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And Scientific Research

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College of Pharmacy

Prevalence of Hepatitis ‘B’ Virus Infection Among Blood Donors in Al-Diwaniyah Governorate – Iraq

Graduation research submitted to college of pharmacy, university of Al-Qadisiyah of the requirements for the degree of B.se of pharmacy.

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Dedication

This Research is dedicated to my fathers, who gave me the greatest gift anyone could give another person which is believing in me, It's also dedicated to my mother, who has been there day after day to make sure my life turned out this way.

Ali

This Research is dedicated to my family, without the inspiration, drive and support they gave me, I might not be the person I am today, dedicated to all my friends who stood by my side and helped me reach my goals, There could never be words in all the world, to express what's owed to you.

Mustafa

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-And if you count the blessing of Allah, never will you be able to count them - So first of all, I thank Allah for blessing me.

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Abstract

Hepatitis B virus (HBV) infection is of global public health concern, this study was conducted to detect the prevalence of hepatitis B virus in Iraq / Al-Diwaniyah Governorate by using enzyme linked immunosorbent assay (ELISA) test, carried out from January 2016 to December 2017, out of a total of 51812 individuals who were donors in the central blood bank at Al-Diwaniyah governorate, Donators ages range from 20-60 years of both genders showed 170 positive cases in a percentage of 0.328%, Seroprevalence of HBV in relation with location, gender and months of the year were also studied, discussed and compared with other global researches.

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ)

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CHAPTER ONE

INTRODUCTION

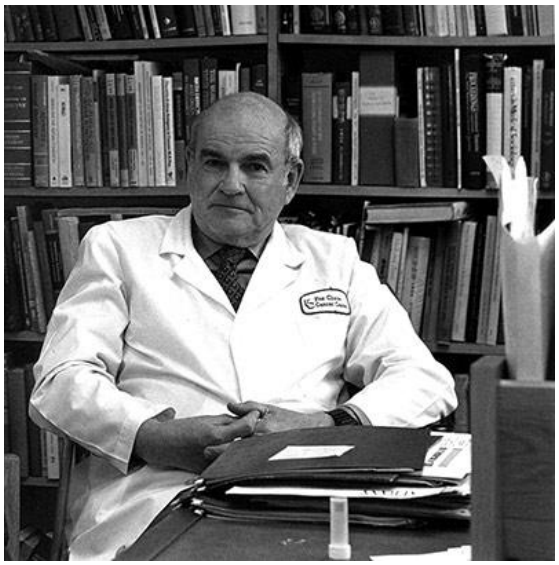
1-INTRODUCTION

“Viral hepatitis," refers to infections that affect the liver and are caused by viruses. It is a major public health issue worldwide. Viral hepatitis includes five distinct disease entities, which are caused by at least five different viruses. Hepatitis A and hepatitis B (infectious and serum hepatitis, respectively) are considered separate diseases and both can be diagnosed by a specific serologic test. Hepatitis C and E comprise a third category, each a distinct type, with Hepatitis C parenterally transmitted, and hepatitis E enterically transmitted. Hepatitis D, or delta hepatitis, is another distinct virus that is dependent upon hepatitis B infection (Dustin et al 2007)

The Centers for Disease Control and Prevention estimated that approximately 400,000–600,000 people were infected with viral hepatitis during the decade of the 1990s. Hepatitis plagued mankind as early as the fifth century BC. It was referenced in early biblical literature and described as occurring in outbreaks, especially during times of war. Toward the end of the nineteenth century, hepatitis was thought to occur as a result of infection of the hepatic parenchyma. The infectious nature of hepatitis was established after World War II. (Shepard et al 2005)

In the mid-1960s Blumberg and colleagues discovered the surface antigen and antibody of hepatitis B. This Nobel Prize-winning

research opened the door to our appreciation of the morphological and immunochemical features of other forms of viral hepatitis.



1-2-Aim of Study

The study aimed to detect the HBV among blood donors by Indirect ELISA at AL-Diwaniyah governorate and the prevalence of HBV was unknown and unclear background of HBV epidemiology of the governorate , for that the present study suggested to carry out to achieve the following objective:-

1- Evaluate the role of HBV in hepatitis infection in blood donors of AL- Diwaniyah governorate.

2- Study some epidemiological characteristic of HBV infections.

3- Study rapid and early detection of HBV infection by using indirect ELISA as Confirmation diagnosis.

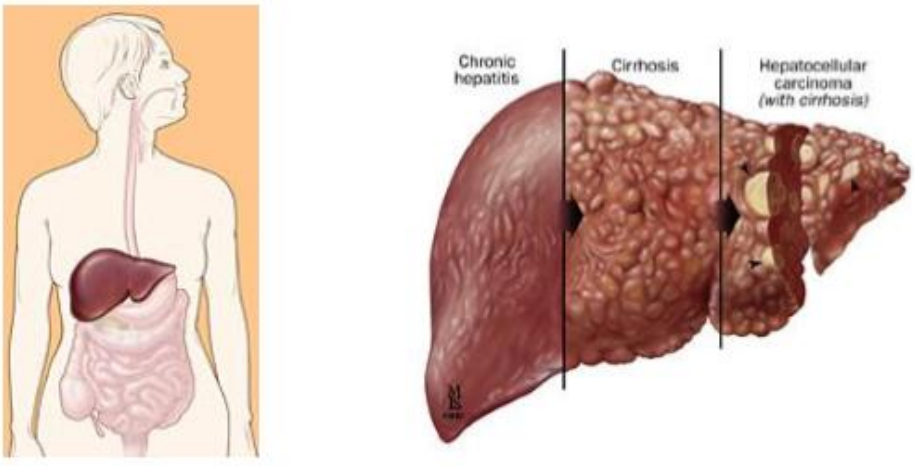
4- Study the factors that affect the prevalence of HBV infection such as Age, Sex, Location and Time

CHAPTER TWO

LITERATURE REVIEW

2-1-THE DISEASE

Hepatitis B is a serious liver disease, caused by hepatitis B virus (HBV) Which is a DNA virus that was first identified in the 1960s, The transmission occurs through human body fluids such as blood and serum. It is becoming an alarming public health problem worldwide.(Nergo et al 2011) Its methods of transmission include through mother to baby (perinatal), sexual contact and the use of improper injection techniques.(Idrees et al 2008) More than two billion amongst the population alive today, would have been infected at some time or other in their lives by the hepatitis B virus (HBV) and approximately 350 million of them are the carriers of the chronically infected disease (Who factsheet No.164 2011) Out of these 25-30% would die as a consequence of the infection (Who Guide 1999). These carriers are at high risk of this serious illness and death from cirrhosis of liver and/or primary liver cancer would kill more than one million of them, per year. They also constitute a reservoir of infected individuals, who perpetuate the infection from generation to generation (Schuppan et al 2003).



1(a) 1(b)

Figure 1(a): Human Viscera showing liver
1(b): Progress of hepatitis B infection.

2-2-The Virus

Hepatitis B is a DNA Virus of the hepadnaviridae family of viruses. It replicates within infected liver cells (hepatocytes) (Dustin et al 2007) The hepatitis B virus particle (virion), also known as the Dane Particle, is a spherical lipid-containing structure that consists of a viral envelope, nucleocapsid and a single copy of double-stranded DNA genome (Houghton et al 1996).

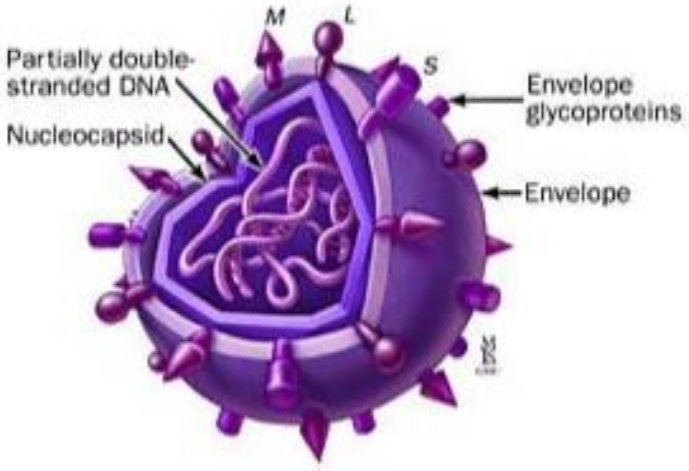


Figure 2: Hepatitis B Virion

The protein of the virion coat is termed "surface antigen" or HBsAg. It is sometimes extended as a tubular tail on one side of the virus particle. The surface antigen is generally produced in vast excess, and is found in the blood of infected individuals in the form of filamentous and spherical particles. Filamentous particles are identical to the virion "tails" – they vary in length and have a mean diameter of about 22nm. The outer surface coat surrounds an inner protein shell, composed of HBc protein (hepatitis-B core protein) or HbcAg (hepatitis B core antigen). This inner shell is referred to as

the core particle or capsid. Finally, the core particle surrounds the viral DNA and the enzyme, DNA Polymerase (Houghton et al 1996)

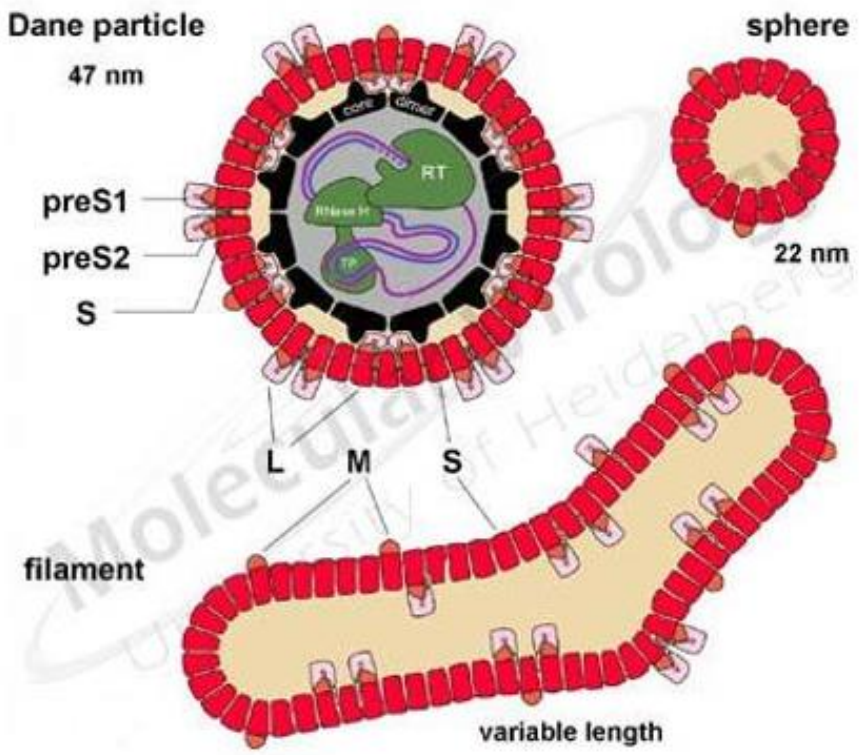


Figure 3: Detailed structure of hepatitis B virion

2-3-Epidemiology

The researchers have divided the world into areas of high, intermediate, and low HBV endemicity. This division is based on the prevalence of HBV markers and the primary modes of HBV transmission (Heller et al 2005)

In areas of high endemicity, the most common route of transmission is perinatal or the infection acquired during the pre-school years. Africa, Asia, East of the Indian sub-continent, the Pacific Basin, the Amazon Basin, the Arctic Rim, and the portions of the Middle East, Asia Minor, and the Caribbean are the areas of high endemicity. (Smith et al 1997)

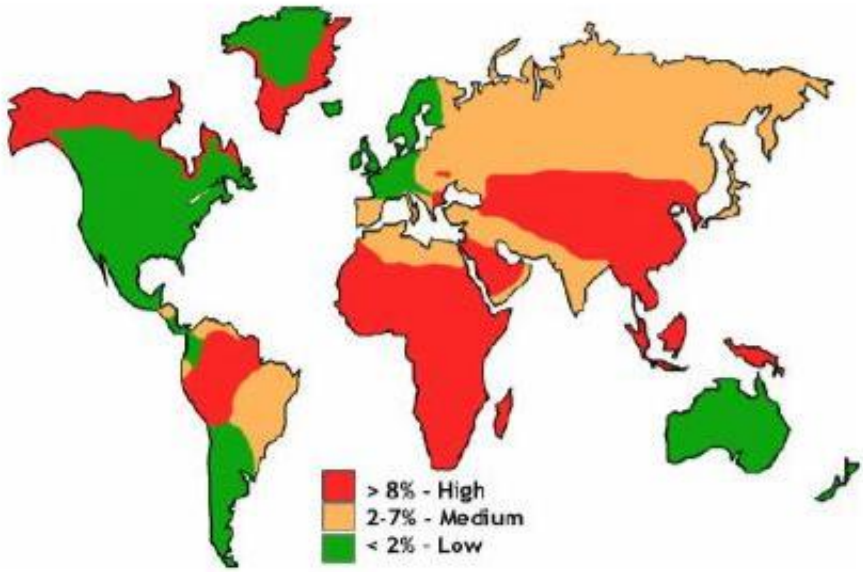


Figure 4: Geographical distribution of hepatitis B infection

Areas of intermediate endemicity generally have an HBV carrier prevalence of 2 to 5%, and 30 to 50% of the population has serological evidence of prior HBV infection. (Theodore et al 2006) In these areas both child to child and adult transmission occur. Acute viral hepatitis with jaundice is a major cause of morbidity. (Van damme et al 2002)

In the areas of low endemicity, perinatal and child to child transmission is relatively uncommon, and most infections occur in

adults through sexual activity, needle sharing during drug abuse, or during occupational exposure to blood. (Memon et al 2002)

2-4-Mode of Transmission

The hepatitis B virus is carried in the blood and other body fluids. The virus is present in the blood, saliva, semen, vaginal secretions, menstrual blood, and to a lesser extent, breast milk, tears and urine of infected individuals. It is usually spread by contact with blood in the following ways. (Heintges et al 1997)

1) Perinatal (mother to child) transmission is one of the most common and serious mode of HBV transmission. Perinatal transmission occurs from mothers, who are positive for both the hepatitis B surface antigen (HBsAg). More than 90% of these women are chronic HBV carriers, although those acutely infected with the virus, during pregnancy, may also transmit to their children, Infected newborns rarely develop acute hepatitis, although reports of fatal fulminant hepatitis have been reported. (Otho et al 1994)

2) Child to child transmission, also called horizontal transmission, is responsible for majority of HBV infections and their carriers. Transmission between children, occurs during social contact through cuts, scrapes, bites and scratches. The skin lesions, such as, impetigo, scabies, abrasions and infected insect-bites, play an important role. These lesions provide a route for the virus to leave the body of the infected children, as well as, one to enter into the body of susceptible children. (Alter 1994)

3) Transmission through an unsafe injection, needle-prick or reuse of unsterile needles, and use of contaminated needles and other medical and dental equipment's. (Heintges et l 1997) Survey in developed countries have revealed, that up to 30% of injections used for immunization, are not found sterile. Disposable syringes are reused and reusable syringes are improperly sterilized, resulting in a significant risk of transmission. Auto-destruct syringes and single use pre-filled devices, can reduce the transmission by averting inappropriate use. In some western countries, needle-sharing by drug abusers is also causative. If sterile needles are not used, it is possible to transmit hepatitis B, through body-piercing, tattooing, drug injection and acupuncture. The hepatitis B virus can also be transmitted by sharing razors, tooth brushes, nail-clippers and earrings. (Nelson et al 2011)

4) Transmission during sexual intercourse through contact with blood or other body fluids.

Hepatitis-B is not transmitted casually. It cannot be spread through sneezing, coughing, hugging or eating food prepared by someone, who is infected. (Heintges et al 1997)

2-5-Signs and Symptoms

The incubation period averages six weeks, but may be as long as six months. About 30% of persons have no sign or symptoms. Signs and symptoms are less common in children than adults. (Rosen 2011) However a large proportion of children may become chronic carriers, compared to adults. Any common symptoms, that might

show up, are usually flu-like symptoms, including fever, fatigue, muscle or joint pain. (Mondelli et al 2005)

Severe symptoms include yellow eyes and skin called jaundice and a bloated or swollen stomach. (Ozaras&Tahran 2009) The symptoms may last several weeks or months. Most acute infection in adults is followed by a complete recovery. However, many children become chronic carriers. People, who recover from acute hepatitis and not becoming chronic carriers, are protected from being infected again, throughout their lives. (Jou&Muir 2008) However, to a limited number of the population, the acute infection could be severe and lead to death. The most serious complications including chronic hepatitis, cirrhosis, liver failure, and liver cancer, occur in people with chronic infection. (Ozaras &Tahran 2009)

2-6-Diagnosis

A simple blood test could easily diagnose hepatitis B infection. The test looks for antigens and antibodies in blood. If recently infected, it will take 4 to 6 weeks, before the virus could be found in the blood (Pawlotsky 1999)

A blood test will show whether the patient:

- Has been infected previously.
- Has an acute hepatitis B infection, presently
- Has recovered from a past infection and is now immune.
- Has a chronic hepatitis B infection and the virus is present in the blood.

Tests			
Hep B surface antigen (HBsAg)	**Hep B surface antibody (Anti-HBs)**	**Hep B core antibody Total (Anti-HBc IgG+IgM)**	**Possible Interpretation / Stage of Infection**
Negative	Negative	Negative	No active or prior infection; not immune — may be good candidate for vaccine; possibly in the incubation stage
Negative	Positive	Negative	Immunity due to vaccination
Negative	Positive	Positive	Infection resolved (recovery), virus cleared; immunity due to natural infection. However, if immunosuppressed, virus can reactivate.
Positive	Negative	Positive or Negative	Acute infection, usually with symptoms; contagious; could also be flare of chronic infection
Negative	Negative	Positive	Acute infection is resolving (convalescent)
Positive	Negative	Positive	Usually indicates an active chronic infection (liver damage likely)
Positive	Negative	Positive	Chronic infection but low risk of liver damage — carrier state

Figure 5 : HBV Tests

2-7-Prevention

The best way to prevent HBV infection is through vaccination. Hepatitis vaccines are highly purified preparations of hepatitis B surface antigen (HBsAg), the glycoprotein that forms the outer coat of the hepatitis B virus. (Wasley et al 2008)

The discovery of the etiologic agents for hepatitis B and the development of safe and effective vaccines for this virus are among the remarkable scientific achievements of the 20th century. The first vaccines to be introduced were made from the blood of infected individuals, which was treated to destroy any live virus. As of date, two kinds of vaccines for hepatitis B are available (Zanetti et al 2003)

1. Plasma – derived vaccines.

2. Recombinant DNA vaccines.

1. Plasma derived vaccines*:* In natural HBV infections, liver cells produce much more HBsAg than is needed to coat viral particles, and the excess HBsAg forms 22nm spherical and long tubular particles. Plasma derived HB vaccines are prepared by purifying HBsAg particles from the plasma of HBsAg positive donors. These vaccines are inactivated to ensure that no infectious viral or other micro-organisms are present, and then are alum adjuvented. Plasma derived vaccines have been available since 1981 and have been used in more than 70 million individuals with an outstanding record of safety and efficacy. (Zanetti et al 2003)

2. Recombinant hepatitis B vaccines are produced from HBsAg derived from yeast or mammalian cells that have replicating plasmids containing the viral HBsAg gene inserted into the cells.

The HBsAg forms spherical particles similar to the natural 22nm spherical particle in both chemical composition and immunogenicity. Recombinant HBsAg for vaccines may be produced in almost unlimited amounts in brewery-like fermentation vats, so there need be no concern that availability of antigen will compromise future vaccine supply. (Zanetti et al 2003)

Hepatitis B immunoglobulin: If hepatitis B immune globulin (HBIG) is given to newborns of HBeAg positive mothers in addition to HB vaccine, the efficacy in preventing the carrier state may be slightly increased. Use of HBIG adds considerably to the cost of treatment, since it is expensive, and since it requires serological testing of mothers to determine their HBsAg status. Such testing is itself expensive, and requires laboratories and prenatal testing programs that are generally unavailable in developing countries. For these reasons it is generally accepted that it is more cost- effective to devote resources to routine infant immunization, and that most developing countries will elect to forego the use of HBIG. (Ghany et al 2009)

2-8-Treatment

Antiviral therapy is not warranted for acute Hepatitis B, because the infection will resolve on its own, in most symptomatic individuals. Acute liver failure may develop in less than 0.5% of adults with acute Hepatitis B. Potential candidates for anti-viral treatment are those with active viral reproduction, elevated liver tests and no signs of advanced liver disease. (Wilkins et al 2010)

The immediate goals of anti-viral therapy in chronic hepatitis B viral infection are to suppress viral reproduction and improve liver tests.

The ultimate goals are to prevent further liver injury and scarring, stop the progression towards cirrhosis, and thereby prevent the complications of cirrhosis, including liver cancer. (D'Souza&Foster 2004)

There are four medications currently approved by the Food and Drug Administration (FDA) for treatment of chronic hepatitis B infection.

1) Interferon-alpha-2b: Interferon-alpha 2b was first shown to be effective in chronic Hepatitis-B virus patients in 1988. In addition to its direct antiviral effects, interferon works against hepatitis B virus by stimulating the body's immune system to clear the virus. For the treatment of chronic hepatitis B, a four to six month course of interferon-alpha is given. (Aronsohn&Reau 2009)

2) Lamivudine: In the last five years, the focus of treatment for chronic hepatitis B virus has turned to nucleoside drugs. A number of nucleoside drugs that are used to treat HIV by slowing down the reproduction of that virus, therefore, have been tried for the treatment of hepatitis B virus. Nucleoside drugs are man-made molecules that closely resemble the biochemical units that make up genetic material (DNA and RNA). The nucleosides, therefore, work as imposters to trick hepatitis B virus genetic material and thereby slow down reproduction. Unlike interferon, the nucleoside class of compounds has no known direct effect on the immune system. (Aronsohn&Reau 2009)

3) Adefovir dipivoxil (Hepsera): inhibits DNA polymerase activity and reverse transcriptase. This drug is administered orally on a daily basis and is typically well tolerated. (Fung et al 2008)

4) Baraclude (Entecavir): is the latest drug approved by the FDA for treatment of chronic hepatitis B. It works by inhibiting the function of Hepatitis B virus polymerase. Side effects include headache, fatigue, dizziness, nausea, and transient elevation in liver enzymes. This drug is taken orally, once daily and the optimal duration of therapy is not yet established. In patients with severe liver dysfunction, a liver transplant may be required. (Fung et al 2008)

Chapter THREE

Materials & Method

3-1- Materials

3-1-1- Equipment's and instruments

- Elisa Reader (BioTek)
- Elisa Washer (BioKit)
- Autoclave
- Oven (Froilabo)
- Low Speed Centrifuge
- Micrometer Stage
- Micropipette
- Multichannel Pipette
- Test tube
- Eppendorf tubes

Figure (6) : Items Used

HBsAg ELISA (FORTRESS DIAGNOSTICS)

Kit Contents;	Volume
Microwell Plate 96 Tests	5 plates (12x8 well strips per plate)
Negative Control	5x1ml
Positive Control	5x1ml
HRP – Conjugate Reagent	5x7ml
Stock Wash Buffer	5x30ml (Dilute 1 to 20 with distilled water before use. Once diluted, stable for two weeks at 2-8◦C).
Chromogen Solution A	5x7ml (Ready to use and once open, stable for one month at 2-8◦C)
Chromogen Solution B	5x7ml (Ready to use and once open, stable for one month at 2-8◦C)
Stop Solution	5x7ml
Plastic Sealable Bag	5 Units
Plate Cover	5 Sheets
Package Inserts	1 Copy

Figure (7) : Kit Used

3-2-Method

1. Patients Samples :- This study was carried out From January 2016 to December 2017, a total of 51812 individuals were donors in the central blood bank at Al-Diwaniyah governorate.
2. A sample of 5ml of fresh blood was drawn from individuals and collected in a sterile plastic tube, left to clot at room temperature then centrifuged at 2000 rpm for 10 min, then serum was collected in sterile tube.
3. Numbering Wells by Setting the strips needed in strip-holder and sufficient number of wells including three Negative controls, two Positive Control and one Blank.
4. 50µl of Positive control, Negative control, and specimen has been added into their respective wells. Then 50µl HRP-Conjugate has been added to each well except the Blank, and mixed by tapping the plate gently
5. Incubating: The plate is covered with the plate cover and incubate for 60 minutes at 37°C
6. Washing: At the end of the incubation, the plate cover is removed and discarded. each well is washed 5 times with diluted Wash buffer.
7. Coloring: 50µl of Chromogen A and 50µl Chromogen B solution has been dispensed into each well including the Blank, and mix by tapping the plate gently. The enzymatic reaction between the Chromogen solutions and the HRP Conjugate produces blue color in Positive control and HBsAg positive sample wells.
8. The reaction is stopped and placed in ELISA reader for conformation (Following Instruction Guide of the kit)

CHAPTER FOUR

Result & Discussion

4-1-Seroprevalence of HBV infection

Hepatitis B virus (HBV) infection is a serious global public health problem that causes both acute and chronic infection with significant complications and sequelae. More than 2 billion people worldwide are estimated to have had hepatitis B virus (HBV) infection, with 350–400 million being chronic carriers of the virus. HBV accounts annually for an estimated 1 million deaths worldwide, and causes acute and chronic liver disease, The lack of information of its sero-prevalence among the general population is an obstacle for formulating effective policies to reduce the burden viral hepatitis. Therefore, this population based serological study was established and conducted in Diwaniyah governorate, where no epidemiological data was available to determine the prevalence and risk factors of HBV infection.

This Study was the results of serological examination by indirect ELISA for detection of antibodies of HBV in central blood bank in Al-Diwaniyah governorate showed that 170 out of 51812 examined donors were positive in a percentage of 0.328% (Table 1).

Total No. Of Samples	No. of Positive Samples	Percent%
51812	170	0.328%

Table (1) : Positive number and total percentage of infected donors

The Small Percentage (0.328%) probably due to sampling from healthy donors only and not receiving the donors that proven to have a pervious infection compared with most researchers that

take sample from various study groups, example (renal dialysis, thalassemic patients, medical staff, polycythaemic patients)

4-2-HBV infection in Relation to Sex Difference

	Positive	Males - %	Females - %
2016	**96**	**62**	**34**
2017	**74**	**51**	**23**
TOTAL	**170**	**113 (66.45%)**	**57 (33.55%)**

Table (2) : HBV infection in Relation to Sex Difference

The Result Shows Higher prevalence of HBV in males with percentage of 66.45% than HBV in females with percentage of 33.55% (Table 2), There have now been studies in several parts of the world regarding Sex Differences in response to Hepatitis B virus in most human populations which all stated that there is a higher prevalence of chronic carriers of hepatitis B virus (persistently HBsAg+) among males than females. Females are more likely than males to produce anti-HBs in response to infection. Diseases associated with increased frequencies of carriers are more prevalent among males than females, Several Researches provided similar data which means that young females were more resistant to infection by a large number of species of bacteria, that once infected they responded to infection better than males, and when given appropriate therapy, they responded better to treatment.

4-3-HBV infection in relation with Location

In this study the prevalence rates of HBV infection in relation with location of different geographical study area is compared between the central city (Al-Diwaniyah) and Other districts such as Al-Shamiyah..etc and the result showed that the percent of

seropositive was 62.35 centrally and 37.65 in other districts combined (Table 3).

	Infection No.	%
Central city	106	62.35
Other Districts	64	37.65

Table (3) : HBV infection in relation with Location

The high percentage of hepatitis spreading in Al-Diwaniyah city over the rest of other regions in the governorate may be because the number of donors from Al-Diwaniyah city is greater that donors from other regions which is more resource-limited and combine a considerable set of barrier to donation, including shortage of healthcare workers, poor medical infrastructures, insufficient screening and poor access to care and treatment.

4-4- HBV infection in relation with Age

The results of sero-prevalence of HBV infection by using indirect ELISA in relation to the different age groups 20-30 years , 31-40 years ,41-50 years and 51->60 years old were 10% , 40.6% , 48.2% and 11.2% respectively, the highest rate of the prevalence was in age groups 31-40 years and 41-50 years 38.2-40.6% and the lowest rate of prevalence was in age group 51->60 years old with 11.2% (Table 4).

Age (Years)	Infection No.	%
20-30	**17**	**10**
31-40	**69**	**40.6**
41-50	**65**	**38.2**
50- >60	**19**	**11.2**
Total	**170**	**100**

Table (4) : HBV infection in relation with Age

The study shows an increasing prevalence of HBV infection with age with peak incidence of infection in 30-50 years' old

4-5-HBV infection percentage in months

Viral hepatitis is an infection that has been reported to be present throughout the year, but some particular months are associated with higher incidences, There is no definite and consistent seasonal pattern has been observed, although evidence points towards summer peak and months of temperature change as our study showed results that the peak of infection was from (April to August) infection ranging from 10-12 in 2016 which represents a 57 patient out of the total 96 in a percentage of 60% and 7-9 in 2017 which represents 39 Infection out of the total 72 in a percentage of 54% , while the rest of the year ranging from 2-8 in 2016 and 3-6 in 2017 in a percentage of 40%, 46% respectively (Table 5).

2016	Total No. of Samples	No. Of positive Samples	2017	Total No. of Samples	No. Of positive Samples
January	1821	4	**January**	1965	4
February	1933	6	**February**	2122	6
March	1724	8	**March**	2117	5
April	2136	10	**April**	2587	7
May	1290	12	**May**	2929	9
June	2350	12	**June**	2040	8
July	2278	11	**July**	2478	7
August	2177	12	**August**	2178	8
September	2751	5	**September**	2261	6
October	2293	2	**October**	1804	4
November	2254	8	**November**	2423	3
December	1974	6	**December**	1927	5
TOTAL	24981	96	**TOTAL**	26831	72

Table (5) : HBV infection percentage in months

This may be due to multiple source of transmission during that time such as summer travel to an endemics area, swimming habits of the population in hot months, increase sexual contact, tattoo, poor hygiene and environmental sanitation and food habits (feco-oral transmission of viral hepatitis).

CHAPTER FIVE

Conclusion & Recommendations

5-1- Conclusion

-HBV infection is an increasingly important public health problem

-The epidemiological features of HBV were associated with age groups, sex and location difference and with different months of the year

-Enhanced understanding of viral, host, and environmental factors that influence disease progression may ultimately improve the management of patients with chronic HBV infection

5-2-Recommendations

Expanding screening for chronic HBV infections would surely identify new cases, but some would be among people with no access to care , Early diagnosis of chronic hepatitis B carries with it the opportunity for treatment and monitoring to reduce the long-term risk of liver cancer and cirrhosis. It also offers the opportunity to vaccinate the patient's uninfected contacts. Such follow-up is very important along with educating the patient about necessary management thought-out life in order to reduce HBV transmission and deaths associated.

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