Serological and Molecular Detection of Hepatitis B virus
in Elmanagil District-Algazeira State.

A Dissertation submitted in partial fulfillment for the requirement of M.Sc degree in Medical Laboratory Science (Microbiology)

By

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June 2017
Dedication

To my dears:

Mother

Father

Brothers

And Sisters
Acknowledgments

First of all thanks to ALMIGHTY ALLAH for every things, and for giving me chance and power to start and complete this study. Thanks to my supervisor: Prof. Yousif Fadlalla for his instruction and suggestion that help me to complete this work with remarkable flexibility. I wish to thank Suhair Ramadan Rehan Sudan University of Sciences and Technology for technical help in the laboratory and Abdalla Ahmed Abdalla for his help in collecting samples and I wish to thank Amel Jafar for materials supplying. Finally I acknowledge after Allah my family, who stimulating me to continue this work and provide me with encouragement and support that make this study possible.
Abstract

The aim of the study was to determine prevalence of Hepatitis B virus among population at Sharafat village on Almanagil province and to compare between ICT and ELISA for surface antigen detection and to detect HBV DNA by PCR. Randomly collected a total of ninety two subjects (n=92) were enrolled in this study. The serum samples were examined by ICT to detect HBsAg, only 6 out of 92 (6.5%) samples were HBsAg positive. ELISA test showed that 30 out of 92 (32.6%) samples were found positive for HBsAg. These 30 samples which was HBsAg positive by ELISA tested by nested PCR and all of them were found positive. The study indicated insignificant (P>0.05) association between HBV infection and risk factors including age, gender, marital status, history of blood transfusion and history of surgical operation. The distribution of positive cases in Gender was 16 (17.4%) of cases males and 14 (15.2%) were females, in Age groups the highest result was (8.7%) in group (5-15) years and there were no infections in age group (56-65), in marital status was 18 (19.6%) married and 12 (13%) were not married, No infections noticed in those had blood transfusion history, in those whose had history of surgical operation it was 9 (9.8%) of cases had history of surgical operation and 21 (22.8%) had no.

The study conclude that prevalence of HBV infection among population at Sharafat village on Almanagil province exceeds the estimated nationwide prevalence. However, that should not ignore this high prevalence and giving special attention to prevent outbreak of hepatitis B.
ملخص الدراسة

كان الهدف من الدراسة هو تحديد نسبة انتشار فيروس التهاب الكبد البائي في مواطني قرية شرفة ريف المناقل في ولاية الجزيرة ومقارنه اختبار ELISA واختبار ICT. وكا كنت الهدف من الدراسة هو تحديد نسبة انتشار فيروس التهاب الكبد البائي في مواطني قرية شرفة ريف المناقل في ولاية الجزيرة ومقارنه اختبار ELISA واختبار ICT.

كان عدد العينات المستخدم في الدراسة (92) جمعت عشوائياً ثم فحص عينات المصل بواسطة الشريحة لتحديد البروتين السطحي فيروس وجد فقط 6 (6.5%) من اصل 92 موجبًا تم اختبارهم باستخدام اختبار الايزا حيث كانت النتيجة موجبة لـ30 (32.6%) عينة من اصل 92 عينة تم اختبارها بطريق عشوائيه انا مصابة بالفيروس

وإنها مصابة بالفيروس كما تم تأكيد هذه النتيجة باستخدام تقنيه تفاعل البلمرة المتعدد وجميعها (30) أعطت نتائج موجبة.

كانت النتيجة المعنوية أكبر من 0.05 وتشير عدم وجود علاقة بين الأصابات والعوامل المؤثرة أي ليس هناك ارتباط مع العمر والتنوع والحالة الزوجية ونقل الدم والعملية الجراحية. وكان توزيع الحالات الموجبة بين الذكور 16 (17.4%) والإناث 14 (15.2%) حالى وبين الاسماع كانت عالية (8.7%) في المجموعه (15) سنة ولم توجد حالة اصابه في المجموعه (56-65). بين المتزوجين وغير المتزوجين كانت نسبة الاصابه 18 (19.6%) و 12 (13%) على التوالي. لم توجد اصابات بين من لهم تاريخ نقل الدم، بين من سبق لهم الخضوع لعمليات جراحية ومن لم يخضع لعمليات جراحية كانت نسبة الاصابات 9 (9.8%) و 21 (22.8%) على التوالي.

ما تحصلنا عليه من هذه الدراسة أن نسبة الاصابات بفيروس التهاب الكبد الوبائي ب في قريه شرفة ريف المناقل قد زادت كثيرا عن النسب المتحده عالمياً لذلك يجب النظر وعدم تجاهل نسبة الانتشار العالمي 32.6% واعطاء المشكله اهتمام خاص لمنع وتشاور وباء انتشار فيروس الكبد البائي.
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Abbreviations

HBV   Hepatitis B virus
ALT   Alanine aminotransferase
AST   Aspartate aminotransferase
cccDNA Covalently Closed Circular DNA
ICT   Immunochromatography test
ELISA Enzyme linked Immune Sorbent Assay
HCC   Hepato Cellular Carcinoma
OHB   Occult hepatitis B
HRP   Horse Radish peroxidase
MHC   Major Histocompatibility Complex
SVR   Sustain virological response
SPSS  Statistical package for Social Science
TMB   Tetramethyl Benzidine
PCR   Polymerase chain reaction
WFI   water for injection
DNA   Deoxy ribonucleic acid
CHAPTER ONE

INTRODUCTION
CHAPTER ONE

INTRODUCTION

1.1 Background

Hepatitis B, one of the major and common infectious diseases of the liver. Hepatitis B virus (HBV) chronically infects approximately 350 million people worldwide. Without intervention, approximately 15% to 40% of chronically infected individuals will eventually develop cirrhosis, end-stage liver disease or hepatocellular carcinoma, or require liver transplantation. The availability and extensive use of the HBV vaccine has dramatically reduced the number of incident infections in worldwide. Effective therapeutic agents have been and continue to be developed to treat chronic infection. The present review provides a comprehensive overview of diagnostic tests for HBV infection and immunity, and elaborates on HBV risk factors, vaccine prevention and therapeutic monitoring (Krajden et al., 2005). Hepatitis means inflammation of the liver. It is most common caused by one of the several viruses, such as hepatitis A virus, hepatitis B virus, hepatitis C virus and other viruses, Toxins, bacterial infections, certain drugs, and heavy alcohol use can also cause hepatitis (Jawetz et al., 2007). HBV is transmitted through percutaneous or parenteral contact with infected blood body fluids, and by sexual intercourse (Ganem and Schneider., 2001).

Transmission rates significantly increase if acute infection occurs at or near the time of delivery, with rates reported as high as 60 percent (Sookoian., 2006). Mother-to-infant transmission represents a basic factor in maintaining chronic HBV infection and usually depends on the degree of maternal infectivity, especially in the prenatal period (Wang et al., 2003). Exposure to the virus varied from 47%–78%, with a hepatitis B surface antigen prevalence ranging from 6.8%
in central Sudan to 26% in Southern Sudan. Studies pointed to infection in early childhood in southern Sudan while there was a trend of increasing infection rate with increasing age in northern Sudan. Hepatitis B virus was the commonest cause of chronic liver disease and hepatocellular carcinoma and was the second commonest cause of acute liver failure in the Sudan (Hatim., 2008).
1.2. Rationale

Hepatitis B virus (HBVs) infection has been recognized as a worldwide health problem. It was estimated that more than two billion people alive today have been infected and 350 million of these suffer from chronic hepatitis B (Krajden et al., 2005). There are numerous of extra hepatic manifestations have been reported in patients with both acute and chronic hepatitis B (arthralgias or arthritis, skin rashes, glomerulonephritis and neuritis), all of which are present in polyarteritis nodosa (PAN) which is the most unique and spectacular extrahepatic manifestation. In the 1970s, the frequency of PAN due to the hepatitis B (HBV) reached 30%. Clinical manifestations reflect this most classic form of PAN, Hepatic manifestations including, ALT (alanine amino trasferase) AST (Aspartate amino tranferase) elevations are mild and usually overlooked.

We conducted this study among population at Sharafat village at Almanagil province on Algazeira State because there were repeated cases from this area. Therefore, this study was aimed to study the prevalence of hepatitis B among population at that area and to identify possible risk factors associated with hepatitis B infection in them.

We chosen ICT in the study because it become used for screening of HBV in most Sudan rural hospital for blood transfusion.
1.3. Objective:

1.3.1. General objective:
To determine the prevalence of hepatitis B virus among population at Sharafat village, province of Almanagil at Algazeira state

1.3.2. Specific objective:
1. To screen for HBV by ICT.
2. To determine the frequency of hepatitis B surface antigen (HBsAg) By sandwich ELISA among population at Sharafat village.
3. To compare between ICT results and ELISA results.
4. To Confirm the ELISA results by PCR.
5. The possible effect of risk factors (age, gender, married, history of surgical operation and blood transfusion).
CHAPTER TWO
LITERATURE REVIEW
CHAPTER TWO

2. Literature Review

2.1 Discovery of the Hepatitis B Virus
The history of modern research on viral hepatitis began in the year 1963, when Nobel Prize winner Baruch S. Blumberg (1925–2011) reported for the first time publicly on the discovery of a new antigen named Australia antigen (AuAg) (Blumberg, 2002). In the years following, AuAg would become the first specific marker of viral hepatitis. Thereafter, viral hepatitis type B became a driving force for the development of modern virus diagnostics and vaccines. This article will recapitulate the major advances in the field of hepatitis B research throughout the last 50 years and point out some perspectives for future research.

2.2. Classification
The hepatitis B virus is classified as the type species of the Orthohepadnavirus, which contains three other species: the Ground squirrel hepatitis virus, Woodchuck hepatitis virus, and the Woolly monkey hepatitis B virus. The genus is classified as part of the Hepadnaviridae family, which contains two other genera, the Avihepadnavirus and a second which has yet to be assigned. This family of viruses have not been assigned to a viral order (Mason et al., 2008).

2.3 Morphology and genome structure
Electron microscopy of HBsAg-positive serum revealed three morphologic forms. The most numerous are spherical particles measuring 22 nm in diameter. These small particles are made up exclusively of HBsAg-as are tubular or filamentous forms, which have the same diameter but may be over 200 nm long and result from overproduction HBsAg. Larger, 42–nm spherical virions (originally referred to as Dane particle) are less frequently observed. The outer surface, or envelope, contains HBsAg and surrounds a 27-nm inner nucleocapsid core that contains
HBcAg. The variable length of a single-stranded region of the circular DNA genome results in genetically heterogeneous particles with a wide range of buoyant densities (Jawetzet al., 2010).

2.3.1. Structure

The HBV core contains besides the HBV genome the HBV polymerase with the primase (pr) and the reverse transcriptase (RT) domain and the cellular protein kinase C alpha (PKC). Note there are two start codons in the PreC/core ORF and the three start codons in the HBs ORF. (National Reference Center for Hepatitis B and D, 2013).

Fig. 2.1: The structural components of HBV (left) and open reading frames (OR) for encoding proteins in the covalently closed form of HBV DNA (right). (National Reference Center for Hepatitis B and D, 2013).
2.4. Evolution

Attempts have been made to relate these observations to HBV evolution. Much of our knowledge about human hepatitis has relied heavily upon information derived from infection (natural and experimental) of non-human primates during the last 50 years. However, there seem to be some difficulties due to the relationship between the human genotypes A–E and G (and even more divergent genotype F) to each other and to other primate-associated genotypes. The origin of HBV in humans is as confusing as that of the hepadna viruses from other primates. Different theories have been proposed by investigators on HBV origins based on the hypothesis that the numbers of nucleotide and amino-acid substitutions over time, the molecular clocks, are indicators of viral evolution (Mizokami and Orito., 1999). A primate origin for HBV infection was proposed by MacDonald. This theory based on the finding of variants in chimpanzees (MacDonald et al., 2000) woolly monkeys (Lanford et al., 1998) and orangutans (Warren et al., 1999), suggested that these viruses co-evolved with their primate hosts over periods of 10–35 million years. This hypothesis has been supported by the observations that areas of high HBV prevalence in humans are those in which contact with, and cross-species transmission from primates are most likely (South America, Sub Saharan Africa and Southeast Asia). Indeed, certain HBV genotypes are specific to these three areas (F, E, B/C, respectively). Moreover, the mixture of HBV genotypes found outside these areas, such as Europe and North America, may have resulted from much more recent epidemic spread (Simmonds, 2001). In contrast, a recent emergence hypothesis for HBV infection indicated that the current wide distribution of HBV in Apes must have arisen through several cross-species or subspecies transmission in the relatively recent past (Starkman et al., 2003). Based on observation of Norder et al. that most of the dendrograms obtained from Gibbon and Chimpanzee strains represented early lineages, assumptions were
made that these viruses were indigenous to their respective hosts and not recent acquisitions from man (genotype F). Therefore, they suggested that either genotype F represents an early cross-species transfer from a non-Ape primate to man, or that a hepadna virus of a common ancestor to man and Apes gave rise to two viral lineages (Norder et al., 1996). Thus, they proposed that the evolutionary history of HBV corresponds to the spread of anatomically modern humans as they migrated from Africa 100 000 years ago (Norder et al., 1994), and different genotypes infecting humans evolved since this dispersal. However, this hypothesis does not explain the origin of the various non-human primate viruses which are interspersed among the human genotypes in the phylogenetic tree. The phylogenetic tree of the various primate HBV variants in now day reflects the phylogeny of the host species, as would be expected for co-speciation (Simmonds , 2001). For example the presence of genotype F in native American populations is inconsistent with the presence of genotype B and C in their genetically nearest relatives, the Mongoloid Northeast Asian. Indeed, there is little relationship between HBV genotype distribution with any of the other human population groups (Southeast Asian, Caucasians, and African population). Alternatively, the HBV genotypes may have evolved later than, and independent of, human migration (Gunther et al., 1999). According to the finding that HBV showed a nucleotide substitution rate of 2.1 \( \cdot 10^{-5} \) substitutions per site per year over a mean observation period of 22 years, Orito proposed that the human genotypes of HBV would have originated from a common ancestor approximately 3000 years ago (Orito et al., 1989). In their study, they showed that three major clusters of HBV (birds, mammals and humans) diverged from their common origin in the same order as that of host evolution. They concluded that the evolution of the hepadna virus family was independent of host-species divergence and for HBV in humans this has taken place much more recently than has divergence of humans. Alternatively, a New World origin for
HBV was proposed by Bollyky, who suggested that HBV originated from the Americas and spread into the Old World over the last 400 years after contact from Europeans during colonization; a genotype F origin. Further, they considered the possibility that if the virus originally entered the Americas from Asia, this may have required a higher rate of nucleotide substitution as it adapted to this naive human population (Bollyky and Holmes, 1999). However, the main problem for this hypothesis is the observation of the widespread distribution of HBV in Old World primate species. A remarkable example is a shared genotype of HBV infecting West African chimpanzees (Takahashi et al., 2000), which showed approximately 11% divergence from the human genotypes A–E. This finding was based on analysis of mutations in the C-terminus region of the core protein (which is well conserved among hepadna viruses) between human genotypes E/F and the chimpanzee one. Interestingly, HBV-E/F and the non-human primate hepadna viruses had a common motif within 20 nucleotides upstream from the stop codon for the core gene, whereas, HBV-A/B/C/D genotypes contained a different motif at this site. It has been revealed that sequences in wild-born Old World primates from Africa and Southeast Asia were unrelated to five human HBV strains (A–E); the conclusion is that the virus was not acquired from humans, and all the Old World non-human primate HBVs were on a common ancestral branch (Robertson, 2001). This finding together with the observation that the closest relative of the woolly monkey HBV is genotype F, led to the speculation that chimpanzees have their own hepadna virus, which resembles the human hepadna virus (genotype F). At present, the problems associated with each of these hypotheses for the origin of HBV prevent a definitive conclusion. Resolution of these issues requires more extensive sequence analysis of HBV in poorly sampled areas as well as combined human and primate studies together with utilizing models of DNA substitution which better describe the process of viral evolution.
2.5. Genome

The viral genome consists partially of double-stranded circular DNA, 3200bp in length. Different HBV isolates share 90-98% nucleotide sequence homology. The full-length DNA minus strand (L or long strand) is complementary to all HBV mRNAs; the positive strand (S or short strand) is variable and between 50% and 80% of unit length.

There are four open reading frames that encode seven polypeptides. These include structural proteins of the virion surface and core, a small transcriptional transactivator (X), and a large polymerase (P) protein that include DNA polymerase, reverse transcriptase, and RNase H activities. The S gene has three in-frame initiation codons and encodes the major HBsAg, as well as polypeptide containing in addition pre-S2 or pre-S1 and pre-S2 sequences. The C gene has two in-frame initiation codons and encodes HBcAg plus the HBe protein, which is processed to produce soluble HBeAg. The particles containing HBsAg are antigenically complex. Each contain group-specific antigen, a, in addition to two pairs of mutually exclusive subdeterminants, d/y and w/r. Thus, four phenotypes of HBsAg have been observed: adw, ayw, adr, and ayr. (Jawetz et al, 2010).

2.5.1. Genotypes

HBV is differentiated into many genotypes, according to genome sequence. To date, eight well-known genotypes (A-H) of the HBV genome have been defined. Moreover, two new genotypes, I and J, have also been identified. Some HBV genotypes are further classified as sub-genotypes. HBV sequence is characterized by > 8% nucleotidedifferences for genotype, and 4%-8% nucleotide differences for sub-genotype. Over 30 related sub-genotypes belonging to HBV genotypes have
been determined to date, but the mechanisms of different pathogenic characteristics of HBV genotypes are not known for certain. Many studies have reported that different genotypes and sub-genotypes show different geographical distribution, and are related to disease progression, clinical progression, response to antiviral treatment, and prognosis. A-D and F genotypes are divided into various sub-genotypes; no sub-genotypes have been defined for E, G and H genotypes.

Genotype A is widespread in sub-Saharan Africa, Northern Europe, and Western Africa; genotypes B and C are common in Asia; genotype C is primarily observed in Southeast Asia; genotype D is dominant in Africa, Europe, Mediterranean countries, and India; genotype G is reported in France, Germany, and the United States; and genotype H is commonly encountered in Central and South America. Genotype I has recently been reported in Vietnam and Laos. The newest HBV genotype, genotype J, has been identified in the Ryukyu Islands in Japan.

Geographic distribution of HBV genotypes may be related to route of exposure. For example, genotypes B and C are more common in high-endemic regions of perinatal or vertical exposure, which plays an important role in viral transmission. Other genotypes are primarily observed in regions of horizontal exposure (Sunbul, 2014). Therefore, genotyping provides an epidemiological clue in the investigation of acquisition, because this lies in the geographical distribution of HBV. Figure 2.2 shows genotype distribution across the world.
2.6. Life cycle

Attachment to liver-specific receptors (heparansulfate proteoglycan and NTCP, leads to endocytosis of HBV and release of HBV core particles. These are transported to the nucleus and arrested at the nuclear pore complex where the HBV genome is released to the nucleus. In the nucleus, the viral DNA is “repaired” to the covalently closed circular (ccc) DNA and complexed with nucleosomes (not shown). In interaction with transcription factors (not shown), the ccc DNA is transcribed to the pregenomic and subgenomic mRNAs. The mRNAs are transported, mainly without splicing, to the cytoplasm. The two subgenomic mRNAs for the three HBs proteins are translated at the endoplasmic reticulum, assemble to subviral HBsAg particles and are secreted via the Golgi apparatus. In
parallel, the pregenomic mRNA is translated in the cytosol to the HBV core protein and the viral polymerase, whereby the three components assemble to the immature core particle. The HBV genomes mature within the core particles via reverse transcription of the pregenomic mRNA to DNA. The mature core particles can migrate again to the nuclear pore complex or are enveloped by the surface proteins and secreted via the multivesicular bodies (MVB). (National Reference Center for Hepatitis B and D, 2013).

2.7. Stability
It is difficult to assess the stability of HBV due to the lack of suitable laboratory culture system (Greenwood et al., 1996). Indirect evidence has been obtained from the study of recipients of blood products treated in various ways and chimpanzee inoculation experiments. Thus it was established that:- Heating to 600 C for 10 hours inactivates the virus also Treatment with hypochlorite. Or 2% glutaraldehyde for 10 min will inactivate the virus. Studies based on the survival of HBs Ag show that it is much more resistant to destruction (Greenwood et al., 1996).

2.8. Incidence/Epidemiology
Viral hepatitis caused 1.34 million deaths in 2015, Globally, in 2015, an estimated 257 million people were living with chronic HBV infection, The epidemic caused by HBV affects mostly the WHO African Region and the Western Pacific Region. Access to affordable hepatitis testing is limited. Few people with viral hepatitis have been diagnosed (9% of HBV-infected persons, 22 million) Among those diagnosed, treatment has
reached only a small fraction. In 2015, 8% of those diagnosed with HBV infection or 1.7 million persons were on treatment.

Among the 36.7 million persons living with HIV in 2015, an estimated 2.7 million had chronic HBV infection Liver diseases are a major cause of morbidity and mortality among those living with HIV and co-infected with viral hepatitis. These people should be diagnosed and provided with appropriate and effective treatment for both HIV and hepatitis as a priority (WHO, 2017).

2.9. HBV in Sudan
Sudan is classified among countries with a high hepatitis B surface antigen (HBsAg) endemicity of more than 8%. Exposure to HBV infection ranges from 47% to 78% with a hepatitis B surface antigen (HBsAg) seroprevalence ranging from as low as 6.8% in central Sudan to as high as 26% in southern Sudan. Identified risk factors for HBV infection in Sudan include living in southern Sudan, parenteral antischistosomal therapy, sexual promiscuity, and scarification which is a common ritual in southern Sudan. There was no association with schistosomal infection or blood transfusion. These rates are comparable to some African countries where seroprevalence of HBsAg was reported at rates of 15.6% in Burundi, 14% in Central African Republic, and 10% in Uganda. Lower rates were however found in other countries such as Tanzania (4.4%), Nigeria (4.98%), and Ethiopia (7%). Seroprevalence of HBsAg among asymptomatic blood donors ranged from 12.3% in southern Sudan to 17.5% in central Sudan. These studies were carried out in the eighties and nineties when screening of blood and blood products was only done in a few blood banks in the capital, Khartoum. In 2002, a national program for screening blood and blood products for HBV and HCV infection was introduced throughout the whole country.
A high seroprevalence of HBsAg was detected in patients with liver cirrhosis ranging from 31%–61% and similar carrier rates of 43%–60% were found in patients with HCC, indicating that HBV infection is perhaps the commonest risk factor for developing HCC (Mudawi, 2008).

2.10. Transmission of HBV

The hepatitis B virus can survive outside the body for at least 7 days. During this time, the virus can still cause infection if it enters the body of a person who is not protected by the vaccine. The incubation period of the hepatitis B virus is 75 days on average, but can vary from 30 to 180 days. The virus may be detected within 30 to 60 days after infection and can persist and develop into chronic hepatitis B. In highly endemic areas, hepatitis B is most commonly spread from mother to child at birth (perinatal transmission), or through horizontal transmission (exposure to infected blood), especially from an infected child to an uninfected child during the first 5 years of life. The development of chronic infection is very common in infants infected from their mothers or before the age of 5 years. Hepatitis B is also spread by percutaneous or mucosal exposure to infected blood and various body fluids, as well as through saliva, menstrual, vaginal, and seminal fluids. Sexual transmission of hepatitis B may occur, particularly in unvaccinated men who have sex with men and heterosexual persons with multiple sex partners or contact with sex workers. Infection in adulthood leads to chronic hepatitis in less than 5% of cases. Transmission of the virus may also occur through the reuse of needles and syringes either in health-care settings or among persons who inject drugs. In addition, infection can occur during medical, surgical and dental procedures, through tattooing, or through the use of razors and similar objects that are contaminated with infected blood (WHO, 2016).
2.11. Pathogenesis and Immunity

The adaptive immune response is thought to be responsible for viral clearance and disease pathogenesis during hepatitis B virus infection. It is generally acknowledged that the humoral antibody response contributes to the clearance of circulating virus particles and the prevention of viral spread within the host while the cellular immune response eliminates infected cells. The T cell response to the hepatitis B virus (HBV) is vigorous, polyclonal and multispecific in acutely infected patients who successfully clear the virus and relatively weak and narrowly focussed in chronically infected patients, suggesting that clearance of HBV is T cell dependent. The pathogenetic and antiviral potential of the cytotoxic T lymphocyte (CTL) response to HBV has been proven by the induction of a severe necroinflammatory liver disease following the adoptive transfer of HBsAg specific CTL into HBV transgenic mice. Remarkably, the CTLs also purge HBV replicative intermediates from the liver by secreting type 1 inflammatory cytokines thereby limiting virus spread to uninfected cells and reducing the degree of immunopathology required to terminate the infection. Persistent HBV infection is characterized by a weak adaptive immune response, thought to be due to inefficient CD4+ T cell priming early in the infection and subsequent development of a quantitatively and qualitatively ineffective CD8+ T cell response. Other factors that could contribute to viral persistence are immunological tolerance, mutational epitope inactivation, T cell receptor antagonism, incomplete down-regulation of viral replication and infection of immunologically privileged tissues. However, these pathways become apparent only in the setting of an ineffective immune response which is, therefore, the fundamental underlying cause. Persistent infection is characterized by chronic liver cell injury, regeneration, inflammation, widespread DNA damage, and insertional deregulation of cellular growth control.
genes which, collectively, lead to cirrhosis of the liver and hepatocellular carcinoma. (Chisari et al., 2010).

2.12. Clinical picture

HBV infection leads to a wide spectrum of liver disease ranging from acute hepatitis (including fulminant hepatic failure) to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC).

The diagnosis of HBV infection and its associated disease is based on a constellation of clinical, biochemical, histological, and serologic findings. (Hollinger et al., 2001).

2.12.1 Acute hepatitis

The incubation period is 1-6 months in the acute phase of hepatitis B infection. Anicteric hepatitis is the predominant form of expression for this disease. The majority of the patients are asymptomatic, but patients with anicteric hepatitis have a greater tendency to develop chronic hepatitis. Patients with symptomatology have the same symptoms as patients who develop icteric hepatitis.

Icteric hepatitis is associated with a prodromal period, during which a serum sickness–like syndrome can occur. The symptomatology is more constitutional and includes the following:

- Anorexia
- Nausea
- Vomiting
- Low-grade fever
- Myalgia
• Fatigability
• Disordered gustatory acuity and smell sensations (aversion to food and cigarettes)
• Right upper quadrant and epigastric pain (intermittent, mild to moderate)

Patients with fulminant and subfulminant hepatitis may present with the following:

• Hepatic encephalopathy
• Somnolence
• Disturbances in sleep pattern
• Mental confusion
• Coma
• Ascites
• Gastrointestinal (GI) bleeding
• Coagulopathy (Nikolaos, 2016).

2.12.2. Chronic hepatitis B infection

Between 350 million and 400 million persons worldwide are chronically infected with hepatitis B virus (HBV). (Lavanchy, 2004) The two primary adverse outcomes of chronic infection are hepatocellular carcinoma (HCC) and cirrhosis, either of which can lead to a liver-related death.
### 2.12.2.1. Phases Of Chronic hepatitis B infection

**Table 2.1. Phases of Chronic Hepatitis B Infection**

<table>
<thead>
<tr>
<th>Immune Tolerant Phase</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Occurs primarily after perinatal infection from HBsAg/HBeAg-positive mother</td>
<td></td>
</tr>
<tr>
<td>• ALT levels are normal</td>
<td></td>
</tr>
<tr>
<td>• HBV DNA _ 200,000 IU/mL (_1 million copies), often above 107-8 IU/mL</td>
<td></td>
</tr>
<tr>
<td>• Liver biopsy is normal or shows only minimal inflammation with no or minimal fibrosis</td>
<td></td>
</tr>
<tr>
<td>• Occurs most frequently in HBV genotype C infection</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Immune Active (Clearance) Phase</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>• HBeAg-positive chronic hepatitis B</td>
<td></td>
</tr>
<tr>
<td>• Elevated ALT levels</td>
<td></td>
</tr>
<tr>
<td>• HBV DNA _ 20,000 IU/mL</td>
<td></td>
</tr>
<tr>
<td>• Anti-HBe–positive chronic hepatitis B</td>
<td></td>
</tr>
<tr>
<td>• Elevated ALT</td>
<td></td>
</tr>
<tr>
<td>• HBV DNA _ 2000 IU/mL</td>
<td></td>
</tr>
<tr>
<td>• Hepatic inflammation with or without fibrosis on biopsy often present in both HBeAg-positive and HBeAg-negative immune active phase</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inactive Phase</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Anti-HBe</td>
<td></td>
</tr>
<tr>
<td>• ALT levels normal</td>
<td></td>
</tr>
<tr>
<td>• HBV DNA _ 2000 IU/mL</td>
<td></td>
</tr>
<tr>
<td>• Hepatic inflammation minimal or absent</td>
<td></td>
</tr>
<tr>
<td>• Hepatic fibrosis may improve over time</td>
<td></td>
</tr>
<tr>
<td>• HBsAg clearance may eventually occur</td>
<td></td>
</tr>
</tbody>
</table>


### 2.12.3. HBV and hepatocellular carcinoma (HCC)

The risk of hepatocellular carcinoma (HCC) increases with increasing level of hepatitis B virus (HBV) in serum (viral load). However, it is unclear whether
genetic characteristics of HBV, including HBV genotype and specific genetic mutations, contribute to the risk of HCC. We examined the HCC risk associated with HBV genotypes and common variants in the precore and basal core promoter (BCP) regions. HBV genotype C and specific alleles of BCP and precore were associated with risk of HCC. These associations were independent of serum HBV DNA level (Yang et al., 2008).

2.12.4. Persistence of HBV

Only a small portion of patients can undergo HBsAg seroclearance with antiviral therapy; this response has been associated with several host and viral factors. Unfortunately, viral rebound often occurs following discontinuation of antiviral therapy. Accumulating evidence has proven that the persistence of covalently closed circular DNA (cccDNA) is the major barrier to eradicating chronic HBV infection. In addition, persistent cccDNA can occasionally be detected even in the liver of patients with resolved hepatitis B infections. More importantly, the persistent cccDNA appears to be replication competent at least in a significant portion of these patients, as evidenced by the fact that HBV reactivation occurs when they receive intensive immunosuppressive agents, such as chemotherapy (Hung-Chih and Jia-Horng, 2014).

2.13. Laboratory Diagnosis

2.13.1. Collection, transport and storage of sample

HBV antigen or antibodies are stable at room temperature for several hour to days, can be stored at -20 °C to – 70°C for many years. Repeat freezing and thawing can lead to degradation of HBV serologic markers. Furthermore, use of hemolyzed sample should be avoided as interferes with detection signals. Plasma is separated
from blood collected in containers with EDTA or citrate dextrose as the anticoagulant (Horvart and Tegtmeier, 2011).

2.13.2. Diagnosis
Diagnosis of hepatitis is made by:

2.13.2.1. Biochemical assessment of liver function. Initial laboratory evaluation should include:
Total and direct bilirubin, ALT, AST, alkaline phosphatase, prothrombin time, total protein, albumin, serum globulin, complete blood count, and coagulation studies. (Hollinger et al., 2001).

2.13.2.2. Diagnosis is confirmed by demonstration of specific Antigens:
Three clinical useful antigen systems have been identified for hepatitis B:
Hepatitis B surface antigen (HBsAg)
Hepatitis B core antigen (HBcAg)
Hepatitis B e antigen (HBeAg)
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).
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. The presence of HBeAg is associated with relatively high infectivity and severity of disease.

Antibodies: Three clinical useful antibody systems have been identified for hepatitis B:
Antibody to HBsAg (anti-HBs) Anti-HBs replaces HBsAg as the acute HBV infection is resolving. Anti-HBs generally persists for a lifetime in over 80% of patients and indicates immunity. (Lavanchy, 2004).

Antibody (anti-HBc IgM and anti-HBc IgG) is the first antibody to appear. Demonstration of anti-HBc in serum indicates HBV infection, current or past. IgM anti-HBc is present in high titre during acute infection and usually disappears within 6 months, although it can persist in some cases of chronic hepatitis. This test may therefore reliably diagnose acute HBV infection. IgG anti-HBc generally remains detectable for a lifetime (Robinson, 1995).

Antibody to HBeAg (anti-HBe) Anti-HBe appears after anti-HBc and its presence correlates to a decreased infectivity. Anti-HBe replaces HBeAg the resolution of the disease (Mahoney and Kane, 1999).

2.13.2.3. Tests specific for complete virus particles or DNA:

DNA polymerase-containing virions, and for hepatitis Delta antigen (HDAg) and hepatitis Delta virus (HDV) RNA in liver and serum are available only in research laboratories (Hollinger et al., 2001)
2.13.2.4. Table 2; Interpretation of the Hepatitis B Panel Tests Results Interpretation

Hepatitis B Virus Serological and Virological Markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>HBV infection, both acute and chronic</td>
</tr>
<tr>
<td>HBeAg</td>
<td>High-level HBV replication and infectivity; marker for treatment response</td>
</tr>
<tr>
<td>HBV DNA</td>
<td>Level of HBV replication; primary virologic marker for treatment response</td>
</tr>
<tr>
<td>Anti-HBc (IgM)</td>
<td>Acute HBV infection; could be seen in flare of chronic hepatitis B</td>
</tr>
<tr>
<td>Anti-HBc (IgG)</td>
<td>Recovered or chronic HBV infection</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>Recovered HBV infection or marker of HBV vaccination; immunity to HBV infection (titer can be measured to assess vaccine efficacy).</td>
</tr>
<tr>
<td>Anti-HBe</td>
<td>Low-level HBV replication and infectivity; marker for treatment response</td>
</tr>
<tr>
<td>Anti-HBc (IgG)</td>
<td>Recovered HBV infection; could lose anti-HBs</td>
</tr>
<tr>
<td>Anti-HBc (IgG)</td>
<td>Chronic HBV infection</td>
</tr>
<tr>
<td>Anti-HBc (IgG)</td>
<td>Latent or occult HBV infection (PCR)</td>
</tr>
</tbody>
</table>

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2809016/table/T1/?report=objectonly

2.14. Prevention

The Advisory Committee on Immunization Practices (ACIP) recommends screening pregnant women (including women previously vaccinated or previously tested) for HBsAg during the first prenatal visit of each pregnancy. Unvaccinated HBsAg-negative pregnant women at continuing risk for HBV exposure should initiate vaccination during pregnancy. Women at risk include
those who are household contacts or sex partners of HBsAg-positive persons, injection drug users, have endstage renal disease, HIV infection, chronic liver disease, diabetes, or other factors. Women who are not screened prenatally, or who continue to be at risk for HBV infection, should be screened or rescreened at presentation for delivery (Noele, 2014).

2.15. Vaccination

Hepatitis B vaccine is produced by recombinant DNA technology, most commonly in yeast. The complete vaccination series consists of three doses of vaccine; the first two doses are usually given 1 month apart, with the third dose 1–12 months later. The WHO recommended schedule for hepatitis B immunization of children consists of a dose within 24 hours of birth followed by a second and third dose of hepatitis B containing vaccines at intervals of at least 4 weeks. A complete series of immunization provides protection for at least 25 years and, according to current scientific evidence, probably for life. Boosters are not recommended for routine immunization programs. Because of the prolonged incubation period of hepatitis B, some protection will be afforded to most travelers following the second dose given before travel however; the final dose should always be given.

A combination vaccine that provides protection against both hepatitis A and hepatitis B should be considered for travelers who may be exposed to both organisms. This inactivated vaccine is administered as follows: day 0; 1 month; 6 months. A rapid schedule of day 0, 1 month and 2 months with an additional dose at 12 months, and a very rapid schedule of day 0, day 7 and day 21 with a booster dose at 12 months, have been proposed by the vaccine manufacturer and approved by national regulatory authorities in some countries. Recommended for Hepatitis B vaccine should be considered for all non-immune individuals travelling to
countries or areas with moderate to high risk of infection. It can be administered to infants from birth (Global Health Report, 2017).

2.16. Treatment

Currently, seven drugs are available: IFN-α, pegylated interferon, lamivudine, adefovir dipivoxil, entecavir, telbivudine and tenofovir. The choice of the drugs should always take into consideration the clinical features of patients, the antiviral efficacy of each drug, the risk of developing resistance, the long-term safety profile, the method of administration and the cost of therapy. Ideal candidates for treatment are hepatitis B e antigen-positive patients with a prolonged phase of immune clearance and hepatitis B e antigen-negative patients with elevated levels of serum HBV DNA, abnormal alanine aminotransferase and histologic evidence of moderate or severe liver necroinflammation and/or fibrosis. NUCs suppress viral replication by inhibiting HBV viral polymerase, whereas interferon therapy works by enhancing the host immune response. The two main treatment strategies are finite therapy with interferon or NUC therapy (for those who maintain an SVR off treatment), or long-term therapy with one or more NUCs, for those with cirrhosis or who do not maintain an SVR sustained virologic response (Di Marco and Craxi, 2009).
CHAPTER THREE
MATERIALS AND METHODS
3.1. Study design
This study was descriptive, cross-sectional and analytical based study.

3.2. Study population
Population with different ages, were the source of samples.

3.3. Study area
The study was conducted at Almanagil province, Sharafat village.

3.4. Sampling
Sampling Technique: Simple random sample
Sample size: A total of ninety two subjects (n=92) were enrolled in this study.

3.5. Selection criteria
3.5.1. Inclusion criteria
Population live at Sharafat countryside Almanagil.

3.5.2. Exclusion criteria
Children below 5 years were excluded.

3.6. Ethical consideration
Each individual invited to participate in the study was informed about the purpose of Study before data collection. The study was approval by Ethical committee, College of Medical Laboratory Science, Sudan University of Science and Technology

3.7. Data collection
The data was collected by structured questionnaire(Appendix).

3.7.1. Interview:
An interview with questionnaire to obtain the clinical data was used for each participant in this study.
3.7.2 Blood samples collection
The area of blood collected was disinfected by (70%) Alcohol. Venous blood (3ml) were taken from each participant by standard procedures and put in plain containers then left to clot for 15 minutes and then centrifuged at 1500 rpm for 5 minutes. The serum was separated and kept at -20°C until used.

3.7.3 Methods
3.7.3.1 Detection of HBsAg ByICT
The ICT tests were produced from ACON laboratories. San Diego, USA (Appendix) and supplied as follows:

a) Test devices contain anti-HBsAg particles coated on the membrane.
b) Disposable specimen dropper.

Materials required but not provided
a) Specimen collection containers.
b) Pipette and disposable tips.
c) Centrifuge
d) Timer

Storage and stability
The kits were stored at room temperature or refrigerated (2–30°C). The test devices were kept in the sealed pouch until used. The expiry date was observed.

ICT test procedure:
Specimens (serum) were allowed to reach room temperature (20–25°C) before running the test. Frozen specimens were completely thawed and mixed well prior to testing. The test devices were removed from the sealed pouch and placed on clean and level surface and used immediately and the test was performed within one hour. Then 3 full drops of serum (approx. 100 μl) were transferred into specimen well (S)
of the testdevice. Results were read after 10 minutes as indicated by the appearance of the red lines.

**Interpretation of results:**

a) Positive test: Two distinct redlines appear, one line should be in the control region© and another line should be in the test region (T).
b) Negative test: When one redline appears in the control test region (T).
c) Invalid test: If no line appeared on the positive control region (C).

### 3.7.3.2 Detection of HBsAg by Sandwich Enzyme linked Immune-Sorbent Assay (ELISA)

**Principle:**
The test is an enzyme-immunoassay based on a sandwich principle. Polystyrene microtiter strip wells have been coated with monoclonal anti-HBs(antibody toHBsAg). Patient serum sample is added to microwells. During incubation, the specific immune-complex formed in case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove serum proteins, second antibody conjugated to the enzyme HRB and directed against a different epitope of HBsAg is added to the wells. During the second incubation step, these HRB conjugated antibodies will be bound to any anti-HBs-HBsAg complexes previously formed during the first incubation, and the unbound HRB conjugate is then removed by washing. After washing to remove unbound HRB conjugate, chromogen solutions containing TMB and urea peroxidase are added to the wells in presence of antibody-antigen-antibody HRB sandwich immune-complex, the colourless chromogens are hydrolyzed by the bound HRBconjugate to a blue coloured product. The blue colour turns yellow after stopping the reaction using stop solution. The colour intensity can be measured and it is proportional to the
amount of antigen captured in the wells and to its maount in the sample respectively. Wells contain samples negative for HBsAg remain colourless.

**Procedure**

**Step1-Reagent preparation**

The reagent and sample were allowed to reach room temperature(18-30 °C for 15-30 minutes.

The stock wash buffer had been diluted **1to20** with distilled water.

**Step2- Numbering of wells**

The strip needed were set in strip holder and sufficient number of wells including one blank(A1) two negative control(B1,C1) and one well as positive control(D1) were numbered.

**Step 3-Adding sample diluent:**

Twenty ul of sample Diluent was added to each well except the blank and mix by tapping the plate gently

**Step 4 adding sample**

hundred ul of positive control, negative control and specimen were added into their respective wells except blank.

**Step 5 Incubation**

The plate was covered with the plate cover and incubated for 60 minutes at 37°C

**Step 6 Added HRP Conjugate:**

Then 50ml of HRP-conjugate were also added to each well except to blank well and mixed by tapping the plate gently.

**Step 7- Incubation**

The plate was covered with the plate cover and incubated for 30 minutes at 37°C.
Step 8- washing
After the end of the incubation the plate cover was removed, each well was washed 5 times with diluted washing buffer each time the well were allowed to soak for 30-60 second. After the washing the plate was turn down onto blotting paper to remove any remainders.

Step9- Coloring
Fifty ul of chromogen A and 50ul of chromogen B were added into each well including blank and mixed by tapping plate. The plate was Incubated at 37°C for 15 minutes, avoiding light. Blue color was developed in positive control and HBsAg positive sample wells.

Step10- stopping the reaction
Fifty ul of stop solution were added into each wells and mixed gently intensive yellow color was developed in positive control and HBsAg positive sample wells.

STEP11 –Measuring the Absorbance
The absorbance was read at 450nm using the ELISA reader.

calculation of the result
The result were calculated by relating each specimen absorbance(A) value to cutoff value(C.O) of the plate.

Calculation of cut-off value
(C.O)=NC×2.1 NC (the mean absorbance value to two negative controls).

Quality control range
The A value of blank well which contains only chromogen and stop solution should be less than 0.080 at 450nm The A value of positive control must be more than or equal 0.800 at 450nm The A value of negative control must be less than 0.100 at 450 nm.
Interpretation of the results

Negative Results
Sample giving A value less than cut-off value are negative for this assay which indicate that no HBsAg antibodies have been detected with this HBsAg ELISA kits therefore the patient is probably not infected with hepatitis B virus.

Positive Results:
Samples giving A value greater than or equal to cut-off value are considered initially reactive which indicate that HBV surface antigen has probably been detected with this HBsAg ELISA kit.

Borderline
Sample with A value to cut-off ratio between 0.9 and 1.00 are considered borderline samples And retest is recommended. repeatedly positive sample can be considered positive for HBsAg

Data Analysis
Collected data were analysis by a computer system using statistic package for social sciences(SPSS) program using the chi-square test statistical significance was set at (p. values<0.05).

3.7.3.3 Detection of HBV DNA by Nested PCR
Methods
DNA extraction by phenol chloroform method
procedure
Five hundred μL of 5% sodium dodecyl sulfat (SDS) was added on Eppindrof tube, then 250 μL of patient’s serum and 20 μL of proteinase K was added and incubated at 56°C for one hour then the sample was left at 95°C for 10 minutes to deactivate proteinase K then 500 μL of phenol: chloroform: isoamylalcohol (25:24:1) were added and centrifuged at12000 rpm for 10 minutes.
The upper layer was transferred into new eppendof tube. The Three steps were repeated twice then 25 μL of 5% sodium chloride were added and mixed. 1 ml of absolute ethanol was then added and well mixed. Then incubated at -20°C overnight. The mixture was centrifuged at 14000 rpm for 15 minutes and the supernatant was discarded and the tube was dried. 200 μL 70% ethanol were then added and shaken up until the pellet disappeared and centrifuged at 14000 rpm for 15 minutes. The supernatant was discarded and the tubes were inverted opened for two hours. 150μL of WFI were added and DNA preserved at -20°C.

**Amplification**

The first-round PCR primers (outer primer pairs) and the second-round PCR primers (innerprimer pairs) were designed on the Hepatitis B virus strain gmc5 nonfunctional surface antigen (S) gene. PCR was carried out in a tube containing 25μL of the reaction mixture made up of 0.2μL of the outer primer (1ul F and 1ul R), Maxime PCR PreMix Kit contain (2.5U of i-Taq DNA polymerase, 2.5Mm each of the four deoxynucleotides, 1x of reaction buffer, 1x of Gell loading buffer), 5 μL of DNA Template was added. Then 13 μL of distilled water. The samples were denatured for two min at 95°C followed by 30 cycles each consisted of 30 s at 95°C, annealing for 30 sat 57°C, and extension one min at 72°C in an Eppendrof thermal cycler. 5 μL aliquot of the first-round PCR product was added to a tube containing the second of inner primer, each of the deoxynucleotides, i-Taq DNA polymerase, PCR buffer and DW as in the first reaction. The samples were amplified as above.
Detection of PCR product

Ten microliter of the reaction product was electrophoresed on a 2% agarose gel, stained by ethidium bromide, and evaluated under UV light. Negative and positive controls were also treated as samples.

Table 3.1: Sequence of the oligonucleotide primers and expected PCR product size

<table>
<thead>
<tr>
<th>Primer direction</th>
<th>5---3 sequence</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer F</td>
<td>TTATCGCTGGATGTGTCTGC</td>
<td>405</td>
</tr>
<tr>
<td>Outer R</td>
<td>TACAGACTTTGGCCCCCAATA</td>
<td></td>
</tr>
<tr>
<td>Nested F</td>
<td>GTTGCCCAGTTTGTCCTCTAA</td>
<td>250</td>
</tr>
<tr>
<td>Nested R</td>
<td>AAGCCCTACGAACCACTGAA</td>
<td></td>
</tr>
</tbody>
</table>

3.8. Data collection and analysis

Processing and analysis of data were carried out by means of the statistical package for the social sciences (SPSS). A descriptive statistic frequency was used to assess the risk. Cross tabulation (chi-square) was used to compare the variable with positive result.
CHAPTER FOUR
RESULTS
RESULTS

Out of 92 samples, 6 (6.5%) were found positive by ICT, 30(32.6%) were found positive for HBsAg by ELISA. The PCR confirmed that 30 positive cases obtained by ICT and ELISA.

4.1 ICT results:
Ninety two serum samples were collected from people at Sharafat village.6(6.5%) samples were found positive for HBsAg respectively when screened by ICT.

4.2 ELISA results:
The previous test samples when tested by ELISA using HBsAgsandwich ELISA, 30 (32.6%) samples were found positive for HBsAg.

4.3 Distribution between risk factors:
Gender: 16 (17.4%) of cases were male and 14 (15.2%) were female.(table 4.1 and figure 4.1).
Age: The highest results (8.7%) were distributed at age group (5-15) years and there were no infections between age group (56-65).
Marital status: 18 (19.6%) of cases were married and 12 (13%) were not married.
Blood transfusion: No infections noticed among transfused people.
Surgical history: 9 (9.8%) of cases had history of surgical operation and 21 (22.8%) had no.

4.4 Comparison between ICT and ELISA tests of HBsAg:
Out of 30 positive tests by ICT and ELISA, there were 24 positive samples that were found negative by ICT test.

4.5 Nested PCR Results: (Detection of HBV-DNA):
Thirty serum samples with HBsAg positive screened by ICT and ELISA were investigated to detect HBV-DNA using the nested PCR. HBV-DNA was detected in 30 samples of HBsAg positive by ELISA.
Table 4.1 Frequency of HBV in males and females

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count</td>
<td>Count</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>% of Total</td>
<td></td>
</tr>
<tr>
<td>HBV</td>
<td>positive</td>
<td>negative</td>
<td>Total</td>
</tr>
<tr>
<td>Male</td>
<td>16</td>
<td>39</td>
<td>55</td>
</tr>
<tr>
<td>% of Total</td>
<td>17.4%</td>
<td>42.4%</td>
<td>59.8%</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>23</td>
<td>37</td>
</tr>
<tr>
<td>% of Total</td>
<td>15.2%</td>
<td>25.0%</td>
<td>40.2%</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>62</td>
<td>92</td>
</tr>
<tr>
<td>% of Total</td>
<td>32.6%</td>
<td>67.4%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

P(0.380) Insignificant level

Fig 4.1 Frequency of HBV in male and female.
(Table 4.2) The highest percentage of population infected by HBV was found in the age 5-15 years and no hepatitis infection was found in the age 56-65 years.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Positive ELISA</th>
<th>Negative ELISA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-15</td>
<td>8</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>8.7%</td>
<td>9.8%</td>
<td>18.5%</td>
</tr>
<tr>
<td>16-25</td>
<td>7</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>7.6%</td>
<td>10.9%</td>
<td>18.5%</td>
</tr>
<tr>
<td>26-35</td>
<td>4</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>4.3%</td>
<td>25.0%</td>
<td>29.3%</td>
</tr>
<tr>
<td>36-45</td>
<td>7</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>7.6%</td>
<td>9.8%</td>
<td>17.4%</td>
</tr>
<tr>
<td>46-55</td>
<td>4</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>4.3%</td>
<td>10.9%</td>
<td>15.2%</td>
</tr>
<tr>
<td>56-65</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>.0%</td>
<td>1.1%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>62</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>32.6%</td>
<td>67.4%</td>
<td>100%</td>
</tr>
</tbody>
</table>

P(0.185)
The distribution of HBV infection on married and non married is shown in (Table 4.3) and (Fig 4.2).

**Table 4.3 Frequency of HBV on married and non married**

<table>
<thead>
<tr>
<th>HBV</th>
<th>positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marid yes</td>
<td>Count</td>
<td>18</td>
<td>47</td>
</tr>
<tr>
<td>Total   no</td>
<td>% of Total</td>
<td>19.6%</td>
<td>51.1%</td>
</tr>
<tr>
<td>Count</td>
<td>12</td>
<td>15</td>
<td>70.7%</td>
</tr>
<tr>
<td>% of Total</td>
<td>13.0%</td>
<td>16.3%</td>
<td>27</td>
</tr>
<tr>
<td>Count   no</td>
<td>30</td>
<td>62</td>
<td>92</td>
</tr>
<tr>
<td>% of Total</td>
<td>32.6%</td>
<td>67.4%</td>
<td>100%</td>
</tr>
</tbody>
</table>

P(0.146)

**Fig 4.2 Frequency of HBV among married people**
(Table 4.4), (Figure 4.3) showed the effect of surgery on the prevalence of HBV.

**Distribution of HBV among who’s had a surgical history**

<table>
<thead>
<tr>
<th>HBV</th>
<th>positive</th>
<th>negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>surgical yes</td>
<td>Count 9</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>9.8%</td>
<td>14.1%</td>
</tr>
<tr>
<td>no</td>
<td>Count 21</td>
<td>49</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>22.8%</td>
<td>53.3%</td>
</tr>
<tr>
<td>Total</td>
<td>Count 30</td>
<td>62</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>32.6%</td>
<td>67.4%</td>
</tr>
</tbody>
</table>

P(0.242)

**Figure 4.3 Frequency of HBV among who’s had surgical process**
Table 4.5 showed no affect of transfusion on the prevalence of HBV

Table 4.5 Effect of blood transfusion on HBV infection

<table>
<thead>
<tr>
<th>Transfusion</th>
<th>HBV</th>
<th>positive</th>
<th>negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>yes</td>
<td>Count</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>.0%</td>
<td>5.4%</td>
<td>5.4%</td>
</tr>
<tr>
<td>no</td>
<td>Count</td>
<td>30</td>
<td>57</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>32.6%</td>
<td>62.0%</td>
<td>94.6%</td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td>30</td>
<td>62</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>32.6%</td>
<td>67.4%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

P(0.110)>0.05

Fig 4.4 Effect of blood transfusion on HBV infection
Table 4.6, Fig 4.5 show a comparison between ICT result and ELISA result and there was a great difference between them.

**Table 4.6 Comparison between ICT and ELISA**

<table>
<thead>
<tr>
<th></th>
<th>+ve ELISA</th>
<th>-ve ELISA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ict</strong></td>
<td><strong>Positive</strong></td>
<td><strong>Count</strong></td>
<td><strong>6</strong></td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td></td>
<td><strong>6.5%</strong></td>
</tr>
<tr>
<td><strong>Negative</strong></td>
<td><strong>Count</strong></td>
<td></td>
<td><strong>24</strong></td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td></td>
<td><strong>26.1%</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>Count</strong></td>
<td></td>
<td><strong>30</strong></td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td></td>
<td><strong>32.6%</strong></td>
</tr>
</tbody>
</table>

P (0.001)

**Fig 4.5 Comparison between ICT and ELISA**
Fig. 4.6: Detection of HBsAg by ELISA test

Fig. 4.7: Ethidium bromide stained agarose gel (2%) electrophoresis of HBV nested PCR products with a band of expected size 250 bp, carried out on DNA samples extracted from serum, Lane 5: 50 bp ladder, with fragment size indicated along the Center of the figure, from the left 3 positive control, 4 negative control, 5 ladder, 6-7-8 serum samples showed Positive results at 250 bp.
CHAPTER FIVE

DISCUSSION
CHAPTER FIVE
DISCUSION

5.1 DISCUSSION

Hepatitis B virus remains a major problem in developing countries. The HB viruses (HBV) express antigens such as HBsAg (surface antigen) on its surface, provoking both cell-mediated and humoral responses (Gill and Beeching, 2004). This study is focused on prevalence of HBV on Almanagil province at Algazeira State in Sudan by molecular and serological diagnosis. The number of 92 serum samples were collected randomly. Out of 92 samples 6 (6.5%) were found positive for HBsAg, when tested by ICT, while 30 samples (32.6%) were found positive for HBsAg. The intensity of the red colour in the test region (T) in ICT may vary depending on the concentration of HBsAg present in the sample. Therefore, any shade of red color that develops in the test region was considered positive., (Elsheik et al., 2007) reported seroprevalence of 5.6% among antenatal care attendants in central Sudan.

When we compared the ELISA and ICT test the ELISA test detected 24 more positive sample that were negative by ICT test. Previous study by (Maaweya, 2002) showed a very high agreement between ICT assay and ELISA test 99.79 % and no statistical significant difference was obtained and both assay were sensitive, specific and reliable tests. The low prevalence of HBV infection when tested by ICT compare to ELISA indicate that ICT test is less sensitive, the test could be used for screening but it should be confirmed by ELISA test. In this study HBV infection was found high among young age group (8.7%), which indicate congenital transmission of HBV from mother to fetus among HBsAg positive patients.
(Ayoola et al., 1981). The present study is in support of this assumption and 26.6% of the positive HBsAg within the age of less than 15 years.

Five persons of cases had history of blood transfusion but all of them show negative result for HBsAg. Screening of blood donors and blood products for HBsAg has been shown to be highly effective in preventing transmission and post-transfusion of hepatitis B virus and eliminate the risk of infection (Zou, 2009). In the present study PCR was covered out 30 positive samples tested for HBsAg by ELISA. (Mas et al, 1990) reported that Hepatitis B virusDNA was found in only two patients out of 29 patients.

Generally, results of this study were considerably higher than that reported at Central Sudan (6.8%) (Mudawi, 2008). The prevalence of HBV was higher among males (17.4%) than females (15.2) similar results were reported by Baig (2009). It was higher in age (5-15) and no infection at age (56-65). Also it higher in the married sample (19.6%) than the unmarried (13%) similar results were reported by Amini et al (1994). And higher for those had no surgical operation (22.8%) than those with history of surgical (8.9%). The result of blood transfusion disagree with the study of Yuen et al (2011) that showed 8.2% of HBV infection came through blood transfusion.
5.2 CONCLUSION

1. High incidence of HBsAg (32.6%) was found in randomly selected sample from Sharafat village at Almanagil and increased among young ages which indicate congenital transmission of disease.

2. In this study we detected the HBV DNA to confirm HBsAg-ELISA positive samples.

3. ICT is less reliable as ELISA for screening for HBsAg.

4. There was a very low agreement between ICT and ELISA and significant difference was observed by
5.3 Recommendations:

1. ELISA should be introduced along side ICT test.
2. Any person should be vaccinated.
3. Blood donors must be tested for HBsAg before transfusion to recipient.
4. Further studies with large number of participants and more advanced techniques.
5. Public awareness of HBV must be increased.
REFERENCES


Hung-Chih Yang and Jia-Horng Kao.(2014);Persistence of Hepatitis B cccDNA in hepatocytes. Emerging Microbes and Infections, e64; doi:10.1038.


National Reference Center for Hepatitis B and D, (2013), Hepatitis B: how it began and where we are now. Gerlich Virol, 10:239.


Noele P, N. (2014). Division of Viral Hepatitis, Vaccine Research and Policy Unit, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, 1600 Clifton Road, MS-G37, Atlanta, GA 30333.


Appendix

Sudan University of Science and Technology

College Of Graduate Studies

Questionnaire

Serological and Molecular detection of Hepatitis B virus in Elmanagil District in Algazeira State.

Name: ........................................ Serial No: ..............................

Gender: ........................................... Age: ..............................

Marital Status:
Married ( ) Single ( )

Previous blood transfusions:
Yes ( ) No ( )

History of Surgical operation:
Yes ( ) No ( )

Symptoms of jaundice:
Yes ( ) No ( )
APPENDIX

Fig. 4.7: Detection of HBsAg by Immune-chromatography Test (ICT).
Fig. 10: Ethidium bromide stained agarose gel (2%) electrophoresis of HBV nested PCR products with a band of expected size 250 bp, carried out on DNA samples extracted from serum, Lane(5) M: 100 bp ladder, with fragment size indicated along the Center of the figure, from 1-8 serum samples showed Positive.
Fig. 11: Ethidium bromide stained agarose gel (2%) electrophoresis of HBV nested PCR products with a band of expected size 250 bp, carried out on DNA samples extracted from serum, Lane M: 100 bp ladder, with fragment size indicated along the Center of the figure, from 1-7 serum samples showed Positive.
**Maxime PCR PreMix Kit (i-Taq)**

**DESCRIPTION**

INReN’s Maxime PCR PreMix kit has not only various kinds of PreMix Kit according to experience purpose, but also a 2X Master mix solution. Maxime PCR PreMix Kit (i-Taq) is the product that is mixed every component i-Taq™ DNA Polymerase, dNTP mixture, reaction buffer, and so on in one tube for 1 x PCR. This is the product that can get the best result with the most convenience system. The first reaction is to dissociate every component for PCR, so we can use PCR just as a template DNA, primer set, and D.W. The second reaction is that it has Gel loading buffer to do electrophoresis, so we can do gel loading without any treatment. In addition, each bands are checked by a thorough O.C. so, its reappearance is high. It is suitable for various samples experience by fast and simple using method.

**STORAGE**

Store at -20°C under this condition, it is stable for at least a year.

**CHARACTERISTICS**

- High efficiency of the amplification
- Ready to use; only template and primers are needed
- Stable for over 1 year at -20°C
- Time-saving and cost-effective

**CONTENTS**

- Maxime PCR PreMix (i-Taq) for 20 x reaction: 96 (480) tubes
- Maxime PCR PreMix (i-Taq) for 50 x reaction: 96 tubes
- 7.5 U of i-Taq™ DNA Polymerase (2,500 U/mL)
- dNTPs (2.5 mM each; 5 mM each)
- Reaction Buffer (10X) 1X
- Gel Loading Buffer 1X

**PROTOCOL**

1. Add模板 DNA and primers into Maxime PCR PreMix tubes (i-Taq).
2. Appropriate amount of dNTP mix template samples.
   - 0.5-10% of first RT reaction volume
   - Primer (dNTP): 10 ng/μl
   - dNTP mix: 10 ng/μl
   - DNA: 10 ng/μl for each cycle.
3. Note: Appropriate amount of primer
   - Primer: 2-50 pM/μl each (sense and anti-sense)
4. Add water into the tubes to a total volume of 20 μl or 50 μl.
5. Perform PCR of samples.

**SUGGESTED CYCLING PARAMETERS**

<table>
<thead>
<tr>
<th>PCR cycle</th>
<th>Temp.</th>
<th>PCR product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>94°C</td>
<td>10^{-2} μm</td>
</tr>
<tr>
<td>30-45°C</td>
<td>2 min</td>
<td>2 min</td>
</tr>
<tr>
<td>Annealing</td>
<td>55-60°C</td>
<td>15 sec</td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>20 sec</td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C</td>
<td>10 min</td>
</tr>
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</table>

**EXPERIMENTAL INFORMATION**

**Comparison with different company kit**

![Comparison of Maxime PCR PreMix (i-Taq) and Company A’s PreMix system by amplifying 1 Kb DNA fragment.](image)

**Comparison of Maxime PCR PreMix (i-Taq) and Company A’s PreMix system by amplifying 500 bp DNA fragment (GAPDH).**

Total RNA was purified from BUN-1 using easy-Blue™ Total RNA Extraction Kit (Cat. No. 17061). And then, the first strand of cDNA was synthesized using Power cDNA Synthesis Kit (Cat. No. 20011). After cloning the DNA mixture as indicated, the RT-PCR reaction was performed.

* Lane M: BUN-100 DNA Marker; lane 1, undiluted cDNA; lane 2, 10-fold diluted cDNA; lane 3, 100-fold diluted cDNA; lane 4, 1:1,000 diluted cDNA; lane 5, 1:10 diluted cDNA; lane 6, 1:100 diluted cDNA; lane 7, Negative control.
**HbsAg Rapid Test Cassette (Serum/Plasma)**

**Package Insert**

*ACON HbsAg Rapid Test Cassette (Serum/Plasma)* is a rapid test for the qualitative detection of Hepatitis B Surface Antigen (HbsAg) in serum or plasma.

**DESCRIPTION**

ACON HbsAg Rapid Test Cassette (Serum/Plasma) is a rapid, qualitative, immunochromatographic assay for the detection of HbsAg in serum or plasma. The test cassette is pre-coated with anti-HbsAg antibodies on the test line region of the cassette. During testing, the test cassette contains a solution of antigens and sample. The sample is dispensed onto the test cassette and the antigens in the sample react with the anti-HbsAg antibodies on the membrane to form a visible line. A positive result is indicated by the presence of HbsAg in the sample, while a negative result is indicated by the absence of a line. The test cassette is supplied with a control line, which serves as a positive control to verify the integrity of the test system.

**PRECAUTIONS**

- **FOR Laboratories Only:** This test is for diagnostic use only. Do not use in clinical diagnosis.
- **Not for use in home use.**
- **Store at room temperature (15-30°C).**
- **Do not freeze.**
- **Use within 1 year of manufacture.**

**EXPERIMENTAL VALUES**

<table>
<thead>
<tr>
<th>Method</th>
<th>Total Results</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA</td>
<td>184</td>
<td>369</td>
<td>369</td>
</tr>
</tbody>
</table>

**REFERENCES:**

1. Blumberg, B. S. *The Discovery of Australian Antigen and Its Relation to Hepatitis, Viral.*

**LIMITATIONS:**

- Accuracy of test results depends on proper specimen collection and handling. Improper collection or handling can affect test results.
- False positives may occur due to cross-reactivity with other antigens.
- False negatives may occur if the specimen contains low levels of HbsAg.

**STORAGE AND STABILITY:**

- Store at room temperature (15-30°C).
- Do not freeze. Do not use after the expiration date.

**IHC**

- **For Laboratories Only:** This test is for diagnostic use only. Do not use in clinical diagnosis.
- **Not for use in home use.**
- **Store at room temperature (15-30°C).**
- **Do not freeze.**
- **Use within 1 year of manufacture.**

**EXPERIMENTAL VALUES**

<table>
<thead>
<tr>
<th>Method</th>
<th>Total Results</th>
<th>Positive</th>
<th>Negative</th>
</tr>
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<td>369</td>
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</table>

**REFERENCES:**

1. Blumberg, B. S. *The Discovery of Australian Antigen and Its Relation to Hepatitis, Viral.*

**LIMITATIONS:**

- Accuracy of test results depends on proper specimen collection and handling. Improper collection or handling can affect test results.
- False positives may occur due to cross-reactivity with other antigens.
- False negatives may occur if the specimen contains low levels of HbsAg.

**STORAGE AND STABILITY:**

- Store at room temperature (15-30°C).
- Do not freeze. Do not use after the expiration date.
Micro centrifuge

PCR Machine (Thermocycler)  Gell electrophore

Microwave