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Detection and Identification of Hepatitis B,C and HIV viruses in Benghazi city

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كشف و تعريف إلتهاب الكبد الوبائي B، C وفيروس نقص المناعة البشرية في مدينة بنغازي

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Summary

Libya is one of the largest countries in Africa and has the longest coast in the Mediterranean basin facing southern Europe. High rates of prevalence of viral hepatitis and HIV infection have been observed in various regions in Africa, however, prevalence of hepatitis B, C and HIV infection in Benghazi, Libya is not well documented. This study was designed to detect and identify the hepatitis viruses B, C and HIV among community in Benghazi city. This study focused on three human resources including adult Libyan nationals, Libyan children and foreigner workers. Also as a selective sources representing the majority of serology referencing including four medical centre are a private laboratory is Al Razi medical Centre and three governmental medical centers including Benghazi Centre of infectious diseases, Children hospital, and the medical Laboratory of Red crescent centre. Benghazi, Libya. In this study, we identified the extent of the viral infection at the local level, where the samples included 800 patients represented of all age groups with 200 samples from each source. The results showed that, the infection with HBV surface antigen (HBsAg) and HCV were (6.4%) and (5%) respectively and HIV virus was (1.9%). The prevalence of HCV increased with age, in contrast the prevalence of HBs Ag

and HIV infection are common in young people. From this study, we concluded that infection with HBV, HCV, and HIV are obvious problem in Libya. A community based study should be planned for targeting at risk and non-at risk subjects to investigate the extent of this problem and its impact on the community with an effort to develop preventive strategies.

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Dedication

I would like to dedicate my project to spirit of my father, to my wonderful Mother, to my husband for his endless love, support and encouragement, to sweet hearts my children, to my brothers and sisters and to all who encouraged me to finish my research.

Abbreviations

Ab	Antibody
Ag	Antigen
AIDS	Acquired Immune Deficiency Syndrome
ALT	Serum Alanine Aminotransferase
BCIDI	Benghazi Centre of Infectious Diseases and Immunity
CD4	Cluster Difference 4
CD8	Cluster Difference 8
DNA	Deoxyribo Nucleic Acid
EIAs	Enzyme Immuno Assay
ELISA	Enzyme Linked Immuno Sorbent Assay
EU	European Union
HAART	Highly active antiretroviral therapy
HbcAg	Hepatitis B core Antigen
HbeAg	Hepatitis B early Antigen
HBIG	Hepatitis B Immune globulin
HbsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus

HCC	Hepatitis cellular carcinoma
HCV	Hepatitis C virus
HD	Haemodialysis
HDV	Human diploid vaccine
HIV	Human Immunodeficiency virus
HRP	Horserdish Peroxidase
IDUs	Injection drug users
IG	Immune Globulin
IGG	Immunoglobulin G
IGM	Immunoglobulin M
IVDAs	Intra venous drugs abusers
MSM	Men who have sex with men
PCR	Polymerase Chain Reaction
PT-NANB	Post transfusion non A non B Hepatitis
RNA	Ribo Nucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
TMB	Tetra methyl benzidine
US	United states
WHO	World health organization

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CHAPTER 1 INTRODUCTION

Hepatitis B virus (HBV), hepatitis C virus (HCV), and HIV infections represent a global public health problem. Transmission of these viruses occurs via blood and blood products transfusion and by sexual contact (Lee, *et al*.2001) Today, along with tuberculosis and malaria, the infections by hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) represent the main problems in the world (Morteza,*et al*.2007) The infections caused by the three viruses are not curable even with the latest available treatments. Effective vaccine is available only for HBV (Zaied, *et al*.2010).

Viral hepatitis has tremendous socioeconomic, healthcare and even political repercussions. A better understanding of the epidemiology of viral hepatitis and the risk factors involved is among the priorities of any nation (Remis,2009 and Kershenobich, *et al*. 2011).

Regards HBV much has been learned about it since its original discovery in 1965. Leading to the availability of hepatitis B vaccine for the prevention of the disease and multiple drugs for the

treatment of its chronic status. Chronic HBV infection is a global major public health problem. Its prevalence and patterns of transmission vary greatly throughout the world. Approximately, two billion individuals worldwide have been infected with HBV and between 350 and 400 million persons have chronic HBV infection. Individuals who have chronic HBV infection from birth have 15% to 30% lifetime risk for developing cirrhosis associated with hepatic decompensation and/or hepatocellular carcinoma, leading to premature death (Blumberg, *et al*. 1965; Lavnchy, 2004 and Keeffe, 2007). The hepatitis B surface antigen (HBsAg) in serum is the first seromarker to indicate active HBV infection, either acute or chronic. A hepatitis B vaccine, available since 1982, has a high efficacy in the prevention of HBV transmission and has brought about remarkable changes in the global epidemiology of HBV infection(Lok and Mcmahon, 2007 and Sood and Malvankar, 2010).

HCV infection is also common worldwide; it is transmitted in a manner similar to HBV (WHO,2009).Hepatitis C virus has been considered one of the most potential pathogens that have hindered the medical community all over the world. Indeed, since its discovery in 1989, hepatitis C virus (HCV) has been recognized as a major cause

of chronic liver disease worldwide (Shepard, *et al*.2005). More than 170 million people are chronically infected with hepatitis C virus (HCV) worldwide (WHO, 2010) the data reported by WHO estimated that the prevalence of HCV infection is 2.2%, and more than one million new cases were reported annually. Furthermore, an estimated 27% of cirrhosis and 25% of hepato-cellular carcinomas (HCC) worldwide occur in HCV-infected people. Such infection increases tremendously among the developing countries particularly at those categories that were considered to be at a potential risk of acquiring hepatitis C virus(Alter,2007).

Patients receiving maintenance haemodialysis (HD) therapy are at increased risk for acquiring these infections and have a higher prevalence of HBV and HCV than the general population (Fabrizi,*et al*. 2002 a and Fabrizi, *et al*.2002 b) Recognition of the risk of nosocomial infection has resulted in recommendations that strict infection control procedures should be followed on HD units; patients with blood-borne virus infections should be isolated from sero-negative patients during dialysis and patients as well as staff should be vaccinated against hepatitis B,C (Fabrizi,et *al*. 2008 and Taal and Van, 2001). Libya provides free access to maintenance HD for end stage

kidney disease through a rapidly expanding network of centers. Although there are no national dialysis practice guidelines or infection control polices enforced by health care authorities, there is general agreement that patients on HD should be screened for HBV and HCV infection before the initiation of HD and monitored every 3–6 months thereafter(Alashek,*et al*.2011).

As well, the global impact of the HIV and AIDS epidemic has spurred its rapid prioritization as one of the most pressing health issues facing the world community. While the epidemiology and social forces affecting its continued proliferation differ between communities and regions, HIV has spread worldwide. In 2012, an estimated 35.3 million people around the world were living with HIV and AIDS and 2.3 million were newly infected with the virus. of those affected by HIV, young people around the world assume a disproportionate burden of the disease, with 40% of new cases of HIV infection occurring amongst them (Abu-Raddad,*et al*. 2010 a and Abu-Raddad,*et al*.2010 b). The epidemiology, natural history, clinical features and outcome of HIV infection have undergone considerable changes in recent years, because of the proportional increase of heterosexual transmission, the progressive rise of patients'

age, and especially the virological monitoring and the introduction of highly active antiretroviral therapy (HAART) (Nicoll and Gill, 1999).

The endemicity of HBV infection varies greatly over the world, from highly endemic areas (> 8% infection rate), to intermediate (2−8%) and low endemicity areas (< 2%). Africa is among the highly endemic areas, but some countries in the north fall in the intermediate category, with an average rate of about 7%, whereas most regions of west and east Africa are highly endemic areas with chronic infection rates of 7−10% (Lavanchy, 2004). African countries have among the highest prevalence rates of HCV in the world, ranging from one to 26% (WHO,1999 and Muhlberger,*et al*.2009). More than 28 million people are chronically infected with HCV in this continent, and it is difficult to speculate about current and future trends (Daw, 2012 and Abdelwahab,*et al*.2013). Libya is one of the largest countries in Africa and has the longest coast in the Mediterranean basin facing southern Europe. High rates of prevalence of viral hepatitis have been observed in various regions in Africa, but the prevalence in Libya is not well documented (Daw and El-Bouzedi, 2014). Libya, a developing country of approximately 6 million people, belongs to the intermediate endemicity countries

(Daw,*et al*.2002 and Elzouki, 2008). HCV has been divided into six major genotypes and a number of subtypes (Simmonds,*et al*.2005). In North Africa, the most common subtype is 1b. In Arabic countries, such as Egypt, Lebanon, Syria, Saudi Arabia, and Kuwait, genotype 4 predominates (Ramia and Eid, 2006). Recently, a comprehensive study was carried out on HCV genotypes in Libya. Hepatitis C virus genotype 4 was the predominant one, followed by HCV genotype 1 and then other less common genotypes (Elasifer,*etal*.2010). In Libya, most of the blood donors are young men (20_40 years of age). It is known that this age group is usually in the high-risk group for drug abuse, unprotected sex, and other unsecure habits for the transmission of the virus. (Kutrani,*et al*.2007).

Chronic HBV infection at birth occurs in approximately 90% of infants who are born to HBsAg- and HBeAg-positive mothers (Elasifer,*et al*.2010). Even if they are not infected during pregnancy, children of HBV-infected mothers have a high risk of acquiring HBV infection by horizontal transmission during the first years of life (Alrowaily,*et al*.2008). On the other hand, the range of transmission of HCV from mother to child during pregnancy is between 0% and 15% (Kassem,*et al*.2000). The prevalence of HBV among pregnant

women worldwide is approximately 5%, ranging from 0.6% in low-endemic regions to more than 20% in high-endemic areas in the Far East and Africa (Petrova and Kamburov, 2010). In contrast, the prevalence of HCV among pregnant women worldwide is between 1% and 8% (Arshad,*et al*.2011). Recent data on the prevalence of hepatitis B and C and HIV viruses and risk factors among the Libyan population are lacking. Such data are important for understanding the burden of viral infection and for predicting future trends. Implementation of surveillance to guide public health policy is needed to efficiently control viral hepatitis spread in Libya, and this requires reliable epidemiological data (Bagasra,*et al*. 2007 and Daw and Elkhammas, 2008).

Finally, most of the epidemiological studies carried out individually based upon seroprevalence of HCV among specific groups. These include blood donors, heath care workers, or patients undergoing haemodialysis. Such studies were not representative of the community; independent scientists usually carried them out, even though some countries lack such studies (Sievert, *et al*. 2011).

1.1 Hepatitis B virus:

Hepatitis is a general term meaning inflammation of the liver and can be caused by a variety of different viruses such as hepatitis A, B, C, D and E. Since the development of jaundice is a characteristic feature of liver disease, a correct diagnosis can only be made by testing patients' sera for the presence of specific anti-viral antigens or antibodies (Hollinger and Liang, 2001). of the many viral causes of human hepatitis, few are of greater global importance than hepatitis B virus (HBV) (Mahoney and Kane, 1999). Hepatitis B is a serious and common infectious disease of the liver, affecting millions of people throughout the world (Chisari and Ferrari, 1997). The severe pathological consequences of persistent HBV infections include the development of chronic hepatic insufficiency, cirrhosis, and hepatocellular carcinoma (HCC). In addition, HBV carriers can transmit the disease for many years (Ganem and Schneider, 2001). Infection occurs very often in early childhood when it is asymptomatic and often leads to the chronic carrier state; more than 2000 million people alive today have been infected with HBV at some time in their lives. of these, about 350 million remain infected chronically and become carriers of the virus (Chisari and Ferrari

1997). Three quarters of the world's population live in areas where there are high levels of infection. Every year there are over 4 million acute clinical cases of HBV, and about 25% of carriers, 1 million people a year, die from chronic active hepatitis, cirrhosis or primary liver cancer (WHO, 2001). Hepatitis B has also been called type B hepatitis, serum hepatitis, homologous serum jaundice (Robinson, 1995).

1.1.1 Causes the disease:

Hepatitis B is caused by the hepatitis B virus (HBV), an enveloped virus containing a double stranded circular DNA genome, and classified within the family hapadnavirus (Robinson, 1994), the virus interferes with the functions of the liver while replicating in hepatocytes. The immune system is then activated to produce a specific reaction to combat and possibly eradicate the infectious agent. Because of pathological damage, the liver becomes inflamed; HBV may be the cause of up to 80% of all cases of hepatocellular carcinoma worldwide, second only to tobacco among Known human carcinogens (VHPB, 1996).

1.1.2 HBV spread:

One should not judge by appearance: most infected people look perfectly healthy and have no symptoms of disease, yet may be highly infectious.HBV is transmitted through percutaneous or parenteral contact with infected blood, body fluids, and by sexual intercourse (Gitlin, 1997). HBV is able to remain on any surface it comes into contact with for about a week, e.g. table-tops, razorblades, blood stains, without losing infectivity (Hollinger and Liang, 2001). HBV does not cross the skin or the mucous membrane barrier. Some break in this barrier, which can be minimal and insignificant, is required for transmission (Robinson, 1995).

HBV is a large virus and does not cross the placenta, hence it cannot infect the fetus unless there have been breaks in the maternal-fetal barrier, e.g. via amniocentesis. Still, pregnant women who infected with HBV can transmit their disease to their babies at birth. If not vaccinated at birth, many of these babies develop lifelong HBV infections, and many develop liver failure or liver cancer later in life (Mahoney and Kane, 1999). Sexual intercourse with multiple partners or with persons who have multiple partners can be dangerous. Hepatitis B is the only sexually transmitted infection for which there

is a protective vaccine (Mahoney and Kane, 1999). All persons who are hepatitis B surface antigen (HBsAg, positive are potentially infectious. The many millions of people around the world who become HBV carriers are a constant source of new infections for those who have never contracted the virus (Robinson, 1995), blood is infective many weeks before the onset of the first symptoms and throughout the acute phase of the disease. The infectivity of chronically infected individuals varies from highly infectious (HBeAg positive)to often sparingly infectious (anti-HBe positive).

1.1.3 Ability of HBV to cause infection:

Susceptibility is general, only people who have been vaccinated successfully or those who have developed anti-HBs antibodies after HBV infection are immune to HBV infection , persons with congenital or acquired immunodeficiency including HIV infection, and those with immune suppression including those with lymphoproliferative disease, and patients treated with immunosuppressive drugs including steroids and by maintenance haemodialysis are more likely to develop persistent infection with HBV, following acute HBV infection, the risk of developing chronic

infection varies inversely with age. Chronic HBV infection occurs among about 90% of infants infected at birth, 25-50% of children infected at 1-5years of age and about 1-5% of persons infected as older children and adults. Chronic HBV infection is also common in persons with immunodeficiency (Ganem and Schneider, 2001).

There is no specific treatment for acute viral hepatitis B (Mahoney and Kane, 1999), hepatitis B is a viral disease, and as such, antibiotics are of no value in the treatment of the infection. The use of adrenocorticosteroids in the management of acute, uncomplicated hepatitis B is not indicated because they have no effect on the resolution of the underlying disease process, and may increase the rate of relapse. Early treatment of acute hepatitis B with steroids may result in the development of persistent infection. Corticosteroid therapy is only to be used in patients with chronic active hepatitis who are symptomatic, HBsAg negative and who have severe histologic lesions in liver biopsies. (Robinson, 1995). The therapeutic effectiveness of interferon on the course and prognosis of acute hepatitis B is not known (Mahoney and Kane, 1999). Haemodialysis, exchange transfusions, cross-perfusion, and immune globulin (IG) containing high titres of anti-HBs (HBIG) do

not affect favourably the course of fulminant hepatitis. Therapy for acute hepatitis B should be supportive and aimed at maintaining comfort and adequatenutritional balance (Mahoney and Kane, 1999). Specific antiviral drugs such as lamivudine, a second-generation nucleoside analogue, are available, and others are under development, but these drugs have not been evaluated for the treatment of acute hepatitis B.

1.1.4 Epidemiology of HBV:

The world can be divided into three areas where the prevalence of chronic HBV infection is high (>8%),intermediate (2-8%), and low (<2%) (VHPB, 1998). High endemicity areas include Southeast Asia and the Pacific Basin (excluding Japan, Australia, and NewZealand), sub-Saharan Africa, the Amazon Basin, parts of the Middle East, the central Asian Republics, and some countries in Eastern Europe. In these areas, about 70 to 90% of the population becomes HBV-infected before the age of 40 and 8 to 20% of people are HBV carriers. (Hollinger and Liang, 2001), in countries such as China, Senegal, Thailand, infection rates are very high in infants, and continue through early childhood. At that stage, the prevalence of HBsAg in serum may

exceed 25%. In other countries such as Panama, Papua New Guinea, Solomon Islands, Greenland, and in populations such as Alaskan Indians, infection rates in infants are relatively low and increase rapidly during early childhood (Hollinger and Liang, 2001). Low endemicity areas include North America, Western and Northern Europe, Australia, and parts of South America. The carrier rate here is less than 2%, and less than 20% of the population is infected with HBV (Mahoney and Kane, 1999). The rest of the world falls into the intermediate range of HBV prevalence, with 2 to 8% of a given population being HBV carriers, the most important mode of HBV transmission globally is perinatal, from the mother to her newborn baby. If a pregnant woman is an HBV carrier and is HBeAg-positive, her newborn baby has a 90% likelihood to be infected and become a carrier. Of these children, 25% will die later from chronic liver disease or liver cancer (Hollinger and Liang, 2001). Another important mode of HBV transmission is from child to child during early life resulting from blood contact (Gitlin, 1997). All patients with acute hepatitis B are HBeAg positive, and therefore highly infectious and careless contact with their blood or body fluids can lead to HBV infection. HBeAg-positive specimens contain high concentrations of

infectious virions and HBV DNA, in contrast to anti-HBe positive samples, in which the number of hepatitis B virions is substantially reduced.

1.1.5 Diagnosis of HBV: (Large-scale screening for HBV infection)

Diagnosis is confirmed by demonstration in sera of specific antigens and/or antibodies. Three clinical useful antigen-antibody systems have been identified for hepatitis B:

- Hepatitis B surface antigen (HBsAg) and antibody to HBsAg (anti-HBs)
- Antibody (anti-HBc IgM and anti-HBc IgG) to hepatitis B core antigen (HBcAg)
- Hepatitis B e antigen (HBeAg) and antibody to HBeAg (anti-HBe)

Tests specific for complete virus particles or DNA and DNA polymerase-containing virions, and for HDAg and HDV RNA in liver and serum are available only in research laboratories (Robinson, 1995). HBsAg can be detected in the serum from several weeks before onset of symptoms to months after onset. HBsAg is present in serum during acute infections and persists in chronic infections. The presence of HBsAg indicates that the person is potentially infectious

(Mahoney and Kane, 1999). Anti-HBs replaces HBsAg as the acute HBV infection is resolving. Anti-HBs generally persists for a lifetimein over 80% of patients and indicates immunity (Hollinger and Liang 2001). Acute hepatitis patients who maintain a constant serum HBsAg concentration, or whose serum HBeAg persists 8 to 10 weeks after symptoms have resolved, are likely to become carriers and at risk of developing chronic liver disease (Hollinger and Liang 2001). A complication in the diagnosis of hepatitis B is the rare identification of cases in which viral mutations change the antigens so they are not detectable.

1.1.6 HBV Host Immune response:

Primary infection leads to an IgM and IgG response to HBcAg shortly after the appearance of HBsAg inserum, at onset of hepatitis. Anti-HBs and anti-HBe appear in serum only several weeks later, when HBsAg and HBeAg are no longer detected, although in many HBsAg-positive patients, HBsAg-anti-HBs complexes can be found in serum (Mahoney and Kane, 1999).

1.1.7 Serological markers of HBV infection:

During HBV infection, the serological markers vary depending on whether the infection is acute or chronic.

Table (1) Serological markers of HBV infection (Gitlin, 1997) (Modified).

Antigens	Antibodies
HBsAg Hepatitis B surface antigen is the earliest indicator of acute infection and is indicative of chronic infection if its presence persists for more than 6 months. It is useful for the diagnosis of HBV infection and for screening of blood. Its specific antibody is anti-HBs.	Anti-HBs This is the specific antibody to hepatitis B surface antigen. Its appearance 1 to 4 months after onset of symptoms indicates clinical recovery and subsequentimmunity to HBV. Anti-HBs can neutralize HBV and provide protection against HBV infection.

1.1.8 Serological test findings at different stages of HBV infection and in convalescence:

Table (2) Hepatitis B virus serological markers in different stages of infection and convalescence. (Zuckerman, 1996) (Modified)

Stage of infection	HBsAg	Anti-HBs
late incubation period	Positive	Negative
acute hepatitis B or persistent carrier state	Positive +	Negative
HBsAg-negative acute hepatitis B infection	Negative	Negative

recovery with loss of detectable anti-HBs	Negative	Negative
healthy HBsAg carrier	Positive	Negative
chronic hepatitis B, persistent carrier state	Positive	Negative
HBV infection in recent past, convalescence	Negative	Positive ++
HBV infection in distant past, recovery	Negative	Positive or Negative
recent HBV vaccination, repeated exposure to antigen without infection, or recovery from infection with loss of detectable anti-HBc	Negative	Positive ++

1.2 Hepatitis C:

Hepatitis C is called type C hepatitis, parenterally transmitted non-A non-B hepatitis (PT-NANB), Non- B transfusion-associated hepatitis, Post transfusion non-A non-B hepatitis, HC (Choo, *et al*. 1989).

1.2.1 Causes the disease:

Hepatitis C is caused by infection with the hepatitis C virus (HCV), an envelope, single stranded, positive sense RNA (Purcell, 1994). The virus infects liver cells and can cause severe inflammation

of the liver with long-term complications (VHPB, 1995). The onset of disease is usually insidious, with anorexia, vague abdominal discomfort, nausea and vomiting, fever and fatigue (Marcellin, 1999). of those exposed to HCV, about 40% recover fully, but the remainder, whether they have symptoms or not, become chronic carriers. of these, 20% develop cirrhosis. of those with cirrhosis, up to 20% develop liver cancer (EASL, 1999).

1.2.2 HCV Spread:

Hepatitis C virus is usually spread by sharing infected needles with a carrier, from receiving infected blood, and from accidental exposure to infected blood. Some people acquire the infection through nonparenteral means that have not been fully defined, but include sexual transmission in persons with high-risk behaviours, although transmission of HCV is less common than that of HBV and HIV (Alter, 1999). HCV is not spread by breast feeding, sneezing, coughing, hugging, sharing eating utensils or drinking glasses, other normal social contact, food or water (Mast, *et al*. 1999). Mother-to-baby transmission is now well documented, but uncommon. Needs a high viraemia (>1 log-) as found in HIV co-infection(EASL. 1999). A

person who has hepatitis C can still get other types of hepatitis, such as hepatitis A or hepatitis B (Mast, *et al*. 1999).

1.2.3 Ability of HCV to cause infection:

Susceptibility is general. Humans and chimpanzees are the only known species susceptible to infection, with both species developing similar disease (WHO, 1997).

1.2.4 Epidemiology of HCV:

HCV infections are common worldwide. It is estimated that about 3% of the world's population have HCV. There are about 4 million carriers in Europe alone (VHPB, 1995), and up until the introduction of anti-HCV screening tests for blood donors, introduced in 1990/1991 in Europe and the United States, it has represented the major cause of transfusion-associated hepatitis (Van, 1999). The incidence of HCV on a global scale is not well known, because acute infection is generally asymptomatic (WHO, 1997). Most European countries report a prevalence of HCV in the general population of between 0.5 and 2% (VHPB, 1995), WHO estimates that about 3% of the world's population has been infected with HCV and that there are more than 170 million chronic carriers who are at risk of developing

liver cirrhosis and/or liver cancer (WHO, 1999). Very high rates of HCV antibody reactivity (>70%) have been reported in injecting drug users and in haemophiliacs. Intermediate prevalence of 20 to 30% have been observed in patients receiving haemodialysis (VHPB, 1995).

1.2.5 Diagnosis of HCV:

Hepatitis C diagnosis depends on demonstration of anti-HCV detected by an EIA. Anti-HCV is generally not detectable in patients with initial signs or symptoms of hepatitis C. Anti-HCV develop in acute infection generally between 2 and 8 weeks after evidence of liver injury. Some persons may not test positive for 6-9 months after onset of illness. Hepatitis C viremia may be detected by RT-PCR within days after infection (Hsu and Greenberg, 1994). An EIA test for HCV core-antigen detection has been established and appears to be suitable for large scale screening of blood donations, whilst its use in clinical monitoring remains to be determined (Nubling, *et al*. 2002). The chronic hepatitis associated with HCV infection acts as a cofactor in increasing the severity of hepatic injury in patients with other chronic liver diseases (Lemon and Brown, 1995). Children should not

be tested for anti-HCV before 12 months of age as anti-HCV from the mother may last until this age. Diagnosis relies on determination of ALT levels and presence of HCV RNA in baby blood after the second month of life (Ruiz, *et al*. 1999).

Table (3) Diagnostic tests for hepatitis C (Ruiz, *et al*. 1999).

EIA result	Suggested action
anti-HCV positive	HCV infection in a patient with a positive EIA test should be confirmed by a qualitative HCV RNA assay. However, confirmation may be unnecessary in a patient who has evidence of liver disease and obvious risk factors for HCV. The immune blot assay is still useful as a supplemental assay for persons screened in nonclinical settings and in persons with a positive EIA who test negative for HCV RNA.
anti-HCV negative	A negative EIA test is sufficient to exclude a diagnosis of chronic HCV infection in immune competent patients, if the test is performed 26 within 4-6 weeks of infection. Rarely, patients on haemodialysis and patients with immune deficiencies may have false negative EIAs. In these patients, an assay for HCV RNA is necessary for diagnosis of chronic infection.

1.2.6 HCV Host Immune response:

The first marker of HCV infection is serum HCV RNA detectable by PCR as early as one week after infection and increasing to 106 – 108 genomes/ml (Marcellin, 1999), different antibodies appear in serum at different intervals from the time of initial inoculation. Anti-core antibodies directed to the nucleocapsid protein are generally the first to appear and can be detected by the time ALT is peaking , infected individuals generally develop antibodies reactive with the core (C) protein as well as several nonstructural protein antigens of HCV within days to weeks after onset of clinical symptoms. However, protective antibodies have not been identified yet (Lemon and Brown, 1995). CD8+ cytotoxic T lymphocytes have been found in the liver of chronically infected patients, suggesting that they are not always capable of eliminating the infection (Nakamoto, *et al*. 2003). HCV specific CD4+ T cells have been identified in the peripheral blood of chronically infected patients. However, HCV persists despite the induction of a broad humoral and cell-mediated immune response. One mechanism of HCV persistence occurs via the generation of immune-escape mutants (Houghton, 1996).

1.2.7 Serological markers of HCV infection:

Serological tests give an insight into the patient's immune response to HCV infection (Mast, *et al*. 1999). Since 1990, serological tests such as enzyme-linked immunoassays (EIAs / ELISAs) are used to screen blood donations and to diagnose HCV infection in symptomatic patients (Houghton, 1996). The EIA test is would be done first, if positive, it should be confirmed e.g. by the same assay but on a second, different sample and if necessary by HCV RNA. If the EIA test is negative or borderline positive, the patient is unlikely to be infected with HCV (Urdea, *et al*. 1997).

Table (4) Serological markers of HCV infection (Mast, *et al*. 1999)

Test/Type	Purpose	Comments
Anti-HCV (antibody) - EIA (enzyme immunoassay) - recombinant immune blot assay (e.g. RIBA™)	Verify if necessary positive EIA with HCV RNA detection Indicates past or present infection, but does not differentiate between acute, chronic or past infection.	Sensitivity >95% Detects anti-HCV in 80% of patients within 5-6 weeks of onset of hepatitis. Late seroconversion can occur. High false-positive rate for EIA in low prevalence populations and in those with auto immunedisorders.
HCV core antigen EIA	Detects presence or absence of virus. Detects virus 1-3 weeks after exposure. Under	Not licensed. Appears to be suitable for large scale screening of blood donations, its use in

· Trak-C	evaluation for the monitoring of patients on anti viral therapy	clinical monitoring remains to determined

1.3 Human immunodeficiency virus (HIV)

HIV is the abbreviation used for the Human Immunodeficiency Virus. HIV attacks the body's immune system. Normally, the immune system produces white blood cells and antibodies that attack viruses and bacteria. The infection fighting cells are called T-cell lymphocytes. Months to years after a person is infected with HIV, the virus destroys all the T-cell lymphocytes. This disables the immune system to defend the body against diseases and tumors. Various infections will be able to develop; these opportunistic infections take advantage of the body's weakened immune system. The infections that normally will not cause severe or fatal health problems will eventually cause the death of the HIV patient (Rombauts, 1997).

1.3.1 Causes the disease:

HIV disease is caused by infection with HIV-1 or HIV-2, both of which cause very similar conditions. They differ in transmission and progression risks, HIV type 1 and HIV type 2 are two distinct

viruses. Worldwide, the predominant virus is HIV-1, and generally, when people talk about HIV without specifying the type of virus they are referring to HIV-1.The relatively uncommon HIV-2 virus is concentrated in West Africa, but has been seen in other countries. It is less infectious and progresses slower than HIV-1. While commonly used antiretroviral drugs are active against HIV-2, optimum treatment is poorly understood (Campbell and Gandhi; 2011 and Ekouevi, *et al*. 2014).

1.3.2 HIV Spread:

The virus is spread (transmitted) person-to-person in any of the following ways: Through sexual contact, through blood by needle sharing, from mother to child, a pregnant woman can spread the virus to her fetus through their shared blood circulation, or a nursing mother can pass it to her baby through her breast milk. the virus is not spread by: casual contact, such as hugging, mosquitoes, participating in sports , touching items that were touched by a person infected with the virus (Moyer, 2013).

HIV is not spread to a person who donates blood or organs. People who donate organs are never in direct contact with the people who

receive them. Likewise, a person who donates blood is never in contact with the person receiving it. In all of these procedures, sterile needles and instruments are used. However, HIV can be spread to a person receiving blood or organs from an infected donor. To reduce this risk, blood banks and organ donor programs check (screen) donors, blood, and tissues thoroughly, only blood, semen, fluids from the vagina, and breast milk have been shown to transmit infection to others, the virus may also be found in saliva, tears, and spinal fluid (Moyer, 2013).

1.3.3 Ability of HIV to cause infection:

Anyone can contract HIV, and while injection drug users (IDUs) are at great risk because of practices related to their drug use, anyone who engages in unsafe sex (e.g., unprotected sex with an infected partner) could be exposed to HIV infection. However, while HIV affects all groups, some are more vulnerable than others, as summarized below, men who have sex with Men: Gay or bisexual MSM are the most severely affected population. MSM account for just a small fraction (2 percent) of the total U.S. population, yet nearly two-thirds of all new infections occurred within this group in 2009, and one-half of all people living with HIV in 2008 were MSM. MSM

within ethnic minority populations are at greatest risk (Wejner, *et al*. 2012).

Injection Drug Users: Injection drug uses (IDUs) has long been associated directly or indirectly with approximately one-third of AIDS cases in the United States. The fact that IDUs made up only 8 percent of new HIV infections in 2010 versus 23 percent in 1994–2000 demonstrates the progress made in HIV prevention and treatment within this population. Still, much work remains; while there may be fewer new infections among IDUs, in 2009, nearly one-half of those who were HIV+ were unaware they were infected (Wejner, *et al*. 2012).

1.3.4 Epidemiology of HIV:

HIV continues to be a major global public health issue. Since 2000, 38.1 million people have become infected with HIV and 25.3 million people have died of AIDS-related illnesses. In 2014, an estimated 36.9 million people were living with HIV (including 2.6 million children) – a global HIV prevalence of 0.8%. The vast majority of this number live in low- and middle- income countries. In the same year, 1.2 million people died of AIDS-related illnesses.25.8

million of people living with HIV are in sub-Saharan Africa, accounting for 70% of the global total. Only four (54%) of all people living with HIV know that they have the virus. In 2014, there were roughly 2 million new HIV infections, 220,000 of which were among children. Most of these children live in sub-Saharan Africa and were infected via their HIV-positive mothers during pregnancy, childbirth or breastfeeding (UNAIDS 2015).

1.3.5 Diagnosis of HIV:

Serological tests, such as enzyme immunoassays (EIAs), detect the presence or absence of antibodies to HIV-1/2 and/or HIV p24 antigen. When such tests are used within a testing strategy according to a validated testing algorithm, HIV infection can be detected with great accuracy. It is important to note that serological tests detect antibodies produced by an individual as part of their immune system to fight off foreign pathogens, rather than direct detection of HIV itself, most individuals develop antibodies to HIV-1/2 within 28 days and therefore antibodies may not be detectable early after infection, the so-called window period. This early period of infection represents the time of greatest infectivity; however, HIV transmission can occur during all stages of the infection. It is best practice to also retest all

people initially diagnosed as HIV-positive before they enroll in care and/or treatment to rule out any potential testing or reporting error (WHO, 2015).

1.3.6 HIV Host Immune Response:

After the virus enters the body, there is a period of rapid viral replication, leading to an abundance of virus in the peripheral blood. During primary infection, the level of HIV may reach several million-virus particles per milliliter of blood (Piatak, *et al*. 1993), this response is accompanied by a marked drop in the numbers of circulating $CD4^+$ T cells. This acute viremia is associated in virtually all people with the activation of $CD8^+$ T cells, which kill HIV-infected cells, and subsequently with antibody production, or seroconversion. The $CD8^+$ T cell response is thought to be important in controlling virus levels, which peak and then decline, as the $CD4^+$ T cell counts rebound. A good $CD8^+$ T cell response has been linked to slower disease progression and a better prognosis, though it does not eliminate the virus (Pantaleo, *et al*. 1997). During the acute phase, HIV-induced cell lysis and killing of infected cells by cytotoxic T cells accounts for $CD4^+$ T cell depletion,

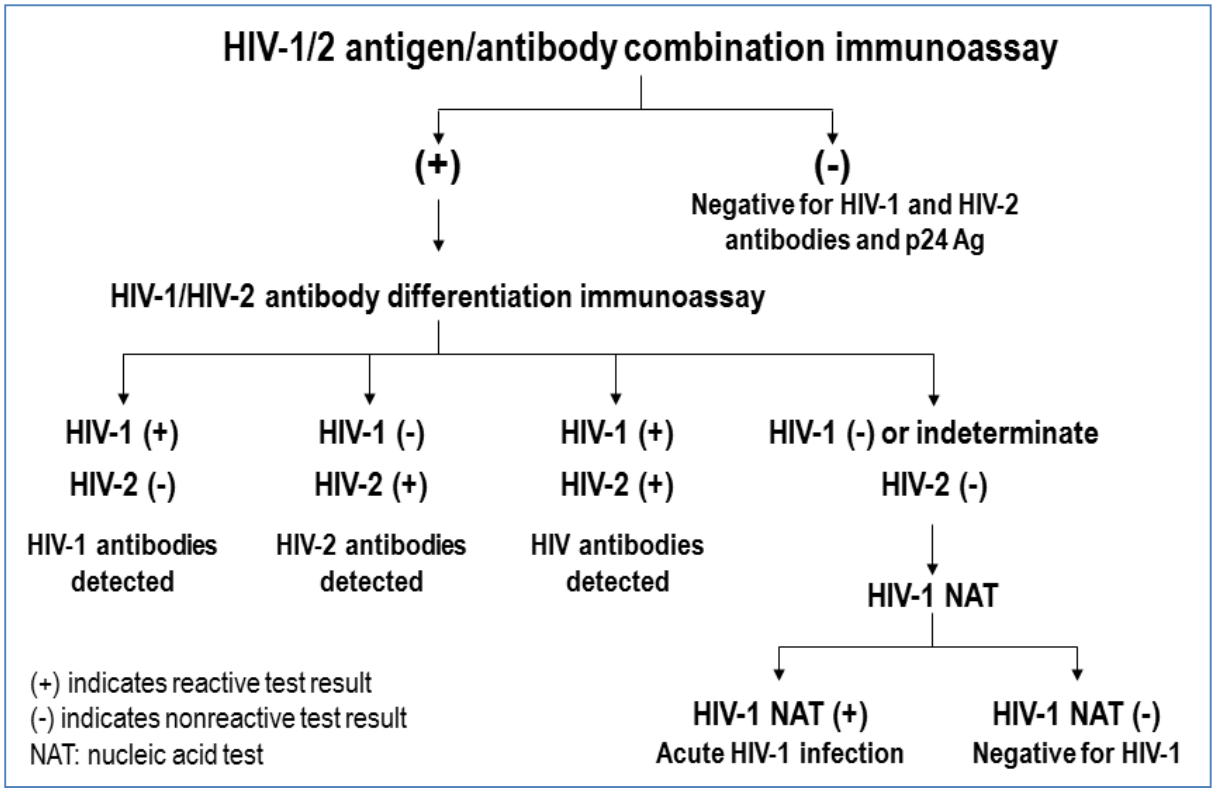
Although apoptosis may also be a factor during the chronic

phase, the consequences of generalized immune activation coupled with the gradual loss of the ability of the immune system to generate new T cells appear to account for the slow decline in $CD4^+$ T cell numbers, however, the symptoms of immune deficiency characteristic of AIDS do not appear for years after a person is infected, the bulk of $CD4^+$ T cell loss occurs during the first weeks of infection, especially in the intestinal mucosa, which harbors the majority of the lymphocytes found in the body (Mehandru, *et al*. 2004). The reason for the preferential loss of mucosal $CD4^+$ T cells is that a majority of mucosal $CD4^+$ T cells express the CCR5 co-receptor, whereas a small fraction of $CD4^+$ T cells in the bloodstream do so (Brenchley, *et al*. 2004). HIV seeks out and destroys CCR5 expressing $CD4^+$ cells during acute infection. A vigorous immune response eventually controls the infection and initiates the clinically latent phase. However, $CD4^+$ T cells in mucosal tissues remain depleted throughout the infection, although enough remain to initially ward off life-threatening infections. Continuous HIV replication results in a state of generalized immune activation persisting throughout the chronic phase (Appay and Sauce, 2008). Immune activation, which is reflected by the increased activation

state of immune cells and release of pro inflammatory cytokines, results from the activity of several HIV gene products and the immune response to ongoing HIV replication. Another cause is the breakdown of the immune surveillance system of the mucosal barrier caused by the depletion of mucosal $CD4^+$ T cells during the acute phase of disease (Brenchely, *et al*. 2006).

1.3.7 Serological markers of HIV:

Figure (1) Serological markers of HIV infection (Branson, et al. 2014)



1.4 The aim of this study:

This is study was aim to:

1. Detection and identification of Hepatitis B, C and HIV viruses among Libyan children .
2. Detection and identification of Hepatitis B, C and HIV viruses among adult Libyan nationals .
3. Detection and identification of Hepatitis B, C and HIV viruses among foreign workers at Benghazi city.
4. Detection and identification of Hepatitis B, C and HIV viruses among patients who have medical history .

CHAPTER 2 LITERATURE REVIEW

In a study has been carried out on the epidemiology of hepatitis B and C infection among a sample of patients referred to the Department of the Infectious and Endemic Diseases at Al Jamahiriya Hospital in Benghazi. of a total 3250 patients, 40% were randomly selected. of these, 51.2%were infected with hepatitis B virus, 46.9% with hepatitis C virus and 1.9% with hepatitis B and C. Younger patients and single patients were more prone to hepatitis B infection, while older patients, married patients and travellers were more prone to hepatitis C. About 20% of hepatitis B cases and 25% of hepatitis C cases were non-Libyans. Males were more affected (65%) than females (35%)(Kutrani, *et al*. 2007).

Another study on prevalence of HBV and HCV infection in the haemodialysis (HD) population of Libya as well as risk factors for infection. Participant median age was 49 years and 58% were male. About 831 patients (34.9%) were sero-positive for HBV and/or HCV (anti-HCV positive 31.1%; HBsAg positive 2.6%; both positive 1.2%). Of the sero-positive patients, 4.7% were known to be infected

before the initiation of HD. The prevalence of HBV, HCV infection varied widely between HD centres from 0% to 75.9%. Sero-positive patients were younger, had longer time on dialysis and more previous blood transfusions. Prospective follow-up revealed an incidence of sero-conversion of 7.7% during 1 year (7.1% HCV; 0.6% HBV). Wide variation in rates of newly acquired infections was observed between dialysis centers. All new HBV cases were referred from centers already treating HBV infected patients. New HCV infections were reported in most centers but the rate of HCV sero-conversion varied widely from 1.5% to 31%. Duration of dialysis, history of previous renal transplant and history of receiving HD in another centre in Libya were significantly associated with sero-conversion(Alashek, *et al*. 2012).

According to another study on Prevalence of hepatitis B and hepatitis C infection in Libya. The overall prevalence of hepatitis B was 2.2% (95% CI 2.1%−2.3%) and was higher among males than females (1.4:1.0). Hepatitis C virus (HCV) prevalence was 1.2% (95% CI 1.1−1.3) and it increased gradually after the age of 30 years (0.7−0.9% for < 30 years; 3.6% for ≥ 60 years). Prevalence of HBsAg was 0.8−0.9% below the age of 10 years, and higher but similar in

older age groups (2.3−2.7%). There was an association between literacy and prevalence of hepatitis, particularly for HCV. Hospital admission, surgical operation, blood transfusion, and intravenous drug use were the main risk factors, and they were associated independently with a higher prevalence rate of viral hepatitis (Daw, *et al*. 2014).

A study on hepatitis B and C virus infections and their related risk factors carried out in Libya: a national seroepidemiological survey Where reports indicated the existence of a high distribution of hepatitis B (HBV) and C virus (HCV) infections has been reported among specific patient groups in Libya; a survey was thus designed to determine the extent of the problem at the national level. A multistage sampling design covering all administrative areas of Libya was applied, covering > 65 000 individuals of all age groups. All subjects gave a blood sample and completed a questionnaire on demographic and risk behavior data. The spread of HBV surface antigen (HBsAg) and anti-HCV were 2.2% and 1.3% respectively. The infection with anti-HCV increased with age, rising gradually after age 30 years, in contrast to a stable spread of HBsAg in all age groups 10+ years. Age-

adjusted risk factors for HCV infection were previous hospitalization, surgical operations, previous blood transfusions and intravenous drug use; for HBV infection only family exposure or contact with HBV case were identified (Elzouki,*et al*. 2013).

Also another study reveal a high rate of hepatitis C virus in the normal Libyan population. The propagation of hepatitis C virus (HCV) in Libya has been investigated by seeking evidence of HCV infection in 266 healthy Libyan subjects (147 females, 119 males; age range 1–78 years), 76 of whom were registered blood donors. None had any history of blood transfusions, surgery, homosexuality, drug misuse or other risk factor for viral hepatitis. Sera from all subjects were tested for anti-HCV antibodies by enzyme-linked immune sorbent assay against synthetic structural and non-structural HCV peptides from the HCV core, envelope, NS1, NS3/NS4 and NS5 regions. Eighteen (6.8 %), all of whom were seronegative for hepatitis B surface antigen (HBsAg), were found to be anti-HCV positive (including five blood donors). The patterns of reactivity against the individual peptides varied between subjects as follows: core (14 subjects), envelope (11), NS1 (9), NS3/NS4 (10), and NS5 (6). Fourteen of the 18 had elevated serum aminotransferase activities but

so also did nine other subjects who were seronegative for both HBsAg and anti-HCV. Twelve of the 18 anti-HCV positive subjects, including three of the five anti-HCV positive blood donors, had circulating HCV RNA detected by the polymerase chain reaction. HCV RNA was also detected in three of the nine anti-HCV negative cases with elevated AST/ALT. The finding that 21 (7 · 9%) of the 266 subjects had evidence of HCV infection indicates that there is a very high frequency of 'community-acquired' HCV in the normal Libyan population, and this has major implications for blood transfusion in that country (Saleh,*et al*.1994).

In a study has been carried out on Maternal and neonatal seroprevalence of Hepatitis B surface antigen (HBsAg) in Tripoli, Libya, HBsAg was detected in 1.5% (23/1,500) pregnant women and in 0.9% (14/1,500) neonates. Although HBsAg was detected at higher rate in pregnant women aged > 25 years [1.8% (22/1,235)] than in pregnant women aged < 25 years [0.4% (1/265)], the difference was not statistically significant (P > 0.05). All HBsAg-positive neonates were born to HBsAg-positive mothers with a rate of maternal transmission at 60.9% (14/23). HBeAg was detected in 21.7% (5/23) and in 7.1% (1/14) of HBsAg-positive pregnant women and neonates,

respectively. Because of the high risk of developing chronic HBV infection at birth among infants born to HBsAg-positive mothers, administration of HBIG in combination with hepatitis B vaccine as post-exposure prophylaxis for such infants is of paramount importance. In addition, universal HBsAg screening of all pregnant women will greatly assist in reducing the maternal transmission of HBV in the country (El-Magrahe,*et al*. 2010).

Another study on nosocomial outbreak of multiple blood borne viral infections.In resource-limited countries, nosocomial transmission of blood borne pathogens is a major public health concern. After a major outbreak of human immunodeficiency virus (HIV) infection in 400 children in 1998 in Libya, they tested HIV, hepatitis C virus (HCV), and hepatitis B virus (HBV) markers in 148 children and collected epidemiological data in a subgroup of 37 children and 46 parents. HIV infection was detected in all children but one, with HCV or HBV coinfection in 47% and 33%, respectively. Vertical transmission was ruled out by analysis of parents' serology. The children visited the same hospital 1–6 times; at each visit, invasive procedures with potential blood transmission of virus were performed.

HIV and HCV genotypic analyses identified a HIV monophyletic group, whereas four clusters of HCV sequences were identified, this is the largest documented outbreak of nosocomial HIV transmission (Yerly,*et al*. 2001).

According to another study on Prevalence and risk factors of hepatitis B and C virus infections among the general population and blood donors in Morocco HCV and HBV-seropositivity was documented in 1.58% and 1.81% out of 41269 and 23578 participants respectively from the general population. Two patients were found to be co-infected. HCV-RNA was detected by PCR in 70.9% of the 195 anti-HCV positive subjects. The anti-HCV prevalence was not different among males and females ($P = 0.3$). It increased with age; the highest prevalence was observed among subjects with >50 years old (3.12%). Various risk factors for acquiring HCV infection were identified; age, dental treatment, use of glass syringes and surgical history. In addition to these factors, gender and sexual risk behaviors were found to be associated with higher prevalence of hepatitis B. The HBV positivity was significantly higher among males than females participants in all age groups ($P < 0.01$). The peak was noticed among

males aged 30–49 years (2.4%). None of the 152 persons younger than 20 years had HBsAg or anti-HCV. The prevalence of anti-HCV and HBsAg among 169605 blood donors was 0.62% and 0.96% respectively (Baha, *et al*.2013).

A study on detection of hepatitis C virus and human immunodeficiency virus in expatriates in Saudi Arabia by antigen-antibody combination assays the study group (N = 875) included expatriate workers of both sexes who were undergoing mandatory pre-employment testing. Detection of anti-HCV antibodies, HCV core antigen, HCV viral RNA, HIV antigens and antibodies was conducted using commercially available kits. Of the 875 samples that were screened for HCV-specific antibodies, four (0.46%) tested positive (two from Pakistan, one from India, and one from the Philippines) and two (0.23%) were equivocal (one from Egypt and one from Nepal). All four samples that were positive for HCV-specific antibodies also tested positive using HCV RNA assay and the HCV antigen-antibody combination assay. The two samples that were equivocal tested positive using the HCV RNA assay and the HCV antigen-antibody combination assay. Of the 875 samples that were tested for HIV

antibodies, only one (0.11%) sample gave repeatedly positive results. The same sample also tested repeatedly positive using the HIV combination assay. These results were subsequently confirmed by HIV western blot assay. A study was carried out in Saudi Arabia indicated that the addition of antigen detection to the screening of HCV and HIV may lower the risk of transmission of these viruses in the host country and contribute to the overall control of HCV and HIV (Alzahrani, *et al*. 2009).

HIV disease among immigrants coming to Italy from outside of the European Union where the study found the epidemiological, clinical and therapeutic features of HIV disease diagnosed in 41 immigrants from outside of the European Union (EU), were compared with those of 123 Italian and EU patients, in a cross-sectional case-control study, with individuals matched according to age and gender. In total 4.15% of our patients came from outside of the EU (51.2% of them from sub-Saharan in Africa), with a proportional predominance of females, and heterosexual and perinatal transmission of HIV disease ($P< 0.0001$ and $P< 0.02$, respectively). Compared with Italian and EU subjects, patients coming from abroad had a shorter duration

of known HIV infection ($P< 0.001$), but only some subjects were aware of their HIV disease prior to immigration, or acquired HIV infection only after coming to Italy (14.6% and 12.2%, respectively). No cases of HIV-2 infection or co-infection were detected in either study group. Compared with controls, patients coming from outside of the EU had a comparable clinical and immunological status, and had similar antiretroviral therapy, which was administered earlier ($P< 0.0001$), and proved better tolerated ($P< 0.04$), than in Italian and EU subjects. The apparently more limited virological response (as expressed by a higher mean plasma viral load, and a lower rate of viral suppression at the last visit (Manfredi, *et al*. 2001).

Astudy on epidemiology and risk factors in a large cohort of pregnant women in lorestan, west of Iran Anti-HBc was found in 28 of 827 pregnant women (overall prevalence, 3.4%; 14 of 523 in urban areas, 2.7%; 14 of 304 in rural areas, 4.6%). of the 28 positive samples, 6 (0.7%) were positive for HBs-Ag. Only 2 samples (0.2%) were anti-HCV-positive. These results underscore the need for prenatal screening for HBV infection in pregnant women and

treatment of newborns from HBsAg-positive mothers (Mohebbi *et al*. 2011).

Another paper studied seroprevalence of and risk factors associated with Hepatitis B, Hepatitis C, and human immunodeficiency virus among prisoners in Iran 1431 prisoners were enrolled, they settled in four prisons in three provinces in Iran, and all of them were men. Imprisonment duration was between one and 10 years in these prisons, and prisoners were aged 25 to 60 years. From 1431 studied prisoners, 1153 persons (80%) had a history of addiction to a narcotic drug, and 401 (28%) of them were the intravenous drug abusers (IVDAs). From 401 IVDAs aforementioned, 236 persons (58.8% of IVDAs and 16.5% of total prisoners) were share needle users. On the other hand, from 1431 studied prisoners, 399 persons (27.8%) had a history of illegal sex, and 113 of them (28.3% of illegal sex users and 7.8% of total) had a history of homosexuality (the meaning of illegal sexual contact was homosexuality and/or sex with another other than wife or husband). In this study, 46 prisoners (3%) were HBsAg positive, 497 prisoners (34.7%) were HCV Ab positive,

92 prisoners (6.4%) were HIV Ab positive, and 8 prisoners (0.5%) had triple infections (Morteza, *et al*. 2007).

In a study has been carried out on prevalence of hepatitis-B surface antigen among blood donors and human immunodeficiency virus-infected patients in Jos, Nigeria. Hepatitis-B surface antigen (HBsAg) ELISA was used to determine the prevalence of HBsAg among 175 blood donors (aged 20-40 years) and 490 HIV-infected patients (aged 17-60 years) in Jos, Nigeria , twenty-five (14.3%) of the blood donors and 127 (25.9%) of the HIV infected individuals were HBsAg seropositive, indicating a higher HBV infection among HIV-infected persons than among healthy blood donors. A slightly higher HBsAg seroprevalence was recorded in the males (14.6%) than females (12.9%) of the blood donors. Among the HIV-infected patients, the males had considerably higher HBsAg seroprevalence than the females (31.8 vs 22.1%) with the highest prevalence of HBsAg occurring in the 51-60 years age group (44%), followed by those of 31-40 years (28.2%). Results confirmed the high endemicity of HBV infection in Jos, Nigeria and the significantly greater

prevalence of HBV infection among HIV -infected patients than among blood donors (Uneke*et al*. 2005).

Hepatitis B, hepatitis C, and HIV studies carried out on correctional populations, the results showed that 2 million persons incarcerated in US prisons and jails are disproportionately affected by hepatitis B virus (HBV), hepatitis C virus (HCV) and HIV, with prevalence of infection two to ten times higher than in the general population. Infections are largely due to sex- and drug-related risk behaviors practiced outside the correctional setting, although transmission of these infections has also been documented inside jails and prisons. Public health strategies to prevent morbidity and mortality from these infections should include hepatitis B vaccination, HCV and HIV testing and counseling, medical management of infected persons, and substance abuse treatment in incarcerated populations (Cindy, *et al*. 2005).

There is a study on incidence of HIV, hepatitis B virus, and hepatitis C virus infections among males in Rhode Island prisons. HIV, hepatitis B virus, and hepatitis C virus prevalence were 1.8%, 20.2%, and 23.1%, respectively. Infections were significantly

associated with injection drug use (odds ratio=10.1, 7.9, and 32.4). Incidence per 100 person-years was 0 for HIV, 2.7 for HBV, and 0.4 for HCV. Conclusions. High infection prevalence among inmates represents a significant community health issue. General disease prevention efforts must include prevention within correctional facilities. The high-observed intraprison incidence of HBV underscores the need to vaccinate prison populations (Grace. *et al*. 2004).

In a study carried out on HIV, Hepatitis B, and Hepatitis C in people with severe mental illness. The prevalence of HIV infection in this sample (3.1%) was approximately 8 times the estimated US population rate but lower than rates reported in previous studies of people with severe mental illness. Prevalence rates of HBV (23.4%) and HCV (19.6%) were approximately 5 and 11 times the overall estimated population rates for these infections, respectively (Stanley, *et al*. 2001).

In addition, a study on prevalence of antibodies to hepatitis B, hepatitis C, and HIV and risk factors in Irish prisoners. Prevalence of antibodies to hepatitis B core antigen was 104/1193 (8.7%; 95%

confidence interval 7.2% to 10.5%), to hepatitis C virus, 442/1193 (37%; 34.3% to 39.9%), and to HIV, 24/1193 (2%; 1.3% to 3%). The most important predictor of being positive for hepatitis B and hepatitis C was a history of injecting drug use. A fifth (104) of 501 injecting drug users reported first injecting in prison and 347 (71%) users reported sharing needles in prison (Allwright, *et al*. 2000).

As it illustrated another study prevalence of HIV, hepatitis B, and hepatitis C antibodies in prisoners in England and Wales. Prisoners in eight of the 135 prisons in England and Wales were surveyed in 1997 and 1998 to study the prevalence of and risk factors for transmission of blood borne viruses in prison. Subjects voluntarily completed a risk factor questionnaire and provided oral fluid specimens for unlinked a nonymous testing for the presence of antibodies to HIV, hepatitis C virus (HCV), and the core antigen of hepatitis B virus (HBC). Almost 8% (4778) of the 60561 prisoners were eligible and four-fifths (3942) of those, eligible took part. Among all those tested (3930), 0.4% (14) were positive for anti-HIV, 8% (308) for anti-HBc, and 7% (293) for anti-HCV (anti-HBc and anti-HCV prevalence were not adjusted for assay sensitivities of 82% and 80%, respectively). 24% (777/3176) of adult prisoners reported

ever having injected drugs, 30% of whom (224/747) reported having injected in prison. Three quarters of those who injected in prison (167/224) shared needles or syringes. Among adult injecting drug users, 0.5% (4/775) had anti- HIV, 31% (240/775) anti-HCV, and 20% (158/775) anti-HBc. The presence of anti-HCV and anti-HBc was associated with injecting inside prison and number of previous times in prison. The results suggest that hepatitis viruses are probably being transmitted in prisons through sharing non-sterile injecting equipment and that a risk of HIV transmission exists. Harm minimization measures for the 6% of prisoners who continue to inject while in prison should be strengthened. (Weild, *et al*. 2000).

Another paper studied Hepatitis B and hepatitis C prevalence among blood donors and HIV-1 infected patients in Florianopolis—Brazil. Information is scarce on the prevalence of hepatitis B (HBV) and hepatitis C (HCV) among voluntary blood donors and patients infected with the human immunodeficiency virus (HIV) in Florianópolis, Brazil. A total of 2,678 serum samples from 2,583 blood donors and 95 HIV-infected patients, collected between April, 1994, and March, 1995, were examined for markers of HBV and HCV. All the samples were analyzed to detect HBV and HCV

markers (HBsAg, anti-HBc, and anti-HCV). Hepatitis B and C prevalence among the studied blood donors reached 9.3% and 1.0%, respectively; 0.7% being seropositive for HBsAg and 9.2% for anti-HBc. It was also verified that 0.1% of blood donors were seropositive for HBsAg alone, 8.6% seropositive for the anti-HBc alone, and 0.6% presented a positive reaction for both of the HBV markers studied. Among HIV-infected patients, prevalence of 69.5% and 54.7% for hepatitis B and hepatitis C, respectively, were observed. of these patients, 18.9% were seropositive for HBsAg, and 66.3% for the anti-HBc. The prevalence of a reaction for HBsAg alone, and for anti-HBc alone was 3.1% and 50.5%, respectively, for HIV-infected patients, whereas 15.8% were seropositive for both of the studied markers. HBV and HCVconfection was 0.1% in blood donors, and 40% of those patients tested seropositive for HIV. Results show prevalence of HBV and HCVinfection to be significantly greater among HIV-infected patients than among blood donors. These observations confirm the high frequency of HIV-infected patient's exposure to these other viruses (Treitinger,*et al*.2000).

Astudy on seroprevalence of hepatitis B, C, and HIV in Malawian pregnant women evidence of HBV and HCV infection

found in 71.7 and 16.5% of women, respectively. Chronic carriage of HBV (HBsAg positive) is high (13%) and in agreement with prevalence reported from highly endemic areas. Exposure to HBV and HCV probably occurred well before adulthood as the prevalence of anti-HBc antibody was high in young mothers <20 years of age (22/27; 81%) (Ahmed *et al*. 1998).

CHAPTER 3 MATERIALS AND METHODS

This study was designed to identify the prevalence of hepatitis viruses B, C and HIV in Benghazi city. This study focused on three human resources including adult Libyan nationals, Libyan children and foreigner workers. Also as a selective sources representing the majority of serology referencing including four medical centre are a private laboratory is Al Razi medical centre and three governmental medical centres including Benghazi centre of infectious diseases, Children hospital, and the medical Laboratory of Red crescent centre. Benghazi, Libya. People who are chosen for virology analysis were selected based on their medical history as well as by a referral from a medical consultation to the serology department. In addition to that, all cases of blood drawing were applied as a response to the desire of the patients, not a voluntary programme, nor a study to a health Libyan organizations. Libyan adult`s samples were tested in Al Razi medical centre, all children´s blood samples were collected and analyzed in children hospital, people who have medical history of the disease were tested in the Benghazi medical centre of infectious diseases, and also, blood samples collection, virology investigation of

the foreigner worker were carried out in the medical centre of Benghazi Red crescent.

3.1 Samples collection and preparations:

All blood samples were collected in accordance with a standard medical techniques. In order to obtain blood serum, all whole blood samples were collected in a covered clean test tubes, the blood samples were allowed to clot by leaving them undisturbed at room temperature for 15-30 minutes, tubes were then centrifuged (5000 rpm for 10 minutes), the resulting supernatant is serum.

Following centrifugation, immediately serum components were transferred in to a clean polypropylene tubes using a Pasteur pipettes, serum samples were maintained at 2-8 C˚ prior analyzing. If serum is not analyzedimmediately, serum should be stored at -20 C˚ .It is important to avoid serum components destructive by not use freeze-thaw cycles if so mix thawed samples throughly by low speed overtaxing 10 times, visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.

3.2 Identification Method:

3.2.1Enzyme Linked Immuno Sorbent Assay (ELISA test)

ELISA is a biochemical assay that uses antibodies and an enzyme –mediated colour change to detect the presence of either antigen (proteins, peptides, hormones, etc.) or antibody in a given sample. Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene microtiter plate) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody that is linked to an enzyme through bioconjugation. Between each step, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are non-specifically bound. After the final wash step, the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample 3EFRV

3.2.2 Polymerase Chain Reaction (PCR test)

Materials and methods has been carried out by Benghazi medical center for infectious disease laboratory .

3.3 Storage Conditions:

Most of serum samples were examined for viral infection directly after samples collection, for short time storage less than 8 hours, serum samples were stored between 18-25 C°.For longer time storage up to 48 hours, samples were stored at 2-8 C°. For long time storage, more than 48 hours, serum samples were stored at – 20 C°.

3.4 Reagents and chemicals used in this study:

3.4.1 Reagents Anti-HBV (Anti- HBsAg) consumed in this study :

see index (1)

3.4.2 The Reagents of Anti-HCV consumed in this study :

see index (2)

3.4.3 Reagents of Anti HIV consumed in this study :

see index (3)

3.5 Methods:

Test method according to the instructions of Kits:

3.5.1 Anti- HBV (HBsAg):

- Used only the number of wells required and formatted the micro plates wells for each control and sample to be assayed .

  Well A1 was left as the blank well. 50µl of the negative control was added to each plate, to wells B1, C1 and D1 and 50 µl of positive controls to wells E1 and F1 . Then added 50 µl of sample to each of the rest of the wells .
- 50 µl of enzyme conjugate was added to each well except the blank well .
- The plate was covered with a lid and incubated at 37C̊ for 30 minutes .
- 350 µl of wash solution was added, decanted (tap and blot) or aspirated . Repeated 5 additional times for a total of 6 washes. An automated micro plate strip washer can be used. At the end of washing ,the plate was inverted and tapped out any residual wash solution onto absorbent paper .

- 50 µl of substrate A was added , then 50 µl of substrate B to each well, including the blank well .
- It was mixed gently for 15 seconds and incubated at 37C° in the dark for 10 minutes without shaking .
- 50 µl of stop solution was added to each well, including the blank well and mixed gently .
- The absorbance was read within 20 minutes at 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfections) in a micro plate reader . Alternatively, the actual absorbance can be obtained by subtracting the absorbance of each well at 450 nm with the absorbance of the blank well at 450 nm .

- Pretended product:

Nonreactive: Samples giving an absorbance less than the cut-off value are considered nonreactive.

Reactive : Samples giving an absorbance equal to or greater than the cut-off value are considered reactive such samples should be retested in duplicate using the original sample source. Samples that are reactive in at least one of the re-tests are presumed to contain HBsAg and should be confirmed by using a confirmatory kit and tests for

other HBV markers. Samples that are non-reactive in both wells on retest should be considered non-reactive .

Figure (2) HBsAg ELISA version 1 kit

3.5.2 Anti-HCV:

- Used only the number of wells required and formatted the micro plates wells for each control and sample to be assayed.

  Well A1 was left as the blank, to each plate 100 µl of the negative controls was add to wells B1, C1 and D1 and 100 µl of positive controls to wells E1 and F1 was added too. 100 µl of sample diluents was added to each of the rest of the wells, Then 10 µl of sample, added to the wells containing sample diluents.
- To obtain well mixed liquid within the plates were placed a shaker at room temperature for 30 sec.
- Plates, then covered with a lid and incubated at 37C° for 30 minutes.
- 350 µl of wash solution added, six washes were carried out on the samples. At the end of washing, all plates were inverted and tappedout on to absorbent paper to remove any residual wash solution.
- 100 µl of enzyme conjugate was added to each well except the blank well.
- The plate was covered with a lid and incubated at 37C° for 30 minutes.
- 350 µl of wash solution was added, decanted (tap and blot) or aspirated. Repeated 5 additional times for a total of 6 washer. An

automated micro plate strip washer can be used. At the end of washing, the plate was inverted and tapped out any residual wash solution onto absorbent paper.

- 50µl of substrate A was added, then 50µl of substrate B to each well including the blank well.
- It was mixed gently for 15 seconds and incubated at 37C° in the dark for 10 minutes without shaking.
- 50µl of stop solution was added to each well, including the blank well.
- It was mixed gently for 15 seconds. It is very important to make sure that the blue color changes to yellow completely.
- The absorbance was read within 20 minutes at 450 nm (using a reference wavelength of 620-630 nm minimize well imperfections) in a micro plate reader. Alternatively, the actual absorbance can be obtained by subtracting the absorbance of each well at 450 nm with the absorbance of the blank well at 450 nm.

Pretended Product:

- Nonreactive: Samples giving an absorbance less than the cut-off value are considered nonreactive.

- Reactive: Samples giving an absorbance equal to or greater than the cut-off value are considered initially reactive, which indicates that antibodies to hepatitis C virus have probably been detected using this ELISA kit. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive for antibodies to HCV and therefore the patient is probably infected with hepatitis C virus. Blood unit positive for HCV antibodies should be immediately discarded.

Figure (3) Anti-HCV ELISA version 1 kit

3.5.3 Anti-HIV:

- Reagents Preparation: The reagents and samples were allowed to equilibrate at room temperature (18-30 C°) for at least 15-30 minutes. The wash buffer concentrate was checked for the presence of salt crystals. If crystals have formed, it could be solubilized by warming at 37 ± 1 C° until crystals dissolve. The wash buffer was diluted 1 to 20 with distilled or deionized water. Only clean vessels can be used to dilute the buffer.
- Numbering wells : The strips needed were set in the strip-holder and numbered sufficient number of wells including three for the negative controls (e.g. B1,C1,D1), two for the positive controls (one for HIV-1 and one for HIV-2, e.g. E1,F1) and one blank (e.g. A1, neither samples nor HRP-conjugate should be added into the blank well). If the results were determined by using a dual wavelength plate reader, the requirement for use of blank well could be omitted .Only sufficient number of strips required for the test were used.
- Adding Samples: 100µ of positive controls, Negative controls, and specimens were added into their respective wells. (Note: A separate disposable pipette tip was used for each specimen, Negative and Positive Control as to avoid cross-contamination).

- Incubating (1): The plate was covered with the plate cover and incubated for 30 minutes at 37 ± 1C°. It was recommended to use a thermostat-controlled water tank to assure the temperature stability and humidity during the incubation .If a dry incubator was used, do not open the door frequently.
- Washing (1): At the end of the incubation, the plate cover was removed and discarded. Each well was washed 5 times with diluted wash buffer. Each time, the micro wells were allowed to soak for 30-60 seconds. After the final washing cycle, the plate was turned down onto blotting paper or a clean towel, and tapped as to remove any residues.
- Adding HRP-Conjugate: 100µl HRP-conjugate was added into each well except the blank.
- Incubating (2): The plate was covered with the plate cover and incubated for 30 minutes at 37 ± 1C°.
- Washing (2): After the end of the incubation, the plate cover was removed and discarded. Each well was washed 5 times with diluted wash buffer as in step 5.
- Coloring: 50 µl of chromogen A and 50 µl chromogen B solutions were dispensed into each well including the blank (Note: Chromogen

A must be added before Chromogen B), the plate as covered with plate cover and mixed by tapping the plate gently. The plate was incubated at 37 ± 1C° for 15 minutes avoiding light. The enzymatic reaction between the chromogen solution and the HRP-conjugate produced blue colour in positive control and HIV ½ Ab positive sample wells.

- Stopping Reaction: The plate cover was removed and discarded. Using a multichannel pipette or manually, 50 µl stop solution was added into each well and mixed gently. Intensive yellow color appeared in positive control and HIV ½ Ab positive sample wells.
- Measuring the absorbance: The plate reader was calibrated with the blank well and the absorbance was read at 450 nm. A dual filter instrument was used, the reference wavelength was set at 600~ 650 nm. The Cut-off value was calculated and the results were evaluated. (Note: The absorbance was read within 10 minutes after stopping the reaction).

Pretended product:

Negative Results: Samples giving an absorbance less than the cut-off value are negative for this assay , which indicates that no HIV 1+2 antibodies have been detected with this kit, therefore the patient is

probably not infected or the blood unit does not contain antibodies of HIV 1+2 and could be transfused. Negative test result does not exclude the possibility of exposure to or infection.

Positive Results: Samples giving an absorbance equal to or greater than the cut-off value are considered initially reactive, which indicates that HIV 1/2 antibodies have probably been detected using this kit. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive for antibodies to HIV 1/2 and therefore the patient is probably infected with HIV 1/2. Any blood unit containing antibodies to HIV 1/2 should be immediately discarded.

Figure (4) Anti-HIV ELISA kit

3.6 Statistical methods used in the study :

This study is based on descriptive statistics in data analysis, inferential statistics, and then dump the data, and processing for statistical analysis using the SPSS-version 21 program and called statistical package for social sciences which is an abbreviation for the word Statistical Package For Sociality Science. Here are some of statistical methods**:**

- The use of descriptive statistics, and frequencies, percentage and represented by some graphs**.**
- inferential statistics were used to test whether character frequencies are different from expected values. It is best used when you expect numbers in different groups to be in particular ratio. The test calculates the chi-squares statistic**.**

CHAPTER 4 THE RESULT

4.1 Distribution of HBV among patients according to the age and gender:

This analysis was including the collection of samples from 4 sources. 800 was the total of the random samples. The distribution of included for 0-13, 14-45 and 46-80 years. In age 0-13 total of samples male and female was 225, 3 male and 2 female have shown infection with HBV with total percentage (2.2%). In age of 14-45, total of sample was 496, 21 male and 21 female are infected with HBV, total percentage (8.5%). In age of 46-80, total of samples was 79, the number of patients are infected with HBV are 2 in male and 2 in female with total percentage (5%) respectively. People with age 14-45 showed the highest percentage of infection with HBV (8.5%), P-value = 0.005, Table (5) and figure (5).

Table: (5) Distribution of HBV among patients according to the age and gender

Age	0-13				14-45				46-80			
Gender	Male		Female		Male		Female		Male		Female	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
-ve	121	97.6	99	98.01	253	92.3	201	90.5	41	95.3	34	94.4
+ve	3	2.4	2	1.98	21	7.7	21	9.5	2	4.7	2	5.6
Total	124	100	101	100	274	100	222	100	43	100	36	100

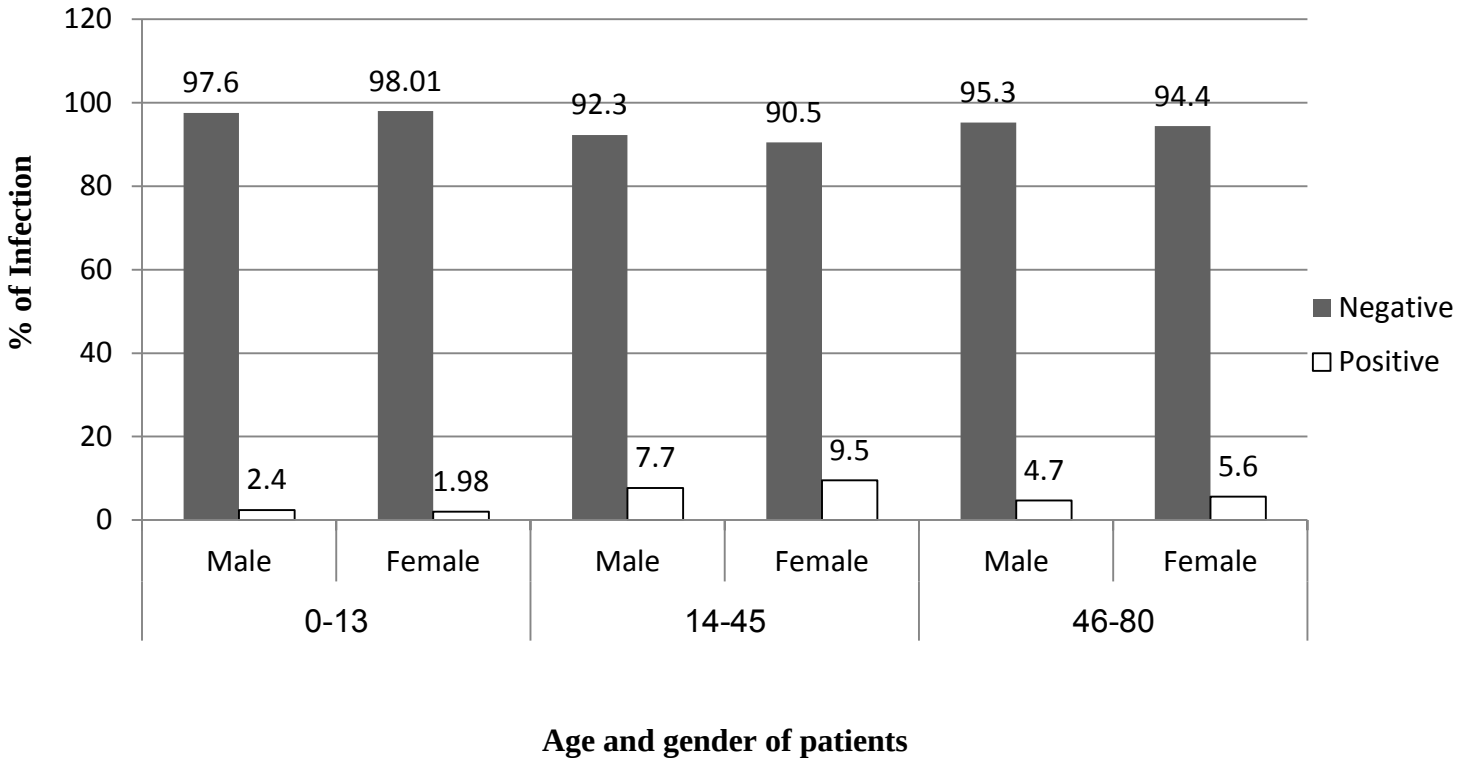


Figure: (5) Distribution of HBV among patients according to the age and gender

4.2 Distribution of HBV infection according to source of samples and age:

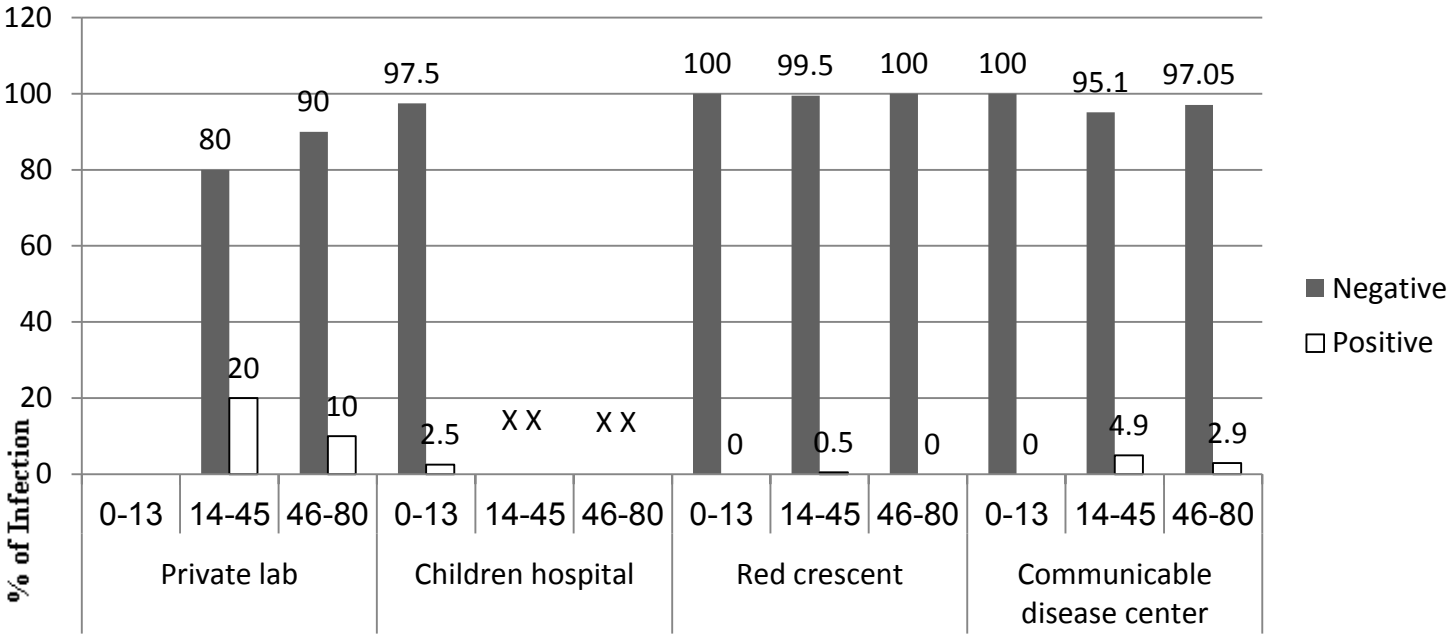
800 is the total of samples from 4 different sources, 200 sample each was included in this section. In the private laboratory, age of 0-13 no patients have attended to the laboratory driving this study, in age 14-45, 170 are representing the total samples and the result showed that 34 patients have infect with HBV (20%), in age 46-80, 30 are the total of samples, with the recorded of infection is 3 patients (10%). In children hospital only age of 0-13 showed patients participated to the hospital, 5 patients infected with the virus (2.5%).

The results obtained from Red Crescent medical laboratory, showed that age 14-45, 183 are total of patients, 1 (0.5%) patient has infected with the virus, non-in the other age.

Results of Benghazi medical centre of infectious diseases revealed in age 14-45 the total samples are 143 where 7 (4.9%) patients are infected with virus, in age 46-80, 34 patients have attended to the center 1 patient (2.9%) was recorded with infection, non-results in age 0-13. private laboratory showed the highest rates to infection for all ages where the ratio of HBV 18.5%, P-value = 0.000, Table (6) and figure (6).

Table: (6) Distribution ofHBV infection according to source of samples and age

Source	Private lab						Children hospital						Red Crescent (foreign workers)						Infectious dis centre			
Age	0-13		14-45		46-80		0-13		14-45		46-80		0-13		14-45		46-80		0-13		14-45	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
-ve	0	0	136	80	27	90	195	97.5	0	0	0	0	2	100	182	99.5	15	100	23	100	136	95.1
+ve	0	0	34	20	3	10	5	2.5	0	0	0	0	0	0	1	0.5	0	0	0	0	7	4.9
Total	0	0	170	100	30	100	200	100	0	0	0	0	2	100	183	100	15	100	23	100	143	100



Source of samples and age

X . No cases provided

Figure: (6) Distribution of HBV infection according to the source of samples and age

4.3 Distribution of HBV infection according to source of samples and gender :

In private laboratory total of samples male and female was 200 where 19 male and 18 female have shown infection with HBV with total percentage (18.5%). In children hospital total of samples was 200, 3 male and 2 female are infected with HBV with total percentage (2.5%). In red crescent laboratory total of samples was 200, only 1 person (male) is infected with HBV with total percentage (0.5%), in Benghazi medical centre of infectious diseases total of samples was 200, the number of patients are infected with HBV are 3 in male and 5 in female with total percentage (4%). Although the rate of infection in all sources with exception Benghazi medical centre of infectious diseases was highest in male than female , however the table ratio of infection with HBV was more in female than in all sources, with total percentage of female 7%, p-value = 0.562 while in male 5.9%. Table (7) and figure (7).

Table (7) Distribution of HBV infection according to source of samples and gender

Source	Private lab				Children hospital				Red Crescent (foreign workers)				Infectious disease centre			
Gender	Male		Female		Male		Female		Male		Female		Male		Female	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
-ve	64	77.1	99	84.6	105	97.2	90	97.8	158	99.4	41	100	89	96.7	103	95.4
+ve	19	22.9	18	15.4	3	2.8	2	2.2	1	0.62	0	0	3	3.3	5	4.6
Total	83	100	117	100	108	100	92	100	159	100	41	100	92	100	108	100

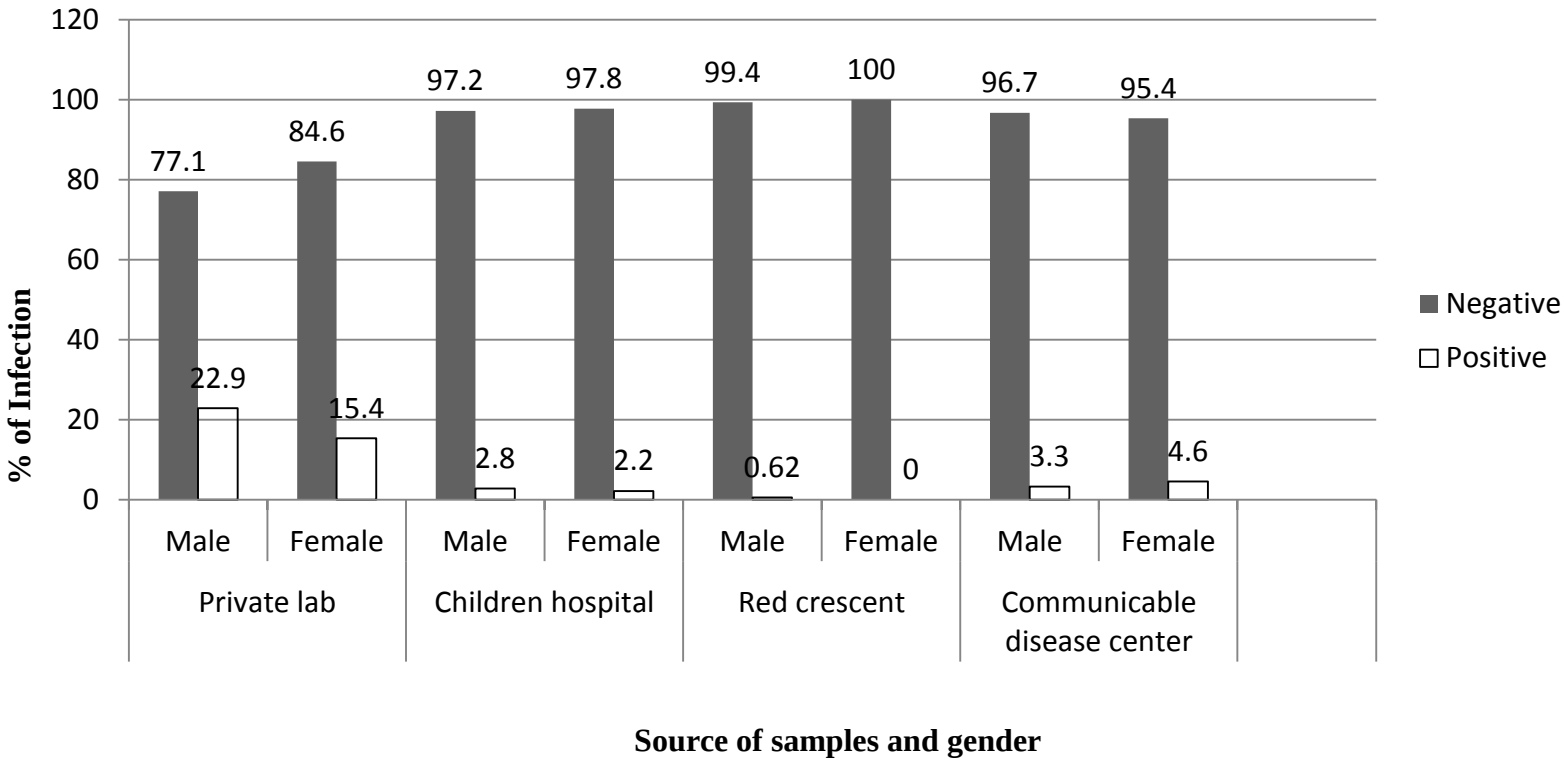


Figure: (7) Distribution of HBV infection according to source of samples and gender

4.4 Distribution of HCV among patients according to the age and gender :

In age 0-13 total of samples male and female was 225, 2 male and 5 female have shown infection with HCV with total percentage (3.1%). In age of 14-45 total of samples was 496, 11 male and 8 female are infected with HCV with total percentage (3.8%). in age of 45-80 years total of samples was 79, the number of patients are infected with HCV are 5 in male and 9 in female with total percentage (17.7%). People with age 46-80 years showed the highest percentage of infection with HCV virus (17.7%), P-value = 0.000. Table (8) and figure (8).

Table: (8) Distribution of HCV among patients according to the age and gender

Age	0-13				14-45				46-80			
Gender	Male		Female		Male		Female		Male		Female	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
-ve	122	98.4	96	95	264	96	213	96.4	38	88.4	27	75
+ve	2	1.6	5	5	11	4	8	3.6	5	11.6	9	25
Total	124	100	101	100	275	100	221	100	43	100	36	100

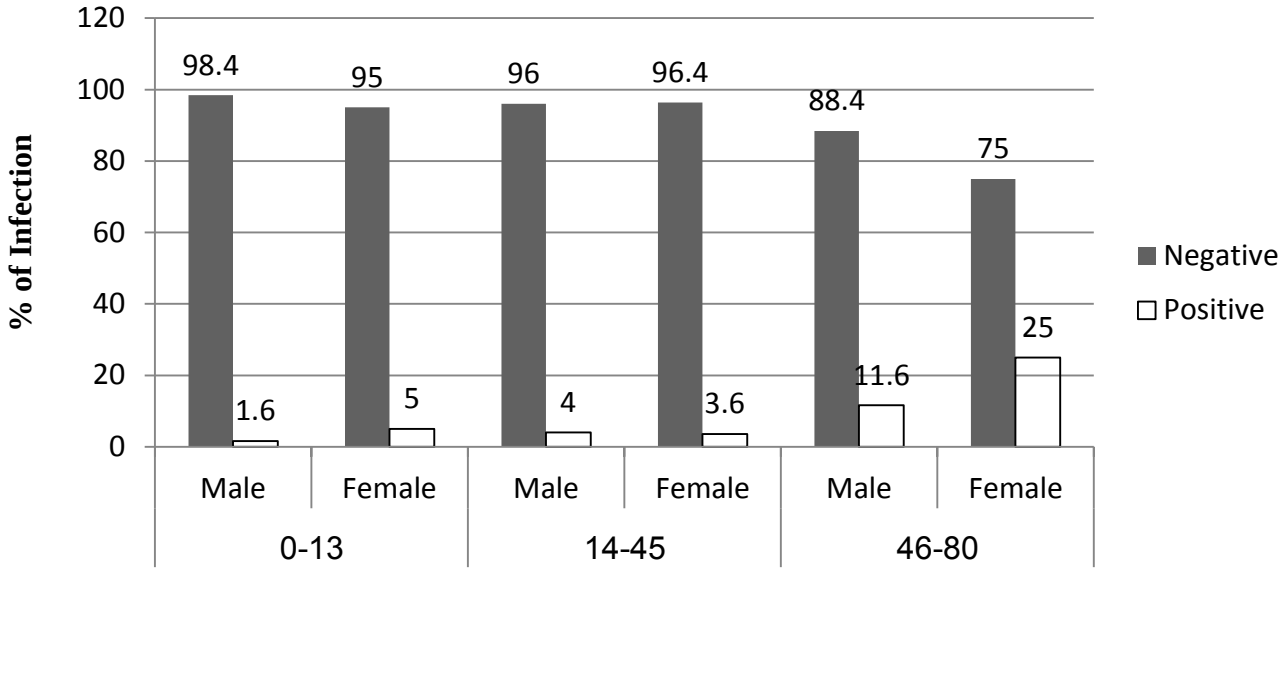


Figure: (8) Distribution of HCV among patients according to the Age and gender

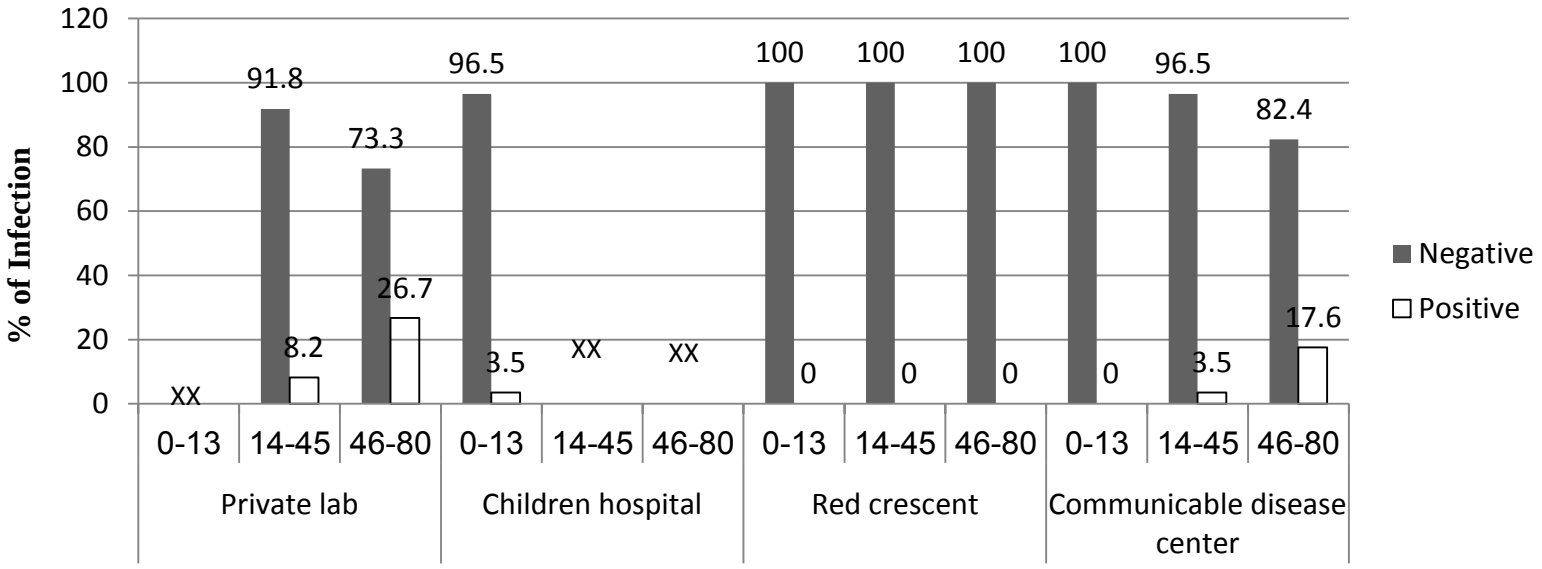
4.5 Distribution of HCV infection according to source of samples and age:

In the private laboratory, age of 0-13 no patients have attended to the laboratory driving this study, in age 14-45, 170 are representing the total samples and the result showed that 14 patients have infect with HCV (8.2%). In age 46-80, 30 are the total of samples, with the recorded of infection is 8 patients (26.7%). In children hospital only age of 0-13 showed patients participated to the hospital, 7 patients infected with the virus (3.5%). Red Crescent medical laboratory it did not recorded any infection with HCV virus for all ages.

The results obtained from Benghazi medical centre of infectious diseases , showed that age 14-45 the total samples are 143 where 5 (3.5%) patients are infected with virus, in age 46-80 years 34 patients have attended to the center 6 (17.6%) was recorded with infection, non-results in age 0-13. Private laboratory showed the highest rates to infection for all ages where the ratio of HCV 11%, P-value = 0.000. Table (9) and figure (9).

Table: (9) Distribution of HCV infection according to source of samples and age

Source	Private lab						Children hospital						Red Crescent (foreign workers)						Infectious dis centre			
Age	0-13		14-45		46-80		0-13		14-45		46-80		0-13		14-45		46-80		0-13		14-45	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
-ve	0	0	156	91.8	22	73.3	193	96.5	0	0	0	0	2	100	183	100	15	100	23	100	138	96.5
+ve	0	0	14	8.2	8	26.7	7	3.5	0	0	0	0	0	0	o	0	0	0	0	0	5	3.5
Total	0	0	170	100	30	100	200	100	0	0	0	0	2	100	183	100	15	100	23	100	143	100



Source of samples and Age

X. NO cases provided

Figure: (9) Distribution of HCV infection according to the source of samples and age

4.6 Distribution of HCV infection according to source of samples and gender :

In private laboratory total of samples male and female was 200 where 11 male and 11 female have shown infection with HCV with total percentage (11%). In children hospital total of samples was 200, 2 male and 5 female are infected with HCV (3.5%). In Red Crescent laboratory from total of samples did not recorded any infection with HCV virus. In Benghazi medical centre of infectious diseases total of samples was 200, the number of patients are infected with HCV virus are 5 male and 6 female with total percentage (5.5%). The rate of infection with HCV virus was more in female than male in all sources. The total percentage of female infected with HCV in all sources was 6.1%, p-value = 0.195. While was 4.1% in male. Table (10) and figure (10).

Table: (10) Distribution of HCV infection according to source of samples and gender

Source	Private lab				Children hospital				Red Crescent (foreign workers)				Infectious disease centre			
Gender	Male		Female		Male		Female		Male		Female		Male		Female	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
-ve	72	86.7	106	90.6	106	98.1	87	94.6	159	100	41	100	87	94.6	102	94.4
+ve	11	13.3	11	9.4	2	1.9	5	5.4	0	0	0	0	5	5.4	6	5.6
Total	83	100	117	100	108	100	92	100	159	100	41	100	92	100	108	100

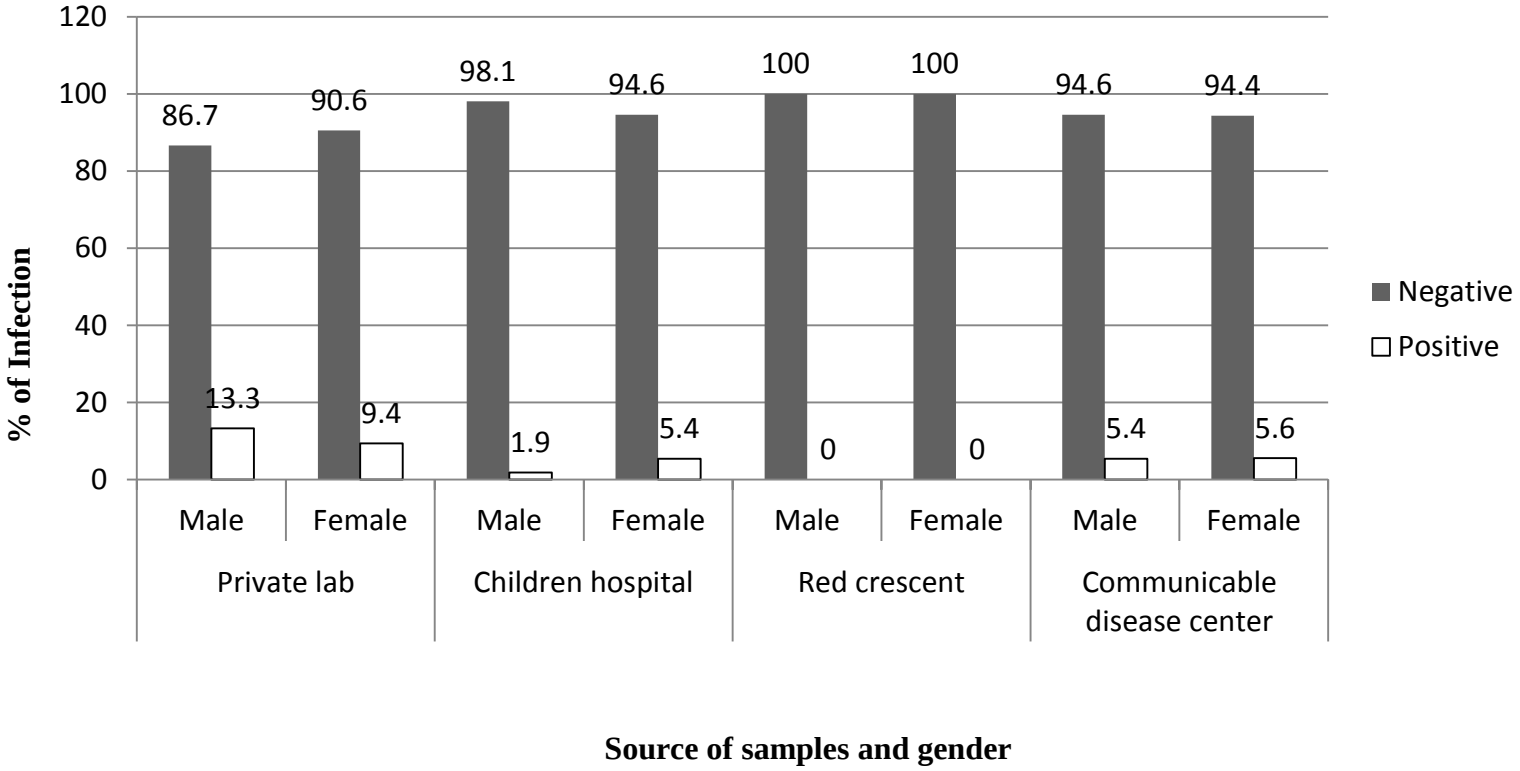


Figure: (10) Distribution of HCV infection according to source of samples and gender

4.7 Distribution of HIV among patients according to the age and gender :

In age 0-13 years total of samples male and female was 225, 2 male and 1 female have shown infection with HIV with total percentage (1.3%). in age of 14-45 years total of samples was 496, 7 male and 5 female are infected with HIV with total percentage (2.4%). In age of 46-80, 79 are total of patients, 1 female (1.3%) has infected with the virus. People with age 14-45 years showed the highest percentage of infection with HIV virus (2.4%), p-value = 0.653. table (11) and figure (11).

Table: (11) Distribution of HIV among patients according to the age and gender

Age	0-13				14-45				46-80			
Gender	Male		Female		Male		Female		Male		Female	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
-ve	122	98.4	100	99	269	97.5	215	97.7	43	100	35	97.2
+ve	2	1.6	1	1	7	2.5	5	2.3	0	0	1	2.8
Total	124	100	101	100	276	100	220	100	43	100	36	100

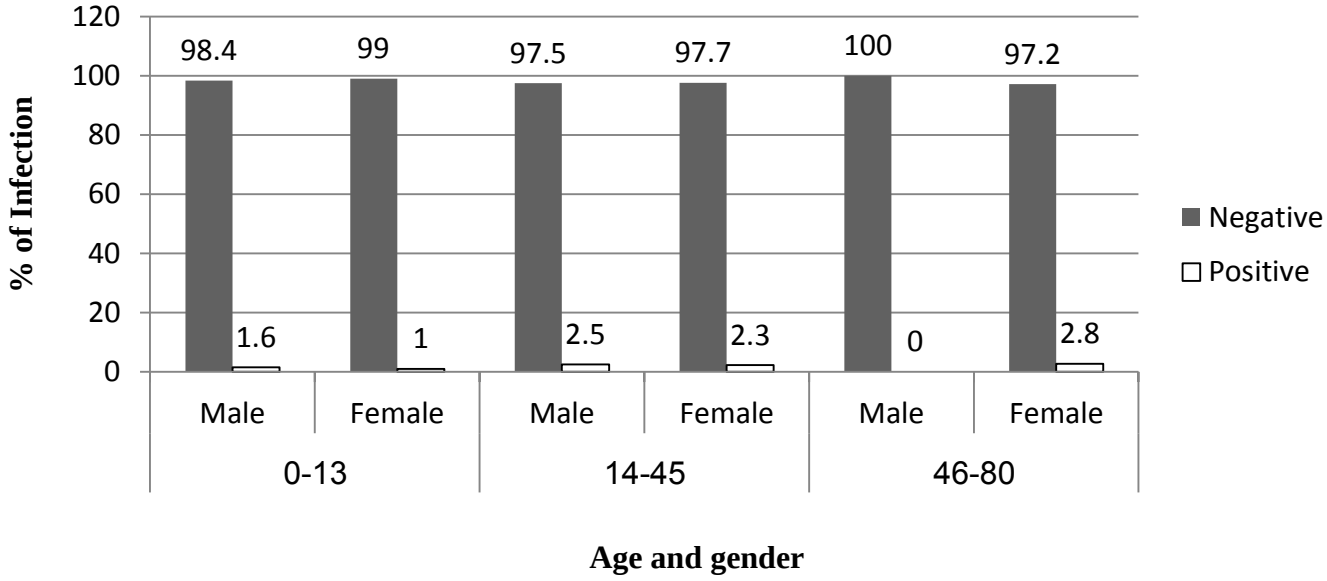


Figure: (11) Distribution of HIV among patients according to the Age and gender

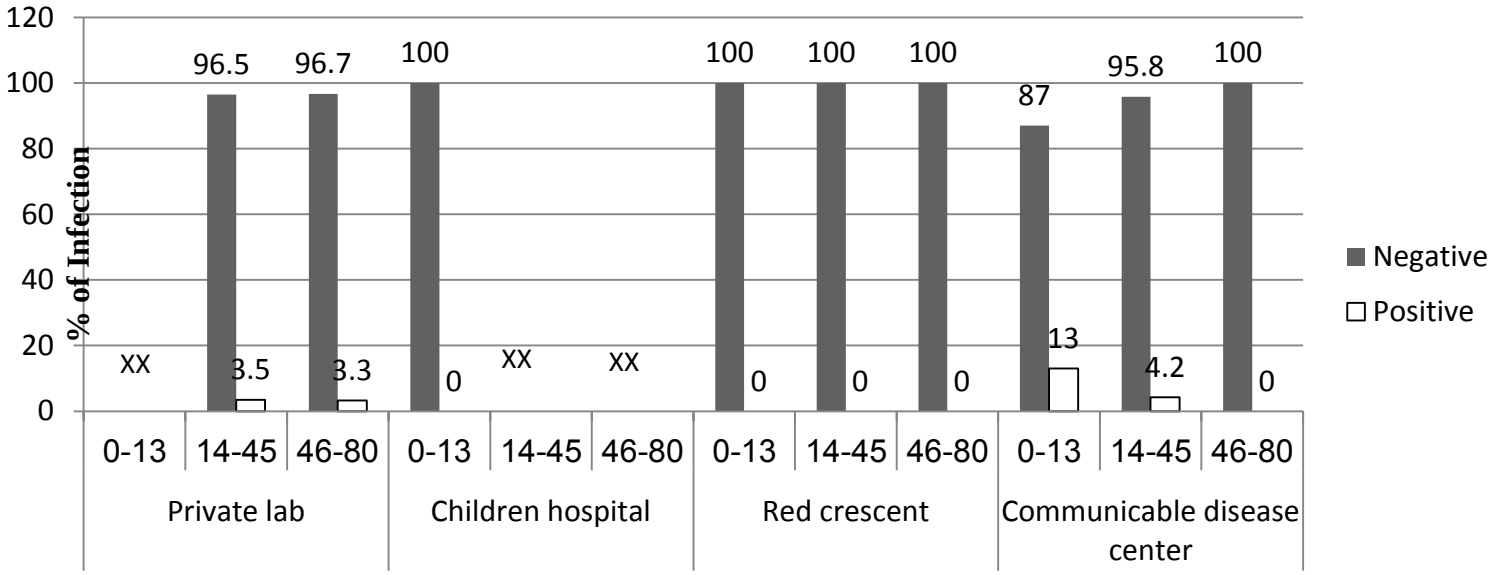
4.8 Distribution of HIV infection according to source of samples and age :

In the private laboratory, age of 0-13 no patients have attended to the laboratory driving this study, in age 14-45, 170 are representing the total samples and the result showed that 6 patients have infect with HIV (3.5%). in age 46-80, 30 are the total of samples with the recorded of infection is 1 patient (3.3%). In children, hospital and Red Crescent laboratory are not recorded any infection with HIV virus.

Results of Benghazi medical centre of infectious diseases revealed in age 0-13 the total samples are 23 where 3 (13%) patients are infected with virus , in age 14-45 years 143 patients have attended to the center 6 (4.2%) patients was recorded with infection , non-results in age 46-80 . Benghazi medical centre of infectious diseases showed the highest rates to infection for all ages where the ratio of HIV (4.5%), p-value = 0.001. Table (12) and figure (12).

Table: (12)Distribution of HIV infection according to source of samples and age

Source	Private lab						Children hospital						Red Crescent (foreign workers)						Infectious disea centre				
Age	0-13		14-45		46-80		0-13		14-45		46-80		0-13		14-45		46-80		0-13		14-45		
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	N
-ve	0	0	164	96.5	29	96.7	200	100	0	0	0	0	2	100	183	100	15	100	20	87	137	95.8	3
+ve	0	0	6	3.5	1	3.3	0	0	0	0	0	0	0	0	0	0	0	0	3	13	6	4.2	
Total	0	0	170	100	30	100	200	100	0	0	0	0	2	100	183	100	15	100	23	100	143	100	3



Source of samples and ag

X. No cases provided

Figure: (12) Distribution of HIV infection according to source of samples and age

4.9 Distribution of HIV infection according to source of samples and gender :

In a private laboratory total of samples male and female was 200 where 3 male and 4 female have shown infection with HIV with total percentage (3.5%). In children, hospital and Red Crescent laboratory are not recorded any infection with HIV virus. In Benghazi medical centre of infectious diseases total of samples was 200, the number of patients are infected with HIV virus are 6 male and 3 female with total percentage (4.5%). The ratio of infection with HIV virus was in male 2%, p-value = 1.000 while the percentage in female 1.95% in all sources. Table (13) and figure (13).

Table: (13) Distribution of HIV infection according to source of samples and gender

Source	Private lab				Children hospital				Red Crescent (foreign workers)				Infectious disease centre			
Gender	Male		Female		Male		Female		Male		Female		Male		Female	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
-ve	80	96.4	113	96.6	108	100	92	100	159	100	41	100	86	93.5	105	97.2
+ve	3	3.6	4	3.4	0	0	0	0	0	0	0	0	6	6.5	3	2.8
Total	83	100	117	100	108	100	92	100	159	100	41	100	92	100	108	100

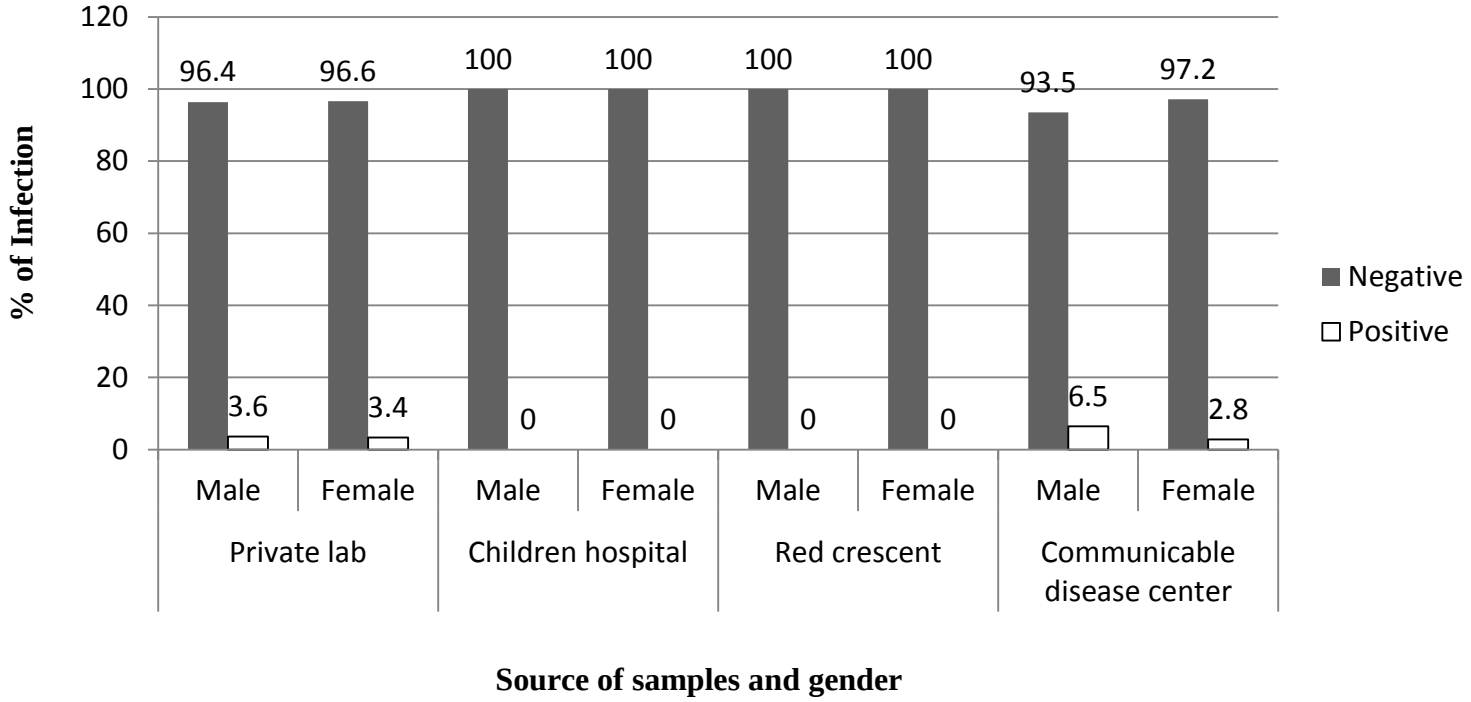


Figure: (13) Distribution of HIV infection according to source of samples and gender

4.10 PCR Results :

In the Center of infectious diseases, it has been assured a group of samples of PCR technology and the results was: It has been assured six samples infected with hepatitis B virus and the result was 100% confirmed. It has been assured six samples infected with hepatitis C virus and the result was 50% confirmed. It has been assured three samples infected with HIV virus and the result was 100% confirmed.

CHAPTER 5 DISSCOSION

Our study demonstrated that, the infection of HBV, HCV, and HIV is rare but may increase the risk of progression of chronic liver disease. This study has found that the seroprevalence of HBsAg among patients is 6.4%, 5% in HCV and 1.9% in HIV. In the same study In the Central Hospital in Tripoli, Libya screened between January 2005 and December 2008 the finding of this study indicated that the seroprevalence of HBsAg was 12.8%, was in HCV 6.9% and HIV was 0.9%. Also in Libya, (Zaied, *et al*. 2010) reported frequency rates of 0.4, 2.6, and 3% for anti-HIV antibodies, HBs-Ag, and anti-HCV antibodies, respectively, which were also reported by (Habas,*et al*. 2009) and (Abudher,*et al*. 2008).Few studies of the prevalence of hepatitis B, C and HIV viruses in Libya explaining viral endemicity and examined specific distribution among population such as children, adults, foreigner workers even genotype (Daw, *et al*. 2014). The epidemiology of these diseases in Europe most of African countries is well studies , Libya classified as the lowest endemicity countries a long with European Mediterranean countries, Tunisia, Algeria, Morocco, Egypt, Chad, Sudan and Niger (Daw, *et al*. 2014). In Libya vaccination against HBV, HCV and

HIV infections has been offered free of charge and become compulsory since 1991 for fants and for children up to age of 12 years, these restrictions and early efforts were adopted by National Prevention program of infection diseases in Libya. In Libya a study was carried out on prevalence of hepatitis B and C infections 2014 showed that the rate of infection was varied from one region to another in both viruses, where they found in Benghazi city is classified as the one of the lowest rate of infection over all Libyan cities, the rate of HBV prevalence was significantly higher in male than females, so the rate of HCV infection among male and females were similar. (Daw, *et al*. 2014). However, this contrasting with the results we obtained, where the rate of HBV, HCV and HIV infection in females were higher than males especially in adults. The prevalence of HBV in group age of 14-45 was significantly higher than group age of 45-80 (8.5%), in contrast the prevalence of HCV showed the rate of incidence is increased with the age (17.7%) (P-value= 0.000),this were similar results to studies in Libya, including a study carried out by Daw, *et al*. 2014 and a study by Kutrani, *et al*. 2007 and Elzouki, *et al*. 2012 and a study in Morocco carried out by Baha, *et al*.2013. While the HIV disease were more rate in age 14-45 (2.2%). In Libya, most of the blood donors are young men (20_40 years of age). It is known that this age group is usually in the high-risk for drug

abuse, sexual activity (Libyan J Med, 2010). Our study was carried out on prevalence of HBV , HCV , HIV viruses among the foreigner workers in both males and females were underwent mandatory test pre-employment as well as in children before study, we found that between 200 samples only one case reported with HBV was from Chad. Another study in Saudi Arabia demonstrated that between 875 samples only four cases (two from Pakistan, one from India, and one from the Philippines) (Alzahrani, *et al*. 2009), also our study showed one case was infected with HIV and hepatitis B virus in this case the confection because of the low immunity of HIV patients which susceptible to wide range of infections. A study concerned with the infection of HBV, HCV viruses in HIV patients results show prevalence hepatitis B and hepatitis C infection to be significantly greater among HIV-infected patients than among blood donors. These observations confirm the high frequency of HIV-infected patient's exposure to these other viruses (Treitinger, *et al*. 2000), also we found another case infected by both hepatitis B and hepatitis C virus this attributed to the route of infection. Regarding the source of samples we found in this study the infection with hepatitis B and hepatitis C virus was predominant in private laboratory with rate (11%) in hepatitis C and (18.5%) in HBV, while the infection with HIV the predominant rate was in infectious disease center (4.5%)

Conclusion :

Reports revealed the presence of high prevalence of infection with Hepatitis B virus (HBV) and Hepatitis C virus (HCV) and Human Immunodeficiency virus (HIV) infection among the general population in Libya, we have this study to identify the extent of the problem at the national level at Benghazi city.

Where the samples included 800 patients of all age groups. It turned out that the prevalence of HBV surface antigen (HBsAg) and HCV were (6.4%) and (5%) respectively and HIV virus was (1.9%) the prevalence of HCV increased with age, in contrast the prevalence of HBsAg and HIV infection are common in young people .

From this study it can be concluded that HBV, HCV, and HIV virus infection are obvious medical problems in Benghazi city. A community-based study should be planned for targeting at risk and non-at risk subjects to investigate the extent of this problem and its impact on the community with an effort to develop preventive strategies.

الملخص :

أشارت الدراسات والتقارير إلي وجود معدلات مرتفعة للإصابة بعدوي فيروس إلتهاب الكبد الوبائي بين عامة السكان في ليبيا HIV وفيروس نقص المناعة البشرية C وفيروس إلتهاب الكبد الوبائي B ، فأجرينا هذه الدراسة وذلك للتعرف علي مدي إتساع نطاق المشكلة علي المستوي الوطني .

حيث شملت الدراسة علي 600 عينة لليبيين و200 عينة لغير الليبيين من جميع الفئات العمرية (6.4%) ومعدل إنتشار Bواتضح ان معدل إنتشار المستضد السطحي لإلتهاب الكبد الوبائي (5%) ومعدل إنتشار فيروس نقص المناعة البشرية Cالمستضد السطحي لإلتهاب الكبد الوبائي يزداد بالتقدم في العمر علي C (1.9%) وقد إتضح ان معدل إنتشار إلتهاب الكبد الوبائي HIV والذي كان منتشر في فئة HIV وفيروس نقص المناعة البشرية Bعكس إلتهاب الكبد الوبائي الشباب أكثر .

تشكل HIV وفيروس نقص المناعة البشرية B,Cمن هذه الدراسة نستنتج إن إلتهاب الكبد الوبائي مشكلة في ليبيا وينبغي التخطيط لدراسة مجتمعية شاملة تستهدف المعرضين والغير معرضين للخطر والتحقق من حجم هذه المشكلة وتأثيرها علي المجتمع مع محاولة وضع إستراتيجيات وقائية .

CHAPTER 6 REFERENCES

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INDEX

- **Index 1 :**

  Reagents Anti-HBV (Anti-HBsAg)

Chemicals and reagents	Manufacturer
HBsAg ELISA Version (1) KIT	Germany
Materials provided with the test kit : • **Control wells**: 1 plate of 96 wells pre-coated with recombinant HCV. • **Enzyme conjugate**: 1 vial containing 11.5 ml of HRP (horseradish peroxidase) labeled mouse anti-human IgG in a stabilizing buffer containing proteins of bovine origin. Contains 0.05% proclin 300 preservative. • **Negative control**: 1 vial containing 1 ml of phosphate buffered solution containing proteins of bovine origin. Contains 0.05% proclin 300 preservative. • **Positive control**: 1 vial containing 1 ml of phosphate buffered solution containing pooled heat-inactivated human serum and plasma positive for anti-HCV and proteins of bovine origin. Contains 0.05% proclin 300 preservative. • **Sample Diluent**: 1 vial containing 11.5 ml of Tris-NaCl buffer and casein. Contains0.02%	

sodium azide preservative. • **Stop solution**: 1 vial containing 7.5 ml of 0.62 mol/l sulfuric acid. • **Substrate A**: 1 vial containing 7.5 ml of hydrogen peroxide. • **Substrate B**: 1 vial containing 7.5 ml or of TMB (3, 3, 5, 5 –tetramethylbenzidine) in a buffer solution. • **Wash solution concentrate**: 1 vial containing 50 ml of 20 times working strength PBS-Tween wash buffer. • 1 copy of instruction for use. • 2 pieces of plate lid. 1 Zip-lock bag	

- **Index2 :**

  Reagents of Anti-HCV

Chemicals and reagents	**Manufacturer**
HCV ELISA Version (1) KIT	Germany
Materials provided with the test kit : • **Coated wells**: 1 Plate of 96 wells pre-coated with mouse monoclonal Anti-HBs. • **Enzyme Conjugate**: 1 Vial containing 7.5 ml of HRP (horseradish peroxidase) labeled sheep polyclonal Anti-HBs in a buffer containing BSA (bovine serum albumin). Contains 0.1% proclin 300® preservative.	

• **Negative control**: 1 Vial containing 1ml of phosphate buffered solution containing proteins of bovine origin contains 0.1% proclin 300® preservative. • **Positive control**: 1 Vial containing 1 ml of phosphate buffered solution containing heat-inactivated human plasma positive for HBsAg and proteins of bovine origin. Contains 0.1% proclin 300® preservative. • **Stop Solution**: 1 Vial containing 7.5 ml of 0.62 mol/l sulfuric acid. • **Substrate A**: 1 Vial containing 7.5 ml of hydrogen peroxide. • **Substrate B**: 1 Vial containing 7.5 ml of TMB (3, 3, 5, 5- tetramethylbenzidine) in a buffer solution. • **Wash Solution concentrate**: 1 Vial containing 30 ml of 20 times working strength PBs-tween wash buffer. • 1 copy of instruction for use. • 1 Piece of plate lid. • 1 Zip-lock bag.	

- **Index 3 :**

  Reagents of Anti-HIV

Chemicals and reagents	**Manufacturer**
HIV ELISA Version (1) KIT	Germany
Materials provided with the test kit : • **Microwell Plate**: Blank microwell strips fixed on a white strip holder. The plate is sealed in aluminum pouch with desiccant.	

Each well contains recombinant HIV 1+2 antigens. The microwell strips can be broken to be used separately. Place unused wells or strips in the plastic sealable storage bag together with the desiccant and return to 2-8 C°. • **Negative Control**: Yellowish liquid filled in a vial with green screw cap. Protein-stabilized buffer tested non-reactive for HIV 1+2 Ab. **Preservatives**: 0.1% proclin 300. Ready to use as supplied. Once open, stable for one month at 2-8C°. • **Positive Control**: Serum-1 (HIV-1) Red-colored liquid filled in a vial with red screw cap. Antibodies to HIV-1 diluted in protein-stabilized buffer. **Preservatives**: 0.1% proclin 300. Ready to use as supplied. Once open, stable for one month at 2-8C°. • **Positive Control** Serum-2(HIV-2) Red-coloured liquid filled in a vial with yellow screw cap. Antibodies to HIV-2 diluted in protein-stabilized buffer. **Preservatives**: 0.1% proclin 300. Ready to use as supplied. Once open, stable for one month at 2-8C°. • **HRP-Conjugate**: Red-coloured liquid filled in a white vial with red screw cap. Horseradish peroxidise-conjugated HIV 1+2 antigens. **Preservatives**: 0.1% proclin 300. Ready to use as supplied. Once open, stable for one month at 2-8C°. • **Wash Buffer**: Colourless liquid filled in a clear bottle with white screw cap. PH 7.4, 20 × PBS (Contains Tween-20 as a detergent). Dilute Before use – The concentrate must be diluted 1 to 20 with distilled or deionized water before use. Once diluted, stable for one week at room	

temperature or for two weeks at 2-8C°. • **Chromogen Solution A**: Colourless liquid filled in a white vial with green screw cap. Urea peroxide solution. Ready to use as supplied. Once open, stable for one month at 2-8C°. • **Chromogen Solution B**: Colorless liquid filled in a black vial with black screw cap. TMP solution (Tetramethyl benzidine dissolved in citric acid). Ready to use as supplied. Once open, stable for one month at 2-8C°. • **Stop Solution**: Colorless liquid filled in a white vial with yellow screw cap. Diluted sulphuric acid solution (2.0 M H2SO4). Ready to use as supplied. • **Plastic Sealable Bag**: For enclosing the strips not in use. • **Cardboard Plate Cover**: To cover the plates during incubation, and prevent evaporation or contamination of the wells. • Instruction Manual.	