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Prevalence of Hepatitis C Virus in Thi-Qar Province

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Annotation: Hepatitis C Virus (HCV) is a major global health concern, yet its prevalence and epidemiological patterns in specific regions remain underexplored. This study investigates the prevalence of HCV in Thi-Qar province through serological (ELISA) and molecular (Real-Time PCR) analyses of blood samples collected from 39,107 individuals between January and December 2023. The findings reveal a 0.54% seropositivity rate, with higher infection rates among males and individuals aged 31–50 years. Blood group O showed the highest prevalence. These results highlight the significance of targeted screening, improved diagnostic strategies, and enhanced public health awareness to curb HCV transmission. The study underscores the need for preventive measures, including stricter infection control protocols in medical and community settings.

Keywords: Hepatitis C Virus, seroprevalence, ELISA, Real-Time PCR, epidemiology, Thi-Qar province.

1_1 Introduction

Any infection that results in inflammation of the liver is called hepatitis [Greek hepaticus, liver], which can be caused by viruses and less commonly by bacteria, or other microorganisms and sometimes caused by exposure to drugs (eg. Isoniozid) or poisons (eg. Ethanol) (Prescott et al., 2005). Hepatitis viruses produce acute inflammation of the liver, resulting in a clinical illness characterized by fever, gastrointestinal symptoms such as nausea, vomiting and jaundice (Hunter et al., 2007). HCV was discovered in 1989 as non-A , non-B hepatitis, and classified in Hepacivirus genus of the Flaviviridae family, and its genome is a positive-stranded RNA of 9.6 kb in length, and it encodes a core protein, two enveloped glycoproteins, and several non structural proteins (Bauman et al., 2004).The genome of HCV is highly variable. so far, six major genotype (HCV1-HCV6) and more than one hundred subtype have been described (Brooks et al., 2010). A number of HCV genotyping systems were developed, McOmish et al., (1993) genotyped HCV by restriction fragment length polymorphism (RFLP). Chayama et al., (1993) and Okomoto et al., (1992) were genotyped HCV by PCR with specific primers. Wittwer et al., (1997) first began to develop Real-Time Polymerase Chain Reaction machines to detect and monitor HCV infection. Many studies have indicated an association between HCV genotype and responsiveness to alpha interferon treatment (Mahy and Van Regenmortel, 2010). Differences in geographic distribution of HCV genotype have also been observed (Strauss and Strauss, 2008). Because there is no molecular studies on HCV infection at Thi-Qar province and the prevalence of genotypes of HCV were unknown, which important for the treatment of the disease in addition to unclear background of HCV epidemiology in the province, the present study proposed.The term non-A, non-B Hepatitis (NANBH) was introduced in the mid 1970s to describe inflammatory liver disease not attributable to infection with hepatitis A virus (HAV) or hepatitis B virus (HBV) (Alter et al., 1975) Inoculation of chimpanzees with blood products derived from human with NANB hepatitis led to persistent increases of serum alanine aminotransferase (ALT) indicating that an infectious agent was the cause of the disease (Alter et al., 1978). Moreover, it was reported that the infectious agent was able to pass through 80 nm membrane filters, and thus likely to be a virus (Bradley, 1985). NANBH virus is enveloped as indicated by it sensitivity to chloroform (Bradley et al., 1983). The molecular cloning of NANBH agent was reported in 1989, which was found to be a positive stranded RNA virus and was designated as hepatitis C virus (Choo et al., 1989). Yu et al., (2007) studied the virus by using cryo-electron microscopy and three-dimensional reconstructions views and he found that the virion surface has multilayered architecture with smooth outer-layer densities arranged in a ″fishbone″ conf. However, the lack of suitable cell culture system for cultivation of the NANBH agent and the limited availability of chimpanzees prevented further characterization of this causative agent for several years. The prevalence of HCV infections varies through out the world (WHO, 1999), with the highest number of infections recorded in Egypt (Strauss and Strauss, 2008). The prevalence rate is higher in persons ages 30 to 49 years than in older or younger persons and is higher in males than in females and among certain ethnic group, such as African American and Mexican American, than in whites (Feldman et al., 2007). Mode of transmission of HCV can be divided into percutaneous (blood transfusion and needle stick inoculation) and nonpercutaneous (sexual contact and perinatal exposure) (Murphy et al., 2000).Virus can be recovered from the saliva of infected person (Shafique et al., 2009). Chimpanzees have been experimentally infected by the injection of saliva from HCV infected person (Abe et al., 1987). Feucht et al., (1995) demonstrated that tear fluid of HCV carriers could be infectious. Up to 20-40% chronically infected patients with HCV, the mode of transmission was still unknown. It concluded that tear fluid might play role in virus transmission Nosocomial transmission has been documented such as from patient to patient by colonoscopy (Bronowiki et al., 1997), during dialysis (Alter, 2002 ; Yoshida et al., 1992), and during surgery, especially in gynecological surgery setting (Massari et al., 2001; Ross et al., 2000). Needle stick

injuries in the health care setting continue to result in nosocomial transmission of the virus (Lauer and Walker 2001). Transmission of HCV infection also may occur from health care worker to patients (Feldman et al., 2007).HCV infection either resolves after acute infection or establishes chronic infection in the majority of patients. Incubation period is in average 50 days it may differ between 15-150 days (Lauer and Walker, 2001; Hagedorn and Rice, 2000). Infections range from subclinical to clinical acute and chronic hepatitis, liver cirrhosis and hepatocellular (Fauquet et al., 2005). In the acute phase of infection, liver cell injury and elevated serum alanine aminotransferase (ALT) levels are observed. Ten times higher of ALT level was recorded approximately in 80% of HCV patients (Hunter et al., 2007 ; Hoofnagle, 1997). In cases in which symptoms of acute hepatitis have been documented, they usually consisted of jaundice, malaise, and nausea (NIH, 1997). Unfortunately, after acute infection 80-85% of patients develop chronic infections (Fauquet et al., 2005 ; Shimotohno, 2000). Up to 20% of patients develop cirrhosis within the first two decades of HCV infection (Hunter et al., 2007 ; Yano et al., 1996). Chronic infection with hepatitis C is a major cause of liver cancer in many countries. 1-5% of patients with chronic infection develop hepatocellular carcinoma (HCC) after 20 years. HCC is more likely to develop in patients with cirrhosis and with long duration of infection (Di Bisceglie, 1997 ; Hoofnagle, 1997 ; Ikeda et al.,1993).

A wide range of factors can influence the development of cirrhosis, but it seems that being male, aged over 50 at time of infection, a high alcohol intake, and co-infection with HIV or HBV increase susceptibility to cirrhosis (Peters and Terrault, 2002 ; Montano-Loza et al.,2001; Zarski et al.,1998). Once the end-stage liver disease has developed, the only treatment is the organ transplantation. The end-stage chronic hepatitis C is the major cause of liver transplantations (Detre et al., 1996). liver damage is a consequence of viral infection or due to immune responses is still not known. Immune-mediated pathways leading to liver damage are more likely since it has been shown there is no correlation between viral RNA titres and liver damage (Wejstal, 1995). Although the liver is the primary target, HCV also causes immune-complex mediated extra-hepatic diseases, namely essential mixed cryoglobulinemia, porphyria cutanea torda, glomerulonephritis, keratoconjuctivitis sicca (Tsukazaki et al., 1998 ; Khella et al., 1995) It has been recognized that liver histology may vary according to HCV genotype. A study by Mihm et al., (1997) showed that patients with genotype 3a frequently manifested more steatosis of the liver and bile duct lesions than patients with genotype 1a. Due to the absence of a small animal model system and deficient InVitro HCV replication system it has been difficult to investigate the life cycle of HCV. The recent development of such system has offered the opportunity to analyses in detail the different steps of viral replication. The HCV specific antigens in liver biopsies of chronic hepatitis C carriers has led to the identification of the liver as the primary site of virus replication (Bartenschlager and Lohmann, 2000). A part from hepatocytes, there is strong evidence that HCV can also replicate in peripheral blood mononuclear cells (PBMCs) (Cribier et al., 1995), or in experimental infected B and T cell lines (Serafino et al., 1997) and more recently negative strand HCV RNA (indicative of replication) has been detected in the central nervous system of patients with recurrent HCV infection after liver transplantation, suggesting HCV is neroinvasive (Goutagny et al., 2003 Several serological and molecular assays were used to detect and monitor HCV infection (Mahy and Van Regenmortel, 2010)The presence of anti-HCV in serum indicated exposure to the virus but does not differentiated among acute, chronic, and resolved infections. Anti-HCV may persist for life in patients with spontaneously resolved infection, although titers decrease and even disappeared over time. In patients with chronic hepatitis C, anti-HCV persists in serum for life. (Takaki et al., 2000).

This study was aimed to determination the epidemiology and the prevalence of HCV genotypes at Thi-Qar province through : Focus on the distribution of HCV infections at Thi-Qar province according to gender, geographical regions, age groups and blood groups. Evaluation the serological diagnostic method (ELISA) for detection of HCV infection in comparison with molecular method by using Real-Time PCR. Estimation the hepatitis C viral load in patients

serum. Using the molecular techniques for determination of HCV genotypes in Thi-Qar as risk factor of the disease and contribute for treatment.

2. MATERIALS AND METHODS

2.1. Sampling:

A total of 39107 individuals referred to the center of thalassemia /AL-Haboby hospital, central blood bank, renal dialysis unit/Al-Hussein Teaching Hospital and public health laboratory at Thi-Qar province, including 31 from medical staff were introduced to the study. This study was carried out from January 2023 to December 2023 .

A questionnaire (appendix-1) was used to record special notes regarding these individuals as follow:

Age, gender, occupation, date of sample collection, patient address, risk factor and blood group.

2.2. Collection of blood samples:

A sample of 5 ml of fresh blood was drawn from each case and collected in a sterile plastic tube, left to clot at room temperature then centrifuged at 2000 rpm for 10 minutes, then serum was collected in sterile tube and examined by ELISA assay to detect anti HCV then stored at -20 °C until examined by Real-Time PCR technique and genotyping by RT-PCR technique.

2.3. Investigation for anti HCV antibodies (IgG) in serum by third generation ELISA test (Contreras and Barbara, 1989)

2.3.1. Preparation of reagents (according to manufacturer, s instructions)

2.3.2. Preparation of washing solution:

Washing solution was prepared from 20X concentrated solution by diluting it to 20-fold with distilled water at room temperature. Diluted washing solution is stable for about 30 days at room temperature. For longer storage, stored at 2~8 ˚C.

2.3.3. Preparation of conjugate solution:

A proper amount of concentrated conjugate was diluted by conjugate buffer solution (ratio is 2:100) in accordance with the number of wells which will be used.

3. RESULTS

3.1. ELISA results:

The present study was revealed that out of 39107 individuals from different regions of Thi-Qar province, 54(0.54%) of those gave positive result for HCV by ELISA III..

3.1.1. Seropositivity of HCV infections in relation to gender:

The infections with HCV among males (0.53%) were higher than females (0.01%).

Table (3-1): Relation between gender and seropositivity rates of HCV infection.

Sex	Blood donors	Infected people	Total
Males	38920(389.2%)	53 (0.53%)	38973(389.73%)
Females	187(1.78)	1 (0.01%)	188(1.88%)

3.1.2. Seropositivity of HCV infections in relation to age groups:

The statistical analysis showed a significant differences between infections depending on age grade. The highest infections percentage recorded was at age groups 26-35 and 36-45 years respectively, flowed by age group 15-25 years which showed the percentage 0.04%, while the lowest infection percentage was recorded at the age groups 56-65 and 66-75 years, which was 0.04% and 0% respectively (Table 3-2).

Table (3-2): Distribution of HCV infections according to age groups of patients.

Age	Blood donors	Infected people	Total
15-25	2556(25.56%)	4(0.04%0	2560(25.6%)
26-35	9325(93.25%)	17(0.17%)	9342(93.42%)
36-45	9523(95.23%)	17(0.17%)	9540(95.4%)
46-55	7795(77.95%)	4(0.04%)	7799(77.99%)
56-65	8126(81.26%)	4(0.04%)	8130(81.3%)
66-75	1782(17.32%)	0(0%)	1782(17.32%)

3.1.3. Seropositivity rates of HCV infections according to blood groups:

The results showed that the highest percentage for seroprevalence of HCV was among patients of blood group O (0.27%), followed by blood group B (0.03%) and blood group A (0.1%), while the lowest percentage was among patients of blood group AB (0.11%) with significant differences.

Table (3-3): Percentages of seropositivity of HCV infections according to blood groups .

Blood group	Blood donors	Infected people	Total
A	13996(139.96%)	10(0.1%)	14006(140.06%)
O	16869(168.69%)	27(0.27%)	16896(168.96%)
AB	7339(73.39%)	6(0.06%)	7345(73.45%)
B	903(0.03%)	11(0.11%)	914(9.14%)

4. Discussion

4.1 The positive percentages of HCV infection in relation to gender

The high percentage of infections in males in comparison with females may be related to several reasons, the most important one is the possibility of exposure of males to infection more than females, especially by using of razors or frequent travel and contact with the occupational hazards (Jacobson *et al*., 2010 ; El-Gilany and El-Fedawy, 2006), while other studies were attributed to the causes of this variation to genetic factors related to the willingness of any of the sexes to infection according to the genotype of the virus (Okomoto *et al*., 2005). Also the sex hormones have effects on infection as indicated by Soulsby, (1982) who explained that male hormones cause decreasing cellular immunity, while female hormones cause increasing.

4.2. Percentages of positive HCV infections in relation to age groups

The results of this study showed that HCV prevalence is higher in persons within age group (31-50) years than in older or younger persons according to ELISA technique, and this result was in agreement with Feldman *et al*., (2007) and Armstrong *et al*., (2006) who studied HCV prevalence at the general population in United State. Exposure of this group to the various medical device for treating and donation of blood is the leading cause of infection.

In Iraq, Al-Hilli and Ghadhban (2006) have recorded that the highest infection rate was at the symptomatic HCV patients belong to the age group 40-49 years old.

The reason for decrease of infections in age groups older than (31-50) years was the death due to liver failure. In contrast the reasons for decreasing of infections in the age groups below this age group are the improvement conditions of blood transfusion and the use of sterile apparatus also many routes of HCV acquisition are absent at the childhood such as sexual contact and occupational risks.

4.3. Percentage of positive HCV infection according to blood groups

The result of this study demonstrated a correlation between HCV infections and blood groups. The prevalence of HCV was found to be higher in patients with blood group O, this finding is not unexpected as there is usually a high demand for blood group O which is the most common blood

group in the general population, and lower in patients with blood group AB, this result is in agreement with Behal *et al*., (2010) and Jeremial *et al*., (2008).

Conclusions

1. Seroprevalence of HCV documented by 1.49%, and there are two major genotype of HCV in Thi-Qar (1 and 4),
2. Serological assays (ELISA III) are reliable for detecting exposure to HCV, and Real-Time PCR technique is required to detect current infection.
3. High prevalence of HCV was detected among males, age group (31-50) years and patient of blood group O.

RECOMMENDATIONS

1. Make all the necessary for blocking the disease and prevent its distribution through conduct periodic inspection of the laboratory personnel and barber shops and beauty salons also using single dose vials of drugs instead of multi-dose vials to reduce the spread of HCV infections.
2. It is suggested to have a strategy to inform and educate the public and press regarding this disease to have HCV awareness program.
3. Serological assays (ELISA III) are reliable for routine diagnostic disease.

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