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Prevalence and Risk Factors of Hepatitis B and C Viruses among Haemodialysis Patients in Gaza Strip, Palestine

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Dedication

This thesis is dedicated to my wonderful parents, who have raised me to be the person I'm today. You have been with me every step of the way, through good and bad times. Thank you for all the unconditional love, guidance, and support that you have always given me, helping me to succeed and instilling in me the confidence that I'm capable of doing anything I put my mind to. Thank you for everything.

Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university of other institute, except where due acknowledgment has been made in the text.

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Abstract

Prevalence and Risk Factors of Hepatitis B and C Viruses among Haemodialysis Patients in Gaza Strip, Palestine

End stage renal disease patients (ESRD) on maintenance haemodialysis (HD) are at high risk of acquiring hepatitis B and C viruses (HBV, HCV) infection. The aim of this study is to investigate the prevalence of HBV and HCV infection and to determine risk factors associated with their transmission among HD patients in Gaza Strip.

During the study all patients attending the four HD centers in Gaza Strip (246 patients) were tested for the presence of hepatitis B surface antigen (HBsAg) by using the second generation enzyme immunoassay and anti-HCV by using the third generation enzyme immunoassay. HCV RNA was detected using reverse transcriptase polymerase chain reaction (RT-PCR). A questionnaire that included possible risk factors was completed by the researcher via patient interview to insure proper data collection.

The overall prevalence of HBV among the four HD centers was 8.1% compared with a prevalence rate of 2.3% among healthy blood donors in Gaza according to the latest report of the Palestinian Ministry of Health (MOH). The main risk factors were HD center ($p=0.05$), history of blood transfusion ($p<0.01$) and treatment abroad ($p=0.01$).

The overall prevalence of HCV among the four HD centers was 22% compared with a prevalence rate of 0.2% among healthy blood donors in Gaza according to the latest report of MOH. The main risk factors were HD center ($p<0.01$), time duration on HD ($p<0.01$), history of blood transfusion ($p<0.01$), treatment abroad ($p<0.01$) and history of blood transfusion abroad ($p<0.01$).

The much higher prevalence of Hepatitis viruses among HD patients compared to normal population of Gaza Strip indicates a causative relation between HD and hepatitis viruses transmission. Therefore extremely careful observation of preventive infection control measures is essential to limit Hepatitis viruses' transmission in HD centers.

Key words: Haemodialysis, HBV, HCV, risk factors, Gaza Strip, Palestine.

ملخص البحث

مدى انتشار و عوامل الخطر للإصابة بفيروسات التهاب الكبد الوبائي من نوع B و C بين مرضى التحال الدموي في قطاع غزة، فلسطين.

يتعرض مرضى الفشل الكلوي الذين يتلقون تحال دموي (ديليزة) إلى خطر شديد للإصابة بالتهابات الكبد الوبائي الفيروسي من نوع B و C. هدفت هذه الدراسة إلى معرفة مدى انتشار هذه الفيروسات بين مرضى التحال الدموي في قطاع غزة و تحديد أهم عوامل الخطر التي تؤدي إلى انتقالها بين هؤلاء المرضى.

شملت الدراسة جميع المرضى الذين ارتادوا وحدات التحال الدموي الأربع في قطاع غزة (246 مريض) خلال فترة الدراسة. تم جمع عينات دم من المرضى وفحصها باستخدام الجيل الثاني من تقنية الإنزيمات المرتبطة بالأجسام المناعية لالتهاب الكبد الوبائي من نوع B و الجيل الثالث من تقنية الإنزيمات المرتبطة بالأجسام المناعية لالتهاب الكبد الوبائي من نوع C و فحص الحمض النووي للفيروس C باستخدام طريقة إكثار الحمض النووي (RT-PCR). كما تم عمل استبيان يحتوي على أسئلة لدراسة عوامل الخطر المتوقعة للإصابة بهذا الفيروسات و تمت تعبئة الإستبانة عن طريق المقابلة الشخصية لضمان صحة البيانات المعطاة.

أظهرت الدراسة أن فيروس الكبد الوبائي من نوع B ينتشر بين مرضى التحال الدموي بنسبة 8.1% مقارنة بنسبة انتشار 2.3% بين المتبرعين بالدم الأصحاء في قطاع غزة، حسب آخر تقارير وزارة الصحة الفلسطينية و بينت الدراسة أيضا أن أهم العوامل المساهمة في انتشار الفيروس هي وحدة التحال ($p=0.05$)، عدد وحدات الدم التي تلقاها المريض ($p<0.01$) و العلاج خارج البلاد ($p=0.01$).

أظهرت الدراسة أن فيروس الكبد الوبائي من نوع C ينتشر بين مرضى التحال الدموي بنسبة 22% مقارنة بنسبة انتشار 0.2% بين المتبرعين بالدم الأصحاء، في قطاع غزة حسب آخر تقارير وزارة الصحة. و بينت الدراسة أيضا أن أهم العوامل المساهمة في انتشار الفيروس هي وحدة التحال ($p<0.01$)، مدة التحال ($p<0.01$)، عدد وحدات الدم التي تلقاها المريض ($p<0.01$)، العلاج في الخارج ($p=0.01$) و تلقي دم في الخارج ($p<0.01$).

تظهر الدراسة أن الارتفاع الشديد للإصابة بالتهابات الكبد الوبائي B ، C بين مرضى التحال الدموي يدل على وجود علاقة بين التحال الدموي و التهاب الكبد الوبائي و لذلك هناك ضرورة لاتخاذ أقصى درجات الرقابة و إتباع التوصيات و الطرق الوقائية السليمة للحد من انتشار الفيروسات بين مرضى التحال الدموي.

الكلمات الدالة: التحال الدموي (الديليزة)، التهاب الكبد الوبائي B ، التهاب الكبد الوبائي C، عوامل الخطر، قطاع غزة، فلسطين.

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List Of Abbreviations

ALT	Serum Alanine Aminotransferase
AST	Serum Aspartate Aminotransferase
BVDV	Bovine Viral Diarrhea Virus
CCC	Covalently Closed Circular
CDC	Centers for Disease Control and Prevention
cDNA	Complementary Deoxyribo Nucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
ER	Endoplasmic Reticulum
ESRD	End Stage Renal Disease
HBeAg	Hepatitis B e Antigen
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HD	Haemodialysis
HVR	Hyper Variable Region
IFN	Interferon
IgM	Immunoglobulin M
IRES	Internal Ribosome Entry Site
ISDR	Interferon Sensitivity Determining Region
kb	Kilobase
KD	Kilodalton

MEIA	Microparticle Enzyme Immunoassay
MOH	Palestinian Ministry of Health
NAT	Nucleic Acid Amplification Technology
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
preC-C	precore-core
preS-S	Presurface-Surface
PTB	Polypyrimidine Tract Binding Protein
RaRp	RNA dependent RNA polymerase
RFLP	Restriction Fragment Length Polymorphism
RIBA	Recombinant Immunoblot Assay
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
TBE	Tick Borne Encephalitis
UAE	United Arab Emirates
USA	United States of America
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

1.1 Overview

Patients undergoing haemodialysis (HD) potentially have an increased risk of infection with parenterally transmitted viral agents due to the impairment of their host immune response and to the multiple transfusion requirements **(1)**. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are important causes of morbidity and mortality in HD patients and pose problems in the management of patients in the renal dialysis units, because chronic renal failure patients do not clear these viral infections efficiently **(2, 3)**.

HBV infection is usually less prevalent than HCV in HD units **(4)**. The introduction of HBV vaccination, the isolation of HBV positive patients and the use of dedicated dialysis machines and regular surveillance for HBV infection dramatically reduced the spread of HBV in this setting **(5)**. On the other hand, the prevalence of HCV infections among HD patients is high and varies from (2% to 60%) between different countries, and between different dialysis centers within a single country **(6)**. Moreover a dual infection with HBV and HCV leads to more aggressive liver disease **(7)**.

The nosocomial transmission of HCV and HBV appears to be an important contributing factor to the spread of these viruses among populations **(8)**. Although the prevalence of HBV has been drastically reduced with the application of control practices, outbreaks are still frequent and routine screening is still necessary **(9)**. HBV is transmitted by percutaneous or permucosal exposure to infectious blood or to body fluids that contain blood and the chronically infected person is central to the epidemiology of HBV transmission. All hepatitis B surface antigen (HBsAg) positive persons are infectious, but those who are also positive for hepatitis B-e antigen (HBeAg) circulate HBV at high titers in their blood (10^{8-9} virions/mL) **(10)**.

With such high virus titers in the blood, other body fluids containing serum or blood also can contain high levels of HBV and are potentially infectious. Furthermore, HBV at titers of 10^{2-3} virions/mL can be present on environmental surfaces in the absence of any visible blood and still result in transmission **(11, 12)**.

HBV is relatively stable in the environment and remains viable for at least 7 days on environmental surfaces at room temperature **(12)**. HBsAg has been detected in dialysis centers on clamps, scissors, dialysis machine control knobs and doorknobs **(13)**. Thus, blood contaminated surfaces that are not routinely cleaned and disinfected represent a reservoir for HBV transmission to HD patients. Dialysis staff members can transfer the virus to patients from contaminated surfaces by their hands or gloves or through use of contaminated equipment and supplies **(13)**.

Several reports from around the world indicated that the frequency of HCV is higher in patients undergoing maintenance HD than in the general population. The reported prevalence of HCV infection in maintenance HD patients varies markedly from country to country and from one center to another, ranging between 8% to 39% in North America, 1% to 54% in Europe, 17% to 51% in Asia, and 1% to 10% in Australia **(14, 15)**. In Iran, the prevalence of HCV varies from 5.5% to 24% and it reaches more than 20% in the Mediterranean area **(14, 16, 17)**. The relevance of HCV infection in patients on HD is usually related to the development of serious liver disease, particularly after renal transplantation **(18)**.

Blood transfusion and the length of time on HD were the main factors involved in HCV transmission to HD patients in the past. Despite the nowadays screening of blood products for HCV and the wide use of erythropoietin, which reduces blood transfusion requirements, some patients still become infected by HCV during HD **(19)**. Screening of HCV is based on the detection of anti-HCV antibodies. However serological assays for detecting anti-HCV antibodies cannot distinguish between patients with active infection and those who have cleared the virus. Moreover, false negative results may be obtained during the

first 4 months after the exposure when no enough antibodies are produced. Due to the absence of an efficient in vitro culture system for HCV or assays capable of detecting viral antigens, direct detection of HCV depends on nucleic acid amplification technology (NAT) techniques such as Polymerase Chain Reaction (PCR) (20).

In Gaza Strip, slightly more than 250 end stage renal disease (ESRD) patients receive HD in four centers at Al-Shifa, Shuhada`a Al-Aqsa, Nasser, and Abu-Yousef Al-Najar Hospitals. There is almost no data about the situation in these centers regarding hepatitis infection, while infection control measures are not applied and/or not strictly followed. In addition, recommendation for the prevention of blood borne infection which include infection control practice specifically designed for the HD setting, routine serologic testing and immunization; surveillance; and training and education programs are not available in most of these centers.

No documented data or previous studies have been reported about the prevalence of hepatitis viruses among HD patients in these centers and this study is the first to address this issue.

1.2 Objectives

The main objective of this investigative work is to estimate the prevalence of hepatitis viruses (B and C) among HD patients in Gaza Strip, using serological and PCR techniques.

The specific aims are as follows:

1. To assess the importance of both serological and DNA based methods in detecting hepatitis B and C viruses.
2. To assess the role of HD setting, patient's practices, blood transfusion, time duration on HD and treatment abroad as major risk factors for hepatitis viruses' transmission.
3. To assess socioeconomic risk factors associated with the above mentioned viral infections.

1.3 Significance

There is a high prevalence of hepatitis viruses among HD patients all over the world. Serious sequelae may occur due to the absence of effective treatment for hepatitis viruses. Lack of data on the prevalence of these viruses and their risk factors among HD patients in Gaza Strip would increase the incidence rate of these viruses. The high cost of the treatment of complications due to infection by these agents adds to the burdens of the devastated health sector.

This study highlights the prevalence of viral hepatitis in HD patients, which may contribute to the improvement of practices in HD centers and the management of hepatitis viruses (B and C) among HD patients. In addition, the determination of risk factors for transmission of these infections would greatly reduce their incidence not only in HD patients but also in their contacts.

CHAPTER 2

LITERATURE REVIEW

2.1 Haemodialysis

ESRD is a chronic condition in which kidney function is impaired to the extent that the patient's survival requires removal of toxins from the blood by dialysis therapy or kidney transplantation. Due in part to a limited availability of kidneys for transplantation, HD is the primary method of treatment and is currently used for approximately 61% of United States of America (USA) ESRD patients **(21)**.

In HD, waste products are removed from the blood by allowing them to pass across a thin semi-permeable membrane into dialysis fluid (dialysate), which is then discarded along with the waste products. Uremic nitrogenous waste, potassium, phosphate, and magnesium move from blood into the dialysate down the concentration gradient, and calcium and bicarbonate (present in higher concentration in the dialysate) move into the circulation. This process is controlled by a monitor, often called a dialysis machine **(22, 23)**.

HD can vary in terms of the setting in which it is carried out (e.g. home, satellite unit or hospital), the type of equipment used (e.g. different HD machines), the duration of sessions (e.g. 4, 6 or 8 hours), the frequency of sessions (e.g. 3, 5, 6 or 7 times per week), the type of membranes used (e.g. those made out of cellulose, modified cellulose or synthetic materials, or the flux of the membrane) and the dialysate (e.g. acetate or bicarbonate)**(22)**.

In spite of numerous therapeutic advances since dialysis therapy became widespread in the 1960s, mortality and hospitalization rates of dialysis patients remain high. In USA, median survival of dialysis patients is less than 4 years; with dialysis patients' average approximately 1.4 hospital admissions per year for an average of 11 hospital days per patient **(24, 25)**.

In Sweden the average 5-year survival rate for patients on HD is 23%. The duration of survival is clearly related to patient age in that the mean 5-year

survival is 64% in patients younger than 65 years of age and only 15% in those older than 65 years. By comparison, the expected survival in the background population is 97% and 75%, respectively **(26)**.

2.1.1 Access for HD

Obtaining and maintaining adequate access to the blood circulation remains a major impediment to the long term success of HD. The fistula, conduit, or catheter through which blood is accessed for HD is often referred to as a "dialysis access" **(27, 28)**.

The placement of large needles (typically 15 gauge) is required to remove blood and to return it after it has passed through the dialyzer. A large, thick-walled fistula can be created by shunting blood from an artery to a vein; the result is the growth and thickening of the venous wall, which then tolerates repeated cannulation **(29)**.

When dialysis is urgently required, a double lumen dialysis catheter is used. Insertion of the catheter into the subclavian vein has fallen into disfavor because such catheters are associated with a high incidence of venous stenosis or thrombosis, which can interfere with the future creation of an arteriovenous fistula or graft in the lateral arm or which may cause chronic edema of the arm. Insertion into the jugular vein is becoming the method of choice because it seems to result in less central venous injury and is a safe procedure, especially when done under ultrasound guidance **(30)**.

Because of their ease of placement, femoral-vein catheters can be inserted in patients who have respiratory distress or coagulopathy, or who require only one or two dialysis treatments. Implantation of a dual lumen cuffed catheter is a good option for patients who have delayed recovery from acute renal failure, who require access for dialysis until a fistula matures, or who lack any other suitable site for graft placement. If carefully maintained, almost half of these catheters remain functional at one year **(31)**.

2.1.2 Infections in HD

Chronic HD patients are at high risk for infection because the process of HD requires vascular access for prolonged periods. In an environment where multiple patients receive dialysis concurrently, repeated opportunities exist for person-to-person transmission of infectious agents, directly or indirectly via contaminated devices, equipment and supplies, environmental surfaces, or hands of personnel. Furthermore, HD patients are immunosuppressed **(32)**.

Historically, surveillance for infections associated with chronic HD focused on viral hepatitis. In USA, centers for disease control and prevention (CDC) began conducting national surveillance for HD associated hepatitis in 1972 **(33, 34)**. Other HD associated diseases and practices not related to hepatitis have been included over the years (e.g., pyrogenic reactions, dialysis dementia, vascular access infections, vancomycin use), and the system is continually updated to collect data regarding HD associated practices and diseases of current interest and importance **(35)**.

Outbreaks of both HBV and HCV infections continue to occur among chronic HD patients. Epidemiologic investigations have indicated substantial deficiencies in recommended infection control practices, as well as a failure to vaccinate HD patients against hepatitis B **(36, 37)**.

2.2 Hepatitis B Virus

2.2.1 Overview

Hepatitis B is the most common blood borne viral infection worldwide; it causes transient and chronic infections of the liver. Transient infections may produce serious illness, and approximately 0.5% terminates with fatal, fulminate hepatitis **(38)**. Chronic infections may also have serious consequences; nearly 25% terminate in untreatable liver cancer **(38)**. It was estimated that a proportion in the order of 30% of the world population (2 billion persons) has been infected with HBV, of which 350 million are being endangered by serious long-term complications of chronic HBV infection, namely cirrhosis and liver cancer. The

toll of these complications was estimated by the World Health Organization (WHO) to be 1 million deaths per annum **(39)**.

HBV in blood or blood contaminated body fluids is transmitted in HD centers by the percutaneous route or direct contact with mucous membranes. In addition, HBV may be viable for at least a week on environmental surfaces at room temperature **(12)**. Reported transmission incidents have involved transfer of HBV from contaminated surfaces via staff members' gloves or hands, or contaminated equipment in dialysis centers **(13)**.

2.2.2 HBV History

The story of HBV started when pathologic studies revealed diffuse hepatic inflammation in persons with acute jaundice, suggesting an infectious cause **(40)**. Studies of human volunteers in the 1930s and 1940s provided convincing evidence of a viral cause with at least two etiologic agents **(41, 42)**.

In 1947, MacCallum and Bauer proposed the current nomenclature of hepatitis A for infectious hepatitis and hepatitis B for "homologous serum" hepatitis **(42)**. Blumberg and Alter described an immunoprecipitin that was present in the serum of a leukemic patient and was detected in a gel diffusion experiment, the patient was an Australian aborigine, and the antigen was named the Australian antigen **(43)**.

The association of the Australian antigen with acute hepatitis B was subsequently demonstrated and led to the development of specific tests for the identification of HBV infection **(44, 45)**.

The viral etiology of hepatitis B was firmly established by electron microscopy and the detection of several viral particles (referred to as Dane particles) that reacted with antisera to Australia antigen **(46)**. It was demonstrated that the Dane particle was HBV, and its surface component was designated hepatitis B surface antigen (HBsAg). The core component contained endogenous DNA and hepatitis core antigen (HBcAg). A third antigen related to infectivity, hepatitis e antigen (HBeAg), was first described in 1972 by Magnius and Espmark **(47)**.

2.2.3 Epidemiology of HBV in General Population

Although the prevalence of HBV carriers varies between countries in the same continent, it can be broadly classified into regions of high endemicity (HBsAg prevalence $\geq 8\%$), intermediate (HBsAg prevalence 2-7%) and low HBV endemicity (HBsAg prevalence $< 2\%$) (Figure 2.1) **(48)**.

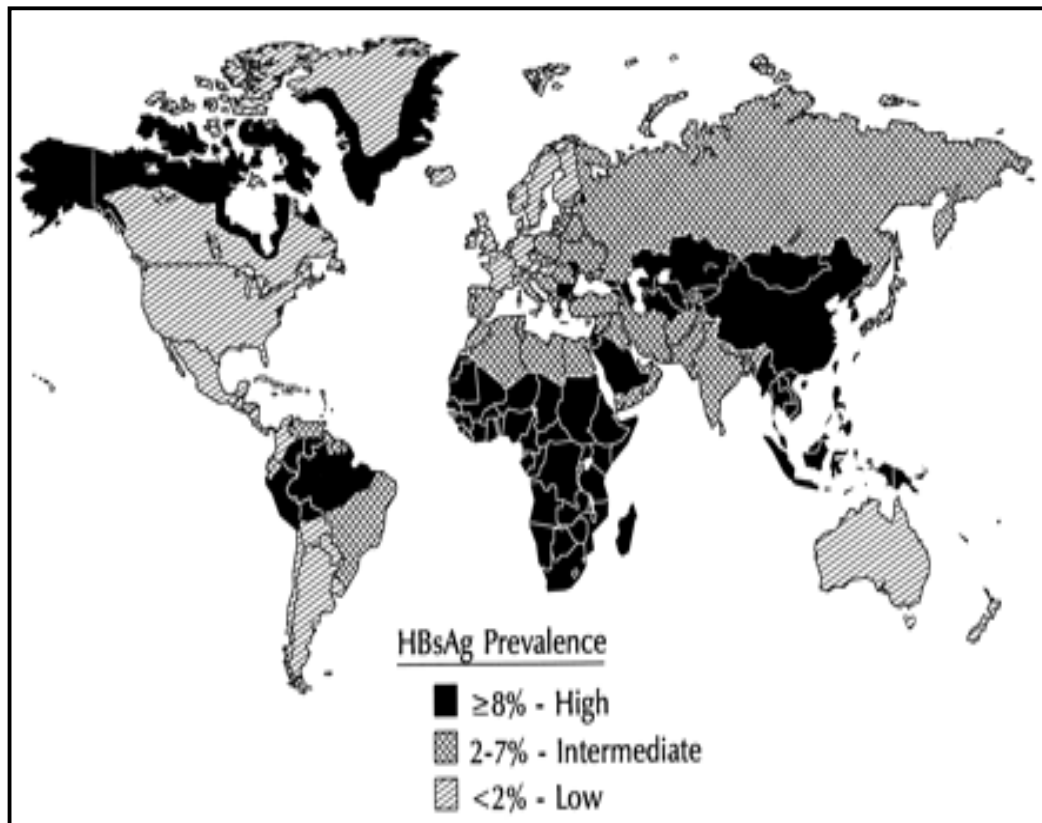


Figure (2.1): Geographical distribution of chronic hepatitis B virus infection (adapted from WHO) (39).

In areas of high endemicity, the lifetime risk of HBV infection is $>60\%$ and most infections occur at birth or during early childhood, when the risk of chronic infection is greatest. Because most early childhood HBV infections are asymptomatic, there is little recognition of acute disease, but rates of chronic liver disease and liver cancer are high **(49)**.

In areas of moderate endemicity, the lifetime risk of HBV infection is 20 to 60% and infections occur in all age groups. Generally, in areas of moderate endemicity 2 to 7% of pregnant women are HBsAg positive, and $<20\%$ of

HBsAg positive women are HBeAg positive; thus perinatal transmission accounts for a small proportion (10 to 20%) of the persons with chronic infection. In areas of low endemicity, the lifetime risk of infection is <20% and most infections occur among adults in well defined risk groups **(49)**.

Studies in the Middle East showed that the prevalence of HBsAg range from 3% to 11% in Egypt, 4% to 5% in Iraq, 2.6% to 10% in Jordan, 2% to 6% in the Libyan Arab Jamahiriya, 2.3% to 10% in Oman, 7.4% to 17% in Saudi Arabia, 16% to 20% in Sudan, 6.5% in Tunisia, 2% to 5% in United Arab Emirates, and 12.7% to 18.5% in the Republic of Yemen **(50)**.

In Gaza Strip the prevalence of HBsAg was found to be 3.5% in the general population and 3.8% in blood donors. The simulation model revealed that the incidence of HBV infection decreased between 1990 and 1999 from 233 to 56 per 100,000 per annum. The decline started in 1994 and continued afterwards, presumably after the introduction of universal vaccination against HBV and screening blood donors for HBV **(51)**. According to the last Palestinian Ministry of Health (MOH) reports in 2005, the prevalence of HBV carriers among blood donors was 2.3% **(52)**.

2.2.4 Prevalence of HBV in Dialysis Population

The risk of acquiring HBV infection has been apparent since HD was first performed in the 1960s. In USA, a large survey of HD centers in 1974 found HBV incidence rates of 6.2% among patients and 5.2% among staff **(35)**. Contaminated dialysis machines, other equipment and environmental surfaces were accused in spreading of HBV among HD patients **(53-55)**.

Furthermore, HD patients are immunosuppressed; which may lead to increase their susceptibility to infections and could explain the observed high frequency among HD patients **(56, 57)**.

As a result of segregation, universal precautions, vaccination, reduced blood transfusions, and screening of organs before transplantation, the incidence of HBV infection decreased to 0.08% for patients and 0.05% for staff within

dialysis units in USA by 1996 **(35)**. These achievements were also supported by better blood bank screening-measures, the introduction of which dramatically decreased the risk of transfusion associated HBV infection **(58)**.

In spite of the reduction of HBV spread within dialysis centers, some isolated outbreaks of HBV infection continue to be reported among HD patients in developed countries **(59, 60)**. The prevalence of chronic HBsAg positivity among HD patients ranged between 0.9% in USA to 29.8% in Brazil (Table 2.1).

Table (2.1): The worldwide prevalence of HBV among HD patients.

Country	Year	Total no. of patients	Result %	Ref.
USA	2001	252739	0.9	61
Japan	1992	607	1.6	62
Switzerland	2000	1713	1.63	63
Maroco	2005	186	2	64
Iran	2005	324	4.6	65
Jordan	2008	427	5.9	66
Kenya	2003	100	8	67
Italy	1991	2,180	9.2	68
Saudi Arabia	2001	67	10	69
Bahrain	2004	81	11.8	70
Pakistan	2004	97	12.4	71
India	2005	75	14.2	72
Taiwan	1996	173	16.8	73
Romania	1999	169	17	74
Greece	2006	49	20.4	75
Span	2005	86	20.9	76
Turkey	2006	188	25	77
Brazil	2006	1095	29.8	78

2.2.5 Classification and Structure of HBV

HBV is the prototype member of the Hepadnaviridae (hepatotropic DNA virus) family. Hepadnaviruses have a strong preference for infecting liver cells, but small amounts of Hepadnaviral DNA can be found in kidney, pancreas, and mononuclear cells. However, infection at these sites is not linked to extra hepatic disease **(79- 81)**.

HBV virions are double-shelled particles, 40 to 42 nm in diameter, with an outer lipoprotein envelope that contains three related envelope glycoproteins (or surface antigens). Within the envelope is the viral nucleocapsid, or core, the core contains the viral genome, a relaxed-circular, partially duplex DNA of 3.2 kb, and a polymerase that is responsible for the synthesis of viral DNA in infected cells. DNA sequencing of many isolates of HBV has confirmed the existence of multiple viral genotypes, each with a characteristic geographic distribution **(46, 82- 85)**.

In addition to virions, HBV-infected cells produce two distinct subviral lipoprotein particles, 20-nm spheres, and filamentous forms of similar diameter. These HBsAg particles contain only envelope glycoproteins and host-derived lipids **(83)**.

2.2.6 Viral genes and Proteins

The HBV genome has only four long open reading frames (ORF). The presurface-surface (preS-S) region of the genome encodes the three viral surface antigens by differential initiation of translation at each of three in-frame initiation codons. The most abundant protein is the 24-KD S protein (which is known as HBsAg) **(82, 83)**.

Initiation at the most upstream start codon generates the M (or preS2) protein, the function of which is unknown. Initiation at the next upstream start-codon yields the L (or preS1) protein, which is thought to play key roles in the binding of the virus to host cell receptors and in the assembly of the virion and its release from the cell **(86, 87)**.

The precore-core (preC-C) region encodes hepatitis B core antigen (HBcAg) and hepatitis B e antigen (HBeAg). These two proteins are also derived by alternative initiation of translation at two in-frame AUG codons (82).

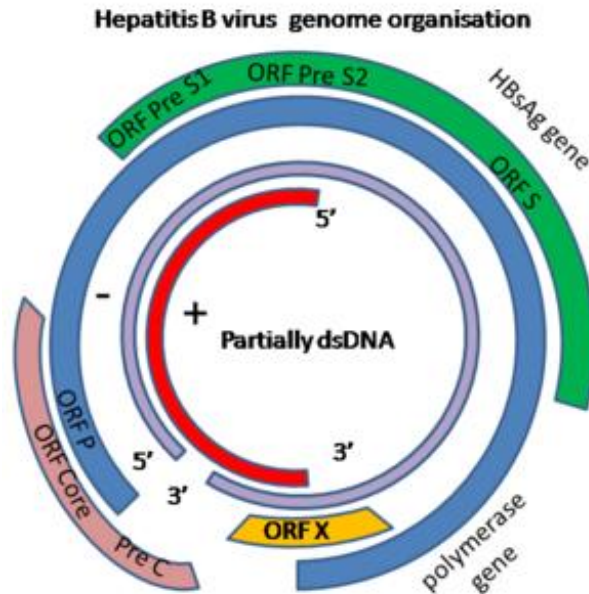


Figure (2.2): Schematic representation of HBV genome organization (adapted from) (49).

The internal AUG encodes the 21-KD C protein, the structural polypeptide of the viral capsid, whereas the upstream AUG directs production of the 24-kD preC protein. The preC region encodes a signal sequence, which directs the chain into the secretory pathway. As the chains traverse the Golgi complex, cleavage by cellular proteases generates HBeAg, a 16-kD fragment that is secreted into the blood (88).

HBeAg plays no role in viral assembly, and its function is not clear. It is not required for viral replication; mutants bearing chain-terminating lesions within the preC region replicate well in culture and, in fact, arise frequently during natural infection (89).

The P coding region is specific for the viral polymerase, a multifunctional enzyme involved in DNA synthesis and RNA encapsidation. The X open reading frame encodes the viral X protein (HBx), which modulates host-cell

signal transduction and can directly and indirectly affect host and viral gene expression. X-protein activity is absolutely required for the *in vivo* replication and spread of the virus **(90)**.

2.2.6 Viral Replication Cycle

In hepadnavirus replication cycle, the cardinal feature is the replication of the DNA genome by reverse transcription of an RNA intermediate **(91)**. Incoming HBV virions are bound by cell surface receptors, the identity of which remains unknown. After membrane fusion, cores are presented to the cytosol and transported to the nucleus. There, their DNA genomes are converted to a covalently closed circular (ccc) form, which serves as the transcriptional template for host RNA polymerase II. This enzyme generates a series of genomic and subgenomic transcripts **(92, 93)**.

All viral RNA is transported to the cytoplasm, where its translation yields the viral envelope, core, and polymerase proteins, as well as the X and preC polypeptides. Next, nucleocapsids are assembled in the cytosol, and during this process a single molecule of genomic RNA is incorporated into the assembling viral core. Once the viral RNA is encapsidated, reverse transcription begins **(94)**. The synthesis of the two viral DNA strands is sequential. The first DNA strand is made from the encapsidated RNA template, during or after the synthesis of this strand the RNA template is degraded and the synthesis of the second DNA strand proceeds, with the use of the newly made first DNA strand as a template **(91, 93, 95)**.

Some cores bearing the mature genome are transported back to the nucleus where their newly minted DNA genomes can be converted to cccDNA to maintain a stable intranuclear pool of transcriptional templates **(92)**. Most cores, however, bud into regions of intracellular membranes bearing the viral envelope proteins. In so doing, they acquire lipoprotein envelopes containing the viral L, M, and S surface antigens and are then exported from the cell, Because HBsAg can inhibit the formation of cccDNA, this may represent negative feedback to HBV replication **(96)**.

2.2.7 Clinical Features

HBV causes both acute and chronic hepatitis. The incubation period ranges from 45–160 days (mean: 120 days), and the onset of acute disease is usually insidious. Infants, young children (aged <10 years), and immunosuppressed adults with newly acquired HBV infection are usually asymptomatic **(97)**.

When present, clinical symptoms and signs might include anorexia, malaise, nausea, vomiting, abdominal pain, and jaundice. Extrahepatic manifestations of disease (e.g., skin rashes, arthralgias, and arthritis) can also occur **(98)**.

The case fatality rate after acute hepatitis B is 0.5%–1%. In adults with normal immune status, most (94%–98%) recover completely from newly acquired HBV infections, eliminating virus from the blood and producing neutralizing antibody that creates immunity from future infection **(97, 99)**.

In immunosuppressed persons including HD patients, infants, and young children, most newly acquired HBV infections result in chronic infection. Although persons with chronic HBV infection are often asymptomatic, chronic liver disease develops in two thirds of these persons, and approximately 15%–25% die prematurely from cirrhosis or liver cancer **(100-102)**.

2.2.8 Diagnosis

Because the clinical symptoms of HBV infection are indistinguishable from other forms of viral hepatitis, definitive diagnosis is dependent on serologic testing for HBV infection. A variety of tests are available to make the diagnosis of HBV infection **(99)**.

2.2.8.1 Antigen Detection

In newly infected persons, HBsAg is present in serum 30–60 days after exposure to HBV and persists for variable periods. Transient HBsAg positivity (lasting <18 days) can be detected in some patients during vaccination **(103, 104)**. Anti-HBc develops in all HBV infections, appearing at onset of symptoms or liver test abnormalities in acute HBV infection, rising rapidly to high levels, and persisting for life. Acute or recently acquired infection can be distinguished

by presence of the immunoglobulin M (IgM) class of anti-HBc, which persists for approximately 6 months **(105)**.

In persons who recover from HBV infection, HBsAg is eliminated from the blood, usually in 2-3 months, and anti-HBs develops during convalescence. The presence of anti-HBs indicates immunity from HBV infection. After recovery from natural infection, most persons will be positive for both anti-HBs and anti-HBc, whereas only anti-HBs develops in persons who are successfully vaccinated against hepatitis B. Persons who do not recover from HBV infection and become chronically infected remain positive for HBsAg (and anti-HBc), although a small proportion (0.3% per year) eventually clear HBsAg and might develop anti-HBs **(102)**.

In some persons, the only HBV serologic marker detected is anti-HBc (i.e., isolated anti-HBc). Among most asymptomatic persons in USA tested for HBV infection, an average of 2% (range: <0.1%–6%) test positive for isolated anti-HBc **(106)**.

A third antigen, HBeAg, can be detected in serum of persons with acute or chronic HBV infection. The presence of HBeAg correlates with viral replication and high levels of virus (i.e., high infectivity). Anti-HBe correlates with the loss of replicating virus and with lower levels of virus. However, all HBsAg-positive persons should be considered potentially infectious, regardless of their HBeAg or anti-HBe status **(35)**.

2.2.8.2 HBV DNA Detection

Detection of HBV DNA has limited usefulness for diagnostic purposes. HBV DNA is detectable in the serum of persons with acute and chronic HBV infection **(99, 107-109)**. Most slot or dot blot hybridization assays can detect HBV DNA unto 1.5×10^6 genomes/ml; branched-DNA hybridization assay detects 7.5×10^5 of HBV DNA per ml **(49)**.

PCR is much more sensitive than direct hybridization and detects HBV DNA levels of approximately 100 to 1,000 genomes per ml; the clinical significance of

detecting HBV DNA by PCR has the same significance as detection of HBsAg and indicates current HBV infection. Monitoring HBV DNA levels is useful in determining the response of chronic HBV infection to treatment, Nucleic acid sequence analysis has been used to identify genetic variants of the virus and to investigate common-source outbreaks of HBV infection **(110, 111)**.

2.2.9 Hepatitis B Vaccine in HD Patients

Hepatitis B vaccine has been recommended for both HD patients and staff members since the vaccine became available in 1982 **(112)**. The recommended primary series of hepatitis B vaccine induces a protective anti-HBs response in 90%– 95% of adults with normal immune status. The major determinant of vaccine response is age, with the proportion of persons developing a protective antibody response declining to 84% among adults aged >40 years and to 75% by age 60 years **(113, 114)**.

Compared with adults with normal immune status, the proportion of HD patients who develop a protective antibody response after vaccination (with higher dosages) is lower. For those who receive the three-dose schedule, the median is 64% (range: 34%–88%) **(115-120)**, and for those who receive a four dose schedule, the median is 86% (range: 40%–98%) **(121-127)**.

Higher antibody response rates could be achieved by vaccinating patients with chronic renal failure before they become dialysis dependent. After vaccination with four doses of recombinant vaccine, a protective antibody response developed in 86% of predialysis adult patients with serum creatinine levels ≤ 4.0 mg/dl (mean: 2.0 mg/dl) compared with 37% of those with serum creatinine levels > 4.0 mg/dl (mean: 9.5 mg/dl), only 12% of whom were predialysis patients **(128)**.

Although no data exists on the response of pediatric HD patients to vaccination with standard pediatric doses, 75%–97% of those who received higher dosages on either the three or four dose schedule developed protective levels of anti-HBsAg **(129-131)**.

2.3 Hepatitis C Virus

2.3.1 Overview

HCV infection is a major cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC) (132). Around 3% of the world population is chronically infected with HCV and there are some 170 million chronic HCV carriers through the world (133, 134). HCV is considered as the major cause of liver disease among patients undergoing maintenance HD and also the major cause of mortality and morbidity in these patients (135- 139).

2.3.2 HCV History

the story of HCV began shortly after the identification of the hepatitis A and B viruses, it became clear that most cases of post transfusional hepatitis could not be attributed to either of these agents. This led to the description of another form of hepatitis, called non-A, non-B hepatitis.

In the early 1980 the putative agent of non-A, non-B hepatitis was characterized as a filterable microorganism believed to be a virus, approximately 50 nm in diameter. Because its infectivity was abolished by treatment with chloroform, it was assumed to have a lipid envelope, It was also inactivated by exposure to formalin, heat, and ultra violet light (140, 141).

These initial studies formed the basis for a molecular biological approach for identifying and characterizing the etiologic agent responsible for the great majority of non-A, non-B viral hepatitis, now called the HCV. The approach taken was to harvest viruses from large volumes of serum of one of the experimentally infected chimpanzees. Because the genomic nature of the putative virus was not known, both DNA and RNA were extracted and converted to complementary DNA (cDNA).

Restriction fragments of this cDNA were cloned into a recombinant bacteriophage vector to form a cDNA library, and these phages were then inserted into *Escherichia coli* capable of transcribing and expressing the

peptides encoded by the restriction fragments. These expression products were screened with the use of sera from patients with chronic non-A, non-B hepatitis. It was assumed that the sera from such patients would contain antibodies against peptide products of the infectious agent. More than one million clones were screened in this way and 5 were found to be consistently and strongly reactive. Identification of other clones with overlapping regions of cDNA eventually allowed investigators to establish the sequence of the entire viral genome in 1989 **(142, 143)**.

2.3.3 Epidemiology of HCV in General population

Worldwide, it is estimated that there are approximately 170 million persons infected with HCV, this is nearly 3% of the world population **(144)**. This range varying from 0.5 to 5% in the general population of western countries and USA while higher rates have been reported in some African and southeast Asian countries **(145-147)**. In some highly endemic areas of the world the prevalence rates range from 10% to 30% **(148)**. In the most highly endemic areas of the world, HCV infection is prevalent among persons older than 40 years but is uncommon in those younger than 20 years **(149,150)**.

A prevalence rate of about 7% has been observed in a subgroup of elderly people in Sardinia and prevalence rates of HCV carriers as high as 11% have been reported in some subgroups of the general population in Southern Italy **(151)**. In the sub Saharan Africa the overall estimated HCV prevalence was 3% (median prevalence 2.2%, range from 0.1% to 13.8%) **(152)**.

In Egypt studies showed that the antibody prevalence in blood donors ranged from 6 to 38% and averaged about 15% **(153-157)**. Other neighboring countries such as Saudi Arabia, Syria and Jordan have HCV prevalence rates of 2.5%, 1% and 1.7% respectively **(158-160)**. In "Israel" the reported prevalence of HCV infection is 0.5% **(161)**. In Palestine the HCV incidence was 4.2 and 5.8 per 100,000 in the years 2002 and 2003 respectively.

In Gaza Strip alone, 21,219 blood samples were screened in 2003 for Anti-HCV antibodies in the blood banks, among which 67 samples were found positive (prevalence rate of 0.3) **(162)**. During the year 2005, only 2 cases of hepatitis C and 194 hepatitis C carriers were reported with an incidence rate of 5.2 per 100,000 compared with an incidence rate of 5.5 in the year 2004, the prevalence rate of hepatitis C was 0.2% among blood donors by serology method according to MOH **(52)**.

2.3.4 Prevalence of HCV in Dialysis Patients

HCV is most efficiently transmitted by direct percutaneous exposure to infectious blood, and like HBV, the chronically infected person is central to the epidemiology of HCV transmission. Risk factors associated with HCV infection among HD patients include history of blood transfusions, the volume of blood transfused, and number of years on dialysis **(163)**. The number of years on dialysis is the major risk factor independently associated with higher rates of HCV infection. As the time patients spent on dialysis increased, their prevalence of HCV infection increased from an average of 12% for patients receiving dialysis <5 years to an average of 37% for patients receiving dialysis >5 years **(164-166)**.

During 1999-2000, CDC investigated three outbreaks of HCV infection among patients in chronic HD centers, in two of the outbreaks, multiple transmissions of HCV occurred during periods of 16–24 months (attack rates: 6.6%–17.5%), and seroconversion were associated with receiving dialysis immediately after a chronically infected patient. Multiple opportunities for cross contamination among patients were observed, including equipment and supplies that were not disinfected between patient use; use of common medication carts to prepare and distribute medications at patients stations; sharing of multiple dose medication vials which were placed at patients stations on top of HD machines; contaminated priming buckets that were not routinely changed or cleaned and disinfected between patients; machine surfaces that were not routinely cleaned and disinfected between patients, and blood spills that were not cleaned up promptly **(35)**.

A Portuguese collaborative study found that in units that reprocessed dialyzers, centers that used a separate room to reprocess dialyzers from anti-HCV positive patients, and those that did not reprocess dialyzers from anti-HCV positive patients had significantly lower incidence rates compared with those that did not follow any specific precautions **(167)**. A Belgian multicenter study reported a reduction in de novo anti-HCV seroconversion rates to 0% for 18 months by strict enforcement of body fluid precautions **(168)**. This occurred despite the absence of disinfection of HD machines after each HD procedure, suggesting that internal contamination of HD machines does not play a major role in nosocomial HCV transmission within HD units.

Okuda et al. found that some nurses withdrew needles for dialysis access in several consecutive HD patients without changing gloves between patients, After education of staff members and application of an adhesive pad at the time of needle withdrawal, no additional cases of acute HCV were recognized for more than 1 year in 730 patients on chronic HD **(169)**. On other hand there are cogent arguments supporting a policy of isolation of HCV positive patients on maintenance HD by rooms, machines, and staff, Prospective trials have reported a reduction in HCV acquisition within HD units by complete isolation of HCV positive individuals **(170, 171)**. In a study by Djordjevic et al. on 170 patients undergoing HD at a single unit who were followed for 4 years, the incidence of HCV seroconversion fell from 12.9% in 1995 to 6.6% in 1998 by complete isolation of HCV infected patients **(171)**. In some European countries, 25% of dialysis centers have adopted this strategy **(172)**.

The prevalence of HCV antibodies among HD patients has been reported to range from 3.3 % in Germany to 76.3% in Indonesia , in Brazil surveys have shown that HD patients have high anti-HCV prevalence rate (13.23% -46.7%), in Arab countries the prevalence range from 12.3% in Lebanon to 75% in Syria, "Israel" has prevalence rate 24.6% (Table 2.2) **(173-196)**.

Table (2.2): Worldwide prevalence of HCV among HD patients.

Country	Year	Total no. of patients	Detection methods	Result %	Ref.
Belgian	2000	1710	Anti -HCV	6.8	173
Brazil	2001	428	Anti- HCV RNA Detection	39 30.6	174
Brazil	2002	434	Anti- HCV RNA Detection	20.3 94.3*	175
Brazil	2002	250	Anti- HCV RNA Detection	8.4 7.6	176
Brazil	2002	795	Anti- HCV	16.5	177
Germany	1991-1992	122	Anti -HCV RNA Detection	3.3 75*	178
Germany	2002	2796	Anti -HCV RNA Detection	6.1 4	179
Greece	2005	366	Anti -HCV RNA Detection	24 31.7	180
India	2003-2004	151	Anti- HCV	9.93	181
India	2005	134	Anti- HCV	5.9	182
Indonesia	1996	76	Anti -HCV	76.3	183
Indonesia	2002	93	Anti- HCV	63.4	184
Iran	2003	838	Anti -HCV	21	185
Israel	1999	65	Anti -HCV RNA Detection	24.6 12.3	186
Italy	1993	77	Anti- HCV RNA Detection	46 37.6	187
Jordan	2002	283	Anti -HCV RNA Detection	34.6 30.6*	188
Lebanon	1996	317	anti -HCV	27	189
Mexico	2004	149	Anti- HCV RNA Detection	6.7 5	190
Netherlands	1995-1996	2108	Anti -HCV RNA Detection	3.8 2.5	191
Peru	1994	221	Anti- HCV	59.3	192
Syria	1998	120	Anti HCV RNA Detection	75 87.5*	193
Tunisia	1995	325	Anti- HCV	42	194
Tunisia	2001	4340	Anti- HCV RNA Detection	19.07 72.3*	195
Venezuela	1996	227	Anti- HCV RNA Detection	71 72*	196

* % HCV RNA positive from anti-HCV positive samples.

However there are strong indications that studies of HD patients which rely solely on serological screening could underestimate the prevalence of HCV infection considerably (197,198). Partial immunosuppression in these patients, resulting in a poor antibody response may be a contributing factor (199).

Such shortcomings could be overcome by determining HCV RNA, which may be required to identify all infected patients (200-202). The prevalence of HCV RNA has been estimated to be between 2.48 % in Germany to 65.62 % in Syria (Summarized in table 2.2) (174-176, 178-180, 186-188, 190, 191, 193,195, 196).

2.3.5 Classification and Structure of HCV

HCV was identified as the main causative agent of post transfusion non-A, non-B hepatitis (142, 143). It's now documented to be heterogeneous in nature and belongs to the genus *Hepaciviruses* and family *Flaviviridae* (203). The genome of HCV comprises a single stranded positive sense RNA of approximately 9.6 kb in length and contains a single ORF that encodes for a non functional polyprotein of approximately 3000 amino acids in length (143).

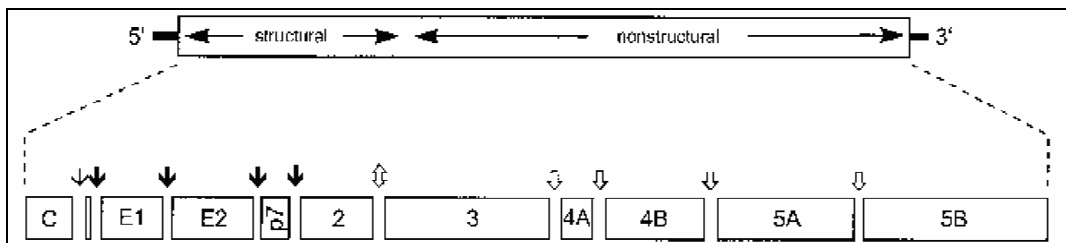


Figure (2.3): Schematic representation of HCV genome organization (204).

This non functional polyprotein is cleaved co- and post-translationally by cellular and viral proteases to result in three structural proteins and six non structural proteins. Structural proteins, encoded in the N terminal region, include the core protein (C), believed to be the viral capsid; envelope proteins (E1 and E2), and p7. the nonstructural proteins, encoded in the C-terminal region, are six proteins NS2, NS3, NS4A, NS4B, NS5A, and NS5B that carry out a number of enzymatic activities, some of which are still not fully elucidated (205, 206).

Core protein is the first structural protein encoded by the HCV ORF; it is highly basic and RNA-binding protein which presumably forms the viral nucleocapsid. Intriguingly, the core protein has been reported to interact with a variety of cellular proteins and to influence numerous host cell functions **(207)**.

The envelope proteins E1 and E2 are extensively glycosylated and form a non-covalent complex, which is believed to represent the building block for the viral envelope **(208)**. Interestingly, the transmembrane domains, located at the carboxyterminus of E1 and E2, are involved in heterodimerization and have endoplasmic reticulum (ER) retention properties. A structure model for E2 based on the envelope protein structure from the related tick-borne encephalitis (TBE) virus was recently reported **(209)**.

The envelope glycoprotein E2 exhibits a great degree of heterogeneity, both in the nucleic acid and protein sequence **(210, 211)**. This region is termed the hyper-variable region-1 (HVR-1) of HCV. In infected individuals, quasi-species arise mainly by accumulating mutations in the HVR-1 induced by strong immune pressure **(212)**.

p7 is a 63 amino acid polypeptide that is often incompletely cleaved from E2. It has two transmembrane domains connected by a short hydrophilic segment **(213)**. P7 of the related bovine viral diarrhea virus (BVDV) is essential for the production of progeny virus, but not for RNA replication **(214)**.

Cleavage of the polyprotein precursor at the NS2–NS3 junction is accomplished in cis by an autoprotease encoded by NS2 and the N terminal one-third of NS3. NS2–3 protease activity is essential for the replication of full length HCV genomes in vivo **(215)**. However, NS2 is dispensable for replication of subgenomic replicons in vitro, recombinant proteins lacking the N-terminal membrane domain of NS2 have been found to retain cleavage activity and may allow further examination of this unique enzymatic activity **(216)**.

A distinct serine protease located in the N-terminal one-third of NS3 is responsible for the downstream cleavage events in the nonstructural region

(217). In addition, an NTPase and helicase domain is found in the C-terminal two-thirds of NS3 **(218)**.

The NS4A polypeptide functions as a cofactor for the NS3 serine protease and is incorporated as an integral component into the enzyme core. The crystal structures of the serine protease and RNA helicase domains of NS3 and, more recently, of the entire NS3–4A complex, have been elucidated; these enzymes are essential for viral replication and have emerged as major antiviral targets **(219)**.

NS4B, a relatively hydrophobic 27 KD protein, is the least characterized HCV protein. The NS4B proteins of HCV, pestiviruses and flaviviruses are similar in size, amino acid composition and hydrophobic properties. No function, however, has yet been ascribed to NS4B in any of these related viruses. It was shown that expression of NS4B is sufficient to induce the formation of a seemingly ER-derived membrane alteration, designated membranous web that may harbor the HCV replication complex **(220)**.

NS5A is a phosphoprotein whose function is the subject of current debate. In its basal phosphorylation state, it is a 56 KD protein, and 58 KD in a hyperphosphorylated form. NS5A of HCV as well as NS5 of the yellow fever virus is phosphorylated by as yet unidentified serine or threonine kinases, suggesting that these proteins share a common function related to their phosphorylation state **(221)**.

HCV NS5A has attracted considerable interest because of its potential role in modulating the interferon (IFN) response. Studies performed in Japan first described a correlation between mutations within a discrete region of NS5A, termed interferon sensitivity determining region (ISDR), and a favorable response to IFN- α therapy **(222)**. In addition, NS5A has been reported to interfere with activity of the double stranded RNA activated protein kinase **(223)**.

HCV replication proceeds via synthesis of a complementary minus-strand RNA using the genome as a template and the subsequent synthesis of genomic plus-strand RNA from this minus-strand RNA template. The key enzyme responsible for both of these steps is the NS5B RdRp (RNA-dependent RNA polymerase). This essential viral enzyme has been extensively characterized at the biochemical and structural level, and has emerged as a major target for antiviral intervention **(224, 225)**.

HCV as RNA virus exhibits a remarkable degree of heterogeneity throughout the length of its nucleic acid **(226)**. Traditionally, this heterogeneity has been classified as quasi-species and genotypes **(227)**. The term quasi-species is applied to the heterogeneity in the nucleic acid sequences of the virus isolated from a single patient, while the term genotypes refers to the differences in the sequences that are observed among isolates from different patients. This heterogeneity arises as a consequence of lack of proof-reading activity of RNA-dependent RNA polymerase during replication of the virus. Over a period of time, these mutations get accumulated at specific regions, leading to what are known as the genotypes. To date at least six genotypes and more than 120 subtypes of the virus have been identified **(228)**.

2.3.6 Replication of HCV

2.3.6.1 Attachment and Entry

CD81 was identified as a putative HCV receptor based on its strong interaction with E2 as well as with virus particles in vitro **(229)**. Also HCV as well as other members of the Flaviviridae family may enter the cell by binding to low-density lipoprotein (LDL) receptors, based on the observation that HCV particles are associated with beta-lipoproteins **(230, 231)**.

While the nature of the HCV receptor is not known, the major envelope glycoprotein E2 is thought to be responsible for initiating virus attachment to the host cell because E2-specific anti-sera can block binding to cells **(232-234)**. The role of E1 is less clear but the presence of a stretch of hydrophobic amino acids tentatively called the E1 fusion peptide, displaying similarities to the fusion

peptides of paramyxovirus and flavivirus suggests that E1 is involved in membrane fusion (235).

2.3.6.2 Polyprotein Translation and Processing

Once inside the cytoplasm the genomic RNA is directly translated. Since HCV most likely does not encode a methyl transferase activity and replicates in the cytoplasm where such cellular enzymes are missing, the genome is not capped, Therefore, translation of the viral RNA is not mediated by a cap dependent mechanism with ribosomes scanning along the RNA up to the first initiator AUG codon, but rather by an internal ribosome entry site (IRES) (236, 237).

Activity of the HCV IRES is influenced by several factors. First, the X-tail at the very 3' end of the HCV genome appears to enhance IRES dependent translation by an as yet unidentified mechanism (238). Second, several cellular factors have been demonstrated to bind to the HCV IRES and in most cases, stimulate translation. Most of these include polypyrimidine tract binding protein (PTB), the La antigen, and heterogeneous nuclear ribonucleoprotein-L (239-242).

The requirements for cellular factors for IRES activity may also explain the dependence on the cell cycle. Using cell lines stably expressing bicistronic reporter constructs with a cap-dependently expressed upstream reporter and a down-stream reporter translated from the HCV IRES it was found that IRES-dependent translation was greatest in mitotic and lowest in quiescent (G_0) cells (243).

2.3.6.3 RNA Replication

Several coprecipitation studies deduced that most or all of the HCV polyprotein cleavage products, in particular NS3-5B, form a replicase complex associated with intracellular membranes that most likely contains cellular proteins too (244-246). The individual steps underlying RNA replication are largely unknown. It is obvious that the NS5B RdRp is the key player catalysing the synthesis of minus and plus strand RNA. In vitro the enzyme prefers a primer dependent initiation

of RNA synthesis, either by elongation of a primer hybridized to an RNA homopolymer or via a copy back mechanism when using heteropolymeric templates **(247-252)**.

However, at least under certain experimental conditions, HCV NS5B, as well as the RdRp of the closely related pestivirus BVDV, can initiate RNA synthesis de novo and it is plausible that this mechanism also operates in vivo **(253-256)**.

2.3.6.4 Virion assembly and release

Although in vitro core protein binds to RNA without detectable specificity, recent evidence indicates a preferential intracellular binding to RNA sequences in the 5' half of the HCV genome **(257)**. Such binding may not only accomplish a selective packaging of the plus stranded genome but also appears to repress translation from the IRES, suggesting a potential mechanism to switch from translation/replication to assembly **(257)**.

Whether the core protein forms a distinct nucleocapsid structure or a rather non-structured ribonucleoprotein complex with the RNA genome is not known. Certainly, core protein interacts with itself and the sequences required for this interaction have been mapped to the amino-terminal 115 residues **(258)**. Within this region a tryptophan-rich primary interaction domain was identified between residues 82 and 102 that is masked in the full-length core protein and revealed only under certain experimental conditions **(259)**.

A feature typical of the HCV E proteins is their retention in the ER compartment when expressed with various heterologous systems in cell culture **(260)**. The retention is achieved by signals in the transmembrane domains of E1 and E2 and it has been shown to be a true retention in the ER. This observation suggests that viral nucleocapsids acquire their envelope by budding through ER membranes **(261, 262)**. In this case the virus may be exported via the constitutive secretory pathway. In agreement with this assumption, complex N-linked glycans were found on the surface of partially purified virus particles suggesting virus transit through the Golgi **(263)**.

2.3.7 Clinical Features

HCV causes both acute and chronic hepatitis **(264)**, it can be transmitted by parenteral, sexual, household, perinatal **(265-267)**. The incubation period ranges from 14-180 days (average: 6-7 weeks) **(264)**.

During the incubation period HCV replicates in the liver causing hepatic cell necrosis and inflammation, HCV RNA is already detected in the serum 2 weeks after exposure while Anti-HCV antibodies become detectable during the acute phase of illness which typically lasts 2-12 weeks. Recovery is characterized by the disappearance of HCV RNA and symptoms **(268)**.

Acute Hepatitis C is self limited in only 15% of cases. Despite broad humoral and cellular immunological host response to HCV, approximately 85% of patients fail to clear the virus by 6 months and develop chronic HCV infection, the mechanisms of HCV persistence are unknown but several possible factors have been pointed out as mutation of HCV during replication, formation of quasi-species, HCV persistence in extrahepatic cells, and modification of cytokine synthesis in the liver or sensitivity of liver cells to these cytokines **(269-271)**.

Patients with chronic HCV and elevated serum alanine aminotransferase (ALT) (significantly increased in case of acute hepatitis C) usually progress to cirrhosis. The mean interval between the time of hepatitis transfusion and cirrhosis diagnosis was 21.2 ± 9.6 years in one study and 20.6 ± 10.1 in another one **(272, 273)**.

Once cirrhosis is established there is a risk of developing major complications secondary to liver failure and portal hypertension such as jaundice, ascites, encephalopathy or variceal hemorrhage. There is also a risk of developing hepatocellular carcinoma and the only practical means of restoring health is liver transplantation **(274)**.

Hepatic failure associated with HCV related cirrhosis is now the most frequent indication for liver transplantation among adults **(275)**. HCV not only cause liver

diseases but also lymphoproliferative disorders, thus it stimulates B-lymphocytes proliferation with the consequent production of various antibodies. The most frequent are cryoglobulins which cause type II mixed cryoglobulinaemia which in some subjects progresses to malignant B cell non Hodgkin's lymphoma **(276, 277)**.

Mixed cryoglobulinaemia is found in 36-50% of patients with chronic HCV infection **(275)**. In addition it is symptomatic in <20% of cases as systemic vasculitis with purpura, arthritis, Raynaud's phenomenon, peripheral neuropathy or glomerulonephritis **(276, 277)**. Many other extrahepatic conditions have been associated with chronic HCV infection, such as Sjogren's syndrome, autoimmune thyroiditis, autoimmune hemolytic anemia, idiopathic thrombocytopenia, polyarteritis nodosa, and idiopathic pulmonary fibrosis **(278-280)**.

2.3.8 Diagnosis of HCV

2.3.8.1 Serology Test

HCV probably circulates in the serum at concentration of between 10^2 and 5×10^7 particles /ml **(281)**, and it is difficult to detect viral antigenaemia, therefore the detection of antibodies to HCV has become a good indicator of past or present infection, the first epidemiological and diagnostics studies of anti-HCV antibodies were carried out in 1989 using tests based on antibodies to c100-3, a 363 aa fusion polypeptide derived from non structural NS4 region of the HCV genome **(143)**. However, these first generation enzyme linked Immunosorbent assay (ELISA) lacked optimal specificity and this test had false positive results, especially when screening low risk groups such as blood donor. False positive results were also common in patients with hypergammaglobulinaemia and in stored samples **(282)**.

The deficiencies in the first generation ELISA were responsible for development of second and third generation ELISA for anti-HCV antibodies, both test incorporated two and three new recombinant proteins respectively, increasing the sensitivity of the assay. The first of these is c22-3 encoded by the putative

core region of the HCV genome, studies show that in many cases antibodies to c22-3 are found earlier than those to c100-3 **(283)**.

The major new additional antigen in third generation ELISA is recombinant NS5 which is derived from the NS5 region, these lead to increase sensitivity with no change in specificity **(284)**.

As with any screening test, the positive predictive value of ELISA for anti-HCV is directly related to the prevalence of infection in the population and is low in populations with an HCV infection prevalence <10% **(285, 286)**. Supplemental testing with a more specific recombinant immunoblot assay (RIBA) of a specimen with a positive anti-HCV result by ELISA prevents reporting of false positive results, particularly in settings where asymptomatic persons are being tested **(35)**.

Results of seroprevalence studies among chronic HD patients have indicated that 57%-100% of ELISA positive results were RIBA positive **(18, 196, 197, 287-290)** and 53%-100% were HCV RNA positive by reverse transcriptase polymerase chain reaction (RT-PCR) testing **(137, 187, 198, 287, 291)**.

2.3.8.2 Nucleic Acid Detection

PCR has been used to detect HCV RNA sequences in both plasma and liver. The procedure involves the extraction of nucleic acids present in the sample and the reverse transcription of the RNA with the aid of random or sequence specific primers to produce a single strand cDNA which is then amplified using HCV specific primers in a PCR **(284)**.

Three possible patterns of viraemia that have clinical relevance have been identified; a transient viraemia accompanying an acute self limiting hepatitis, persistent viraemia with progression to chronicity, and intermittent viraemia characterized by an initial loss of HCV RNA but which recurs after some months **(283)**.

False positive results usually occur as a consequence of nucleic acid contamination. Rigorous precaution therefore, must be used with negative

control included at each step. In addition various anticontamination measures based on amplicon inactivation have also been developed **(281, 292)**.

False negative results are usually due to lack of sensitivity and hence failure to detect weakly positive samples, other reasons for lack of detection are the loss of HCV RNA during specimen processing and the existence of intermittent viraemia **(293)**. Several methods for distinguishing HCV genotypes have been developed some of them are PCR based, including direct sequencing, detection of PCR products with type specific probes, PCR with type specific primer, and analysis of restriction fragment length polymorphism (RFLP) **(294-296)**.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Population

All of the 4 governmental HD centers of Gaza Strip were included in this study. The first center at Al-Shifa hospital in the center of Gaza city serves patients from the north and Center of Gaza Strip; the second in Shuhada`a Al-Aqsa Hospital at Dair El-Balah city serves patients from the middle of Gaza Strip; the third at Nasser hospital in Khan Yunis city serves patients from Khan Yunis and its neighboring camps; and finally the fourth center at Abu-Yousef Al-Najar Hospital in Rafah city serves patients from Rafah.

All patients attending the four HD centers between August and September 2006 were interviewed and blood samples were collected in the location (246 patients: 131males and 115 females with mean age 46.7 years).

3.2 Setting

Nucleic acid extraction was carried out at the molecular biology laboratories of Al Remal clinic, while RT-PCR experiments were carried out at the molecular biology laboratory of the Islamic University of Gaza. Biochemical tests were carried out at the clinical chemistry department, while serological tests were carried out at the virology department of Al-Shifa hospital central laboratory.

3.3 Questionnaire and Data Collection

A close ended and multiple choice based questionnaire was designed in Arabic and completed by the researcher via patient interview to ensure proper data collection and prevent any misunderstanding. The collected data included age, sex, education level, residency, time spent on HD, the number of blood units transfused, HBV vaccination, personal habits, history of infection, and treatment abroad (Refer to appendix (1) for the complete questionnaire).

3.4 Ethical Considerations

The study principles and protocols were submitted and approved by the Islamic university, the committee of Helsinki (declaration of Helsinki the most widely accepted guideline and medical research involving human subject) and MOH.

Verbal consent was obtained from all patients after the principle of the study and its possible outcomes were explained to all subjects. All personal information of the study subjects and result were dealt with confidentiality.

3.5 Reagents and Equipment

3.5.1 Reagents

The reagents consumed in the study are listed in table (3.1).

Table (3.1): Reagents consumed during the study.

Chemicals and reagents	Manufacturer
• HBsAg (V2) KIT	ABBOTT, Germany
• HCV version 3.0 kit	ABBOTT, Germany
• Serum alanine aminotransferase (ALT) test reagent	Diasys, Germany
• Serum aspartate aminotransferase (AST) test reagent	Diasys, Germany
• QIAamp Viral RNA extraction Mini Kit: ○ Buffer of (AVL, AW1, AW2 & AVE) ○ Carrier RNA (poly A)	Qiagen, Germany
• QIAGEN One Step RT-PCR Kit: ○ One step RT-PCR enzyme mix: Contains the QIAGEN products Omniscript, Reverse Transcri- ptase, Sensiscript Reverse Transcriptase, and HotStarTaq DNA Polymerase. ○ Storage buffer 20mM Tris.Cl, 100mM KCl, 1mM dithiothreitol (DTT), 0.1mM EDTA, 0.5% (v/v) Nonidet p-40, 0.5% (v/v) Tween20, 50% glycerol (v/v) and stabilizer. The pH 9.0 at 20°C. ○ 5x QIAGEN one step RT-PCR buffer: the same balanced combination of potassium chloride (KCl) and ammonium	Qiagen, Germany

Chemicals and reagents	Manufacturer
sulphate (NH ₄) ₂ SO ₄ , Tris Cl, 12.5 mM MgCl ₂ , DTT; PH 8.7. ○ DNTP Mix: 10mM of each of dATP, dCTP, dGTP and dTTP. ○ RNase-free water.	
• PCR master mix 2X : 50 units /ml of tag DNA polymerase pH 8.5, 400 µM of each dATP, dGTP, dCTP, dTTP, 3mM MgCl ₂	Promega, USA
• RNase inhibitor. 40 units/ µl.	Promega, USA
• Ethyl alcohol 96%.	Local
• Agarose Molecular Biology grade.	Promega, USA
• 100 bp DNA ladder.	New England Bio Labs, USA
• Ethidium bromide	Promega, USA

3.5.2 Primers Sequences

The primers listed in table (3.2) were designed to specifically bind the 5'UTR of the HCV genome that is highly conserved among the different genotypes (297).

Table (3.2): Primers sequences used for HCV detection.

Primer ID	Sequence (5' TO 3')*	Location	Product size (bp)
HCV - Ext (S)	CCCTGTGAGGAACTWCTGTCTTCACGC	-299	298
HCV - Ext (AS)	GGTGACGGTCTACGAGACCT	-1	
HCV - Int (S)	TCTAGCCATGGCGTTAGTRYGAGTGT	-264	235
HCV - Int (AS)	CACTCGCAAGCACCCCTATCAGGCAGT	-29	

*To include sequence degeneracy, W = A or T, R = A or G, Y = T or C .

3.5.3 Equipment

The equipment used in the study is listed in table (3.3).

Table (3.3): Equipment used during the study.

Instruments	Manufacturer
AxSYM autoanalyzer (version 5.20)	ABBOTT, Germany
Konelab 60 autoanalyzer	Thermo electron, Finland
Thermal-cycler, mastercycler gradient	Eppendorf, Germany
Electrophoresis chambers and power supply	Biorad, USA
Microcentrifuge	Sanyo, UK
UV-transilluminator	Hoefer, USA
Microwave oven	L.G, Korea

3.6 Methods

3.6.1 Patients and Samples Collection

During the period from August to September 2006, blood samples were collected from 246 patients attending the four HD centers in Gaza Strip, 130 samples were collected from Al-Shifa hospital, 30 from Shuhada`a Al-Aqsa Hospital, 34 from Nasser hospital, and 52 from Abu-Yousef Al-Najar Hospital. Two blood samples were collected from each patient, in plain tube, prior to dialysis to prevent the interference of heparin with downstream applications. Serum separated from one tube was subjected within two hours to ALT, AST, HBsAg, and anti-HCV antibodies tests. Serum separated from the second tube was frozen at -70°C in a sterile, DNase, RNase free tightly capped tube until used for PCR analysis.

3.6.2 Biochemistry

ALT and AST levels were analyzed for all samples using (Konelab 60 autoanalyser machine), the normal ranges for ALT and AST are from 0 to 40 IU /ml and 0 to 37 IU /ml respectively **(298)**.

3.6.3 Virology

HBsAg and anti-HCV antibodies were determined for all samples using (AxSYM machine) in the laboratory of the Al-Shifa hospital.

For HBsAg determination, AxSYM HBsAg (V2) kit was used which is based on the microparticle enzyme immunoassay (MEIA) technology. Non reactive samples were considered negative for HBsAg and not tested further, while a reactive sample was retested to confirm the result. A repeatedly reactive sample was considered positive and not further tested. Positive and negative serum samples were included in each run as controls to ensure proper serology results.

For anti-HCV antibodies determination, AxSYM HCV version 3.0 kit was used which was also based on MEIA technology. The kit is designed to detect serum antibodies against putative structural and non structural proteins of the HCV genome. Non reactive samples were considered negative for HCV, while reactive samples were retested to confirm the result and repeatedly reactive samples were considered positive. Positive and negative serum samples were included in each run as controls to ensure proper serology results.

Serologically positive HCV samples were tested individually by nested RT-PCR technique, while negative samples were pooled in tens and tested by the same technique.

3.6.3.1 Viral RNA Extraction

Viral RNA was extracted from serum samples using the QIAamp viral RNA extraction kit according to the manufacturer recommendations. Briefly, 140 μ l from each sample were first brought to room temperature before being lysed under highly denaturing conditions, to inactivate RNases and to keep viral RNA integrity.

The lyses buffer contains carrier RNA to increase the yield. The viral RNA was extracted by 96% ethanol and subsequently loaded onto the QIAamp spin columns that are designed to bind the RNA selectively. The columns were

washed by ethanol-containing buffers and RNA was eluted in a special RNase-free buffer. The RNA samples were either immediately processed for PCR or stored at -20°C for later PCR Experiments.

3.6.3.2 HCV nested RT- PCR Amplification

Both cDNA synthesis and PCR amplification of the target sequences were performed in a single tube using the QIAGEN one step RT-PCR kit according to the manufacturer instructions. This allows for enrichment of the target sequence and increases the specificity using a sequence specific primer for cDNA synthesis and amplification. Moreover this would reduce the loss of sample and reduce the possibilities of contamination.

The reactions were carried out in $25\mu\text{l}$ reaction volumes using $10\mu\text{l}$ RNA in the presence of $0.6\mu\text{M}$ of each HCV external primers (HCV- Ext (S) and HCV- Ext (AS)), $400\mu\text{M}$ of each dNTP and 5 units RNase inhibitor. The reaction cycling conditions were: 1 cycle at 50°C for 30 minutes, 1 cycle at 95°C for 15 minutes followed by 40 cycles of 95°C for one minute, 55°C for one minute and 72°C for one minute. Finally the reactions were allowed to complete at 72°C for 10 minutes and hold at 4°C **(299)**.

The products were analyzed by running onto 2% agarose gel with 100bp ladder and stained with ethidium bromide. The appearance of a 298 bp band was considered a positive result **(299)**.

In all cases a second nested PCR was performed to improve the detection capacity and specificity of the PCR test. The second PCR reactions were carried out in $25\mu\text{l}$ volumes using $5\mu\text{l}$ DNA template from 1st PCR in the presence of 1X PCR master mix, $0.4\mu\text{M}$ of each HCV internal primers (HCV –Int (S) and HCV- Int (AS)). The reaction cycling conditions were 1 cycle at 95°C for 5 minutes, followed by 34 cycles of 95°C for one minute, 55°C for one minute and 72°C for one minute. Finally the reactions were allowed to complete at 72°C for 10 minutes and hold at 4°C **(299)**.

The products were analyzed by electrophoresis onto 2% agarose gel with 100bp ladder and stained with ethidium bromide. The appearance of a 235 bp band was considered a positive result.

In each reaction set a positive and negative control was included, a water sample was used as a negative control to exclude possible contamination and positive PCR HCV serum as positive control to ensure proper PCR product.

3.6.3.3 Pooling of Serum Samples

A pooling strategy was developed in this study, in which ten serum samples from different patients (negative anti-HCV) were pooled together in one tube. Two hundred μl serum from each sample were combined together in a single 2ml microcentrifuge. The tubes were ultracentrifuged for two hours at (21.000 xg) and cooling at 4°C. A pellet was visible and the supernatant was reduced to approximately 150 μl by removal of most of the liquid. The pellet was resuspended in the remaining serum and viral RNA was extracted for PCR amplification. The samples of a positive pool are either reanalyzed individually or subdivided into 3 smaller pools.

In order to make sure that the detection limit of RT-PCR is not reduced each of three previously known HCV positive samples were introduced separately into a pool of another 9 negative samples. The positive samples had different viral loads (2.7×10^6 copies/ml, 6.8×10^4 copies/ml and 6.8×10^1 copy/ml). Viral RNA was extracted from the three pools and analyzed by nested RT-PCR for HCV. Viral RNA was also extracted from the original non-pooled serum samples and analyzed by RT-PCR in parallel to assess detection capacity of the procedure.

3.7 Data Analysis

The data was collected, summarized, tabulated and analyzed using the statistical package for social sciences (SPSS) 13 software. Differences in proportions were assessed by a chi-square test, p-value < 0.05 was considered statistically significant.

CHAPTER 4

RESULTS

This study focuses on determining the prevalence of both HBV and HCV among HD patients, detecting the main risk factors associated with these agents. The results of this study highlight the value of using a combination of traditional and molecular techniques in the diagnosis of hepatitis viruses especially HCV. To the best of our knowledge, this is the first study in Palestine investigating the prevalence of hepatitis viruses and their related risk factors among HD patients.

4.1 Patients Description

The study was conducted on HD patients attending HD centers in Gaza Strip. As inclusion criteria, the study accepted only those patients attending HD center and undergoing HD for more than one month, patients who underwent HD for less than one month were excluded.

4.1.1 Patients Distributions

The study was conducted on 246 HD patients attending four HD centers in Gaza Strip (See materials and methods 3.1).

One hundred thirty patients from Al-Shifa hospital in the center of Gaza City, 30 patients from Shuhada`a Al-Aqsa Hospital in Dair EI-Balah, 52 patients from Nasser hospital in Khan Yunis city, and 34 patients from Abu-Yousef Al-Najar Hospital in Rafah city were included in the study (Figure 4.1).

4.1.2 Patient's Age

Patient's age ranged between 6 to 80 years; 20 patients were less than 19 years old, 71 patients between 19 and 40 years; 101 patients between 41 and 60 years; and 54 patients were over 60 years (Figure 4.2). The mean age for all patients was 46.7 years with standard deviation (SD) ± 17.5 years.

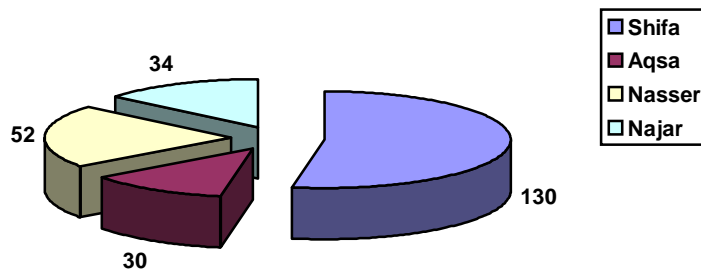


Figure (4.1): Distribution of HD patients among HD centers in Gaza Strip.

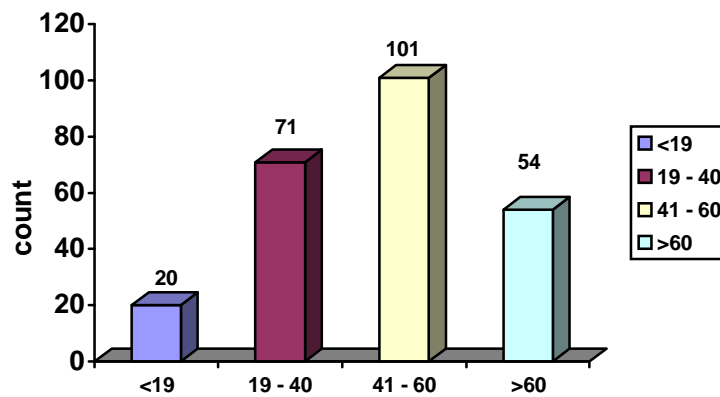


Figure (4.2): Distribution of HD patients according to their age.

4.1.3 Patient's Sex

From the 246 patients tested, there are 131 male and 115 female (Figure 4.3). For male patients the mean age was 44.7 years, while female patients mean age was 49 year.

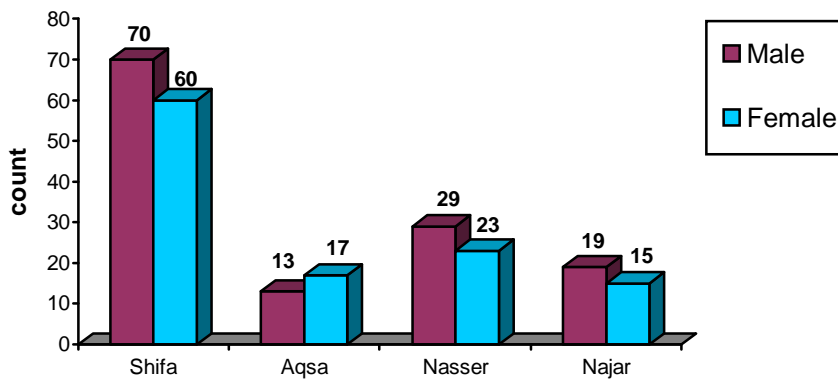


Figure (4.3): Distribution of patient sex among HD centers.

4.1.4 Patients Residence Distribution.

Patients of Al-Shifa hospital were from three regions of Gaza Strip. Eighty seven patients were from Gaza governorate, 42 were from north governorate, and 1 patient was from the middle governorate. All of Al-Aqsa Hospital Patients (30 patients) were from the middle governorate, Nasser hospital patients were distributed as 45 patients from Khan Yunis governorate and 7 patients from Rafah governorate and all the patients of Al-Najar Hospital (34 patients) were from Rafah governorate (Figure 4.4).

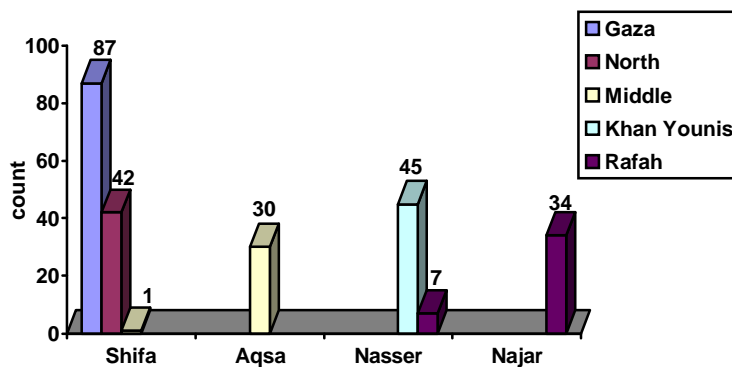


Figure (4.4): Distribution of patient's residence among HD centers.

4.1.5 Patients Education Level.

The level of education of patients was classified into five categories, illiterate, elementary, preparatory, secondary, and university degree. Sixty five patients were illiterate which constituted the highest percentage among HD patients (26.4%). The distribution of patient's education level among HD centers is illustrated in figure 4.5.

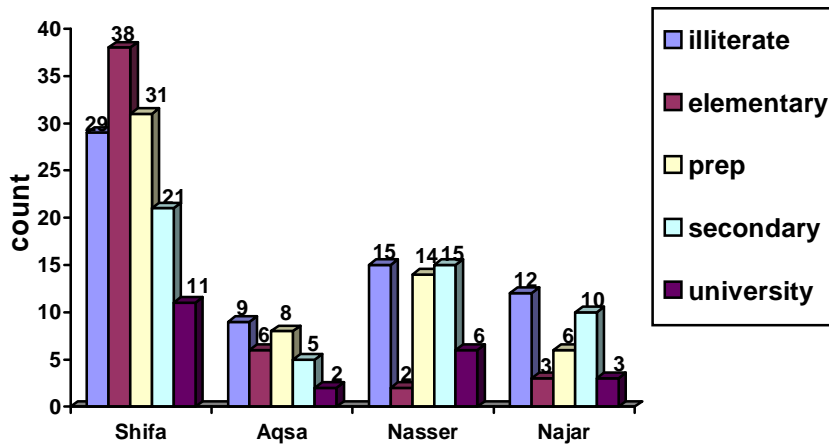


Figure (4.5): Patients education level distributed by HD Centers.

4.1.6 Patients Marital Status.

From all the study population we found that 176 patients were married which constituted 71.5% while the rest were single. Distribution of HD patient's marital status among HD centers is illustrated in figure 4.6.

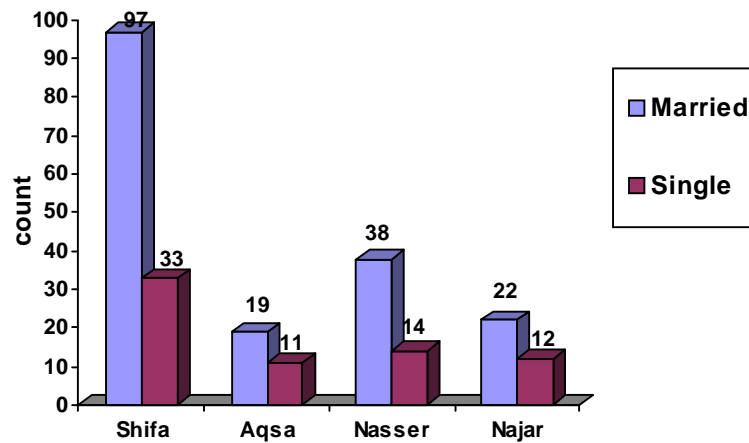


Figure (4.6): Distribution of patients' marital status by HD centers.

4.2 Haemodialysis Centers Services.

This section describes the services provided by the all HD centers.

4.2.1 Distribution of Patients among HD Shifts.

HD centers operate in three shifts; the morning shift is from 8 to 12 A.M, the noon shift from 12 A.M to 4 P.M, and the third in the afternoon shift from 4 to 8 P.M. Only Al-Aqsa center works in two shifts in the morning and in the noon due to the low number of patients attending this center.

The study found that 43.9% of patients underwent HD in the morning shift, 39.4% at the noon shift and 16.7% at the afternoon shift. Distribution of HD patients among the three shifts in the four centers is illustrated in figure 4.7.

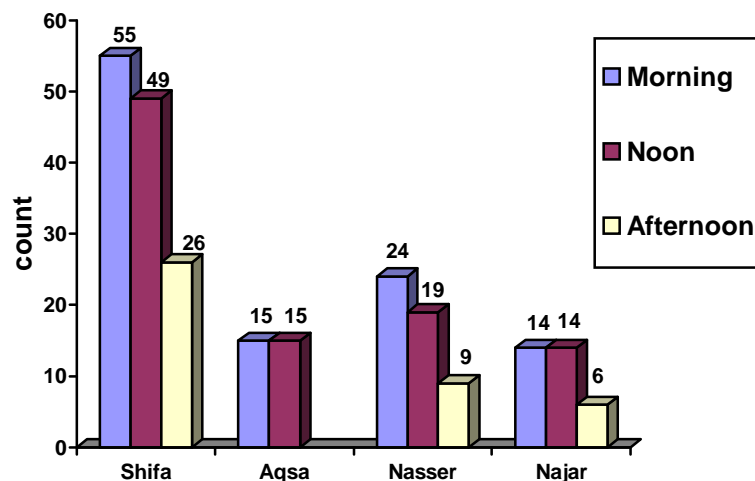


Figure (4.7): Distribution of patients among three shifts in the four HD centers.

4.2.2 Frequency of HD Sessions

Physicians prescribe the frequency of HD sessions for each patient according to patient's condition. Most patients need from one to three sessions per week. Most of the patients undergo two HD sessions per week (54.9%), followed by three HD session (43.5%) and the minimum number of sessions was one per week (1.6%). Figure 4.8 illustrates the frequency of HD sessions in the four centers.

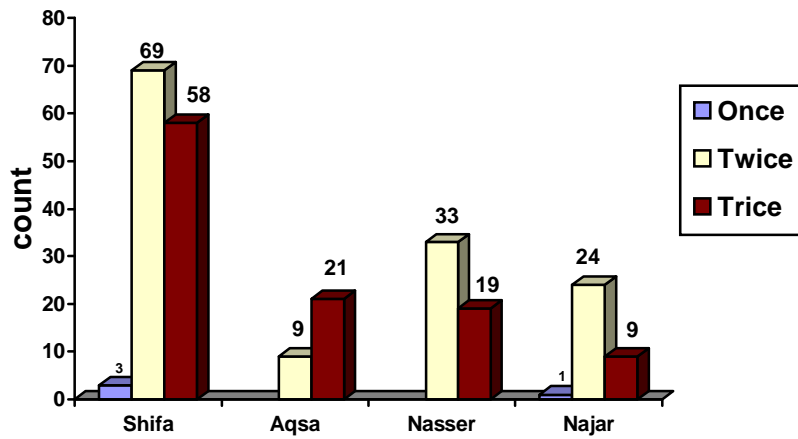


Figure (4.8): Frequency of HD sessions in the four HD centers in Gaza Strip.

4.2.3 Duration of HD Sessions

Physicians prescribe the duration of each HD session according to several factors including age, weight and response of patient to treatment. The average time duration per session ranged between 2 hours to 4 hours. Most patients underwent HD for three hours each session (44.3%), followed by four hours (30.1%), three and half hours (22%), two hours (2.8%) and finally two and half hours (0.8%). Duration of HD sessions distribution among HD patients in the four centers of Gaza Strip is illustrated in figure 4.9.

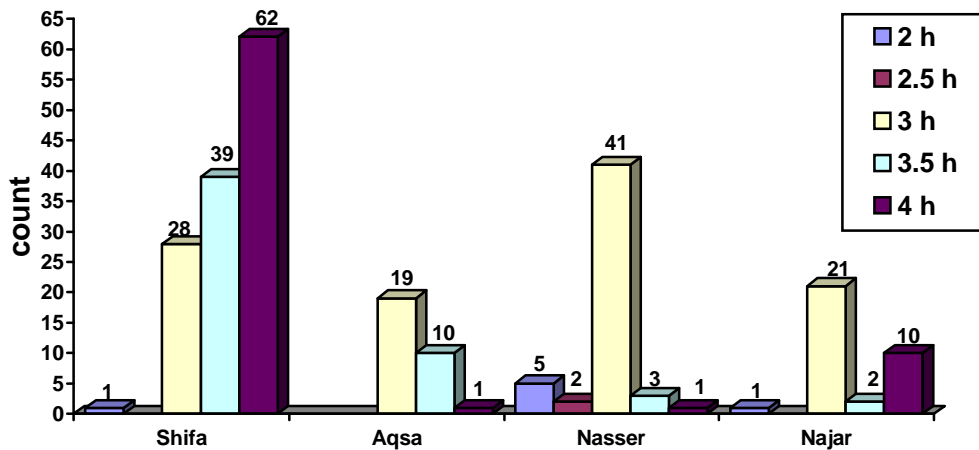


Figure (4.9): Duration of HD session distribution among HD patients.

4.2.4 HD Areas and Working Criteria within HD Centers

In all of the centers, new cases of ESRD patients are tested for HBsAg and anti-HCV before initiation of HD and according to the result they are directed to the suitable HD area and machine within the center.

In all HD centers except for Abu-Yousef Al-Najar center, two HD areas are maintained; one area is dedicated for hepatitis B and C negative patients, while in the second area, separate machines are dedicated for HBV positive patients and another for HCV positive patients.

As noticed by the researcher, emergency patients are not tested as usual; they are referred directly to anti-HCV positive machine, assuming that it will not transmit the virus to the patient. In all centers, patients are used to bring their personal bed linens, towels with them, assuming that this would reduce the pathogens transmission.

4.2.5 Infection Control Measures

To control the spread of Hepatitis viruses among HD patients, the CDC published universal recommendations that must be implemented by all HD centers to achieve a comprehensive infection control program (35). The components of such program include infection control practice specifically designed for HD setting, including routine serologic testing and immunization, surveillance, training and education.

In an attempt to assess for some of these factors the following measures were investigated:

4.2.5.1 Hepatitis B Virus Vaccination

HBV vaccine is recommended for both HD patients and Staff. Vaccinated patients must be tested for the presence of anti-HBs annually. If anti-HBs is <10 mIU/ml, the patient is considered susceptible and needs booster doses (35).

We found that only 35 patients (14.2%) of 246 patients were vaccinated. Six patients didn't know if they were vaccinated or not. The highest percentage of vaccination was found at Abu-Yousef Al-Najar center (38.2%), followed by Shuhada`a Al-Aqsa center (16.7%) and Nasser center (15.4%) while the lowest percentage was found at Al-Shifa center (6.9%) (Figure 4.10). None of the HD patients in the four centers was tested for the efficiency of the vaccine nor received booster doses.

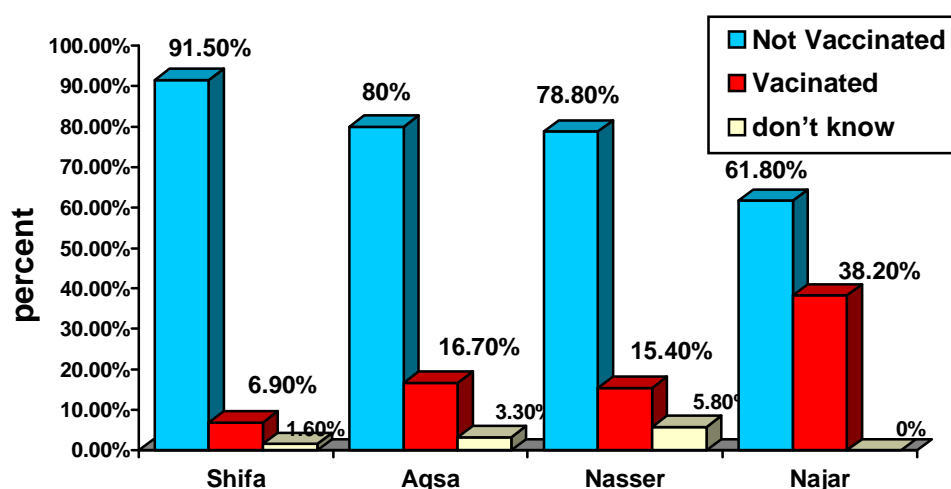


Figure (4.10): Distribution of HD patients by HBV vaccination.

4.2.5.2 Periodical Testing for Hepatitis Viruses Infection

According to CDC recommendation, negative HBV patients must be tested for the presence of HBsAg each month and negative anti-HCV patients must be tested for anti-HCV semiannually.

By investigating these criteria we found that only 89 patients (36.2%) were tested periodically for hepatitis B and C viruses. One hundred and six patients (43.1%) were not tested at all or not tested periodically since the first test at the beginning of HD.

The highest percentage of patients who were periodically tested for hepatitis B and C viruses was found at Shuhada`a Al-Aqsa center (86.7%), then Abu-Yousef Al-Najar center (47.1%), and Nasser center (30.8%), while the lowest

percentage was found at Al-Shifa center (23.8%). Number of periodically tested patients in each HD centers compared with non tested patients is illustrated in (figure 4.11).

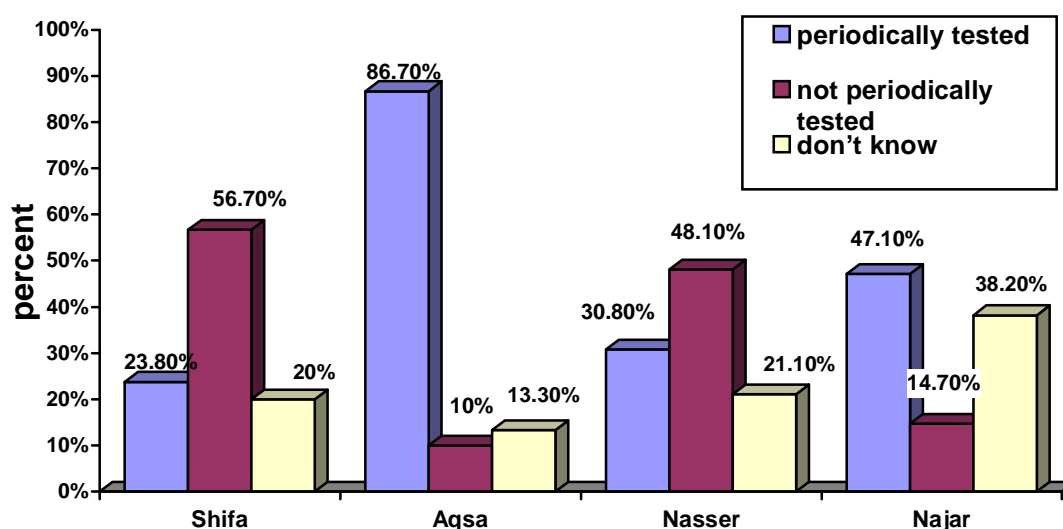


Figure (4.11): Distribution of HD patients by periodical testing for HBsAg and anti-HCV.

4.2.5.3 Periodically Testing for Liver Enzymes Tests

HD patients must be tested monthly for ALT enzyme which has a direct relationship with viral hepatitis especially HCV. In our study, only 90 patients (36.6%) were tested periodically for ALT and AST enzymes activity, while 104 patients (42.3%) were not tested at all or not tested periodically and 52 patients (21.1%) didn't know if they were tested or not .

The highest rate for testing was at Shuhada`a Al-Aqsa center (100%), followed by Abu-Yousef Al-Najar center (38.2%), then Nasser center (28.8%). The lowest percentage was found at Al-Shifa center (24.6%).

Numbers of periodically tested patients for ALT and AST enzymes compared with non tested patients in each HD centers are illustrated in figure 4.12.

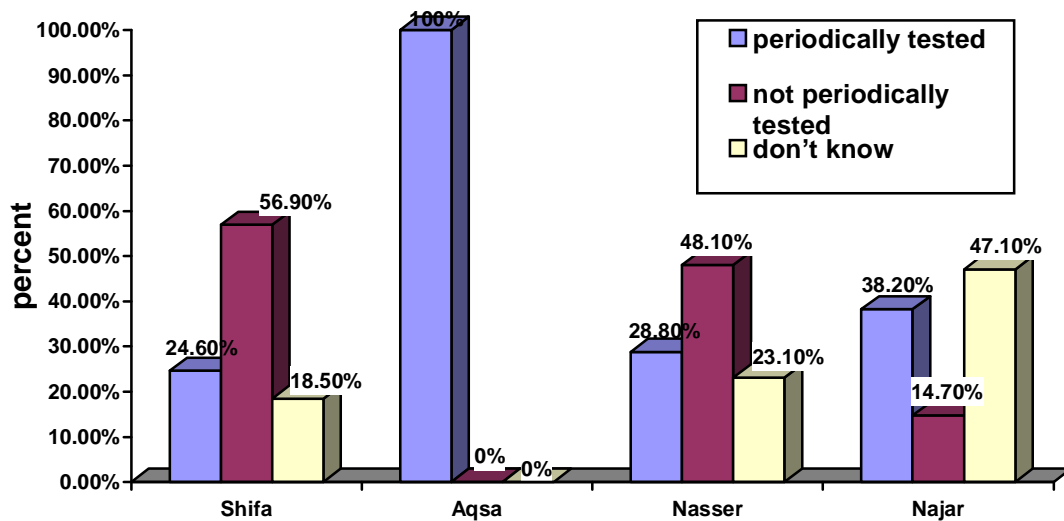


Figure (4.12): Number of periodically tested patients for ALT and AST enzymes compared with non tested patients in each HD centers.

4.3 Medical History of HD Patients

Most HD patients are usually subjected to a range of medical management procedures. The following sections investigate some of these procedures that are usually associated with hepatitis viruses' transmission.

4.3.1 History of Blood Transfusion

Out of the total study population, 143 patients (58.1%) received at least one blood transfusion. Patients with a history of blood transfusion were grouped into four groups according to the number of blood units received. The first group consisted of patients who received less than 5 units of blood (42%), the second group between 5 to 10 blood units (31.5%), the third between 11 to 15 blood units (14%), and the fourth with more than 15 blood units (12.5%).

The total number of patients who received blood transfusion in each of the four centers is illustrated in figure 4.13, while the groups distribution according to the total number of blood units transfused in the total HD centers is illustrated in figure 4.14.

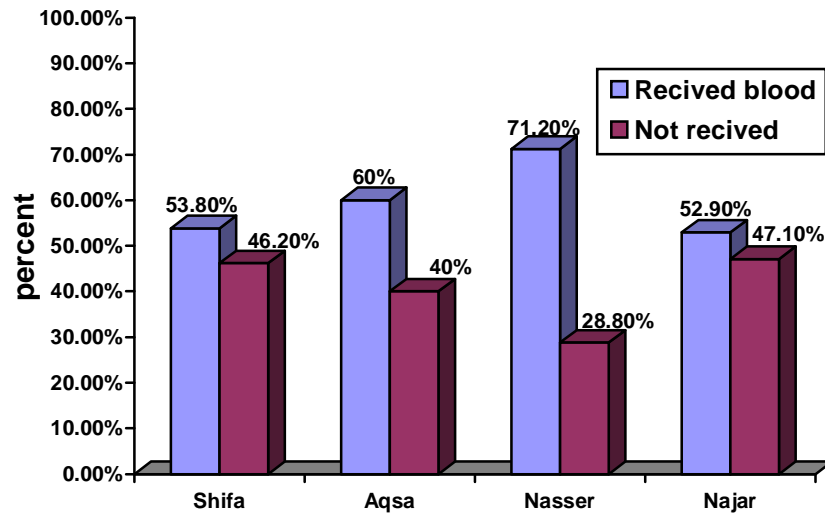


Figure (4.13): Number of patients who received blood transfusion in each of the four HD centers of Gaza Strip.

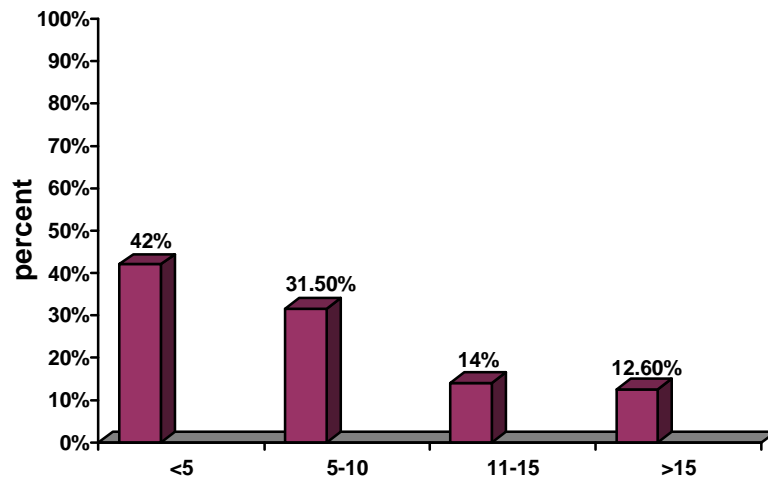


Figure (4.14): Distribution of patients according to number of blood units transfused.

4.3.2 History of Surgical Operation among HD Patients

Surgical operations are considered risk factors for the transmission of hepatitis viruses. As a prerequisite for HD procedure, all patients underwent surgical procedure to make a fistula (shunting blood from an artery to a vein).

Out of the total study population, 72% of the subjects underwent surgical operation (s) before starting HD, while the other 28% had only a fistula.

4.3.3 History of Treatment, Surgical Operation, and Blood Transfusion Abroad.

The study shows that among the 246 tested patients 152 patients (61.8%) were treated abroad. From these, 54 patients (35.5%) had a surgical operation abroad, 2 patients (1.3%) received blood transfusion abroad, and 68 patients (44.8%) had a surgery and received blood abroad. Twenty eight patients (18.4%) neither had a surgery nor received blood abroad.

Distribution of patients treated abroad among HD centers is illustrated in figure 4.15, while the distribution of patients who underwent surgical operation abroad or received blood transfusion abroad is illustrated in figure 4.16.

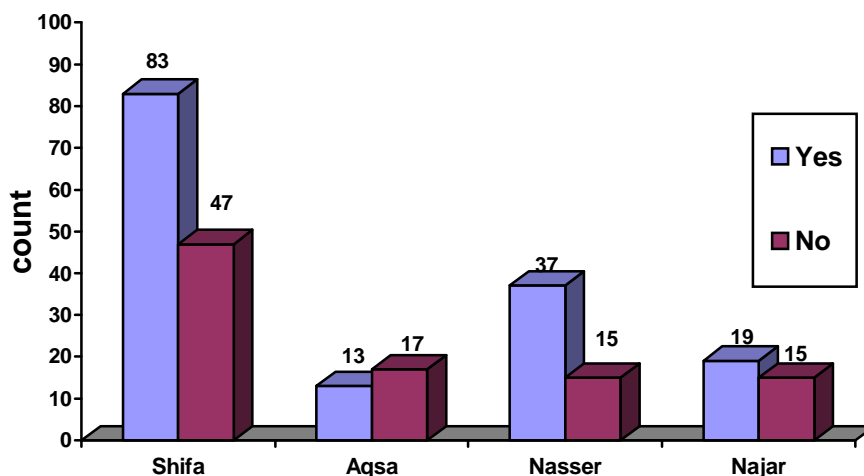


Figure (4.15): Distribution of patients treated abroad among HD centers.

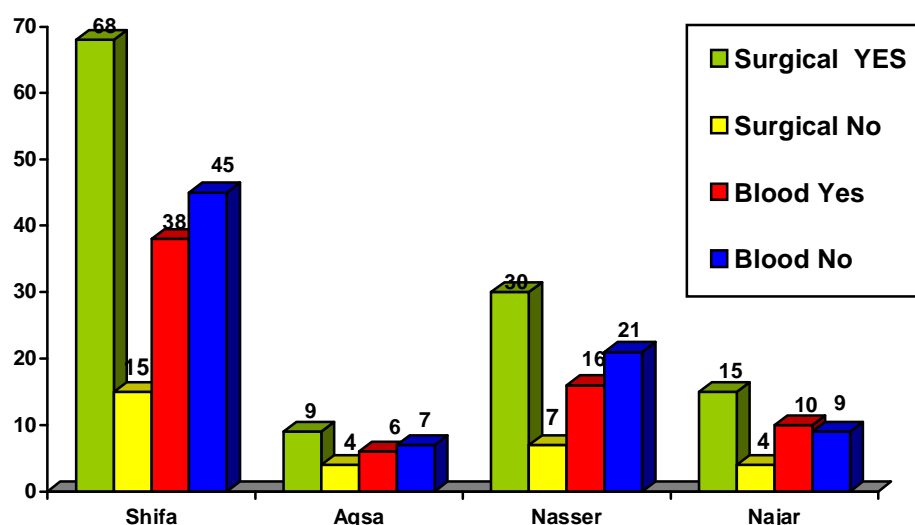


Figure (4.16): Distribution of patients making surgical operation abroad or received blood transfusion abroad among the HD centers.

4.3.4 Health Conditions of HD Patients in the Last Year

The most prevalent infections among HD patients were urinary tract infection (56.5%), respiratory tract infection (46.3%), and Pharyngitis (43.1%). Fever has been found in 57.7% of patients and only 28% of patients were admitted to hospital during the last year (Table 4.1).

Table (4.1): Health status of HD patients during the last year.

Infection/ Symptom	% of patients	Infection/ Symptom	% of patients
Fever	57.7	Gastroenteritis	32.5
Urinary tract infection	56.5	Abdominal cramp	31.3
Respiratory tract infection	46.3	dermatitis or skin rash	29.3
Pharyngitis or mouth infection	43.1	Nausea	26.4
Recurrent infection	41.5	Loss of appetite	24.8
Vomiting	35.8	Jaundice	6.5
Diarrhea	35.4		

4.4 HBsAg Prevalence among HD Patients

The overall prevalence of HBV among HD patients in Gaza Strip was found to be 8.1%, (Al-Shifa hospital 11.5%, Nasser hospital 9.6%, and both Shuhada`a

Al-Aqsa and Abu-Yousef Al-Najar Hospitals 0%), the proportional attributable risk was 71% (Figure 4.17).

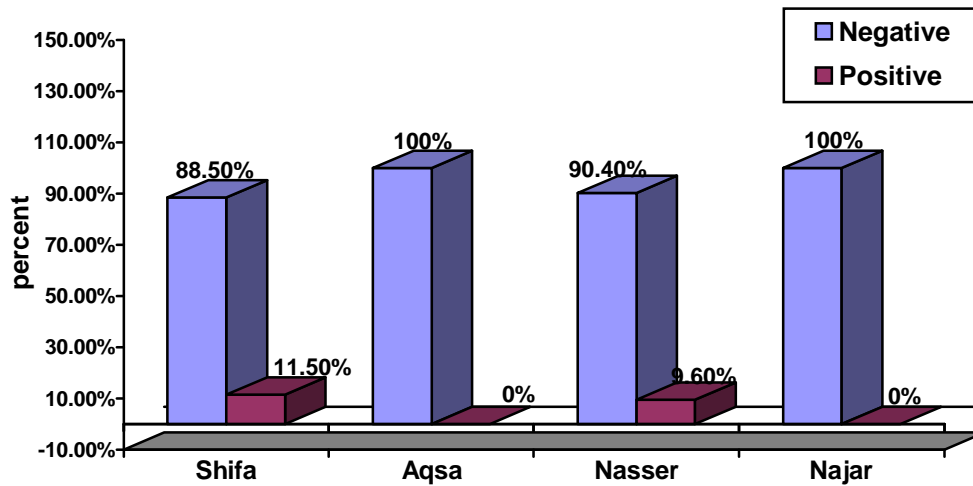


Figure (4.17): Distribution of HBV among HD centers in Gaza Strip.

4.5 Prevalence of HCV among HD Patients

HCV was tested by serological and NAT techniques. Patients with positive results for any of the tests or both of them were considered positive.

4.5.1 Prevalence of Anti- HCV

The prevalence of anti-HCV was 17.9%, (Al-Aqsa Hospital 26.7%, Nasser hospital 23.1% Al-Shifa hospital 18.5%, and Al-Najar Hospital 0%) (Figure 4.18).

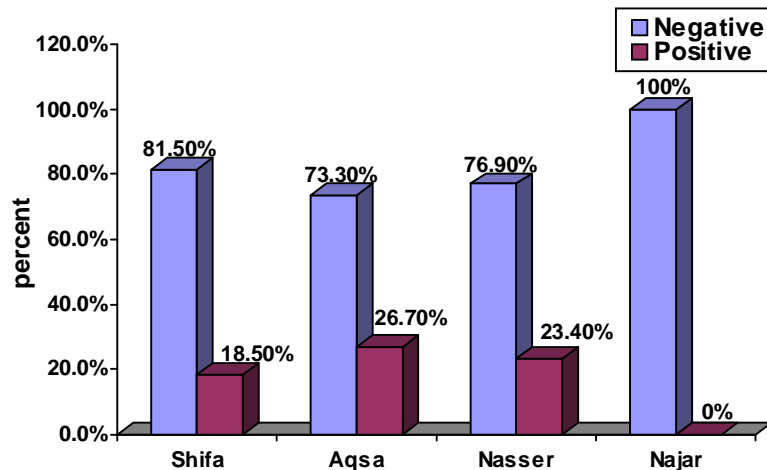


Figure (4.18): Distribution of HCV among HD centers in Gaza Strip by using serology test.

4.5.2 Pooling of Serum Samples

Results didn't show any difference in detection between the original and the pooled samples (Figure 4.19). All of the negative plasma samples in the study were tested by using this technique. Using this technique, 10 new cases of HCV were detected among negative anti-HCV patients (Figure 4.20).

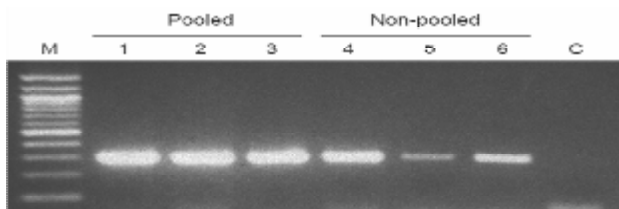


Figure (4.19): Results of initial RT-PCR experiment from Pooled samples (See materials and methods).

(M: 100bp ladder, lanes 1, 2, 3 pooled samples, 4, 5, 6 non pooled samples, C: negative control).

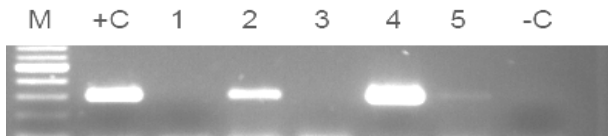


Figure (4.20): Representative results of pooled patient's samples.

(M: 100bp ladder, +C: positive control, 2, 4, 5 positive pooled samples, 1, 3 negative pooled samples, -C: negative control).

4.5.3 Prevalence of HCV Nucleic Acid

HCV RNA was extracted from serum specimens of each patient, and the RNA was then subjected to both reverse transcription of a complementary strand and to PCR amplification in a single test. The appearance of a 298 bp band was considered a positive result (Figure 4.21).

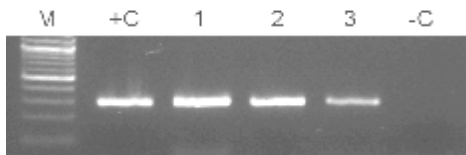


Figure (4.21): Representative results of 1st RT-PCR from different patients.

M: 100bp ladder, +C: positive control, lanes: 1, 2, 3 positive patients' samples (298 bp), -C: negative control.

In all cases, a second nested PCR was performed to improve the detection capacity and specificity of the PCR test. The appearance of a 235 bp band was considered a positive result (Figure 4.22) (See material and method).

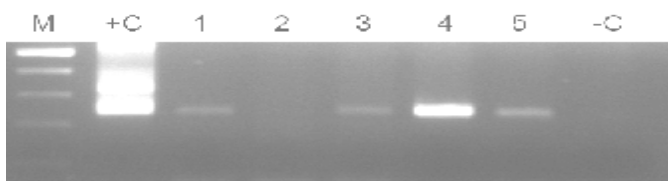


Figure (4.22): Representative results of 2nd (nested) PCR from different patients.

(M: 100bp ladder, +C: positive control, lanes 1, 3, 4, 5 positive patients samples (235 bp), lanes 2 negative patient sample, -C: negative control).

The prevalence of HCV RNA was 19.1%, (Al-Aqsa Hospital 36.7%, Nasser hospital 23.1%, Al-Shifa hospital 18.5%, and Al-Najar Hospital 0%) (Figure 4.23).

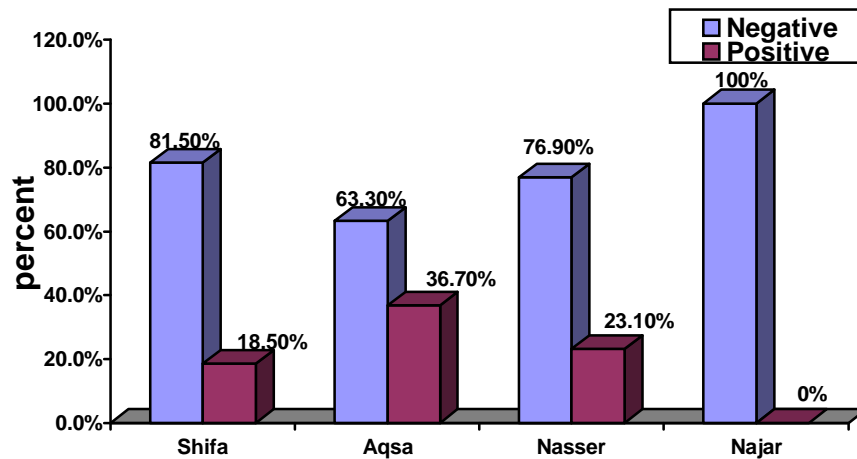


Figure (4.23): Distribution of HCV among HD centers by using NAT test.

4.5.4 Overall Prevalence of HCV

By combining the results of both serological and NAT methods, 54 patients were found to be HCV positive (22%). Thirty seven patients (68.5%) were detected by the two methods, 10 patients (18.5%) by nested RT-PCR method alone and 7 patients (13%) by serology method alone (Table 4.2).

Table (4.2): Overall prevalence of HCV among HD patients in Gaza Strip.

Character	HCV RNA positive	HCV RNA negative	Total
anti- HCV positive	37	7	44
anti- HCV negative	10	192	202
Total	47	199	246

The highest prevalence of HCV was found at Al-Aqsa Hospital (36.7%), followed by Nasser hospital (28.8%), then Al-Shifa hospital (21.5%), and Al-Najar Hospital (0%), the proportional attributable risk was 99% (Figure 4.24).

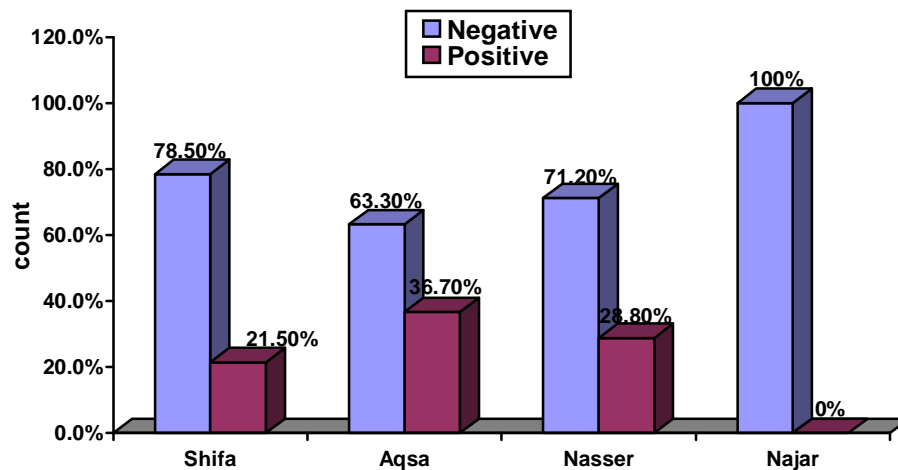


Figure (4.24): Overall prevalence of HCV among HD centers in Gaza Strip.

4.6 Mixed Hepatitis B and C Viruses Infection

During the study, four cases were identified as positive for both HBV and HCV, two cases at Al-Shifa center, and the other two cases at Nasser hospital.

4.7 Risk factors associated with acquiring hepatitis B and C viruses

4.7.1 Relationship between HBV, HCV and the Patients Age.

The mean age for HBV positive patients was 39.2 ± 14.6 year, while for HBV negative patients was 47.4 ± 17.6 year, there was statistically significant relationship between HBV and age of patients ($p=0.04$) (Table 4.2).

On the other hand, no statistically significant relationship was found between HCV and age of the patients ($p=0.11$). The mean age for HCV positive patients was 43.4 ± 15.5 and for HCV negative patients was 47.6 ± 17.9 year (Table 4.2).

4.7.2 Relationship between HBV, HCV and Patient Sex

There is statistically significant relationship between HBV and sex of patient ($p=0.01$). Male patients were found to be more susceptible to HBV than female patients. On the other hand, for HCV no relationship was found with patient sex (Table 4.2).

4.7.3 Relationship between HBV, HCV and Education level

Patients on HD have variable education levels. No significant relationship was found between HBV and the level of education ($p=0.9$). On the other hand, a statistically significant relationship was found with HCV ($p<0.01$), most patients with HCV were found in the preparatory level (40.7%) followed by the Elementary level (20.4%) (Table 4.2).

4.7.4 Relationship between HBV, HCV and Smoking

No significant relationship was found between HBV and smoking ($p=0.51$) while with HCV there was a statistically significant relationship ($p<0.01$) in which smoker patients appear to be more susceptible to HCV than non smokers (Table 4.2).

Table (4.3): Relationship between HBV and HCV infection and age, sex, education level, and smoking among HD patients.

Variable		HBV Positive	HBV Negative	HCV Positive	HCV Negative
Age	Mean Age	39.2 ± 14.6	47.4±17.6	43.4±15.5	47.6±17.9
	p Value	0.04		0.11	
Patient Sex	Male	16	115	30	101
	Female	4	111	24	91
	p Value	0.01		0.70	
Education Level	Illiterate	4	61	7	58
	Elementary	4	45	11	38
	Prep	6	53	22	37
	Secondary	4	47	7	44
	University	2	20	7	15
	p value	0.9		< 0.01	
Smoking	Yes	2	14	8	8
	No	18	212	46	184
	p Value	0.5		0.01	

4.7.5 Relationship between HBV, HCV and HD Centers.

A statistically significant relationship was found between HBV ($p=0.05$), HCV ($p<0.01$) and HD centers. For HBV the highest prevalence of positivity was found at Al-Shifa center 11.5%, followed by Nasser center 9.6%, while both Shuhada`a Al-Aqsa and Abu-Yousef Al-Najar centers 0% (Table 4.3).

For HCV the highest prevalence was found at Al-Aqsa center 36.7%, followed by Nasser center 28.8%, then Al-Shifa center 21.5%, and Al-Najar center 0% (Table 4.3).

4.7.6 Relationship between HBV, HCV and Time Duration on HD

Varying time duration on HD is spent by patients, ranging from 1 month to 276 months. The study found a statistically significant relationship was not present between HBV and HD duration time ($p=0.68$). The mean time duration for negative HBV patients was 34.3 ± 36.7 months and for HBV positive patients was 37.7 ± 36.8 months.

On the other hand, a statistically significant relationship was found between HCV and time duration on HD ($p<0.01$). The mean time duration for HCV negative patients was 30.3 ± 33.7 months while for positive HCV was 49.6 ± 42.4 month (Table 4.3).

Table (4.4): Relationship between HBV, HCV infection, and HD centers and time duration on HD among HD patients.

Variable		HBV Positive	HBV Negative	HCV Positive	HCV Negative
HD Center	Al-Shifa	15	115	28	102
	Al-Aqsa	0	30	11	19
	Nasser	5	47	15	37
	Al-Najar	0	34	0	34
	p Value	0.05		< 0.01	
Time Duration on HD (month)	Mean Time	37.7	34.3	49.6	30.3
	p value	0.68		< 0.01	

4.7.7 Relationship between HBV, HCV and History of Blood Transfusion.

There was a statistically significant association between the number of blood units transfused and hepatitis virus infection (HBV ($p < 0.01$) and HCV ($p < 0.01$). The risk of infection increased sharply if patient received more than 15 blood units for HBV infection, and 10 blood units for HCV infection (Table 4.4).

4.7.8 Relationship between HBV, HCV and Treatment Abroad.

One hundred and fifty two patients on HD were treated abroad before or during HD. Most treatments were done in neighboring Arab countries, mostly Egypt. Some patients have history of kidney transplantation operated in countries such as Pakistan and Iraq. Also it's important to mention that some cases have been treated in more than one country (Tables 4.4, 4.5).

For both HBV and HCV a highly statistically significant relationship was found with history of treatment abroad, (HBV ($p = 0.01$) and HCV ($p < 0.01$) (Table 4.4).

According to the results for HBV, no statistically significant relationship was found with the countries where patients were treated ($p = 0.3$). On the other hand, a statistically significant relationship was found between HCV and countries where patients were treated ($p < 0.01$). For example patients treated in Egypt showed the highest prevalence of HCV infection among other patients (70.4%) (Table 4.5).

4.7.9 Relationship between HBV, HCV and surgical operation abroad

During their treatment abroad, 122 patients (80.3%) out of 152 patients treated abroad had surgical operation. No statistically significant relationship with surgical operation abroad was found for both HBV and HCV ($p = 0.7$) and ($p = 0.9$) respectively (Table 4.4).

4.7.10 Relationship between HBV, HCV and blood transfusion abroad

During there treatment abroad 70 patients (46.1%) out of 152 patients received blood. For HBV there was no statistically significant relationship with blood transfusion ($p = 0.7$) while for HCV there was a statistically significant relationship ($p < 0.01$) (Table 4.4).

Table (4.5): Relationship between HBV, HCV infection and number of blood units transfused, treatment, surgical operation, and blood transfusion abroad among HD patients.

		HBV Positive	HBV Negative	HCV Positive	HCV Negative
Number of Blood Units Transfused	0	7	96	19	84
	1- 5	4	56	6	54
	5-10	2	43	9	36
	10-15	1	19	10	10
	<15	6	12	10	8
	p value	< 0.01		< 0.01	
Number of Patients Treated Abroad	Yes	18	134	45	107
	No	2	92	9	85
	p value	0.01		< 0.01	
Surgical Operation Abroad	yes	14	108	36	86
	no	4	26	9	21
	p value	0.7		0.95	
Blood Transfusion Abroad	yes	9	61	29	41
	no	9	73	16	66
	p value	0.70		< 0.01	

Table (4.6): Relationship between HBV, HCV infection and treatment abroad countries.

country	HBV		HCV	
	positive	negative	positive	negative
Egypt	15	86	26	75
Saudi Arabia	0	6	3	3
Kuwait	0	1	0	1
Iraq	1	0	0	1
Jordan	1	10	1	10
India	0	1	0	1
Pakistan	0	7	3	4
"Israel"	0	9	0	9
Both Egypt and Jordan	0	3	2	1
Both Egypt and Iraq	1	4	5	0
Both Egypt and Saudi Arabia	0	5	4	1
Both Egypt and Pakistan	0	1	1	0
Both "Israel" and Jordan	0	1	0	1
Total	18	134	45	107
p Value	0.3		< 0.01	

4.7.11 Relationship between HBV, HCV and liver enzymes

ALT and AST are enzymes mainly elevated in case of liver disease such as hepatitis. This study found that both HBV and HCV had a strong relationship between ALT and AST levels with hepatitis infection.

HBV positive patients had ALT mean value of 22.4 ± 20.9 IU/ml while negative patients 13.4 ± 11.8 IU/ml. There was a highly statistically significant relationship ($p < 0.01$). Also HBV positive patients had AST mean value of 23.5 ± 15.3 IU/ml while negative HBV patients 15.2 ± 11.2 IU/ml. There was a highly statistically significant relationship ($p < 0.01$) (Table 4.6).

Positive HCV patients has ALT mean value of 19.9 ± 19.1 IU/ml while negative HCV patients has ALT mean value of 12.53 ± 10.3 IU/ml ($p < 0.01$). Also positive HCV patients had AST mean value of 22.9 ± 16.9 IU/ml while negative HCV patients has AST mean value of 13.9 ± 9.00 IU/ml ($p < 0.01$) (Table 4.6).

Table (4.7): Relationship between HBV, HCV infection and liver enzymes among HD patients.

Liver Enzymes		HBV Positive	HBV Negative	HCV Positive	HCV Negative
	ALT mean		22.4	13.4	19.9
p Value		< 0.01		< 0.01	
AST mean		23.5	15.2	22.9	13.9
p Value		< 0.01		< 0.01	

CHAPTER 5

DISCUSSION

Viral hepatitis remains a major hazard for both patients and medical staff of HD units **(300-302)**. HCV became the major form of viral hepatitis among HD patients especially after the decline in incidence of HBV infection due to several factors including vaccination and screening of transfused blood for HBV **(303-305)**.

In Gaza Strip, the prevalence of hepatitis viruses among HD patients was not previously investigated and their related risk factors were not assessed. This study investigates the prevalence of hepatitis viruses B and C among HD patients using serological and molecular techniques. It also aims at identifying factors currently involved in hepatitis viruses' transmission to HD patients.

The risk of acquiring HBV infection has been apparent since HD was first performed in the 1960. A large survey of HD centers in USA (1974) found HBV incidence rates of 6.2% among patients and 5.2% among staff **(35)**.

As a result of segregation, universal precautions, vaccination, reduced blood transfusions, and screening of organs before transplantation, the incidence of HBV infection decreased to 0.08% for patients and 0.05% for staff within dialysis units in the United States by 1996 **(35)**.

In spite of the reduction of HBV spread within dialysis centers, some isolated outbreaks of HBV infection continue to be reported among HD patients in developed countries **(59, 60)**.

In this study, the overall prevalence of HBV in HD patients in Gaza Strip was 8.1% (ranging from 11.5% in Al-Shifa center, to 0% in both Shuhada`a Al-Aqsa and Abu-Yousef Al-Najar centers) compared with a prevalence rate of 2.3% among healthy blood donors in Gaza according to last report of the MOH **(52)**. This prevalence is higher than that in the USA (0.9%), Japan (1.6%), Switzerland (1.63%), Casablanca (2%), Saudi Arabia (3.7%), Iran (4.6%),

Jordan (5.9%) and Kenya (8%), but lower than Italy (9.2%), Saudi Arabia (Najran) (10%), Bahrain (11.8%), Pakistan (12.4%), India (14.2%), Taiwan (16.8%), Romania (17%), Tunisia (19.5%), Greece (20.4%), Spain (20.9%), Turkey (25%) and Brazil (29.8%) **(61-78, 306)**.

The four tested HD centers were heterogeneous when comparing the use of disposable needle for artery-vein fistula, number of reuse of lines and dialysis equipments, type of dialysis machine, machine hygiene, machine sterilization, type of dialysis rooms regarding infected patients, lines and dialysis equipments, reprocessing rooms and the patients/care workers ratio.

Statistically significant differences in prevalence between the four HD centers are obvious ($p=0.05$). Such a result was observed in previous published study in Jordan **(66)**. This difference in prevalence may be due to variation in the degree of implementation of the universal precautions to prevent the appearance of nosocomial transmission; such as avoidance of sharing multidose vials or blood contact equipment, routine screening of blood products and routine vaccination of dialysis patients.

In our study no statistically significant relationship was found between HBV and time duration on HD, this observation was in agreement with a previous report in Moldavia **(74)**, while other studies in Jordan and Brazil showed a direct relationship between HBV and time duration on HD **(66, 307)**.

In this study, the prevalence of HBV was increased significantly with increasing the number of blood units transfused ($p<0.01$), this is in agreement with studies worldwide **(54, 175, 308)**, on the other hand, other studies showed no relationship **(66, 74, 307)**.

From 1967 to 2005, Gaza Strip was occupied by "Israel" which hindered the development of health services in the Strip. Most patients with chronic diseases or who are in need for major surgeries were treated abroad, mostly in neighboring countries. Some of them (e.g., kidney transplantation patients) were treated in countries such as Pakistan and India. Also some patients during

their treatment underwent HD. We found a statistically significant relationship between treatment abroad and HBV infection ($p=0.01$). A previous study showed that traveling abroad is associated with increased risk of hepatitis virus infection and this risk is expected to be increased when traveling to endemic areas **(309)**. Other authors previously reported hepatitis virus seroconversion in patients returning from a holiday and dialyzed in units with high prevalence **(310, 311)**.

In our study no statistically significant relationship was found between HBV prevalence and surgical operation or blood transfusion abroad. This result contradicts results from previous studies shown that surgery is a risk factor for acquiring hepatitis infection in the general population **(312-314)**. This contradiction may be due to differences in the type of surgery, the level of medical services and other factors related to the countries where surgeries are performed.

Moreover, there was no statistically significant relationship between HBV and the country of treatment. It is noteworthy to mention that most of the HBV positive patients were treated in Egypt which has prevalence rate of 3% to 11% **(50)**.

A statistically significant relationship was found between HBV infection and age of the patients (47.4 ± 17.6 years versus 39.2 ± 14.7 years), wherein patients less than 40 years old were found more susceptible to HBV than older patients. This is in agreement with a study done in Gaza among the general population, which found that the highest percentage of HBsAg among age group of 30 to 39 years **(51)**. This may be due to the fact that most of the Gazian population are young (mean age 15.4 years) **(52)**. On the other hand, other studies showed no statistically significant differences in ages between HBV positive and negative patients **(74)**.

The results also showed that males HD patients had higher HBV prevalence than females. This may be related to the fact that males in Palestine are more socially active than female. Furthermore, they are more exposed to male-

related risk factors for HBV than female (e.g. hairdressing and circumcision). This result is in agreement with other studies in general populations **(315- 318)**.

The study found no relationship between HBV prevalence among HD patients and education level, smoking, residence and marital status. A previous study among general population in Taiwan showed that HBV is more prevalent among married and smoking persons **(319)**.

The present study found that only 14.2% of the total HD patients were vaccinated against HBV (ranging from 38.2% at Abu-Yousef Al-Najar center to 6.9% at Al-Shifa center). This low percentage of vaccination probably increased the risk of HBV infection. It's highly recommended to vaccinate both patients and staff at the HD center **(35)**. In a case control study, the results showed the risk of HBV infections was 70% lower among immunized HD patients **(104)**.

HCV infection is a major cause of liver disease among the general population leading to chronic active hepatitis with or without cirrhosis in 50% of cases **(320)**. If left untreated chronic hepatitis C progress to cirrhosis and in certain countries it is a major cause of primary hepatic carcinoma **(321)**. In HD centers HCV is also a major problem and is prevalent both in pre-dialysis population and more significantly in patients on maintenance HD **(164, 183, 196, 322-325)**.

HCV infection is usually asymptomatic **(326)** and can be diagnosed by serological methods and amplification of HCV RNA by RT-PCR **(327)**. The later distinguishes between viraemic and non viraemic HCV patients and also is used for HCV genotyping **(328)**.

In this study, the prevalence of anti-HCV among HD patients was 17.9% (ranging from 26.7% in Al-Aqsa center to 0% in Al-Najar center). This percentage is much higher when compared with a prevalence rate of 0.2% among healthy blood donors in Gaza according to last report of MOH. This anti-HCV prevalence is higher than Germany (3.3%-6.1%), Netherlands (3.8%), India (5.9%-9.93%), Mexico (6.7%) and Brazil (8.4%); and lower than Tunisia (19.1%), Iran (21%), Greece (24%), "Israel" (24.6%), Lebanon (27%), Jordan

(34.6%), Peru (43.7%), Italy (46%), Indonesia (63.4-76.3%), Venezuela (71%), Syria (75%) and Egypt (80%) **(176, 178- 193, 195, 196)**.

Partial immunosuppression in HD patients resulting in a poor antibody response to Hepatitis viruses infection **(199)** which make serological screening of HCV underestimate. Such short coming could be overcome by detecting HCV RNA **(200-202)**.

In this study HCV RNA was detected in 19.1% of HD patients (ranging from 36.7% in Al-Aqsa center to 0% in Al-Najar center). This result is lower than Brazil (30.6%), Greece (31.7%), and Italy (37.6%) and higher than Germany (2.5-4%), Mexico (5%), Israel (12.3%) and Tunisia (13.8%) **(174, 178-180, 186, 187,190, 195)**.

HCV RNA was detected in 84.1% of the anti-HCV positive patients, this deviation between PCR and ELISA results was also previously reported in many studies, Syria (87.5%), Brazil (94.3%), Jordan (30.6%), Venezuela (72%), and Tunisia (72.3%) **(175, 188, 193,195, 196)**.

The possible explanations for this Difference in percentage of viraemic patients includes false positive serology, intermittent viraemia, or the level of viraemia in these patients below the lower limit of PCR detection **(188)**.

It has been reported that (7-68%) of HD patients have intermittent viraemia with a period of undetectable HCV RNA for up to 4 weeks **(328, 330-332)**. Also the viral load is relatively low in this group of patients and long term maintenance HD decreases the HCV RNA level but doesn't produce clearance of viraemia **(333-335)**.

On the other hand, HCV RNA was detected in serum of 10 patients of the anti-HCV negative (4.95%). Again, several studies have shown that serological assays alone are not sufficient for diagnosis of HCV infection and the detection of HCV RNA is required to identify all infected patients **(197, 336)**. So detection of HCV RNA is more reliable than serology in detecting ongoing HCV infection in HD patients who may not mount an adequate antibody response **(197, 199,**

336). Our results among seronegative patients was higher than studies in center of Recife, Brazil (0%), Netherlands (0.23%) **(176,191)**, but lower than another study in central Brazil (10.3%) **(174)**.

Because HCV infections are of special public health concern and the infected person is a potential reservoir for its transmission, we considered patients positive for either ELISA or PCR as positive for HCV.

The overall prevalence of HCV among Gazian HD patients thus is (22%) (Ranging from 36.7% in Al-Aqsa center to 0% in Al-Najar center). This is higher than in Germany in which the over all prevalence was between (3-7%) **(179, 191)** and lower than in Brazil (46.7%) **(182)**.

HCV prevalence was different between HD centers, and as stated earlier, this difference in prevalence may be due to different degrees of commitment to universal precautions taken in each center, the same result was shown in previous studies in Jordan and Tunisia **(188, 195)**.

Duration on HD was found to be a statistically significant risk factor for HCV infection in HD setting. The risk of HCV infection increased with increasing the time duration. In HD population under investigation, the mean duration time for HCV negative patients was found to be 30.3 ± 33.7 months and for HCV positive patients was 49.6 ± 42.4 months. Other studies from different regions in the world have shown similar results **(62, 164, 165, 169, 196, 325, 336-344)**.

This effect may be due to nosocomial transmission of HCV as indicated by other studies **(169, 345-347)**. Epidemiological and molecular studies have shown the role of HD environment for dissemination of HCV between patients **(188, 348-350)**. A previous study showed that HCV RNA was resistant to drying at room temperature for at least 48 hours **(351)**.

Another study detected HCV on a HD machine used for HCV negative patients **(352)**. Actually in our study it was not unusual to find staff taking care of susceptible and infected patients in the same shift. Additionally they didn't

routinely discard gloves after use; this practice may facilitate the dissemination of HCV RNA between HD patients.

Blood transfusion was found to be a highly statistically significant risk factor in our study. The risk increased with the increase in the number of blood units which were transfused ($p < 0.01$). This result was in agreement with many studies in the world **(62, 136, 179, 325, 353-359)**. It's important to note that, in blood banks all blood units are tested for anti-HCV, and only 80% of positive HCV patients can be detected during the first 15 weeks after exposure, in $\geq 90\%$ within 5 months and in $\geq 97\%$ within 6 months and in rare instances seroconversion can be delayed until 9 months after exposure **(360-362)**. Accordingly, false negative ELISA results can mistakenly lead to transfusion of HCV positive blood unit.

In this study, no relationship was found between HCV prevalence and sex of the patients. This was in agreement with other studies **(185, 363)**; while some studies showed that anti-HCV was detected in female more commonly which could be due to females being more exposed particularly during labor **(310, 364, 365)**. In contrast, HCV was found to be more prevalent in males **(325, 366)**. Also no statistically significant relationship was found between HCV prevalence and patient's age; this finding is supported by other studies in different region of the world **(185, 363)**.

A direct statistically significant relationship was found between HCV and education level in which most of HCV positive patients were in the preparatory level followed by elementary. Previous studies among general population worldwide showed that HCV is prevalent among less educated level people **(319, 367)**.

In this study a direct relationship was found between HCV prevalence and smoking, in which smoker patients are more susceptible to HCV than non smokers. This may be due to bad practices in Gaza Strip where people are smoking water pipe (Shisha) in groups. Water pipe is passed from one person to another; the mouth piece maybe wiped but is not changed between users,

which theoretically result in exposure to blood from individuals with gingivitis for example. Also smokers are highly associated with increased visit to out patient physician and hospital services, a result that was indicated in reports among general population **(319, 368)**.

Again due to inadequate health services in Gaza Strip due to "Israel" occupation most ESRD patients were treated in other countries especially neighboring Arab countries as Egypt. A highly statistically significant relationship was found between HCV prevalence and treatment abroad and blood transfusion abroad while no statistically significant relationship was found with surgery operation abroad.

Previous studies showed that outbreaks of HCV transmission have been linked to a number of medical procedures, including the use of contaminated multidose vials **(369-372)**, surgical **(373-375)** and gastrointestinal endoscopy **(375)**. As mentioned before, traveling abroad is associated with increased risk of hepatitis virus infection especially when traveling to high endemic area **(309)**. We found a direct and strong correlation between HCV and country where the patient was treated ($p < 0.01$). Most patients were found to be treated in Egypt which has one of the highest prevalence rate of HCV in the world **(153-157, 376, 377)**. Previous study "unpublished" found a direct relationship of HCV and treatment in Egypt using genotyping method **(299)**.

The striking difference in prevalence and risk factors seen between HBV and HCV (with similar molecular size and transmission routes in general and the HD population) was also described by others **(324, 378-381)**.

Co-infection with both HBV and HCV was reported in 4 patients (1.6%) of the total HD population which is low when compared with other studies in developed and developing countries: (Moldavia (10%), Venezuela (41%), Japan (54.8%), Poland (92.3%)) **(62,74,337,379)**.

ALT and AST are used for screening for liver disease both in general population **(382)**, and in HD patients **(338,383)**. It has been reported that serum

aminotransferases were decreased in HD patients **(384,385)** and the cutoff value of AST and ALT in detecting viral hepatitis should be set at lower levels in such population **(385)**. Due to the poor correlation of serum ALT with liver histology and serum viral load, clinical decisions based only on the ALT may be misleading. It has been suggested that AST also is a useful predictor of liver pathology **(386)**.

In our study a direct and strong correlation between the activity of liver enzymes and both HBV and HCV prevalence was found. Early studies suggested that patients with chronic renal failure have vitamin B₆ deficiency, and hypoamino transferasemia was caused by deficiency of B₆ which is the coenzyme of this enzyme reaction **(387-390)**. Other studies suggested that low serum B₆ levels were also caused by increased requirement **(391,392)** and accelerated clearance of pyridoxal phosphate **(393)**.

Previous studies suggested that ALT levels, even that not exceeding upper limit of normal are higher in anti-HCV positive patients in comparison with anti-HCV negative patients **(386, 394, 395)**. Therefore recriteria for normal reference of ALT and AST is proposed for HD population. On the other hand, some studies suggested that serum aminotransferases aren't reliable marker for HCV screening or for evaluation of hepatitis activity in HD patients since they are frequently normal **(5, 396, 397)**.

CHAPTER 6

CONCLUSIONS and RECOMMENDATIONS

6.1 Conclusions

Hepatitis B and C viruses infection are very frequent among HD patients in Gaza Strip (HBV 8.1%, HCV 22% compared with 2.3% and 0.2% in the blood donors respectively) as in many regions of the world. HD center, blood transfusion and treatment abroad appears to be the major risk factors for both viruses, in which nosocomial infection are important source for both viruses.

For HBV infection other risk factors including, younger age (less than 40 years), HBV was also found to be more prevalent among male patients. Further studies among total population are needed to investigate this factor.

For HCV infection other risk factors including, duration on HD which is associated with acquisition by nosocomial transmission, HCV prevalence was found higher among smoker patients, and also among preparatory and highest level of education.

There was a striking difference in prevalence between HBV and HCV despite the common route of transmission and similar features.

The study emphasized the need for applying both of PCR and ELISA testing for detecting of HCV in HD setting.

The study also showed that applying pooling strategy for low prevalence viruses such as HCV is effective and lowers the expenses associated with RT-PCR.

The study highlights the importance of HBsAg vaccination for HD patients. Also the study highlights the importance of testing the patients for liver enzymes and hepatitis viruses periodically according to universal recommendations.

Finally the study proved the role of HD centers in transmission of hepatitis viruses and the urgent need for implementation of a comprehensive infection control program.

6.2 Recommendations

1. Implementation of a comprehensive infection control program, the components of such program include infection control practices especially designed for HD setting, including routine serological testing and immunization, surveillance, training and education. These practices should be carried out routinely for all patients in the HD centers.
2. In each HD center, policies and practices should be reviewed and updated to ensure that infection control practices recommended for HD are implemented and rigorously followed.
3. Intensive efforts must be made to educate HD staff members regarding these practices.
4. All susceptible patients must be vaccinated against HBV, vaccinated patients must be tested for anti-HBs (1-2 months after last dose),
5. Hepatitis positive patients must dialyze in a separate room using separate machines, equipment, instrument and supplies.
6. Staff members caring for hepatitis positive patients should not care for negative hepatitis virus patients at the same time.
7. Patient must be tested monthly for ALT and AST liver enzymes which has a direct relation with viral hepatitis infection.
8. Negative HBV patients must be tested for the presence of HBsAg each month, and for negative anti-HCV, patients must be tested for anti-HCV semiannually.
9. Adoption of the PCR method to test HD patients for HCV because of its increased sensitivity compared to ELISA and because it reflects the current situation of positive patients.

10. Continuous training and education are recommended for both staff and patient or patient family, the training courses must contain information such as proper hand hygiene techniques, proper use of protective equipment, mode of transmission of blood borne viruses and infection control practices.
11. Decrease the dependence on blood product in treatment and use of erythropoietin to correct renal anemia.
12. Reduce the dependence on traveling abroad for treatment, and when needed should be restricted to countries with low hepatitis virus endemicity.
13. Further studies are recommended to investigate the role socioeconomic risk factors in transmission of hepatitis viruses, HD workers practice in transmission of infection among HD patients; finally further studies are needed to define hepatitis virus's genotypes between HD patients.

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Appendix 1



اسم المستشفى-----
اسم المريض-----
رقم المريض-----
الوقت و التاريخ-----

استبيان

أخي المريض:

يهدف هذا البحث إلى فهم المخاطر التي يتعرض لها مرضى غسيل الكلى و يركز بشكل أساسي على الفيروسات المسببة لالتهاب الكبد الوبائي من نوع B,C والتي غالبا ما تؤدي إلى مضاعفات خطيرة يمكن التخلي عنها بإتباع إجراءات وقائية بسيطة داخل وحدة الغسيل.

إن تعاونك معنا في إتمام الاستبيان و جمع العينات سوف يعود بالفائدة عليك و على المجتمع بأكمله و سوف يقلل من المخاطر التي يتعرض لها مرضى الغسيل الكلوي.

إن المعلومات التي سيتم جمعها ستعامل بصورة سرية ولن يطلع عليها أحد كما أننا سوف نزودك و في نهاية البحث بنتيجة فحوصاتك إذا رغبت في ذلك.

الباحث/ عبد القادر يوسف العطل

الاسم (يمكن حجه):-----

الرقم التسلسلي:

العمر:-----

الجنس ذكر أنثى

الوظيفة:-----

مكان الإقامة:-----

التعليم: ابتدائي عداي انوي جامعي فأكثر

ممارسة الرياضة: نعم لا

تدخين: نعم لا

متزوج: نعم لا

هل تعاني من البدانة نعم لا

الساعة التي تقوم بها بالغسيل-----

هل هذا الموعد ثابت متغير

هل تغير موعدك عدة مرات نعم لا

منذ متى تجري غسيل كلوي:-----

عدد مرات الغسيل الأسبوعية: ١ ٢ ٣ ٤ ٥

مدة كل غسلة:-----

هل أنت معتاد على مكان واحد منذ بدء الغسيل أم انتقلت من مكان لأخر:-----

هل تلقيت تطعيما للفيروس من نوع **B** نعم لا

إذا كان الجواب نعم متى كان ذلك:-----

هل تم التأكد من نجاعة التطعيم نعم لا

هل تلقيت جرعات تنشيطية نعم لا

إذا كان الجواب نعم متى كان ذلك:-----

هل أجريت أي عمليات جراحية في الفترة السابقة نعم لا

----- إذا كان الجواب نعم فمتى كان ذلك-----

هل يتم عمل فحص دوري لالتهاب الكبد الوبائي لك نعم لا

----- إذا كان الجواب نعم فمتى تم آخر فحص-----

هل تنقل دم بشكل مستمر نعم لا

----- إذا كان الجواب نعم فكم مرة تتلقى الدم شهريا-----

----- كم مرة تلقيت دم في السابق-----

----- ما هي كمية الدم المنقولة لك في كل مرة-----

هل تتناول الطعام أو الشراب أثناء الغسيل نعم لا

هل تتعاطى أي أدوية عن طريق الحقن نعم لا

هل تتعاطى علاج الارثروبيوثين نعم لا

هل يتم عمل وظائف الكبد لك بصورة دائمة نعم

هل تعاني من أي أمراض مزمنة نعم لا

----- إذا كان الجواب نعم فاذكرها -----

هل تحضر أدواتك الشخصية عند كل غسيل (مثل المخدة) نعم لا

هل تقوم بزيارة أطباء الأسنان بشكل دائم نعم لا

هل سافرت للعلاج في الخارج نعم لا

----- إذا كان الجواب نعم فما هي الدولة التي سافرت لها-----

هل أجريت أي عمليات جراحية هناك نعم لا

هل تلقيت دم هناك نعم لا

هل تعاني من الالتهابات بشكل متكرر نعم لا

هل عانيت خلال فترة الغسيل للسنة الحالية من:

التهابات في الصدر نعم لا

لا	<input type="checkbox"/>	نعم	<input type="checkbox"/>	التهابات في المسالك البولية
لا	<input type="checkbox"/>	نعم	<input type="checkbox"/>	الإسهال
لا	<input type="checkbox"/>	نعم	<input type="checkbox"/>	القيء
لا	<input type="checkbox"/>	نعم	<input type="checkbox"/>	الصفار
لا	<input type="checkbox"/>	نعم	<input type="checkbox"/>	فقدان الشهية
لا	<input type="checkbox"/>	نعم	<input type="checkbox"/>	الغثيان
لا	<input type="checkbox"/>	نعم	<input type="checkbox"/>	مغص
لا	<input type="checkbox"/>	نعم	<input type="checkbox"/>	الالتهابات المعوية
لا	<input type="checkbox"/>	نعم	<input type="checkbox"/>	ارتفاع درجة الحرارة
لا	<input type="checkbox"/>	نعم	<input type="checkbox"/>	الحبوب و الالتهابات الجلدية
لا	<input type="checkbox"/>	نعم	<input type="checkbox"/>	التهابات العظام
لا	<input type="checkbox"/>	نعم	<input type="checkbox"/>	التهابات الفم و الحنجرة
لا	<input type="checkbox"/>	نعم	<input type="checkbox"/>	هل يتم الإشراف الطبي عليك من قبل طبيب ثابت

هل أقمت في المستشفى في الفترة القربية نعم لا

إذا كان الجواب نعم فمتى كان ذلك-----

ما هو سبب الإقامة-----

هل لديك أي مشاكل أو ملاحظات أخرى-----

شكرا لحسن تعاونك معنا

Appendix 2