

Prevalence of hepatitis A,B & C in Kirkuk city

Najat A.Zaman , Sohaib S.Al-Salehy , Omer M.Ali

Department of Biology , College of Science , University of Kirkuk , Kirkuk , Iraq

Abstract

This is a retrospective study included (1766) patient with different symptoms of hepatitis in addition to blood donors attended central public health lab. in kirkuk city, the investigations carried out from October 2008 to March 2009. Of (229) patients investigated for Anti-HAV: 69(30.13%) showed positive results and the peak of positive results was during January 18(45%). Among the positive cases 31(13.54%) were male while 38(16.59%) were females. The high frequency of the Anti-HAV positivity is observed among the (1-5) age group.

Of (1525) patients investigated for the presence of HBsAg, it has been found that 44(2.88%) were positive and the peak of positive results was during March 13(4.47%). Among the positive cases 34(2.23%) were males while 10(0.65%) were females. The high frequency of HBsAg positivity observed among the (25-30) age group and the high frequency of HBsAg positivity was 28(1.84%):blood donors.

Anti-HCV observed among 12(0.79%) and the peak was during November 6(2.25%). Of 8(0.53%) of positive cases were male while 4(0.26%) were females. The high frequency of positive cases observed among (5-10) age group and the frequency of positivity observed among Thalaciemic patients 6(0.39%).

Introduction:-

Viral hepatitis (A,B,C,D,E & G) is a global public health problem, which is responsible for major chunk of morbidity and mortality. Viral hepatitis occurs endemically and sporadically throughout the world depending on the endemicity of infection [22].

Hepatitis A is endemic in all parts of the world although the exact incidence is difficult to estimate because of the high proportion of asymptomatic and anicteric infection as well as differences in surveillance and differing patterns of disease [12].

Hepatitis A virus (HAV) is a distinct member of picorna virus. It is a 27-32 nm spherical non-enveloped practical containing a linear single – stranded RNA genome approximately 7500 nucleotides in length and coding for three major structural polypeptides. Only 17 amino acids has been predicted from the nucleotide sequence of the virus [13].

HAV is exceptionally stable it is either resistant, stable at PH 3.0 & relatively resistant to inactivation by heat [14]. There is evidence that HAV is inactivated by Chlorine at a concentration of 1mg/L for 30 mins [21]. The pathological features that are constant in all type of viral hepatitis consist of parenchymal cell necrosis and periportal inflammation [16].

Clinical disease with jaundice is uncommon in infants and young children and the infection may pass unnoticed in this group. Severe hepatitis is correspondingly more common in older persons [24].

The onset of jaundice is often preceded by gastrointestinal symptoms such as nausea, vomiting, anorexia & mild fever, jaundice may appear within a few days of the prodromal period but anicteric hepatitis is more common [21]. Incubation period for HAV is 10-15 days (average 25-30). The onset of disease tends to occur within 24 hours, complete recovery occurs in most hepatitis A cases [10].

HAV is widespread throughout the world outbreaks of type A hepatitis are common in families and institutions, summer camps, day care centers, central intensive care units and among military troops [13].

HAV can be detected in the liver, stool, bile and blood of naturally infected humans by immunoassays, nucleic acid. Hybridization assay or PCR. Peak titer of HAV are

detected in the stool about 1-2 weeks prior to the first detectable liver [2]. Specific serological tests for hepatitis A antigen (HA-Ag) and antibodies include radioimmunoassay and enzyme linked immunosorbent assay. Hepatitis A antibody (anti-HAV) is always demonstrable by such assays during the early phase of the illness and titers increase rapidly [8].

Recovery from infection is associated with life long immunity [3]. Liver biopsy is not usually required in acute hepatitis [4].

Hepatitis B virus HBV is the major member of the hepadnaviruses. HBV is a small, enveloped DNA virus. The virion also called (Dane particle) is 42 nm in diameter. Hepatitis B virus can cause acute or chronic, symptomatic or asymptomatic disease. Which of these occurs seems to be determined by the persons immune response to the infection [19]. Detection of both the HBsAg and the HBeAg components of the virion in the blood indicates the existence of an ongoing active infection. HBsAg continues to be released in to the blood even after virion release has been ended and until the infection resolves [7]. The major source of infections virus is blood but HBV can be found in semen , saliva, milk, vaginal and menstrual secretions and amniotic fluid. The most efficient way to acquire HBV is through injection of the virus into the bloodstream. Common but less efficient routes of infection are sexual contact and birth [20]. A vaccine for HBV has been available since 1982 [15 ; 5].

Hepatitis C virus HCV accounts for 90% of the cases of NANBH virus infection and is the major cause of post transfusion hepatitis [2]. HCV is a flavivirus with a positive-sense RNA genome and is enveloped. As a flavivirus HCV has an inclination to establish non cytolytic persistent infections and therefore chronic disease [6]. HCV can cause acute infection but is more likely to establish chronic infection [25]. The diagnosis of HCV infection is based on the ELISA detection of antibody. The reverse transcriptase- polymerase chain reaction technique a variation of the PCR technique can detect HCV RNA in seronegative people and may become a key tool in the diagnosis of HCV infection [18; 20; 26]. Recombinant interferon- alpha is the only known

effective treatment for HCV [20]. There are no vaccines available to protect contact of patients with hepatitis C [11].

Hepatitis D virus (delta agent) responsible for causing 40% of the fulminant hepatitis infection. HDV is unique in that it uses HBV and target cell proficient and produce its one protein. The HBsAg is essential for packaging the virus. HDV similar to HBV, is spread in blood. The person can be co-infected with HBV and delta agent, a person with chronic HBV can also superinfected with delta agent. The HDV increase the severity of HBV infections. Fulminant hepatitis is more likely to develop in people infected with HDV. Hepatitis D virus can be detected by ELISA or RIA. There is no specific treatment for HDV [20].

Hepatitis E virus is ET-NANBH, which is clinically indistinguishable from hepatitis A disease and the disease is usually is mild except in pregnant women, who appear to be exceptionally susceptible to severe disease and excessive mortality. The HGV/GBV-C is a recently characterized flavivirus, that may cause acute and chronic hepatitis and the HGV/GBV-C is a recently characterized flavivirus, that may cause acute and chronic hepatitis and the precise role of HGV/GBV-C in human disease is currently under investigation [22].

Material & Methods:-

The total number of patients involved in present study was (1754). Their ages ranged from (1-60) years. They were suffering from different symptoms of hepatitis and attended central public health laboratory in Kirkuk. In addition to blood donors during period from October 2008 to March 2009, (229) subjects were tested for anti-HAV by primary method, (1525) subjects were tested for hepatitis B surface antigen (HBsAg). (1525) subjects were tested Anti-HCV using enzyme linked immunosorbent assay (ELISA):

1-Collection Blood Sample:-

Three milliliter of blood were collected in sterile plain tube and allowed to clot at room temperature for one hour then centrifuged at 3000 rpm for ten minutes. The resulted serum was collected and either used immediately or stored in freezer.

2-Detection of Anti-HAV:-

By using the red colloidal particles to label the antibodies as indicator and the Millipore filtering membrane coated with antigen as the carrier. Affected by filtration and condensation, the antigen antibody reaction is enabled to go on rapidly. When the serum tested is added to the Millipore membrane previously coated with HAAG, if there was HAV IgM antibody, the HAAG-Anti-HAV IgM colloidal gold complex will be formed on the membrane as red dots which were visible to the naked eyes. It takes about 2 to 4 minutes for the whole reaction to be carried out.

3-Detection of HBsAg:-

Hepanostika HBsAg ultra is an ELISA based on a "sandwich". Upon completion of the assay, the development of color indicates the presence of HBsAg, while on low color development suggests the absence of HBsAg.

Specifically, well of micro ELISA strips are coated with Anti-HBs(murine and human monoclonals). Subsequently HRP- labeled Anti -HBs (ovine) conjugate

is added and incubation is continued. If HBsAg is present, a solid phase antibody / HBsAg / enzyme labeled antibody complex is formed. Following washing and incubation with TMB (tetramethylbenzidine) substrate a blue color is produced. The enzyme reaction is stopped by the addition of a sulfuric acid solution, which change the color to yellow. When HBsAg is present in the sample, an intense color develops. However, if the sample is free of HBsAg, no or little color forms after the addition of substrate. Within limits, the amount of HBsAg in the sample is proportional to the degree of color development.

An optional feature of this test is that the addition of controls, samples and conjugated can be measured. This is called Sample and Reagent Addition Monitoring (SARAM). For this purpose colors have been added to several reagents.

4- Detection of Anti-HCV:-

This Anti-HCV test employs solid phase, indirect ELISA method for detection of antibodies to HCV in two step incubation procedure. Polystyrene microwell strips are pre-coated with recombinant, highly immunoreactive antigens corresponding to the core and the non-structural regions of HCV specific antibodies, if present, will be bound to the solid phase pre-coated HCV antigens. The wells are washed to remove unbound serum proteins, and rabbit anti-human IgG antibodies (anti-IgG) conjugated to the enzyme horseradish peroxidase (HRP-Conjugate) are added. During the second incubation step, these HRP- conjugated antibodies will be bound to any antigen-antibody (IgG) complexes previously formed and the unbound HRP-conjugate is removed by washing. Chromogen solution containing tetramethylbenzidine (TMB) and urea peroxide are added to the wells and in presence of the antigen-antibody-anti-IgG (HRP-immunocomplex), the colorless chromogens are hydrolyzed by the bound HRP conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antibody captured in the wells, and to the amount of antibody in the sample respectively. Wells containing samples negative for anti-HCV remain colorless.

Results-

During present study 69(30.13%) revealed positive for anti-HAV and the positivity peaked during January(45%) (table 1). Among the positive cases of anti-HAV 31(13.54%) were males, while 38(16.59%) were females (table 2). The distribution of the positivity with age groups. It has been found that high frequency of anti-HAV among the age group (1-5) years (table 3). About HBV: 44(2.88%) revealed positive results for HBsAg (table 4), according to sex it was observed that 34(2.23%) were males, while 10(0.65%) were females (table 5). High frequency of the positive cases of HBsAg is observed during March 13(4.47%) (table 4). The distribution of the positivity of HBsAg among the risk groups: 28(1.842%) blood donors, 4(0.26%) Haemodialysis, 2(0.12%) Barbers, no positive results reported among thalassemia patients and midwives (table 6). The high frequency of HBsAg positive cases found among the age group (25-30) years (table 3).

Anti-HCV positive cases were 12(0.79%) and the positivity peaked during November 6(2.25%) (table 7). Among the positive cases 8(0.53%) were male, while 4(0.26%) were female(table 8). The distribution of the positive cases among risk group we observed 6(2.24%): Thalaseimia patients, 3(1.37%): blood donors and 1(0.34%): midwives: No positive results found among haemodialysis and Barbers (table 9).

Discussion:-

The problem of viral hepatitis in all world is still a serious one specially to health care workers who have more chance for contact with the infectious virus of hepatitis than others. The present study revealed that 69(30.13%) were positive for Anti-HAV, while other study done in Baquba city showed that the prevalence of Anti-HAV was (25.3%) [23].

According to the positivity of HBsAg this study revealed that 44(2.88%) were positive and this dissimilar to a realer study done in Baquba city which shows that (7.9%) were positive for HBsAg [23]. Other study done in southeastern of Turkey which show that positivity for

HBsAg was (8.2%) in rural area and (6.2%) in the urban area [9].

Other study done in Saudi Arabia revealed that HBsAg positivity among dialysis patient where (4.4%) which dissimilar with present study which revealed (0.26%) positive for HBsAg among dialysis [1].

According to the seroprevalence of Anti-HCV the present study revealed that 12(0.79%) were positive , other study done in Baquba city dissimilar with the present study that shows the prevalence of Anti-HCV was (7.9%) [23].

HAV transmit by faeco0oral route may be this one of the reasons that lead to high frequency of HBsAg positively among the age group of (20-25) may be also due to transmission route of this virus by sexual parenteral route. HCV called postransfusion virus in present study high frequency observed among thalaciemic patients because of receiving them many blood units which increase the possibility of receiving infected unit when the getting the blood units without testing for anti-HCV.

Table(1):Number and percentage of anti-HAV according to months

Monuth	Total patient	+VE	percentage	- VE	percentage
October	5	0	0	5	100
November	68	7	10.29	61	89.71
December	69	28	40.58	41	59.42
January	40	18	45	22	55
February	10	4	40	6	60
March	37	12	32.43	25	67.57
Total	229	69	30.13	160	69.87

Table(2): The correlation between presence of Anti HAV and sex

Monuth	Male	percentage	Female	percentage
October	0	0	0	0
November	4	5.88	3	4.41
December	15	21.74	13	18.84
January	7	17.50	11	27.50
February	2	20	2	20
March	3	8.11	9	24.32
Total	31	13.54	38	16.59

Table(3) : The correlation between presence of anti HAV, HBsAg and Anti-HCV and age

Group	anti HAV	HBsAg	anti-HCV
5- 1year	24	0	2
10- 5	23	0	3
15- 10	14	0	2
20- 15	2	2	0
25- 20	3	5	0
30- 25	2	11	1
35- 30	0	4	0
40- 35	0	5	1
45- 40	0	6	1
50- 45	0	8	1
55- 50	0	3	1
60- 55	0	0	0
>60	1	0	0

Table (4): Number and percentage of HBsAg. according to months

Monuth	Total patient	+VE	percentage	-VE	percentage
October	201	7	3.48	194	96.52
November	267	6	2.25	261	97.75
December	272	10	3.68	262	96.32
January	277	3	1.08	274	98.92
February	217	5	2.30	212	97.70
March	291	13	4.47	278	95.53
Total	1525	44	2.88	1481	96.98

Table(5): The correlation between presence of HBsAg and sex

Monuth	Male	percentage	Female	percentage
October	4	1.99	3	1.49
November	4	1.50	2	0.75
December	10	3.68	0	0
January	3	1.08	0	0
February	5	2.3	0	0
March	8	2.75	5	1.72
Total	34	2.23	10	0.65

Table(6): The correlation between presence of HBsAg and risk group.

Mon	Risk group									
	Barbers		Thalasiemia		Blood donors		Haemodialysis		Midwife	
	NO	%100	NO.	%100	NO	%100	NO	%100	NO	%100
Oct.	1	0.50	0	0	2	1.00	3	1.49	0	0
Nove.	0	0	0	0	3	1.125	0	0	0	0
Dec.	0	0	0	0	6	2.2	1	0.37	0	0
Jan.	0	0	0	0	3	1.08	0	0	0	0
Febr	0	0	0	0	4	1.84	0	0	0	0
Mar.	1	0.34	0	0	10	3.44	0	0	0	0
Total	2	0.13	0	0	28	1.84	4	0.26	0	0

Table (7): Number and percentage of Anti-HCV according to months

Monuth	Total patient	+VE	percentage	-VE	percentage
October	201	3	1.49	198	98.51
November	267	6	2.25	261	97.75
December	272	2	0.74	270	99.27
January	277	0	0	277	%100
February	217	0	0	217	%100
March	291	1	0.34	290	99.66
Total	1525	12	0.79	1513	99.21

Table(8): The correlation between presence of Anti-HCV and sex

Monuth	Male	percentage	Female	percentage
October	2	0.99	1	0.5
November	4	1.5	2	0.75
December	2	0.74	0	0
January	0	0	0	0
February	0	0	0	0
March	0	0	1	0.34
Total	8	0.53	4	0.26

Table(9): The correlation between presence of Anti-HCV and risk group.

Mon.	Risk group									
	Barbers		Thalasiemia		Blood donors		Haemodialysis		Midwife	
	NO	100%	NO	100%	NO	100%	NO	100%	NO	100%
Oct.	0	0	0	0	2	1	0	0	0	0
Nov.	0	0	4	1.50	1	0.37	0	0	0	0
Dec.	0	0	2	0.74	0	0	0	0	0	0
Jan.	0	0	0	0	0	0	0	0	0	0
Feb.	0	0	0	0	0	0	0	0	0	0
Mar.	0	0	0	0	0	0	0	0	1	0.34
Total	0	0	6	0.39	3	0.2	0	0	1	0.07

References:-

1. Abul Kashem, Ibrahim N., Mirvat M., Mohamad R., Jaisimha N., Rezaul K., MP Divakaran, Abdul Shakoar T. (2003). Hepatitis C Virus Among Hemodialysis Patients in Najran: Prevalence is More Among Multi-Center Visitors. Saudi Journal of Kidney disease & transplantation Vol.:14:2 : 206-211.
2. Alter, H.J.(1988). Transfusion-associated non-A, non-B, hepatitis. The first decade. In Viral Hepatitis and Liver Disease (ed. A.J. Zuckerman, pp, 537-542. Liss. New York. studies in non-human primates. In Viral Hepatitis and Liver Disease (ed, A. J. Zuckerman), pp 138-147, Liss, New York.
3. Bradley, D.W., Krawczynski. K., Cook, E.H. Jr. (1998). Enterically transmitted non-A, non-B, hepatitis: etiology of disease and laboratory
4. Bradley, D.W., McCaustland. K.A., Cook, E.H. (1985). Posttransfusion non-A, non-B hepatitis in chimpanzees. Physicochemical evidence that the tubule-forming agent is a small enveloped virus. Gastroenter. 88, 773-779.
5. Brechot, C, Degos, F, Lugassy, C,. Hepatitis B virus DNA in patients with chronic liver disease and negative tests for hepatitis B surface antigen. N Engl J M ed 1985; 270-312.
6. Choo, QL, Kuo, G, Weiner, AJ,. Isolation of a c DNA clone derived from a blood borne non-A, non-B viral hepatitis genome. Scie.1989;244-359.
7. Chu, CM, Liaw, YF, Pao, CC, Huang, MJ. The etiology of acute hepatitis superimposed upon previously unrecognized asymptomatic HBsAg carriers. Hepat. 1989; 9-452.
8. Decker, R.H., Overby, L.R., Ling, C.M. Fronser, G., Deinhardt, F and Boggs, J. Serologic studies of transmission of hepatitis A virus in human. J. Infect Dis 139(1979)74.
9. Dursun Mehmet, Ertem Meliksah, Yilmaz Serif, Saka Gunay, Ozekinic Tuncer and Simsek Zeynep.(2004). Prevalence of Hepatitis B Infection in the Southeastern Region of Turkey: Comparison of Risk Factors for HBV Infection in Rural and Urban Areas. Jpn. J. Infect. Dis., 58, 15-19.
10. Emerson, S.U., Huang, Y.K., McRill, C. Mutation both the 2B-gene and 2C-gene of hepatitis A virus are involved in adaptation to growth in cell culture. J.of Vir., 60; 650-654.
11. Fattovich, G., Giustina, G., Degos, F. (1997). Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. Gastroenter., 112,463-472.
12. Hen, E.J., Portmann Bc; Naoumov, NV; Smith-HM (1987). Long term outcome of hepatitis infection. N-Engl-J-Med; 334(13):20-815.
13. Gust, I.D., Coulepis, A.G., Feinstone, S.M. (1983). Taxonomic classification of hepatitis A. Intervirology, 20, 1-7.
14. Halliday, M.L., Kang, L.Y., Zhou, T.K. (1991). An epidemic of hepatitis A attributable to the ingestion of raw clams in Shanghai, China. Jou. of Infectious Dis. 164; 852-859.
15. Hendricks, DA, Stowe, BJ, Hoo, BS. Quantitation of HBV DNA in human serum using a branched DNA (b DNA) signal amplification assay. Am J Clin pathol 1995; 104:537.
16. Hollinger, FB, Ticehurst JR. (1996). Hepatitis A virus. In: Field Virology. 3rd. Fields BN. (editors). Lippincott-Raven.
17. Miriam J. Alter, Deanna Kruszon-Moran, Omana V. Nainan, Geraldine M. McQuillan, Fengxiang Gao, Linda A. Moyer, Richard A. Kaslow, and Harold S. Margolis (1999). The Prevalence of Hepatitis C Virus Infection in the United States, 1988 through 1994. Vol. 341:556-562.
18. Martinot-Peignoux, M, Marcellin, P, Gournay, J. Detection of and quantitative of serum HCV-RNA by branched. DNA amplification in anti-HCV positive blood donors. J.Hepatol (1994): 20: 676.
19. Monti, G, Galli, M, Invernizzi, F,. Cryoglobulinemias :a multi center study of the early clinical and laboratory manifestations of primary and secondary disease. GISC. Italian Group for the study of Cryoglobulinemias QJM(1995); 88:115.
20. Trick R.Murray.Ken S. Rosenthal, Gorge S. Kobayashi and Micheal A. Paller.(1998).medical microbiology.3rd ed:481-483.
21. Ng, L.H., and Lemon, S.M. (1992). Antigenic structure of human hepatitis-A virus defined by analysis of escape mutants against murine monoclonal antibodies: Journal of Virology, 66, 2208-2216.
22. Jiv Bajjal, Nikhil Patel, S.A.Kolhapure. Evaluation of efficacy and safety of Liv.52 DS tablets in acute viral hepatitis: A prospective, double-blind, randomized, placebo-controlled, phase III clinical trial. Medicine J. 2004;12(5),41-53

23 ba J. Jawad Al-Zubiadi. (2007). Serological study of hepatitis A virus in Baquba city, athesis MSc. College of education, Dyala university.

24 mons, JN, Pilot-Matias TJ, Leary TP, et al. Identification of two flavivirus –like genomes in the GB hepatitis agent. Proceedings of the national Academy of Sciences U.S.A. 1995;92:3401-3405.

25 Villano, SA, Vlahov, D, Nelson, KE et al. Persistence of viremia and the importance of long term follow-up after acute hepatitis C infection. Hepat. 1999;29:908.

26 Younossi, Z, McHutchison, J. Serological test for HCV infection. Viral hepatitis Reviews 1996; 2:161.

الملخص

شملت الدراسة (1754) مريضا مصابين باعراض مختلفة لالتهاب الكبد الفايروسي بالاضافة الى متبرعي الدم الذين راجعو مختبر الصحة العامة المركزي في كركوك مابين بداية شهر تشرين الاول 2008 الى نهاية شهر اذار 2009. تم فحص (229) مريضا منهم للبحث عن الاجسام المضادة للفايروس نمط A واطهر 69(30.13%) نتائج ايجابية الفحص للاجسام المضادة تجاه الفايروس نمط A وكان عدد الذكور ضمن الحالات الموجبة 31(13.54%) اما الاناث 38(16.59%) اما توزيع الحالات الموجبة ضمن الفئات العمرية فكانت اكثر تكرارا ضمن الفئة العمرية (1-5) سنة وكانت اكثر النتائج ايجابية الفحص تكرارا في شهر كانون الاول. اما بالنسبة لوجود HBsAg ف لوحظ انه 44(2.88%) مريضا اظهروا ايجابية الفحص وكان 34(2.23%) ذكور اما الاناث 15(0.65%). وكانت الفحوصات ايجابية اكثر تكرارا في شهر اذار. اما توزيع الاصابة فكانت اكثر تكرارا مع الفئة العمرية (28-30) سنة. وفيما يخص مجاميع الاختطار كانت اكثر شيوعا ببيتين متبرعي الدم 28(1.84%).

بالنسبة للاجسام المضادة لـ HCV لوحظ انه 12 حالة (0.79%) كانت ايجابية الفحص وكان عدد الذكور 8(0.53%) اما الاناث 4(0.26%) وكانت ايجابية الفحص اكثر تكرارا في شهر تشرين الثاني. اما تكرار الإصابة فكانت ضمن الفئة العمرية (5-10) سنة واكثر تكرارا ضمن مرضى فقر الدم البحر الابيض المتوسط 6(0.39%).