in this population, the relatively low efficacy of current medication and its low tolerability should prompt early and efficient clinical management.

In conclusion, HBV/HCV dual infection is a complex clinical/virological entity. This co-infection appears to be associated with the most severe forms of chronic liver disease and it is an important risk factor for hepatocellular carcinoma development. Different, often dynamic virological profiles may be observed that are strictly related with the activity of one or both the viruses overtime. Thus, a careful longitudinal evaluation of the HBV and HCV viremia levels is mandatory for a correct diagnosis and proper therapeutic approach.

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Table 1
Distribution of hepatitis type B according to the month

Month	Test	2014 (Positive)	Test	2015 (Positive)	Total (%)
January	143	1	381	4	5 (5.8)
February	151	2	197	8	10 (11.6)
March	202	4	211	5	9 (10.5)
April	273	2	18	2	4 (4.7)
May	234	6	57	5	11 (12.8)
June	195	2	78	2	4 (4.7)
July	115	1	55	1	2 (2.3)
August	99	1	102	4	5 (5.8)
September	265	3	299	7	10 (11.6)
October	179	3	145	6	9 (10.5)
November	141	2	117	5	7 (8.1)
December	167	5	161	5	10 (11.6)
Total	2164	32 (37.2)	1821	54 (62.8)	86 (100)

Table 2
Distribution of hepatitis type C according to the month

Month	Test	2014 (Positive)	Test	2015 (Positive)	Total (%)
January	143	17	381	41	58 (9.7)
February	151	11	197	46	57 (9.5)
March	202	55	211	51	106 (17.7)
April	273	39	18	4	43 (7.2)
May	234	19	57	11	30 (5)
June	195	12	78	8	20 (3.3)
July	115	14	55	3	17 (2.8)
August	99	16	102	11	27 (4.5)
September	265	56	299	47	103 (17.2)
October	179	33	145	22	55 (9.2)
November	141	43	117	19	62 (10.4)
December	167	9	161	12	21 (3.5)
Total	2164	324 (54.1)	1821	275 (45.9)	599 (100)

Table 3
Prevalence of hepatitis type B andtype C among different patient groups

Patients	Hepatitis B		Hepatitis C	
	No.	+ve (%)	No.	+ve (%)
Renal Transplant Group	1681	11 (12.8)	1681	129 (21.6)
Dialysis Group	1237	15 (17.4)	1237	97 (16.2)
Health Care Workers	264	31(36)	264	141(23.5)
Dentistry Group	188	10 (11.6)	188	9 (1.5)
Laboratory Group	149	6 (7)	149	92 (15.4)
Blood Bank Group	122	4 (4.7)	122	26 (4.3)
Marriage Group	120	5 (5.8)	120	41(6.8)
Internal Medicine	86	3 (3.5)	86	30 (5)
Other Vocations	79	1(1.2)	79	18 (3)
Barbers Group	59	0 (0)	59	16 (2.7)
Total (%)	3985	86 (100)	3985	599 (100)

–Mean of Hepatitis B(8.6), Mean of Hepatitis C(59.9), *P Value* < 0.01

Table 4
Distribution of hepatitis B and C according to patient sex

Sex	Total Type B (%)	Total Type C (%)	Total %
Male	69(80.2)	372 (62.1)	441 (64.4)
Female	17(19.8)	227 (37.9)	244 (35.6)
Total (%)	86(100)	599 (100)	685 (100)

–Mean of sex (type B): 43, Mean of sex (type C): 299.5, *P Value* < 0.01.

Table 5
Distribution of hepatitis type B according to patient age

Age	2001	2002	Total (%)
0 -11	1	3	4 (4.7)
12-23	4	14	18 (20.9)
24-35	8	26	34 (39.5)
36-47	13	5	18 (20.9)
48-59	5	4	9 (10.5)
60-71	0	1	1(1.2)
72-84	1	1	2 (2.3)
Total %	32 (37.2)	54 (62.8)	86 (100)

Mean of age: 12.28(36-47).

Table 6
Distribution of hepatitis type C according to patient age

Age	2001	2002	Total (%)
0 -11	69	42	111(18.5)
12-23	180	165	345(57.6)
24-35	48	40	88(14.7)
36-47	17	22	39(6.5)
48-59	7	5	12(2)
60-71	2	1	3(0.5)
72-84	1	0	1(0.2)
Total %	324 (54.1)	275 (45.9)	599(100)

Mean of age: 85.57(36-47).

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