Detection of Chronic HBV infections by Serological and Molecular Markers in Non-Hospitalized Patients

By

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Department of Microbiology

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Quaid-i-Azam University Islamabad

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A thesis submitted in partial fulfillment of the requirements for the Degree of

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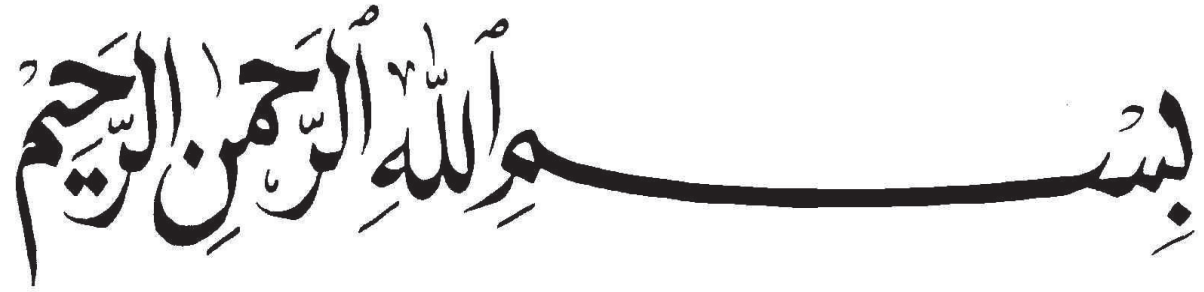

IN THE NAME OF

ALLAH ALMIGHTY

THE MOST COMPASSIONATE,

THE MERCIFUL

DEDICATION

I dedicate my this research work

To

My Loving Parents

Muhammad Arshad

DECLARATION

The material contained in this thesis is my original work. I have not previously presented any part of this work elsewhere for any other degree.

Muhammad Arshad

CONTENTS

Sr. No.	Title	Page No.
1	List of Abbreviations	i
2	List of Appendices	iii
3	List of Figures	iv
4	Acknowledgements	v
5	Abstract	vii
6	Chapter 1. Introduction	1
7	Chapter 2. Review of Literature	7
8	Chapter 3. Materials and Methods	24
9	Chapter 4. Results	28
10	Chapter 5. Discussion	42
11	Conclusion	50
12	Future prospects	51
13	Recommendations	52
14	References	53
15	Appendices	60

LIST OF ABBREVIATIONS

Abbreviations	Description
A	Adenine
ALT	Alanin Transaminases
Anti HbsAg	Antibody against Hepatitis B virus Surface Antigen
Anti-HBc	Antibody against Hepatitis B virus core Antigen
Anti-Hbe	Antibody against Hepatitis B viral e Antigen
APIN	Aids Prevention Initiative in Nigeria
AST	Aspartame Amino Transferases
BP	Base pair
C	Codes for pre Core precursor protein
CAH	Chronic Active Hepatitis
cccDNA	Covalently Closed Circular DNA
CDC	Centre for Disease Control
DNA	Deoxy ribo nucleic acid
DOAJ	Directory of Open Access Journals
dsDNA	Double stranded DNA
EIA	Enzyme Immune Assay
ELISA	Enzyme Linked Immune Sorbent Assay
G	Guanine
HbcAg	Hepatitis B virus core antigen
HbeAg	Hepatitis B virus e antigen
HbsAg	Hepatitis B virus surface antigen
HBV	Hepatitis B virus
HCC	Hepato Cellular Carcinoma
HCV	Hepatitis C virus
HDV	Hepatitis D virus

HEV	Hepatitis E virus
HIV	Human immune deficiency virus
IDP	Internally Displaced People
ISH	In-Situ Hybridization
KPK	Khyber Pakhtunkhwa
LFH	Laminar Flow Hood
LFT	Liver Function Test
M	Marker
mRNA	Messenger RNA
OBI	Occult B Infection
P	Open reading frame in HBV genome, which codes for the viral polymerases
P value	Probability Value
PCR	Polymerase chain reaction
PEPFAR	President's Emergency Plan for AIDS Relief
PH	Power of Hydrogen Ions
qPCR	Quantitative Polymerase Chain Reaction
RNA	Ribo Nucleic Acid
RT PCR	Real Time Polymerase Chain Reaction
S	Open reading frame in HBV genome, which codes for the surface antigen and viral envelope
ssDNA	Single Stranded DNA
TBE	Tris, Boric acid, Ethidium Bromide
TH	Triple Hepatitis
UV	Ultraviolet
WHO	World Health Organization
X	Codes for transcriptional activators viral proteins.

LIST OF APPENDICES

Appendix	Title	Page No.
Tab 01	Primers used for the amplification of HBV DNA	27
Tab 02	Percentages for different Markers for HBV infection.	35
Tab 03	Percentage for the PCR positive samples along with the percentage for HBcore IgM and HBcore IgG (PCR positive) samples.	35
Tab 04	Gender and Age wise Discrimination of the patients included in the study.	61
Tab 05	HBV acute and chronic infections along with the occurrence of Jaundice in these infections.	61
Tab 06	Risk factors associated with HBV infection	62
Tab 07	Symptoms associated with HBV infection	63
Appendix B	Calculations for the PCR Reaction Mixture Preparation:	65

LIST OF FIGURES

Fig. No	Title	Page No
Figure 01	Percentage of male and females patients and age wise distribution of patients	29
Figure 02	Symptoms of HBV infection and number of patients having the symptoms.	31
Figure 03	Factors aiding to HBV infection and their percentages	33
Figure 04	Agarose Gel Electrophoresis image. Image shows the PCR amplified product of HBV DNA.	37
Figure 05	Agarose Gel Electrophoresis image. This image shows the PCR amplified product of HBV DNA of only sample number 3 and 5.	38
Figure 06	Agarose Gel Electrophoresis image showing no amplification of the HBV DNA.	39
Figure 07	HBV Acute and Chronic HBV infections.	41
Figure 08	Different markers for the infections of HBV and their percentages.	64

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ABSTRACT

Total of 212 samples taken from the patients were screened for HBV infection. Out of the total samples 100% of the samples were positive for HbsAg and had ALT levels raised. According to our study 25% of the samples were positive for Hbcore IgM and 63.67% for Hbcore IgG. DNA was detected by polymerase chain reaction in 24.5% of screened samples. Furthermore, 11.3% were negative for both IgM and IgG. A gender based difference in the rate of infection can clearly be seen in this study, as 54.2% of male population is infected with HBV as compared to females which were 45.7% respectively. (55.6%), of the patients infected with HBV had the range of age in between 15-35 years. Different risk factors such as dentist visit, blood transfusions, illegal intravenous drug use and unhygienic surgical procedures were also included in this study. Either replicative phase of HBV or recovery rates for both acute and chronic infection cannot be predicted in this study since anti-HBs or anti- Hbe were not tested for these samples. Out of the total PCR positive samples HBV DNA was detected in 40.38% of the tested HBcore IgM positive samples and 53.84% in the HBcore IgG positive samples. This study shows higher prevalence of chronic hepatitis B infection in non-hospitalized patients. Clearly young patients having young age (15-35 years) are at the higher risk to be infected with hepatitis B virus.

Chapter No.1

Introduction

Introduction

Hepatitis B virus is one of the major cause of liver associated diseases worldwide, which affects about 350 million people and causes about 50,000 to 1.2 million deaths each year (Norder *et al.,* 1994*).* HBV belongs to the family of *Hepadnaviriadae* and ranges in size from 40-42nm. It has a circular partially and double stranded DNA genome of approximately size of 3.2kb. The genome of HBV consist of four open reading frames i.e. S, P, X and C. The S codes for HbsAg and the envelope, i.e. Pre-S1 and Pre-S2, P codes for the viral polymerases and X codes for transcriptional activators viral proteins. Similarly, C reading frame codes for pre-Core precursor protein, HbeAg and HbsAg (Arauz *et al*., 2002).

HBV is usually present in body fluids like blood, semen and saliva. In 2002 it has been reported that HBV can remain live outside the blood for several days, without losing its ability to cause infections (Workowski and Berman 2002). Moreover HBV is resistant to organic chemicals and also to heat and PH. HBV is 100 times more infectious than HIV virus and is transmitted through various routes like unsafe sexual contact, intravenous drug use, and prenatal transmission. Horizontal transmission of HBV includes contact with infected surfaces (Khan *et al.,* 2011). Blood transfusion is also one of the main sources of HBV transmission. In addition organ transplantations can also aid to transmission of HBV, mainly due to poor economical situation either due to less or no awareness about HBV in Pakistan (Masroor and Sohail 2007).

Hepatitis B Virus is detected in human blood by antibody test based on HbsAg. Although screening for HbsAg has greatly reduced the transfusion associated HBV infections, it still remains the most frequently transmitted virus. The occult B infection has also a significant role in bone marrow and organ transplantations (Liu *et al.,* 2010).

Incubation period or prodormal stage for HBV infection range from 45 to 180 days. The clinical consequences of the HBV infection may vary among age groups. Jaundice is the result in about 10% of children those are infected with HBV. Symptoms of HBV infection include Anorexia, Nausea and Malaise (Mahoney 1999). Other symptoms may

include flu like problems and fatigue. In general most of the adult population infected with HBV can fully recover. However, some patients may progress to chronic infection, which is often asymptomatic after 6 months due to loss of HbsAg. It is estimated that about 20% of the adult may progress to fulminant hepatitis and to hepatocellular carcinoma (HCC) (Hassan *et al.,* 2011). The remaining patients may fully resolve the infection and recover in about 2 years. Progression rate f HBV infections into chronic infections are quite high in children's at the age of 4 years. Majority of the clinical outcome of acute HBV infection are related to the host immune response.

HbsAg is the first serological marker of the HBV infection which is detected within 2 to 12 weeks of HBV infection. The HBV surface antigen can precede the appearance of symptoms like increased ALT level and abnormalities of the hepatic biochemistry in 6 to 8 weeks. The recovering patients may loss HbsAg in 12-20 weeks after viral infection. An increase in the Amino Transferases may appear in this case. In the same way antibody against Hbcore protein (anti-HBc IgM) can be detected after 2 weeks of HbsAg detection that indicates an Acute HBV infection and IgM can be detected up to six months after the onset of acute hepatitis (Massimo *et al.,* 2002). After six months IgG may appear that remains detectable up to indefinite period of time. Another serological marker known as HbeAg appears in the acute stage of viral infection, detection of this antigen indicates that virus is in replicative form. If HbeAg remain for 20 weeks then there are high chances of progression to the chronic phase (Gitlin, 1997).

HBV infection is classically diagnosed with presence of HbsAg and antibody to Hbcore protein, i.e. (anti-HBc) that appears in acute infection and remains after the clearance of the virus. The appearance of antibodies to the surface antigen that is (anti-HBs) indicate the revival of the patient and immunity towards infection (Tonekaboni *et al.,* 2000). Nucleotide changes in the gene encoding the HbsAg gene can interfere with the antigenic property of the protein and detection of HbsAg therefore, antibodies against core protein and surface antigen have resulted in the increase attention because these can serve as a detection marker for the HBV chronic infection. This could also suggest that people having such condition could be the carriers for HBV. HBV DNA levels are believed to be

low in those patients which are HbsAg negative. The molecular biology techniques are useful for the detection of HBV infections. These techniques can be used for the detection of infections in which the HbsAg is absent and having antibody against core antigen. These techniques can also be useful in detecting infections in which the antibody to core antigen is absent. This type of infection in which the HbsAg level is undetectable but the HBV DNA can be detected is known as "occult" infection (Raimondo *et al*., 2007). Occult infection can be clinically very important because several studies suggested that OBI can result in the progression and establishment of chronic HBV infections having HCV infection. Prevalence of the occult HBV infection is mostly varied and it depends largely on the population in which the infection is mostly prevalent. (Vitale *et al.,* 2008).

As we know that the presence of HbeAg shows the presence of replicative form of the virus and hence an active HBV infection. Some studies reported that there are some HBV infections in which virus are present in replicative form without production of HbeAg. This is due to the mutation in the core promoter and precore region. The mutation occurs from G to A nucleotide, which can result in the formation of a stop codon that is premature and halts the synthesis of HbeAg (Okamoto *et al.,* 1994). Several transfection studies showed that some mutations lead to low synthesis of the HbeAg that causes low transcription of mRNA reduction up to 50% to 70% (Jammeh *et al.,* 2008). A study reported that the Hepatitis e antigen that is expressed in the hepatocytes can be a target for therapy. Incase these antigens which are expressed in the infected hepatocytes are not produced; viruses in the infected hepatocytes might escape the immune responses. (Utama *et al*., 2011).

Presence of HBV is strongly associated with acute and chronic hepatitis, as the acute form of the disease is for short period of time, it can result in severe form of liver diseases like fulminant hepatitic failure and carcinoma of the liver cells known as hepatocellular carcinoma, (HCC). However patients having acute infections are easy to treat for the HBV infections as compared to chronic infections. Chronic HBV infections are long lasting and can result in severe hepatitis which is very difficult to treat. The most

chronic HBV infections could lead to the HCC. ALT levels were low in acute infections while it was found much higher in patients with chronic infections, similarly the survival rate are low in the chronically infected patients as compared to the acute ones. Patients infected with acute infections have the better chances for self recovery in comparison to the patients with chronic infection (Yoshiba *et al.,* 1993)

The chronically infected patients are not able to eliminate the HBV after fulminant hepatic failure, and they require the artificial liver system which involves the plasma exchange system membranes. Haemodiafiltration can also be utilized in this regard. Studies showed that the survival rate was higher in acute infection as compared to chronic infections, (73% vs. 39%). Some patients having chronic infections also developed fulminant hepatic failure after withdrawal of steroid or chemotherapy or cancer chemotherapy (Inoue *et al*., 1998).

Presence of the antibodies to the core region of the HBV virus can also be the source of detection of HBV infections. Especially when there is absence of other markers such as surface antigen known as HbsAg and antibody against the surface antigen known as Anti-Hbs. Incase anti-HBc is present in the absence of surface antigen and anti-surface antibody it is known as isolated anti-HBc. Studies have shown that the detection of the isolated anti-HBc can be helpful in analyzing the HBV infections. So it is useful to screen these patients for preventing HBV transmission (Ramezani *et al.,* 2009).

Super or co-infections of HBV with HCV and HDV are also reported. As HDV virus can only cause infection in the presence of HBV. HDV requires the aid of HBV for coat and surface antigen synthesis. Several studies had proposed that most infections with HDV are through parental and sexual routes. Super infections involving HBV and HDV can result in liver exacerbations, quick progress into the chronic infection, liver failure and death of the infected patient. It has been estimated that about 5% of the patients have super or co infection with HDV leading to about 15 million people infected with HDV worldwide (Khan *et al*., 2011).

HCV co infections with HBV are also reported. Pakistan is endemic in case of HBV infections. About 3 percent of the population is infected with HBV. The exposure rates of HBV in Pakistan are not clearly defined (CDC USA 2007).

According to the WHO standards defining endemicity of hepatitis countries with infection rates between 3-5% of general population are considered as moderate and above 5% as highly endemic. Approximately 4% of the general population in Pakistan is infected with HBV (Zuberi; 1998). Sources of transmission for HBV infection are mainly blood and body secretions. According to an estimate 80% of the acute cases of HBV are cleared within 6 months and rest of the cases progress towards chronic stage of infection. If a virus remains detectable in the blood, longer than six months, it reflects that person has a "chronic infection". As above mentioned majority of the cases of acute hepatitis will recover within six months however, exceptions do exist, as 90% of the infants and 50% of young children having HBV infections are at the highest risk of developing chronic infections (Kumar *et al.,* 2006). It is estimated that from the endemic areas 50% of the cases diagnosed as primary infections are in fact acute exacerbations of chronic hepatitis. A complete and reliable differentiation between acute and chronic infections should be considered as the most important step prior to the initiation of proper treatment for the infection. In the case of acute hepatitis infection antiviral therapy is not recommended unless otherwise sever infection prevails (Orenbuch *et al*., 2008). There are some important serological and molecular parameters those can differentiate between these two conditions but due to the high costs and lack of facilities these test are not practiced in routine diagnosis in many areas of Pakistan.

Most of the data is about the prevalence of hepatitis that includes studies on healthy children, vertical transmission, pregnant women, healthy individuals, army recruits, blood donors, health care workers, use of unsafe injections, high risk groups, patients with provisional diagnosis of hepatitis, patients with chronic liver disease, and genotypes of HBV (Alam *et al.,* 2007). Conclusively, it was highlighted in the review that there is a lack of community-based epidemiological work on this subject. In future, large scale community based epidemiological data by using sophisticated procedures of screening

might bring a better picture about the prevalence of acute and chronic HBV in Pakistan. In this study quantitative analysis of different serological parameters, including IgM anti-HBc are evaluated for the detection of acute or chronic HBV infections in non hospitalized patients.

Aims and Objectives:

1) To detect the HBV Chronic infections using Serological and Molecular Markers in Non- Hospitalized Patients.
2) To relate the infection with associated risk factors.
3) To co relate the serological and molecular markers and differentiate the infection.

Chapter 02

Review of Literature

Hesham and Rajab, 2008 stated that with the course of time our knowledge about Hepatitis has increased. One of its etiological agent is HBV. After its discovery in 1963 until now, much progress has been made in the context of HBV, Its mechanisms of infections, its structure and treatment. But there are certain areas where we still cannot progress, which is due to the astonishing nature of the HBV virus. About 2 billion populations which is one third population of the world are infected with HBV virus and 3 million population is infected each year. As HBV causes cirrhosis and hepatocellular carcinoma, due to which, about 1 million people are infected and that means HBV takes a life in every thirty seconds.

Francis 1999, conducted a study in which he stated that HBV is member of the family of *hepadnaviridae* and genus *Orthohepadnavirus* and a causative agent for acute as well as chronic liver hepatitis. Along with humans HBV is also isolated from other hosts like birds, rodents, non human primates like chimpanzee, gorilla, orangutan, gibbon, and woolly monkey. Structure of HBV virus is, enveloped and partially double stranded DNA virus. The circular genome of HBV is about 3200 bp which contain open reading frames, four in number and which codes for the polymerase enzyme (P), surface antigen (S). A nucleocapsid proteins (C), and (X) and transcriptional transactivator proteins. The open reading frames show overlapping and hence they encode more than one protein product. (Mario and Edward *2002*). The size of HBV ranges from 42-45 nm. It is a small human infecting virus. HBV antigen known as surface antigen can also exist in the serum in spherical and tubular forms but they are non infectious and they have size of about 22nm.

Shazia *et al.,* in 2008 conducted a study to investigate the occurrence of HBV genotypes in the healthy females belonging to Karachi Pakistan. As HBV is the causative agent for both acute and chronic HBV infection. There is a lot of genetic variability in the genome of HBV, because there are 8 genotypes of HBV (A-H). Each of the HBV genotype has a specific geographical distribution. Previous studies had indicated that the genotype A and D are mostly prevalent in the Sindh of Pakistan. Blood samples were collected from 4000 healthy female patients. These patients were volunteer students. The obtained samples were screened for the HbsAg and antibodies against surface antigen

that is anti-Hbs. The antigen and antibody detection was done with the process of immune chromatography and ELISA. After that Genotyping of the concerned samples was done. They found that out of 4000 patients 180 (4.5%) patients were positive for surface antigen. While 20 (0.5%) of the patients were positive for the anti-Hbs antibody. After this they carried out the genotyping of 180 HbsAg positive serum samples by PCR in which the sequencing analysis were done for 21 samples. Out of the total 180 samples 150 samples showed the D genotype of the HBV, 29 of the patients had co-infection of B and D genotype, while 1 patient showed had co-infection of genotype C and D. They concluded that the genotype D appears to be the most prevalent genotype in Karachi within the healthy female population.

Alam *et al.,* 2007 carried out a study in which he pointed out that the infection rate of HBV is continuously escalating in Pakistan. They focused on the epidemiological point of view of HBV, which is the requirement for having clear picture of HBV infection. The sample size they selected was 1300 and they carried out the serological analysis of the sample which includes the detection of HbsAg, HbeAg, anti-HbcAg and anti-Hbs. They compared the epidemiological status, like residence, age of the patient and the economical condition of the patient with the serological markers. They found that only 4% of the patients were positive for the surface antigen and the mean age of the patient was 23.5 ± 3.7 years. The antibodies to the HbeAg were found in 9.3% and 12% against the core antigen of HBV. Similarly antibodies against the surface antigen were present in 33.4% of the patients. They further found that seropositivity rate of HbsAg was significantly associated (p = 0.03) with the residing locality indicating high infection in rural areas. They found an inverse relation between the antibody titer against the HbsAg and increasing age. The titer was decreasing as the age of the patient increased. They concluded that the prevalence rate of HBV was quite high which indicates that educational programmes, mass awareness and vaccination strategy is urgently needed.

Khan *et al.,* in 2011 carried out a study regarding the prevalence and risk factors responsible for HBV infection in the internally displaced persons (IDPs). These IDPs were misplaced due to the war against terrorism in the Malakand division of Northern Pakistan. They stated that HBV is the one of the major health issue in Pakistani

population. HBV is one of the main cause of acute and chronic infections which may lead to the liver fibrosis, cirrhosis and hepatocellular carcinoma. They reported that HBV infection is mainly associated with areas of low economic status in Pakistan. The collected samples from 950 patients which were IDP, these persons were suspected to have HBV infection. These patients belonged to the different districts of Malakand division like Dir (lower), Swat and Buner. The infected patients mostly belonged to the district Dir lower. The infection risk (29.13%) was mostly found in the older patients that is 46-60 years. Lower infection rates were found in children's and it was 11.9% having age less than 15 years. The risk factors in the study found to be syringes, blades, unsafe sexual contact and needles use. This study concluded that the rural northern areas of the Pakistan have a high degree of HBV infection. This infection is gender dependent and also age dependent. This may be due to the exposure rate or the associated risk factors. Proper awareness about the transmission, risk factors and immunization is needed in these areas of Pakistan.

Ali *et al.,* 2011 carried out a study in which he estimated that the carrier rate for HBV in Pakistan is 3-5%. They stated that about 7-9 million people in Pakistan are carriers for HBV. They collected all the data regarding the awareness status of HBV in Pakistan, risk factors, prevalence of HBV and the HBV genotypes in Pakistan. They collected all the published data from 1998 to 2010 from the Directory of Open Access Journals (DOAJ), Pub Med, Google Scholar and PakMediNet. The total number of studies they found, which were done were 106. They estimated that 4.3318% ± 1.644%, of hepatitis B virus infection prevails in general population. they also reported that (3.93% ± 1.58%) percentage of healthy blood donors were infected, military recruits (4.276% ± 1.646%), patients with liver diseases (27.54% ± 6.385%), prisoners (5.75% ± 0.212%), patients with HCC (22% ± 2.645%), multiple transfused patients (6.223% ± 2.121%), surgical patients (7.397% ± 2.012%), patients with cirrhosis (28.87% ± 11.90%), healthcare persons (3.25% ± 1.202%). They found that the mostly prevalent genotype of HBV in Pakistan is the genotype D (63.71%). Finally they concluded that a strategy regarding the Mass vaccination and awareness should be conducted immediately in those populations in which the rate of HBV infection is more than 5%.

Khan *et al.,* in 2011 carried out a study in the Punjab province of Pakistan to investigate the epidemiology and risk factors for the HBV infection. The sample size included in the study was 4890. Real time PCR was used to detect the status of the infection. A questionnaire was prepared for all the patients and all information about the status and risk factors for the patients were included. Out of the total sample size 3143 samples were positive for Hepatitis B infection and gender wise the percentage for infection was 68.15% for males and 31.85% for females. As most of the studies conclude the high percentage of infection in males. This study also reported that the rate of male infection was high in males as compared to females and the rate of positivity for males was 2.14:1. The infection rate was high in older persons as compared to younger age that is 34.9 3% in the age of 21-30 years, 23.8 3% in age of 31-40 years, and 13.39% of the patients having age of 11-20 years. They found that the rate of infection declines with age as groups 41-50 (16.13 %) and 51-60 (7.09 %), while children's having age of 0-10 years are less infected having percentage of 1.49%. Very old aged patients like having age of greater than 60 years had the infection rate of 1.65%. The key risk factors involved in the HBV transmission to other subjects included barber having percentage of 23.60%, blood transfusions 4.04%, injection history of 26.19% and dental risk aiding the part of 11.2%. They concluded that males are mostly exposed as compared to females and the patients having younger age are most likely to be infected as compared to older age patients. Syringes reuse, barber and dentist visits were the main contributing risk factors for the HBV infection. Finally government should take solid steps for awareness and vaccination programmes to control the rate of infection in the Punjab province of Pakistan.

Poorolajal and Majdzadeh in 2009 conducted a study in Iran regarding the chronic infection prevalence. Demographic factors for the infection were also included in the study. The prevalence of HBV chronic infection was found to be 1.7%.; 0.8% (95% CI: 0.6% to 0.9%) in blood donors and 3.2% (95% CI: 2.3% to 4.1%) in intravenous drug users. It varied from zero to 1.5% in beta thalassemic patients. Mass vaccination Programmes during 1993 had greatly reduced the HBV infections in children's and adolescents. This reduction is due to the effectiveness of the immunization

programmes and it is contributing to the decrease in the prevalence of chronic HBV infection in general population of Iran.

Helmy and Sebayel in 2006 reported a study regarding the isolated core antibody known as anti-HBc presence having co infection with HCV virus. They screened all the patients having chronic HCV infection at King Faisal Specialist Hospital and Research Center for the HbsAg and antibody to the surface antigen known as anti-Hbs. The screening of the antibody against the hepatitis B core antigen known as anti-Hbcore antibody. A total of one hundred sixty nine patient were both negative for both the surface antibodies and surface antigen. Results from the pathology department showed that 59 patients had cirrhosis which was proved by biopsy; patients having chronic active hepatitis were 110. Out of these patients 50% were positive for anti-HBc antibody. Patients having chronic hepatitis infection had the percentage rate of isolated antibody and also cirrhosis was 64.5%. Total of 25 patients were found positive for the DNA presence by qualitative PCR. While 3 patients had occult HBV infection.

Renata *et al*., 2006 conducted a study in Brazilian patients to find out the prevalence of HBV infection in hemodialysis patients and the risk factors associated with the HBV infection. They also tried to find out the HBV genotypes, which were distributed in the concerned patients. They included a total of 1095 patients. These patients were interviewed in 15 dialysis units in different hospitals. They screened the blood serum samples for the presence of serological markers through ELISA. Those samples which were positive for HbsAg were further tested for the presence of HBV DNA, using the technique of PCR. The genotyping of the concerned samples was carried out using restriction fragment length polymorphism. Prevalence rate for HBV was 29.8%. Multi analysis showed that different factors such as the Male gender, the time elapsed in dialysis and blood transfusions were almost related with the HBV infection. The HBV DNA was found in 65.4% (17/26) of those samples which were HBV surface antigen positive. Out of these 17 HBV DNA samples 13 samples were genotyped and the results showed that the genotype D (61.5%) was the most prevalent genotype. The genotype was prevalent in only 7.7% of the concerned samples.

Jerzy *et al.,* 2010, designed a study regarding the determination of HbsAg levels during different phases of HBV infection. This study was conducted in European patients which were positive for surface antigen. They included 226 HBV infected patients. They were only infected with HBV. These patients were not receiving any antiviral therapy. The phases of HBV infection were, immune tolerance phase in which the HbeAg is positive (n=30), the immune clearance phase (n=48) and low replicative phase (n=68). Categorization of the all patients was done according to the above phases of HBV replication. The cases of acute HBV infection were (n=12). The quantified surface antigen was compared with HBV DNA, HBV genotypes and other clinical parameters. The results showed that the HBV surface antigen ratio was higher in the immune tolerant patients. In acute hepatitis infection the relation of the HbsAg was weak. The co relation was also missing in some phases of HBV infection. In the HBV genotype D, the HbsAg was showing a co relation with HBV DNA levels. Those patients which were in low replicative phase of HBV infection showed increase in the HbsAg levels as the cut off value was 3500IU/L. From this study it was concluded that the HbsAg levels show significant differences during the different phases of HBV infection and the concerned genotypes. These findings of this study could be helpful in understanding the natural history of the HBV infection. HbsAg can be used as quantitative, diagnostic tool for predicting the reactivation of HBV infection.

Inoue *et al.,* in 1998 compared the properties of the patients who had developed fulminant hepatic failure which was due to the HBV acute infections, with those patients which developed the hepatic failure during their carriage of HBV. 11 of the patients had ALT levels raised than the normal (mean ± SD: 4943 ± 2867 vs. 1157 ± 678 IU/L, $P < 0.01$). The bilirubin levels were low in the patients (15.3 ± 4.4 vs. 28.1 ± 14.3 mg/100 ml, $P < 0.01$). The detection of HbsAg was mostly found in the chronic infection as compared to the acute infections that is (55% vs. 100%, $P < 0.05$, the viral DNA polymerases was also present in less proportion in acute infection as compared to chronic infections, (0% vs. 46%, $P < 0.05$). Same was the situation with the HbeAg, which was present in the acutely infected patients in percentage of 9%, and in chronically infected patients it was present in 46% of the patients having the probability value of ($P < 0.05$).

as mutation are common in the pre core region of HBV so in this study they found that there was about 62% of the chronically infected patients had mutation in the core region of the HBV genome, similarly 91% of the acutely infected patients had also mutation in the core region of the HBV genome. The remaining 38% of the chronically infected patients don't have any mutations in the core region, rather than they have mutations in the promoter region. From this study they concluded that the HBV which is not capable of producing the e antigen avoids the host immune response and due to this the main reason that the fulminant hepatic failure occur after an infection with acute hepatitis. The other factor in the HBV variants which can play a crucial role in the fulminant hepatic failure is the mutations in the promoter regions, which can result in rapid progression towards chronic infection.

Efrat *et al.,* in 2008 conducted a study regarding the assays which can be made in use for the proper management and diagnosis of the patient. They stated that HBV is one of the serious health issue which is faced by all world. Those countries where HBV is endemic exacerbations for the chronic HBV infection are also common. The problem is, that these patients are miss diagnosed, because they are considered to have acute HBV infections. Dealing with HBV infections it is very necessary that we have the correct knowledge of status of the infection in the regard of starting treatment for the HBV infections. As we know that the acute HBV infection does not require any treatments, so in this regard the liver exacerbations are get benefited. The routine diagnosis and laboratory findings cannot make us clear between these two conditions. As we know that the presence of levels which are quite high of anti-Hbe antibodies, HbsAg and HBV DNA are the markers for the chronic infections, while the titers for the IgM antibody with avidity index are indicative for the acute infections.

Thompson *et al.,* 2010, proposed a study in which they tested the hypothesis that the titers of the HbeAg and that of HbsAg may not depend on the viral replication in vivo. As the antibody titers may also help in the therapy of the HBV chronic infection, however the relationship between the antibody titers with the viral load circulating, those replicative HBV which are intrahepatic and the viral variants which are emerging, are not understood. Therefore a total of 149 chronically infected patients were included in which

the HbeAg positive patients were 71, and the HbeAg negative patients were 78. The HbeAg and surface antigen were quantified by enzyme immune assay. The virus was characterized by serum HBV DNA levels, genotypes for HBV, core mutations, core promoter and precore mutations. The intrahepatic levels of covalently closed circular DNA (cccDNA) and the HBV DNA. Patients in whom the e antigen was positive the HbsAg was positively co related in the chronic HBV infections with HBV DNA and the DNA which is present in the hepatic cells. In those patients where the HbeAg was negative in chronic HBV, the surface antigen was poorly co related with the DNA of HBV. Conclusively the study reported that the relation between the surface antigen and titer and the DNA levels in the serum and intrahepatic markers which is resultant of the HBV replication is different between the patients who are HbeAg positive and those who are HbeAg negative. The HbeAg levels fall independently of the replication of the virus. The main reason behind this could be that there are HbeAg defective mutants of the viruses which emerge before the seroconversion.

Susan *et al.,* 2011 conducted a study regarding the screening of HBV infections using the serological and molecular markers that are mostly the HbsAg, anti-HBc antibodies and by using the DNA amplification technique known as PCR. Those patients which were in window period of their infection were not identified because they had not developed any antibodies against the antigens. The nucleic acid testing or identification of infections by nucleic acid testing can be helpful in providing safety and efficacy to the screening processes. What the team actually did was, that they perform the process of nucleic acid testing in blood donations which were 3.7 million; they made progress in the work by the evaluation of the markers like HBV DNA, HbsAg and anti-HBc. The samples having both DNA and surface antigen positive were analyzed. Features like serological characteristics, biochemical and molecular features of those samples which were positive for HBV were determined. The sexual partners of the infected patients were also sampled. Other donors who were seronegative for HCV and HIV were also included in the study. 9 of the patients were found positive for the HBV DNA and out of these patients 6 patients had already received vaccine against the HBV. In these patients the infection was developed and was already resolved. Of the all HBV DNA positive

patients, 4 had acquired the infection by sexual contact. Liver injury was developed in patients which were 2 in number and they were unvaccinated. In total of the 6 vaccinated donors in 5 donors a non-A genotype was identified which was dominant. A sub genotype known as A2 was present in the unvaccinated donors. Out of 75 patients who were identified as seronegative blood donations, 9 were HBV infections, 15 for HCV and 2 HIV infections. Results indicated that the nucleic acid testing can detect triple infections of HBV, HCV and HIV. These infections were detected at the window period when there was no seroconversion. HBV vaccination was protective against HBV infections.

Merican *et al.,* 2000 carried out a study regarding the chronic infection in Asian countries. They proposed that about 50 million new cases of HBV chronic infections are diagnosed every year. Out of all HBV chronic infections 5-10 of the patients are adults and infants are infected at the percentage of 90%. All of these infections progress to chronic infection. Out of all HBV infection 75% of the infection are present in Asia. HBV is one of the major cause of the hepatocellular carcinoma (HCC), cirrhosis and chronic Hepatitis infection. The Indonesian population positive for the HbsAg was 4.6%. in 1994 . 21% of the patients were showing positive results for the HbeAg and 73% of the patients were positive for the antibody against e antigen. The percentage of cirrhosis and HCC was 44% and 45%. In Philippines the prevalence of HbsAg showed 2 types of age related prevalence, which means that the mode of transmission was different. The Thailand studies showed that about 8-10% population of males is positive for the surface antigen, similarly the percentage of HbsAg positivity for females was 6-8%. Those patients which had cirrhosis were 30% and the HCC were 50-75%. Taiwan studies showed that 75-80% of the chronic infections were HbsAg positive. The HbsAg was also found in patients having cirrhosis and HCC and the ratio was 34% and 72%. China, 73% of the patients had chronic HBV infection, while 78% and 71% had cirrhosis and HCC. Singapore had the prevalence rate of infection dropped because of the HBV vaccination programme. 4.5% of seroprevalence was found in unvaccinated individuals having age of 5 years. Healthy volunteers in Malaysia having mean age of 34 years, 5.24% of the volunteers were positive for HbsAg in 1997. Endemic areas of Asia constitute the

infection in which the patients are HbeAg positive and they also had high levels of the virus, the liver inflammation is lower in these patients. Mutations in the virus which results in the halting of the production of HbeAg, this phenomenon result in more severe liver disease and high rates of chronic infections. HBV vaccination is still one of the main tool to avoid the infections with HBV infections and HBV related cirrhosis and hepatocellular carcinoma.

Mukerjee *et al*., 2010 carried out a study in which they tried to find out the relationship between the load of different markers such as HbsAg and HBV DNA, with the percentage of the immune system cells in the blood of periphery. This study was designed in India. As the HBV chronic infections are the consequences of the mal functioning of the cellular mediated immunity in the immune system. As we know that the markers for the replication of HBV such as HbsAg, HBV DNA, core antibodies are important for measuring the HBV infection. We don't have a clear idea about the status or position of the immune system regarding the chronic HBV infections. Such studies regarding India have not been reported previously. They carried out the evaluation of 31 patients for the Hbe antigen (HbeAg), anti-Hbe and ALT levels. These were analyzed through ELISA and other procedures. Real time PCR Taq Man assay was used for the determination of serum HBV DNA levels. The surface antigen levels were measured with the third generation kit for ELISA. The analysis for the peripheral immune cells was carried out by multifluorometric flow cytometry analysis and for this purpose 21 healthy samples were used as controls. Results in this study showed that 93.5% of the patients were negative for the HbeAg and were positive for the anti-HbeAg. The mean viral load, surface antigen and ALT levels were 4.20 ± 1.96 log copies/ml, 5.98 ± 4.62 log IU/ml, and 74.5 ± 110 IU/ml. The T cells and cytotoxic T cells populations were reduced during HBV infection. Levels of the B cells, natural killer T cells, T helper to cytotoxic cells remained unchanged. Conclusively the suppression of the peripheral T cells in the patients, in which the e antigen was negative, was affected by the HBV viral load. The surface antigen levels were independent of the HBV DNA levels.

Nigeria has one of the greatest burdens of HBV endemicity as well as pediatrics due to the HIV. Emmanuel *et al.,* carried out this study in 2013, to address the co infections of HBV-HIV present in children's. Children's undergoing treatment at the clinics of pediatrics APIN Plus/Harvard PEPFAR program in the time of in between June 2008 to June 2012 were included. Results of the study indicated that the mean age of the children's in the study was 7.53±4.23years. Total of 395 subjects are included in the study. Out of these 7.8% of the patients were showing positive results of HBV infection. There was no triple infection of HIV-HBV and HCV in any of the infected samples. The rate of high HIV-HBC infections was found in the age range of 11-15 years. Those patients which did not have any HBV vaccination were less than 15%. The patients having immune suppression had p values of 0.00, 0.01, and 0.00. The co-infection of HIV-HBV does not have any effect on the characteristics like gender, clinical stage regarding WHO, absolute median CD4 cells count, the mean viral load, the ALT levels and the toxicity of the. Conclusively this report for the pre-ART screening of the patients which are dually infected with HBV and HIV concluded, a high rate of sero prevalence of HBV was found in those children's, which were already infected with HIV.

Cadona *et al.,* 2011 carried out a study in which a characterization of the infection caused by the HBV among the piaora community, which is an Amerindian group. Occult HBV infection is a type of HBV infection which negative for all the serological markers, except for the presence of DNA. They characterized the infections of HBV which has significant evidence of exposures. In total of 150 serum samples the prevalence of anti-HBc was 17% and that of the surface antigen was 1.3%. Out of these samples 70 samples were checked for the presence of DNA. 36% of the samples were found to be positive for HBV DNA by the process of PCR in the core region of the HBV genome. 2 patients out of 70 were positive for the HbsAg which indicated an overt type of HBV infection. In the remaining 68 samples, 23 samples exhibited the occult B infection (OBI). In these 13 were HBV DNA, and 10 HBV DNA positive, out of 43 anti -HBc negative e (23%), with a statistical significance of p = 0.03. from the sequencing analysis, which was done for the core region showed that all of the strains of HBV belonged to the genotyped F3. The nucleotide identity between the occult HBV isolates was 96-100%. One of the isolate

showed the presence of wild type of variant in which there was a premature stop codon at the core region. There was deletion of about 28 nucleotides at the core region.

Qingrun *et al.,* 2007 carried out a study for designing effective universal viral primers for the amplification of HBV genome. the study was carried out in the regard that due to the heterogenic characteristics of the viruses, it is one of the main problem for the amplification of the HBV DNA. They selected the conserved sites for designing primers for HBV genome. HBV sequences alignment in public databases and a programme named BxB in Perl script was utilized for the proper designing of the HBV primers. After designing of the primers the PCR results of the primers was compared with that of most popularly and mostly used primers for the amplification of the full length genome. The set of four walking primers showed improvements. These designed primers were applicable for the amplification of most of the subtypes of HBV genotypes. Conclusively the study reported that the method of primer design described over here can be used for designing sub type specific primers for the subtypes of viruses. The BxB programme, which is based on the alignment of multiple sequences, can be used as tool alone or it can be also used in combination with other design software employed for the primer design.

Alavian *et al.,* 2007 conducted a study regarding the sero epidemiology and modes of transmission of HBV in Pakistan and Iran. As HBV infection has gained the endemic situation in the Middle East and is the cause of severe morbidity and mortality. Therefore some strict strategies should be brought in to work to prevent, diagnose and manage the HBV infections. The endemic situation for HBV is low in Iran while in Pakistan the situation of HBV endemicity is intermediate. The risk factors that are responsible for the current situation in Pakistan is the transfusions, vertical transmissions, therapeutic injections, traditions like ear and nose piercing are important risk factors for the HBV endemicity in Pakistan. In children the prevalence of HBV is countable in Pakistan. While in Iran the factors responsible for the HBV situation are intravenous drug use, and sexual contact. To avoid or control the HBV transmission in both countries it is necessary that the transmission of HBV within the family should be controlled. Vaccination can help us to control the infection related to therapeutic injections.

Similarly the women which are pregnant should be screened for infection with HBV, as they can be one of the major source of transmission of HBV into the new born. Vaccination programmes in all of the provinces in the Pakistan and creating awareness about HBV infection in Iran, screening of the infection should be done by collaboration of both the countries. It is necessary for both the countries to have effective vaccination programmes and control over the addiction in the neighboring countries.

Liu *et al.,* 2010 conducted a study regarding the investigation of occult HBV infections in the Nanjing province of China. As the incidences of the HBV infection have decreased after the introduction of the diagnostic procedures employed for the detection of HBV in the blood donors. The detection limit in the study for the process of nested PCR was 20 copies/ml of HBV DNA. The rate of HBV occult infection in the blood donors was found to be 0.13% (5 of 2972). The sequencing analysis f the data showed that the HBV sequences were different from each other, from which the false positive results were excluded. The phylogentic studies of the concerned samples showed that the mostly found genotype was genotype B, a deletion of single base was observed in the S region of HBV DNA. The antigenic determinant a was common in all the subjects and no mutations were observed. 5 donors were positive for the antibodies against the core antigen and were negative for the antibody against the hepatitis B surface antigen. From this study it was concluded that the occult HBV infection in the blood donors in Nanjing China is quite high. This study could be helpful in the eradication of occult HBV infection in post transfusion in China.

Caballero *et al*., 1995 designed a study in 119 patients to evaluate the diagnostic efficacy of the in situ hybridization for the HBV infections. The HBV DNA in liver and its pathological effects were also included in the study. Biopsy of the liver of 119 patients showed that 55 of the patients were HbsAg seropositive, while 64 patients were HbsAg sero negative. In the sero positive patient's in situ hybridization was positive in 26 (47%) patients and negative in 29 (53%) patients. The transaminases levels were high in the ISH positive patients. The HbsAg positive ratio and B core antigen positivity ratio in liver was same in both of ISH patients and ISH negative patients. The activity index for the histology was at higher level in the ISH positive patients (11 vs. 7, $p<0.001$). 6 patients

out of 12 were positive for HBV DNA by PCR technique. Those patients who were seronegative for the surface antigen the in situ hybridization was negative in 57 subjects, while ISH was positive in only 7 patients. 5 of the 7 cases were positive for the anti-Hbs antibody. The study suggested the ISH technique is one of the valuable techniques which can be employed for the detection of nucleic acids of HBV nucleic acids in liver. The study also suggested that the HBV DNA cannot be always used as marker for HBV replication as some patients who were seronegative for the surface antigen having chronic liver disease and they also had DNA in their hepatocytes.

Man-Fung Yuen and Ching-Lung Lai in 2001 reviewed the treatment for the chronic HBV infections; they discussed the major drugs that can be used for the therapy of chronic HBV infections. There are various agents for the chronic HBV infections treatment. Some of these are used in single therapy while other is used in combination of two or more, which is also known as combination therapy. The first group includes immunomodulators which alters the host immune responses against those antigens which are expressed on liver cells. The immune modulators like thymosin alpha1, interferon alpha, pegylated interferon and vaccines which are therapeutic in action. The second groups of agents used for the therapy include analogues for nucleosides and they suppress the replication of HBV, which further block the re infection of those hepatocytes which are healthy. Nucleoside analogues like lamivudine mainly suppress the HBV replication and it has immunomodulatory effect. Other nucleoside analogues like entecavir, Emtricitabine and beta-L-2'-deoxythymidine are also used for the treatment of chronic HBV infections

Merat *et al.,* 2009 carried out a cross sectional study in which he found the HbsAg prevalence, anti-core antibody and factors in the populations of three provinces in Iran. He found that 3% of the Irani population was chronically infected with HBV. Current studies regarding the Iranian population in both urban and rural population do not agree, as there is too much variation in both. They randomly selected 6583 cases from different provinces of Iran named Hormozgan, Tehran and Golestan. The age of the patients varied in between 18 and 65 years. The samples were tested for different markers such as HbsAg and anti-HB core antibody. Prevalence of the HbsAg and Anti-

Hbcore antibody in the three provinces of Iran was 2.6% and 16.4%, the study showed no significant differences in male and female patients. As a nationwide vaccination programmed for the newborns were carried out in 1992, but the HBV infection still remains the main cause or reason for the chronic hepatitis in Iran which should be considered for coming 30 to 50 years.

Study conducted by Mendy *et al.,* 2005 in which they tried to develop a PCR assay based on the real time quantitative method for monitoring the viral load in the serum of the infected patients. These patients were infected chronically and they were carriers. These patients were already enrolled in trails for therapeutics. The real time quantitative PCR assay was done by using SYBR-Green signaling system for detection. The primers which were specific for the S gene were also included a real time quantitative PCR assay for monitoring the HBV serum viral load in chronic carriers which were enrolled in therapeutic trials. The quantitative real time PCR assay was done using the SYBR-Green signal selection system and primers, which were specific to the S gene was also used. The process of thermal cycling was done detection system used was ABi 5700. Calibration of the assay was done according to the standard for HBV which is international standard. Viral load in carriers which were treated was studied for about one year. The patients were treated with lamivudine. The co relation between the HBV DNA levels and e antigen status was monitored in patients which were untreated. Results indicated that viral loads dropped during therapy with lamivudine or after short period of time. Results showed that the DNA levels for HBV is one of the most reliable indicator of viral presence and they found DNA in 77% of the patients which were all negative for HbeAg. This method of HBV detection is one of the most reliable, reproducible and accurate. The quantitative PCR is used for monitoring the efficiency of therapy for HBV and could be helpful in studying the HBV history in a particular area, which is endemic in case of HBV infection.

Ghadir *et al.,* 2012 concluded that in acute or chronic HBV infected patients hepatitis D virus (HDV) viral co-infection or super infection may also found because HDV is a defective RNA virus that do not cause infection alone and depend on the surface antigen (HbsAg) of HBV for its replication. They noticed that in Iran very

negligible data available regarding the routes of HDV transmission. They further summarize different risk factors involved in HDV infected population of Iran, including traditional phlebotomy, family history, tattooing, blood transfusion, endoscopy, dental interventions, and surgery and war injury. They included a total of 3690 samples randomly from different region including 7 rural clusters and 116 urban clusters. They have screened all the individuals with HBs antigen and all of the positive HBV cases were processed for anti-HDV. Only 48 subjects (1.3%) were found positive for HBV infection and only one sample out of the all samples show co-infection with HDV and were positive for the surface antigen. hepatitis B infection and only one of HbsAg positive case had HDV infection. They found that the prevalence of HDV was 0.03% in Qom Province. They also found that HDV prevalence in HbsAg positive subjects was 2%. They noticed that the only case which was anti-HDV positive had a history of dental surgery, tattooing and surgery. They concluded that there is no such symbolic, there was no significant association between HDV infection surgery history, or dental surgery and tattooing. They found that in Qom province the prevalence of hepatitis D is the lowest similar to a study in Babol (north of Iran). They found that only two patients HDV RNA were confirmed positive for PCR. They further verified and confirmed. Phylogentic studies which show that both RNA positive patients carried HDV having genotype 1. They finally concluded 0.32% prevalence of HDV was noticed in Korea which was very low. They analyzed that this data showed that only HDV genotype 1 was noticed and detected in inactive HbsAg carriers. They suggested that in chronic HBV infection. Co-infection with HDV in Korean patients may not have any important or significant impact.

Study conducted by Shaikh *et al.,* 2012, which focused on the occurrence of triple infection regarding HBV, HDV and HCV. The study was carried out at the Hepatology Clinic of Chandka Medical College, Larkana from January 2008 to June 2011. Sample size was 1713; blood samples were drawn for detection of anti-HDV Antibodies, anti-HCV antibodies HBV DNA, HCV RNA and HDV RNA and liver function tests (LFT). Ultrasound of all patients was performed. Serological, biochemical and radiological profile of Triple Hepatitis (TH) positive and negative patients were compared, Of 1713 patients, anti-HCV Abs was detected in 420 (24.5%), anti-HDV Ab in 1116 (65.1%),

HBV DNA in 308 (18%), HCV RNA in 148 (8.6%) and HDV RNA in 896 (52.3%). 268 (15.6%) had triple hepatitis (HBV+HCV+HDV). 220 (82.1%) of TH positive patients were male ($p<0.000$). LFT were significantly elevated and ultrasonological examination showed advanced chronic liver disease in triple hepatitis. Triple Hepatitis infection was found in 15.6% HBs Ag positive patients. More males were affected and infected patients had advanced liver disease.

Chapter 03.

Materials and Methods

Materials and Methods

Material and Equipment Required:

- Micro plate reader
- Automatic micro plate washer with Incubator
- Centrifuge machine
- Pipette 10ul – 100 µl
- Pipette 100ul – 1000 µl
- ELISA kit
- Purified water/Distilled water
- Disposable Pipette tips (yellow & blue)
- A lid for polystyrene 96-well plates or a protective film for plates
- Tissue paper
- Gloves
- Lab coat
- Stop watch
- Waste container
- Primers
- PCR Tubes
- White and yellow tips
- Methanol
- LFH
- PCR tubes rack
- Tip box
- Ice boxes
- Master mix
- Sample DNA
- Thermo cycler
- Agarose
- TBE buffer
- Ethidium bromide

- Loading dye
- Gel tray
- Gel buffer and tank buffer
- Gel tank
- UV transilluminator
- Gel documentation equipment (Biored)

Total 212 samples from non-hospitalized patients were included in the study. A questionnaire was filled after the collection of blood from the patient. The questionnaire contained all the information about the history, complications of the patient. Similarly it also contained the information like exposure and contact of the patients with the various agents or persons, which could transmit the viral disease. Complications like anorexia, malaise, fever etc were also included.

The samples taken from the patients belonged to the different districts of KPK Pakistan. Majority of the patients included in the study had the range of age in 15-35 years. The collected samples were stored. After that the serum was extracted from the blood samples. The samples were analyzed for the serological markers.

Serological Analysis

The surface antigen (HbsAg) was analyzed through Enzyme immune assay (EIA). The kit (ELISA) used for the analysis was MBS ITALY. The contents of the kit were Strip Micro plate, Positive Control, Negative Control, Conjugate, Substrate A, Substrate B, Wash Solution, Stop Solution. The whole process was done according to user manual protocol. The whole procedure and the precautionary measures for the analysis were followed, and results were noted.

Procedure for ELISA

All the reagents were brought to room temperature at least for one hour before use. The samples were thawed before the test. The A1 well was left blank. 50 ul of negative control was dispensed in the next three wells (A2, A3, A4). Then 50ul of the positive

controls and 50ul of the samples were added in the respective wells. 50ul of conjugate was added in all wells except the A1 well. The contents of the well were gently mixed by tapping on edge of the plate. The plate was covered with plate lid and was incubated for 60 minutes in microplate incubator at $37^{O}C$. The plate lid was removed and washed 5 times with working washing solution in microplate washer. 50 µl of substrate A and 50 µl of substrate B all the wells including A1 were added. After this they were incubated for 15 minutes and at 37 ^{O}C in microplate incubator. The lid of incubator was covered to avoid light. After that 50ul of stopping reagent was added in all wells. Then the reader was blanked at 620-630 nm for measuring the microplate background then read the absorbance of samples and controls at 450 nm in microplate reader. The microplate was read in 30 minutes after dispensing the stop solution.

The antibody to core protein of HBV was also measured with the help of Enzyme Immune Assay (EIA). The kit used for the process was from MBS ITALY, and it was also an ELISA kit. The contents of the kit were same to that of the above kit. Procedure followed for ELISA was same to the above. The prescribed procedure was followed for the detection of the concerned antibodies. Results obtained, were noted and analyzed. Both the antibodies that are anti-coreIgM and anti-coreIgG were measured in the same way and were analyzed.

DNA Extraction

DNA from the samples was extracted for the amplification purpose. The kit used for the extraction of viral DNA was “Saceae”. DNA was extracted according to the user manual protocol. The extracted DNA was stored at -20 degree centigrade.

PCR Amplification of the samples

The DNA was amplified by using the process of PCR. Two set of primers were used for the amplification of DNA, these were FA1-L and FA1-R, the sequences of the primers were as, forward primer TTTCACCTCTGCCTAATCATCTC, and the sequence of the reverse primer was TCTTGTTCCAAGAATATGGTG. Similarly the second pair of

primer sequences was as under, forward primer, FA3-L, CTGCTGGTGGCTCCAGTT and the sequence of the reverse primer was as FA3-R GCCTTGTAAGTTGGCGAGAA. PCR mixture was prepared by taking 4ul of master mix, 0.6ul of forward primer, 0.6ul of reverse primer and 9.8ul of PCR water for each sample. The quantity of each sample taken was 10ul. PCR conditions were as, initial denaturation at 95°C for 2 min 30 s, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min, finally extension at 72°C for 10 min.

Primer Name	Primer sequence	Length bp	GC content	Amplicon size(bp)
FA1-L	TTTCACCTCTGCCTAATCATCTC	23	43.5	1014
FA1-R	TTT ACCTCTGCCTAATCATCTC	22	40.9	
FA3-L	CTGCTGGTGGCTCCAGTT	18	61.1	1059
FA3-R	GCCTTGTAAGTTGGCGAGAA	20	50	

Table 01.Primers used for the amplification of HBV DNA

After the process of PCR amplification the PCR product was analyzed on Agarose Gel Electrophoresis. The gel prepared had the concentration of 1%, and was prepared by weighing 0.4 gram of agarose and dissolving it in 40 ml of 1X TBE buffer. The buffer was prepared in deionized water. The gel was prepared and 2ul of ethidium bromide was added to the gel and was kept for solidification. After solidification the gel was placed in the gel tank containing 1X TBE buffer. Samples were loaded by mixing the 5ul of the samples with 1ul of loading dye and were loaded to the wells of the gel. 45 min was the total time for gel running. Voltage applied was 100V and the current was 400milli Amperes. After completion of gel running, the gel was observed in the UV transilluminator for the presence of the bands of DNA. The picture of gel was taken with gel documentation equipment and the band of 1000bp size was observed.

Chapter 04.

Results

Results

Samples from non-hospitalized patients were collected. The patients belonged to different districts of KPK, Pakistan. The blood taken as a sample was then transported to the laboratory for further analysis. Total of 212 samples were included in the study. Serum was separated from the blood by means of centrifugation. After that the samples were subjected for the serological analysis using various techniques like Enzyme Immune Assay. After the serological analysis, DNA was extracted from the samples using a kit; the extracted DNA was stored at -20 degree centigrade. Molecular studies of the samples were carried out using the polymerase chain reaction (PCR). DNA of the concerned samples was amplified using specific set of primers. After amplification the band of correct size was observed.

Both male and females subjects were included in the study. They were divided into age groups. The first age group ranged from 1-15 years and the percentage of infection in this group was 24%. The age range in the second group was 16-35 year and the percent of the patients infected in this group was 55.6%. In other group the percentage of the patients infected was 24% and the age of the patients infected was 36 plus years. Our study reported that the percentage of Males that were infected with HBV was 54.24%. According to our study high percentage of female infection was also found, as the percentage of infection in females was 45.75%.

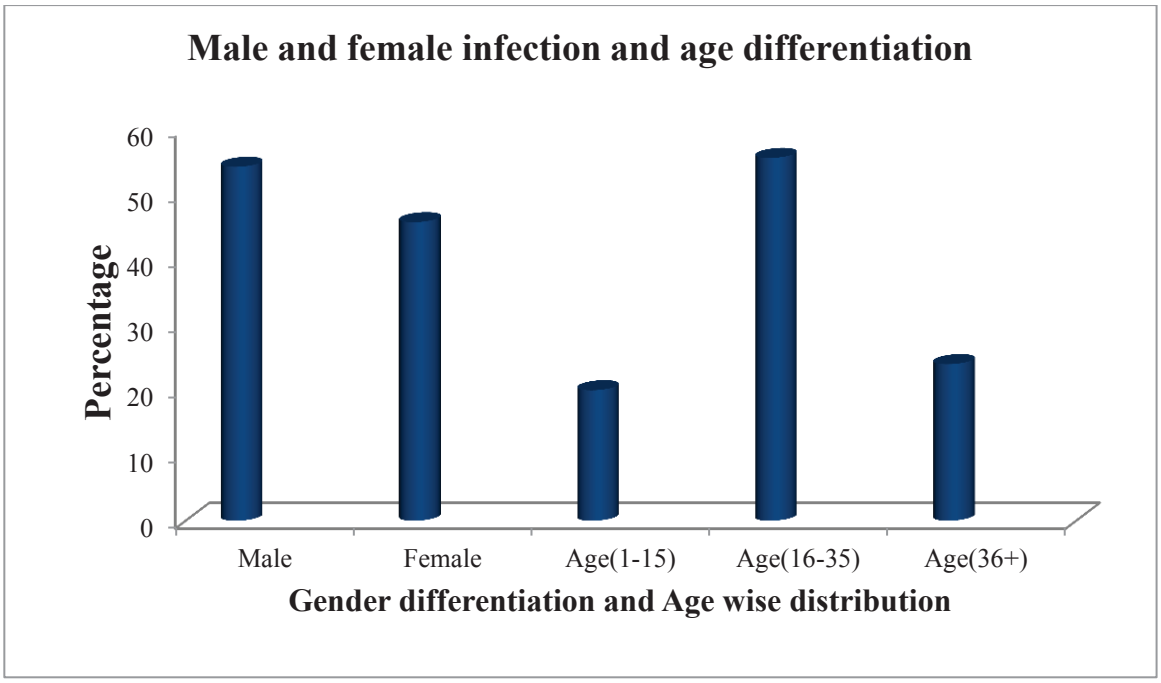

Figure 01. Shows the percentage of male and females patients included in the study. The figure also shows the percentage of age patients. The figure shows that the infection is high in the age of 16-35 years.

Table showing all the Data of the patients which were included in the study. This data is further analyzed in the results.

Key:

G= Gender, **HP**= Hospitalization, **JU**= Jaundice, **AD**= Abdominal Discomfort, **AN**= Anorexia, **DU**= Dark Urine, **FT**= Fatigue, **F**= Fever, **M**= Malaise, **SM**= Splenomegaly, **IN**=Injection, **IV**= Intravenous Infusions, **BPV**= Beauty Parlor Visit, **S**= Surgery, **BT**= Blood Transfusion, **DV**= Dentist Visit, **BV**= Barber Visit, **SKP**= Skin Piercing, **TP**= Tattoos or Apunctures. **HBs**= HbsAg, **ALT**= Alanin Transaminases, **IIU**= Illegal Injection Use, **IgM**= HBcoreIgM, **IgG**= HBcoreIgG, PCR.

H= High levels.

Data representation of the patients included in the study.

S. N	G	HP	JU	A D	AN	DU	FT	F	M	SM	IN	IV	BPV	S	BT	DV	BV	SKP	TP	IIU	HBs	ALT	IgM	IgG	PCR
1	M	-	+	+	-	-	+	-	+	-	+	+	-	-	-	-	+	+	-	-	+	H	+	-	-
2	M	-	+	+	+	-	+	+	+	-	+	+	-	-	-	+	+	-	-	-	+	H	-	+	-
3	F	-	-	+	+	+	+	+	+	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
4	M	-	-	+	+	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+	H	-	+	-
5	F	-	-	+	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	+	H	+	-	+
6	M	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	H	-	+	-
7	M	-	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	+	H	+	+	+
8	F	-	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
9	M	-	+	+	-	-	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	H	-	-	-
10	M	-	+	+	+	-	+	-	+	-	-	+	-	-	-	+	+	-	-	-	+	H	+	+	-
11	M	-	-	+	-	-	+	-	-	-	+	+	-	+	-	-	-	+	-	+	+	H	-	+	-
12	M	-	-	-	+	-	-	-	-	-	+	+	-	+	-	+	-	-	-	-	+	H	-	+	-
13	M	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	+	H	-	-	-
14	F	-	+	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	-	-	+	H	-	+	-
15	M	-	-	+	-	-	+	-	+	-	+	+	-	-	-	+	-	-	-	-	+	H	+	+	+
16	M	-	-	-	+	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-	+	H	-	+	-
17	F	-	-	+	+	-	+	-	+	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
18	F	-	-	+	+	+	+	+	+	-	+	+	-	-	-	+	-	+	-	-	+	H	-	+	-
19	M	-	-	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-	-	+	H	-	+	+
20	F	-	-	+	-	-	-	-	+	-	+	+	-	+	-	-	-	+	-	-	+	H	+	+	-
21	M	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	+	-	-	-	+	H	+	+	-
22	F	-	-	+	+	-	+	-	+	-	-	+	-	+	-	+	-	+	-	-	+	H	-	+	-
23	F	-	-	-	+	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
24	M	-	-	+	-	+	+	-	-	-	+	+	-	-	+	-	-	-	-	-	+	H	-	+	-
25	M	-	-	+	-	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	+	H	-	+	+
26	F	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	H	+	+	-

27	M	-	-	-	+	-	+	+	+	-	+	+	-	-	-	+	-	-	+	-	+	H	-	+	-
28	M	-	-	+	-	-	+	+	+	-	+	-	-	-	-	-	-	-	+	-	+	H	+	+	-
29	M	-	-	+	+	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-	+	H	+	+	+
30	M	-	-	+	-	-	+	+	+	-	+	+	-	+	+	+	+	-	-	-	+	H	-	+	-
31	F	-	+	+	-	-	+	+	+	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
32	M	-	-	-	+	-	+	+	+	-	-	+	-	-	-	-	+	-	-	-	+	H	+	+	-
33	M	-	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-	-	+	H	+	+	-
34	M	-	-	+	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	-
35	F	-	-	-	+	-	+	-	+	-	+	+	-	-	-	+	-	+	-	-	+	H	-	+	-
36	M	-	+	+	+	-	-	-	+	-	-	+	-	-	-	+	+	-	-	-	+	H	+	+	-
37	M	-	-	+	+	-	-	+	+	-	-	+	-	+	+	-	+	-	-	-	+	H	+	+	+
38	F	-	+	+	+	+	-	+	-	-	+	+	-	-	-	-	-	+	-	-	+	H	-	-	-
39	F	-	+	+	+	+	-	+	+	-	+	+	-	+	-	-	-	+	-	-	+	H	-	-	-
40	F	-	+	+	+	-	+	-	+	-	+	-	-	+	-	+	-	+	-	-	+	H	-	+	-
41	F	-	-	+	+	-	+	+	-	-	+	+	-	+	-	-	-	+	-	-	+	H	-	+	+
42	F	-	-	+	+	-	+	-	-	-	+	+	-	-	+	-	-	+	-	-	+	H	-	-	-
43	F	-	-	+	-	+	+	+	-	-	+	+	-	-	-	-	-	+	-	-	+	H	-	-	+
44	M	-	+	+	+	-	-	+	+	-	-	+	-	+	-	-	+	-	-	-	+	H	+	+	+
45	F	-	-	+	+	+	+	+	+	-	-	+	-	-	-	-	-	+	+	-	+	H	+	+	-
46	M	-	-	+	-	-	+	+	+	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
47	M	-	-	+	+	-	-	+	+	-	+	+	-	-	-	+	+	-	-	-	+	H	-	+	-
48	M	-	-	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	-	+	H	-	+	-
49	M	-	-	-	+	-	-	+	+	-	+	-	-	-	-	+	+	-	-	-	+	H	+	+	+
50	F	-	-	+	+	-	+	+	-	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
51	M	-	-	+	-	-	+	+	+	-	+	+	-	-	+	-	+	-	-	-	+	H	-	+	-
52	M	-	-	+	-	-	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	-
53	M	-	-	+	-	-	+	+	+	-	+	+	-	+	-	+	+	-	-	-	+	H	-	+	+
54	M	-	+	+	+	+	-	+	+	-	+	+	-	-	-	-	+	-	-	-	+	H	+	+	-
55	F	-	-	+	+	-	+	-	+	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	+
56	F	-	+	+	+	-	+	+	+	-	-	+	-	-	-	-	-	+	-	-	+	H	-	+	-

57	M	-	-	-	-	+	+	-	+	-	+	+	-	+	-	+	+	-	-	-	+	H	-	+	-
58	M	-	-	+	+	+	+	-	+	-	+	+	-	-	-	-	-	-	-	-	+	H	+	+	-
59	M	-	-	+	+	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-	+	H	-	+	-
60	M	-	-	+	+	+	+	-	+	-	+	+	-	-	-	-	+	-	-	-	+	H	+	-	+
61	M	-	-	+	+	+	+	-	+	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	-
62	M	-	-	-	+	+	+	-	+	-	+	-	-	-	-	+	+	-	-	-	+	H	+	+	-
63	M	-	-	+	-	+	+	+	+	-	+	+	U-k-w-	-	-	+	+	-	-	-	+	H	-	+	-
64	F	+	-	+	-	+	-	+	+	-	+	+	-	-	-	+	-	+	-	-	+	H	-	+	+
65	M	-	-	-	+	+	+	+	-	-	+	+	-	-	-	-	+	-	-	-	+	H	+	+	-
66	F	-	-	+	-	-	+	+	-	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
67	F	-	-	+	-	+	+	+	-	-	+	-	-	+	-	-	-	+	+	-	+	H	-	+	-
68	M	-	-	+	+	+	-	-	-	-	-	+	-	+	+	-	+	-	-	-	+	H	-	+	-
69	F	-	-	+	+	+	+	+	-	-	+	-	-	-	-	+	-	+	-	-	+	H	-	+	-
70	M	-	-	+	+	-	+	-	+	-	+	-	-	+	-	-	+	-	-	-	+	H	-	+	-
71	M	-	-	+	+	+	-	+	-	-	+	+	-	+	-	+	+	-	-	-	+	H	+	+	-
72	M	-	-	-	+	-	+	-	+	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	+
73	M	-	-	+	+	-	+	+	-	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	+
74	F	-	-	-	+	+	+	-	-	-	+	+	-	+	-	+	-	+	-	-	+	H	+	+	-
75	F	-	-	-	+	-	+	-	+	-	+	+	-	-	-	-	-	+	-	-	+	H	+	+	-
76	M	-	+	+	+	+	+	-	+	-	-	+	-	-	-	+	+	-	-	-	+	H	-	+	-
77	F	-	-	-	-	+	+	-	+	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
78	F	-	-	+	-	-	-	+	+	-	+	+	-	-	-	+	-	+	-	-	+	H	+	+	+
79	F	-	-	+	+	-	+	-	+	-	-	+	-	+	+	+	-	+	-	-	+	H	-	-	-
80	F	-	-	-	+	+	+	+	-	-	+	+	-	-	-	+	-	+	-	-	+	H	-	+	-
81	M	-	+	+	+	-	+	+	-	-	+	+	-	+	-	-	-	-	-	-	+	H	-	+	-
82	F	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	H	-	+	-
83	F	-	+	+	-	-	+	+	-	-	+	+	-	+	-	-	-	+	-	-	+	H	-	+	-
84	M	-	+	+	+	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	+	H	-	+	-
85	F	-	-	+	+	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	+	H	-	-	+

86	M	-	-	+	-	+	+	-	+	-	+	+	-	-	-	+	+	+	+	+	+	H	-	+	-
87	M	-	+	+	-	+	+	-	+	-	-	+	-	-	-	+	+	-	-	+	+	H	-	+	-
88	M	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	H	-	+	-
89	M	-	+	+	+	+	-	+	+	-	+	+	-	-	-	-	+	-	-	-	+	H	+	+	-
90	M	-	-	+	+	-	+	+	+	-	+	+	-	+	-	+	+	-	-	-	+	H	+	+	-
91	F	-	-	+	+	+	-	-	-	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
92	M	-	-	+	+	-	-	+	+	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	-
93	F	-	-	+	+	+	+	-	-	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
94	F	-	-	+	+	-	+	+	-	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	+
95	F	-	-	+	-	-	+	+	+	-	+	-	-	+	-	U-k-w-	-	-	-	-	+	H	+	+	+
96	M	-	-	+	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	+	H	-	+	-
97	F	-	-	+	+	-	+	+	+	-	+	+	-	+	-	-	-	-	-	-	+	H	-	+	-
98	F	-	-	+	+	+	+	+	-	-	-	+	+	-	-	-	-	+	-	-	+	H	-	+	-
99	M	-	+	+	-	-	+	-	+	-	+	+	-	-	-	+	+	-	-	-	+	H	-	+	-
100	M	-	+	+	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	H	+	+	-
101	F	-	-	+	+	-	+	-	+	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
102	M	-	-	+	+	+	-	-	+	-	+	+	-	-	+	-	+	-	-	-	+	H	-	+	-
103	F	-	-	+	+	-	+	-	+	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
104	M	-	-	+	+	-	-	+	+	-	+	+	-	-	-	-	-	-	-	-	+	H	-	+	-
105	M	-	+	+	+	+	-	-	+	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	+
106	M	-	+	+	+	-	+	-	+	-	+	-	-	-	-	-	+	-	-	-	+	H	+	+	-
107	F	-	+	+	+	-	-	+	+	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
108	M	-	+	+	+	-	+	-	+	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	-
109	M	-	-	+	+	-	-	+	+	-	+	+	-	-	-	-	+	+	-	-	+	H	+	+	+
110	F	-	-	-	+	-	+	-	+	-	+	+	-	+	+	-	-	+	-	-	+	H	-	+	-
111	F	-	+	+	+	+	+	-	-	-	+	+	-	-	-	+	-	+	-	-	+	H	-	+	-
112	F	-	+	+	+	-	+	+	-	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
113	M	-	+	+	+	-	+	-	+	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	+
114	M	-	+	+	+	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	+	H	-	+	-

115	M	-	+	+	+	-	+	-	+	-	+	-	-	-	-	-	+	-	-	-	+	H	-	+	-
116	M	-	+	+	+	+	+	+	+	-	+	+	-	+	-	-	+	-	-	-	+	H	-	+	-
117	M	-	-	+	-	+	+	-	+	-	+	-	-	-	-	+	+	-	-	+	+	H	+	+	-
118	F	-	-	+	-	-	+	+	+	-	+	+	-	-	-	+	-	+	-	-	+	H	-	-	-
119	F	-	+	+	+	-	+	-	-	-	+	+	+	+	-	+	-	+	-	-	+	H	-	+	+
120	F	-	+	+	-	+	-	+	+	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
121	M	-	-	+	+	+	+	-	-	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	+
122	F	-	-	-	+	+	+	-	+	-	-	+	+	-	-	+	-	+	-	-	+	H	-	+	-
123	F	-	-	-	+	+	-	+	-	-	+	+	-	+	-	-	-	+	-	-	+	H	+	+	-
124	F	-	-	+	+	-	+	-	+	-	+	+	-	+	-	-	-	+	-	-	+	H	-	+	-
125	M	-	-	+	+	-	+	-	-	-	+	+	-	-	-	-	+	-	-	-	+	H	+	+	-
126	F	-	-	+	-	+	+	+	-	-	+	+	-	-	-	+	-	+	-	-	+	H	-	+	-
127	M	-	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-	-	-	+	H	-	+	-
128	F	-	-	+	-	-	+	+	+	-	+	-	-	-	-	+	+	-	-	-	+	H	+	+	-
129	M	-	-	+	+	+	+	+	-	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	-
130	M	-	-	-	+	+	+	+	+	-	+	-	-	-	-	+	-	-	-	-	+	H	-	+	-
131	F	-	-	+	-	+	-	+	+	-	+	+	-	-	+	-	-	-	-	-	+	H	-	+	+
132	F	-	-	-	-	-	+	+	+	-	+	-	-	+	-	-	+	-	-	-	+	H	-	+	-
133	F	-	-	+	+	-	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	H	-	-	-
134	M	-	+	+	+	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	+	H	-	+	-
135	F	-	+	-	+	-	-	+	-	-	+	+	-	-	-	+	-	+	+	-	+	H	-	+	-
136	F	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	-
137	M	-	-	+	-	-	+	+	-	-	-	-	-	-	-	+	-	+	-	-	+	H	-	+	-
138	M	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	+	H	-	+	-
139	F	-	-	+	+	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	H	-	+	+
140	F	-	+	+	+	+	+	-	-	-	+	+	-	-	-	-	-	+	-	-	+	H	+	+	-
141	F	-	-	-	+	-	+	+	-	-	+	+	-	-	-	+	-	+	-	-	+	H	-	+	-
142	M	-	+	-	+	-	-	-	-	-	+	+	-	+	-	+	+	-	-	-	+	H	-	-	-
143	M	-	+	+	+	+	-	+	-	-	+	+	-	-	-	-	+	-	-	+	+	H	-	-	-
144	M	-	-	+	+	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	+	H	-	+	-

145	M	-	-	+	+	-	+	+	-	-	+	+	-	+	-	-	-	-	-	-	+	H	+	+	+
146	F	-	+	+	-	-	+	+	-	-	+	+	-	+	-	+	-	+	-	-	+	H	-	+	-
147	M	-	+	+	+	-	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	-
148	M	-	-	-	+	+	-	-	-	-	+	-	-	+	-	+	+	-	-	-	+	H	-	+	-
149	M	-	+	+	-	+	+	+	+	-	+	+	+	-	-	-	+	-	-	-	+	H	+	+	+
150	M	+	+	+		+	-	+	+	-	+	+	-	+	-	-	+	-	-	-	+	H	+	-	-
151	F	-	-	-	+	-	-	-	+	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
152	M	-	+	+	+	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	+	H	+	+	-
153	M	-	-	+	+	-	+	+	+	-	+	+	-	-	-	-	+	+	-	-	+	H	+	+	-
154	F	-	-	-	+	+	+	+	-	+	+	-	-	+	+	+	-	+	-	-	+	H	-	+	+
155	F	-	-	+	+	+	+	-	-	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	+
156	F	-	-	+	+	-	+	-	-	-	+	+	-	+	-	+	-	+	+	-	+	H	-	+	-
157	F	-	-	-	+	-	+	+	+	-	-	+	-	+	-	+	-	+	-	-	+	H	-	-	-
158	F	-	-	+	+	+	+	-	+	-	+	+	-	-	-	+	-	+	-	-	+	H	-	-	-
159	F	-	-	+	-	+	+	-	+	-	+	+	-	-	-	+	-	+	-	-	+	H	+	+	+
160	F	-	-	-	+	-	+	+	-	-	+	+	-	-	-	+	-	+	-	-	+	H	-	+	-
161	M	-	+	+	-	+	-	-	+	-	+	+	-	-	-	+	+	-	-	-	+	H	+	+	-
162	M	-	-	+	-	+	-	-	-	-	+	+	-	+	-	-	+	-	-	-	+	H	-	+	-
163	M	-	-	+	-	+	+	-	+	-	+	+	-	-	+	+	+	-	-	-	+	H	-	+	-
164	F	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	-	+	-	-	+	H	-	+	-
165	M	-	+	+	-	+	+	-	+	-	+	+	-	-	-	+	+	-	-	-	+	H	-	+	+
166	F	-	+	+	+	-	+	-	-	-	+	+	+	-	-	+	-	+	+	-	+	H	-	+	-
167	F	-	-	+	+	-	+	+	+	-	-	+	-	-	-	-	-	+	-	-	+	H	-	+	-
168	F	-	+	-	+	+	+	-	+	-	+	+	-	+	-	+	-	+	-	-	+	H	-	+	+
169	F	-	-	-	-	+	+	-	+	-	+	-	-	-	-	-	-	+	-	-	+	H	-	+	-
170	M	-	-	+	-	+	+	+	+	-	+	-	-	-	-	+	-	+	-	-	+	H	+	+	+
171	M	-	-	-	-	+	+	-	+	-	+	+	-	+	-	+	+	-	-	-	+	H	+	+	+
172	M	-	-	-	-	-	+	-	+	-	+	+	-	+	-	-	+	-	-	-	+	H	-	+	-
173	M	-	-	-	+	-	-	+	-	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	-
174	M	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	-	+	H	-	+	-

175	F	-	-	+	-	-	+	-	-	-	+	+	-	-	-	+	-	+	-	-	+	H	+	-	-
176	M	-	-	+	-	-	+	-	+	-	+	+	-	+	+	+	+	-	-	-	+	H	+	+	+
177	M	-	+	+	-	+	+	+	-	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	-
178	M	-	-	+	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	H	-	+	-
179	F	-	-	+	-	+	+	-	+	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	-
180	F	-	+	+	+	-	-	+	-	-	+	+	-	-	-	+	-	+	-	-	+	H	-	+	+
181	M	+	+	-	+	-	+	+	+	-	+	+	-	-	-	+	-	+	-	-	+	H	-	+	-
182	M	+	-	-	+	+	+	+	+	-	+	+	-	-	-	+	-	-	-	-	+	H	-	+	-
183	F	-	-	+	+	-	+	-	+	-	+	+	-	-	-	-	-	+	-	-	+	H	+	+	+
184	F	-	+	-	+	+	+	+	+	-	+	+	-	-	-	+	-	+	-	-	+	H	-	+	-
185	F	-	-	+	+	+	+	-	-	-	+	+	-	-	-	-	-	+	-	-	+	H	-	+	+
186	F	-	-	+	-	+	+	+	+	-	+	-	-	-	-	+	-	-	-	-	+	H	-	+	+
187	M	-	-	+	+	+	-	+	+	-	-	+	-	-	-	+	+	-	-	-	+	H	-	+	-
188	F	-	-	+	+	+	-	+	-	-	+	+	-	-	-	+	-	+	-	-	+	H	-	+	+
189	F	-	-	+	-	-	+	-	+	-	+	+	-	+	+	-	-	-	-	-	+	H	-	+	-
190	F	-	+	+	-	-	+	+	+	-	+	+	-	+	-	+	-	+	-	-	+	H	-	-	-
191	F	-	-	+	-	-	-	-	+	-	+	+	-	-	-	+	-	+	-	-	+	H	-	+	+
192	M	-	-	+	+	+	+	+	-	-	+	+	-	+	+	+	-	-	-	-	+	H	-	-	-
193	F	-	-	-	+	-	+	-	+	-	+	+	-	-	-	-	-	+	-	-	+	H	-	-	-
194	M	-	-	+	+	-	+	-	+	-	+	+	-	-	-	-	+	-	-	-	+	H	+	+	+
195	M	-	-	+	+	-	-	+	+	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	-
196	M	-	-	+	-	-	-	+	+	-	+	+	-	-	-	-	+	-	-	-	+	H	-	-	-
197	M	-	-	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+	-	-	+	H	-	+	-
198	F	-	-	+	+	+	+	-	-	-	-	+	-	-	-	-	-	+	-	-	+	H	-	+	+
199	F	-	-	+	+	+	+	+	+	-	+	+	+	-	-	+	-	+	-	-	+	H	-	-	-
200	F	-	-	+	-	-	+	+	+	-	+	+	-	-	-	-	-	+	-	-	+	H	-	-	-
201	F	-	-	-	-	+	+	+	+	-	+	+	-	+	-	+	-	+	-	-	+	H	+	+	+
202	F	-	-	+	+	-	+	+	-	-	+	+	+	-	-	-	-	+	+	-	+	H	-	-	-
203	F	-	-	+	+	-	+	+	-	-	+	+	-	-	-	+	-	+	+	-	+	H	-	+	+
204	F	-	-	+	-	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-	+	H	-	+	-

205	M	-	-	+	-	+	-	+	+	-	+	+	-	-	-	-	+	-	-	-	+	H	-	-	-
206	M	-	-	+	-	+	+	-	+	-	+	+	-	-	-	-	+	-	-	-	+	H	-	+	+
207	M	-	-	+	+	+	+	-	-	-	+	+	-	-	-	+	-	-	-	-	+	H	-	+	-
208	F	-	-	+	+	-	+	-	+	-	+	+	-	-	-	-	+	-	-	-	+	H	-	-	+
209	M	-	-	+	+	-	+	+	+	-	+	+	-	-	-	+	-	-	-	-	+	H	-	+	-
210	M	-	-	+	+	+	+	+	+	-	+	+	-	-	-	+	+	-	-	-	+	H	-	+	-
211	M	-	-	+	+	-	+	+	+	-	-	+	-	-	-	+	+	-	-	-	+	H	+	+	-
212	F	-	+	+	-	+	+	+	+	-	+	+	-	+	-	-	-	+	-	-	+	H	+	+	+

Signs and Symptoms Analysis

There are different symptoms of HBV infection. Symptoms can help us in the diagnosis of the HBV infections. We also included different symptoms of HBV infection in our study. Abdominal discomfort can be one of the symptoms of HBV infection. In the patients included in our study 168 (79.24%) had abdominal discomfort. Unknown pain RHC was found to be present in 80 (37.73%) patients. HBV infection related Anorexia was found to be present in 140 (66.03%) patients. Similarly fatigue in the HBV infected patients was present in 154 (72.24%). Symptoms of fever appeared in 114 (53.77%) of the patients. Malaise was also the symptom included in the study which was prevalent in 131 (61.69%) of the patients. Dark urine and splenomegaly was found to be associated in 94 (44.33%) and 05 (2.35%). Similarly vomiting resulted in 104 (49.05%) patients.

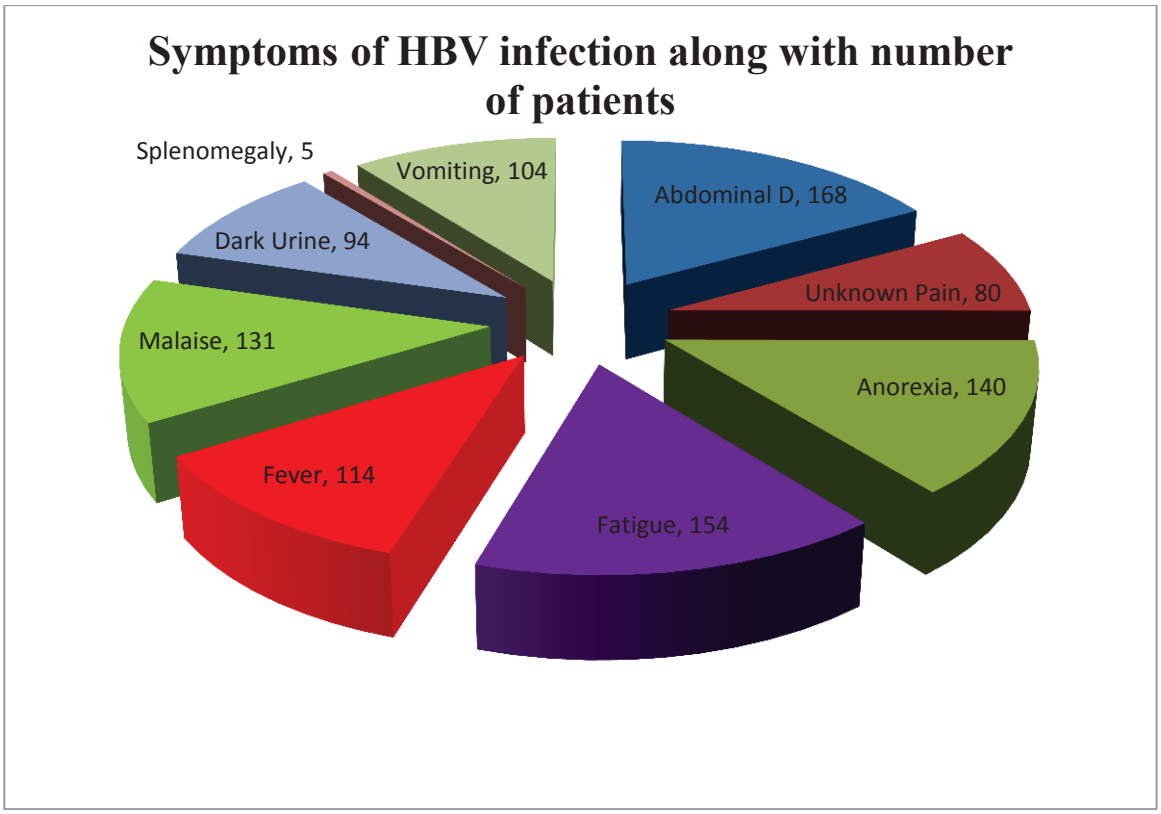

Fig 02. Shows the symptoms HBV infection and number of patients having the symptoms.

This Pie chart shows distribution of different symptoms for HBV infection like, Fatigue, Fever, Malaise, Dark Urine, Splenomegaly, Vomiting, Abdominal Discomfort, Unknown Pain and Anorexia.

Risk Factors responsible for HBV infection

The factors which can predispose one to HBV infection such as surgery, blood transfusion, barber visit and dentist visit were also included in the study. The percentage of the patients which underwent surgery were 54 (25%). Similarly the patients which received blood transfusion were (17) 8.01%. Those patients who visited barber were 40% and the percentage of patients who visited the dentist was also 110 (51.8%). The injection use was in total of 186 (87.7%) patients. Hospitalization was in 99 (46.69%). The intravenous infusions were done in 178 (83.96%) patients. Visits to beauty parlor was reported in 18 (8.49%) of patients. Skin piercing was done in 89 (41.98%) of the patients. Tattoos or apunctures in the skin were 11 (5.18%) of the patients. The illegal drug injection users were 16 (7.54%). Similarly the house hold contact was in 140 (66.03%) of the patients infected with HBV. As we know that jaundice is one of the indicative factors for the HBV infection. The percentage of jaundice positive patients was 26.4%. Jaundice was present in the acutely infected patients with the percentage of 26.31%. While the jaundice in the chronic patients was 25.5%.

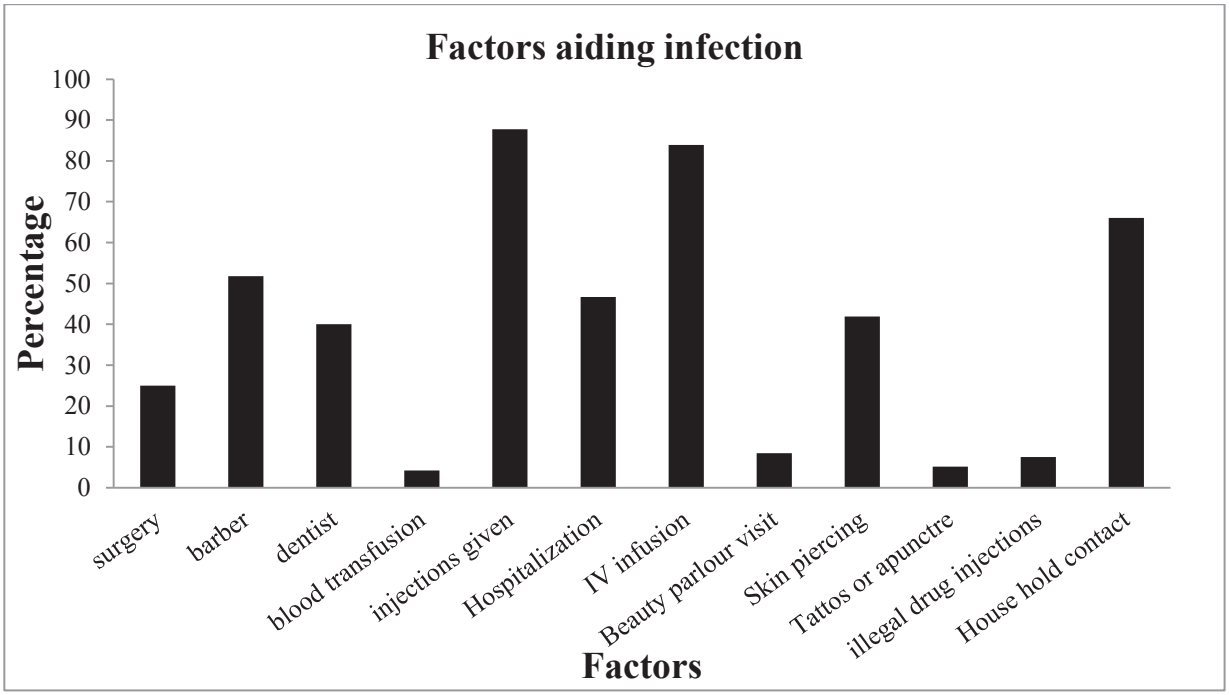

Figure 03. Factors aiding to HBV infection and their percentages

This graph shows the percentages for factors that can aid in causing the HBV infection; here the factors are surgical operations, barber visits, dentist visits, blood transfusions, injections, hospitalizations, intravenous infusions, beauty parlor visits, skin piercing, tattoos or apunctures, illegal drug injections and house hold contact.

Serological Analysis

The sample size included in the study was 212. The serological analysis showed that the ALT level of all the concerned samples was high, as ALT levels are elevated during injury or damage to the hepatocytes. The percentage of ALT level in our study was 100%. As the ALT are the alanin transferases which are the liver enzymes secreted by the liver cells when there is injury to the liver cells.

The second marker which was analyzed for the infection was the HBV surface antigen known as HbsAg, as the HbsAg is the first marker which is available in the serum after infection with HBV. HbsAg can show the different forms of HBV infections, like acute infection, chronic infection and another infection in which the surface antigen is negative is known as occult infection. In our study the HbsAg was positive for all the samples. The percentage of the samples which were positive for HbsAg was 100%. This percentage shows that all the infections were of Hepatitis B virus.

Anti-Hbcore IgM is an antibody which is produced against the core antigen of HBV. This antibody is also an indicative for the acute infection and can remain in the serum for up to 6 months. In our study the 25% of the samples were positive for the Anti-Hbcore IgM antibody. This parameter can also be regarded for the acute infections, in other words we can say that the 25% infections were acute infections. Of the total PCR positive samples, 40% were positive for the acute infection.

The other parameter which is usually employed for the detection of chronic HBV infection is anti-HbcoreIgG. As our study was based on the detection of chronic infections, so this study showed that about 63.67% of the patients were chronically infected. The PCR results showed that 53.84% of the PCR positive samples were chronic HBV infections**.** The percentage of the chronic infections was high.

S.No	ALT levels	HbsAg	Anti-Hbcore IgM	Anti-Hbcore IgG	Negative for IgM& IgG	PCR
N	212	212	53	135	24	53
percentage	100	100	25	63.67	11.32	24.5

Table 01. Percentages for different Markers for HBV infection.

S.No	Total PCR positive samples	HBcore IgM PCR positive samples (out of total PCR +ve samples)	HBcore IgG PCR positive samples (out of total PCR +ve samples)	Both HBcore IgM and IgG (-) but PCR positive
N	53	21	28	03
Percentage	24.5	40.38	53.84	5.76

Table 02. Percentage for the PCR positive samples along with the percentage for HBcore IgM and HBcore IgG (PCR positive) samples.

PCR Amplification of the Concerned Samples

The most important and commonly used marker for the detection of HBV infection now a days is by detection through polymerase chain reaction (PCR). As PCR is one of the well known technique and a very sensitive technique because it can detect DNA quantities of up to 1pg.We also used the process of PCR for the molecular studies of the HBV. In our study the total percentage of PCR positive samples in was 24.5% and as early stated, most of the positive samples were from chronic infections.

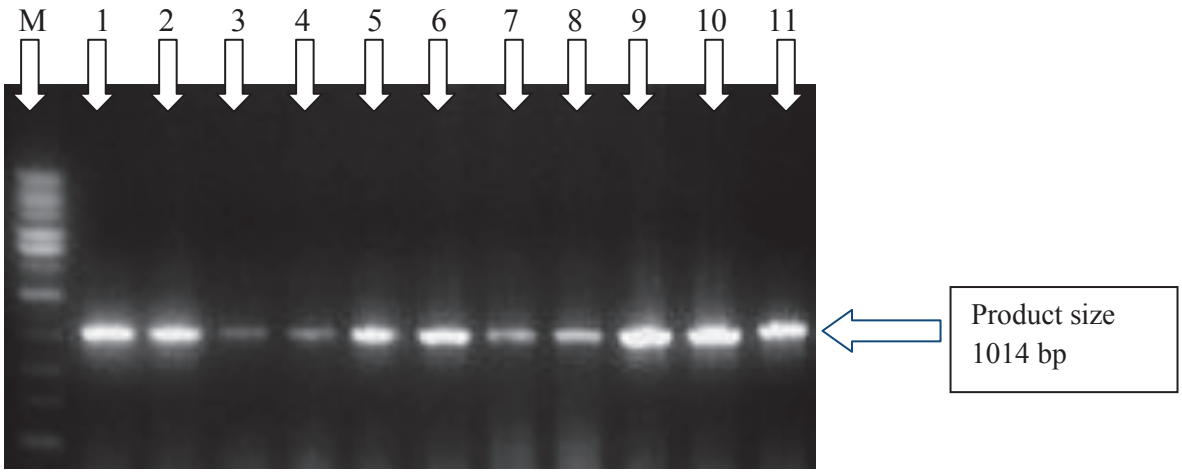

Figure 04. Agarose Gel Electrophoresis image. This image shows the PCR amplified product of HBV DNA. The size of the Amplicon was 1014bp. M shows the DNA marker of 1kb size. Lane 1-11 indicates the PCR product of the samples.

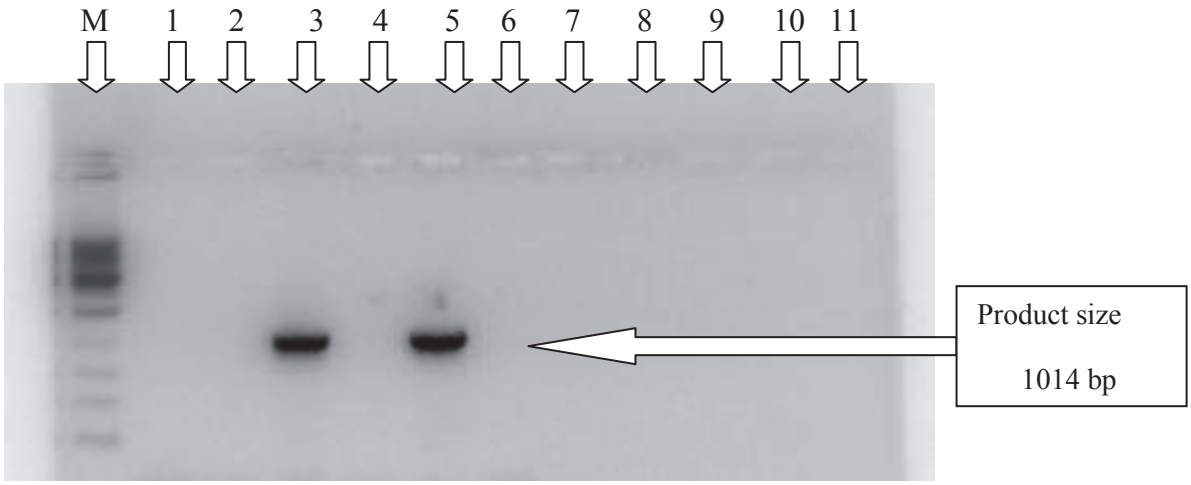

Figure 05. Agarose Gel Electrophoresis image. This image shows the PCR amplified product of HBV DNA of only sample number 3 and 5. The size of the Amplicon was 1014bp. M shows the DNA marker of 1kb size. Lane 1-11 indicate the samples. Only two samples are positive and the rest are negative.

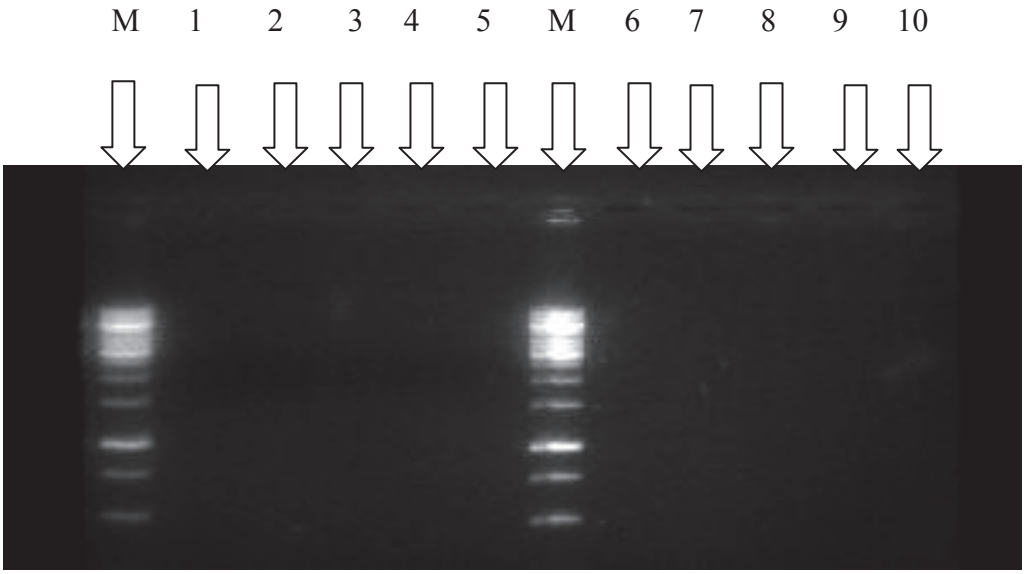

Figure 06. Agarose Gel Electrophoresis image showing no amplification of the HBV DNA. Both M shows the marker. Lane 1-5 and 6-10 shows samples which are not amplified.

Acute and Chronic Infections of HBV

HBV infections were also classified into Acute and Chronic infections. The cut off value for the HBcore IgM was 1. In this study 51 out of 53 HBcore IgM positive had the titer value above one and hence they were regarded as acute infections. Similarly the cut off value for the HBcore IgG was also 1. Interestingly all the samples which were HBcore IgG positive had the antibody titer value above 1. In this study 11.3% of the patients were negative for both HBcore IgM and HBcore IgG.

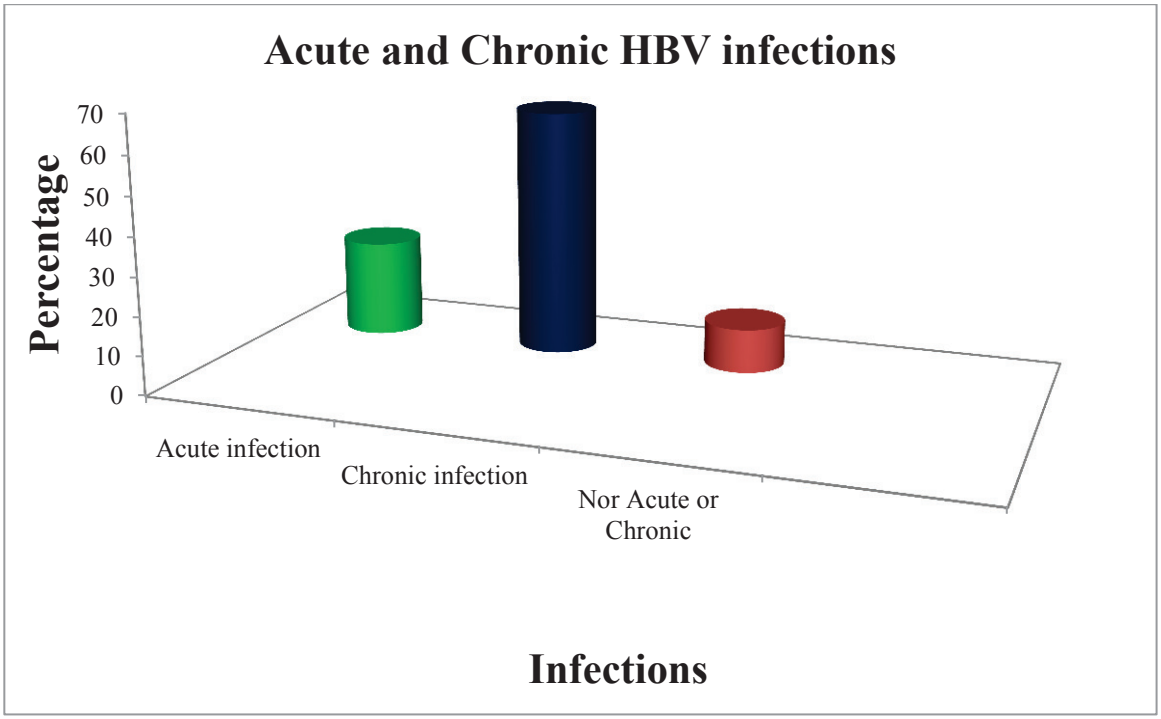

Fig 07. HBV Acute and Chronic HBV infections.

Chapter 05.

Discussion

Discussion

According to the WHO standards defining endemicity of hepatitis countries with infection rates between 3-5% of general population are considered as moderate and above 5% as highly endemic. Approximately 4% of the general population in Pakistan is infected with HBV (Zuberi 1998). Sources of transmission for HBV infection are mainly blood and body secretions. According to an estimate 80% of the acute cases of HBV are cleared within 6 months and rest of the cases progress towards chronic stage of infection. If a virus remains detectable in the blood, longer than six months, it reflects that the person has a chronic infection. However as mentioned above majority of the cases of acute hepatitis will recover within six months however, exceptions do exist, as 90% of the infants and 50% of young children having HBV infections are at the highest risk of developing chronic infections. It is estimated that from the endemic areas 50% of the cases diagnosed as primary infections are in fact acute exacerbations of chronic hepatitis. A complete and reliable differentiation between acute and chronic infections should be considered as the most important step prior to the initiation of proper treatment of the infection. In the case of acute hepatitis infection antiviral therapy is not recommended unless severe infection prevails. There are some important serological and molecular parameters that can differentiate between these two conditions but due to high costs and lack of facilities these tests are not practiced in routine diagnosis in many areas of Pakistan.

Recently, a remarkable effort has been made by WHO team (Bosan *et al*., 2010) that brought important information in public domain, reflects the situation related to hepatitis (A-E) in Pakistan. This information is based on two hundred and three published articles and abstracts (Bosan *et al*., 2010). Most of the data is about the prevalence of hepatitis that includes studies on healthy children, vertical transmission, pregnant women, healthy individuals, army recruits, blood donors, health care workers, use of unsafe injections, high risk groups, patients with provisional diagnosis of hepatitis, patients with chronic liver disease, and genotypes of HBV. Conclusively, it was highlighted in the review that there is a lack of community-based epidemiological work on this subject. In

future, large scale community based epidemiological data by using sophisticated procedures of screening might bring a better picture about the prevalence of acute and chronic HBV in Pakistan.

The current study focuses on the detection of Chronic HBV infections by serological and molecular markers in non-hospitalized patients from different districts of Khyber Pakhtunkhwa Pakistan. Acute and chronic infections are main causes of morbidity and mortality in Pakistan as well as all over the world. Diagnosis and detection of these HBV infections are very important from health and economical point of view. Khyber pakhtunkhwa (KPK) is located in the North West of Pakistan having considerable population. Certain disasters like terrorism, floods and earthquakes had contributed a lot to the poor economy of the people of KPK. Current situation can result in increased infections such as acute and chronic HBV infections.

As earlier studies from the particular region, which were carried out, focuses on the prevalence of HBV in particular areas and secondly the genotypic studies which focuses on the prevalence of specific genotypes in the particular regions and in Pakistan. The present study can serve as a model for the acute and chronic infections. Our study reported that in non hospitalized patients the ratio of acute and chronic HBV infections were 25% and 63.67% respectively. Studies conducted by I Merican *et al.,* in (2000) show that in Asian population about 50 million cases of HBV infections are diagnosed annually. Out of which 5-10% of the adults and 90% of the infants are chronically infected. Similarly other studies regarding chronic HBV infections had reported high infection rates of chronic HBV infections.

According our study the percentage of chronic infection occurrence in the non hospitalized patients were about 63.67% in a subset of population which belonged to the different districts of KPK Pakistan. These results also coincide with the other studies which also show the rate of occurrence of chronic HBV infection, both at national and international level.

As the signs and symptoms of a disease can play a crucial role in its diagnosis and its treatment. In our study we have also determined the signs and symptoms of the

patients which were included in the study. As we know that abdominal discomfort can also be one of the indicative for the HBV infection so in our study the abdominal discomfort was present in 168 (79.24%) subjects. Unknown pain was found in 80 (37.73%), Anorexia in 140 (66.03%) of the patients respectively. Fatigue and fever were found in 154(76.24%) and 114(53.77%) of the total patients. Malaise is also a symptom of HBV infection and it was found in 131(61.79%) of the patients. Dark urine was reported in 94 (44.33%) of the patients. Splenomegaly occurred in 05 (2.35%) of the patients. Vomiting was found in 104 (49.05%) patients. The symptoms of HBV infection can vary and it may also depend on the type of HBV infection and also on the genotype of HBV. Different patients can show varying degree of symptoms for HBV infections.

Our study also reported the infection rate in male and female patients which was 54.4% and 45.6% respectively. Rauf *et al* in (2012) reported 49.1% infection in male patients and 50.80% in female patients. Attaullah *et al.,* 2011 reported that the percentage of male and female patients was 78.5% and 21.49% respectively. These studies co-incides with our study as the percentage of infections in male is high. Nevertheless the results of Rauf *et al*., (2012) contradict our study. A high percentage of infection has been reported in females in our study. Though most of the studies had confirmed the high rate of infection in males, yet the rate of infection in female population is also quite high. The age of the patients included in the study ranged from 15-35 years (55.6%), while less than 15 (20%) and (24%) had an age of more than 35 years. Amjad *et al.,* in (2012) reported that the prevalence rate of HBV infection in patients was 38.5%, having the age of 21-30 years. While other groups, <10 and > 50, had the infection rates of 3.14% and 7.14% respectively. The data show that most of the patients had age between 15-35 years which is a quite young age. HBV chronic infections at younger age are not considered as a good sign for the health of a community. The difference in the infection rates between the different ages may due to the risk factors for the HBV infections.

The ALT levels in the all patients included in the study were high. Cut off value for the ALT was 45U/L. ALT level of all the patients was high. ALT levels in the HBV infections can vary with the course of time and also with the type of infection, which are acute infection, chronic infection or Occult HBV infection. Han *et al*., in (2008) reported

that ALT levels in the chronic infections can remain persistently normal however it is also reported that the ALT levels can also remain high during the chronic infections. Elevated ALT levels show immune-mediated inflammation and a higher rate of hepatitis B virus E antigen (HbeAg), seroconversion. As in our study the levels of ALT are high in these non hospitalized patients, which are contradictory to the above study. During HBV chronic infections ALT levels can remain high or it may vary.

Jaundice is also indicative for the infection or damage of the hepatocytes. In our study we determined that 26.88% of the patients had jaundice, the percentage of jaundice in acute infections was 26.31% and in chronic patients the jaundice was 25.5%. Jaundice can only develop in about 30% people having HBV infection, majority of the people do have infection but they don't have any jaundice. (Daniel, 2009).

There are many factors that can increase the risk for HBV infections, which include, surgeries, blood transfusions, unsafe sexual contact, barber visits, dentist visit and many more. In our study we found that the 25% of the patients have already gone through surgery. 40% of the patients have visits to the barber shop for shaving or other purposes. 51.8% of the patients have visited the dentist for treatment. While about 8% of the patients had already blood transfusions histories. The injection use was found to be 87.7% of the infected patients. Hospitalization of the patients also got the share of 46.49%. The infusions which were given intravenously were reported to be in 83.96% of the patients. Patients visiting beauty parlor were found to be 8.49%. Nevertheless 41.98% of the patients had skin piercing through needle or some other way. Tattoos making or apunctures in the skin was reported in 5.18% of the patients. Illegal drug use and house hold contact was found to be 7.54% and 66.03%.

Different studies regarding HBV risk factors have shown different results as Oliveira *et al.,* in (2000) reported that percentages of the different risk factors of HBV infections vary greatly. They reported that the intravenous drug users, males having homosexual contacts and the needle sharing are the main risk factors for the HBV transmission in Brazil. Similarly Mahomet *et al*., in (2004) reported age of the patient as one of the risk factor associated with HBV infection. Areas such as urban or rural areas

and the education status of the particular community in these urban and rural areas can also one of the major risk factor for the HBV infection. Khan *et al.,* in (2011) reported the risk factors which were involved in the HBV transmission. Blood transfusions were 4.04%, barber visits 23.60%, injections history in 26.19% of the patients and dental risk aiding the part of 11.2% respectively. They concluded that males are mostly exposed as compared to females and the patients having younger age are most likely to be infected as compared to older age patients. Syringes reuse, barber and dentist visits were the main contributing risk factors for the HBV infection. This study also co incides with our study. Many other studies have also reported about the blood transfusions, barber shops and dentist visits as risk factors for HBV infections.

HbsAg is the first serological marker which appears in the serum after infection with HBV. Interestingly in our study all the samples were HbsAg positive. As HbsAg can remain positive for the whole period of HBV infection or it may disappear from the serum after some time as in Chronic or Occult infection. Literature studies suggest that the lifelong positivity of HbsAg in HBV positive patients may be due to the integration of viral DNA into the host chromosome. This can result in the continuous production of HbsAg. HbsAg levels were also higher in HbeAg positive patients than those who were HbeAg negative. Jerzy *et al.,* (2010).

We did not perform the HbeAg assay for our concerned samples so we don't have a picture regarding the co relation between HbeAg and HbsAg. Several studies had reported the HBV surface antigen positivity for the HBV infections. Inoue *et al.,* in 1998 showed that the HbsAg was positive in all of the chronic infections. This is exactly in correspondence to our study.

HBV infection is sometimes miss diagnosed with the surface antigen, as in some cases there are conditions in which the surface antigen is negative for the infection so then we can use the other markers like antibodies to the core antigen of the virus and by directly detecting the DNA in the serum.

Anti-Hbcore IgM is the serological marker for the Acute HBV infection. Patients having IgM as marker of infection almost clears the virus, this was reported by C

Lavarini *et al.,* 1983. Our study reported that 25% of the patients had core IgM in their sera, which displays that this percentage of the patients was infected acutely. The cut off value for the core IgM was 1. Samples having the HBcore IgM antibody titers values above 1 were regarded as chronic infection. 51 out of the 53 HBcore IgM positive samples had the titer value above 1. This percentage clearly showed that these samples were infected acutely. Of the total PCR positive samples the ratio for the PCR positive samples for HBcore IgM was 40%, which demonstrated that the virus is still in replicative form. Japhet *et al.,* (2011) reported the presence of anti-HBcore IgM in 12(13.0%) of the 92 blood donors. Similarly Wolfram *et al.,* (1986) reported that about 30% cases were positive for coreIgM, which is not much different from our study. The infections in which the total core antibodies, i.e., core IgM and coreIgG were positive were regarded as acute. The HBcore IgG also appears in the preliminary stage of infection but it can remain after to progressing to the chronic infection.

HBV chronic infections, is one of the most serious issue regarding health facilities. As HBV chronic infections are characterized by the presence of HbsAg for more than six months and development of antibodies against the core region of the virus that is anti-HBcore IgG Antibody. When this antibody appears in the sera of patients then the infection can be regarded as chronic and it may be resolved. Later on this antibody is replaced by Anti-Hbs which remain in the patient sera for whole life. As to our study there was a high infection rate of HBV chronic infection in the non-hospitalized patients and it was 63.67%. Samples having the HBcore IgG antibody titers values above 1 were regarded as chronic infections. Cut off value for HBcore IgG was 1. In our study all the patients which were positive for anti-HBcore IgG had the titer values above 1. The marker interpreted for the HBV chronic infection was mainly the anti-HBcore IgG with the surface antigen positive for more than six months. Rajesh, *et al.,* in (2003) reported the presence of anti-HBc antibody up to 42% in patients who were infected with HIV virus. Studies conducted by Tadjiev in (2010) concluded that the HBcore IgG was found in the 50 (100%) of the infected subjects. The variations in the chronic infection rates can depend on various factors, like age of patient, socioeconomical condition. In our study we found that 21(9.90%) of the total patients were negative for both the Anti-HBcore IgM

and Anti-HBcore IgG. These samples were also negative for PCR. Studies indicated that these samples may be in the incubation period of infection. Three of samples were negative for the serological markers but were positive for the PCR. This situation may be developed due to the immunocompromised condition of the patient, due to which there is no antibody production against the HBV in them. These samples may also reflect a recent infection in the concerned samples, as the PCR is positive for these samples.

PCR assay is one of the most reliable assay for the detection a of the HBV infection. We also carried out the process of PCR for the molecular detection of viral DNA in the serum. As we all know PCR is the most reliable source for the detection of HBV infection, because PCR actually tells us about the quantity or quality of the DNA present in the serum. Presence of DNA shows the active form of virus which is also replicating, or the virus may not be in the replicative form but it is present as a such in the serum, which is the carrier state of the infection, at this stage the virus waits for the opportunistic moment and when it gets the chance it causes an infection.

What we have determined that the percentage of PCR positive patients was 24.5%. C Brechot *et al.,* (1993) reported the presence of HBV DNA, which were amplified through PCR were 93% n=30, in the active chronic hepatitis B infection while in asymptomatic HbsAg carriers 57 % n=40, contained the suspected DNA. Similarly C Rodrigues *et al.,* 2001, reported the percentage for the chronic infection which were PCR positive and it was found that among them the percentage was 63% in one group, 62% in the second group and 75% in the third group. Lahiri *et al.,* 2007 conducted a study regarding the HbeAg (-) chronic HBV infections and they concluded that the prevalence of e antigen negative chronic HBV infection was 8.3% in Indian population.

The DNA detection of HBV through PCR process depends on various conditions like, the unavailability of DNA in the serum. The HBV DNA is present in the liver in cccDNA form that is covalently closed circular DNA. The cccDNA is not available in the serum of the patient and is present in the hepatocytes. The second reason for the low detection is the sensitivity of the PCR reaction. The primers and the annealing temperatures can also have a role in this regard. The HBV DNA may be also unavailable

because it may be integrated into the host chromosome and it is not detectable by the PCR assay. Mutated forms of the viruses can also aid to the low detection of the virus by avoiding the amplification, as the region of concern is mutated so we don't have the desired amplification.

PCR amplification of the HBV genome in the serum had advantage over the other serological markers because in certain infections like occult infections, or infections in which the HbsAg is missing from the serum, PCR can be used to truly identify an infection. As the serological marker for the replicative HBV is HbeAg, but there are some mutations which had resulted resistance in the virus because in this case they don't develop the e antigen. So in this condition PCR can be the main player to find out the infection. One of the limitation in our study was that we did not perform the HbeAg or anti- HbeAg assay, which shows us the replicative forms of HBV virus. So we can tell that we could not predict the replicative forms of the virus which is active in the liver and is causing infection. Presence of DNA detected by the PCR can be used to indicate the replicative forms of virus, because the patients we analyzed had surface antigen positive and ALT level raised. The second parameter which we have not done in our study was the antibody to surface antigen that is anti-Hbs antibody. As this antibody indicates you the recovery from the infection, so due to this limitation we could not detected the patients who were recovering.

Conclusion

Conclusions

- Our study show higher prevalence of chronic HBV infection in the non hospitalized patients from the KPK.

- A considerable percentage for the acutely infected non hospitalized patients was also reported.

- Regarding our study patients having age in between 15-35 years in the KPK region were mostly infected.

- Different risk factors like blood transfusions, intravenous drug use, visit to dentist, visit to barber shop, skin piercing, house hold contact and surgical operations care the source of increased risk for HBV infections in that particular region of KPK.

- In comparison to other methods for the detection of HBV infections, PCR is one of the most reliable assay for the detection of HBV infection.

Future prospects

Future prospects

- The genotypic study for the concerned samples should be carried out to find out the prevalence of specific genotypes in KPK.

- Development of hepatocellular carcinoma in the population of KPK infected with HBV should be determined.

- Co infection of HIV virus with HBV should be studied in the population of KPK.

- Genetic predisposition to the HBV in the people of KPK is necessary to be sorted out, to assess the HBV infections in that particular region.

Recommendations

Recommendations

- According to CDC Atlanta, people having chronic HBV infection should be treated with lamivudine.

- Injection use, intravenous infusions and unhygienic household contacts should be reduced to avoid HBV infections.

- Mass vaccination programmes should be initiated at national level to assess the HBV infection in Pakistan.

- The Prime Minister Control programme for hepatitis should notice the situation of chronic infection at KPK and should take solid steps to reduce the burden of HBV in KPK.

- Awareness among the public is necessary about preventive measures and the consequences of HBV infection.

- Screening of HBV infection at district level should be initiated by the health ministry of KPK to find out the prevalence of HBV in districts of KPK**.**

References

References

Alam, M., Zaidi, S., Malik, S., Naeem, A., Shaukat, S., Sharif, S. & Butt, J. (2007). Serology based disease status of Pakistani population infected with Hepatitis B virus. *BMC Infectious diseases*, 7(1), 64.

Alavian, S. M. M., Fallahian, F., Lankarani, K. B. (2007). Comparison of Seroepidemiology and Transmission. *Hepatitis Monthly,* 7,4, 233-2238.

Ali, A., Nisar, M., Idrees, M., Ahmad, H., Hussain, A., Rafique, S., Sabri, S., Rehman, H, U., Ali, L., Wazir, S. & Khan, T. (2012). Prevalence of HBV infection in suspected population of conflict-affected area of war against terrorism in North Waziristan FATA Pakistan. *Infection* MEEGID*,* 12. 1865–1869.

Ali, M., Idrees, M., Ali, L., Hussain, A., Rehman, I., Saleem, S. & Butt, S. (2011). Hepatitis B virus in Pakistan: A systematic review of prevalence, risk factors, awareness status and genotypes. *Virol J*, 8, 102.

Arauz, R. P., Norder, H., Robertson, B. H. & Magnius L.O. (2002). Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. *J Gen Virol,* 83, 2059-2073.

Attaullah, S., Rehman, S. U., Khan, S., Ali, I., Ali, S. & Khan, S. N. (2011). Prevalence of Hepatitis B virus genotypes in HbsAg positive individuals of Afghanistan. *Virol J,* 8:281.

Bosan, A., Qureshi, H, Bile, K. M., Ahmad, I., & Hafiz, R. 2010. A review of hepatitis viral infections in Pakistan. *J. Pak. Med. Assoc*, 60(12),1045-58.

Brechot, C. (1993). Polymerase chain reaction for the diagnosis of viral hepatitis B and C. *Gut ; supplement,* S39-S44.

Caballero, T., Caballero, M. A., Ruiz-Extremera, A., Barrios Del Pino, Y., O'Valle, F., Salmeron, F. J., & Sánchez Salgado, G. (1995). Detection of hepatitis B virus in the liver by in situ hybridization (ISH) in HbsAg seropositive and seronegative patients. *Histol Histopathol,* 10: 265-270.

Cardona, N. E., Loureiro, C. L., Garzaro, D. J., Duarte, M. C., García, D. M., Pacheco, M. C. & Pujol, F. H. (2011). Unusual presentation of hepatitis B serological markers in an Amerindian community of Venezuela with a majority of occult cases. *Virol J*, 8(1), 1-7.

Daniel, C. Hepatitis B virusinfection.About.com.http://hepatitis.about.com. Updated, July 16, 2009.

Elgouhari, H. M., Tamimi, T. I. A. R. & Carey, W. D. (2008). Hepatitis B virus infection: understanding its epidemiology, course, and diagnosis. *CCJM*, *75*(12), 881-889.

Ferreira, R.,Teles, S. Dias, M. A.,Tavares, V. R., Silva, S. A., Gomes, S. A., Yoshiba, C. FT. & Martins, R. M. B. (2006). Hepatitis B virus infection profile in hemodialysis patients in Central Brazil: prevalence, risk factors and genotypes. *Mem Inst Oswaldo Cruz, Rio de Janeiro*, 6, 689-692.

Gerlich, W. H. A., Uy. A., Lambrecht, F. & Thomssen. R. (1986). Cutoff levels of immunoglobulin M antibody against viral core antigen for differentiation of acute chronic and past hepatitis B virus infections. *J. Clin. Microbiol*, 24(2):288.

Ghadir, M. R., Belbasi, M., Heidari, A., Sarkeshikian, S. S., Kabiri, A., Ghanooni, A. H., & Alavian, S. M. (2012). Prevalence of hepatitis d virus infection among hepatitis B virus infected patients in Qom province, center of Iran. *Hepatitis Monthly*, 12(3), 205.

Gitlin, N. (1997). Hepatitis B: diagnosis, prevention, and treatment. *Clinical Chemistry*, 43:8, 1500 –1506.

Hakim, S,T., Kazmi, S.U., & Bagasra, O. (2007). Seroprevalence of Hepatitis B and C Genotypes among Young Apparently Healthy Females of Karachi-Pakistan. *Libyan J Med*, 071123.

Han, K. H. & Kim, D. Y. (2008). Chronic HBV infection with persistently normal ALT b. Not to treat. *Hepato Into*, 2:185–189.

Helmy, A., & Al-Sebayel, M. I. (2006). Isolated antibody to hepatitis B core antigen in patients with chronic hepatitis C virus infection. *WJG*, 12(27), 4406.

Inoue, K., Yoshiba, M., Sekiyama, K., Okamoto, H., & Mayumi, M. (1998). Clinical and molecular virological differences between fulminant hepatic failures following acute and chronic infection with hepatitis B virus. *J Medical Virol*, 55(1), 35-41.

Jammeh, S., Tavner, F., Watson, R., Thomas, H. C., Karayiannis, P. (2008). Effect of basal core promoter and precore mutations on hepatitis B virus replication. *J Gen Virol,* 89: 901-909.

Jaroszewicz, J., Serrano, B. C., Wursthorn, K., Deterding, K., Schlue, J., Raupach, R. & Cornberg, M. (2010). Hepatitis B surface antigen (HbsAg) levels in the natural history of hepatitis B virus (HBV)-infection: a European perspective. *Journal of hepatology*, 52(4), 514-522.

Khan, A, U., Waqar, M., Akram, M., Zaib, M., Wasim, M., Ahmad, S., Niaz, Z., Ali, S., Ali, H., Idrees, M. & Bajwa, M, A, (2011). True prevalence of twin HDV-HBV infection in Pakistan: a molecular approach. *Virol J*, 8:420.

Khan, F., Akbar, H., Idrees, M., Khan, H., Shahzad, K., & Kayani, M. A. (2011). The prevalence of HBV infection in the cohort of IDPs of war against terrorism in Malakand Division of Northern Pakistan. *BMC infectious diseases*, 11(1), 176.

Khan, F., Shams, S., Qureshi, I. D., Israr, M., Khan, H., Sarwar, M. T., & Ilyas, M. (2011). Hepatitis B virus infection among different sex and age groups in Pakistani Punjab. *Virol J*, 8, 225.

Kumar, M., Jain, S., Sharma, B. C. & Sarin, S. K. (2006). Differentiating acute hepatitis B from the first episode of symptomatic exacerbation of chronic hepatitis B. *DDS,* 51: 594-599.

Lahiri, K. K., Sahni, A. K., Gupta, R.M., Duhan, S. D., Kapila, K. & Jena, B. J. (2007). Hepatitis B e Antigen Negative Chronic Hepatitis in Indian Patients : A Reality, *Mjafi,* . 4-8.

Lavarini, C., Farci, P., Chiaberge, E., Veglio, V., Giacobbi, D., Bedarida, G. & Caporaso, N. (1983). IgM antibody against hepatitis B core antigen (IgM anti-HBc): diagnostic and prognostic significance in acute HbsAg positive hepatitis. *BMJ, (Clinical research ed.)*, 287(6401), 1254.

Liu, Y., Li, P., Li, C., Zhou, J., Wu, C., & Zhou, Y. H. (2010). Detection of hepatitis B virus DNA among accepted blood donors in Nanjing, China. *Virol J*, 7(193), 193.

Mahoney, F. J., (1999). Update on Diagnosis, Management, and Prevention of Hepatitis B Virus Infection. *Clin. Microbiol. Rev*, 351–366.

Mehmet, D., Meliksah, E., Serif, Y., Gunay, S., Tuncer, O., & Zeynep, S. (2005). Prevalence of hepatitis B infection in the southeastern region of Turkey: comparison of risk factors for HBV infection in rural and urban areas. *Jpn J Infect Dis*, 58(1), 15-9.

Mendy, M. E., Kaye, S., van der Sande, M., Rayco-Solon, P., Waight, P. A., Shipton, D. & McConkey, S. J. (2006). Application of real-time PCR to quantify hepatitis B virus DNA in chronic carriers in The Gambia. *Virol J*, 3(1), 23.

Merat, S., Rezvan, H., Nouraie, M., Jamali, A. R. S. I. A., Assari, S., Abolghasemi, H. & Jafari, N. (2009). The prevalence of hepatitis B surface antigen and anti-hepatitis B core antibody in Iran: a population-based study. *Arch Iran Med*, 12(3), 225-31.

Merican, I., Guan, R., Amarapuka, D., Alexander, M. J., Chutaputti, A., Chien, R. N. & Xu, D. Z. (2000). Chronic hepatitis B virus infection in Asian countries. *JGH*, 15(12), 1356-1361.

Mukherjee, R. M., Reddy, P. B., Arava, J., Rao, P., & Reddy, D. (2010). Relationship between serum HbsAg level, HBV DNA level, and peripheral immune cells in patients with chronic hepatitis B virus infection. *Hepatic Medicine: Evidence and Research*, *2*, 157-162.

Noorali, S., Hakim, S. T., McLean, D., Kazmi, S. U., & Bagasra, O. (2008). Prevalence of Hepatitis B virus genotype D in females in Karachi, Pakistan. *J Infect Developing Countries,* 2(05), 373-378.

Norder, H. ,Courouce, A.M. & Magnius LO. (1994). Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology*, 198:489-503

Okamoto, H., Tsuda, F., Akahane, Y., Sugai, Y., Yoshiba, M., Moriyama, K., Tanaka, T., Miyakawa, Y. & Mayumi, M. (1994). Hepatitis B virus with mutations in the core promoter for an e antigen-negative phenotype in carriers with antibody to e antigen. *J Virol*, 68: 8102-8110.

Oliveira, M. L. A., Bastos, F. I., Telles, P. R., Yoshida, C. F. T., Schatzmayr, H. G., Paetzold, U. & Schreier, E. (1999). Prevalence and risk factors for HBV, HCV and HDV infections among injecting drug users from Rio de Janeiro, Brazil. *BJMBR*, 32(9), 1107-1114

Olutola, A. (2013). Prevalence and Clinical and Immunoviralogical Profile of Human Immunodeficiency Virus-Hepatitis B Coinfection among Children in an Antiretroviral Therapy Programme in Benue State, Nigeria. *ISRN pediatric,* 125-131.

Orenbuch-Harroch, E., Levy, L., & Ben-Chetrit, E. (2008). Acute hepatitis B or exacerbation of chronic hepatitis B-that is the question. *WJG*, 14(46), 7133.

Poorolajal, J. & Majdzadeh, R. (2009). Prevalence of chronic hepatitis B infection in Iran, a review article. *JRMS,* 14-20.

Raimond, G., Pollicino, T.C., Acciola, I. & Squadrito, G. (2007). Occult hepatitis B virus infection. *J Hepatol,* 46:160 – 170.

Rajesh, T., Gandhi, Wurcel, A., Lee, H., McGovern, B., Boczanowski, M., Gerwin, R., Corcoran, C. P., Szczepiorkowski, Z., Toner, T., Daniel, E. Cohen, D. E., Sax, P. E. & Ukomadu, C. (2003). Isolated Antibody to Hepatitis B Core Antigen in Human Immunodeficiency Virus Type-1–Infected Individuals. *CID,* 36:1602–5.

Ramezani, A., Mohraz, M., Banifazl, M., Eslamifar, A. & Aghakhani, A. (2009). Significance of Hepatitis B Core Antibody as the Only Marker of Hepatitis B Virus Infection in High Risk Patients. *IJP,* 4 (2), 80 – 84.

Rauf, A., Nadeem, M. S., Ali, A., Iqbal, M., Mustafa, M., Muzammal, M., Latif, M. Z., Ahmed, N. & Shakoori, A. R. (2010). Prevalence of hepatitis B and C in internally displaced persons of war against terrorism in Swat, Pakistan. *European Journal of Public Health*, 1–5.

Shaikh, B. A., Muhammad, S. K. & Shah, I. A.(2012). *Medical forum monthly, A journal for all specialist*, 422-426.

Stramer, S. L., Wend, U., Candotti, D., Foster, G. A., Hollinger, F. B., Dodd, R. Y. & Gerlich, W. (2011). Nucleic acid testing to detect HBV infection in blood donors. *N Eng J Med*, 364(3), 236-247.

Tadjiev, B. (2010). Clinical diagnostic and molecular genetic features of chronic mixed hepatitis B+C in Uzbekistan. *Medical and Health Sci J,* 47-51.

Tariq, H. W. Z., & Ahmad SZ, (1998). Acute hepatitis B, serological confirmation. *Pak AMJ,* 48(2):102-105.

Thompson, A. J., Nguyen, T., Iser, D., Ayres, A., Jackson, K., Littlejohn, M. & Locarnini, S. A. (2010). Serum hepatitis B surface antigen and hepatitis B e antigen titers: disease phase influences correlation with viral load and intrahepatic hepatitis B virus markers. *Hepatology*, 51(6), 1933-1944.

Tonekaboni, S. S., Waters, J. A., Jeffers, S., Gehrke, R., Ofenloch, B., Horsch, A., Hess, G., Thomas, H. C. & Karayiannis, P. (2000). . Effect of variation in the common ‘‘a’’ determinant on the antigenicity of hepatitis B surface antigen. *J Med Virol,* 60:113 – 121.

Utama, A., Siburian, M. D., Purwantomo, S., Intan, M. D. B., Kurniasih, T. S., Gani, R. A., & Achwan, W. A. (2011). Association of core promoter mutations of hepatitis B virus and viral load is different in HbeAg (+) and HbeAg (-) patients. *WJG*, 17(6), 708.

Vitale, F., Tramuto., Orlando, A., Vizzini, G., Meli, V., Cerame, G., Mazzucco . W., Virdone, R., Palazzo, U., Villafrate, M. R., Tagger. A. & Romano. N. (2008). Can the Serological Status of Anti-HBc Alone Be Considered a Sentinel Marker for Detection of Occult HBV Infection. *J Med Virol*, 80:577 – 582.

Weinberger, K. M., Bauer. T., Ohm, B. S. & Jilg, W. (2000). High genetic variability of the group specific a determinant of hepatitis B virus surface antigen (HBsAg) and the corresponding fragment of the viral polymerase in chronic virus carriers lacking detectable HbsAg in serum. *J Gen Virol,* 81:1165 – 1 174.

Workowski, K. A., & Berman, S. M. (2006). Sexually transmitted diseases treatment guidelines, *CDC,* 55, 1-94.

Yoshiba, M., Sekiyama, K., Iwamura, Y. & Sugata, F. (1993). Development of reliable artificial liver support plasma exchange in combination with hemodiafiltration using high performance membranes. *Digestive Diseases and Sciences,* 38:469–476.

Yuen, M.F. & Lai, C. L. (2001). Treatment of chronic hepatitis B. *The Lancet Infectious Diseases*, 191-196.

Zhang, Q., Wu, G., Richards, E., Jia, S. G., & Zeng, C. (2007). Universal primers for HBV genome DNA amplification across subtypes: a case study for designing more effective viral primers. *Virol J*, 4(1), 92.

Zuberi, S. J. (1998). An overview of HBV/HCV in Pakistan. *Pak. J.Med*, Res, 12-18.

Appendices

Appendix A

Primer Name	Primer sequence	Length bp	GC content	Amplicon size(bp)
FA1-L	TTTCACCTCTGCCTAATCATCTC	23	43.5	1014
FA1-R	TTT ACCTCTGCCTAATCATCTC	22	40.9	
FA3-L	CTGCTGGTGGCTCCAGTT	18	61.1	1059
FA3-R	GCCTTGTAAGTTGGCGAGAA	20	50	

Table 01.Primers used for the amplification of HBV DNA

S.No	ALT levels	HbsAg	Anti-Hbcore IgM	Anti-Hbcore IgG	Negative for IgM& IgG	PCR
N	212	212	53	135	24	53
percentage	100	100	25	63.67	11.32	24.5

Table 02. Percentages of different Markers for HBV infection.

S.No	Total PCR positive samples	HBcore IgM PCR positive samples (out of total PCR +ve samples)	HBcore IgG PCR positive samples (out of total PCR +ve samples)	Both HBcore IgM and IgG (-) but PCR positive
N	53	21	28	03
Percentage	24.5	40.38	53.84	5.76

Table 03. Percentage for the PCR positive samples along with the percentage for HBcore IgM and HBcore IgG (PCR positive) samples.

S.No	Male	Female	Age, 1-15	Age, 16-35	Age, 36+..
N	115	97	43	115	51
%	54.24	45.75	20.2	54.24	24

Table 04. Gender and Age wise Discrimination of the patients included in the study.

S.no	Chronic infections	Acute infections	Jaundice	Jaundice in Acute infections	Jaundice in chronic patients
N	141	57	56	15	36
%	66.5	26.8	26.4	26.31	25.5

Table 05. HBV acute and chronic infections along with the occurrence of Jaundice in these infections.

Risk Factor	Yes	%	No	%
Injections given	186	87.73 %	26	12.26 %
Hospitalization	99	46.69 %	113	53.30 %
Surgery	54	25.47 %	158	74.52 %
Blood transfusion	17	8.01 %	195	91.98 %
IV infusion:	178	83.96 %	34	16.03 %
Dentist visit:	84	39.62 %	128	60.37 %
Visit to barber (men only):	110	51.88 %	102	48.11 %
Visit to beauty parlor	18	8.49 %	194	91.50 %
Skin piercing:	89	41.98 %	123	58.01 %
Tattoos or acupuncture:	11	5.18 %	201	94.81 %
Illegal injection drug use:	16	7.54 %	196	92.45 %
Household contact::	140	66.03 %	72	33.96 %

Table 06. Risk factors associated with HBV infection.

.

Symptoms	Yes	%	No	%
(1) ❑Abdominal Discomfort	168	79.24 %	44	20.75 %
(2) ❑Unknown pain RHC	80	37.73 %	132	62.26 %
(3) ❑ Anorexia	140	66.03 %	72	33.96 %
(4) ❑Fatigue	154	72.64 %	58	27.35 %
(5) ❑ Fever	114	53.77 %	98	46.22 %
(6) ❑Malaise	131	61.79 %	81	38.20 %
(7) ❑ Dark urine	94	44.33 %	118	55.66 %
(8) ❑Splenomegaly	05	2.35 %	207	97.64 %
(9) ❑ Vomiting:	104	49.05 %	108	50.94 %

Table 07. Symptoms associated with HBV infection.

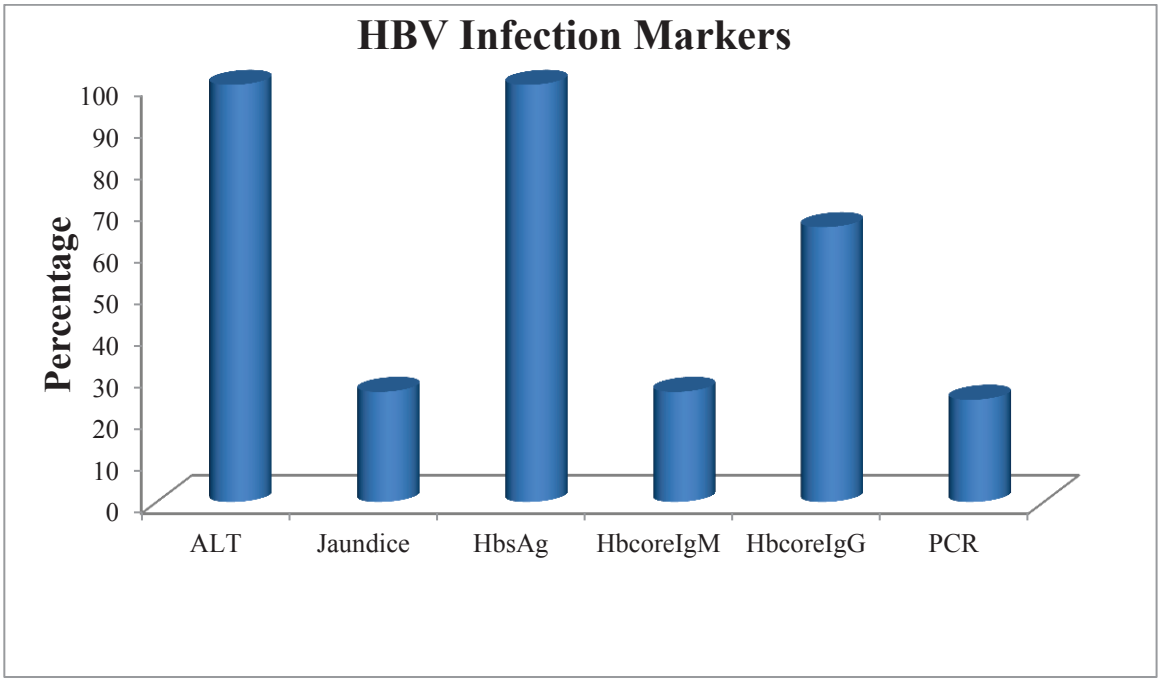

Fig 08. Different markers for the infections of HBV and their percentages

Appendix B

Calculations for the PCR Reaction Mixture Preparation:

Master Mix= 4UI/sample

Forward Primer= 0.6UI/sample

Reverse Primer= 0.6UI/sample

PCR Water= 9.8 UI/sample

Sample= 10UI

Calculations for the making of 1X Agarose GEL:

Agarose = 0.4 gm in 40 ml of 1X TBE Buffer

Ethidium Bromide= 2UI

Loading Dye= 1UI/sample

Sample Loaded= 5UI.

10 X TBE buffer preparation:

TBE buffer contain, Tris base, Boric Acid, EDTA,

For Making 10 X TBE buffer in 1000 ml

Tris base= 108g

Boric Acid= 55g

EDTA= 7.5g

The 10X TBE buffer was then diluted to 1X by adding 900ml of deionized water and 100ml of 10X TBE buffer.