وزارة التعليم العالي والبحث العلمي

جامعة ديالى

كلية التربية للعلوم الصرفة

قسم علوم الحياة

الانماط الجينية لفيروس التهاب الكبد نمط (ب) ونمط (ج) بين المرضى المصابين بانيميا البحر المتوسط و الفشل الكلوي و واهبين الدم

أطروحة مقدمة

إلى مجلس كلية التربية للعلوم الصرفة بجامعة ديالى كجزء من متطلبات نيل

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Chapter One

1.1 Introduction

Viral hepatitis is a systemic disease primarily involving the liver. Most cases of acute viral hepatitis in children and adult are caused by one of the following agents: hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D (HDV), hepatitis E virus (HEV), hepatitis G virus (HGV) and Transmitted transfusion virus (TTV). The hepatitis can be self-limiting or can progress to fibrosis, cirrhosis and liver cancer (Arie *et al*., 2005; Obeagu *et al*., 2018).

Hepatitis B virus (HBV) is a partially double-stranded DNA virus, a species of the genus *Orthohepadnavirus* and a member of the *Hepadnaviridae* family. It is the causes of acute or chronic HBV infection worldwide (Ryu, 2017).

HBV was the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine was available. HBV cause acute and chronic hepatitis often progress to permanent carrier state and hepatocellular carcinoma (HCC) (Dieter *et al*., 2021). HBV was transmitted by contact with blood or body fluids of an infected people (Bineeta *et al*., 2018).

Hepatitis B virus infection constitutes a major economic and public health problem throughout the world, particularly in the developing countries including the Middle East, where it is responsible for considerable morbidity and mortality and the development of a chronic carrier state, liver cirrhosis that may progress to hepatocellular carcinoma (Chang, 2007; Abdul-Hakeem *et al*., 2016). A global prevalence of chronic HBV infection found that the HBs Ag seroprevalence was 3.61% worldwide with highest endemicity in countries of African, West Pacific

and South America regions (Schweitzer *et al*., 2015). Infection in infancy and early childhood is strongly associated with progression to chronic HBV infection, contributing to a significant proportion of chronic liver diseases (Rukunuzzaman and Karim, 2015). It has been estimated that more than one third of the World's population was infected with HBV leading to the deaths of 1 - 2 million people annually, and there are more than 350 million chronic carriers of hepatitis B virus (Chen and Tian, 2019; Zhang *et al*., 2019).

Acute and chronic of HBV infections are a common health problem in Iraq. In the pre-vaccine era, the prevalence of the HBs Ag was 3-4 % among normal healthy population, made Iraq among the countries of intermediate zone of endemicity (Omer and Al-Douri, 1984). However, later study found that the national prevalence of HBsAg dropped down to 0.6% and correlated positively with age (Ataalla *et al*., 2011). In another large population study conducted in Basrah, reportedly 2.3% blood donors had serological evidence for HBV infection; of that 0.2% showed a positive result for both anti-HBc antibody and HBs Ag (Al-Rubaye, 2016).

In Diyala province, the first seroepidemiological study of HBV infection among blood donors and risky groups was conducted covering the period from 1989 to 2002. It was found that the prevalence of HBV positivity rate among blood donors was 1.5%, and the highest positivity rate was among hemophilia patients (42.5%) (Hasan *et al*., 2006).Previous study was conducted in 2018 in Diyala province was reported the total HBV positive was (5.12%) (Hasan *et al*., 2018). Another seroprevalence study to explore the HBV infections in Diyala province during the period from 2003 to 2008, found that the prevalence of HBs Ag was 3.9% (Al-Jebori *et al*., 2010).

HBV is divided into four major serotypes (adr, adw, ayr, ayw) based on surface antigenic epitopes. Genotypes differ by at least 8% of the

sequence and have distinct geographical distributions and it has been associated with anthropological history. Within genotypes subtypes have been described: these differ by 4–8% of the genome. There are eight known genotypes labeled A through H. A possible new "I" genotype has been described. Two further genotypes have been recognized. Several subtypes are also recognized. There are at least 24 subtypes (Olinger *et al*., 2008). These genotypes have a distinct geographical distribution that may affect the disease severity, course and likelihood of complications, response to treatment and possibly vaccination (Anna *et al*., 2019).

There is no seasonal trend for HBV infection and no high predilection for any age group, although there are definite high-risk groups such as parenteral drug abusers, institutionalized persons, health care personnel, multiply transfused patients, organ transplant patients, hemodialysis patients and staff, highly promiscuous persons, and newborn infants born to mothers with hepatitis B (Chen and Tian, 2019; Zhang *et al*., 2019).

Hepatitis B vaccine has an outstanding record of safety and effectiveness. The HBV vaccine is the first anti-cancer vaccine because it helps reduce HCC**,** since chronic HBV and HCV causes 80% of all liver cancer, which is the second most common cause of cancer death worldwide (Kao, 2015). The road to the HBV vaccine began in 1968, since the Blumberg's finding that the HBs Ag is a part of the virus that causes serum hepatitis. In 1981, the Food and Drug Administration (FDA) approved a more sophisticated plasma-derived HB vaccine for human use. It was the first commercial inactivated type vaccine for HBV. The blood-derived HBV was withdrawn from the marketplace in 1986 when the world's first recombinant vaccine was invented which is still in use today (Orlando *et al*., 2015; Romano *et al*., 2015; Neil *et al*., 2017).

Hepatitis C virus (HCV), a member of the *Hepacivirus C* species, is a small (55–65 nm in size), enveloped, positive-sense single-stranded RNA

virus, classified as family *Flaviviridae*. The hepatitis C virus is the cause of hepatitis C and the causes of hepatocellular carcinoma (HCC) and lymphomas in humans (Rusyn and Lemon, 2014).

Based on genetic differences between HCV isolates, the HCV species is classified into six genotypes (1–6) with several subtypes within each genotype (represented by lower-cased letters) Subtypes are further broken down into quasispecies based on their genetic diversity. Genotypes differ by 30–35% of the nucleotide sites over the complete genome. The difference in genomic composition of subtypes of a genotype is usually 20–25%. Subtypes 1a and 1b are found worldwide and cause 60% of all cases (Ohno *et al*., 2007; Hye *et al*., 2019). The identification of HCV genotypes is very important in selection of antiviral treatment. Genotype is an important indicator of sustained viral response rates. Antigenic differences between the genotypes may cause problems in the serological diagnosis of the infection (Nakano *et al*., 2011; Rose *et al*., 2013). HCV genotypes have also been shown to be a major tool for investigating outbreaks and for understanding the epidemiology of the HCV infection (Geert *et al*., 2016).

The HCV causes both acute and chronic hepatitis, ranging in severity from a mild illness lasting a few weeks to a serious, lifelong illness, and it is the major cause of liver cancer. HCV is a blood-borne virus; the most common modes of infection are through exposure to blood and blood products (Falade-Nwulia *et al*., 2019).

Globally, The WHO estimated that 71 million people were living with HCV infection in 2015, accounting for 1% of the world population. The infection is unevenly distributed in different countries, with prevalence in the general population ranging from 0.5 to 6.5%. In Western countries and Australia this rate ranges from 0.5 to 1.5%; it reaches 2.3% in countries of south-east Asia and in eastern Mediterranean regions (WHO, 2017), 3.2%

in China, 0.9% in India, 2.2% in Indonesia and 6.5% in Pakistan (Petruzziello *et al*., 2016). WHO estimated that in 2016, approximately 399 000 people died from hepatitis C, mostly from cirrhosis and HCC (Mehmet *et al*., 2018).

In both developed and developing countries, high risk groups include injecting drug users, recipients of unscreened blood, hemophiliacs, renal dialysis patients, thalassemia patients and person with multiple sex partners who engage in unprotected sex. In developed countries, it is estimated that 90% of persons with chronic HCV infection are current and former injecting drug users and those with a history of transfusion of unscreened blood or blood products (Zaltron *et al*., 2012).

The previous studies were conducted in different provinces of Iraq to found the prevalence of HCV were reported results, for instance, at Duhok City /Iraq found that the prevalence of HCV was 11.05% (Al-Khaffaj and AlGhazal, 2019). On other hand, the results in study conducted in Iraq by Hamied *et al*., (2010) that reported the HCV prevalence in Baghdad province (8.3%) and more than the findings obtained Tarky *et al*., (2013) in all over Iraqi governorates (0.4%).In study was enrolled in Karbala Governorate, Iraq was reported 3.6% HCV prevalence in 2016 (Mohammed *et al*., 2019).While, in Babylon (Al-Juboury *et al*.,2010) governorate showed a prevalence rate of HCV infection (0.5%). However, cross sectional study was conducted on 160 patients with chronic renal failure and on regular hemodialysis in the dialysis unit at Adiwaniyah Teaching Hospital, Adiwaniyah Province / Iraq. HCV was detected in 34 (21.2 %). These results were revealed by Wisam, (2020).

In Diyala province, is lucky by the large number of studies covering different aspects of HCV since 1989 (Hasan *et al*., 2005; Al-Jebori *et al*., 2010). Addidtionally, previous study in Diyala province reported 26.4% prevalence of HCV infection (Tareef *et al*., 2011). Another study in Diyala

province was conducted to determine the hepatitis C virus infection rate among unpaid blood donors and certain risky population (healthcare workers, thalassemia, and hemodialysis patients). The results showed that the higher hepatitis C virus infection rate was recorded among thalassemia patients (26.2%), followed by hemodialysis patients (21.7%) and Healthcare workers (3.3%), while the lowest infection rate was recorded among blood donors (1.1%) (Nadhim, 2012).

Unlike hepatitis A and B, there is currently no vaccine to prevent HCV infection. Research was in progress but the high mutability of HCV genome complicates vaccine development (WHO, 2004; David *et al*., 2013).

1.2 Aims of the Study

The present study was conducted in attempts to achieve the following goals:

1. Serological and molecular detection of HBV and HCV from high risk population: the thalassemia, renal dialysis patients (vaccinated) and blood donors (non-vaccinated) compared to non-risky or low risky population.
2. Typing of HBV and HCV from non-risky population as control group by partial sequencing of viral genes.
3. Exploration of the effect of socio-demographic factors included age, gender, residence, level of education and vaccination with HBV vaccine on infection rate of HBV and HCV.

الخلاصة

أجريت الدراسة الحالية للفترة من كانون الأول / 2019 إلى حزيران / 2021 في محافظة ديالى. تمت الموافقة علميا وأخلاقيا من قبل اللجنة العلمية في كلية التربية للعلوم الصرفة واللجنة العلمية لدائرة صحة ديالى. تهدف الدراسة الحالية إلى الكشف المصلي والجزيئي عن فيروس التهاب الكبد نمط B و التهاب الكبد نمط جـ من السكان المعرضين لمخاطر عالية ؛ وهي الثلاسيميا والغسيل الكلوي والمتبرعين بالدم مقارنة بأفراد مجموعة السيطرة، والتنميط الجيني لالتهاب الكبد نمط ب و نمط جـ عن طريق التسلسل الجزئي للجينات الفيروسية.

شملت الدراسة الحالية 360 مشاركا. كان النطاق العمري 3-69 سنة. الغالبية من الرجال 76.9٪. تم تقسيم مجتمع الدراسة إلى أربع مجموعات: 90 مريضًا مصابًا بالفشل الكلوي تم تطعيمهم بلقاح HB وخضعوا لغسيل كلوي ، 90 مريضًا مصابًا بالثلاسيميا مؤكدة مختبريًا تم تطعيمهم بلقاح HB ، و 90 مجموعة من المتبرعين بالدم لم يتم تطعيمهم بلقاح HB و 90 فردًا كعنصر تحكم لم يتم تطعيمهم بلقاح HB. تم إعادة ترميز المعلومات الاجتماعية والديموغرافية بالإضافة إلى حالة التطعيم ضد التهاب الكبد B للمشاركين. تم إجراء الكشف المصلي عن فيروس التهاب الكبد B و HCV في عينات المصل باستخدام تقنية ELISA للواسمات HBs Ag ، و HBc IgM ، و HBc IgG ، و anti-HBe Ab ، و Anti-HCV Ab.

أظهرت نتائج الدراسة الحالية أن المعدل الإيجابي العام لعلامات HBs Ag و HBc IgG و HBc IgM و Anti-HBe IgG و Anti-HCV Abs كان 6.7٪ 6.1٪ و 0.8٪ و 2.5٪ و 10. ٪ على التوالى.

وجدت النتائج أن نسبة HBs Ag الإيجابية بين مرضى غسيل الكلى ومرضى الثلاسيميا والمتبرعين بالدم والأفراد الأصحاء كانت 4.4٪ و 6.7٪ و 7.8٪ و 7.8٪ على التوالي. كانت نسبة anti-HBc IgG بين مرضى غسيل الكلى 17.8٪ أعلى معنويا من مرضى الثلاسيميا بنسبة 6.7٪. ومع ذلك ، كان جميع الأفراد في المتبرعين بالدم و مجموعة الاصحاء سلبيين. أظهر anti-HBc IgM أن معدل الإيجابية كان غير معنوي بين مجموعة غسيل الكلى ومجموعة الثلاسيميا (2.2٪ مقابل 1.1٪). لم يكن أي من الأفراد في المتبرعين بالدم والافراد الاصحاء إيجابيين تجاه مضاد anti-HBc IgM. كان معدل الإيجابية anti-HBe IgG مختلفًا إحصائيًا بين مرضى غسيل الكلى ومرضى الثلاسيميا (5.6٪ مقابل 4.4٪). لم يكن أي من المشاركين في المتبرعين بالدم والمجموعات السليمة إيجابيين لمضاد