Chronic hepatitis B infection in the immigrant communities of East London

Dias, Aruna

CHRONIC HEPATITIS B INFECTION IN THE IMMIGRANT COMMUNITIES OF EAST LONDON

Dr Aruna Dias

BSc MBBS MRCP

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Abstract

Worldwide there are 350 million people with chronic hepatitis B infection and globally it causes up to half of the liver cancer deaths and one third of deaths from cirrhosis. Only a fraction of sufferers will develop these complications. Various studies have implicated socio-demographic, biochemical and viral factors in disease progression but research has been limited to local populations in endemic countries. Our aim was to study the prevalence and factors associated with advanced disease of hepatitis B infection in immigrants living in East London.

I completed a retrospective analysis of notes and electronic health records of 1209 immigrant patients attending hospitals in East London, 217 of whom were from Bangladesh and Pakistan. Screening of volunteers attending local mosques using oral mucosal transudate swabs and national statistics data allowed us to calculate prevalence rates in these populations. Those 13 patients from Bangladesh and Pakistan admitted over 30 months with decompensated disease were men aged >40. Age, sex, ALT, smoking, alcohol and diabetes were significant predictors for cirrhosis and decompensated disease but not viral markers. Similar analyses were performed for other ethnicities with similar outcomes. The scale of under diagnosis of hepatitis B for all ethnicities was estimated and the reasons explored. This work has scrutinised the epidemiology of chronic hepatitis B in East London and the difficulties encountered exploring it. We provide differing results to published studies and suggestions for how this domain can be examined further.

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ABBREVIATIONS

µl	Micro litres
A&E	Accident & Emergency department
AASLD	American Association for the Study of Liver Diseases
AFP	Alpha feto protein
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine transaminase
Anti-HBc	Antibody to hepatitis B core antigen
Anti-HBe	Antibody to hepatitis B e antigen
Anti-HBs	Antibody to hepatitis B surface antigen
BCP	Basal core promoter
cccDNA	Covalently closed circular DNA
CD	Cluster of differentiation
CDC	Centres for Disease Control
CFU	Colony forming units
CHB	Chronic hepatitis B (infection)
CRC	Clinical research centre
CT	Computed tomography
CTL	Cytotoxic T lymphocytes

DBS	Dried blood spot
DEFRA	Department for Environment, Food and Rural Affairs
DNA	Deoxyribonucleic acid
dsDNA	Double stranded DNA
EASL	European Association for Study of the Liver
ELISA	Enzyme linked immunosorbent assay
EU	European Union
EVR	Early virological response
GMO	Genetically modified organism
GP	General practitioner
HAV	Hepatitis A virus
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B e antigen
HBIG	Hepatitis B immunoglobulin
HBsAg	Hepatitis B surface antigen
HBV DNA	Hepatitis B virus deoxyribose nucleic acid level
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV RNA	Hepatitis C virus ribonucleic acid level
HCV	Hepatitis C virus

HDV	Delta virus
HIV	Human immunodeficiency virus
HPA	Health Protection Agency
hrs	Hours
HSE	Health and Safety executive
IFNα	Inteferon alpha
IFNγ	Interferon gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
im	Intramuscular
IMP	Investigational medicinal product
INR	International normalised ratio
IPS	International passenger survey
IU/ml	International units per millilitre
IVDU	Intravenous drug user
L	Litres
LFS	Labour force survey
LFTs	Liver function tests
MHRA	Medicines and Healthcare products Regulatory Agency

mins	Minutes
ml	Millilitres
MRI	Magnetic resonance imaging
MTA	Material transfer agreement
NA(s)	Nucleoside/Nucleotide analogues
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NINo	National insurance number
NK	Natural killer cells
nm	Nanometres
NUH	Newham University Hospital
OMT	Oral mucosal transudate
ONS	Office for National Statistics
ORF	Open reading frame
PD-1	Programmed death-1
PEG-IFN	Pegylated interferon 2a/b
pgRNA	Pregenomic RNA
PT	Prothrombin time (seconds)
RCT	Randomised control trial
REC	Research and ethics committee

RIA	Radioimmunoassay
RLH	Royal London Hospital
RNA	Ribonucleic acid
RVR	Rapid virological response
S. typhi	Salmonella typhoid
SAE	Serious adverse event
sc	Subcutaneous
secs	Seconds
SSA	Site specific assessment
SVR	Sustained virological response
Th1	T-helper cells type 1
Th2	T-helper cells type 2
TIM	Total international migration
TNFα	Tissue necrosis factor alpha
UK	United Kingdom
ULN	Upper limit of normal (range)
US	United States of America
USA	United States of America
w/v	Weight per volume
WHO	World Health Organisation

1 Introduction

1.1 Classification of human hepatitis viruses

Many viruses affect the liver such as Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Herpes Simplex virus (HSV), human immunodeficiency virus (HIV), yellow fever virus and Dengue fever virus but they do not have the liver as their primary target of infection. The human hepatitis viruses are hepatotrophic with the liver being the primary target of cellular damage and viral replication. There are five main human hepatitis viruses and these are defined virologically into RNA and DNA viruses. Hepatitis A and E are transmitted via the faecal-oral route, whilst hepatitis B, C, and D are transmitted parenterally. Hepatitis B (HBV) differs from the other parenterally transmitted hepatitis viruses in that it is a DNA virus, whilst the others are RNA viruses.

HBV belongs to the Hepadnaviridae family of viruses. Human HBV shares a degree of structural, genomic and clinical sequelae with other animal hepatitis viruses from the same family: ground squirrel hepatitis virus (GSHV),[1] woodchuck hepatitis virus (WHV),[2] and duck hepatitis B virus (DHBV).[3] In all these species, presence of the virus can ultimately lead to the development of chronic liver disease and hepatocellular carcinoma albeit with differing degrees of oncogenic potential.

1.2 Discovery of HBV

Hepatitis B was first isolated by Baruch Blumberg and his co-workers in Philadelphia in 1965. They identified a viral antigen in serum that was "introduced by transfusions". As this virus originally came from the sera of an Australian Aborigine it was termed Australia Antigen (AuAg).[4] In 1970, David Dane and colleagues described the structure of the complete HBV particle.[5] In 1973 the world health organisation (WHO) renamed AuAg as

hepatitis B antigen (HBAg) and this subsequently was known as hepatitis B surface antigen (HBsAg).

1.3 Structure of HBV

The infectious HBV virion is 42-47 nm in diameter and is also known as the *Dane particle* after its discoverer (see Figure 1-1). It is composed of an outer envelope and inner icosahedral nucleocapsid. The outer envelope is composed of a lipid bilayer and embedded within are the surface proteins which may or may not be glycosylated. There are 3 types of surface proteins which are also known as surface antigens: small, medium, and large, and are typically found in a ratio of 4:1:1 in Dane particles.[6] As the small antigen is present on the surface of all HBV particles it is termed *hepatitis B surface antigen* (HBsAg). The role of medium proteins has not been fully determined yet. Large proteins, on the other hand, are thought to be essential for infection and viral morphogenesis.[7]

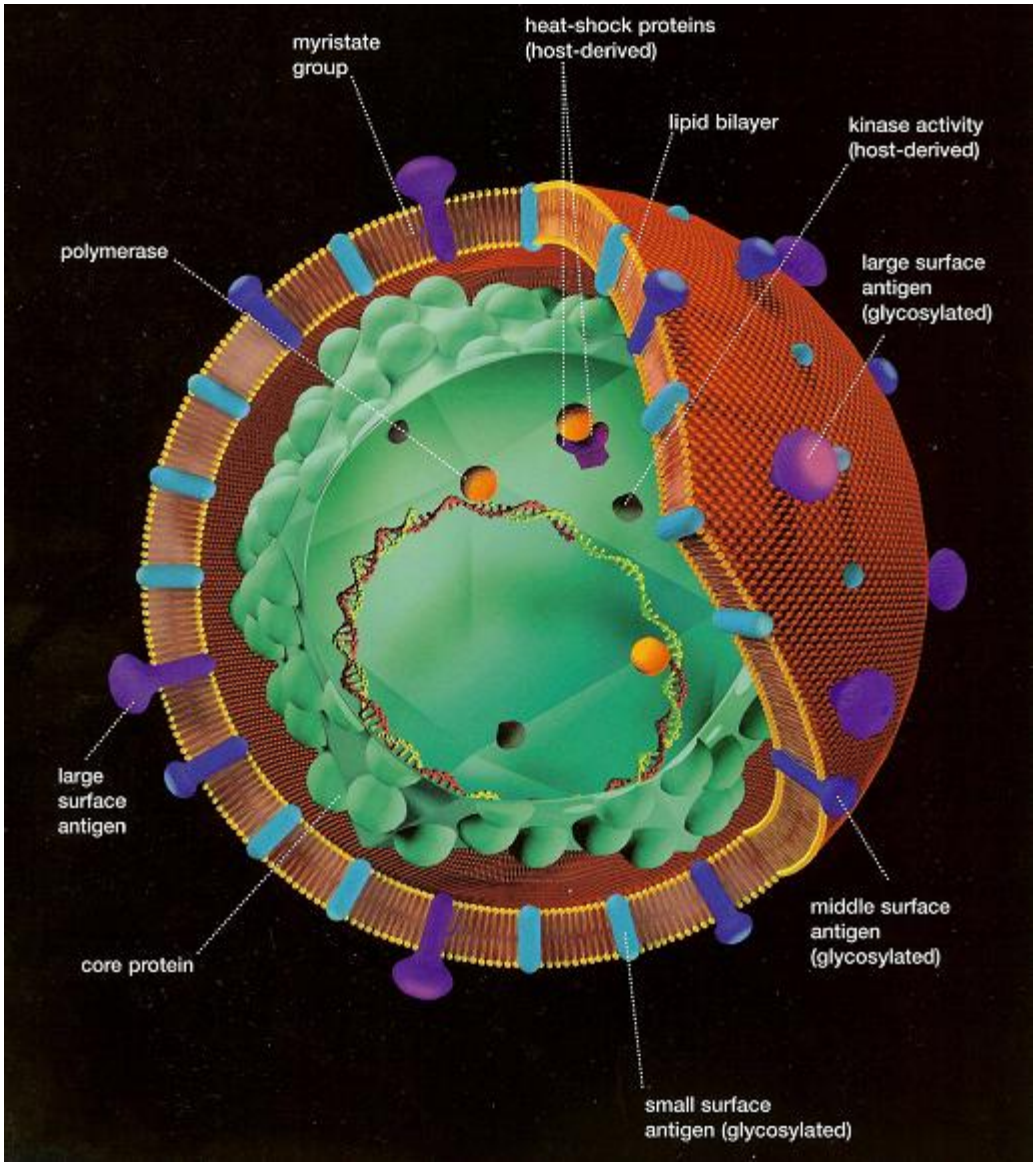

Figure 1-1 Structure of HBV virus (taken from Lai CL, Locarnini S, editors. Hepatitis B Virus. 2 ed. London: International Medical Press; 2008.)

The main structural protein of the inner nucleocapsid is the phosphorylated C or core protein. This was originally detected serologically and referred to as *hepatitis B core antigen* (HBcAg).[8] Within this core is found the P or polymerase protein, the viral DNA and a protein kinase. In vitro studies have shown that the protein kinase is involved with

phosphorylating C protein[9;10] but that recombinant C and P proteins do not possess this enzyme which suggests that it is derived from the host.

In addition to producing the Dane particle, HBV also releases 2 types of sub-viral particles which exist as 20 nm spheres or filaments (see Figure 1-2). They are composed entirely of S proteins and lipid membrane derived from the host[11;12] with no inner nucleocapsid. The S proteins are mainly small surface proteins with some medium proteins but very few large proteins. Whilst these particles are non-infectious due to the lack of nucleic acid, they are highly immunogenic and efficiently induce a neutralizing anti-HBs antibody response. There may be as many as 10,000 to a 1,000,000 fold excess of these spheres compared to Dane particles. Filaments are produced in smaller numbers than spheres and have the same diameter but are of variable length. The reason for there being so many spheres produced when they are not infectious has not been fully ascertained but one theory maybe that they absorb neutralizing antibody and so shield the virions from the host defences.[13]

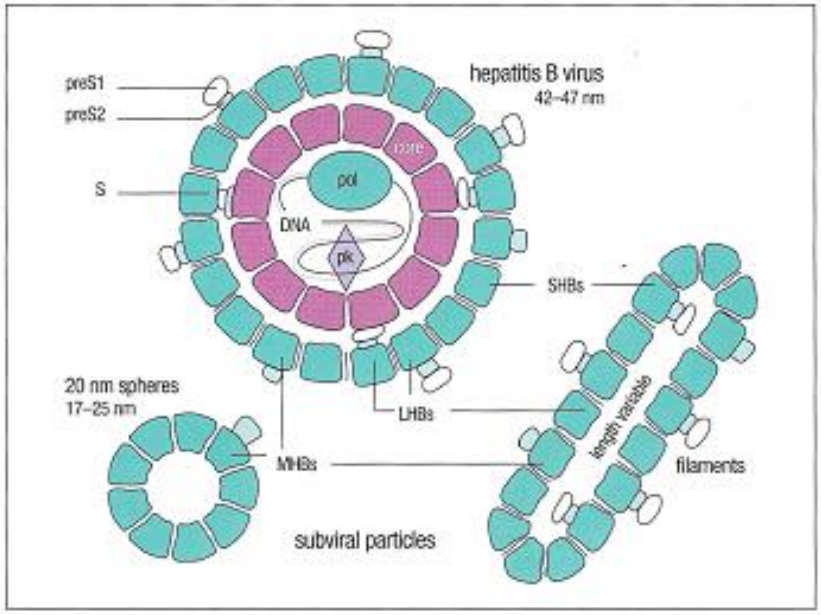

Figure 1-2 Schematic diagram of HBV Dane particle and sub-viral particles (taken from Kann M. Structure and Molecular Virology. In: Lai CL, Locarnini S, editors. Hepatitis B Virus. 2 ed. London: International Medical Press; 2008. 2.1-2.15)[7]

1.4 Viral Genome

HBV has a relatively short genome of 3200 kilobases arranged as relaxed, circular, partially double stranded DNA (dsDNA).[14] The DNA consists of a complete negative strand that is paired with an incomplete complimentary positive strand that is of variable length. The structure is kept circular by base pairing between the 2 strands. The 5' end of the negative strand is covalently attached to the viral polymerase protein, whilst the 5'end of the positive strand is covalently linked to a RNA oligonucleotide. Both of these additional structures are essential for initiating DNA synthesis. In addition to these, there are direct repeat (DR) sequences which are also important in viral replication (see Figure 1-3).

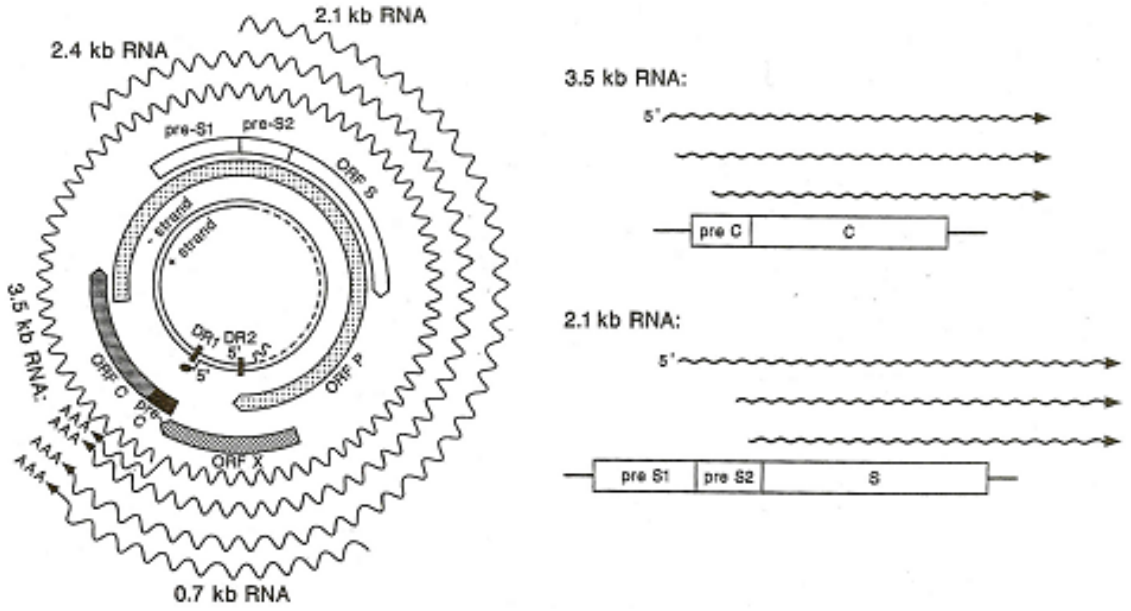

Figure 1-3 Structure of viral genome (taken from Ganem D, Schneider RJ. Hepadnaviridae: The Viruses and Their Replication. In: Knipe DM, Howley PM, editors. Fundamental Virology. 4 ed. Philadelphia: Lippincott Williams & Wilkins; 2001. 1285-1332)[13]

The genetic information within the small HBV genome is organised in a very compact fashion. All of the nucleotides are functionally arranged within the coding region or reading frame.[13] There are four major proteins in HBV: surface (S), core (C), polymerase (P) and X protein (X) and each of these has their own open reading frame (ORF). This compactness is maximised by having overlapping ORFs that utilise more than half of the nucleotides in different ORFs so that they can transcribe different messenger RNAs (mRNAs). These mRNAs can then subsequently be translated into more than one viral protein. For instance, from Figure 1-3, it can be seen that whilst the S gene encodes for HBsAg, the entire length of its sequence is used in another ORF to encode part of the P gene. This has implications for the development of mutations as any changes to the S gene will invariably have an effect on the P gene.

Another feature of the HBV genome is having ORFs with multiple start sites. The surface ORF codes for the surface proteins but it is elongated upstream at its 5' end by a 400 nucleotide preS region.[15] This region can be subdivided further into preS1 and preS2 regions each of which has its own AUG initiation codon. Translation of just the S region results in small protein production; translation of preS2 and S results in medium protein formation; and translation of preS1, preS2 and S is necessary to form large surface proteins.

A similar phenomenon exists with the core ORF which has a C region and a 33 nucleotide region upstream from its 5' end called preC.[16] Translation of the combined C and preC regions results in a protein that is similar to but different from core protein both structurally and functionally called hepatitis B e antigen (HBeAg). The precore sequence directs the protein to the endoplasmic reticulum and Golgi where proteases cleave part of the C-terminus peptide residues before it gets secreted out of the host cell. The specific function of HBeAg is not fully understood but it is not essential for viral assembly, replication or infection.[17] It may play a role with immune regulation and inducing "tolerance" of the host defences towards HBV infection.[18]

Core protein is translated from the C region of the core ORF but it is transcribed by a different mRNA to e mRNA. The core-polymerase (C/P) mRNA codes for both the core and polymerase proteins and is the largest of the mRNA transcripts at 3.5kb in length. The C/P mRNA also serves another function as a reverse transcriptase for the entire DNA negative strand and so is also referred to as pregenomic RNA, which is crucial for viral replication to occur. In addition to the reverse transcriptase, the polymerase protein also consists of an RNase H enzyme which serves to destroy the RNA template so that synthesis of the DNA positive strand can occur. This feature, Hepadnaviridae share with retroviruses but they differ in other respects such as there is no integrase activity; the HBV DNA is episomal and independent of the host DNA.

1.5 Viral Replication

The life cycle of HBV is intricate and unusual. It is not fully understood, but from animal studies especially with duck hepatitis B virus (DHBV), it is thought to involve a number of steps (see Figure 1-4).

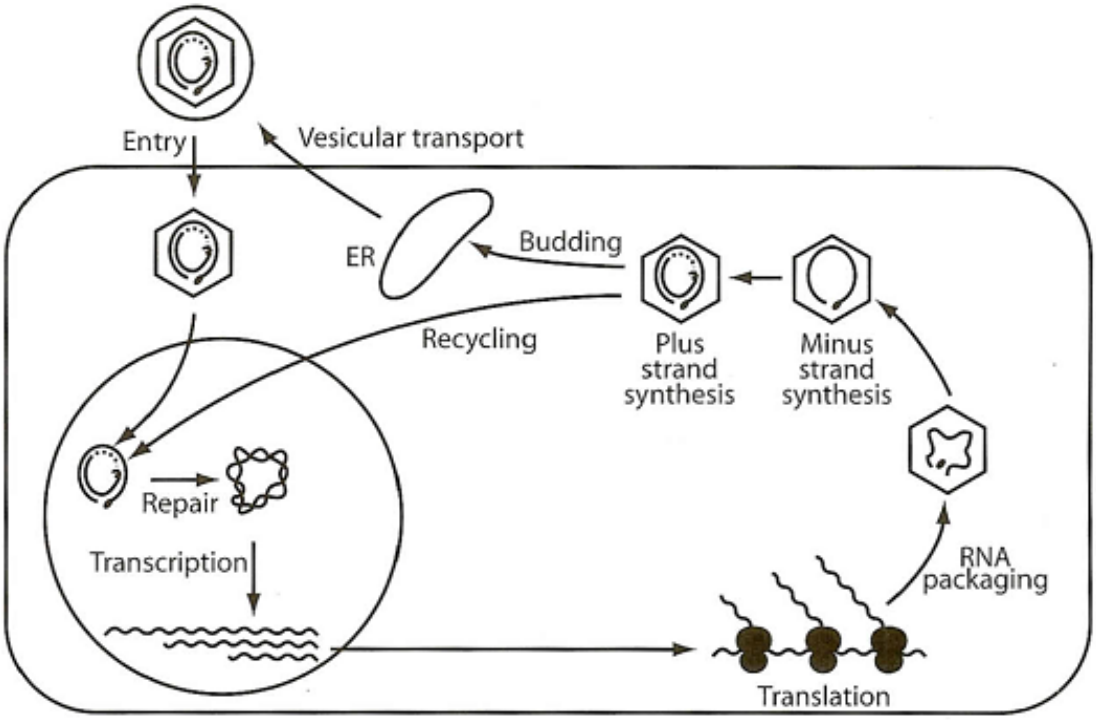

Figure 1-4 Viral Replication (taken from Ganem D, Schneider RJ. Hepadnaviridae: The Viruses and Their Replication. In: Knipe DM, Howley PM, editors. Fundamental Virology. 4 ed. Philadelphia: Lippincott Williams & Wilkins; 2001. 1285-1332)[13]

1.5.1 Viral binding and entry

The first step in viral infection is attachment of the virus to the host cell. The preS1 domain, which ultimately produces large hepatitis B surface proteins, has been strongly implicated in attaching the virus to the host cell because sub-viral particles, rich in preS1 peptides, have been shown to bind to cells whilst particles only containing S domain don't. If the preS1 sequence was subsequently added to the S domain then binding ability was restored. Further animal studies have shown that for infection to occur, with both HBV and HDV particles, there needs to be post-translational N-terminal myristylation of the large protein and that this can be blocked if a N-terminal myristoylated peptide corresponding to amino acids 2–48 of this pre-S1 domain is used. [19-21] However only recently have Yan *et al* have confirmed that the viral preS1 receptor

binding region interacts with a multiple transmembrane transporter called sodium taurocholate cotransporting polypeptide (NTCP). NTCP is predominantly found in the sinusoidal/baso-lateral membrane of hepatocytes and partakes in the enterohepatic circulation of bile salts. They showed that blocking NTCP could prevent HBV and HDV infection and that non-susceptible hepatocellular carcinoma cells could be made susceptible if they were transfected with human and tree shrew NTCP. Similarly if part of the amino acid sequence of non-functional monkey NTCP was replaced with human NTCP then this bestowed the ability to be infected with human HBV and HDV.[22]

Following attachment to the hepatocyte, there are at least two methods by which the virus can enter the cell. The first is by direct membrane fusion using a fusion peptide in a similar fashion to alphaviruses.[23] The other method is via receptor-mediated endocytosis.[24]

1.5.2 Transport of viral genome within hepatocyte

In order for replication to occur, the viral genome needs to be delivered to the cell nucleus. How this happens has not been conclusively determined. There may be passive diffusion or microtubule-dependent mechanisms. Having reached the nucleus, there is uncertainty as to how the virus traverses the nuclear pores as the nucleocapsid appears too large. One theory is that there is uncoating of the genome in the cytosol and the DNA travels into the nucleus. Once inside the relaxed circular genomic DNA (rcDNA) is then converted, via a number of complex steps, into plasmid-like covalently closed, circular DNA (cccDNA). Whilst the exact details of how this occurs are unknown, what has been determined is that there is repair of the single-stranded gap in the positive DNA strand, removal of the 5'-terminal structures (RNA and P protein), and covalent ligation of the strands to form completely double-stranded DNA.[13]

1.5.3 Transcription and translation of viral mRNA

There are two main types of mRNA. One of which is shorter than genomic length and is referred to as subgenomic RNAs. They code for the large, medium and small surface proteins and the X protein. The other type is greater than genomic length: one of them codes for HBeAg and the other one codes for the core and pol proteins. This latter mRNA is referred to as pregenomic or pgRNA. All transcription occurs in the nucleus and is regulated by a number of promoters and enhancers.

Transcription is carried out by host RNA polymerase II using cccDNA as a template. It results in the synthesis of a full-length positive RNA strand as well as multiple overlapping mRNAs. From this they are transported to the cytoplasm to be translated into the four main viral proteins. The pol protein produces the DNA polymerase necessary for the formation of the complimentary negative DNA strand.

1.5.4 Encapsidation of pgRNA and polymerase into nucleocapsids

Viral packaging or encapsidation occurs in the cytoplasm and is initiated by the binding of viral polymerase to a unique stem-loop structure at the 5' end of the pgRNA. This encapsidation signal (ε) is relatively small (about 100 nucleotides in size) and consists of a number of repeats that are folded to give a distinct hairpin loop shape that has a bulge on one side and conserved loop at the top with the stem structure being maintained by base pairing (see Figure 1-5). All viral RNAs have ε at their 3' ends but only pgRNA has it at its 5' end, and for some unknown reason, only the 5' copy of ε is functional; this could explain why pgRNA is involved with DNA synthesis and subgenomic RNAs are excluded.

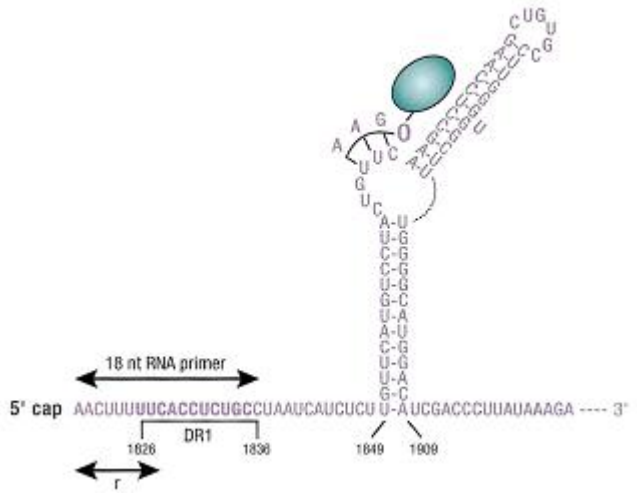

Figure 1-5 Structure of the encapsidation signal (taken from Jilbert AR, Mason WS, Kann M. Hepatitis B Virus Replication. In: Lai CL, Locarnini S, editors. Hepatitis B Virus. 2 ed. London: International Medical Press; 2008. 4.1-4.13[25]

Polymerase (pol) and ε form a complex that begins upstream of a region on the pgRNA called DR1 and continues beyond DR2, another downstream DR1 (called DR*1) and ends at the 3' end polyadenylation site. In a process that is not fully determined, the complex interacts with core protein to manufacture core protein dimers. These dimers then become assembled to form an immature nucleocapsid.

1.5.5 Reverse transcription and synthesis of DNA

DNA synthesis occurs in the cytoplasm and is thought to occur either just prior to or just after encapsidation. It is initiated by reverse transcriptase from the pol protein and starts with pgRNA acting as a template for negative DNA strand synthesis. It begins with the pol-oligonucleotide complex from ε translocating to the DR*1 sequence (at the 3' end) of pgRNA. The negative strand is elongated by travelling towards the 5' end of the pgRNA. As it does so, the pgRNA template is degraded by another enzyme from pol called RNase H; this is necessary so that the negative DNA strand can act as a template for the positive

strand. All that remains of the pgRNA template after RNase H activity is a small sequence of nucleotides which includes the DR1 sequence. This is important because this RNA sequence is translocated to DR2 and used as a primer for positive DNA strand synthesis. Elongation of the positive DNA strand begins from the 3’ end of the negative DNA strand. Ultimately circular DNA is produced of which the positive DNA strand will have a variable length compared to the negative strand. (See Figure 1-6)

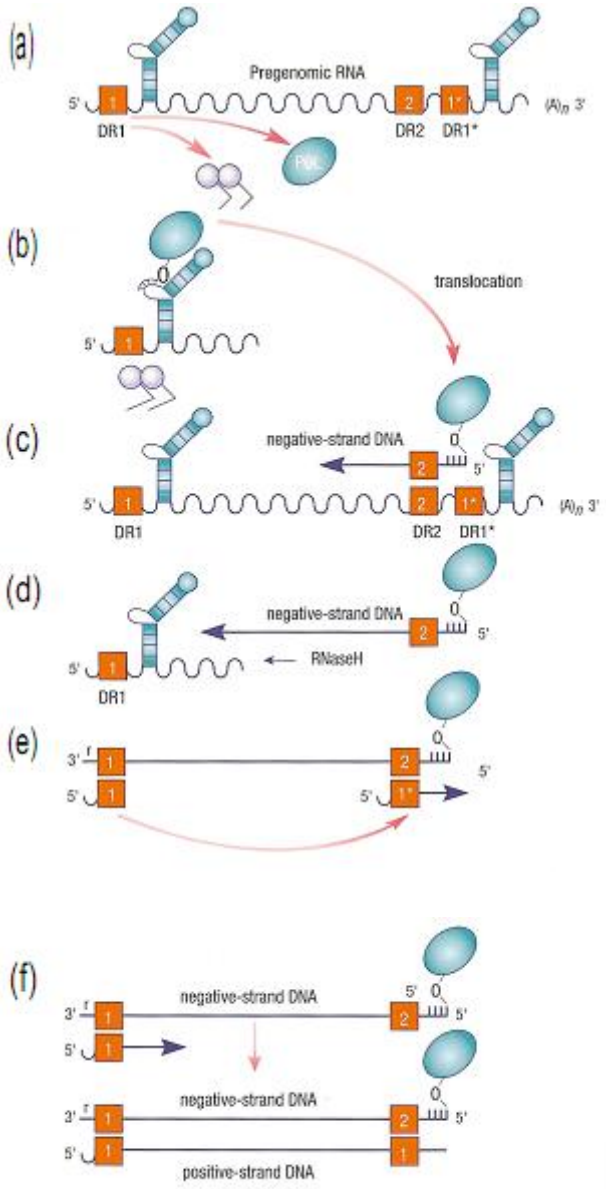

Figure 1-6 Replication of HBV (taken from Jilbert AR, Mason WS, Kann M. Hepatitis B Virus Replication. In: Lai CL, Locarnini S, editors. Hepatitis B Virus. 2 ed. London: International Medical Press; 2008. 4.1-4.13[25]

1.5.6 Envelopment

After viral replication has been completed, the nucleocapsid can follow one of two final pathways. The first is to be transported back to the nucleus to establish and maintain a reservoir of cccDNA. This is most likely to occur with early infection when there are relatively small quantities of cccDNA and other viral proteins. The cccDNA will act as a template for positive strand DNA which will remain in place even when the infected hepatocyte divides through mitosis. cccDNA is very stable and is immune to antiviral drugs that target reverse transcriptase and clear viral DNA from the cytoplasm.

The other pathway is export of the virion from the hepatocyte. In order for this to happen, it needs to be enclosed with an envelope developed from the S gene proteins: surface, M and L. Assembly occurs in the endoplasmic reticulum and Golgi apparatus where a lipid layer and the transmembrane proteins are added. This final stage of the viral life cycle, where there is release of new virions and subviral particles (that do not contain a nucleocapsid), is completely dependent on the S gene and in particular the surface and pre-S1 proteins.

1.6 Pathogenesis

Hepatitis B infection can result in either acute or chronic disease states, both of which are associated with a spectrum of clinical manifestations. Acute infection can present as: asymptomatic infection, anicteric hepatitis, icteric hepatitis or fulminant hepatitis. Chronic infection can vary from an asymptomatic carrier state to chronic, active hepatitis, compensated cirrhosis, decompensated liver disease or hepatocellular carcinoma. 5-10% of infections acquired in adulthood develop chronic infection compared to 30-90% of children infected before the age of 5.[26;27]

HBV *per se* is not directly cytopathic to cells. The damage that occurs is thought to be due to the interplay between viral replication and the immune response. Age has a role to

play as demonstrated by experiments with neonatal ducks[28] and woodchucks[29] which demonstrated that whilst only tiny amounts of viral inoculum were required to cause infection, there were threshold values whereby either transient or chronic infection occurred. Similarly with older animals it required larger viral loads to induce chronic infection. It is unclear why the immune response of children and young animals to HBV is so weak when they mount adequate response to other viral infections.

Following viral infection, each hepatocyte contains 5-50 copies of cccDNA that acts as a reservoir for new virion production and these continue to infect the rest of the liver. Animal studies have propounded that infection usually occurs via cell-to-cell contact and increases exponentially such that almost the entire liver of 10^{12} hepatocytes could be infected in about 7 weeks.[30] There is more uncertainty regarding the question of how the immune system clears the liver of cccDNA to prevent re-infection, and why some patients do not clear the virus and subsequently become chronically infected.

HBV infection has an incubation period of 2-3 months (but can be up to 6 months) before the onset of symptoms, which coincides with detection of HBV antigens in the liver and serum, and with the initiation of immune clearance of the infection.[31] What actually triggers the immune response is not fully known but it is thought that there is a multi-specific polyclonal cytotoxic T lymphocyte (CTL) response to several HBV antigens. This could explain why patients who experience fulminant hepatitis B suffer massive immune-mediated destruction of hepatocytes despite there being a very low viral load. Similarly those individuals who clear HBeAg either spontaneously or with interferon treatment often experience an exacerbation in their liver disease with a rise in the liver transaminases (ALT).[32] The initial response of the immune system appears to be critical in determining whether the infection is transient or remains chronic.

The proportion of CTLs to infected hepatocytes is so small that other parts of the immune system must be involved. Natural Killer cells have been implicated in resolution of HBV-infected hepatocytes.[31;33] They have the effect of killing infected cells by direct

cell-cell contact and by releasing inflammatory cytokines such as interferon gamma (IFNγ) and tissue necrosis factor alpha (TNFα).

Resolution of transient infection is thought to occur through turnover of previously infected hepatocytes in a piecemeal fashion as there is no significant decrease in hepatocyte mass during this time. Elimination of cccDNA is not fully understood. It may be that there is dilution of the cccDNA to progeny hepatocytes when the infected hepatocytes die, or that cytokines directly destroy cccDNA or even that cccDNA is unstable and its synthesis is inhibited during resolution which ultimately leads to its disappearance.[26] That said it is very rare to get complete eradication of cccDNA even after recovery of acute hepatitis as the disease can reactivate many years later during periods of immunosuppression or with liver transplantation.[34]

1.7 Histopathology of HBV Infection

Analysis of the liver histology gives an indication of how effectively the liver deals with viral replication and the immune response against the virus. In the normal liver, hepatocytes are arranged in plates to form a network that extends between the portal tracts and terminal hepatic veinules. These plates are separated by sinusoids, which are lined with endothelial cells and phagocytic Kupffer cells. The hepatocytes are arranged in anastomosing plates that are 1 cell thick with the layer of hepatocytes that is closest to the portal area called the limiting plate, which is an important anatomical landmark with progressive chronic inflammation.

In acute HBV, there are non-specific changes with the immune system effectively destroying the cells that harbour viral antigens to produce a spotty necrosis that is pancinar in distribution. Apoptosis is the major underlying mechanism causing acidophilic and ballooning degeneration of hepatocytes that results in swelling and destruction with phagocytosis of the cellular remnants. In severe cases there may also be simultaneous loss of adjacent hepatocytes which is referred to as bridging necrosis. Most cases of

acute infection resolve 1-3 months after the onset of symptoms resulting in no remaining hepatocellular injury.

Some patients experiencing transient infection present with acute (fulminant) hepatic failure. In these cases there is sub-massive hepatic necrosis with extensive loss of hepatocytes and collapse of the supporting connective tissue stroma. There may be bridging necrosis with portal-portal or portal-central linkage, with the bridges being composed of collapsed reticulin, newly laid down collagen fibres, hypertrophied Kupffer cells and other inflammatory cells. The hepatocyte damage is zonal and usually centrilobular. If the patient survives the acute injury there is usually regeneration of the liver but there may be some residual nodules.

A characteristic feature of chronic HBV infection is the presence of ground glass hepatocytes.[35] These are liver cells which have an eosinophilic, granular, glassy cytoplasm when stained with different immunohistochemical techniques using light microscopy. Such cells contain large amounts of cytoplasmic HBsAg and are found in patients who are in the non-replicative phases where there is more HBV DNA integrated into the hepatocyte genome and less HBcAg being produced.

With chronic infection, inflammation tends to spread out from the portal tracts. Expansion beyond this area to involve the limiting plate is referred to as interface hepatitis or piecemeal necrosis. As the necro-inflammatory process progresses and engages more hepatocytes, collagen fibres are laid down which can condense and eventually contract to leave scars. It is the contraction of these fibrous septa, the formation of regenerative nodules and the development of fibrous bridges (either portal-portal, central-central or central-portal), which leads to parenchymal architectural distortion that ultimately destroys the acinar structure and leads to cirrhosis.

Often patients will suffer episodes of exacerbation with an increase in severity of the inflammation and associated fibrosis, followed by periods of quiescence in which the injury and inflammation decrease and the fibrosis activity may even appear to have

lessened. Even if cirrhosis develops then this should not be seen as a condition that does not change. If the underlying cause persists the hepatic parenchyma will continue to be transformed into increasing amounts of scar tissue and eventually clinical decompensation can ensue. The opposite is also true and if the instigating disease subsides or is treated, then fibrosis may regress to a degree. During seroconversion there may be a flare of the disease and histology may demonstrate increased severity of injury, but once this is concluded, inflammation and activity will diminish.

The stage of HBV infection refers to the modification of structure or loss of function as the disease progresses. As the disease advances, the patient goes from a normal liver to a stage of fibrous portal expansion that extends between vascular structures, develops bridging fibrosis and then results in the appearance of parenchymal nodules surrounded by fibrous tissue. When there is complete loss of architecture and composition consists entirely of nodules and fibrosis, then cirrhosis has developed.

The grade or activity corresponds to the severity of one or more clinical, functional or histological features. It gives an indication of how rapidly the disease will progress through the stages, with a low-grade disease being slowly progressive while a high grade disease is rapidly progressive. Histological grading of HBV involves assessing the severity of the hepatocellular injury and inflammation and deciding whether the activity is mild, moderate or severe. There are four major scoring systems for grading and staging chronic inflammation which were initially developed for use in patients with chronic HCV infection[36-39] (see Table 1-1 and Table 1-2).

Histological Grading	Metavir[36]	Batts-Ludwig[37]	Knodell (I-III)[38]	Ishak[39]
Minimal hepatitis	A1	Grade 1	0-3	0-3
Mild hepatitis	A1	Grade 2	3-5	3-6
Moderate hepatitis	A2	Grade 3	6-9	7-9
Severe hepatitis	A3	Grade 4	10-12	10-15
Severe hepatitis with bridging necrosis	A3	Grade 4	14-18	16-18

Table 1-1 Various scoring systems for grading liver inflammation

Histological Stage	Metavir[36]	Batts-Ludwig[37]	Knodell (I-III)[38]	Ishak[39]
No fibrosis	F0	Stage 0	0	0
Portal fibrosis (minor)	F1	Stage 1	1	1
Portal fibrosis (major)	F1	Stage 1	2	2
Bridging fibrosis (minor)	F2	Stage 2	3	3
Bridging fibrosis (major)	F3	Stage 3	3	4
Incomplete cirrhosis	F4	Stage 4	4	5
Cirrhosis	F4	Stage 4	4	6

Table 1-2 Various scoring systems for grading liver fibrosis

1.8 Natural History of Chronic Hepatitis B

The clinical course of chronic HBV infection is long and complicated (see Figure 1-7). Although it is determined by the interaction of viral replication and the host immune response, it is affected by other factors such as patient's age, alcohol consumption

and co-infection with other viruses, such as hepatitis C. Men have an greater risk of HCC and disease progression than women especially after the age 45.[40] By understanding the natural history, informed decisions can be made about disease monitoring, and when to initiate (or even terminate) treatment. Whilst there is some debate as to the actual number (and naming) of phases that HBV infection undergoes, it is accepted that there is a period whereby viral replication and liver damage is more prominent followed by a period where there is a degree of remission.[41;42] Similarly there is a difference between patients acquiring the disease perinatally and in early childhood than those that acquire it at a later time in their lives.

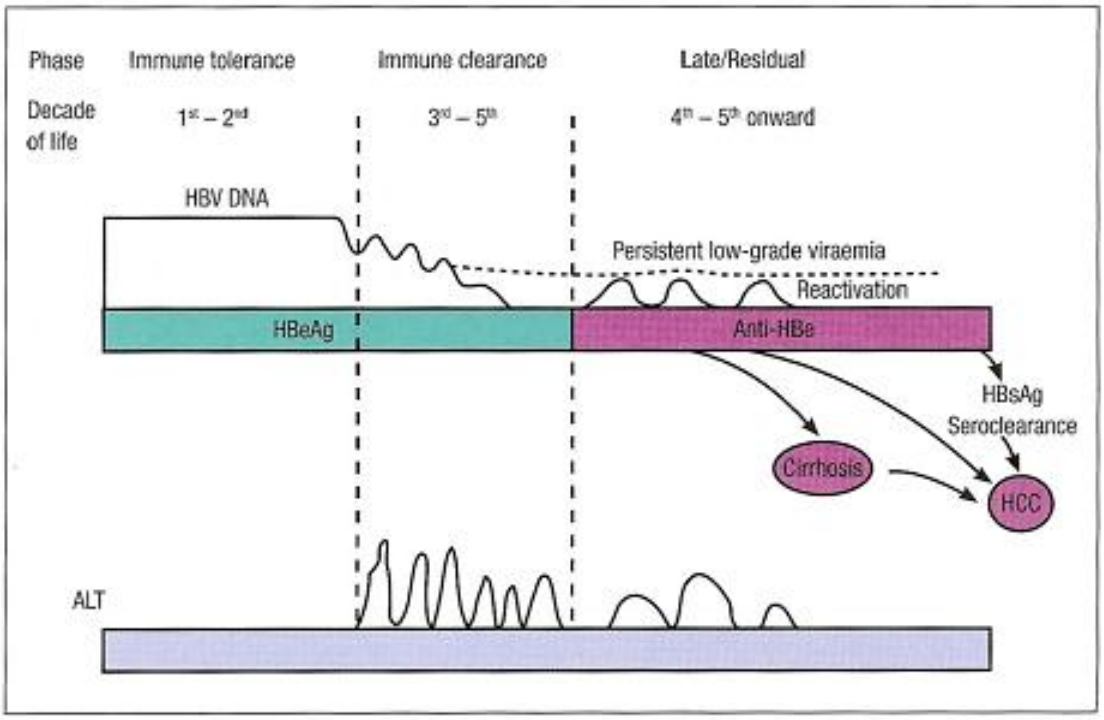

Figure 1-7 Natural history of the phases of chronic HBV infection (taken from Yuen MF, Lai CL. The Natural History of Chronic Hepatitis B. In: Lai CL, Locarnini S, editors. Hepatitis B Virus. 2 ed. London: International Medical Press; 2008. 12.1-12.11[43]

The first phase is called the immune tolerance phase and occurs in individuals infected in early childhood, especially perinatally. Patients typically are HBeAg positive and have very high amounts of HBV DNA (usually 10^8-10^{11} copies/ml) but little in the way of active disease as evidenced by normal ALT and liver histology.[44]

The lack of liver damage in the face of active viral replication would suggest that the immune system does not recognise the virus as a threat. HBeAg has been implicated with inducing tolerance as it has been shown to traverse the placenta whilst not stimulating the production of anti-HBe.[45] This phase can last up to three decades in some individuals. Liver biopsy at this stage typically shows minimal liver damage and treatment is not usually indicated.

Individuals infected beyond early childhood tend to enter the immune active or immune clearance phase shortly after infection rather than the 20-30 year wait experienced with perinatal infection patients. This phase is characterised by the host recognising the virus as foreign and mounting a concentrated immune-mediated attack. The trigger for this phase developing is not known. Whilst this response is usually not strong enough to eliminate the virus it does cause hepatocyte damage, release of ALT, and reduction in DNA levels with suppression of the virus. There is also 10-20% chance of spontaneous seroconversion of HBeAg to anti-HBe.[46] Depending on the strength and duration of the response, there may be significant fibrosis, cirrhosis or even liver decompensation. For this reason, anti-viral treatment should be considered. Interferon can be used, even if compensated cirrhosis has developed (Childs-Pugh A), but should be avoided in decompensated cirrhosis (Childs-Pugh B & C) because it can cause a flare in ALT levels that may lead to further decompensation, sepsis and death.[47] Factors associated with an increased prospect of HBeAg seroconversion include genotype B, older age, and elevated ALT as this suggests a heightened immune response.[43;47]

However, recently there have been questions about whether teenagers and young adults are still in a "true" immunotolerant phase because their viral loads tend to be slightly lower (10^6-10^8 copies/ml) than younger children but the ALT levels tend to slowly increase. It may be that there is some immune pressure and some disease progression.[48;49] The longitudinal REVEAL-HBV study from Taiwan, showed that persistence of high viral loads (>10^4 copies) over a long length time and admittedly in older adults, even in the absence of elevated ALT and being HBeAg positive, was associated with increased risk of HCC.[50]

The loss of HBeAg and the occurrence of anti-HBe usually signifies entry into the third phase. This phase has been given a number of different names: residual phase,[43] immunosurveillance phase[51] and inactive phase.[52;53] In the vast majority of patients there is an corresponding reduction in viral load (from undetectable to 10^3 - 10^5 copies/ml), near normalisation of ALT and improvement of histological grade. All this would suggest that the patient is of low infectivity and low risk of transmitting the virus to others. In a long term follow up of patients who had HBeAg seroconversion over 65% remained in remission. However, the remainder either returned to being HBeAg positive or remained HBeAg negative but with elevated ALT.

Some patients develop HBeAg negative chronic hepatitis whereby there is active replication and increased viral load, elevated ALT and deterioration of histology but with undetectable HBeAg. These patients may be thought of as entering a fourth phase where there is immune escape due to a mutation that fails to produce HBeAg. In these cases there appear to be mutations in the precore or core promoter regions.[54-57]

In addition to HBeAg seroconversion happening, there can also be HBsAg seroconversion. This has an annual incidence of 0.4-2% rate for Caucasians and 0.1-0.8% for Asians.[58;59] Predictors of spontaneous loss of HBsAg include older age and persistent inactive hepatitis.[60] In younger individuals (aged less than 50) this is associated with a reduced risk of developing complications like hepatocellular carcinoma. Unfortunately, undetectable levels in serum does not mean complete eradication of the virus, as 10 year follow up studies have found that 100% have intrahepatic HBV DNA and 73% have intrahepatic cccDNA.[61]

1.9 Laboratory Diagnosis of Hepatitis B

Identification of HBsAg is the characteristic marker of HBV infection. Typically it is detected in the serum 6-10 weeks after exposure to the virus and predates clinical symptoms or biochemical changes. In addition to HBsAg, other markers of acute HBV

infection also detected include HBeAg and HBV DNA (see Figure 1-8). Hepatitis B core antigen is not readily detected in the serum.

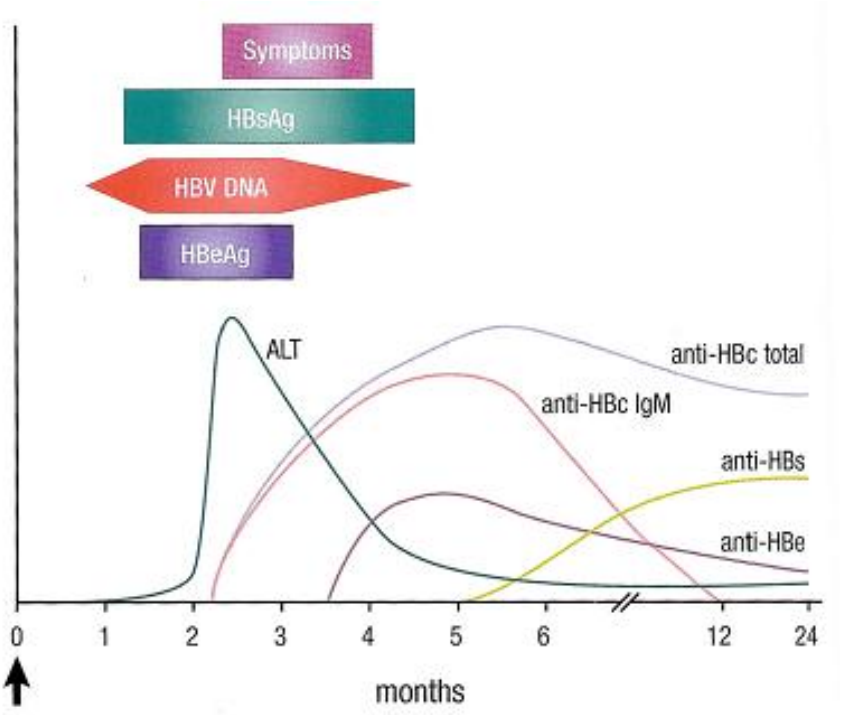

Figure 1-8 Changes in viral markers and ALT over time with acute HBV infection (taken from Bowden S. Laboratory Diagnosis of Hepatitis B. In: Lai CL, Locarnini S, editors. Hepatitis B Virus. 2 ed. London: International Medical Press; 2008. 11.1-11.16[62]

The development of symptoms signifies the host immune response and the production of antibodies to these viral antigens. The first of these to appear is antibody to core antigen, anti-HBc. Initially anti-HBc immunoglobulin M (IgM) is detected and this peaks during early recovery before waning after 3-12 months; this coincides with the appearance of anti-HBc immunoglobulin G (IgG). Whilst IgM is a marker of acute hepatitis, it can occur with flares of chronic hepatitis and may persist for around 2 years; total anti-HBc will persist indefinitely and be an indicator of previous HBV infection. Next to appear is antibody to e antigen, anti-HBe, and this is coupled with a dramatic decrease in HBeAg and HBV DNA. There may also be an increase in ALT at the time of HBeAg seroconversion as the immune system eliminates infected hepatocytes. Last to transpire is the production of the

neutralising antibody to surface antigen, anti-HBs, whose occurrence implies immunity to HBV.

Perseverance of HBsAg for greater than 6 months denotes chronic HBV infection. Whereas greater than 95% of immunocompetent adults with genuine acute HBV clear HBsAg spontaneously,[63] those patients who develop chronic HBV infection have a less than 1% rate of clearance per year.[59] With persistence of infection, there is an improvement of ALT levels although not usually to normal, and there may be a flare up if seroconversion, especially with HBeAg, happens (see Figure 1-9).

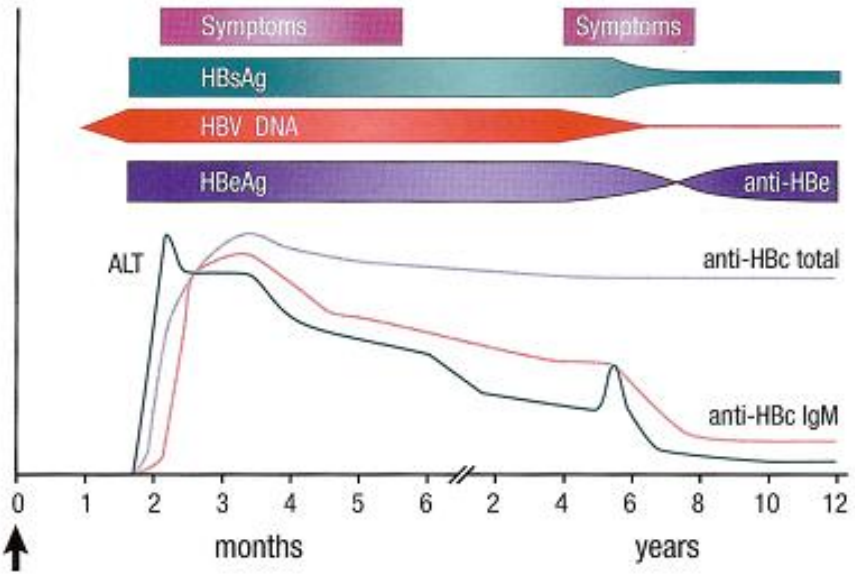

Figure 1-9 Changes in viral markers and ALT over time with chronic HBV infection (taken from Bowden S. Laboratory Diagnosis of Hepatitis B. In: Lai CL, Locarnini S, editors. Hepatitis B Virus. 2 ed. London: International Medical Press; 2008. 11.1-11.16[62]

The natural history of chronic HBV infection is incompletely understood (see earlier). HBeAg existence is allied with high HBV DNA levels and has been thought of as signifying high infectivity. With the production of anti-HBe there is a decline in HBV DNA. However, in a proportion of patients that are now HBeAg negative, there may still be high amounts of HBV DNA (typically greater than 10^4 copies/ml) that leads to severe liver disease, even if anti-HBe is present. In these cases some form of mutation

has occurred: 1) G to A substitution at nucleotide 1896 in the precore region leading to a stop codon be created to prevent translation of HBeAg[64] 2) nucleotide substitutions at position 1762 and 1764 in the basal core promoter region that may reduce transcription of mRNA that encodes HBeAg.

It is important to evaluate HBV DNA as this can serve a number of functions: acting as a marker of disease activity and so assessing those individuals who should be treated - persistent HBV DNA $>10^5$ copies/ml is an independent risk factor for cirrhosis and hepatocellular carcinoma[50;65]; identifying those patients who are unlikely to respond to treatment[66]; determining those patients who have a suboptimal response to treatment (< 2 $\log_{10}$ drop in HBV DNA and continues to stay above 10^4 copies/ml after 6 months of treatment); and identifying the emergence of viral resistance[41] (> 1 $\log_{10}$ increase in HBV DNA from baseline after starting treatment).

In most cases, presence of anti-HBs signifies immunity to HBV, but there are some individuals who are HBsAg positive as well as being anti-HBs positive.[67] HBV DNA is still present and so they must be regarded as patients with chronic HBV infection but in whom anti-HBs fails to act as a neutralising antibody to HBsAg. Another scenario involves babies born of HBV positive mothers, who are given hepatitis B immune globulin (HBIG) at birth and in spite of supposedly adequate amounts of antibody, go on to develop hepatitis B infection and chronic liver disease. These cases of "vaccine escape" occur because of mutations in the S and other genes during viral replication due to a variety of causes. For instance, HBV reverse transcriptase does not have a proof reading ability and it has been estimated that there are 1.4-3.2 $x10^{-5}$ nucleotide substitutions per site per year, which is more than ten times the error rate of other DNA viruses. Because of the compact structure of HBV, the S ORF is completely overlapped by the Pol ORF and this gene codes for reverse transcriptase. A mutation in this domain can be transmitted to the other domains including the S domain. Resistance to the reverse transcriptase (nucleoside) antagonist lamivudine (3TC) can result from a number of mutations in the Pol gene. One of these, rtV173L, is associated with the sE164D mutation in the "a" determinant region of the S gene.[68] The "a" determinant is a highly conserved region that is about 99-170 amino acids

long and is common to all hepatitis B genotypes. It is the anti-HBs antibody domain and used in all vaccinations against HBV. The most common alteration occurs at amino acid 145 where glycine is replaced by arginine. Other changes can occur from deletions, substitutions or insertions of various amino acids in this a determinant region, all of which can alter the conformational structure and prevent the production of functional HBsAb. These transformations may occur either spontaneously or through selection of various quasi-species by using oral anti-viral agents or HBIg as treatments.[69-71]

1.10 Point of Care Testing for HBV

Currently serological confirmation of HBV infection is through using solid phase immunoassays. The principle involves anti-HBS antibody being immobilized to a physical support such as micro well, plastic tube or bead. The test sample, supposedly containing HBsAg is added and this antigen-antibody complex is identified by another antibody attached to a radioisotope (radioimmunoassay, RIA) or enzyme (enzyme immunoassay, EIA). This technique has high specificity and sensitivity which allows for the screening of multiple blood samples in a relatively short time but expense and the ability to access reference laboratories can preclude use in rural, resource-deprived areas where often there is a high prevalence of the disease.

Since the late 1970s there have been attempts to use rapid "point of care" tests that can identify cases with much smaller quantities of blood and within a matter of minutes but with variable degrees of accuracy.[72] There are various types: microfiltration methods, that allow easy separation of blood[73]; chromatogenic tests, where a colour change on a nitrocellulose test strip can confirm infection and give a result in about 15 minutes[74-76]; and dried blood spot testing (DBS), which involves using a lancet to draw a single drop of capillary blood that is collected on a piece of filter paper and is then allowed to dry.[77;78] DBS has many obvious advantages including easier sampling, minimal problems with transportation (such as not having to struggle with breaking tubes or requiring cold storage), and more straightforward

sample processing without the need for centrifuging and separating sera. More recent studies have shown it to be an accurate test for HBsAg with sensitivities and specificities > 95%.[79;80] When used in substance misuse clinics in Wales, albeit testing users for HCV IgG, researchers found that clients much preferred DBS than venous blood sampling.[81] This would suggest the potential use of DBS in large scale population screening for blood borne viruses. Despite over time improving the sensitivity and specificity, even with the different genotypes and possible mutations to HBsAg, currently none of these testing kits have been approved either by the US Food and Drug Administration (FDA) or European Union.[82]

Although more acceptable, DBS involves a degree of pain when the skin is pricked and the risk of needle stick injury still exists. HBV exists in other body tissues and fluids. A completely different method that involves testing oral fluid obviates both these problems. HBV can be detected in saliva, the gingival cervical fluids and oral mucosal transudate (OMT), albeit in much lower quantities, and these together collectively make up oral fluid.[83;84] As OMT represents an ultra-filtrate of blood, it can be used to screen for blood borne viruses. Currently there are FDA approved, over-the-counter, OMT kits for diagnosing HIV (OraQuick® Orasure Technologies Inc., Bethlehem, Pennsylvania, USA). but no FDA approved or CE marked tests for HBV. There have been a few studies that have shown good sensitivity and specificity (>95%) in identifying HBV and HCV using OMT but none of these kits are FDA approved or CE marked.[85-87]

1.11 Clinical Manifestations of HBV Infection

Of the patients who contract acute HBV, only about 30% will develop clinical symptoms. When symptoms occur they progress in a sequential manner. Typically there is an incubation of about 2-6 months before there is the development of the prodromal phase. This consists of non-specific symptoms such as lethargy, malaise, nausea and anorexia. Next to arise is the icteric phase, which can last 1-3 months and is characterised by jaundice, dark urine and right upper quadrant pain. After this

phase has taken place there is usually complete resolution of symptoms, especially in those who do not progress onto chronic HBV. In less than 1% of patients acute, or even hyper-acute, liver failure can transpire and this is exemplified by the presence of hepatic encephalopathy between 8 and 28 days from the onset of jaundice (hyper-acute is when encephalopathy occurs within 7 days).[88] Here the prognosis is poor and may require liver transplantation as a treatment option.

Chronic HBV does not display any specific clinical syndromes. Patients may present with decompensated cirrhosis as embodied by portal hypertension (ascites, variceal haemorrhage and hepatorenal syndrome) and encephalopathy, or with hepatocellular carcinoma (HCC). In a proportion of patients, HBV infection can also be associated with other extrahepatic manifestations. These are thought to be related to circulating immune complexes and can result in the non-specific serum sickness-like prodrome that occurs with acute hepatitis as well as with arthralgia, arthritis and skin rashes. There are 2 other specific linked complications that have been linked to HBV infection: a) polyarteritis nodosa, which can result in neuropathy, vasculitis, renal disease, arthritis and Raynaud's phenomenon[89;90] and b) membranoproliferative glomerulonephritis.[91;92]

1.12 Epidemiology of HBV Infection

In the past it has been difficult to ascertain accurate numbers of HBV infected individuals. Diagnosis relied on detecting anti-HBc for evidence of previous infection and HBsAg for current infection. It has been estimated that almost 2 billion people have been previously infected with HBV and that approximately 350 million are currently chronic carriers of the disease.[93] Approximately 600,000 to 1.2 million individuals die each year from HBV due to acute or chronic HBV or HCC.[94] HCC is the 5th commonest cancer worldwide and its incidence is increasing with approximately 300,000 to 500,000 new cases each year.[95]

Prevalence of HBV varies across the world, with some areas having low endemicity (0.1 - 2% of the population such as USA, Canada, Western Europe, and Australasia), to

areas of intermediate endemicity (2 - 7% such as the Mediterranean, Central and Latin America, the Middle East, Central Asia and the Indian Subcontinent), to areas of high endemicity (8-20% such as China, Far East, Pacific Islands, and Sub-Saharan Africa)(see Figure 1-10).[96] In countries where the prevalence rate is low, the infection is typically acquired during adulthood, infection is usually transmitted horizontally and is more likely to present with a symptomatic acute illness. This is in contrast to high prevalence regions where the infection is transmitted vertically and is obtained during the perinatal period or early childhood.

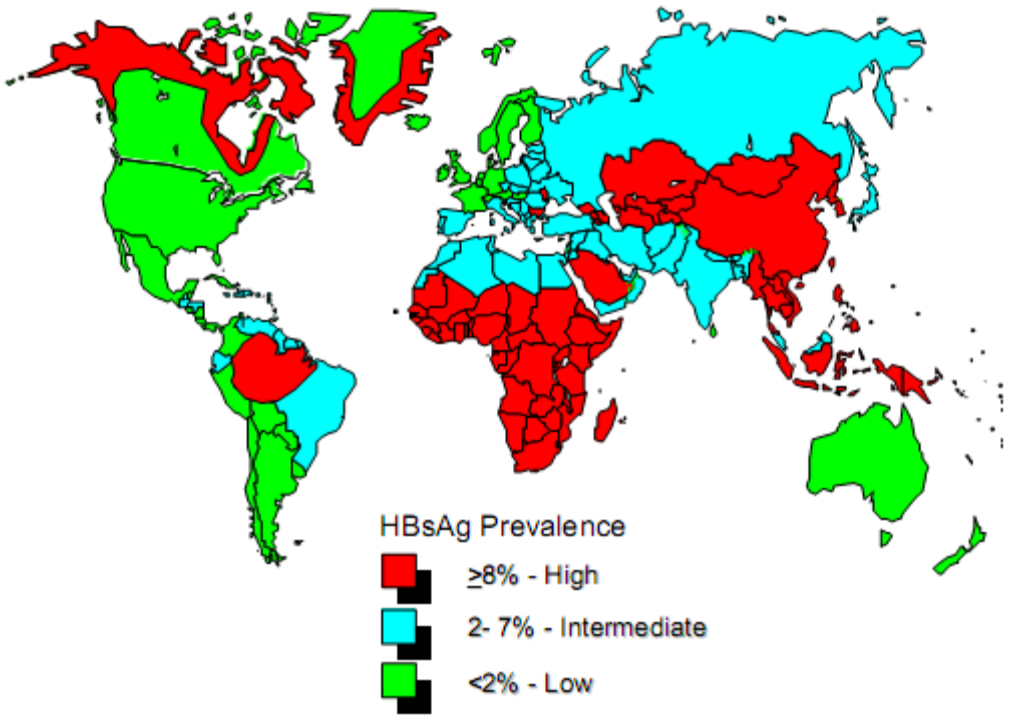

Figure 1-10 Map showing worldwide prevalence of chronic HBV infection (taken from Lavanchy D. Chronic viral hepatitis as a public health issue in the world. Best Pract Res Clin Gastroenterol 2008; 22(6):991-1008[96]

HBV can be carried in a number of body fluids: blood, saliva, semen, vaginal secretions and menstrual blood. It has also been detected, albeit to a lesser extent, in sweat, tears, breast milk and urine. It is resistant to breakdown and can survive outside the human body. The commonest horizontal routes of transmission include

via blood transfusion, sexually, percutaneously (especially with intravenous drug users (IVDU) or through an accidental needle stick injury) and via infected devices such as with patients undergoing haemodialysis. Even though it is theoretically possible, there is no evidence of HBV being spread to humans by HBV infected "blood-sucking" insects such as mosquitoes or bedbugs.[97]

A characteristic feature of low endemic areas is that there is a low perinatal HBV infection rate. Typically this can be as low as 10% when compared to high endemic areas such as the Far East where transmission rates via this route can be as high as 90%.[27;98] One reason for this may be the increased proportion of pregnant women who are HBeAg positive and so also have high viral loads. It is thought that most infection occurs when the baby is exposed to maternal blood during passage through the birth canal or in the time period soon afterwards. However it can also occur with preterm labour and spontaneous abortion. In spite of this, there is no convincing evidence that elective caesarean section prevents the maternal transmission of HBV.[99] In Africa there is evidence to suggest that transmission occurs in very early childhood via child-to-child contamination.[100;101] Newborn immunoprophylaxis with HBIG and HBV vaccine has been shown to be very effective, which goes against the idea of intrauterine infection. Even though HBsAg has been detected in colostrum, breast feeding is not contraindicated in infected mothers and has not been shown to spread the disease to the newborn.[102;103]

There are 3 types of HBV vaccine which differ only from where they are derived from: plasma, yeast or mammalian cells (recombinant). All of these vaccines consist of the S envelope protein but not the M or L proteins. Enclosed within the S protein is a region known as the "a determinant" which is common to all HBV genotypes; this region is the reason why the vaccine is highly immunogenic and provides protection against all HBV genotypes. The vaccine is given in 3 doses over a 6 month period and the "amount" of neutralising anti-HBs can be measured in the blood. In 1992, the WHO endorsed the plan that all countries with high endemicity (>8%) should incorporate HBV vaccination into their national immunization programs by 1995, and that all other countries should do this by 1997. By 2011, 179 countries had adopted a

universal vaccination program, whilst 14 countries operated a "selective" vaccination program. The UK is one of the countries that does not operate a universal HBV vaccination program (see Figure 1-11). Although not all countries that have adopted a universal vaccination program start vaccination at birth there has been a significant improvement in the prevalence of HBV. Taiwan introduced a vaccination program in 1984 and although there is 80-90% coverage there has been a major improvement. After 20 years the chronic HBV carrier rate of children under 15 years had dropped from 9.8% to 1.2% and the incidence of HCC had dropped from 1.02 per 100,000 to 0.3 per 100,000.[104;105] In low endemicity countries, the beneficial effects of universal vaccination are unlikely to be seen for 20-30 years as infection tends to occur in adolescence and adulthood and via sexual or percutaneous routes.

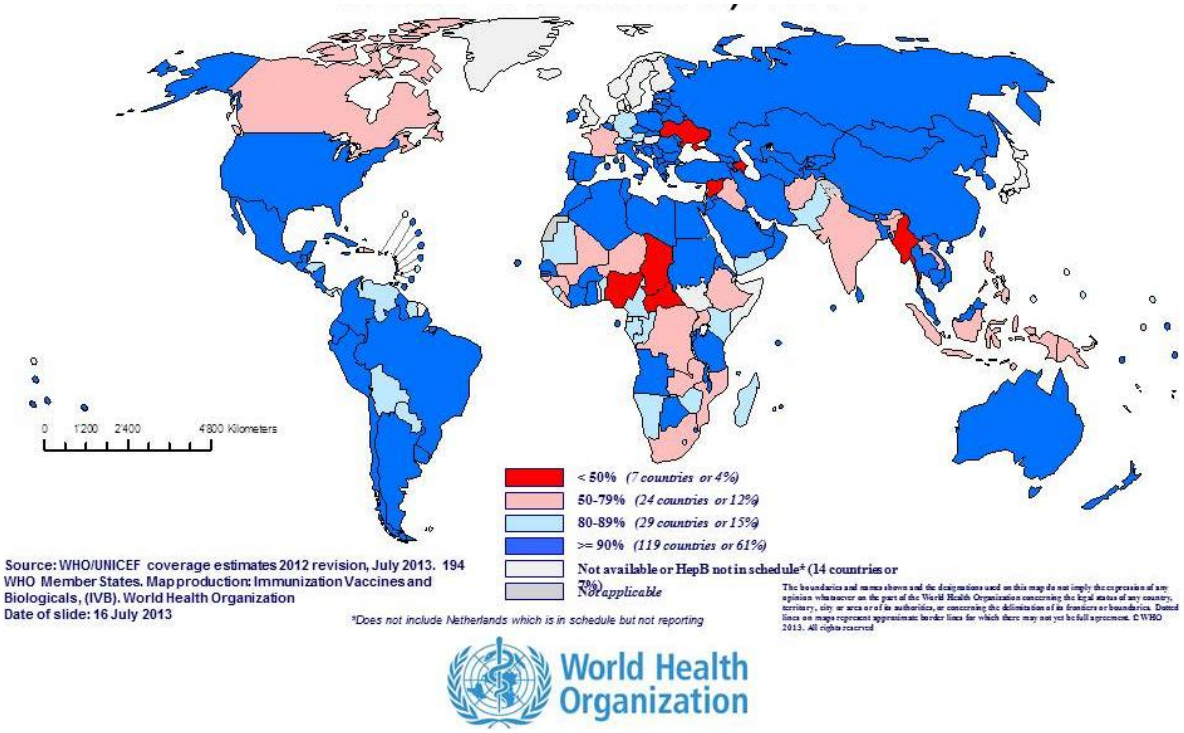

Figure 1-11 Map showing countries that have adopted universal HBV vaccination programs (taken from World Health Organization (www.who.int/immunization_monitoring/diseases/hepatitis/en/index.html))

1.13 HBV Genotypes

HBV can be organized into at least 10 genotypes, A-J, based on there being >8% inter group diversity throughout the complete genome. Traditionally there were 4 serotypes (adr, adw, ayr, and ayw) which were based on the unifying "a" determinant of the surface gene and 2 mutually exclusive sub-determinants "d/y" and "r/w".[106] However, the trend has been to shift from serotypes and classify HBV according to genotype. The first genotypes to be identified were A-D[107] and they also happen to be the most prevalent genotypes. They were then followed by the discovery of genotypes E and F,[108] G[109], H[110], I[111] and J.[112] In a similar manner to hepatitis C genotypes, the HBV genotypes are distributed geographically in a characteristic fashion that correlates with ethnicity and mode of transmission. This distinctive allocation can be seen in Figure 1-12 (Fung *et al* 2004).[113] Genotype A is more

prevalent in North America, Northern Europe, Australasia, Africa and the Indian Subcontinent. Genotypes B and C are predominantly found in the Far East. Genotype D is found in Southern Europe, the Middle East, Africa and the Indian Subcontinent. Countries, such as the USA, have been shown to contain genotypes A-G which reflects the underlying ethnic spectrum: genotype A is more prevalent amongst Caucasian and black patients, whilst B and C are characteristic of individuals of Far Eastern extraction.[114]

Figure 1-12 Map showing geographical distribution of the various HBV genotypes (taken from Fung SK, Lok AS. Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? Hepatology 2004; 40(4):790-792)[113]

The genotypes may occur in combination (for instance genotype A and G)[115] or even as recombination or mixture of 2 genotypes such as C and D. [116]Genotypes can be further subdivided into sub-genotypes if there is >4% divergence in the entire genomic sequence and most of these are distributed geographically (see Table 1-3).

Genotype	Subtype(s)	Geographic location
A	A1	Sub-Saharan Africa
	A2	Northern Europe
	A3	Western Africa
B	B1	Japan
	B2–B5	China, East Asia, Indonesia, Philippines, Taiwan, Vietnam
	B6	Alaska, Greenland, Northern Canada
C	C1–C3	China, Korea, Taiwan, Southeast Asia
	C4	Australia
	C5	Philippines, Vietnam
D	D1–D5	Africa, Europe, India, Mediterranean countries
E		West Africa
F	F1–F4	Central America, South America
G		France, Germany, USA
H		Central America
I		Laos, Vietnam
J		Japan, Ryukyu

Table 1-3 Geographic distribution of HBV genotypes and subtypes (adapted from Tanwar S, Dusheiko G. Is there any value to hepatitis B virus genotype analysis? *Curr Gastroenterol Rep* 2012; 14(1):37-46.)[117]

There are both clinical and virological differences between genotype as well as within genotype. Genotype A has at least 3 sub-genotypes of which A1 is predominantly found in Sub-Saharan Africa and A2 is found more in Europe. A1 is associated with HBeAg seroconversion earlier but a greater risk of developing HCC in Black Africans at an earlier age.[118-120] As mentioned earlier, a substitution at position 1896 between guanosine to adenosine can lead to the prevention of HBeAg being expressed. In genotype A the nucleotide cytosine, rather than uracil, is carried at position 1858 and this restricts the base pairing with adenosine at 1896. Instead genotype A is

associated with core promoter mutations such as substitutions at 1762 (A-to-T) and 1764 (G-to-A), which has been linked with suppressing HBeAg expression.

Genotype	B	C	A	D	E-J
Clinical characteristics					
Modes of transmission	Perinatal /Vertical	Perinatal /Vertical	Horizontal	Horizontal	Horizontal
Tendency of chronicity	Lower	Higher	Higher	Lower	ND
Positivity of HBeAg	Lower	Higher	Higher	Lower	ND
HBeAg Seroconversion	Earlier	Later	Earlier	Later	ND
HBsAg seroclearance	More	Less	More	Less	ND
Histologic activity	Lower	Higher	Lower	Higher	ND
Clinical outcomes (cirrhosis and hepatocellular carcinoma)	Better	Worse	Better	Worse	Worse in genotype F
Response to interferon α	Higher	Lower	Higher	Lower	Lower in genotype G
Response to nucleos(t)ide analogues	No significant differences among genotypes A to D				ND
Virologic characteristics					
Serum HBV DNA level	Lower	Higher	ND	ND	ND
Frequency of precore A1896 mutation	Higher	Lower	Lower	Higher	ND
Frequency of basal core promoter T1762/A1764 mutation	Lower	Higher	Higher	Lower	ND
Frequency of preS deletion mutation	Lower	Higher	ND	ND	ND

[a]Due to peculiar distribution of HBV genotype in Asian and Western countries, available data demonstrates only comparisons between genotype B and C or genotype A and D.
Abbreviations: HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; ND, no data available.

Table 1-4 Comparison of clinical and virological features of HBV genotypes (adapted from Liu CJ, Kao JH. Global perspective on the natural history of chronic hepatitis B: role of hepatitis B virus genotypes A to J. *Semin Liver Dis* 2013; 33(2):97-102.).[121]

Genotypic variations may have an effect on disease progression. Most studies have not compared all the genotypes against each other but mainly A versus D and B versus C (see Table 1-4). Genotype A has a greater risk of developing CHB after acute infection but less risk of cirrhosis and HCC than genotype D. Genotype B can exist as two subtypes: Ba, comprising B2-B5 and Bj, comprising B1 and B6. B1 has been implicated with developing fulminant hepatic failure, whilst individuals with Bj have a higher chance of developing HCC and cirrhosis earlier. Genotype C is associated with longer time for HBeAg seroconversion and more mutations, especially PreS deletions, than genotype B. This may mean progressive liver damage that does not correlate with viral replication activity and greater risk of HCC with a worse outcome.[117;121;122] However, other factors known to affect disease progression, such as age of

acquisition, mode of transmission, mutations, HBV DNA level, and environmental factors like alcohol intake and exposure to aflatoxin need to be taken into consideration as well.[123;124]

A meta analysis of IFNa and PEG-IFNa treatment for CHB showed that the best responses in terms of HBeAg seroconversion and HBsAg loss have been with genotype A and also in HBeAg positive genotype B patients compared to genotypes D and C respectively.[125] However, the same meta analysis could not find any significant differences in terms of treatment response between genotypes regarding nucleoside (lamivudine, telbivudine and entecavir) or nucleotide (adefovir) anti-viral agents but did not examine tenofovir. A recent study by Marcellin *et al* reported that 10% of HBeAg positive CHB patients treated with tenofovir for 5 years had HBsAg loss. This occurred in significantly more in Caucasian patients with genotype A and (to a lesser degree D). However this did not happen in Asian patients, most of whom are genotype B and C.[126] Currently genotype status is not routinely utilised to determine decisions to initiate treatment.

1.14 Disease Progression of HBV

There are a number of independent factors associated with severe disease (cirrhosis, decompensated liver disease and HCC). These can be broadly subdivided into 3 groups: viral factors, host factors and environmental or other factors[127] (see Figure 1-13).

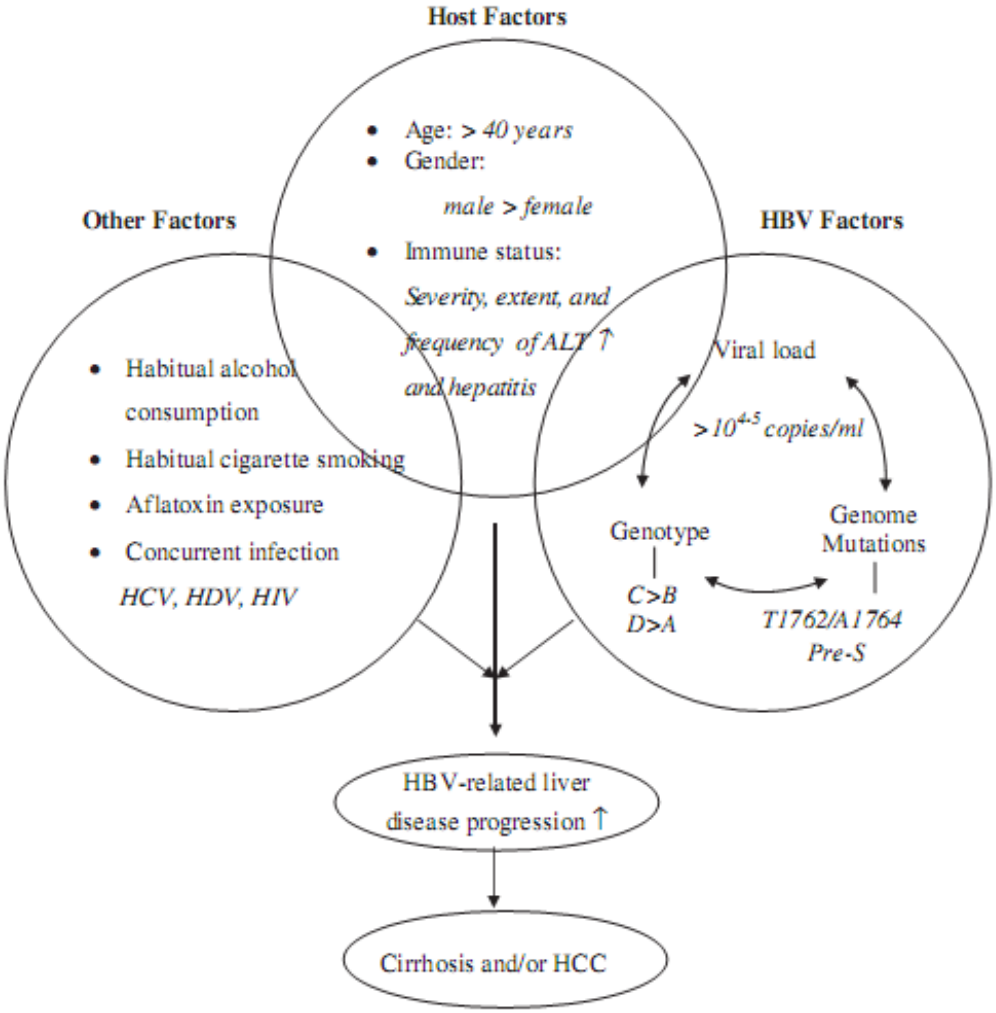

Figure 1-13 Risk factors associated with progression of disease in patients with chronic HBV infection (taken from Liaw YF, Sollano JD. Factors influencing liver disease progression in chronic hepatitis B. Liver Int 2006; 26(Supplement 2):23-29[127]

A number of studies have shown that active viral replication, as evidenced by HBeAg positivity and high viral load, lead to cirrhosis and as HCC is a complication of cirrhosis, they are also implicated in the development of HCC**.** One retrospective study found that the incidence of cirrhosis in patients who remained HBeAg positive was 3.5% per year and that these patients had significantly higher incidence of cirrhosis and HCC compared to those who underwent HBeAg seroconversion.[128] Another study was performed by Yang and colleagues following up 11893 men for the development of HCC.[129] Compared to men who were HBsAg and HBeAg negative, the relative risk of developing HCC in those who were HBsAg positive was 9.6 and 60.2 in those who were both HBsAg and HBeAg positive. A criticism of this study is that

HBeAg status was only checked on entry into the study. Hsu et al found that in 283 patients who underwent HBeAg spontaneous seroconversion, the greatest risk to developing cirrhosis occurred in those who subsequently returned to being HBeAg positive; similarly those who remained in remission had a very low risk of developing cirrhosis. However, they also noticed that up to a third of patients developed HBeAg negative hepatitis with elevated ALT and HBV DNA, and that a significant proportion of these patients developed cirrhosis and HCC. This would suggest that whilst seroconversion may confer a more favourable prognosis, there is still a risk of developing HCC in patients with active disease.[130;131]

The bearing of viral load on cirrhosis risk was demonstrated by a Taiwanese study involving 3582 HBV individuals. 85% were HBeAg-negative and were followed up for a mean duration of 11 years. The incidence of cirrhosis rose in a dose-dependent manner with entry level HBV DNA level and was independent of HBeAg status or ALT level. The adjusted relative risk of cirrhosis for HBV DNA level of 10^4 copies/ml was 2.5 but this increased to 6.5 for patients with entry HBV DNA levels of $>10^6$ copies/ml.[65] Using the same population, another study found that this dose-dependent relationship also existed for predicting which patients developed HCC; this was especially the case in those individuals who were HBeAg negative, had normal ALT levels and not cirrhotic at study entry. They determined the adjusted hazard ratio to be 2.3, 6.6 and 6.1 for individuals with entry HBV DNA levels of 10^{4-5}, $<10^{5-6}$, and $>10^6$ respectively. Similarly a reduction in HBV DNA was associated with a reduction in the development of HCC.[50] Similar findings have been reported by other studies.[132;133]

In addition to viral load and HBeAg status, other viral factors such as genotype and the presence of mutations have been linked to progressive disease. As previously mentioned, a number of investigators have found that compared to genotype B infection, genotype C is associated with a more aggressive disease that has less chance of spontaneous seroconversion.[134-137] There is less data on other genotypes but one study from the Indian Subcontinent has suggested that genotype D is more associated with worse liver disease and HCC than genotype A[138]; although this suggestion has subsequently been challenged.[139] A number of mutations have been

discovered to be associated with advanced disease: precore (PC) $G_{1896}A$ mutation[140]; basal core promoter (BCP) $A_{1762}T$, $G_{1764}A$ mutations[141]; pre-S deletion mutations.[142] A recent study has suggested that rather than occur as single mutations, they may co-exist in a complex pattern to induce progressive disease.[143]

As most chronic HBV infections are acquired vertically or in early childhood, the age of the patient roughly corresponds to duration of the disease. Since the immune clearance phase tends to occur after 20-30 years, it is likely that the complications of cirrhosis and HCC will happen after this time period. A number of studies, using both Far Eastern subjects[50;133;144] and Europeans,[145;146] have shown that age is an independent risk factor for cirrhosis and HCC. Another host factor that has been implicated with advanced disease is male sex. Cross-sectional studies[147;148] and follow-up studies[50;65;129] have consistently shown that males are more associated with severe disease.

The immune response to the virus plays an important role in persistence, as well as clearance of the disease. During the immune clearance phase, there is inflammation and necrosis of the liver but these may be temporary. Raised ALT is a marker of active inflammation. Various studies have shown that elevated enzymes (greater than 0.5-1.0 x upper limit of normal, ULN) are significantly associated with liver-related mortality - not only with viral hepatitis but also in patients with no known liver disease.[43;149] However, a large study by Yuen et al, following up 3233 chronic HBV Chinese patients, found that patients with the highest risk of developing advanced liver disease had "mildly elevated" ALT (1-2 x ULN); whilst patients with ALT >6 x ULN had significantly lower risk of complications. They postulated that very high ALT rises resemble the situation of acute HBV and so do not usually lead to chronic damage. The mildly elevated ALT, on the other hand, suggests a more insidious attack on the liver leading to persistence of the infection.[150]

Other "environmental" factors have been implicated with HBV disease progression. Cross-sectional studies in Far Eastern individuals have suggested there is a role for

aflatoxin,[151] persistent alcohol intake,[152;153] cigarette smoking[129;153] and co-infection with other viruses such as hepatitis C,[154] hepatitis D[155] and HIV.[156]

1.15 Treatment of HBV

Complete elimination of HBV is not possible because cccDNA cannot be eradicated from infected hepatocytes. Therefore the ultimate goal of treatment is to improve quality of life and prevent the development of cirrhosis, decompensated disease and HCC. To do this there needs to be sustained suppression of the virus to undetectable levels. Achievement of this can lead to normalisation of ALT; loss of HBeAg, and possible loss of HBsAg; improvement of liver fibrosis; and the prevention of complications from occurring. The rationale for reducing the viral load to imperceptible amounts is so that there is much less chance of resistance developing.[157] Having said this, there is as yet no conclusive, randomised controlled trial evidence that any therapeutic option prevents the development of HCC.[158] There are studies which have shown reversal of fibrosis and potentially cirrhosis with oral anti-virals but there is no conclusive evidence that they can avert HCC.[159-161] This is because the development of advanced disease occurs many years after the diagnosis of HBV and none of the studies have looked at long term benefits of treatment.

Not all patients with chronic HBV infection need antiviral treatment. Patients who would benefit from treatment include: patients with decompensated liver disease and those with compensated cirrhosis; those with HBV receiving immunosuppressive or chemotherapy as they are at risk of experiencing an exacerbation of their hepatitis; and babies born of mothers with HBV, especially those who are HBeAg positive, with HBIG. Other individuals who may benefit from treatment include patients in the immune active or immune clearance phase with elevated DNA levels (>2000 IU/ml or 10^4 copies/ml), raised ALT (> 2x ULN) and marked fibrosis on liver biopsy (Metavir F2). These patients also should be older than 30 as younger individuals have a higher chance of spontaneous seroconversion. Those who do not routinely require antiviral treatment include: patients in the immune tolerant phase, who are characterised by

high DNA levels, but normal ALT and minimal inflammation and fibrosis on liver biopsy; and patients in the immunosurveillance or inactive phase, who are characterised by having low DNA and ALT levels with minimal inflammation and fibrosis on liver biopsy. These patients do, however, require continued follow up.[47;157;158;162]

There are 3 main types of treatment: interferons (conventional Interferon alpha (IFNα) and pegylated Interferon, (PEG $IFN_{2\alpha}$)), nucleosides (lamivudine, telbivudine, emtricitabine and entecavir) and nucleotides (adefovir and tenofovir). Interferons are given as subcutaneous injections and have the advantages of being of finite duration (approximately 12 months) and not inducing resistant mutants. Unfortunately they are not well tolerated due to being associated with numerous side effects and should not be used in patients with decompensated disease. They are most effective in younger patients, with genotypes A and B,[163] who have high ALT levels, low DNA levels ($<10^7$) and high inflammation on liver biopsy.[157;164] NAs are oral drugs that are better tolerated but are usually given for long term therapy and can lead to the development of resistant strains. Lamivudine and telbivudine are safe, rapid and potent suppressors of HBV DNA but have high rates of drug resistance (low genetic barrier). Adefovir has the advantage of being useful in patients with lamivudine resistance but is not as potent. The newer drugs, tenofovir and entecavir, have high potency and are much less prone to the development of resistance, especially with wild-type viruses, and can be used as first line monotherapy (see Figure 1-14). Emtricitabine is not used alone in the treatment of HBV, but instead is combined with lamivudine and tenofovir in a single tablet for the treatment of HBV-HIV co-infection.

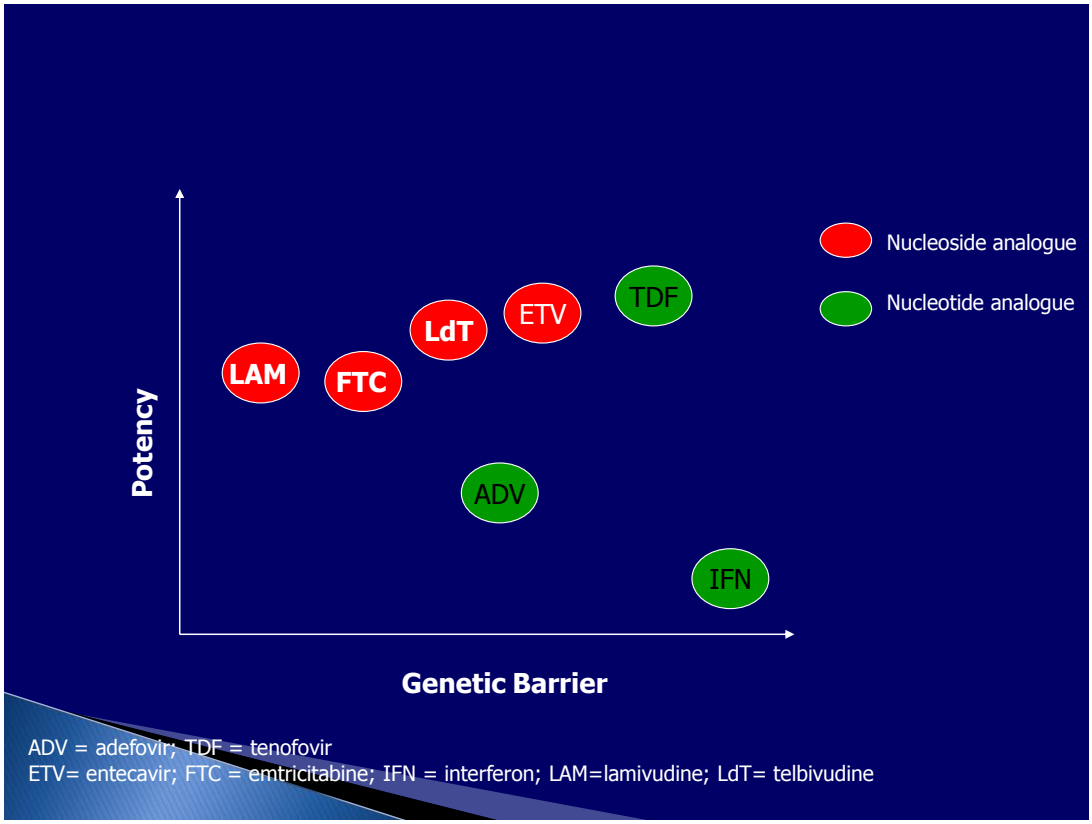

Figure 1-14 Antiviral activity and genetic barrier for resistance of current anti-HBV agents (slide courtesy of Dr Ines Ushiro-Lumb)

1.16 Monitoring Treatment

Recent EASL guidelines for the management of CHB recommend that patients being treated with PEG-IFN have monitoring of their full blood count (anaemia, leucopaenia, and thrombocytopaenia), liver function tests (hepatic flares) and thyroid function tests (thyroiditis) every three months in addition to safety whilst they on their twelve month course of treatment.[165] HBV DNA should be checked at 6 and 12 months during treatment and 6 and 12 months post treatment (and also HBeAg and HBeAb status for those with HBeAg positive disease). Successful treatment is characterised by sustained off-treatment normal ALT and HBV DNA <2000 IU/ml, and HBeAg seroconversion with HBeAg positive disease. If HBV DNA becomes undetectable then there is a better chance of HBsAg loss and HBsAg should be checked yearly. There is still the potential that HBeAg reversion can still occur so this needs to be regularly checked. Lately a "futility" rule has been suggested whereby

PEG-IFN treatment could be stopped if there has been no decline in HBsAg levels by three months because there is very little chance that therapy will be successful.[166;167]

NAs should be monitored every three months for HBV DNA and LFTs to assess for treatment response as well as for the development of resistance in the form of virological or biochemical breakthrough. Virological response occurs when there is no detectable HBV DNA during continuous treatment, whilst partial virological response exists when there is still detectable HBV DNA during treatment. The first indication of viral resistance is the appearance of virological breakthrough, which is defined as a 1 $\log_{10}$ increase in the HBV DNA from nadir in a patient that had an initial virological response. The rate of developing resistance depends on a number of factors: pre-treatment DNA levels, the time taken to achieve viral suppression, the duration of treatment and whether the patient had been exposed to antiviral treatment.[168] 30% of cases of virological breakthrough are due to treatment non-compliance.[47] Following on from this comes biological breakthrough, characterised by an increase in ALT, and then genotypic and phenotypic resistance. With lamivudine resistance, the commonest mutation results in substituting methionine in the YMDD (tyrosine-methionine-aspartate-aspartate) motif of the reverse transcriptase region of the polymerase for valine or isoleucine; the resulting mutation is known as rtM204V/I. This mutation is also effective against other nucleosides and has the ability to be archived so that should the patient be started on lamivudine at a later date the resistance to effect will be maintained. If resistance should develop then either switching or adding a different class of drug should be tried. For instance with lamivudine or telbivudine resistance the strategy should be to switch to a nucleotide like tenofovir. If there is entecavir resistance then switch to or add in tenofovir. So far there is no evidence of tenofovir resistance in treatment naive patients but if it should happen, EASL guidelines recommend adding or switching to entecavir.[165]

As NAs have the potential for causing renal impairment, more so with nucleotides, serum creatinine and creatinine clearance should be monitored every three months. Renal dysfunction is more likely to occur in patients with pre-existing intrinsic renal disease, poorly controlled diabetes and/or hypertension, contemporaneous use of

nephrotoxic drugs or solid organ transplant (because of the potential nephrotoxic effect of anti-rejection medication). In this situation, entecavir is a safer option than tenofovir.

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2 Materials & Methods

2.1 General Introduction

All the work carried out by myself was performed either at the Royal London Hospital (RLH) or at Newham University Hospital (NUH) as this is where the patients attending the outpatient clinics were. This occurred between October 2006 and September 2009.

2.2 Validation of oral mucosal transudate in chronic hepatitis B

2.2.1 Introduction

Blood-borne viruses such as HIV, HBV and HCV are under-diagnosed in the population. A major reason for this is that diagnosis relies on plasma or serum samples confirming the presence of an antibody (HIV antibody or HCV IgG) or antigen (HBsAg). There are a number of problems associated with relying on blood tests. Many individuals, especially children, object to having blood tests because of the pain caused in penetrating the skin; there is also the difficulty in accessing veins, especially in patients who are current or previous IVDUs; and there is the potential for a "needle stick" injury because of accidental puncture with an infected (blood) needle.

In the present study we wanted to adopt a completely different method that involves testing oral fluid which did not require taking blood samples and was completely painless. Saliva predominantly contains salivary immunoglobulin A (sIgA), whereas OMT contains a mixture of sIgA, IgG and IgM and more concentrated amounts of antigens derived from various pathogens like HBV.[169] Those studies that have managed to identify HBV and HCV with satisfactory sensitivity and specificity (> 95%) used the Orasure® collection device (Orasure Technologies Inc., Bethlehem, Pennsylvania, USA).[85-87] This is the same kit we used in the present study with HBV patients.

2.2.2 Aims

To validate immunoassays to identify hepatitis B surface antigen (HBsAg) and antibody to hepatitis B core antigen (anti−HBc) using the OraSure® oral fluid collection device with oral mucosal transudate.

2.2.3 Methods

2.2.3.1 Patients

One hundred patients, known to have chronic hepatitis B and who were attending the hepatology clinics at Barts and the London Hospital, were invited to take part in the study. The patients were of all ethnicities and all were aged over 18. They were selected by their attendance of the hepatology clinic on the day of testing and asked if they would be willing to take part in a study to diagnose HBV. All patients gave full, informed consent to taking part in the study and having the results analysed in another laboratory. They were given a patient information sheet and the study was explained to them in English or in whatever language they understood with the use of interpreters if necessary. Local research and ethics committee approval for testing patients with hepatitis B using OMT had been sought and given (see appendix 1).

The only inclusion criterion was that patients had to be chronic HBV carriers by being HBsAg positive on two or more occasions six months apart prior to testing (i.e. to differentiate them from patients with acute HBV infection that had now cleared the virus). Sixty eight patients were male of which 47 were HBeAg negative. Thirty two patients where female of which 21 were HBeAg negative. The other demographics of the patients are shown in Table 2-1.

Demographic	Mean	Range
Age	40	18-78
ALT/IU	55	11-240
HBV DNA/ log IU/ml	4.00	0-9.72

Table 2-1 Age, biochemical and virological characteristics of subjects enrolled into study

2.2.3.2 Materials

Each OMT testing kit consisted of two components: two Orasure® (Orasure Technologies Inc., Bethlehem, Pennsylvania, USA) mouth swabs and a storage vial as shown in Figure 2-1 below. The costs of providing the testing kits, transporting the samples, and the analysis was provided by Altrix Healthcare Limited, Warrington, UK.

The swab is a nylon stick that has a cotton pad attached to one end of it. The pad contains an electrolyte solution of 3.5% sodium chloride, 0.1% gelatine, 0.1% sodium benzoate, and 0.1% potassium sorbate buffered to pH 7.2. When placed in the lower mouth areas of the buccal mucosa, the hypertonic milieu of the pad allows passive transport of the interstitial fluid to this adsorbent area. Upon attaining the OMT, the swab is removed from the mouth, snapped off midway along its length at a specific point and then inserted into the storage vial.

The storage vial is made of plastic, is about 8cm in length, has a top that can be screwed off and contains preservative in the bottom of it. The preservative is made of 0.01% chlorohexidine digluconate and 0.5% Tween-20. To increase the volume of sample taken, two swabs are inserted into the mouth on either side and then put into a single storage vial.

Figure 2-1 Orasure® oral mucosal transudate swab and storage vial.

http://www.orasure.com/products-infectious/products-infectious-oralfluid.asp

2.2.3.3 Procedure

Testing involved inserting 2 Orasure® swabs into either side of the mouth between the buccal mucosa and the lower, lateral gingival surface. All of the tests were administered by myself or another research fellow who had been trained in how to use the testing kit. Each swab was left in-situ for two minutes and not longer than five minutes before removing the swab from the patient's mouth. The stick was then snapped at the correct point and inserted into the vial. Each patient was assigned a unique test number and each test kit had a unique barcode number. Each vial had a

barcode sticker on it and this matched the barcode sticker on a paper request form. The request form was labelled with the unique test number, barcode sticker, patient's date of birth and the date of collection. There were no other identifying markers put on the request form to the analysing laboratory. Samples were put in a sealed envelope and were sent by courier to the central reference laboratory at the West of Scotland Specialist Virology Centre, Gartnavel Hospital, Glasgow.

Analysis of the samples and interpretation of the results were performed by Dr Sheila Cameron and Professor William Carman. The method of processing the samples was done according to the standard operating procedure described in a previous paper.[170] The current ELISA assays to detect HBV in plasma or serum are the Murex HBsAg (v3) and the Murex anti-HBc (total) (Abbot diagnostics Europe, Wiesbaden, Germany). Using plasma/serum samples, the procedure that they followed is summarised in Table 2-2. For the present study these assays were "adapted" within Professor Carman's laboratory to detect HBsAg and anti-HBc using OMT samples.

Plasma/Serum	**HBsAg**	**Anti-HBc**
Sample amount/µl	75	50
Incubation period	60 min at 37°C	30 min at 37°C
Washes	-	x5
Conjugate	+ 50 µl conjugate for 30 min at 37°C	+ 50 µl conjugate for 30 min at 37°C
Washes	x5	x5

Table 2-2 Summary of procedure for identifying HBsAg and anti-HBc in plasma/serum

There were three experiments. The first was to adapt the current Murex HBsAg and anti-HBc assays to detect HBsAg and anti-HBc in OMT. This was done by altering the various parameters of sample amount, incubation period and conjugate until the

highest sensitivity was achieved. The OMT was taken from three samples of patients known to be positive for HBsAg and anti-HBc.

There are a number of other commercially available assays for detecting HBsAg and anti-HBc using plasma/serum samples. Three of these other ELISA based kits are:

1) BioMérieux Hepanostika HBsAg Ultra & anti-HBc Uni-Form (BioMérieux, Craponne, France)
2) Bio-Rad Monolisa HBsAg ULTRA & anti-HBc PLUS (Bio-Rad, Marnes-la-Coquette, France)
3) Dade Behring Enzygnost HBsAg 5.0 & Anti-HBc monoclonal (Dade Behring, Deerfield, Illinois, USA)

Experiment two involved comparing the "adapted" Murex assay with these three testing kits for the amount of false negative HBsAg and anti-HBc. As all the 100 samples were from patients known to be positive or reactive to HBsAg and anti-HBc, there should ideally be 0 false negatives in the adapted Murex assay.

Experiment three involved adapting the three commercial kits from BioMérieux, Bio-Rad and Dade Behring in a similar fashion to that done with the Murex assay i.e. by altering sample amount, incubation period and conjugate. Then the proportion of false negatives for HBsAg and anti-HBc between the four adapted assays was compared.

2.2.4 Results

Experiment 1

Table 2-3 shows the adaptations done to improve the sensitivity of detecting HBsAg and anti-HBc in the Murex assays using OMT. Over the three samples, the only

modification to the procedure with HBsAg was increasing the number of washes from 5 to 6. The sample volume was the same at 75 µl.

There were significant differences when adapting the assay for anti-HBc. The optimal sample volume was 100 µl. In order for the sensitivity to improve the samples required overnight incubation at room temperature. In addition the number of washes was also increased.

OMT	**HBsAg**	**Anti-HBc**
Sample amount (µl)	75	100
Incubation period	60 min at 37°C	Overnight at room temperature
Washes	-	x12
Conjugate	+ 50 µl conjugate for 30 min at 37°C	+ 50 µl conjugate for 60 min at room temperature
Washes	x6	x6

Table 2-3 Summary of procedures to "adapt" Murex HBsAg and Anti-HBc assays for optimal detection of HBsAg and anti-HBc in OMT

Experiment 2

Using the "adapted" procedure for the Murex assay, the numbers of false negatives for HBsAg and anti-HBc were 6 and 2 respectively. From Table 2-4 it can be seen that apart from the BioMérieux HBsAg assay, all of the other assays had many more false negatives compared to the adapted Murex assays for both HBsAg and anti-HBc.

Commercial Kit	**BioMérieux**		**Bio-Rad**		**Dade Behring**	
Assay type	HBsAg	Anti-HBc	HBsAg	Anti-HBc	HBsAg	Anti-HBc
False negatives	6	32	13	18	8	49

Table 2-4 Number of false negatives for HBsAg and anti-HBc using OMT samples using plasma/serum (unadapted) commercial assays

Experiment 3

Table 2-5 and Table 2-6 encapsulate the process by which all the commercial assays for HBsAg and anti-HBc were adapted for use with OMT.

Adapted HBsAg assay	**Murex**	**BioMérieux**	**Bio-Rad**	**Dade Behring**
Sample volume (µl)	100	100	100	100 + 25 conjugate 1
Incubation period 1	60 minutes at 37°C			
Conjugate volume (µl)	50	50	50	100 conjugate 2
Incubation period 2	60 minutes at 37°C			
Substrate volume (µl)	100	100	100	75
Incubation period 3	In the dark at room temperature for 30 minutes			
Stopping solution volume (µl)	50	100	100	75

Table 2-5 Summary of procedures for adapting HBsAg assays for detecting HBsAg in OMT

Adapted anti-HBc assay	**Murex**	**BioMérieux**	**Bio-Rad**	**Dade Behring**
Sample volume (µl)	100			
Incubation period 1	Overnight at room temperature			
Conjugate volume (µl)	50	50	200	100
Incubation period 2	60 minutes at room temperature			
Substrate volume (µl)	100			
Incubation period 3	In the dark at room temperature for 30 minutes			
Stopping solution volume (µl)	50	100	100	100

Table 2-6 Summary of procedures for adapting anti-HBc assays for detecting anti-HBc in OMT

Table 2-7 and Table 2-8 show the number of false negatives and sensitivities for the four assays before and after adaptation for OMT to detect HBsAg and anti-HBc

respectively. With HBsAg, the BioMérieux adapted assay had no change in sensitivity compared with the un-adapted assay and this may have been because the procedure for adaptation is very similar to how the original BioMérieux assay is produced. That said there was a significant improvement in sensitivity with adapting the BioMérieux anti-HBc assay. The most sensitive adapted HBsAg assay was the Dade-Behring, with the Murex having a sensitivity of 94.3%. The Murex was the most sensitive anti-HBc assay.

An interesting finding from the results is that the identified false negatives were not the same samples in all the assays. Of the six false negative HBsAg in the Murex assay, only 3 of them matched the six false negatives of the BioMérieux assay. The one false negative of the Dade Behring HBsAg assay was positive in all the other assays. In contrast, the two false negatives of the Murex anti-HBc assay were all negative in the other three assays.

HBsAg assay	**Murex**	**BioMérieux**	**Bio-Rad**	**Dade Behring**
Un-adapted false negatives (sensitivity)	-	6 (94.3)	13 (88.5)	8 (92.6)
Adapted false negatives (sensitivity)	6 (94.3)	6 (94.3)	8 (92.6)	1 (99)

Table 2-7 Number of false negatives and sensitivities (%) for un-adapted and adapted assays testing for HBsAg in OMT

Anti-HBc assay	**Murex**	**BioMérieux**	**Bio-Rad**	**Dade Behring**
Un-adapted false negatives (sensitivity)	-	32 (75.7)	18 (84.8)	49 (67.1)
Adapted false negatives (sensitivity)	2 (98)	14 (87.7)	5 (95.2)	6 (94.3)

Table 2-8 Number of false negatives and sensitivities (%) for un-adapted and adapted assays testing for anti-HBc in OMT

2.2.5 Discussion

The aim of the study was to validate an adapted immunoassay to identify HBsAg and anti-HBc in OMT samples and in this respect, it was successful. The adapted Murex assay had sensitivities of 94.3% and 98% for identifying HBsAg and anti-HBc respectively. The Murex assays had similar sensitivities to the other 3 immunoassays for recognizing HBsAg and was the best at finding anti-HBc.

Ideally the sensitivity of both immunoassays would be approaching 100% as all the samples came from patients known to have HBV. As mentioned in the results there was some disparity between the false negative samples detected by the various assays. With future studies, using samples with different volumes, incubation times, or altering the number of washes may improve this.

There have been other studies looking at detecting HBV using different oral fluid collection devices. A study by Hutse et al collected the saliva from 43 patients who were HBsAg positive and 73 who were HBsAg negative using the Oracol collection device (Malvern Medical Developments, Worcester, UK) and analysed by a modified ETI-MAK-4 ELISA. They found sensitivity of 90.7% and specificity of 100%.[171]

The study by Thieme et al used the Orasure® collection device to test oral fluid in 29 patients who were HBsAg positive and 29 patients who were HBsAg negative. They used a Murex ELISA but the sample volumes were either 200µl or 400µl. They had 100% sensitivity and specificity.[85] Compared to the present study, their study has much smaller numbers and uses different sample volumes and procedures.

A criticism of the present study is that because there is no negative control arm, that is we did not screen patients who are known not to have HBV, we cannot comment on false positives and hence specificity. At the time of the ethics application, there was another on-going study that was looking at this issue. That study involved screening GP practices for HBV and HCV by conventional blood tests (for HBsAg, anti-

HBc and HCV IgG) as well as using the Orasure® collection device. We anticipate that with a hepatitis B prevalence of around 2% in this GP population 490 subjects who are proven to be HBsAg negative by blood testing will be screened with the Orasure testing system. Thereby we will obtain a clear assessment of the rate of false positives using the Orasure testing system. However this on-going study will not provide information regarding the false negative rates as too few patients with hepatitis B will be screened. The current study is therefore designed to complement our on-going studies by assessing the false negative rate with this testing system.

We felt that sensitivity of 94.3% for HBsAg using the adapted Murex assay that had a 98% sensitivity for detecting anti-HBc was acceptable for screening an asymptomatic population for HBsAg. This level of sensitivity would not be sufficient if the task was screening blood samples for transfusion or trying to confirm a diagnosis in patients referred for hepatitis B testing. However in our view the slight loss of sensitivity with the assay was more than offset by the advantage in high throughput community screening.

In our sample of 100 patients, 2 had HBV DNA 0 and 10 had viral loads <1.30 log IU/ml. Unfortunately it was not possible to ascertain from the analysing laboratory which patients had the false negatives and see if this correlated with HBV DNA. At the time of the study HBsAg quantication of serum samples was not being carried out. It would have been useful to have values of HBsAg determined in serum and OMT at the same time, giving the opportunity to do limiting dilution analysis in a subset of samples. This would give the added information about the sensitivity level when using OMT compared to serum. Future studies could have compared using OMT with paired blood samples and DBS samples, as a control point of care test, to see which diagnostic method is superior.

2.3 Statistical Analysis

All the data collected was categorical and so non-parametric tests such as Chi square, Fisher's exact test and logistic regression were performed. Statistics were calculated using computer software (SPSS version 16, SPSS Inc., Chicago, IL, USA and GraphPad Prism version 5, GraphPad Software Inc., La Jolla, CA, USA).

Before starting the research I went on courses at Cambridge University about statistics methods & testing and on how to use SPSS. I approached Dr Janice Thomas, statistician at QMUL for statistical advice on a number occasions regarding test choice. Following analysis, I discussed the results with her and she was satisfied with the tests chosen, their analysis, and the conclusions drawn.

3 Preliminary Studies

3.1 General introduction

This thesis examines chronic hepatitis B infection in the immigrant communities of East London. The focus of the study is looking at the epidemiology of the disease, and specifically the prevalence and disease burden. In order to accomplish this, a number of preliminary studies had to be carried out. These were done by other individuals and organisations but I played an important role in their design, collection of samples, and execution.

The first of these studies is collection of demographic data from the Office of National Statistics (ONS). This involved using the 2001 UK census data to determine the number of immigrants of various nationalities, including Bangladeshi and Pakistani, who were residing in East London.

The second study is the 'Mosque Screening Study'. This involved targeted screening of Bangladeshi and Pakistani individuals who attended local mosques in East London. Screening was done using validated OMT test kits. Based on these results, the prevalence of hepatitis B in people attending mosques was calculated.

Although not a separate study, the next section concerns the demographics of the Bangladeshi and Pakistani population. These were derived from utilising the Mosque Study results and applying them to the ONS data for East London.

3.2 Office for National Statistics data

The Office for National Statistics (ONS) is part of the UK Statistics Authority, which is an independent government department that answers directly to Parliament. It collects and publishes data on the population, economy and society of England and Wales at national, regional and local levels over time. Every 10 years there is a national census which all households in the UK have to respond to by law as accurately as possible. At the time of this study, statistics were retrieved from the 2001 UK national census.

The census contains a vast amount of socio-demographic information about the UK population including details about age, sex, ethnicity, and country of birth. Country of origin was not a category in the 2001 census and so country of birth was used as a surrogate marker for this. All this information is in the public domain and can be readily accessed on the internet (http://www.nomisweb.co.uk/). However, the data that is publicly available is not specific enough to calculate the prevalence of HBV in the Bangladeshi and Pakistani populations. The data can be manipulated to give at most 3 variables in a particular region such as local authority: age versus sex versus ethnicity. Even then, this age data is arranged in large groups: 0-15, 16-64, and 65 and over.

There are tables which have been commissioned by other researchers and these are available on request to the ONS. These include table CO182: country of birth by sex and age, and table CO954: country of birth and age by ethnic group by sex. The problems with these tables are that they give very gross figures which lack the ability to give much detail. In table CO182, the age ranges were 0-15, 16-39, 40-64, 65-74 and 75 and older for males and females according to each London authority. In table CO954, the age ranges were every 5 years (i.e. 1-4, 5-9, 10-14, etc.) for males and females depending on the country of birth (i.e. Bangladesh) but could only classify individuals as Asian/Asian British versus other ethnic group and this was at national

level. There was no currently commissioned table that had the 4 variables: age, sex, country of birth and ethnicity.

I negotiated with the ONS census commissioning team, explaining that we needed a single table with those 4 variables and to a highly specific degree (5 year age bands) in order to work out prevalence of HBV in particular populations (Bangladeshis, Indians and Pakistanis) of East London. The only way to achieve this would be to commission and pay for our own bespoke table that would have all this information. Their disclosure team scrutinised our request and agreed to give us the data to the level of detail we required. Table C1056 consists of age (5 year age bands) and country of birth by sex and by ethnic groups at a local authority level. The layout of table C1056 is shown below in Table 3-1.

00BB Newham	TOTAL ETHNIC GROUPS	Asian or Asian British: Indian	Asian or Asian British: Pakistani	Asian or Asian British: Bangladeshi	Other Ethnic Groups
Males 16 to 20					
016 Bangladesh	576	3	3	565	5
093 India - 093A Kashmir	271	257	5	3	6
155 Pakistan	277	0	267	3	7
United Kingdom	6,608	1,253	838	611	3,906
Rest of World	1,815	53	15	11	1,736
Males 21 to 25					
016 Bangladesh	686	4	3	666	13
093 India - 093A Kashmir	496	473	7	4	12
155 Pakistan	563	0	551	0	12
United Kingdom	5,816	995	596	291	3,934
Rest of World	2,548	81	24	14	2,429

Table 3-1 Part of commissioned ONS table C1056 showing age and country of birth by sex by ethnic groups (Source: 2001 Census Commissioned Table. Crown copyright 2009. Crown copyright material is reproduced with the permission of the Controller of HMSO)

Table C1056 shows the number of Bangladeshi, Indian and Pakistani individuals of both sexes born in Bangladesh, India, Pakistan, UK and the rest of the world. The mosque study mentioned above did look at viral hepatitis in Indians and that was why the ethnic Indians and those born in India are included. This data also improves the accuracy of identifying the number of Bangladeshis and Pakistanis born outside the UK as some were born in India.

Data from the migration department of ONS suggest that there has been less than 2% migration of Bangladeshis and Pakistanis to the London Boroughs of Newham and Tower Hamlets (which are the two East London local authorities involved in the research study) between 2001 and 2006 (unpublished data kindly provided by ONS and based on official immigration statistics). Given the low rates of migration we assumed that the impact of inward and outward migration would be minimal and therefore this factor was ignored. To evaluate population density at the time of our study we completed a simple numerical calculation i.e. those individuals who would have been in the 16-20 age group in 2001 would now be in the 21-25 age group in 2006. The corrected population numbers for Bangladeshis and Pakistanis born abroad (first generation immigrants) and those born in the UK (second generation immigrants) are shown in Table 3-2 and Table 3-3 below.

	Male Bangladeshi		Female Bangladeshi	
Age range	**Born abroad**	**Born in UK**	**Born abroad**	**Born in UK**
16-20	1221	4044	1154	4032
21-30	4898	3714	6204	4054
31-40	6893	705	6689	820
41-50	4342	146	3169	103
51-60	1200	33	2594	52
61-70	1890	43	1586	24
71-80	1427	25	635	19
81 and over	197	28	132	32
Total	**20847**	**8738**	**21009**	**9136**

Table 3-2 Population of Bangladeshis living in the two boroughs of Newham and Tower Hamlets according to age, sex and whether they were born in the UK or abroad

	Male Pakistani		Female Pakistani	
Age range	**Born abroad**	**Born in UK**	**Born abroad**	**Born in UK**
16-20	232	936	205	837
21-30	904	1540	808	1453
31-40	1668	690	1128	780
41-50	1153	58	1165	67
51-60	734	28	696	22
61-70	327	11	361	9
71-80	228	15	158	9
81 and over	48	13	37	15
Total	**5062**	**3291**	**4353**	**3192**

Table 3-3 Population of Pakistanis living in the two boroughs of Newham and Tower Hamlets according to age, sex and whether they were born in the UK or abroad

As individuals aged under 16 were not included in the study, their numbers are not included in the above tables. The results show that the youngest age groups of both sexes and ethnicities were born in the UK. Similarly with the 31-40 age group and older age groups more immigrants were born abroad. As expected with an immigrant population, as people get older their age groups have fewer numbers but that those born abroad still outnumber their UK born counterparts. These findings are typical of mass immigration trends into the UK from Commonwealth countries and made easier by the popularity of air travel. In these 2 boroughs, Bangladeshis outnumber Pakistanis. The borough of Tower Hamlets in particular has a very large Bangladeshi population. Bangladeshis and Pakistanis, amongst other ethnic groups, tend to congregate in particular towns and districts, and this could explain the large number of Bangladeshis here. Other UK towns, such as Bradford, have large Pakistani populations.

3.3 Mosque study

3.3.1 Aims

The aims of the mosque study were to estimate the prevalence of HBV and HCV in immigrants of Indian, Bangladeshi and Pakistani origin living in various parts of England.[172]

3.3.2 Methods

3.3.2.1 Patients

The study took place between January 2006 and December 2008, and involved screening healthy individuals for viral hepatitis in mosques, temples, community centres and some GP surgeries. This initially started in East London but was later extended to 4 other English regions: West London, Sandwell and Walsall in the West Midlands, and Bradford in West Yorkshire. These areas were picked because of their high concentration of immigrants from Bangladesh, India, and Pakistan. In total, 52 centres were used to screen for viral hepatitis. Ethical approval and permission from the mosque, temple, and community leaders was sought and granted before screening proceeded. Adverts were placed locally in the screening centres on the day of testing offering free viral hepatitis testing. Screening was performed by the research team along with various medical students and volunteers who had been trained in how to use the screening devices. I played a minor role in screening individuals in two East London mosques. All patients were given a patient information sheet in their primary language and asked to sign a consent form. They gave informed consent that should they test positive for HBV or HCV by oral fluid then they would have a confirmatory blood test and if they tested positive for HBsAg or HCV IgG they would be referred to a local treating physician and be managed according to local treatment guidelines. If patients were unwilling to have a confirmatory blood test then the result would be forwarded to their GP.

Free HBV and HCV testing with oral fluid (OMT) was available to all persons attending the screening centre aged 16 or over. However, only those with Bangladeshi, Indian, or Pakistani ethnic origin were included in the final analysis. Persons from other countries such as Somalia or Afghanistan, who opted to be tested, underwent exactly the same procedure and were informed of a positive result in exactly the same way. Apart from contact information, which was known only to the screeners and principal investigator, various demographic data was also collected. These included age, gender, city and country of birth, country of birth of parents (if person was born in the UK), length of stay in the UK (if person was born abroad), and other medical conditions, specifically diabetes mellitus.

A total of 5162 people attending all of the sites underwent screening, with 4833 coming from the study countries and being included in the analysis. The number of males was 2970 (61%) and the age range was 16 to 103 with a median of 48. The number of first generation immigrants was 4381, of which 726 were from Bangladesh, 1197 were from India and 2458 were from Pakistan. In East London 1975 people were screened, 1387 in Bradford, 644 in Walsall, 448 in Sandwell and 379 in West London.

3.3.2.2 Materials & Procedure

OMT Screening was done using the Orasure® collection device (Orasure Technologies Inc., Bethlehem, Pennsylvania, USA) using exactly the same procedure as was described above in the methods section of the validation study. Testing involved two mouth swabs that were left in-situ in either side of the mouth for two minutes before inserting into the storage vial. As with the previous study, the samples were coded anonymously with a unique identification number and a unique barcode sticker was applied to the storage vial and sample request form. The completed samples were sealed in an envelope and were couriered to the West of Scotland Specialist Virology Centre, Gartnavel Hospital, Glasgow, which acted as the central reference laboratory. Analysis of the OMT was again carried out by Dr Sheila Cameron and Professor

William Carman using the same operating procedure with the adapted Murex ELISA assay described above. As with the validation study, all the financial costs associated with provision of kits, transportation to the central laboratory and virological analysis were borne by Altrix Healthcare Limited, Warrington, UK.

3.3.2.3 Virological analysis

OMT samples were tested for HBsAg and HCV IgG. The study investigators noticed that with the initial 1000 cases, there were a number of HBsAg OMT false positives whose corresponding serum samples were negative (for HBsAg). To counteract this, the HBsAg positive OMT samples were also tested for anti-HBc. Only those OMT samples which tested positive for both HBsAg and anti-HBc were assumed to be from HBV positive persons. These people were then contacted to have confirmatory blood tests to see if they had HBV. Those who declined to have confirmatory blood tests were assumed to be HBV positive if they had positive/reactive HBsAg and anti-HBc OMT samples. It took about one week for the results of the OMT analysis to be relayed to the study investigators. Upon receiving the information the study team contacted the individuals concerned and requested that they attend for confirmatory blood tests. All participants were informed that if they were not contacted within 20 days of being tested then they should assume that the tests for HBV and HCV were negative.

The subjects that did screen positive had their blood tests for HBsAg and HCV IgG analysed by the virology laboratory at the Royal London Hospital.

3.3.3 Results

A total of 57 individuals were diagnosed with HBV and 75 with HCV. The vast majority of cases were first generation immigrants as shown in Table 3-4. In 2nd generation immigrants there was 1 case of HBV and 2 cases of HCV. With HBV infection there were much higher odds of contracting the infection if born in Bangladesh or Pakistan.

The results for HCV are different in that the odds of infection are higher if born in Pakistan.

Ethnicity	**Bangladeshi**	**Indian**	**Pakistani**
HBV	11(1.5)	1 (0.1)	44 (1.8)
HCV	4 (0.6)	2 (0.2)	67 (2.7)

Table 3-4 Overall number of cases and prevalence (%) of HBV and HCV in first generation immigrants attending screening centres.[172]

Figure 3-1demonstrates the distribution of individuals screened in the mosque study. 3558 Bangladeshi and Pakistani individuals were screened, of which 3184 were first generation immigrants born abroad in Bangladesh and Pakistan and 374 were second generation immigrants born in the UK.

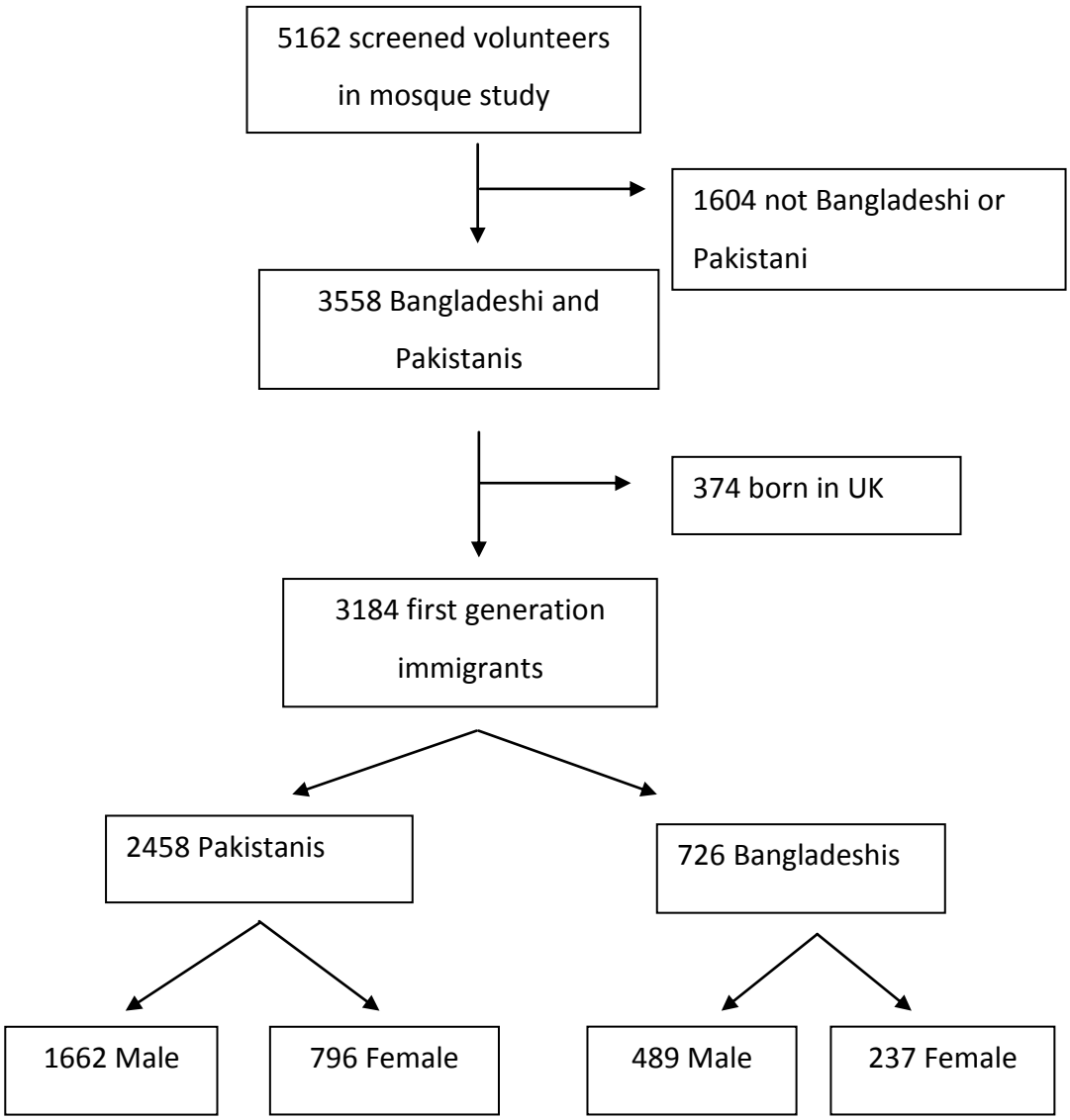

Figure 3-1 Ethnicity, immigration and gender status of volunteers seen in mosque study

Figure 3-2 and Figure 3-3 show the age distribution of the first generation immigrant Bangladeshi and Pakistani population that was screened during the Mosque Study. The mean age of Bangladeshi males was 48.0 years (range 16-103 years) and females was 44.5 years (17-89 years); and Pakistani males was 47.7 (16-103 years) and females was 46.7 (16-103 years). Very few individuals of either sex who were screened were under 20 years old or over 80 years old. This may be due to these

individuals either not attending the mosques for health or social reasons or that they declined to enter into the prevalence study.

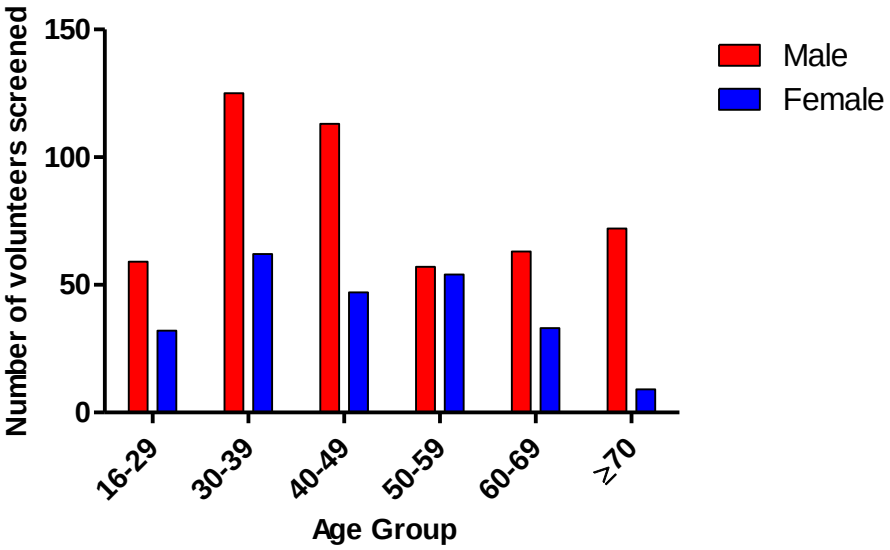

Figure 3-2 Age and sex distribution of first generation Bangladeshi volunteers screened in mosque study

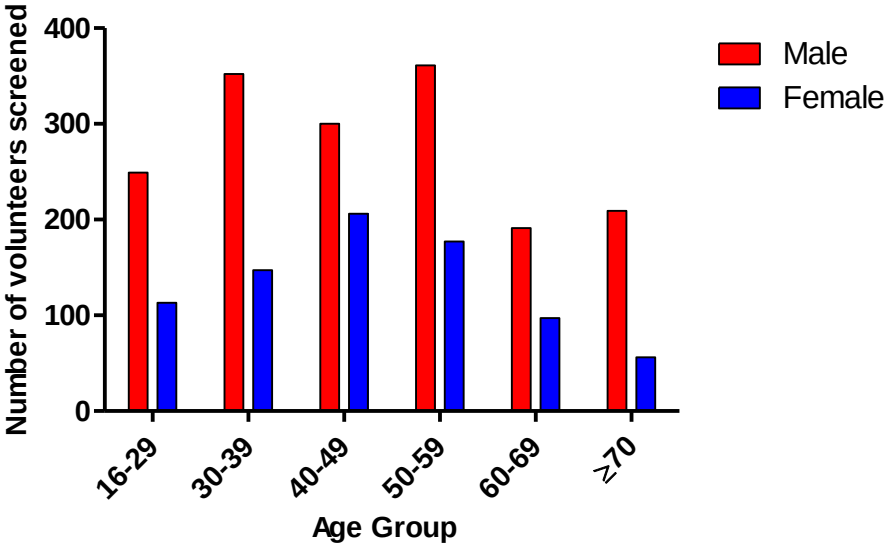

Figure 3-3 Age and sex distribution of first generation Pakistani volunteers screened in mosque study

Figure 3-4 and Figure 3-5 shows the age and sex distribution for second generation Bangladeshis and Pakistanis attending the mosques who agreed to be screened. The most notable difference between these figures and Figure 3-2 and Figure 3-3 is that there were far fewer UK-born individuals attending (and being screened at) the mosques. Those that did attend were much younger, with very few (less than 10%) being over 40 years old. The mean age of these Bangladeshi males was 29.3 years (range 16-65 years) and females was 30.2 years (20-42 years); and Pakistani males was 29.0 (16-66 years) and females was 27.3 (16-51 years).

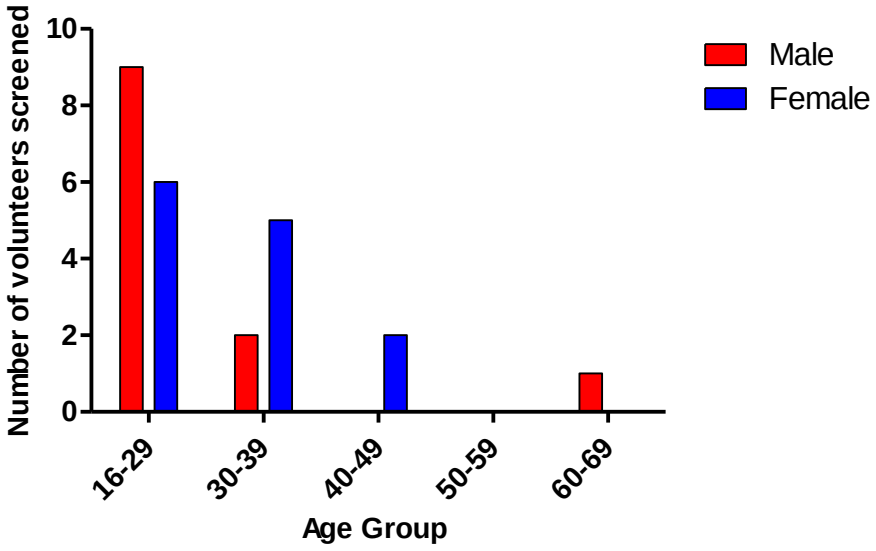

Figure 3-4 Age and sex distribution of second generation Bangladeshi volunteers screened in mosque study

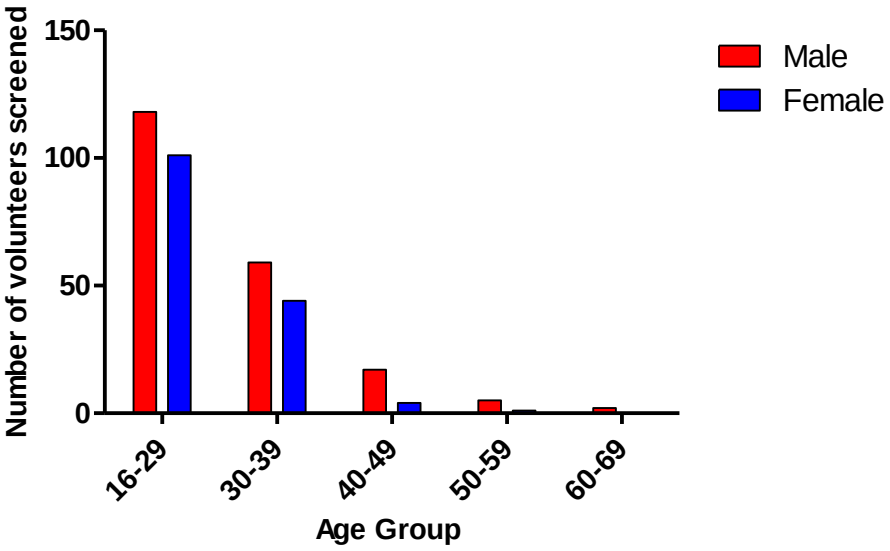

Figure 3-5 Age and sex distribution of second generation Pakistani volunteers screened in mosque study

Figure 3-6 shows the age distribution of the 55 first generation Bangladeshi and Pakistani individuals who screened positive for HBV. 11 Bangladeshis tested positive for HBV, of which 10 were male, and 44 Pakistanis tested positive, of which 31 were male.

Only two of the second generation immigrants tested positive for HBV: one Pakistani and one Bangladeshi. Apart from Bangladeshi females, the peak prevalence for HBV was in the 40-49 age group for Pakistani males and females and Bangladeshi males. The prevalence of HBV was four times higher in the first generation Pakistani screened population than for the Bangladeshis.

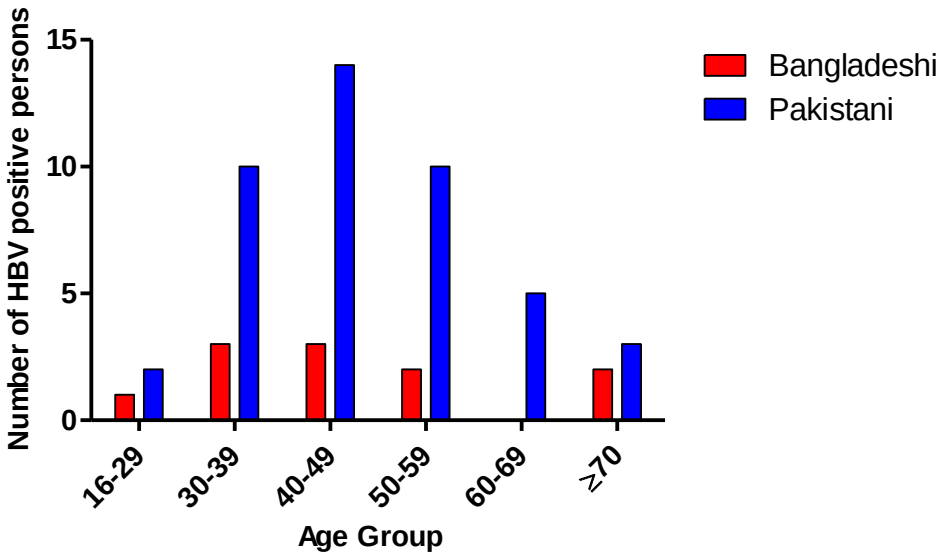

Figure 3-6 Age distribution of HBV in screened first generation Bangladeshi & Pakistani volunteers of both sexes

Figure 3-7and Figure 3-8 show the prevalence of HBV in the first generation screened Bangladeshi and Pakistani population for each of the different age groups for both sexes. The overall prevalence of HBV in both sexes is also shown; the prevalence of HBV being higher in males compared to females.

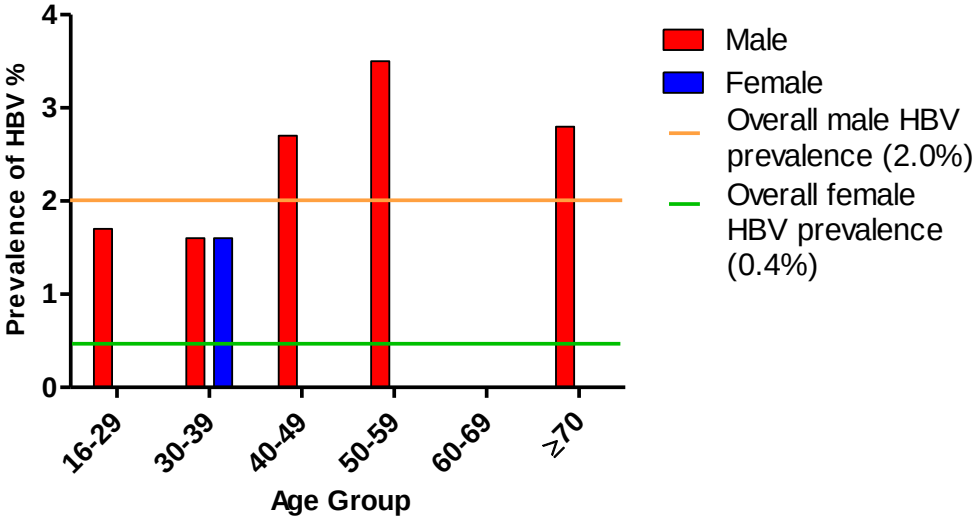

Figure 3-7 Prevalence of HBV across age groups for first generation screened Bangladeshi volunteers

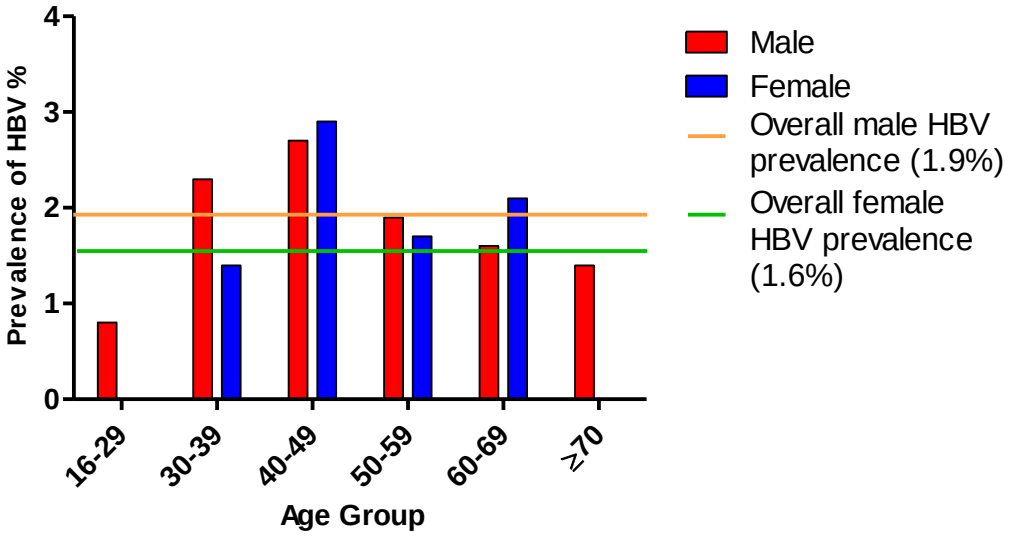

Figure 3-8 Prevalence of HBV across age groups for first generation screened Pakistani volunteers

3.4 Demographics of HBV in Bangladeshis and Pakistanis living in East London

3.4.1 Introduction

At the time of this study only the Mosque study above had estimated the prevalence of HBV in Bangladeshi and Pakistani immigrants.[172] Recently there has since been another study conducted in the North-East of England which screened the Chinese and South-Asian communities using dried blood spot testing. In this study the prevalence of HBV was estimated to be 3.1% in Pakistanis and 0.5% in Bangladeshis.[173]

Tower Hamlets and Newham are boroughs in North East London with large immigrant communities (see Figure 3-9). In Newham, there are approximately equal numbers of Bangladeshis and Pakistanis and together they make up 17% of the population. In Tower Hamlets, there is a very large Bangladeshi community which comprises a third of the population; this is in contrast to Pakistanis who comprise 1% of the population.

The two populations are served by two hospitals, Barts and the London (BLT) and Newham University Hospital (NUH), which provide free NHS care to all residents. The proximity of the hospitals to the local population and the referral patterns of local primary care physicians (who refer patients to local physicians) ensure that the vast majority of patients with liver disease are referred locally to either of these hospitals.

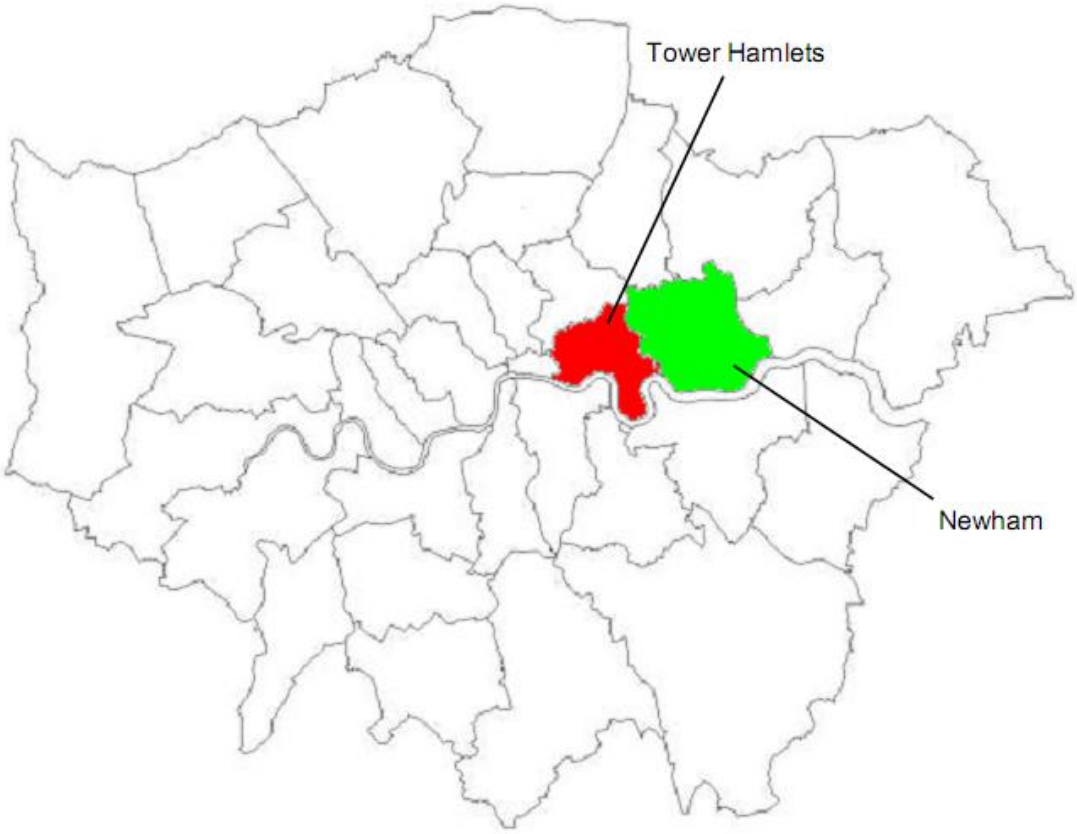

Figure 3-9 Map showing the London Boroughs of Newham and Tower Hamlets

3.4.2 Aims

To determine the prevalence of chronic HBV in hospital based patients from Bangladesh and Pakistan living in East London.

3.4.3 Methods

3.4.3.1 Patients

Data from the 2001 ONS census was used to provide information on the population living in Tower Hamlets and Newham who were born in Pakistan or Bangladesh.

Migration data from ONS indicates that there has been less than a 2% change in the Pakistani/Bangladeshi population in the UK between 2001 and 2006 and the 2001 census data was therefore deemed to be accurate. The Mosque Study showed that, by multivariable analysis there was no difference in the prevalence of HBV between people originating from Pakistan or Bangladesh and there was no difference in prevalence in the different regions in the UK. There was, however, a difference in prevalence between men and women and a difference in prevalence between the different ages. To allow for aging of the population all ages were expressed as 2006 ages (i.e. 5 years was added to the age of the individuals identified in the 2001 census). The proportion of people of different ages with chronic HBV was derived by simple calculation. The analysis of the prevalence of HBV in this dataset was performed solely by myself.

Over a 30 month period from January 2006 to June 2008 I sought to identify every patient with HBV that presented to the 2 hospitals either as an outpatient clinic attendee or who was admitted as inpatient. I achieved this by contacting the coding departments of both hospitals and identifying all patients admitted with a diagnosis of hepatitis, decompensated liver disease or liver cancer and corroborating the details with the patient notes, which were searched by hand. Outpatients were identified from clinic waiting lists and checking the electronic patient record systems of both hospitals. Confirmation of HBV status was done by checking the virology records on the hospital pathology system. Of the 1243 patients (of all ethnicities) attending the out-patient clinics with a diagnosis of chronic HBV, 268 patients were of Bangladeshi and Pakistani ethnicity. Patients with co-infections such as delta, HCV and HIV were noted but were not included in the prevalence analysis.

3.4.3.2 Statistical Analysis

All the data collected was categorical and so non-parametric tests such as Chi square, Fisher's exact test and logistic regression were performed. Statistics were calculated using computer software (SPSS version 16, SPSS Inc., Chicago, IL, USA and GraphPad Prism version 5, GraphPad Software Inc., La Jolla, CA, USA).

3.4.3.3 Ethical Approval

Ethical approval for this study was granted by Oxfordshire REC A as an amendment to the study: Case finding and prevalence of chronic viral hepatitis in South Asians living in the UK – Number 06/Q1604/185.

3.4.4 Results

3.4.4.1 Prevalence of HBV in Bangladeshi and Pakistani immigrants living in East London

Table 3-5 through Table 3-8 show the estimated predicted prevalence of HBV, for the different age groups, in the first generation immigrant Bangladeshi and Pakistani populations of East London for males and females respectively. As only two second generation immigrant volunteers tested positive for HBV (1 Pakistani and 1 Bangladeshi), we cannot determine the prevalence in the second generation populations. Similarly there was only one female first generation Bangladeshi who tested positive and this explains why there are only estimated prevalence numbers in the 30-39 age group. This is almost certainly not the case but it was not possible to estimate prevalence of HBV in this sector of the population based on the small numbers of individuals who agreed to be screened. The estimated numbers were calculated by multiplying the prevalence for each age group with the population numbers for each age group derived from the 2001 census data. Because there are

large confidence intervals for the prevalence of HBV in the different age groups, this translates into much wider estimates for the true numbers of infected individuals in the East London population.

Age	16-29	30-39	40-49	50-59	60-69	≥70
Prevalence of HBV (%) (and 95% confidence interval)	1.7 (± 3.3)	1.6 (± 2.2)	2.7 (± 3.0)	3.5 (± 4.8)	0	2.8 (± 4.8)
Population in East London	6119	6893	4342	1200	1890	1624
Estimated number of people who are HBsAg positive	104 (0-306)	110 (0-262)	117 (0-247)	42 (0-100)	NA	45 (0-123)

Table 3-5 Estimated HBV burden in male Bangladeshi first generation immigrants in East London

Age	16-29	30-39	40-49	50-59	60-69	≥70
Prevalence of HBV (%) (and 95% confidence interval)	0	1.6 (± 3.1)	0	0	0	0
Population in East London	7358	6689	3169	2594	1586	767
Estimated number of people who are HBsAg positive	NA	118 (0-314)	NA	NA	NA	NA

Table 3-6 Estimated HBV burden in female Bangladeshi first generation immigrants in East London

Age	16-29	30-39	40-49	50-59	60-69	≥70
Prevalence of HBV (%) (and 95% confidence interval)	0.8 (± 1.1)	2.3 (± 1.6)	2.7 (± 1.7)	1.9 (± 1.4)	1.6 (± 1.8)	1.4 (± 1.6)
Population in East London	1136	1668	1153	734	327	276
Estimated number of people who are HBsAg positive	9 (0-22)	38 (0-65)	31 (11-52)	14 (4-25)	5 (0-12)	4 (0-8)

Table 3-7 Estimated HBV burden in male Pakistani first generation immigrants in East London

Age	16-29	30-39	40-49	50-59	60-69	≥70
Prevalence of HBV (%) (and 95% confidence interval)	0	1.4 (± 1.9)	2.9 (± 2.3)	1.7 (± 1.9)	2.1 (± 2.8)	0
Population in East London	1013	1128	1165	696	361	195
Estimated number of people who are HBsAg positive	NA	16 (0-37)	34 (7-61)	12 (0-25)	8 (0-18)	NA

Table 3-8 Estimated HBV burden in female Pakistani first generation immigrants in East London

3.4.5 Acute hepatitis B

During the study time period, 268 patients with hepatitis B were seen in both of the 2 hospitals, of which 7 were deemed to have had acute hepatitis B (see Table 3-9). These patients presented with the clinical syndrome of an acute hepatitis: jaundice, elevated ALT and were HBsAg positive. In addition virological testing showed that all these patients were HBcAb (hepatitis B core antibody) IgM positive. In 5 patients, there was HBsAg seroconversion on outpatient follow up. 2 patients were lost to follow up so it is conceivable that they may have had an acute flare of pre-existing hepatitis B. Many patients with acute hepatitis B do not get referred by their GPs or present to the emergency department and so the true numbers of patients with acute hepatitis B may be higher, especially as the majority were born abroad. Although acute hepatitis B is a notifiable disease many patients from immigrant communities may be reluctant to present to health practitioners, particularly if their immigration status is unclear. None of these patients smoke or drank alcohol, or was diabetic. Of these 7 patients, 1 patient tested positive for HCV but none of them were tested for HIV or delta co-infection at the time of presentation. Acute HBV can have severe complications but these did not occur in our cohort, and as our focus was on severe disease in chronic HBV infection we did not include these cases in our further analysis.

Age	Sex	Ethnicity	Immigration status
32	F	Bangladesh	First generation
31	F	Bangladesh	First generation
45	M	Bangladesh	First generation
24	M	Pakistan	First generation
54	M	Bangladesh	First generation
52	F	Bangladesh	First generation
32	M	Bangladesh	Second generation

Table 3-9 Demographics of Bangladeshi and Pakistani patients with acute hepatitis B admitted to hospital

3.4.6 Chronic hepatitis B

Figure 3-10 shows the distribution of all patients diagnosed with HBV of Bangladeshi and Pakistani ethnicity attending the two London hospitals, either as out-patients or who were admitted as inpatients between 1 January 2006 and 30 June 2008. Of the remaining 261 patients with chronic HBV, 10 patients had HCV co-infection and 1 had HDV co-infection. One of the HCV co-infected patients also had cirrhosis. None of these patients had HIV co-infection. As both HCV and HDV co-infections can cause cirrhosis or decompensated disease, these patients were also excluded from the remainder of the study as it would have been impossible to determine the contribution played by their co-infection. All further analysis of severe disease will focus on mono-infected chronic HBV Bangladeshi and Pakistani patients.

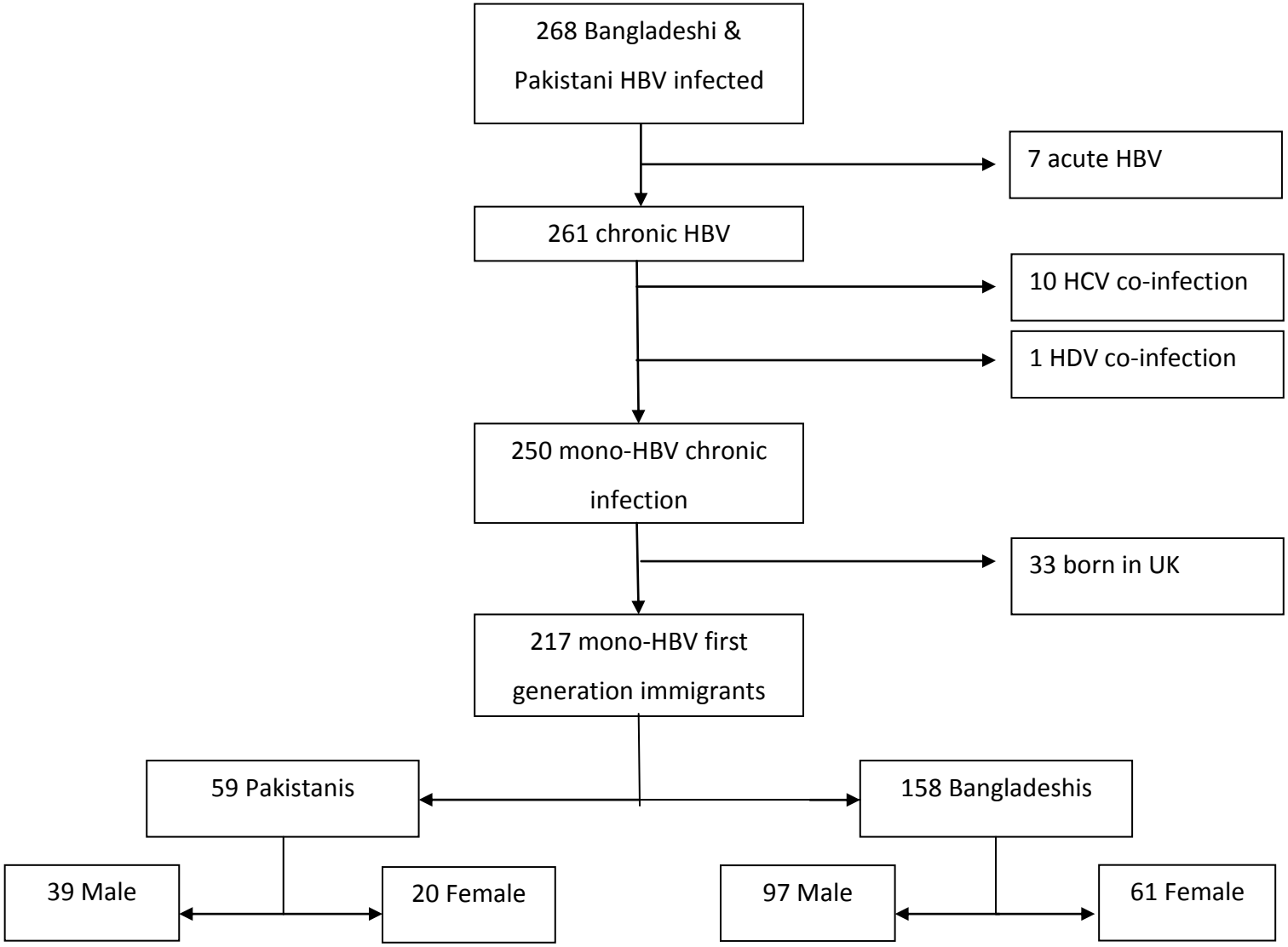

Figure 3-10 Distribution of HBsAg+ patients attending hospital

250 patients had chronic HBV but of this, more than 85% were first generation (born abroad) immigrants. Of the 33 second generation (born in the UK) immigrants, 12 were males (6 Bangladeshi) with a mean age of 26 (range 17-34); and 21 were females (15 Bangladeshi) with a mean age of 29 (range 18-45). The combined male and female age distribution is displayed in Figure 3-11. Compare this with Figure 3-12 which shows the age distribution of first generation immigrants. Because only two of the second generation volunteers who attended screening in the mosques tested positive for HBV, it was not possible to calculate the prevalence in this group and therefore it was not possible to estimate the numbers of HBV infected second generation immigrant patients who should be attending hospital. Hence it is impossible to say with any certainty whether the 33 patients, who were being investigated in hospital, are an under- or overestimate of the true population numbers of HBV in second generation Bangladeshi and Pakistani immigrants.

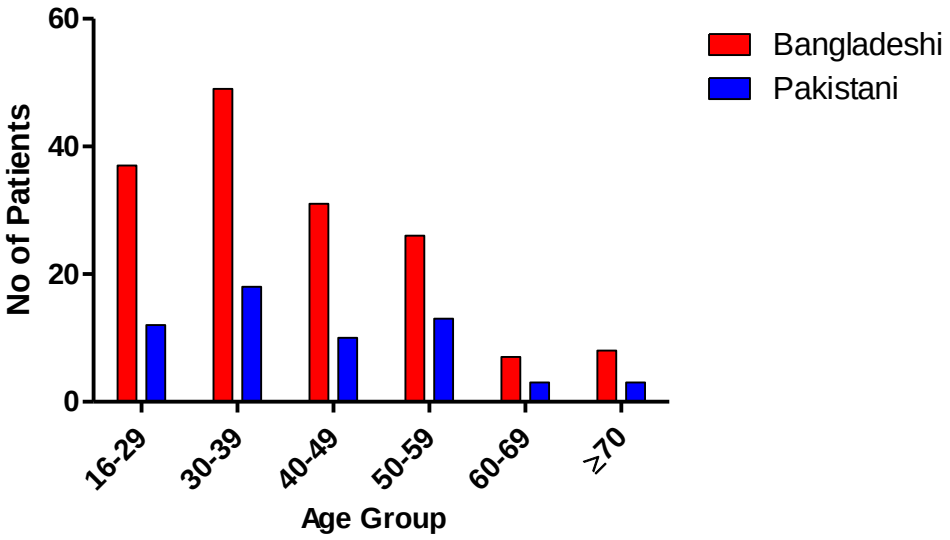

Figure 3-11 First generation immigrant Bangladeshi and Pakistani chronic HBsAg+ males & females living in East London and attending hospital

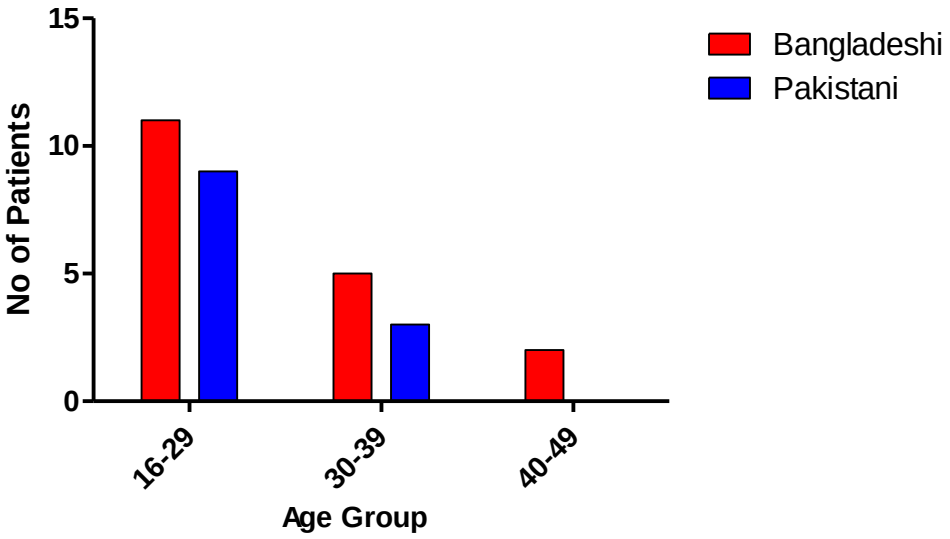

Figure 3-12 Second generation immigrant Bangladeshi and Pakistani chronic HBsAg+ males & females living in East London and attending hospital

4 Hepatitis B in Bangladeshi and Pakistani population of East London

4.1 Introduction

There are approximately 350 million people chronically infected with hepatitis B virus (HBV) around the world. In a landmark longitudinal study by Beasley et al, they showed that the incidence of HCC and cirrhosis in Taiwanese men, was intimately linked with chronic HBV infection.[174] After tobacco, HBV is thought to be the second commonest carcinogen.[175] There are varying estimates as to how many deaths per year are attributable to HBV but the recent Global Disease Burden Study 2010 proposed approximately 785,000 deaths worldwide, including half of all liver cancers deaths and a third of all cirrhosis deaths.[176]

Further studies, primarily using patients from the Far East, have demonstrated that viral factors such as HBeAg seropositivity,[128;129;131] elevated viral load (HBV DNA),[50;65] and genotype C[133;136] are associated with advanced disease states of cirrhosis and HCC. In addition to these, other authors have established that host factors are also significantly associated with cirrhosis and HCC: increasing age and male sex[50;177;178]; elevated ALT[150]; alcohol[152;153] and smoking.[129;153;179] In hepatitis C (HCV) infection there is also an association with disease progression and diabetes,[180;181] however, there is a lack of evidence for a similar relationship with HBV.[182]

The World Health Organization (WHO) has documented the prevalence of HBV in various countries to assess endemicity.[101] Far more difficult a task is to calculate the prevalence and disease burden in immigrant populations in low endemic countries such as the UK. Some have attempted to predict HCC incidence due to HBV by assuming that the HBV prevalence in the immigrant group was equivalent to the prevalence of HBV in the country of origin and then utilising incidence rates based on longitudinal studies.[183;184] Other studies have attempted to delineate prevalence in immigrant groups by looking at prevalence of serum HBsAg in refugees.[185;186] Another option has been to target screening at specific "high risk" groups (individuals from high endemic areas, specifically those from Eastern Asian countries like China)

through invitation to health fairs.[187-191] Fattovich *et al*[192] carried out a systematic review of incidence rates and prognostic factors associated with progressive disease (cirrhosis, decompensation and HCC). They concluded that the only adequate, longitudinal studies to be included in their review were from Western countries (North America, West and Southern Europe) and the Far East. They did not focus on immigrants, let alone those from the Indian Subcontinent.

The prevalence of HBV in Bangladesh has been estimated to be 5.5-5.8%.[193;194] Another review has purported the prevalence of HBV in Pakistan to be 3.3% (in healthcare workers) to 7.4% (in surgical patients).[195] The same study states that the predominant genotype in Pakistan is genotype D with 63%, followed by genotype A with 10%, genotype C with 7.5% and genotype B with 5%. In Bangladesh, genotype D is still the most predominant type with 50% prevalence, but genotype C is common with 37.5%, and genotypes A and B with 2.5%.[196]

4.2 Aims

There is little information on the outcome of chronic HBV in people from Bangladesh and Pakistan and there is no data on the outcome in Bangladeshi and Pakistani immigrants who have settled in the UK. The aim of this study was to:

1. To identify the risk factors associated with the development of cirrhosis and decompensated liver disease in first generation immigrants (i.e. those born abroad) infected with chronic HBV.
2. To determine the disease burden of chronic HBV in immigrants from Bangladesh and Pakistan living in East London.

4.3 Methods

4.3.1 Patients

Patients with CHB attending NUH and RLH were identified from the electronic patient records and hospital notes as described in Chapter 3.

To determine the burden of end stage liver disease we assumed that all patients with cirrhosis and decompensated liver disease (due to hepatic encephalopathy, ascites or variceal haemorrhage, and HCC) would present to one of the two regional hospitals and a database of such patients was created by myself. Over a 30 month period from January 2006 to June 2008 hospital and virology records for every patient admitted with end stage liver disease were evaluated and patients with decompensated liver disease due to chronic HBV infection in the absence of delta, HCV, and HIV co-infection were noted.

For patients with uncomplicated disease the age at final attendance was recorded. For patients with complications the age at which these developed was used and for patients requiring therapy the age at which therapy was introduced was recorded.

As with previous studies, risk factors for progression of disease were identified and assessed for statistical significance. These included host factors such as age, sex, ethnicity, smoking and drinking history, and the presence of diabetes; HBV factors such as eAg status, HBV DNA, genotype and whether anti-HBV treatment had been given; and other factors such as ALT. Most of the risk factors utilise nominal values and so the data collected were assigned to different categories such as: current or ex-smoker versus non-smoker; alcohol drinker versus no alcohol (teetotal); diabetic versus non-diabetic; cirrhotic versus non-cirrhotic. Parameters with continuous data such as age, HBV DNA viral load and ALT at diagnosis were also assigned to categories: under 40 years (16-39) versus over 40 years (≥40); low DNA (≤ 4.0 log IU/L) versus high DNA (> 4.0 log IU/L); and low ALT (≤ 40 IU/L) versus high ALT (> 40 IU/L). Cirrhosis was diagnosed by liver biopsy (either percutaneous or transjugular) or by confirmatory findings on triphasic CT liver or MRI liver with the patient having clinical and endoscopic features of portal hypertension (low albumin, low platelets, splenomegaly, and presence of varices or ascites).

4.3.2 Virological Analysis

A single, central laboratory provides virological support to both hospitals and patients with HBV are routinely evaluated for HBsAg, HBeAg, anti-HBe antibody and delta virus superinfection. HBV DNA levels are measured using standard, commercial assays. In this chapter I shall refer to patients who are HBsAg positive as infected with HBV.

Analysis of genotypes was performed by Dr Samreen Ijaz, clinical scientist at Health Protection Agency (HPA), Colindale, London. She carried out PCR amplification and sequence analysis across the entire HBsAg region, the overlapping polymerase region, part of the X region (last 60 amino acids which cover the basal core promoter), the entire precore and core regions. Patients with cirrhosis and advanced disease were identified and their frozen stored plasma or serum samples were retrieved. The samples were allowed to defrost at room temperature for 2 hours, centrifuged and then had 0.5 ml aliquoted from them. The new samples were then put into new sample tubes, sealed, packed in dry ice and sent to the HPA by courier. To act as a control group, samples from patients who were age and sex matched immigrant Bangladeshis and Pakistanis but without cirrhosis or advanced disease, were also sent in exactly the same way as the patients with cirrhosis and advanced disease. The ratio of controls to disease patients was 2:1.

4.3.3 Statistical Analysis

All the data collected was categorical and so non-parametric tests such as Chi square, Fisher's exact test and logistic regression were performed. Statistics were calculated using computer software (SPSS version 16, SPSS Inc., Chicago, IL, USA and GraphPad Prism version 5, GraphPad Software Inc., La Jolla, CA, USA).

4.3.4 Ethical Approval

Ethical approval for this study was granted by Oxfordshire REC A as an amendment to the study: Case finding and prevalence of chronic viral hepatitis in South Asians living in the UK – Number 06/Q1604/185.

4.4 Results

4.4.1 Characteristics of Bangladeshi and Pakistani chronic HBV patients attending hospital

The social and viral demographics for male and female HBV infected patients who attended hospital, either as out-patients or were admitted as inpatients, according to the various age groups are shown in Table 4-1, Table 4-2, Table 4-3, and Table 4-4. They show that all of the infected men aged over 40 and virtually all the infected women over 40 were born in their home countries of Bangladesh and Pakistan. Of note is the lack of alcohol intake in both sexes, but especially in women, which is probably related to the prevalent Islamic beliefs practised in both countries and in first generation immigrants from these countries.

Age Group/yrs	Ethnicity		Immigration status		Smoker		Alcohol drinker		Diabetes	
	Bangladeshi	Pakistani	First Generation	Second Generation	Never	Current or Ex	No	Yes	No	Yes
16-29	22	12	25	9	27	5	30	2	34	0
30-39	34	12	43	3	27	14	39	2	44	2
40-49	20	8	28	0	21	7	26	2	22	6
50-59	16	9	25	0	17	7	23	1	13	12
60-69	4	1	5	0	3	2	4	1	2	3
≥70	7	3	10	0	6	4	10	0	4	6

Table 4-1 Social demographics of chronic mono-infected male HBsAg+ Bangladeshi and Pakistani first and second generation immigrants presenting to secondary services in East London

Age Group/yrs	ALT at diagnosis		eAg status at diagnosis		HBV DNA at diagnosis		Ever had treatment		Presence of cirrhosis	
	<40	>40	Negative	Positive	<4 log IU/L	>4 log IU/L	No	Yes	No	Yes
16-29	14	20	27	6	20	14	28	6	32	2
30-39	18	28	36	10	28	18	20	16	41	5
40-49	12	16	18	7	13	13	18	10	24	4
50-59	6	19	20	5	12	11	19	6	14	11
60-69	3	2	4	1	4	1	3	2	2	3
≥70	4	6	8	2	4	4	5	5	5	5

Table 4-2 Viral demographics of chronic mono-infected male HBsAg+ Bangladeshi and Pakistani first and second generation immigrants presenting to secondary services in East London

Age Group/yrs	Ethnicity		Immigration status		Smoker		Alcohol drinker		Diabetes	
	Bangladeshi	Pakistani	First Generation	Second Generation	Never	Current or Ex	No	Yes	No	Yes
16-29	26	9	24	11	34	1	35	0	34	1
30-39	23	9	24	8	30	2	32	0	30	2
40-49	13	2	13	2	15	0	15	0	12	3
50-59	10	4	14	0	12	2	14	0	9	5
60-69	3	2	5	0	5	0	5	0	0	5
≥70	1	0	1	0	1	0	1	0	1	0

Table 4-3 Social demographics of chronic mono-infected female HBsAg+ Bangladeshi and Pakistani first and second generation immigrants presenting to secondary services in East London

Age Group/yrs	ALT		eAg status at diagnosis		HBV DNA at diagnosis		Ever had treatment		Presence of cirrhosis	
	<40	>40	Negative	Positive	<4 log IU/L	>4 log IU/L	No	Yes	No	Yes
16-29	26	8	29	5	16	8	32	3	35	0
30-39	26	5	30	2	17	10	27	5	31	1
40-49	12	3	14	1	8	4	14	1	15	0
50-59	10	4	13	1	6	4	14	0	14	0
60-69	4	1	5	0	3	2	5	0	5	0
≥70	1	0	1	0	0	1	1	0	1	0

Table 4-4 Viral demographics of chronic mono-infected female HBsAg+ Bangladeshi and Pakistani first and second generation immigrants presenting to secondary services in East London

4.4.2 Demographics of chronic HBV in 1st generation immigrant population

Figure 4-1 to Figure 4-4 show the distribution of chronic HBV infection in the first generation immigrant Bangladeshis and Pakistanis who attended the two East London hospitals. There are twice as many chronic HBV infections in Bangladeshi males and three times as many in Bangladeshi females as there are in Pakistanis, 97:39 (Figure 4-1 and Figure 4-3) and 61:20 respectively (Figure 4-2 and Figure 4-4). All of the graphs show a skew in disease prevalence towards younger age although this is more prominent with Bangladeshis compared to Pakistanis. The differences in patient numbers between Bangladeshis and Pakistanis probably relates to the difference in the numbers of immigrants from the two countries.

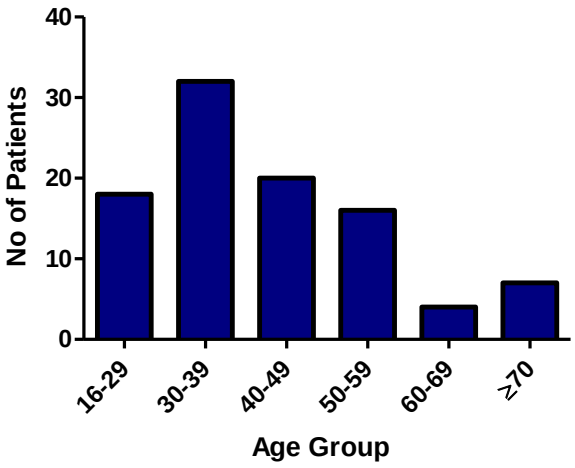

Figure 4-1 First generation immigrant Bangladeshi chronic HBsAg+ males living in East London and attending hospital

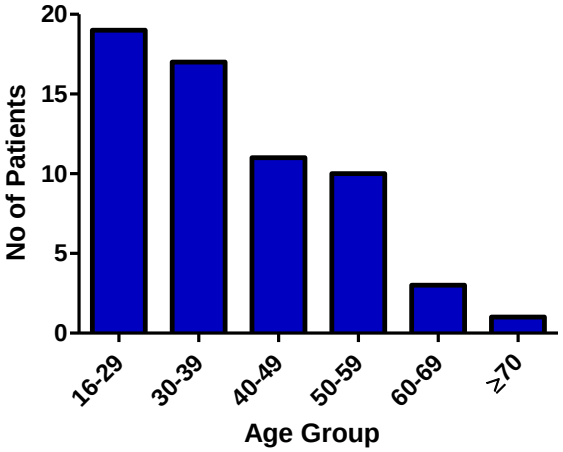

Figure 4-2 First generation immigrant Bangladeshi chronic HBsAg+ females living in East London and attending hospital

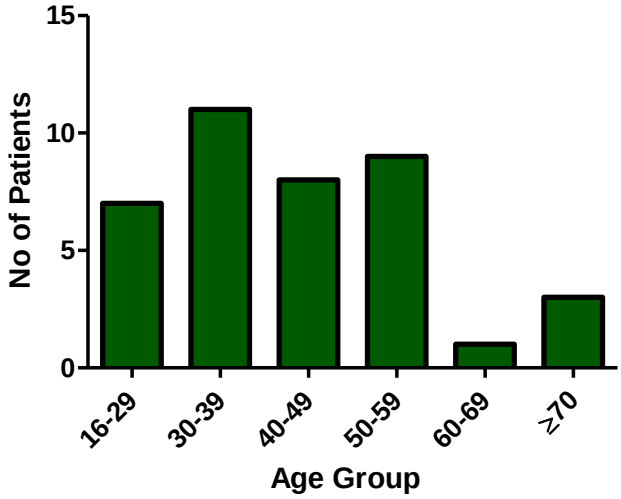

Figure 4-3 First generation immigrant Pakistani chronic HBsAg+ males living in East London and attending hospital

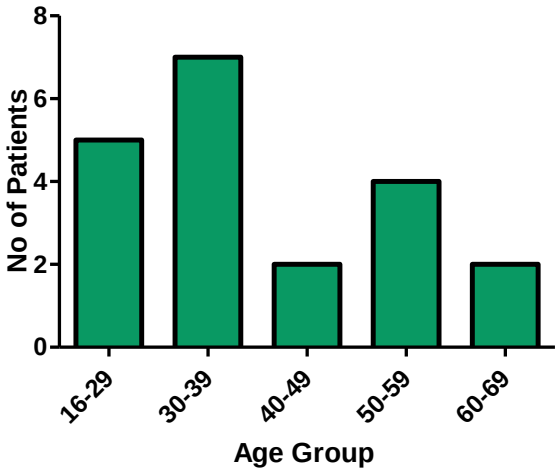

Figure 4-4 First generation immigrant Pakistani chronic HBsAg+ females living in East London and attending hospital

The number of first generation Bangladeshi and Pakistani patients who are estimated to have chronic HBV (along with confidence intervals) are shown in Table 3-5 through to Table 3-8 for males and female. These numbers were determined from the calculated prevalence. The actual numbers of males and females who are infected with chronic HBV and being seen in hospital are shown in Table 4-5 through to Table 4-8. Also displayed in these tables are the estimated proportions of chronic HBV patients being seen in hospital. In most age groups, less than half of the expected number of patients are attending hospital. If the estimated numbers are assumed to be accurate then the overall proportion of Bangladeshis seen is 23% for males and 57% for females. Compared to Bangladeshis, the overall Pakistani proportions differ slightly with 39% of males and 29% of females attending liver services.

Age	16-29	30-39	40-49	50-59	60-69	≥70
Estimated number of people who are HBsAg+	104	110	117	42	NA	45
Actual number of HBsAg+ people attending hospital	18	32	20	16	4	7
Proportion attending hospital (%)	36	45	26	62	NA	18

Table 4-5 Proportion of chronic HBsAg+ first generation immigrant Bangladeshi males attending hospital

Age	16-29	30-39	40-49	50-59	60-69	≥70
Estimated number of people who are HBsAg+	NA	107	NA	NA	NA	NA
Actual number of HBsAg+ people attending hospital	19	17	11	10	3	1
Proportion attending hospital (%)	NA	16	NA	NA	NA	NA

Table 4-6 Proportion of chronic HBsAg+ first generation immigrant Bangladeshi females attending hospital

Age	16-29	30-39	40-49	50-59	60-69	≥70
Estimated number of people who are HBsAg+	9	38	31	14	5	4
Actual number of HBsAg+ people attending hospital	7	11	8	9	1	3
Proportion attending hospital (%)	78	29	26	64	20	75

Table 4-7 Proportion of chronic HBsAg+ first generation immigrant Pakistani males attending hospital

Age	16-29	30-39	40-49	50-59	60-69	≥70
Estimated number of people who are HBsAg+	NA	16	34	12	8	NA
Actual number of HBsAg+ people attending hospital	5	7	2	4	2	0
Proportion attending hospital (%)	NA	44	6	33	25	NA

Table 4-8 Proportion of chronic HBsAg+ first generation immigrant Pakistani females attending hospital

4.4.3 Differences between first generation immigrant Bangladeshis and Pakistanis in East London

Before identifying risk factors associated with cirrhosis and advanced disease, the two first generation immigrant populations were compared to see if there were any significant differences in a variety of independent variables. Frequencies were

determined and then non-parametric descriptive statistics were performed comparing Bangladeshis and Pakistanis.

Table 4-9 shows these comparisons with the 2x2 contingency tables, p values, odds ratio and 95% confidence intervals. Initially Chi-square tests were employed with each of the 2x2 contingency tables, but some of these did not meet all the conditions required for using this test - in each cell, all the expected values must be greater than 5 and in this study some of the sample sizes were too small to meet the test criteria. To ensure that there was congruity with all the risk factors, Fisher's exact test was utilised for all of the contingency tables to generate an exact p value.

Age 40 was used as an arbitrary figure for distinguishing between older and younger individuals. An ALT of 40 was selected to separate 'high ALT' from 'low ALT'. A value of 40 is regarded as the upper limit of normal in many biochemistry laboratories for patients without underlying liver disease, though it has been suggested that this value is too high (especially for women), and that many patients with significant liver inflammation have a 'normal ALT' i.e. below 40[47;197]. All the other risk factors were divided into distinct groups.

HBe antigen status at diagnosis was statistically different between Bangladeshis and Pakistanis with more Bangladeshi patients being HBeAg positive at the time of diagnosis. Another difference between the two communities was having patients on anti-HBV treatment, those currently having treatment and those who have had it in the past: more Bangladeshis than Pakistanis were having or had treatment. These may be true differences but more likely they are a reflection of the local population. The majority of HBV patients seen in RLH are from the local Tower Hamlets population, which has a very large Bangladeshi population and a very small Pakistani population. In Newham, the Bangladeshi and Pakistani populations are similar in size (about 8% each) so proportionally more Pakistanis would be seen in NUH. At the time of the study, all treatment for HBV was initiated at RLH. In order to get treatment, patients originally seen in NUH would have had to have been referred to RLH. Delays

in diagnosis, referral or because of patient non-attendance at RLH for treatment with Pakistani patients may account for this disparity in treatment.

At the time this study was carried out routine testing of HBV genotype was not available. Genotype has been implicated in the development of cirrhosis and HCC. There is a possibility there may have been a difference between the 2 ethnicities but this could not be tested in all of our patients.

Risk Factor	Category	Bangladesh	Pakistan	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Age	≤40	89/217	31/217	0.647	1.17	0.64 - 2.12
	>40	69/217	28/217			
Sex	Female	61/217	20/217	0.637	1.19	0.64 - 2.24
	Male	97/217	39/217			
ALT at diagnosis	Low (≤40)	81/215	32/215	0.648	0.87	0.47 - 1.59
	High (>40)	76/215	26/215			
eAg at diagnosis	Negative	124/212	55/212	0.002	0.15	0.03 - 0.63
	Positive	31/212	2/212			
HBV DNA at diagnosis	Low (≤4 log)	84/194	33/194	0.40	0.72	0.37 - 1.41
	High (>4 log)	60/194	17/194			
Cirrhosis	No cirrhosis	134/217	53/217	0.386	0.63	0.24 - 1.63
	Cirrhosis	24/217	6/217			

Decompensated disease	Stable Disease	149/217	55/217	0.75	1.20	0.36 to 4.07
	Decompensated/HCC	9/217	4/217			
On anti-HBV treatment (Rx) now	Not on Rx now	124/217	54/217	0.029	0.34	0.13 to 0.91
	Currently on Rx	34/217	5/217			
On anti-HBV Rx ever	Never had Rx	113/217	51/217	0.022	0.39	0.17 to 0.90
	Have had Rx	45/217	8/217			
Smoking	Never smoked	123/213	49/213	0.700	0.81	0.37 to 1.78
	Smoker	31/213	10/213			
Alcohol drinker	No alcohol	148/213	57/213	1.000	0.87	0.17 to 4.42
	Alcohol	6/213	2/213			
Diabetes	Non-diabetic	126/217	46/217	0.851	1.11	0.54 to 2.30
	Diabetic	32/217	13/217			

Table 4-9 Differences between first generation immigrant Bangladeshis and Pakistanis with chronic HBV infection according to various risk factors

4.4.4 Cirrhosis in first generation immigrant Bangladeshis and Pakistanis

This section examines various risk factors associated with cirrhosis in the immigrant Bangladeshi and Pakistani population of East London. Risk factors such as age (Figure 4-5 and Table 4-10); sex (Figure 4-6 and Table 4-11); ALT at diagnosis (Figure 4-7 and Table 4-12); being on anti-HBV treatment at the present time (Figure 4-8 and Table 4-13); ever being on anti-HBV treatment (Figure 4-9 and Table 4-14); smoking (Figure 4-10 and Table 4-15); and diabetes (Figure 4-11 and Table 4-16) were significant using univariate analysis. Alcohol (Figure 4-12 and

Table 4-17) was not statistically significant as a risk factor for cirrhosis, but is represented graphically for comparison with the other risk factors. Other independent risk factors such as HBeAg status at diagnosis and HBV DNA viral load at diagnosis were not statistically significant.

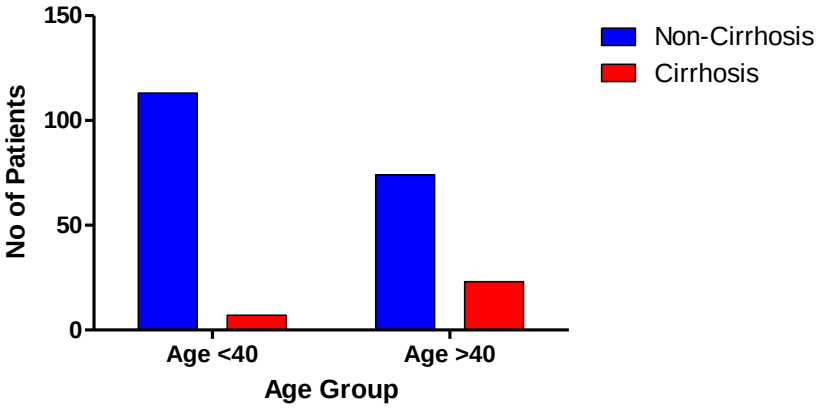

Figure 4-5 Age vs cirrhosis in first generation immigrant Bangladeshis and Pakistanis

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Age	<40	113/217	7/217	0.001	5.02	2.05 - 12.29
	>40	74/217	23/217			

Table 4-10 Univariate statistics for age vs cirrhosis in first generation immigrant Bangladeshis and Pakistanis

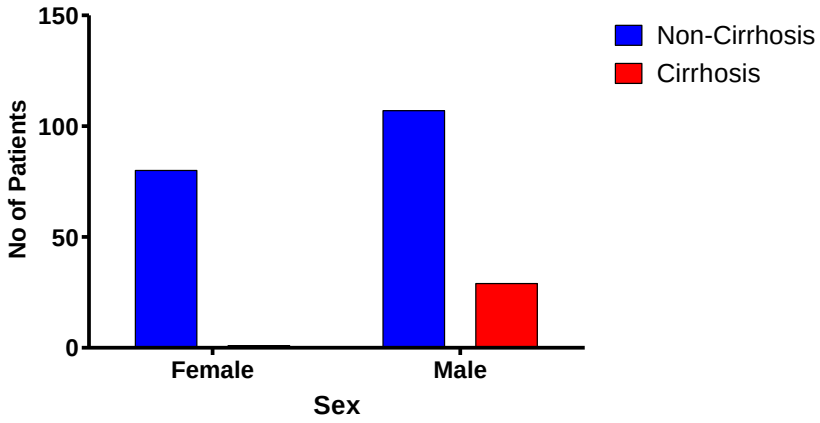

Figure 4-6 Sex vs cirrhosis in first generation immigrant Bangladeshis and Pakistanis

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Sex	Female	80/217	1/217	< 0.001	21.68	2.89 - 162.60
	Male	107/217	29/217			

Table 4-11 Univariate statistics for sex vs cirrhosis in first generation immigrant Bangladeshis and Pakistanis

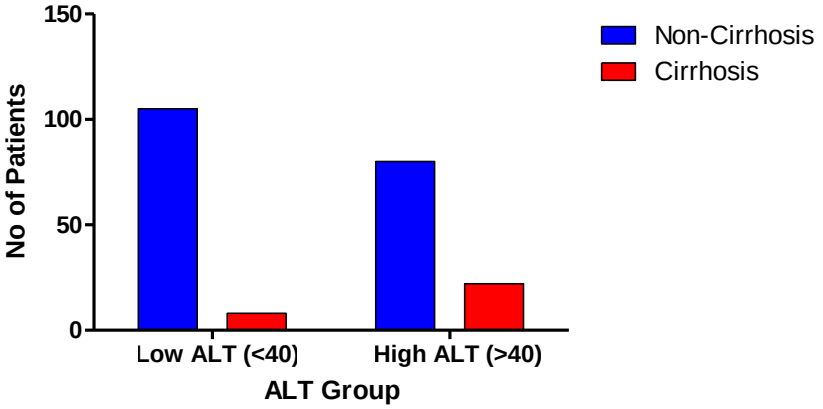

Figure 4-7 ALT vs cirrhosis in first generation immigrant Bangladeshis and Pakistanis

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
ALT at diagnosis	Low (<40)	105/215	8/215	0.004	3.61	1.53 - 8.53
	High (>40)	80/215	22/215			

Table 4-12 Univariate statistics for ALT vs cirrhosis in first generation immigrant Bangladeshis and Pakistanis

Figure 4-8 and Figure 4-9 suggest that treatments both now and in the past are significantly associated with cirrhosis. The clinical significance of these results is difficult to explain but it is probable that those patients who have developed cirrhosis are much more likely to be commenced on antiviral therapy than those who have not developed severe fibrosis.

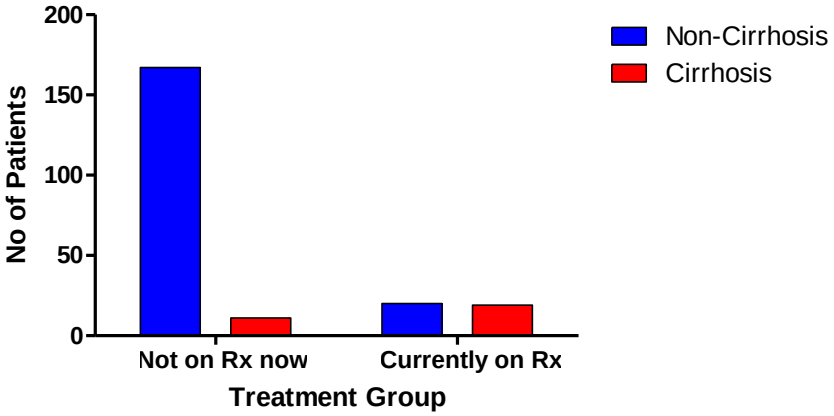

Figure 4-8 Treatment now vs cirrhosis in first generation immigrant Bangladeshis and Pakistanis

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
On anti-HBV treatment (Rx) now	Not on Rx now	167/217	11/217	<0.001	14.42	6.01 - 34.62
	Currently on Rx	20/217	19/217			

Table 4-13 Univariate statistics for treatment now vs cirrhosis in first generation immigrant Bangladeshis and Pakistanis

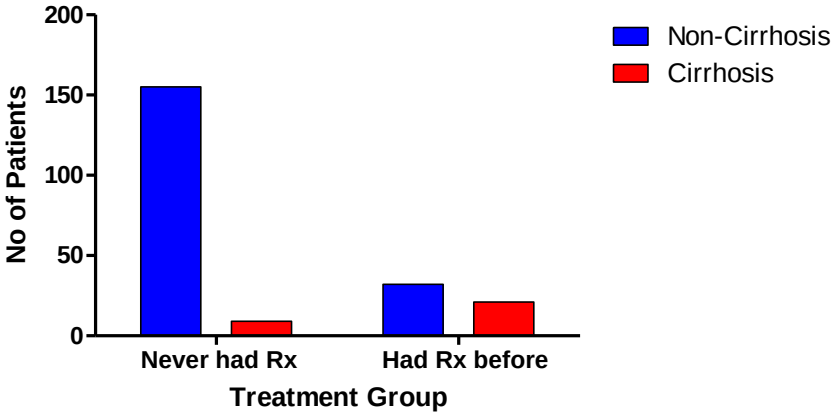

Figure 4-9 Treatment ever vs cirrhosis in first generation immigrant Bangladeshis and Pakistanis

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
On anti-HBV Rx ever	Never had Rx	155/217	9/217	< 0.001	11.30	4.74 - 26.95
	Have had Rx	32/217	21/217			

Table 4-14 Univariate statistics for treatment ever vs cirrhosis in first generation immigrant Bangladeshis and Pakistanis

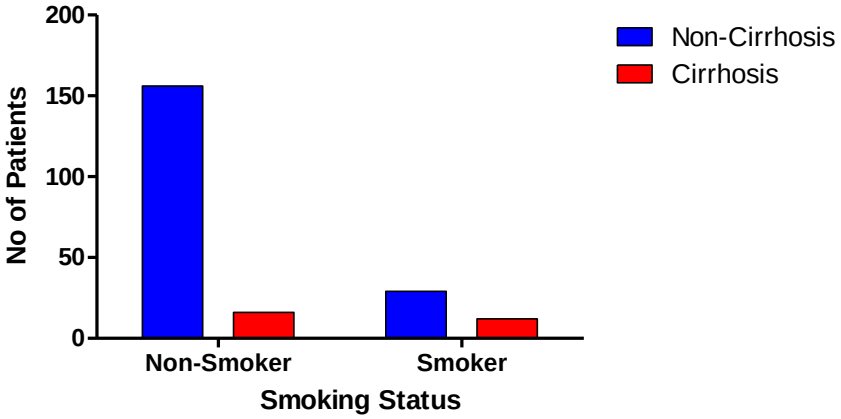

Figure 4-10 Smoking vs cirrhosis in first generation immigrant Bangladeshis and Pakistanis

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Smoking	Never smoked	156/213	16/213	0.002	4.03	1.73 - 9.41
	Smoker	29/213	12/213			

Table 4-15 Univariate statistics for smoking vs cirrhosis in first generation immigrant Bangladeshis and Pakistanis

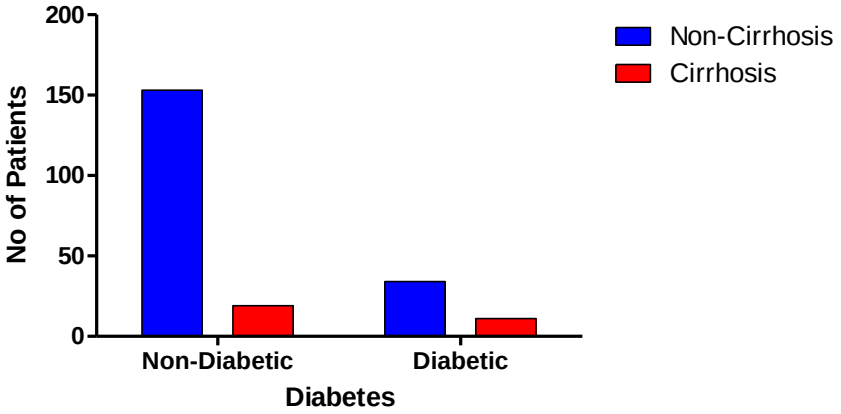

Figure 4-11 Diabetes vs cirrhosis in first generation immigrant Bangladeshis and Pakistanis

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Diabetes	Non-diabetic	153/217	19/217	0.029	2.61	1.14 - 5.98
	Diabetic	34/217	11/217			

Table 4-16 Univariate statistics for diabetes vs cirrhosis in first generation immigrant Bangladeshis and Pakistanis

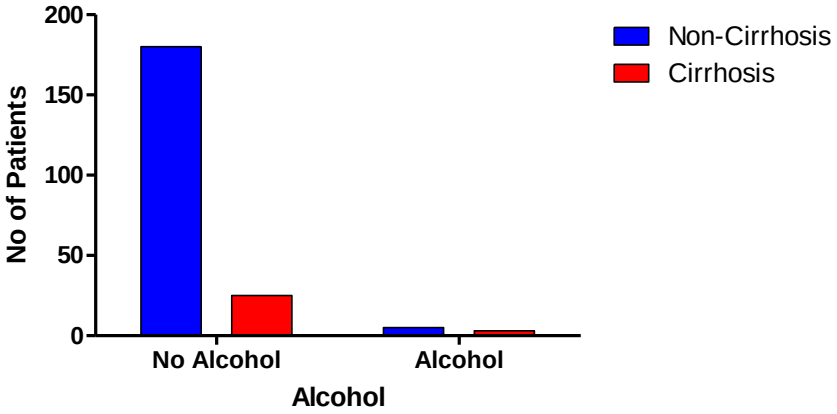

Figure 4-12 Alcohol vs cirrhosis in first generation immigrant Bangladeshis and Pakistanis

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Alcohol drinker	No alcohol	180/213	25/213	0.073	4.32	0.97 - 19.20
	Alcohol	5/213	3/213			

Table 4-17 Univariate statistics for alcohol vs cirrhosis in first generation immigrant Bangladeshis and Pakistanis

As age group, sex, ALT group, smoking and diabetes were significant predictors of cirrhosis by univariate analyses, they were also tested using multivariate analysis. As mentioned before, treatment, either currently or in the past, was not utilised because of its uncertain clinical significance. Because of the binary nature of the outcome (i.e. presence of cirrhosis or not) binary logistic regression was employed using the forced enter method.

Table 4-18 shows that only age and sex show statistically significant associations with cirrhosis when the variables are controlled for. The odds ratio of age >40 was 4.57 and of male sex was 11.74 but both are associated with wide 95% confidence intervals suggesting that neither is very good at giving an accurate estimate of the true population odds.

	Sig.	Odds Ratio Exp(B)	95% Confidence Interval for EXP(B)	
			Lower	Upper
Age group	<.01	4.57	1.56	13.43
Sex	<.02	11.74	1.48	93.43
ALT	.16	2.21	.83	5.92
Smoking	.08	2.35	.92	6.00
Diabetes	.81	1.14	.40	3.22

Table 4-18 Logistic regression showing significant variables for predicting cirrhosis with odds ratio and confidence intervals in Bangladeshis and Pakistanis

4.4.5 Decompensated disease in first generation immigrant Bangladeshis and Pakistanis

Decompensated disease refers to patients with HCC or decompensated cirrhosis. Below are the 2x2 contingency tables, p values, odds ratio and 95% confidence intervals of various risk factors associated with decompensated disease in the immigrant Bangladeshi and Pakistani population of East London. Risk factors such as age (Figure 4-13 and Table 4-19); sex (Figure 4-14 and Table 4-20); ALT at diagnosis (Figure 4-15 and Table 4-21); being on anti-HBV treatment at the present time (Figure 4-16 and Table 4-22); smoking (Figure 4-17 and Table 4-23); alcohol (Figure 4-18 and Table 4-24); and diabetes (Figure 4-19 and Table 4-25) were significant using univariate analysis. Other independent risk factors such as e-antigen status at diagnosis and HBV DNA viral load at diagnosis, or ever being on anti-HBV treatment were not statistically significant.

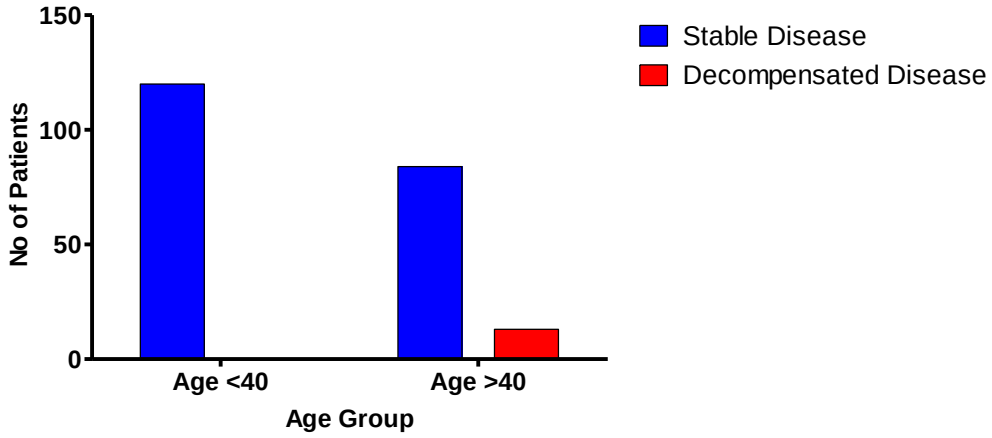

Figure 4-13 Age vs decompensated disease in first generation immigrant Bangladeshis and Pakistanis

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Age	<40	120/217	0/217	<.001	38.50	2.26 - 657.1
	>40	84/217	13/217			

Table 4-19 Univariate statistics for age vs decompensated disease in first generation immigrant Bangladeshis and Pakistanis

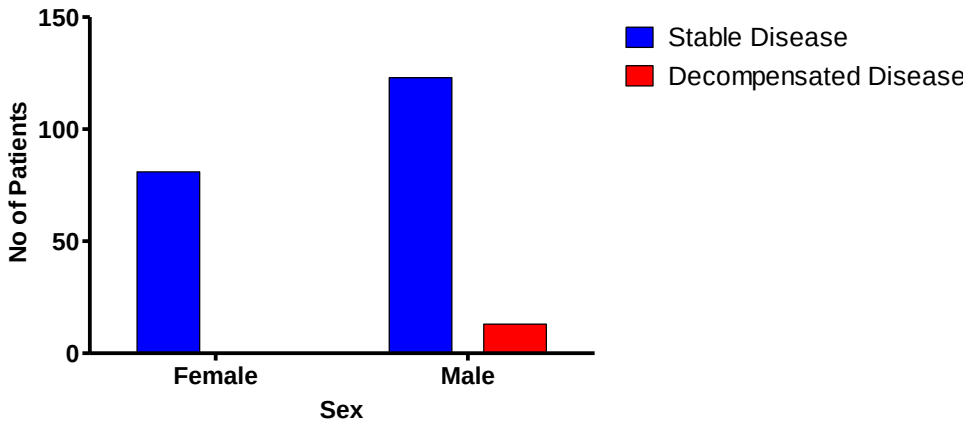

Figure 4-14 Sex vs decompensated disease in first generation immigrant Bangladeshis and Pakistanis

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Sex	Female	81/217	0/217	0.002	17.80	1.04 - 304
	Male	123/217	13/217			

Table 4-20 Univariate statistics for sex vs decompensated disease in first generation immigrant Bangladeshis and Pakistanis

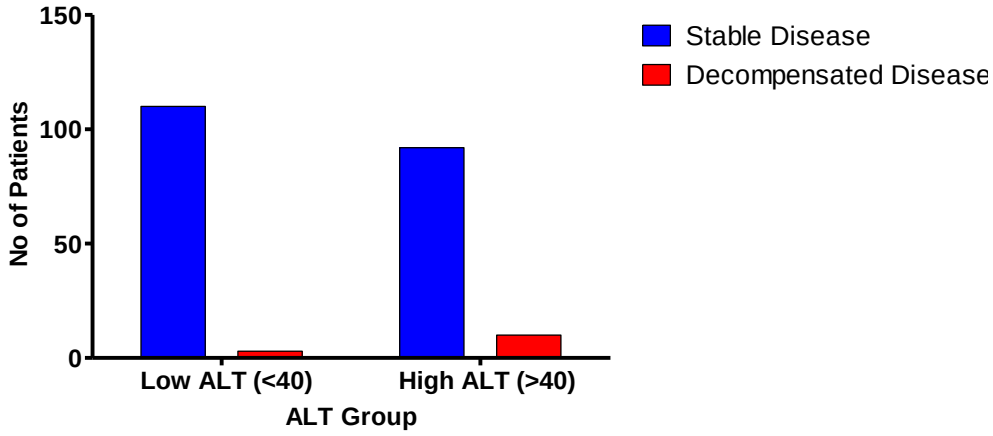

Figure 4-15 ALT vs decompensated disease in first generation immigrant Bangladeshis and Pakistanis

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
ALT at diagnosis	Low (<40)	110/215	3/215	0.042	3.99	1.07 - 14.92
	High (>40)	92/215	10/215			

Table 4-21 Univariate statistics for ALT vs decompensated disease in first generation immigrant Bangladeshis and Pakistanis

Figure 4-16 shows that a significant proportion of patients with HCC or decompensated cirrhosis are receiving antiviral therapy. It is not clear why some patients with advanced disease were not receiving therapy and it is probable that such patients may have left the area and be undergoing therapy elsewhere.

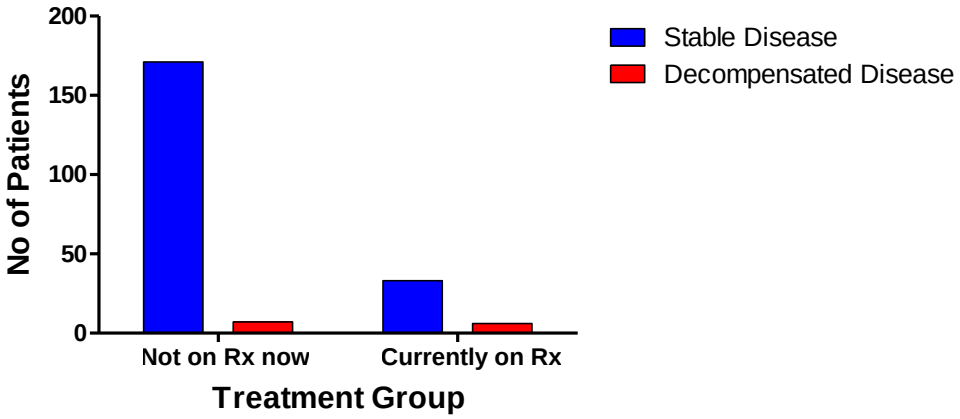

Figure 4-16 Treatment now vs decompensated disease in first generation immigrant Bangladeshis and Pakistanis

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
On anti-HBV treatment (Rx) now	Not on Rx now	171/217	7/217	0.015	4.44	1.40 - 14.06
	Currently on Rx	33/217	6/217			

Table 4-22 Univariate statistics for treatment now vs decompensated disease in first generation immigrant Bangladeshis and Pakistanis

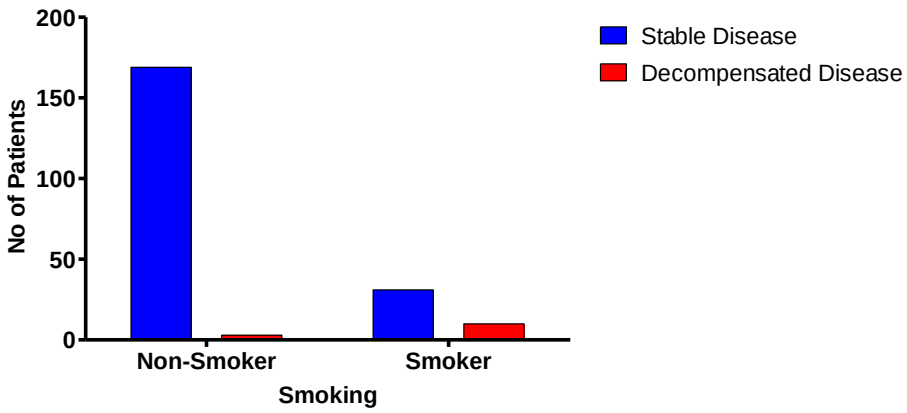

Figure 4-17 Smoking vs decompensated disease in first generation immigrant Bangladeshis and Pakistanis

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Smoking	Never smoked	169/213	3/213	< 0.001	18.17	4.73 - 69.83
	Smoker	31/213	10/213			

Table 4-23 Univariate statistics for smoking vs decompensated disease in first generation immigrant Bangladeshis and Pakistanis

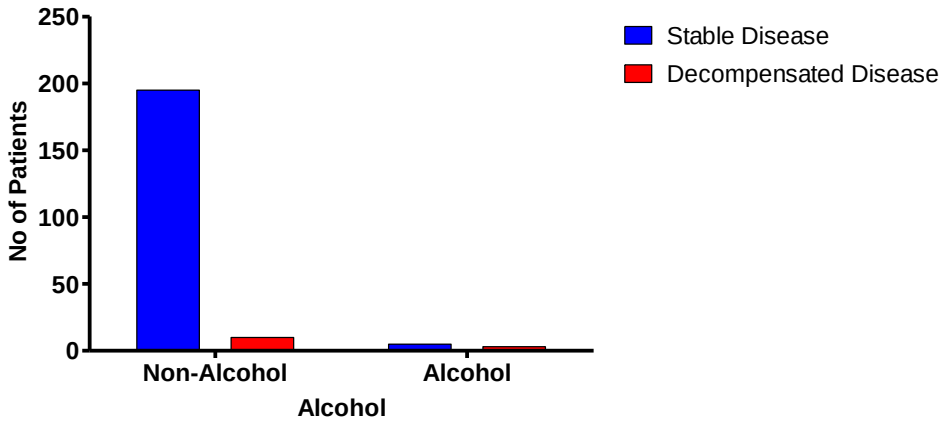

Figure 4-18 Alcohol vs decompensated disease in first generation immigrant Bangladeshis and Pakistanis

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Alcohol drinker	No alcohol	195/213	10/213	0.008	11.70	2.44 - 56.04
	Alcohol	5/213	3/213			

Table 4-24 Univariate statistics for alcohol vs decompensated disease in first generation immigrant Bangladeshis and Pakistanis

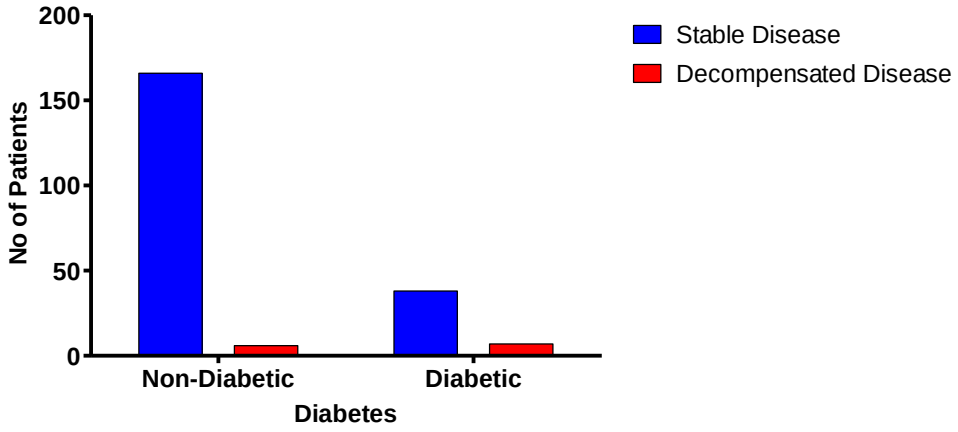

Figure 4-19 Diabetes vs decompensated disease in first generation immigrant Bangladeshis and Pakistanis

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Diabetes	Non-diabetic	166/217	6/217	0.007	5.10	1.62 - 16.04
	Diabetic	38/217	7/217			

Table 4-25 Univariate statistics for diabetes vs decompensated disease in first generation immigrant Bangladeshis and Pakistanis

The significant univariate predictors of decompensated disease: age group, sex, ALT group, smoking, alcohol and diabetes, were analysed using multivariate analysis (see Table 4-26). Again this was done using binary logistic regression as the outcome measure was either presence of decompensated liver disease or not, and this was with the forced enter method. In this case, only smoking was a statistically significant predictor with a good level of significance and high odds ratio but a large 95% confidence interval.

	Sig.	Odds Ratio Exp(B)	95% Confidence Interval for EXP(B)	
			Lower	Upper
Age group	1.00	30298832.0	.00	
Sex	1.00	43376323.0	00	
ALT	.40	2.08	.39	11.26
Smoking	<.002	12.05	2.56	57.00
Diabetes	.76	1.27	.28	5.76
Alcohol	.99	.00		

Table 4-26 Logistic regression showing significant variables for predicting decompensated disease with odds ratio and confidence intervals in Bangladeshis and Pakistanis.

4.4.6 HBV genotypes

To examine the impact of HBV genotype we studied 31 patients with cirrhosis and 13 patients with decompensated disease. However analysis was only possible in 20 patients with cirrhosis and 7 with decompensated disease. In 8 patients no samples were available and in 9 patients the low levels of HBV DNA precluded successful amplification. Control samples were matched in age and sex to the samples from decompensated disease and cirrhosis patients. There were 50 cirrhosis controls and 37 decompensated disease controls.

The distribution of genotypes A-D for cirrhosis and decompensated disease are shown in Figure 4-20 and Figure 4-22. The predominant genotypes in the Indian Subcontinent had been thought to be A and D but C appears to be fairly common as well. Two of the patients with liver cancer did not have underlying cirrhosis and in both cases they had genotype D. Regarding cirrhosis patients, there was a statistically

significant difference between non-genotype D and genotype D infected individuals (see Figure 4-21 and Table 4-27). It may be that genotype D is protective against the development of cirrhosis. However, as the odds ratio and the lower limit of the confidence interval are below 1 it is uncertain whether this could be indicative of the true HBV population in Bangladeshis and Pakistanis.

Table 4-27 shows that none of the other genotypes were significantly associated with cirrhosis. Table 4-28 shows that none of the genotypes were significantly associated with the development of decompensated disease.

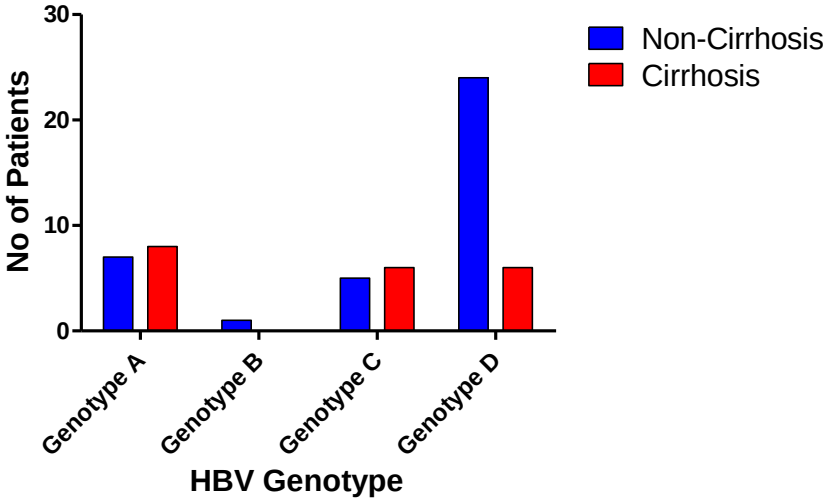

Figure 4-20 Genotype vs cirrhosis in first generation Bangladeshi and Pakistani immigrants

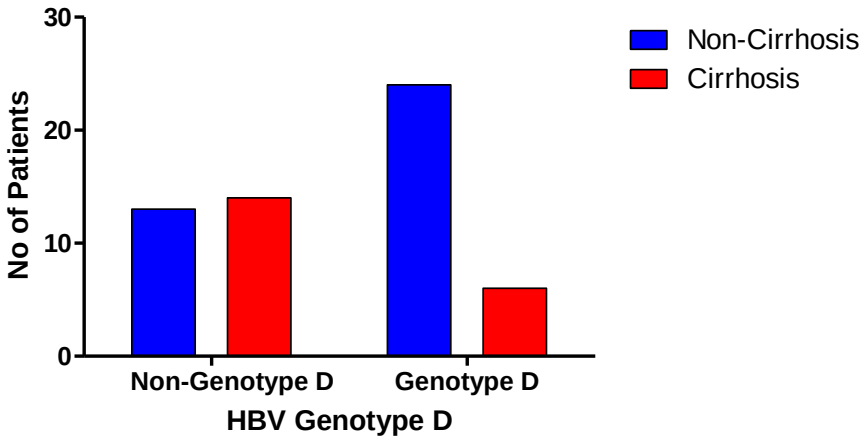

Figure 4-21 Presence of Genotype D vs cirrhosis in first generation Bangladeshi and Pakistani immigrants

Genotype	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Genotype A	Non Genotype A	30/57	12/57	0.117	2.86	0.85 - 9.64
	Genotype A	7/57	8/57			
Genotype C	Non Genotype C	32/57	14/57	0.307	2.29	0.63 to 8.34
	Genotype C	6/57	6/57			
Genotype D	Non Genotype D	13/57	14/57	0.015	0.23	0.07 - 0.75
	Genotype D	24/57	6/57			

Table 4-27 Genotypes in patients with cirrhosis and their clinical significance in first generation immigrant Bangladeshis and Pakistanis with chronic HBV infection

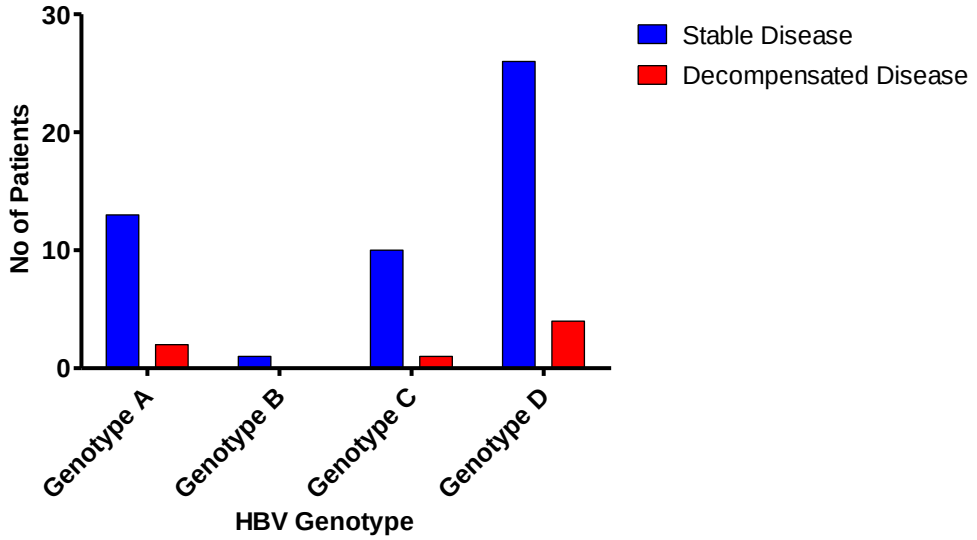

Figure 4-22 Genotype vs decompensated Disease in first generation Bangladeshi and Pakistani immigrants

Genotype	Category	Stable Disease	Decompensated disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Genotype A	Non Genotype A	37/57	5/57	1.000	1.14	0.20 - 6.60
	Genotype A	13/57	2/57			
Genotype C	Non Genotype C	40/57	6/57	1.000	0.67	0.07 - 6.19
	Genotype C	10/57	1/57			
Genotype D	Non Genotype D	24/57	3/57	1.000	1.23	0.25 - 6.08
	Genotype D	26/57	4/57			

Table 4-28 Genotypes in patients with Decompensated disease and their clinical significance in first generation immigrant Bangladeshis and Pakistanis with chronic HBV infection

4.4.7 Disease burden of HBV in first generation immigrant Bangladeshi and Pakistani population

Table 4-29 and Table 4-30 show the disease burden of HBV based on estimated prevalence calculations for Bangladeshis and Pakistanis. For the purposes of the analysis, patients with decompensated disease were divided according to their age being above or below age 40 - all of these patients were male (see Table 4-19). As mentioned previously, screening of mosque volunteers in the various centres revealed 41 males that had HBV. This gives an overall screened point prevalence of 1.7% for under 40 years and 2.0% for over 40 years. This point prevalence was then multiplied by the estimated population prevalence (see Table 3-5 and Table 3-7) to give the expected population prevalence. The annual admission rates for Bangladeshis and Pakistanis with decompensated disease (based on our calculations of how many people are estimated to be HBV positive) are shown in Table 4-29 and Table 4-30. From our data, we estimated that 208 first generation immigrant Bangladeshi males under 40 would be infected with HBV and there would be 208 over 40. The corresponding male Pakistani numbers are 48 aged under 40 and 50 aged over 40. No HBV individuals, from either ethnicity, aged under 40 were admitted to hospital with decompensated liver disease or HCC. However, in the 30 month time period, 2006 to 2008, 13 individuals (9 Bangladeshi and 4 Pakistani) aged over 40 were admitted to hospital with advanced liver disease. Of those with decompensated disease, the majority were e antigen negative, but as already mentioned e antigen status was not statistically significantly associated with progressive disease. The calculations suggest that approximately 1.4% of male first generation Bangladeshi patients with chronic HBV aged over 40 will be admitted to hospital with advanced liver disease per year. The corresponding rate for male first generation immigrant Pakistanis is approximately double that rate at 2.7% per year.

Age	< 40	> 40
Population census	13012	9056
Screened population	185	305
Screened population point prevalence	3 (1.6%)	7 (2.3%)
Expected population prevalence	208 (1.6% x 13012)	208 (2.3% x 9056)
Admissions to hospital 2006-8	0	9
Annual admission rate %	0	1.4

Table 4-29 Disease burden in male Bangladeshi 1st degree immigrants chronically infected with HBV

Age	< 40	> 40
Population census	2804	2490
Screened population	601	1061
Screened population point prevalence	10 (1.7%)	21 (2.0%)
Expected population prevalence	48 (1.7% x 2804)	50 (2.0% x 2490)
Admissions to hospital 2006-8	0	4
Annual admission rate %	0	2.7

Table 4-30 Disease burden in male Pakistani 1st degree immigrants chronically infected with HBV

4.5 Discussion

4.5.1 Prevalence, disease burden and predictive factors

This study examined the prevalence of chronic HBV in the immigrant populations from Bangladesh and Pakistan living in East London based on those attending hospital. The prevalence of HBV in 1st generation Bangladeshi and Pakistani males and females according to age is shown in Table 3-5 through Table 3-8. Also shown in these tables are the estimated numbers of patients who are thought to have HBV based on the calculated prevalence. Cirrhosis and decompensated liver disease (decompensated cirrhosis and HCC) only affected males and so the disease burdens for male Bangladeshis and Pakistanis are shown in Table 4-29 and Table 4-30.

The study examined the risk factors associated with cirrhosis and decompensated disease. Age group, sex, ALT group, smoking and diabetes were significantly associated with the development of cirrhosis through univariate analyses. This suggests that they are all important independent factors in predicting cirrhosis. Using binary logistic regression multivariate statistics there was a significant association with older age and male sex. For decompensated disease, univariate analysis demonstrates that age group, sex, ALT group, smoking, alcohol and diabetes are all statistically significant independent factors. Multivariate analysis showed that only smoking was significant albeit with a very large 95% confidence interval.

The role of gender and age in the progression of HBV is a complex and unresolved issue. Many studies from all around the world have shown that the prevalence of HBV is greater in males.[198] Since transmission in high and medium endemic countries (like Bangladesh and Pakistan) is vertical and equal number of boys to girls are born that would suggest females are better at eliminating the virus. Longitudinal follow up studies of CHB patients have shown that cirrhosis and HCC occur more frequently and at an earlier age in males.[50;145] Females have increased numbers of $CD4^+$ lymphocytes and a greater proportion of $CD4^+$:$CD8^+$ than males and this may have some influence on why autoimmune liver diseases (autoimmune hepatitis and primary biliary cirrhosis) are more common in females.[199;200] Studies looking at HCV infection, have

shown that females have higher rates of spontaneous viral clearance, lower biochemical values and lower rates of fibrosis. This may be due to gender differences in the immune response and/or the defensive anti-fibrotic properties of oestrogens.[198;200;201] There is a lack of evidence as to whether these phenomena apply to HBV.

In spite of not knowing the mechanism by which disease progression in males occurs it does have potential public health implications. African and Asian (Far East) men are at higher risk of developing HCC and it has been proposed that they are screened at age 40.[120] Various "HCC risk calculators" have also recently been created, all of which have age and gender incorporated as independent risk variables, to predict the cumulative risk of developing HCC in CHB patients.[202-204] The problem with these are that they have only been validated in Taiwan, Korea, China and Hong Kong and not tested in other populations. However, based on our results it would suggest that screening this particular immigrant population for HBV would be clinically justified.

In our study, there are many more individuals of both sexes who are HBeAg negative than HBeAg positive in all the age groups (see Table 4-2 and Table 4-4). In the immune tolerance phase of CHB infection patients are typically HBeAg positive with high viral loads and normal ALT values. The immune surveillance phase follows the immune tolerant and immune active phases and is when patients are typically HBeAg negative, HBeAb positive, have lower viral loads and normal ALT values. In our study only 2 patients were young (28 and 30), had normal ALT, were HBeAg positive and had high viral loads: 8.4 and 5.3 log IU/ml respectively. Usually the immune tolerant phase is associated with viral loads >8 log IU/ml. All of the individuals aged under 20 (6) and 41 (out of 51) aged 21-30 were HBeAg negative and HBeAb positive and the majority had normal ALT (<40 IU/L) and low viral loads (<4.0 log IU/ml) at diagnosis. This finding raises questions about which phase of the disease patients are actually in or whether these phases are accurate but blood tests at one point in time are not diagnostic. The roles of genotype and HBsAg levels are not known. Our finding of predominant HBeAg negative disease has also been replicated in a recent study from Bangladesh where 89/167 young CHB patients aged 12-20 were HBeAg negative.[205]

4.5.2 Bias and confounding

The prevalence and disease burden results of this study are heavily dependent on the screening study undertaken by Uddin et al[172]. In that study, mosques and community centres were targeted for screening. It was assumed that as most Bangladeshis and Pakistanis were Muslim and that they would attend these places. It was also assumed that they would attend a mosque that was located in the borough of London that they lived in. i.e. that a Pakistani male who lived in Newham would attend a mosque in Newham or in the adjoining borough of Tower Hamlets rather than travel further afield. Similarly it was assumed, that they would attend their local hospital for follow up and admission if they developed decompensated disease. These assumptions are based on the fact that these two London boroughs have an abundance of mosques and that NHS healthcare in the UK is free. As we relied on volunteers attending mosques, this could possibly account for the low numbers of Bangladeshi women who were screened and tested positive for HBV. There are also a number of Bangladeshi and Pakistani individuals who are Muslim but do not attend mosques. Despite both Bangladesh and Pakistan being Islamic states (where Islam is the official religion), there will be a number of individuals who belong to other religious faiths and so will not attend mosques; these individuals would not have been targeted during the screening process.

A recent unpublished study[206] has evaluated different methods of screening for viral hepatitis in the Pakistani population of East London. They compared three approaches: distribution of testing cards in mosques, opportunistically screening Pakistani patients attending GP surgeries, and specifically writing to and telephoning patients of Pakistani origin to offer screening for HBV and HCV unless they explicitly expressed that they did not want to be contacted by telephone. There was very poor uptake of the 5000 patients offered screening when visiting the mosque with none subsequently visiting their GPs for testing. 17 out of 1163 (1.5%) in the opportunistic arm were screened but none were found to be positive. Of the 1134 patients in the

other arm, only 600 patients could be contacted and were eligible for testing. So far 232 have been screened and 1% are HBsAg positive. This study highlights the difficulty in case finding infected individuals. It may be that screening of primary care may be the most cost-effective method. Further large scale studies need to be carried out to see if this increases the confidence of the observed prevalence numbers.

We calculated the prevalence of first and second generation immigrants based on the 2001 census data that we got from the Office of National Statistics which is publicly available on request. Whilst it is mandatory to fill in the census form back in 2001 it was not a criminal offence and so it may well be that there was a degree of under reporting of actual numbers. However, under reporting is probably a pan-UK phenomenon and not specific to these two London boroughs even though they do have more ethnic minority populations than the national average.

The introduction of universal screening for hepatitis B during pregnancy in the UK was proposed in 1998 but was not fully implemented until April 2000[207]. Figure 3-10 shows that 33 second generation immigrants were HBsAg positive. All of these individuals would have been born before antenatal screening of hepatitis B occurred. The parents of these individuals may have been first generation immigrants and may have contracted HBV abroad. If this were the case, then to all intents and purposes, the transmission of the disease in these patients would be the same as if they were first generation immigrants. Some hospitals did vaccinate babies of HBV infected pregnant women prior to 1998 but there was no routine screening policy in the two NHS hospitals involved in this study. Therefore it is not possible to say whether there was vaccine failure unless the baby was specifically given HBV vaccination.

Because this was a retrospective study, the results are heavily dependent on the accuracy of patient notes and electronic records. This has an impact on determining the significance played by the various independent factors. ALT, HBV DNA and HBeAg status were all taken from the earliest recorded visit that the patient made to the hospital. In some cases, the patients may have been seen on a number of occasions before these tests were requested. Smoking was found to be significantly associated

with both cirrhosis and decompensated disease but the notes mostly did not elaborate on how much or for how long the patient smoked. Included in the smoker group were patients who were ex-smokers but it was not possible to work out from the notes how long they had abstained. A similar story goes for alcohol: how much alcohol is deemed harmful for progression of HBV-related liver disease. One of the unusual features about studying this particular population was that alcohol was less of an issue because of religious beliefs (alcohol is banned by the Muslim faith).

We do not know the role of anti-viral medications or their combinations on the progression of disease in our cohort. It may have been that medication was started because patients had more advanced (fibrosis) disease and was used as a preventative measure against developing decompensated disease or that some individuals were started on it to try and halt the progression of the disease.

4.5.3 Comparison with other studies

The most famous study looking at factors associated with cirrhosis and HCC due to HBV is the REVEAL-HBV study (Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus) carried out by Chen and colleagues in Taiwan[50]. They followed up 3653 individuals aged 30-65 from 7 townships with HBV mono-infection and detectable HBV DNA over an 11-13 year period to see who developed HCC. They found that 15% of individuals who had HBV DNA >6 log copies/mL at study entry had developed HCC by the 13th year of the study. There appeared to be an increasing risk of HCC with higher levels of DNA and this was still significant using multivariate statistics when factors such as ALT, e-antigen status and presence of cirrhosis were controlled for. The authors purport that increased age, male sex, smoking, and alcohol drinking were also significantly associated with HCC but do not elaborate on the strength of this relationship. Regarding HBV DNA in our study, we used international units per millilitre (IU/mL) where 1 IU/mL ≈ 5.8 copies/mL. Due to financial and time constraints, this study could not follow up patients for the same duration to see whether similar results would be produced.

Also individuals in the REVEAL study were not offered treatment and referral to a treatment centre only occurred if cirrhosis or HCC was diagnosed. Our study showed similar significant findings with age, sex, smoking, alcohol and ALT. Their study looked at individuals in a region of high endemicity where the predominant genotypes were B (1520/2405) and C (801/2405).[208] This is in contrast to our study which looked at immigrants from an area of intermediate endemicity where genotype B is rare and genotypes A, C and D are more common.

To date, only the recent study by McPherson et al has also screened South Asian immigrants for HBV (and HCV) in mosques in North East England. In their study they used volunteers attending the mosques and used DBS rather than OMT. Those patients that tested positive for HBsAg on DBS were then invited for specialist outpatient review, however none accepted this offer.[173]

Studies that do not rely on volunteers run the risk of selection bias. Studies on pregnant mothers or blood donors may not give representative figures because these groups may be self-selecting to seek medical help. Because of misconceptions of how HBV has been contracted, there is a lot of stigma attached to being diagnosed with the disease and individuals often do not want to know or have their families know.[209] Another source of bias with studies using immigrants concerns socio-economic status. First generation immigrants may be more affluent than the indigenous population back home and so may have the means to leave the country. Refugees, on the other hand, may have suffered more hardships and so may be at greater risk of contracting various illnesses including blood borne viruses.

4.5.4 Conclusion

The results of this study show that various factors are associated with progression to decompensated disease. Future studies are needed to determine the strength of this relationship. Follow up of 3 years is too short to fully understand what will happen to these patients. There needs to be accurate recording of baseline values and regular follow up to see the effects of time and also treatment on disease evolution. Better education of the immigrant population to eliminate feelings of stigmatisation will result in more people agreeing to be screened and ultimately improve prevalence statistics.

5 Hepatitis B in non-Indian Subcontinent immigrants in East London

5.1 Introduction

Hepatitis B is a global pathogen with almost 2 billion people having been infected with the virus in the past. The world can be subdivided based upon the distribution of cases into areas of high, intermediate and low endemicity (see chapter 1).[96;210] The regions of HCC high incidence are Eastern and South-Eastern Asia, Middle and Western Africa and the Pacific islands, which are also the high HBV endemic areas. In Asia, HBV kills more people through HCC and cirrhosis than Tuberculosis, malaria and HIV/AIDS.[176;211] Most of the infections in these regions would have been transmitted vertically, peri-natally or in early childhood (especially in African children) rather than horizontally (via blood or sexually). There are environmental factors associated with HCC such as alcohol, tobacco and aflatoxin and these pose a greater risk in individuals infected with HBV. Aflatoxin is produced by *Aspergillus sp* and is found in foodstuffs like corn and peanuts that are harvested and allowed to mature in hot, humid conditions. The risk of HCC can go up 60-fold in those HBV patients who show urinary metabolites of aflatoxin.[212;213]

Social, economic and political (including wars and conflicts) reasons, coupled with the convenience of air travel have made migration easier and more common. Western Europe (including the UK) and North America are popular destinations with most migrants coming from the intermediate and high HBV prevalence areas of Asia and Africa. In Europe there is also increased migration from Eastern Europe due to relaxation of border controls. Studies have shown that in low endemic countries areas the majority of infected individuals are immigrants who contracted the disease in their country of origin.[214-216] This finding has led to the argument for the screening of the immigrant population for hepatitis B as they pose a potentially significant health burden.[217;218] A Dutch study proposed that this might be a cost-effective strategy. The authors suggested that a single one-off screen could potentially reduce liver-related mortality by 10% by detecting and treating infected patients earlier.[219]

There have been a number of attempts both in the UK and in other countries to estimate the prevalence of CHB in the immigrant population. Most of these have come from blood transfusion audits and antenatal screening.[220-222] Some studies have looked at particular ethnicities (Chinese, South Asian and Somalian) attending clinics, health fairs or places of worship.[172;173;223;224] A report by the Hepatitis B Foundation UK has given various estimates of the prevalence of CHB in the UK within various ethnic groups based on country of birth.[225] In this study most of these estimates are based on numbers from CDC, Department of Health and from other studies estimating the prevalence of HBV in the original country of origin (for example Custer *et al*[101]). Using 0.25% prevalence in UK born individuals and estimating that 6500 cases enter the UK every year, they calculated that in 2007 there were 326,388 cases of CHB, of which 193,888 came from foreign born immigrants.

Since the REVEAL study was carried out examining the natural history of CHB in affected individuals in Taiwan, there have been very few longitudinal studies and none in immigrant populations.[208] It is by no means clear that the natural history of HBV in immigrants will follow that seen in their country of origin. Recently, two fairly large, multi-centre, cross-sectional studies have looked at the viral and demographic characteristics of CHB in immigrant groups. An Italian study (SIMIT) looking at CHB infected patients compared 900 immigrants with 2800 native Italians from 74 centres. They found the proportions of HBV in the immigrant groups were younger, had a greater proportion of females, but had less advanced disease (cirrhosis and HCC) and were also less likely to be on treatment.[226] A UK study (CUSHI-B) identified 698 patients being followed up in 15 centres of which 80% were born abroad. Their results were different in that 20% of patients had cirrhosis and third were on treatment, although not always on the correct first line treatment and a number of these had antiviral resistance.[227] The burden of HBV in the UK is unknown. In the UK between 2006-2010, 3406 liver transplants were performed, of which 16% were carried out on patients with viral hepatitis and 3% were due to HBV cirrhosis.[228] Given their greater prevalence of HBV, it is more likely that immigrant groups rather than Caucasians will present with end-stage liver disease.

London is the largest city in the UK. The East London boroughs of Newham and Tower Hamlets are the most ethnically diverse regions. There are many individuals from areas of high and moderate HBV endemicity such as Sub-Saharan Africa, the Far East, the Indian Sub-Continent, and Eastern Europe. The impact of this diversity on the prevalence and outcome of viral hepatitis is unknown.

5.2 Aims

The aim of this study was to compare and contrast the severity of disease due to viral hepatitis in immigrant and British populations in the multi-ethnic boroughs of Newham and Tower Hamlets who were attending hospital.

5.3 Methods

5.3.1 Patients

Every individual diagnosed with HBV who presented as an outpatient to the hepatology clinics or as an inpatient to Barts and the London (BLT) and Newham University Hospitals (NUH) was traced by accessing the out-patient letters and virology database. Over a 30 month period from 1 January 2006 to 30 June 2008, all the hospital and virology records for these patients were reviewed. BLT and NUH are the two local hospitals providing services to the boroughs of Tower Hamlets and Newham and there are no alternative health care providers. NHS care is provided free of charge to the patient and we therefore, assumed that patients with liver disease would attend their local hospital and that those attending would be representative of the infected population.

For patients with uncomplicated disease the age at final attendance was recorded. For patients with complications, such as the development of decompensated cirrhosis or hepatocellular carcinoma (HCC), data was censored at the age at which these developed and for patients requiring therapy the age at which therapy was

introduced was recorded and the disease process was assumed to stabilise at that time.

The diagnosis of cirrhosis was made on histological or radiological criteria. Decompensated disease referred to the presence of complications of cirrhosis such as variceal haemorrhage, ascites, spontaneous bacterial peritonitis, hepatic encephalopathy or HCC. Cases were confirmed by review of the hospital notes and clinic letters available on the hospital electronic patient record; reports from endoscopy and radiology; analysis of histology reports and laboratory values; and evaluation of multidisciplinary outcome letters.

As with previous studies, risk factors for progression of disease were identified and assessed for statistical significance. These included host factors such as age, sex, ethnicity and ALT; HBV factors such as eAg status, HBV DNA, genotype and whether anti-HBV treatment had been given; and other factors such as alcohol, smoking and the presence of diabetes. Most of the risk factors utilise nominal values and so the data collected were assigned to different categories such as: current or ex-smoker versus non-smoker; alcohol drinker versus no alcohol (teetotal); diabetic versus non-diabetic; cirrhotic versus non-cirrhotic. Parameters with continuous data such as age, HBV DNA viral load and ALT at diagnosis were also assigned to categories: under 40 years (i.e. 16-39) versus over 40 years (i.e. ≥40); low DNA (≤ 4.0 log IU/L) versus high DNA (> 4.0 log IU/L); and low ALT (≤ 40 IU/L) versus high ALT (> 40 IU/L).

5.3.2 Virological Analysis

A single, central laboratory provides virological support to both hospitals and patients with HBV are routinely evaluated for HBsAg, HBeAg, anti-HBe antibody and delta virus superinfection. HBV DNA levels are measured using standard, commercial assays. In this chapter I shall refer to patients who are HBsAg positive as infected with HBV.

5.3.3 Statistical Analysis

All the data collected was categorical and so non-parametric tests such as Chi square, Fisher's exact test and logistic regression were performed. Statistics were calculated using computer software (SPSS version 16, SPSS Inc., Chicago, IL, USA and GraphPad Prism version 5, GraphPad Software Inc., La Jolla, CA, USA).

5.3.4 Ethical Approval

Ethical approval for this study was granted by Oxfordshire REC A as an amendment to the study: Case finding and prevalence of chronic viral hepatitis in South Asians living in the UK – Number 06/Q1604/185.

5.4 Results

5.4.1 Chronic HBV in patients of all ethnicities

5.4.1.1 Demographics

The boroughs of Newham and Tower Hamlets are in East London. Both of these areas are densely populated. From the 2001 census, the total population of Newham is 243,884 and that of Tower Hamlets is 196,100. Compared to the rest of London (and England), these two boroughs have a younger and more ethnically diverse population. The under 16 population in Newham is 63,841 and in Tower Hamlets it is 44,890. In both boroughs, white British form a minority of the population but in Tower Hamlets, Bangladeshis form more than 50% of under sixteen year olds.

Figure 5-1 and Figure 5-2 show the corresponding distribution of ethnicities in those aged over 16 years for the boroughs of Newham and Tower Hamlets, respectively. The population of Newham is 180,430 and Tower Hamlets is 151,210. In Newham the proportion of adult white British compared to children under 16 has decreased from 38% to 24%, whilst in Tower Hamlets the adult white British population is almost

twice that of the younger population, 47% to 24%. In Newham no one ethnic minority predominates, with there being large Afro-Caribbean and Indian Subcontinent populations. The Black African and Caribbean population account for around a quarter of the adult population in Newham, but less than 10% in Tower Hamlets. The Bangladeshi population is the predominant ethnic minority in Tower Hamlets and comprises over a third of the total adult population.

Immigrants from the Far East, especially China, form 2-3% of the population in the young and adult population of both boroughs. It was not possible to further characterise the Black African, Black Caribbean, Western European, Eastern European or "Other" populations as these groups are not separated in the output data from the national census. Data on country of birth for these populations is not collected but data on 'born in the UK' or "Not born in the UK' is available. Data on country of birth was therefore inferred from the census data and we assumed that people born abroad who listed their ethnicity as "Non-English" were born in the country relating to their ethnic origin. For the Indian sub-continent more information is available and an in-depth analysis of these populations is provided in earlier chapters.

In line with our ethical approval this study focussed on adults with HBV (defined as greater than 16 years old).

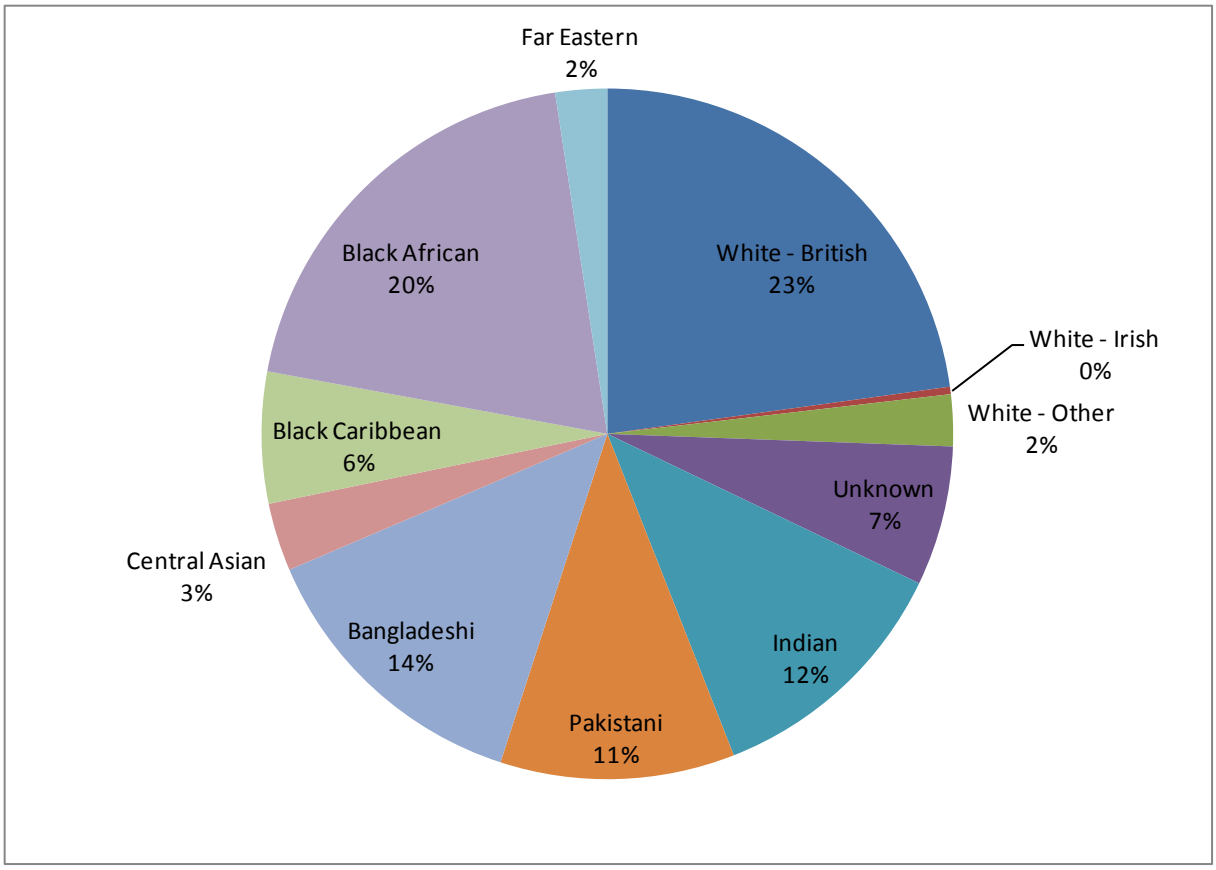

Figure 5-1 Ethnic distribution of individuals aged over 16 years in Newham

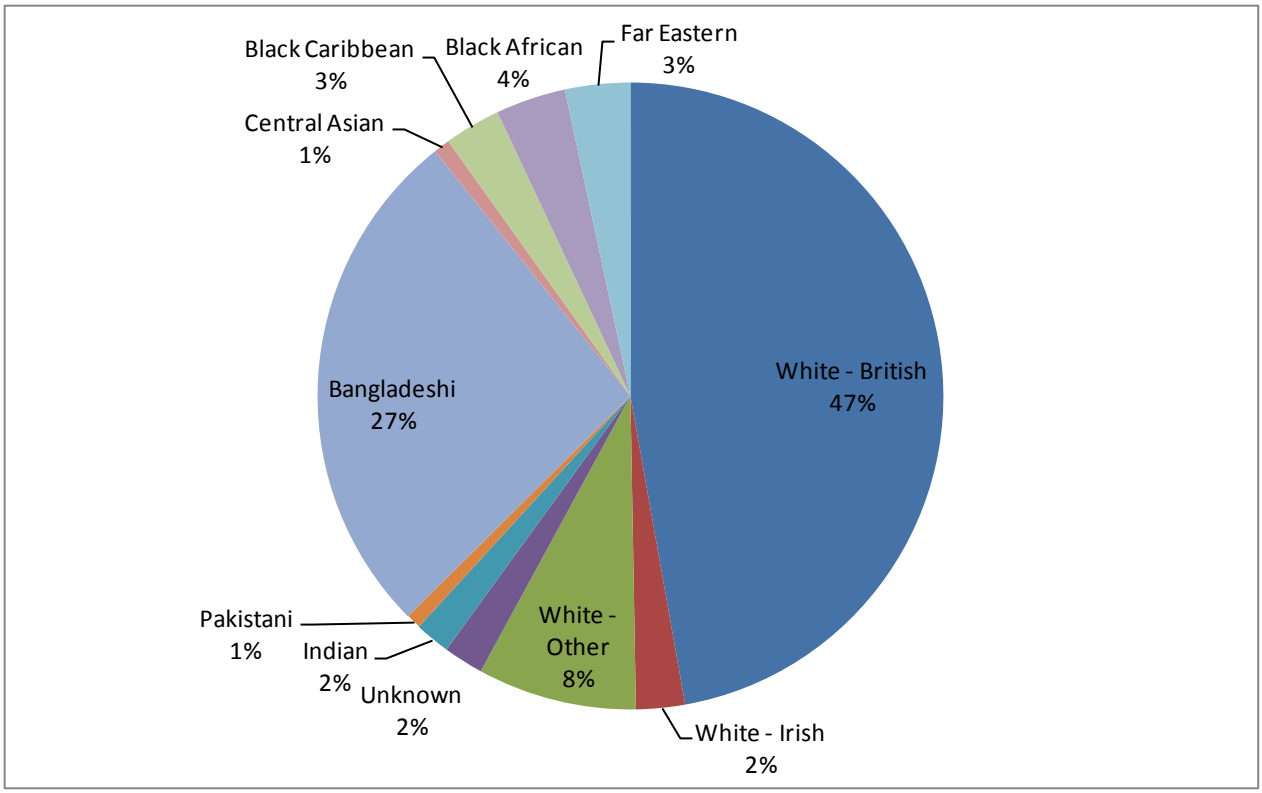

Figure 5-2 Ethnic distribution of individuals aged over 16 years in Tower Hamlets

5.4.1.2 HBV in patients attending hospital

Over the period, 1st January 2006 to 30 June 2008, 1243 patients with HBV were seen in the hepatology and gastroenterology clinics at the 2 local hospitals, NUH and BLT.

Figure 5-3 and Figure 5-4 show the age and sex distribution of patients with both forms of HBV (acute and chronic) according to their ethnicity, including people born in the UK and abroad. For ease of comparison the arbitrary distinction between older and younger age groups was set at 40 (i.e. 16-39 versus ≥40).

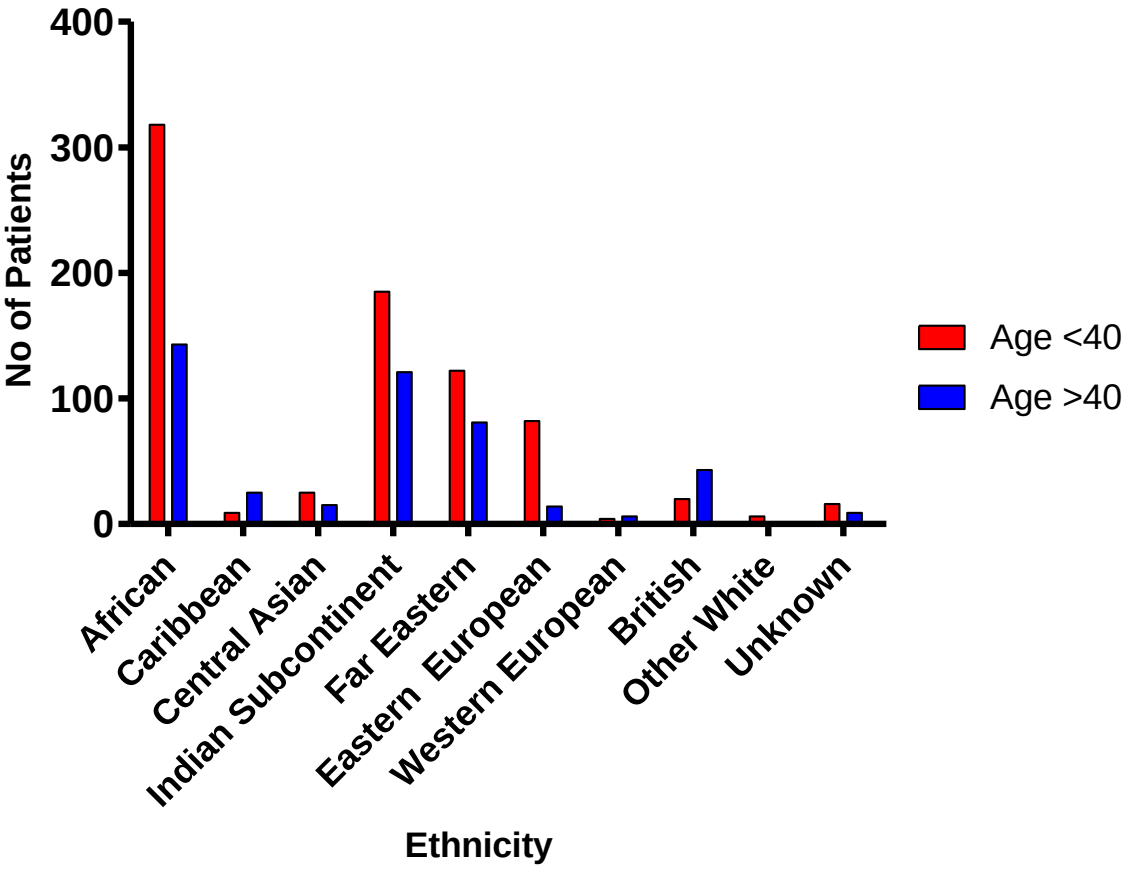

Figure 5-3 Age distribution of acute and chronic HBV amongst patients of all ethnicities attending hepatology clinics

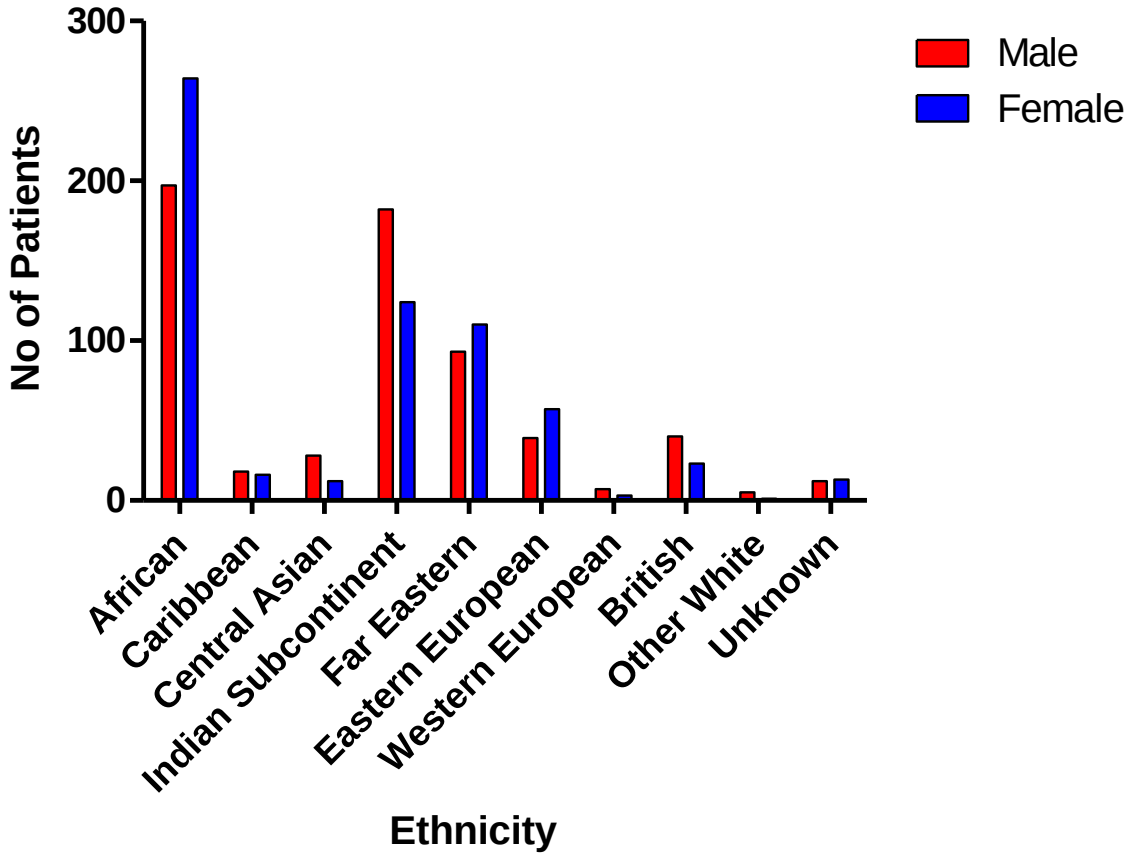

Figure 5-4 Sex distribution of acute and chronic HBV amongst patients of all ethnicities attending hepatology clinics

Acute

Forty-three patients were deemed to have had acute HBV, based upon the clinical symptoms of acute infection (see Chapter 4). The distribution of these patients is shown graphically in Figure 5-5. Twelve of the patients with acute HBV were British. Thirty-one patients had HBsAg seroconversion on follow up. Twelve patients were lost to follow up. Approximately two-thirds of these patients were non-British. As alluded to in chapter 4, the identification of patients with acute HBV relies upon presentation to hospital and the numbers identified may be a significant underestimate of the total disease burden. Although non-attendance may have been influenced by language, cultural or legal/immigration issues we assume that the proportions attending are representative of the proportions who are infected.

All but one of the 43 patients was tested for HCV, 11 were tested for HIV and 8 for HDV. 2 patients were co-infected with HIV and both were African. One Bangladeshi patient, already described in chapter 3, had HCV co-infection and there were no HDV co-infections.

The prevalence of acute HBV in White British is 8.7 per 100,000 compared to 23.4 per 100,000 for Non-British using the estimated population numbers from Table 5-1. This would suggest that White British patients are less likely to get acute HBV that is severe enough to require hospitalisation than other ethnicities.

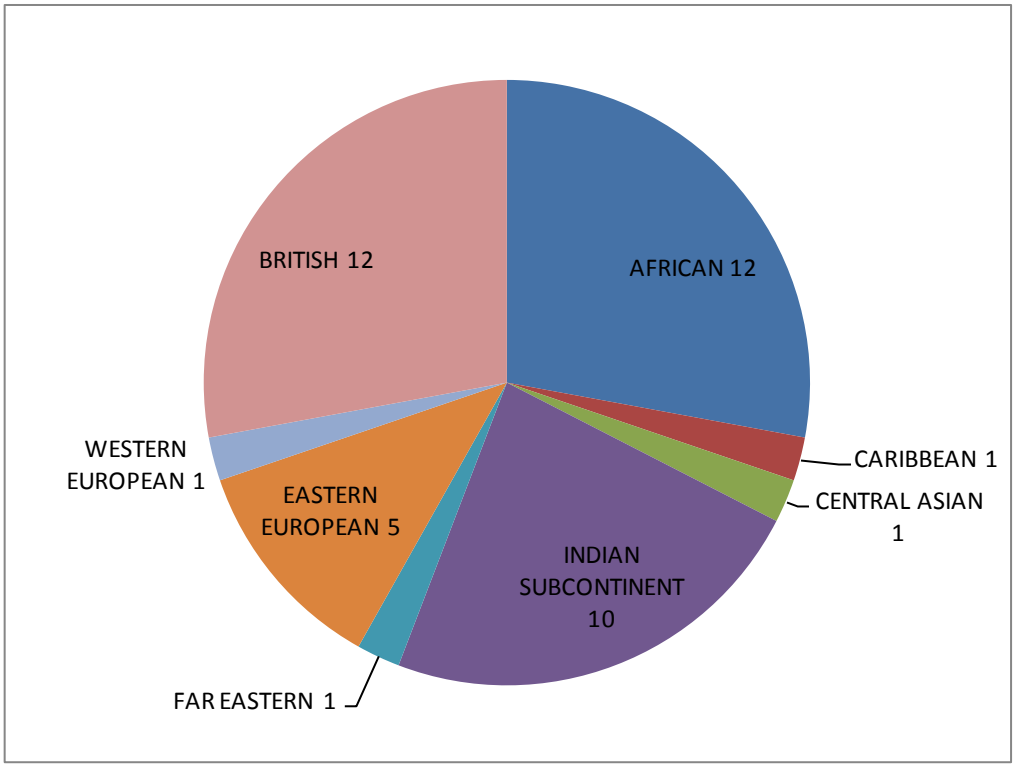

Figure 5-5 Distribution of acute HBV patients according to ethnic group seen in hepatology clinics

Chronic HBV

Figure 5-6 shows the distribution of the 1200 patients with chronic HBV infection. Of these, there were 42 patients with co-infections: 16 HIV, 21 HCV and 5 HDV co-infections. 8 of these patients had cirrhosis and 1 had decompensated cirrhosis, possibly due to HBV but conceivably also due to HCV or HDV. None of these co-infected patients were subsequently used in the analysis of chronic HBV disease in various ethnic groups.

Results from chapter 4 showed that the bulk of chronic liver disease from HBV is carried in first generation immigrants, who bring the disease (and its complications) with them from their home country. We will apply this same assumption to other first generation immigrants who have come to the UK in the rest of the study (see Figure 5-6).

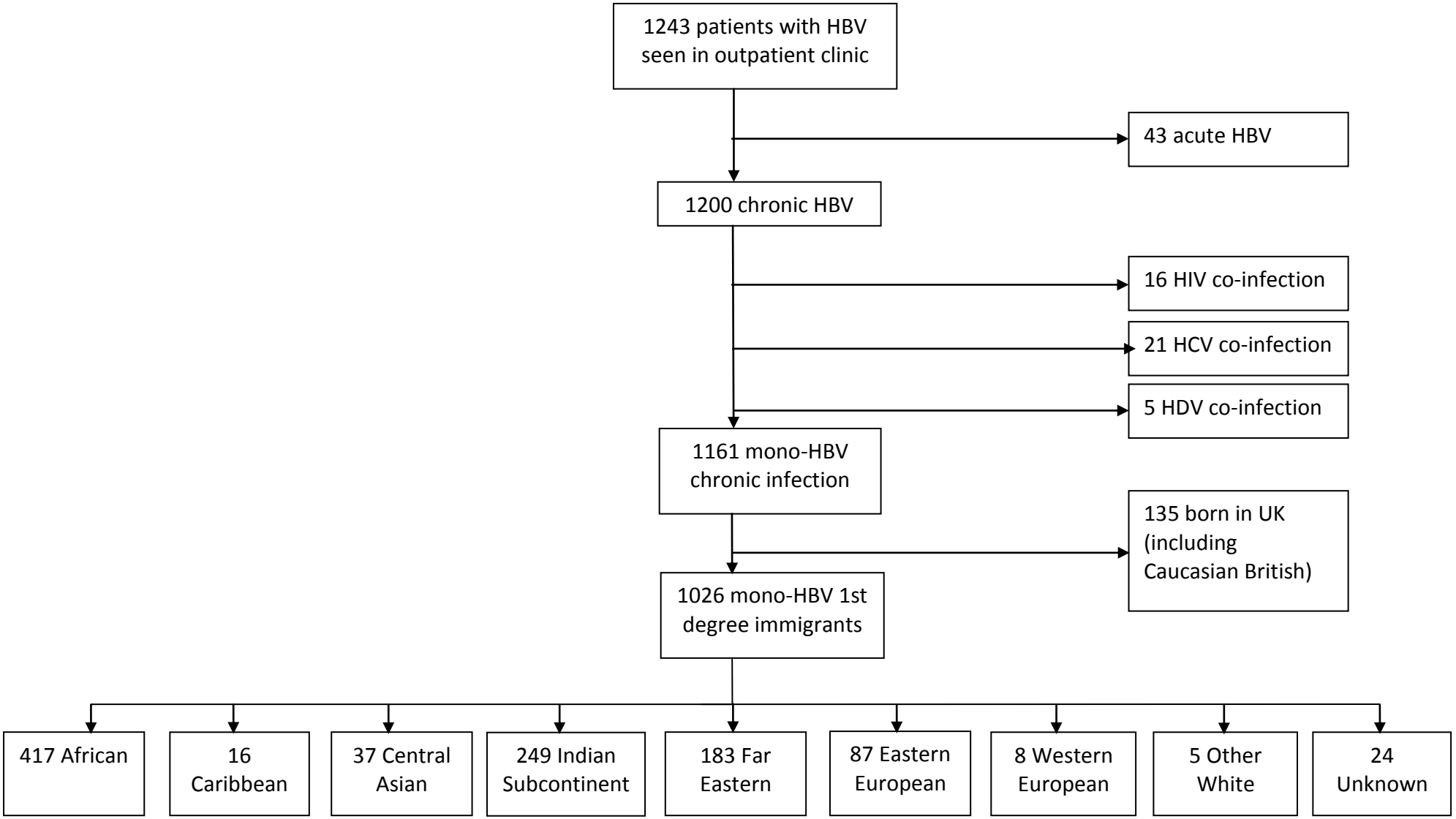

Figure 5-6 Distribution of HBV patients in the study

Figure 5-7 shows the percentage proportions by ethnicity attending the hepatology outpatient and inpatient services of both hospitals. Also included are British patients who form 5% of total patients seen.

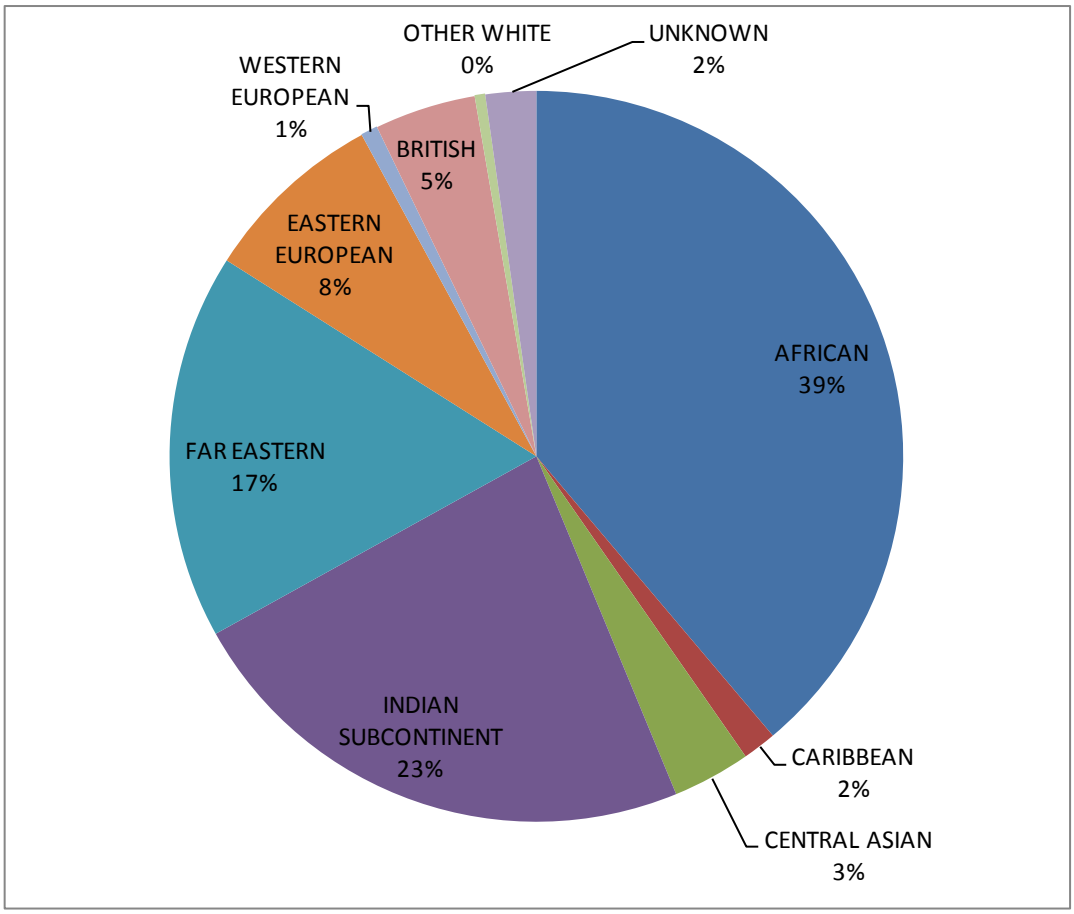

Figure 5-7 Proportion of British and first generation immigrant chronic HBsAg+ patients attending hospital

Figure 5-8 shows that the majority of patients are young and the distribution is similar to Figures 4-13 to 4-16 in Chapter 4 which looked at infected first generation patients from Bangladesh and Pakistan. Unfortunately there are no available statistics for other ethnicities comparing population with age.

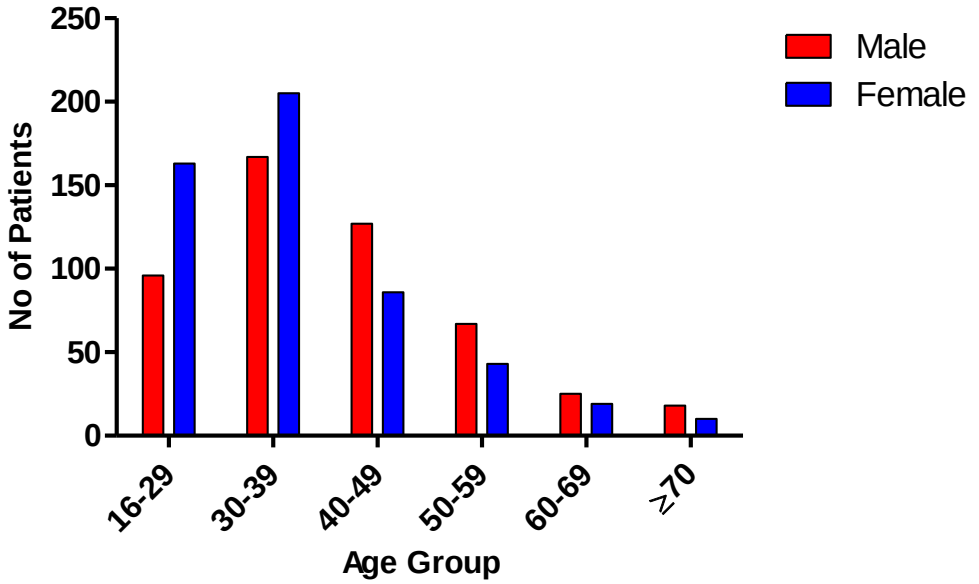

Figure 5-8 First generation immigrant chronic HBsAg+ patients of all ethnicities attending hospital by sex and age

Table 5-1 shows the distribution of CHB by regional ethnicity. The second column shows the estimated adult population (aged over sixteen) of first generation immigrants living in the two London boroughs from the ONS 2001 census. As Indians, Bangladeshis and Pakistanis are the predominant ethnic minorities there is more accurate data on their country of birth and ethnicity. With the other ethnicities, the numbers are based on country of birth and include all individuals of both sexes aged over 16.

The calculated prevalence of CHB in the local population (column 4) is based on the number of patients from each ethnicity diagnosed with CHB who attend hospital divided by their estimated population in the boroughs of Newham and Tower Hamlets. The prevalence of diagnosed HBV in the non-immigrant British population is very low, along with individuals from Western Europe and the Other White ethnic groups and this may reflect the different routes of transmission of the disease in these countries (via sex and infected blood transmission) compared to individuals from developing countries (more vertical and perinatal transmission). The Indian

Subcontinent prevalence is slightly lower than that in Chapter 4 and that is because it takes into account Indians who have a large population but fewer patients with HBV.

The regional HBV prevalence values are taken from Kowdley et al's study.[229] These numbers are from pooled data which is also subject to wide variations within each country let alone within the region. Other caveats include who the samples are from: antenatal screening, prisoners, refugees, children and whether or not they include patients with acute hepatitis.[230] The penultimate column estimates the total number of patients of each regional ethnicity who have CHB. The value is calculated by multiplying the prevalence in the original region with their respective local population numbers. The final column is the estimated amount of undiagnosed CHB in the local population because they have not been seen in hospital and formally diagnosed with HBV. The percentages refer to the proportion of CHB patients not attending hospital. Only a small fraction of the total CHB population of all ethnicities is being followed up in secondary care. This also appears to be the case for Caucasian British where less than 10% of the true CHB population is being seen. The Central Asian values showing apparent good pick up of HBV cases are probably more of a reflection of the low numbers in the area and the low numbers that have presented to clinic rather than us finding all the patients with CHB from this ethnicity.

Ethnicity	Estimated local population	Diagnosed CHB patients attending hospital	Calculated prevalence of CHB in local population (% ± 95% CI)	Estimated prevalence in original region (%)[229]	Estimated numbers of CHB in local population	Estimated numbers of HBV infections not attending hospital (%)
African	28713	417	1.5 ± 0.1%	10.3	2957	2540 (86%)
Caribbean	6330	16	0.3 ± 0.1%	4.5	285	269 (94%)
Central Asian	1098	37	3.4 ± 1.1%	3.4	37	0
Indian Subcontinent	63553	249	0.4 ± 0.1%	3.6	2288	2039 (89%)
Far Eastern	8611	183	2.1 ± 0.3%	9.0	775	592 (76%)
Eastern European	4455	87	2.0 ± 0.4%	3.3	147	60 (41%)
Western European	11377	8	0.1 ± 0.1%	0.6	68	60 (88%)
British[1]	137407	47	0.0 ± 0.0%	0.5	687	640 (93%)
Other White	6899	5	0.1 ± 0.1%	0.6	41	36 (88%)
Unknown	1555	24	1.6 ± 0.6%			

Table 5-1 Estimated prevalence of people diagnosed with chronic HBV amongst first generation immigrants living in the boroughs of Newham and Tower Hamlets based on hospital attendance along with estimated numbers of undiagnosed HBV infections based on prevalence rates in original region and estimated numbers not attending hospital. [1]British patients included here are Caucasian (as opposed to Black or Asian British) and are not immigrants.

5.4.1.3 Risk factors for cirrhosis

Figure 5-9 shows the distribution of cirrhosis amongst the first generation immigrants of the various ethnic groups. Table 5-2 demonstrates that three ethnic groups in particular appear to have an increased risk of developing cirrhosis: African, Indian Subcontinent and Far Eastern. The Indian Subcontinent patients have already been discussed in detail in Chapter 4. As the prevalence of chronic HBV in Indian first generation immigrants is low, as determined by our community prevalence study[172], no further analysis of this ethnic group will be performed. The proportion of Caribbean patients developing cirrhosis is high but due to the overall small numbers of this population further analysis of this group is unlikely to be meaningful. The converse is true for Eastern European patients; whilst there are only 3 out of 87 cases developing cirrhosis, this group forms about 8% of patients attending hospital. Possible risk factors for developing cirrhosis will be examined in the African, Far Eastern and Eastern European ethnic groups.

The risk factors for developing cirrhosis in those ten British (non-immigrant) patients will be dealt with in a separate section.

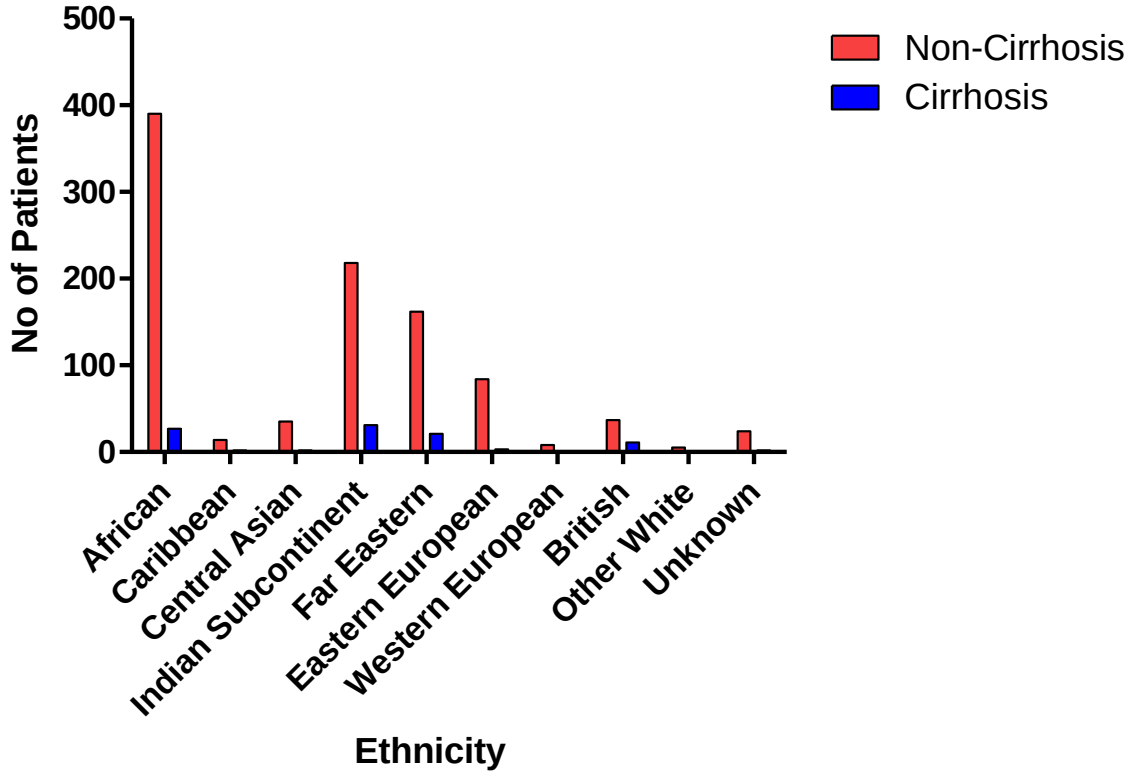

Figure 5-9 Distribution of cirrhosis amongst first generation immigrant patients with chronic HBV of all ethnicities attending hepatology clinics

Ethnicity Group	Non-Cirrhosis	Cirrhosis	Proportion with Cirrhosis (%)
African	390/417	27/417	6.5
Caribbean	14/16	2/16	12.5
Central Asian	35/37	2/37	5.4
Indian Subcontinent	218/249	31/249	12.4
Far Eastern	162/183	21/183	11.5
Eastern European	84/87	3/87	3.4
Western European	8/8	0/8	0
British[1]	37/47	10/47	21.3
Other White	5/5	0/5	0
Unknown	22/24	2/24	8.3

Table 5-2 Distribution of cirrhosis amongst first generation immigrant patients with chronic HBV of all ethnicities attending hepatology clinics. [1]British patients included here are not immigrants.

This section shows the 2x2 contingency tables, p values, odds ratio and 95% confidence intervals of various risk factors associated with cirrhosis in the first generation immigrant HBV patients of all ethnicities seen in the hepatology clinics. Risk factors such as age (Figure 5-10 and Table 5-3); sex (Figure 5-11 and Table 5-4); ALT at diagnosis (Figure 5-12 and Table 5-5); being on anti-HBV treatment at the present time (Figure 5-13 and Table 5-6); ever being on anti-HBV treatment (Figure 5-14 and Table 5-7); smoking (Figure 5-15 and Table 5-8); and diabetes (Figure 5-17 and Table 5-10) were significant using univariate analysis. Alcohol (Figure 5-16 and Table 5-9) was not statistically significant as a risk factor for cirrhosis, but it is represented graphically for comparison with the other risk factors. Other independent risk factors such as e-antigen status at diagnosis and HBV DNA viral load at diagnosis were not statistically significant. Seven patients with liver cancer did not have underlying cirrhosis and for the purposes of this analysis are not included in this section.

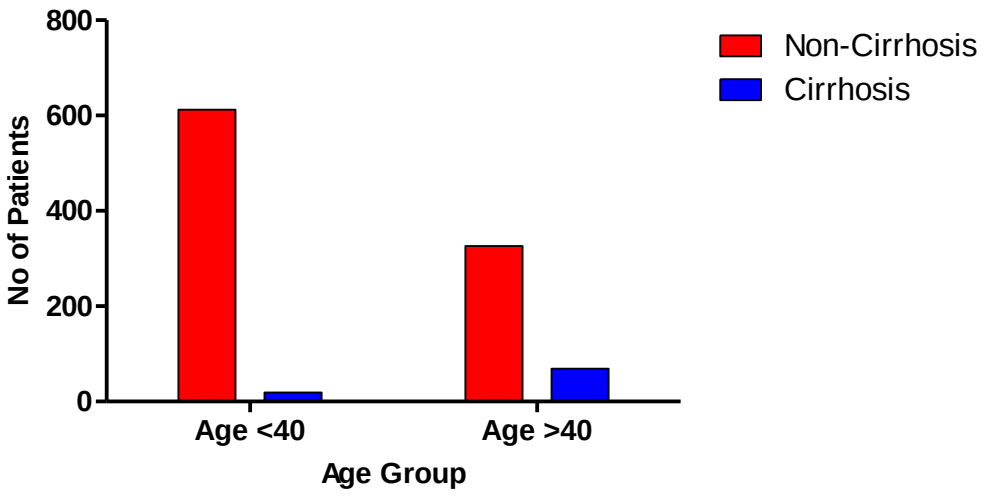

Figure 5-10 Age vs cirrhosis in first generation immigrant HBV patients of all ethnicities

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Age	<40	612/1026	19/1026	<0.0001	6.82	4.03 to 11.53
	≥40	326/1026	69/1026			

Table 5-3 Univariate statistics for age vs cirrhosis in first generation immigrant HBV patients of all ethnicities

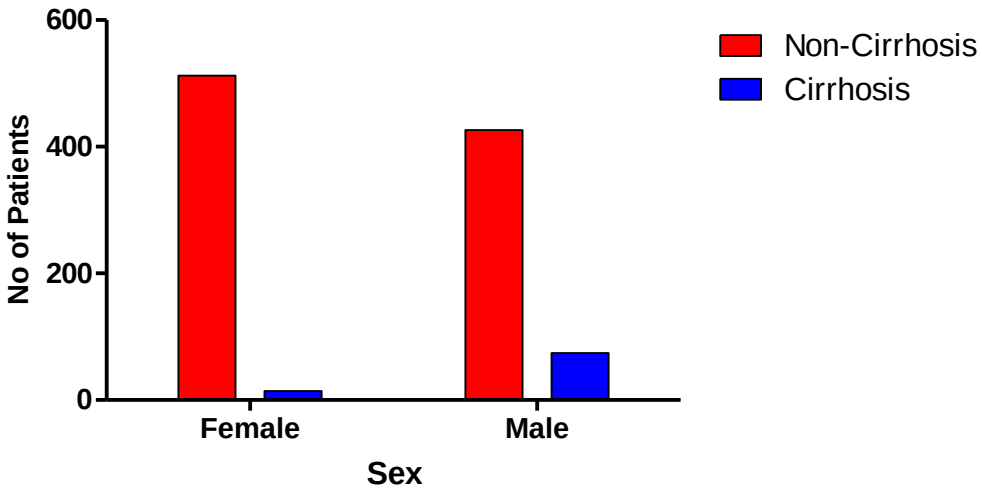

Figure 5-11 Sex vs cirrhosis in first generation immigrant HBV patients of all ethnicities

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Sex	Female	512/1026	14/1026	<0.0001	6.35	3.54 to 11.41
	Male	426/1026	74/1026			

Table 5-4 Univariate statistics for sex vs cirrhosis in first generation immigrant HBV patients of all ethnicities

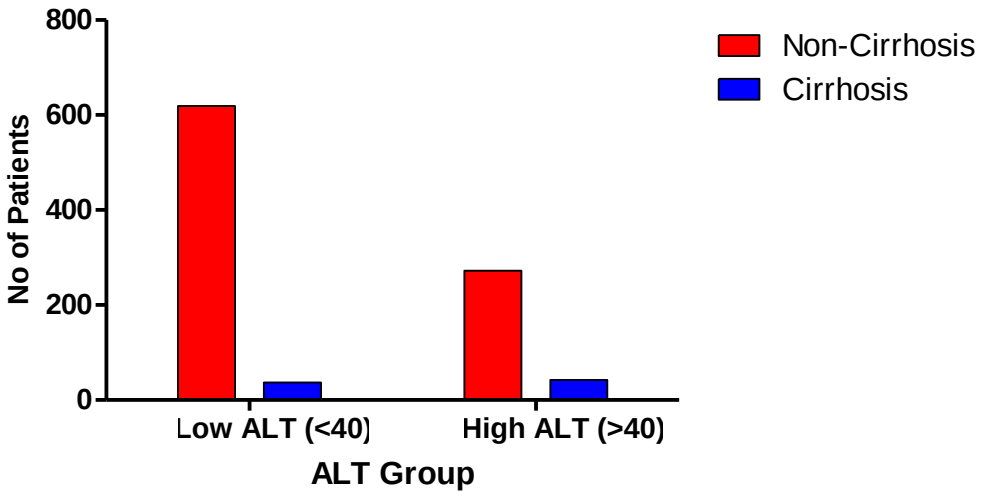

Figure 5-12 ALT vs cirrhosis in first generation immigrant HBV patients of all ethnicities

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
ALT at diagnosis	Low ALT (≤40)	616/970	40/970	<0.0001	2.71	1.74 to 4.23
	High ALT (>40)	267/970	47/970			

Table 5-5 Univariate statistics for ALT vs cirrhosis in first generation immigrant HBV patients of all ethnicities

As discussed in chapter 4 with Bangladeshi and Pakistani cirrhotic patients, Table 5-6 and Table 5-7 show a similar phenomenon in that treatments both now and in the past are significantly associated with cirrhosis. As previously discussed this is most probably related to an increased tendency to treat patients with cirrhosis rather than to any effect of therapy on the development of cirrhosis.

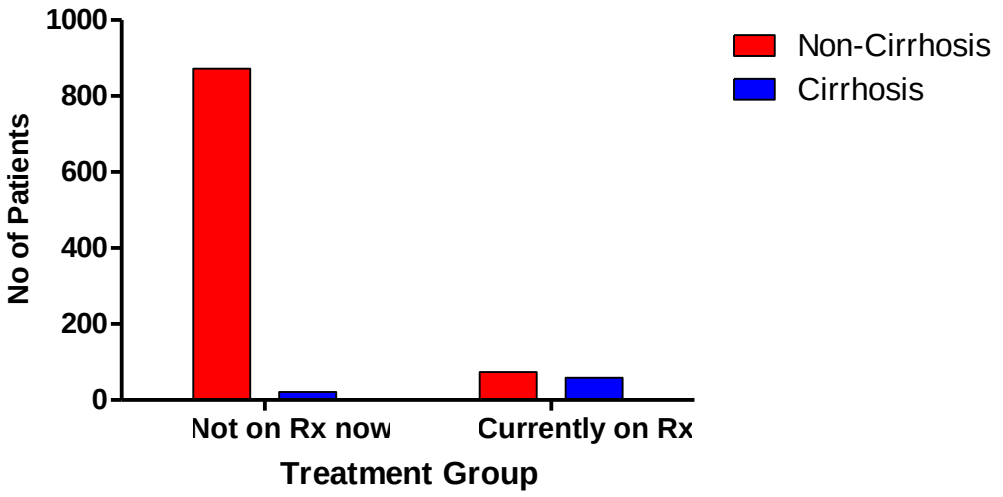

Figure 5-13 Treatment now vs cirrhosis in first generation immigrant HBV patients of all ethnicities

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
On anti-HBV treatment (Rx) now	Not on Rx now	865/1026	28/1026	<0.0001	25.39	15.27 to 42.21
	Currently on Rx	73/1026	60/1026			

Table 5-6 Univariate statistics for treatment now vs cirrhosis in first generation immigrant HBV patients of all ethnicities

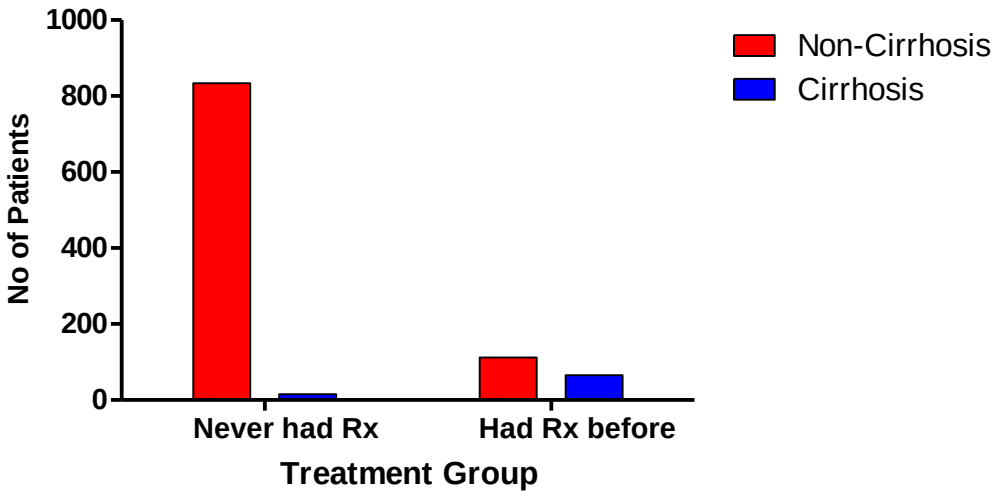

Figure 5-14 Treatment ever vs cirrhosis in first generation immigrant HBV patients of all ethnicities

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
On anti-HBV treatment (Rx) ever	Never had Rx	827/1026	22/1026	<0.0001	22.35	13.27 to 37.66
	Had Rx before	111/1026	66/1026			

Table 5-7 Univariate statistics for treatment ever vs cirrhosis in first generation immigrant HBV patients of all ethnicities

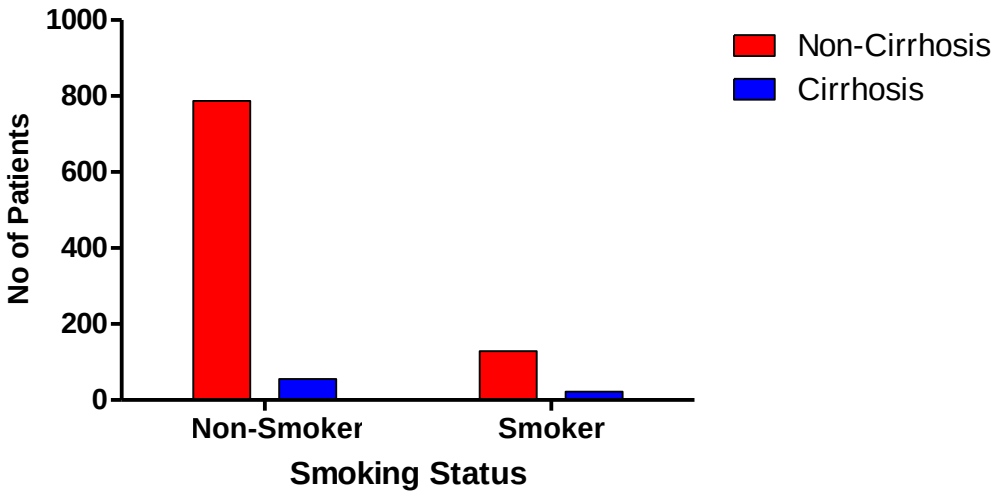

Figure 5-15 Smoking vs cirrhosis in first generation immigrant HBV patients of all ethnicities

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Smoking	Non-Smoker	783/993	59/993	<0.001	2.76	1.68 to 4.55
	Smoker	125/993	26/993			

Table 5-8 Univariate statistics for smoking vs cirrhosis in first generation immigrant HBV patients of all ethnicities

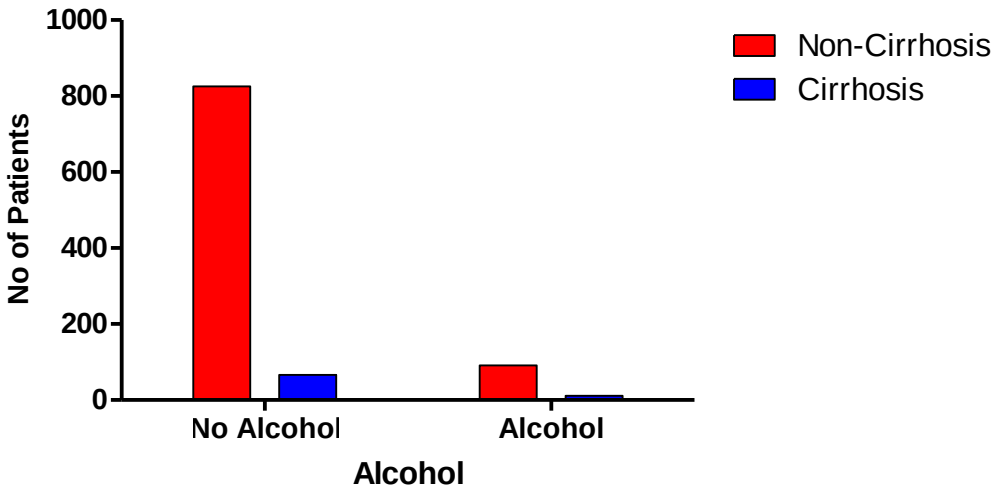

Figure 5-16 Alcohol vs cirrhosis in first generation immigrant HBV patients of all ethnicities

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Alcohol	No Alcohol	820/993	71/993	0.09	1.78	0.96 to 3.28
	Alcohol	88/993	14/993			

Table 5-9 Univariate statistics for alcohol vs cirrhosis in first generation immigrant HBV patients of all ethnicities

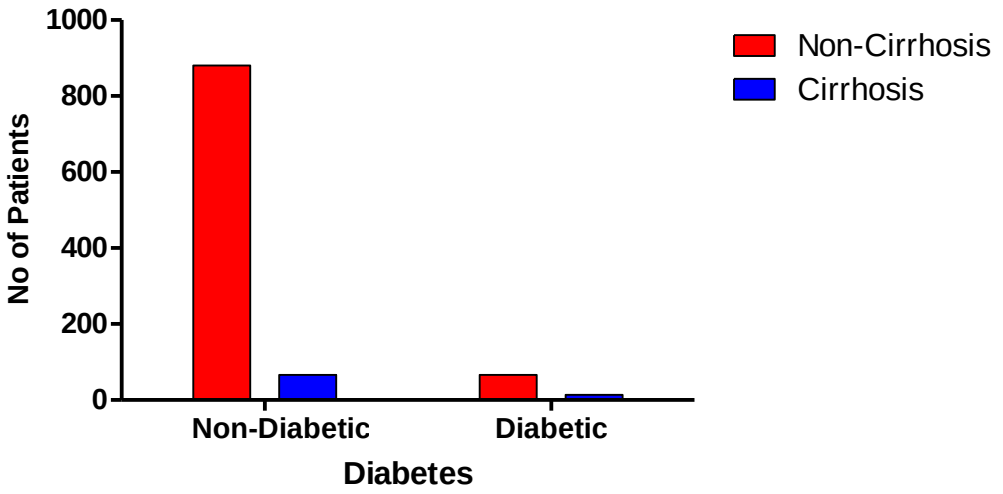

Figure 5-17 Diabetes vs cirrhosis in first generation immigrant HBV patients of all ethnicities

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Diabetes	Non-Diabetic	876/1026	70/1026	<0.0001	3.63	2.04 to 6.48
	Diabetic	62/1026	18/1026			

Table 5-10 Univariate statistics for diabetes vs cirrhosis in first generation immigrant HBV patients of all ethnicities

In addition, multivariate analysis using logistic regression with relevant factors was performed to see if a model for developing cirrhosis could be developed. As age group, sex, ALT group, smoking and diabetes, were significant from univariate analyses, they were used for multivariate analysis. As mentioned before, treatment, either currently or in the past, was not utilised because of its uncertain clinical significance. Because of the binary nature of the cirrhosis outcome, binary logistic regression using the forced enter method was used.

Table 5-11 shows that age over 40, being male and having an elevated ALT at diagnosis are significant predictors for developing cirrhosis in chronic HBV in first generation immigrants even when the other significant univariate variables are taken into account. With age >40, male sex and elevated ALT, the lower limits of the confidence intervals are greater than 1 which gives us confidence that the direction of the relationship we have observed is true in the population.

	Sig.	Odds Ratio Exp(B)	95% Confidence Interval for EXP(B)	
			Lower	Upper
Age group	<.0001	5.27	2.97	9.34
Sex	<.0001	3.53	1.82	6.84
ALT	<.05	1.68	1.01	2.82
Smoking	.45	1.25	.70	2.23
Diabetes	.99	.99	.49	2.00

Table 5-11 Logistic regression showing significant variables for predicting cirrhosis with odds ratio and confidence intervals in first generation immigrant HBV patients of all ethnicities.

5.4.1.4 Risk factors for decompensated disease

The decision to be referred for specialist follow up depends on a number of factors: patient's knowledge and understanding of the condition and its consequences; financial constraints; cultural and social beliefs; and ease of access to healthcare. Most patients with compensated disease, including cirrhosis without complications, are asymptomatic and may not seek medical help. Even though healthcare is free to

patients there may be referral bias by primary care physicians who may not appreciate the severity or investigate patients with asymptomatic HBV infection. As a result there is probably under-representation of HBV in outpatient clinics. However, once patients develop decompensated disease such as variceal haemorrhage, ascites, or HCC, they are likely to be physically unwell and be more amenable to seek medical help from the nearest hospital. These numbers are probably a better reflection of decompensated disease in our cohort.

Figure 5-18 shows the distribution of decompensated disease amongst the first generation immigrants of the various ethnic groups. Table 5-12 demonstrates that the same 3 ethnic groups that have increased risk of developing cirrhosis, are also the ones associated with an increased risk of developing decompensated disease: African, Indian Subcontinent and Far Eastern. The Indian Subcontinent patients have already been discussed in detail in Chapter 4 and so no further analysis of this ethnic group will be performed. There is only one Eastern European patient who developed decompensated disease and so no further analysis of this ethnic group was undertaken. Possible risk factors for developing decompensated disease will be examined in the African and Far Eastern ethnic groups.

The risk factors for developing decompensated disease in those 6 British (non-immigrant) patients will be dealt with in a separate section.

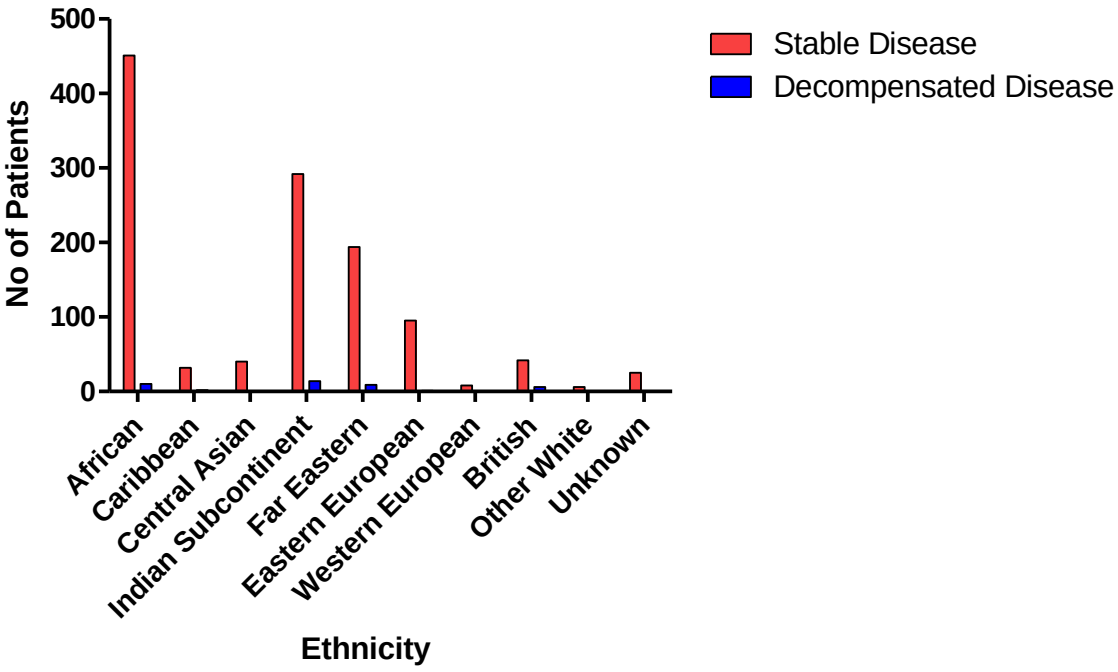

Figure 5-18 Distribution of decompensated disease (decompensated cirrhosis or HCC) amongst patients with chronic HBV of all ethnicities attending hepatology clinics

Ethnicity group	Stable disease	Decompensated disease	Proportion with Decompensated disease (%)
African	408/417	9/417	2.2
Caribbean	15/16	1/16	6.3
Central Asian	37/37	0/37	0
Indian Subcontinent	235/249	14/249	5.6
Far Eastern	174/183	9/183	4.9
Eastern European	86/87	1/87	1.1
Western European	8/8	0/8	0
British[1]	41/47	6/47	12.5
Other White	5/5	0/5	0
Unknown	24/24	0/24	0

Table 5-12 Distribution of decompensated disease amongst patients with chronic HBV of all ethnicities attending hepatology clinics. [1]British patients included here are Caucasian (as opposed to Black or Asian British) and are not immigrants

Below are the 2x2 contingency tables, p values, odds ratio and 95% confidence intervals of various risk factors associated with decompensated disease in the first generation immigrant HBV patients of all ethnicities. Risk factors such as age (Figure 5-19 and Table 5-13); sex (Figure 5-20 and Table 5-14); ALT at diagnosis (Figure 5-21 and Table 5-15); being on anti-HBV treatment at the present time (Figure 5-22 and Table 5-16); ever being on anti-HBV treatment (Figure 5-23 and Table 5-17); smoking (Figure 5-24 and Table 5-18); alcohol (Figure 5-25 and Table 5-19); and diabetes (Figure 5-26 and Table 5-20) were significant using univariate analysis. Other independent risk factors such as e-antigen status at diagnosis and HBV DNA viral load at diagnosis were not statistically significant.

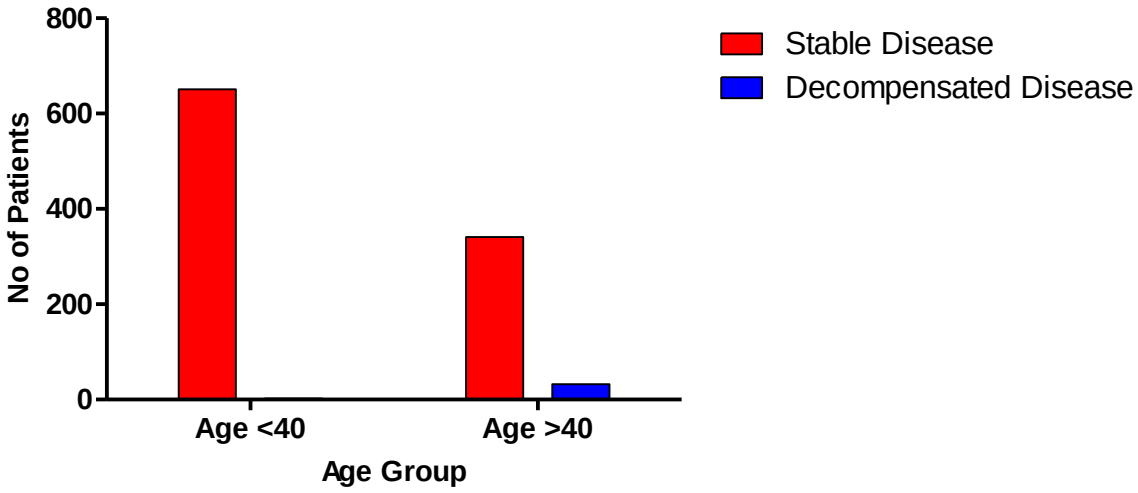

Figure 5-19 Age vs decompensated disease in first generation immigrant HBV patients of all ethnicities

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Age	Age <40	651/1026	2/1026	< 0.001	30.55	7.27 to 128.3
	Age ≥40	341/1026	32/1026			

Table 5-13 Univariate statistics for age vs decompensated disease in first generation immigrant HBV patients of all ethnicities

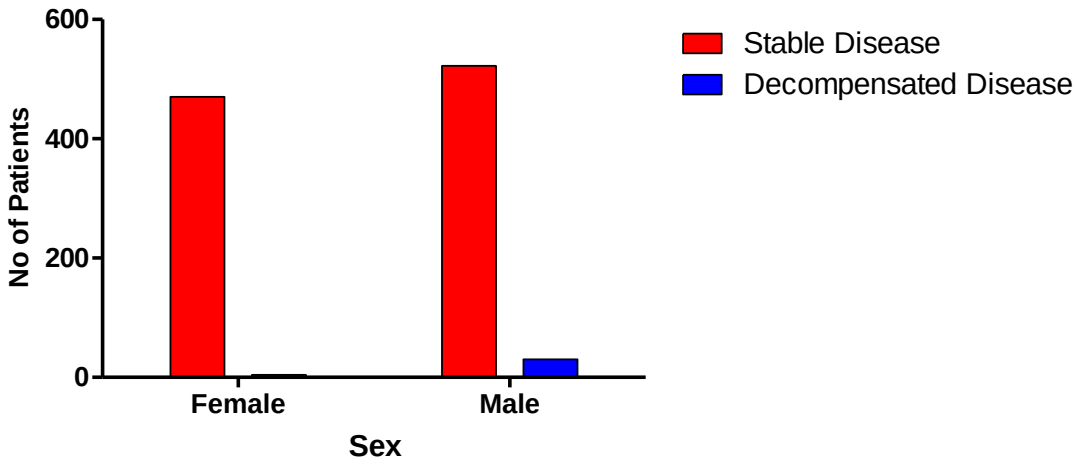

Figure 5-20 Sex vs decompensated disease in first generation immigrant HBV patients of all ethnicities

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Sex	Female	470/1026	4/1026	< 0.0001	6.75	2.36 to 19.32
	Male	522/1026	30/1026			

Table 5-14 Univariate statistics for Sex vs decompensated disease in first generation immigrant HBV patients of all ethnicities

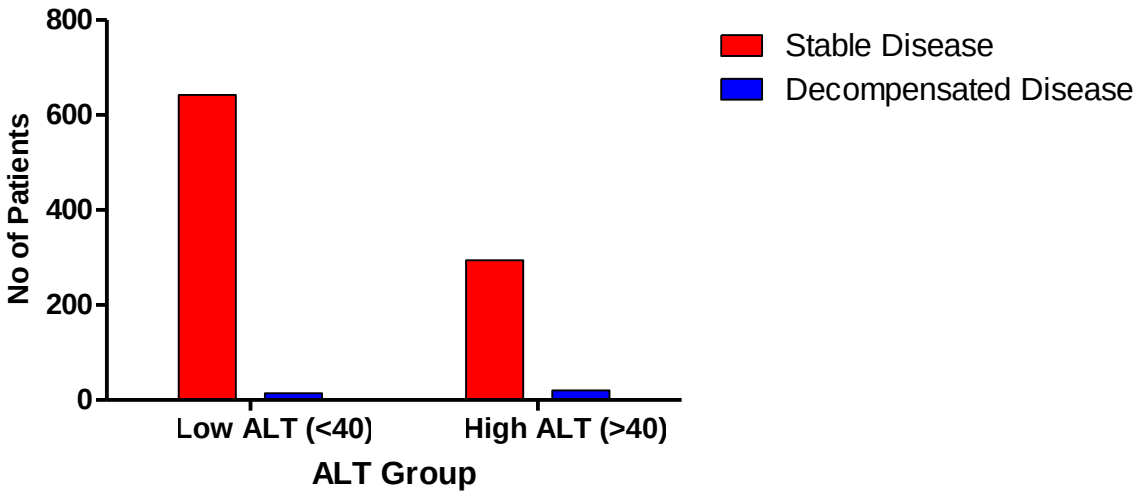

Figure 5-21 ALT vs decompensated disease in first generation immigrant HBV patients of all ethnicities

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
ALT at diagnosis	Low ALT (≤40)	642/970	14/970	<0.001	3.12	1.55 to 6.26
	High ALT (>40)	294/970	20/970			

Table 5-15 Univariate statistics for ALT vs decompensated disease in first generation immigrant HBV patients of all ethnicities

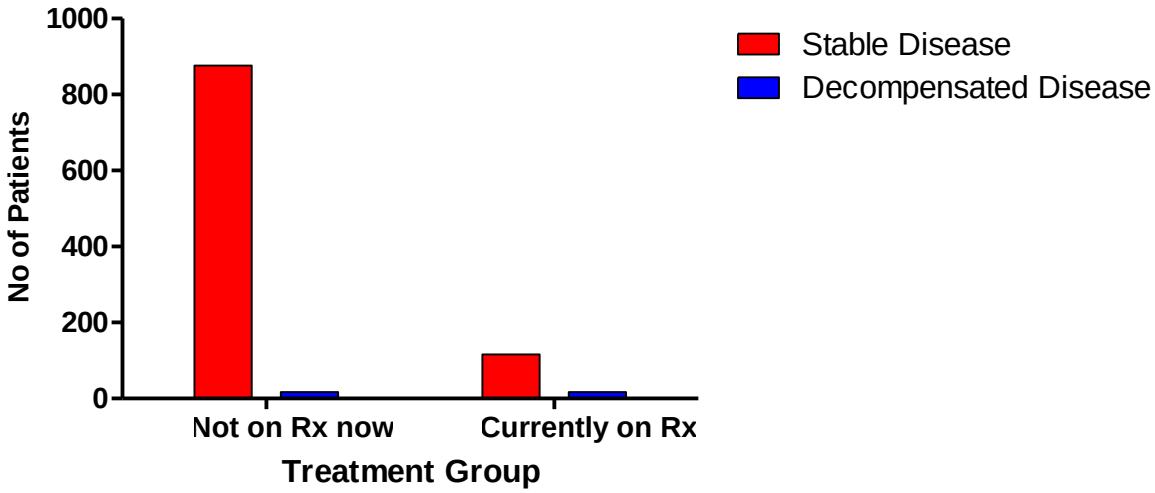

Figure 5-22 Treatment now vs decompensated disease in first generation immigrant HBV patients of all ethnicities

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
On anti-HBV treatment (Rx) now	Not on Rx now	876/1026	17/1026	< 0.001	7.55	3.75 to 15.20
	Currently on Rx	116/1026	17/1026			

Table 5-16 Univariate statistics for treatment now vs decompensated disease in first generation immigrant HBV patients of all ethnicities

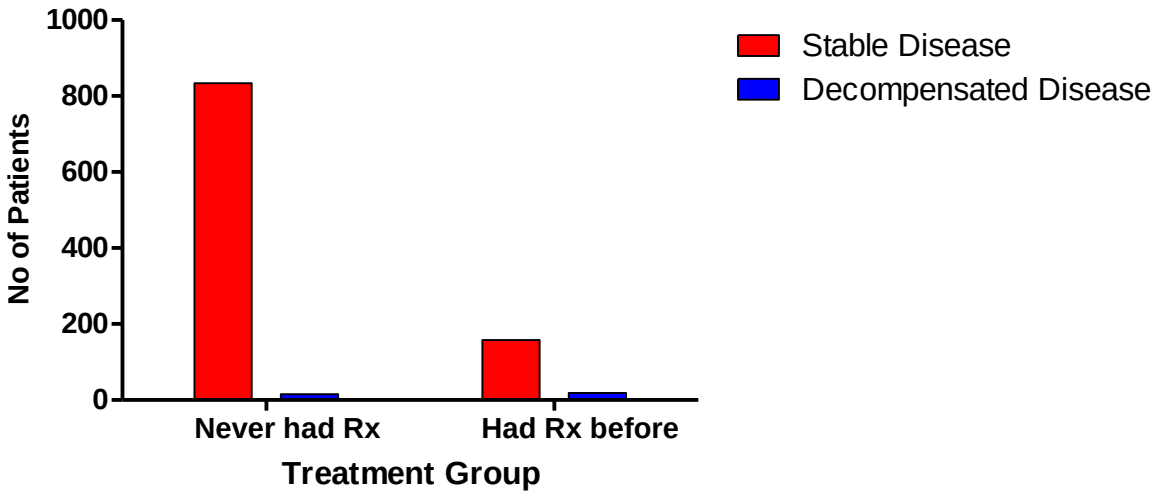

Figure 5-23 Treatment ever vs decompensated disease in first generation immigrant HBV patients of all ethnicities

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
On anti-HBV treatment (Rx) ever	Never had Rx	834/1026	15/1026	< 0.001	6.69	3.33 to 13.44
	Had Rx before	158/1026	19/1026			

Table 5-17 Univariate statistics for treatment ever vs decompensated disease in first generation immigrant HBV patients of all ethnicities

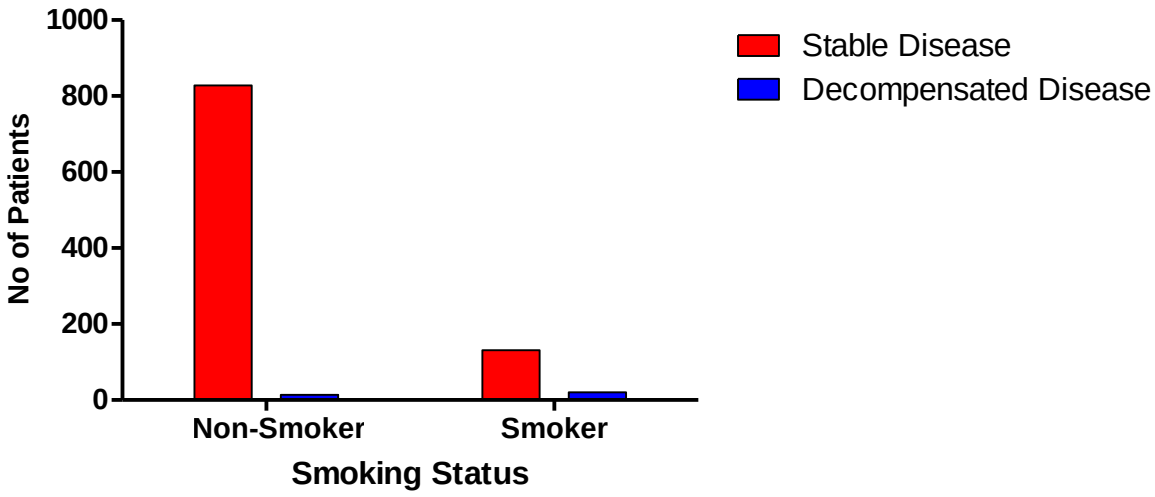

Figure 5-24 Smoking vs decompensated disease in first generation immigrant HBV patients of all ethnicities

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Smoking	Non-Smoker	828/993	14/993	< 0.001	9.03	4.45 to 18.32
	Smoker	131/993	20/993			

Table 5-18 Univariate statistics for smoking vs decompensated disease in first generation immigrant HBV patients of all ethnicities

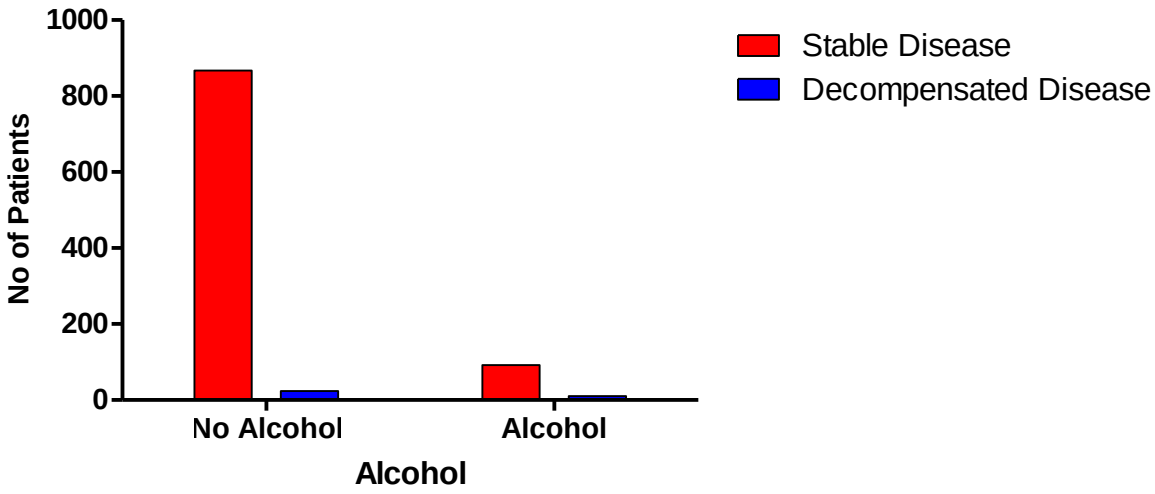

Figure 5-25 Alcohol vs decompensated disease in first generation immigrant HBV patients of all ethnicities

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Alcohol	No Alcohol	867/993	24/993	< 0.001	3.93	1.82 to 8.47
	Alcohol	92/993	10/993			

Table 5-19 Univariate statistics for alcohol vs decompensated disease in first generation immigrant HBV patients of all ethnicities

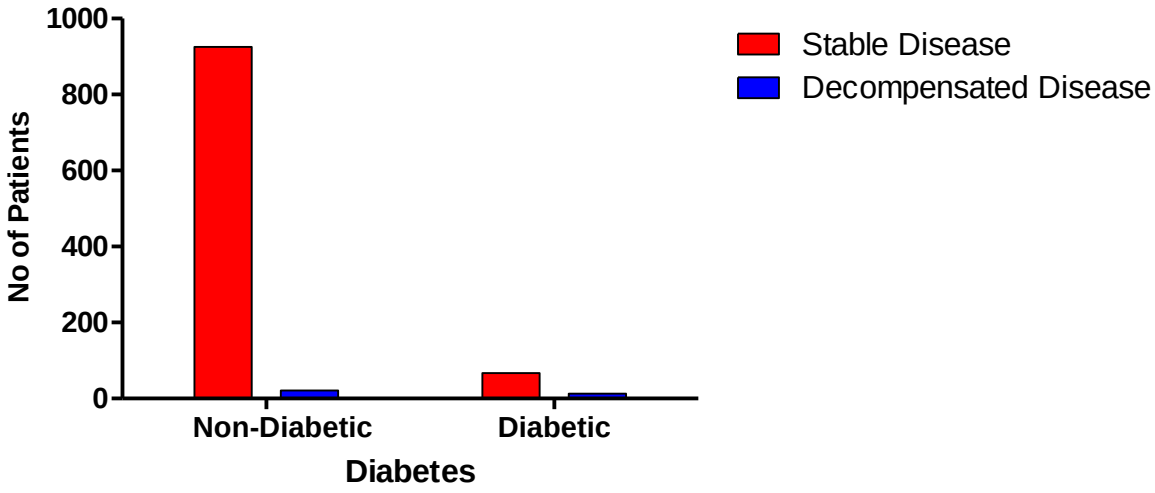

Figure 5-26 Diabetes vs decompensated disease in first generation immigrant HBV patients of all ethnicities

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Diabetes	Non-Diabetic	925/1026	21/1026	< 0.001	8.55	4.10 to 17.82
	Diabetic	67/1026	13/1026			

Table 5-20 Univariate statistics for diabetes vs decompensated disease in first generation immigrant HBV patients of all ethnicities

As age group, sex, ALT group, smoking, alcohol, and diabetes were significant from univariate analyses, they were used for multivariate analysis. As mentioned before, treatment, either currently or in the past, was not utilised because of its uncertain clinical significance. A binary logistic regression using the forced enter method was used (see Table 5-21).

Unlike with the multivariate analysis of cirrhosis, in this case age >40, smoking and the presence of diabetes were significant predictors of decompensated liver disease even when the other factors were controlled for. Age group was the most statistically significant, with the highest odds ratio but also had a very wide 95% confidence interval. This suggests that the odds ratio is not a very accurate estimate of the true population odds.

	Sig.	Odds Ratio Exp(B)	95.0% CI for EXP(B)	
			Lower	Upper
Age group	<.0001	18.69	4.29	81.32
Sex	.13	2.44	.77	7.77
ALT	.11	1.90	.87	4.15
Smoking	<.001	4.00	1.73	9.24
Diabetes	<.02	2.85	1.24	6.54
Alcohol	.23	1.79	.69	4.67

Table 5-21 Logistic regression showing significant variables for predicting decompensated disease with odds ratio and confidence intervals in first generation immigrant HBV patients of all ethnicities.

5.4.2 African chronic HBV patients

5.4.2.1 Demographics

Africa is a vast continent made up of a number of individual countries. 417 patients originated from at least 24 countries. To delineate as clearly as possible where in Africa, these patients came from, the continent was arbitrarily divided into 5 regions which are shown in Figure 5-27. Central Africa referred to countries such as Cameroon, Chad, Democratic Republic of Congo and Nigeria. Eastern Africa referred

to Ethiopia, Kenya, Somalia and Uganda. Northern Africa referred to Egypt, Morocco and Syria. Southern Africa referred to Angola, Namibia, South Africa and Zimbabwe. Western Africa referred to Gambia, Ghana, Liberia and Sierra Leone. In 126 cases, the exact African country of origin could not be identified from the notes. The majority of identified patients came from Ghana (74), Somalia (72) and Nigeria (69).

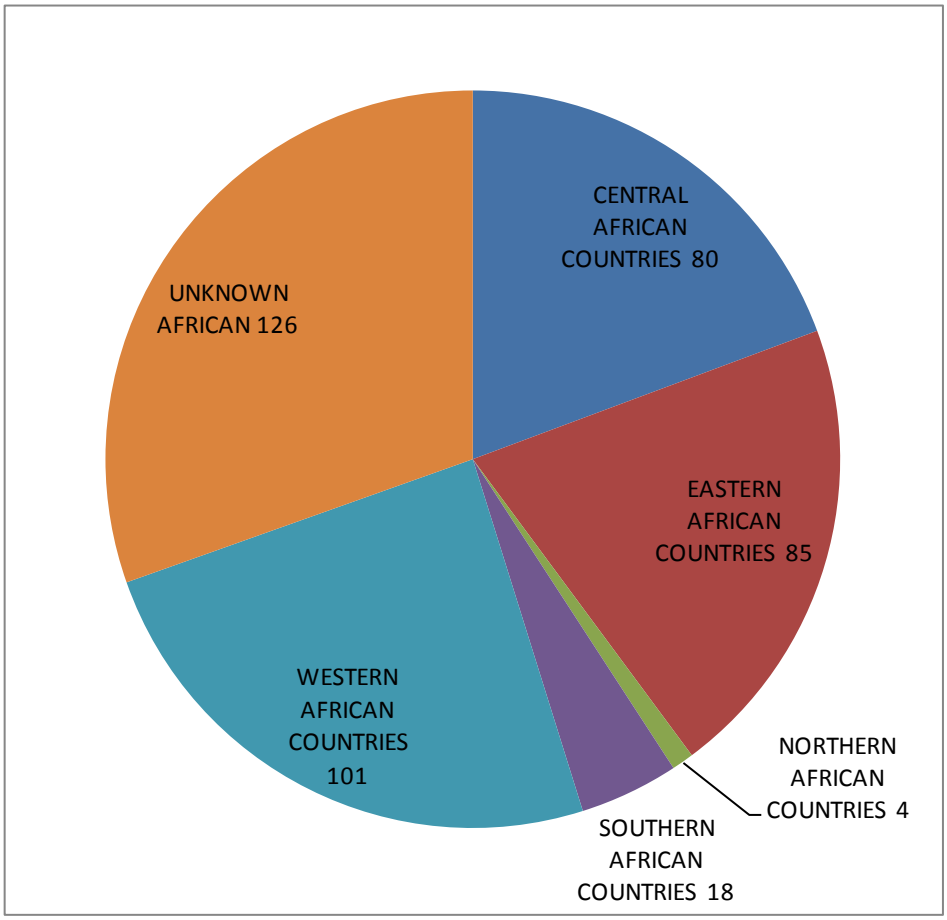

Figure 5-27 Distribution of first generation African immigrant patients with chronic HBV attending clinics

5.4.2.2 Risk factors for cirrhosis

For the sake of comparison with first generation immigrant Bangladeshis and Pakistanis studied in Chapter 4, the same risk factors for cirrhosis were also analysed in the first generation immigrant African population.

This section shows the 2x2 contingency tables, p values, odds ratio and 95% confidence intervals of the statistically significant various risk factors associated with cirrhosis in the first generation immigrant African patients seen in the hepatology clinics. Risk factors such as age (Figure 5-28 and Table 5-22); sex (Figure 5-29 and Table 5-23); and ALT at diagnosis (Figure 5-30 and Table 5-24) were significant using univariate analysis. Being on anti-HBV treatment at the present time and ever being on anti-HBV treatment were also statistically significant but as mentioned before the clinical significance of this is uncertain and so they are not displayed graphically. Smoking, diabetes and alcohol were not significant risk factors for cirrhosis, along with e-antigen status at diagnosis and HBV DNA viral load at diagnosis.

Only 1 patient with liver cancer did not have underlying cirrhosis and for the purposes of this analysis he was not included in this section.

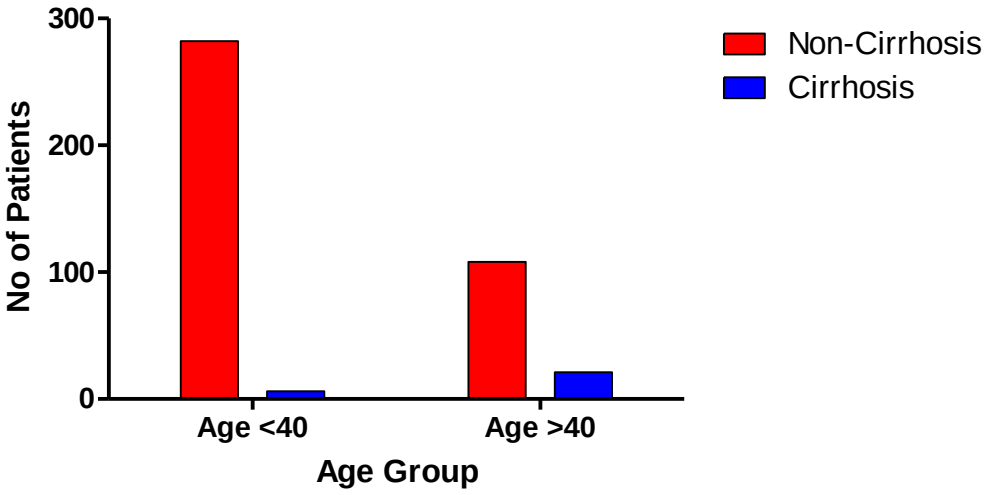

Figure 5-28 Age vs cirrhosis in first generation immigrant African patients

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Age	<40	282/417	6/417	<.001	9.14	3.59 to 23.26
	>40	108/417	21/417			

Table 5-22 Univariate statistics for age vs cirrhosis in first generation immigrant African patients

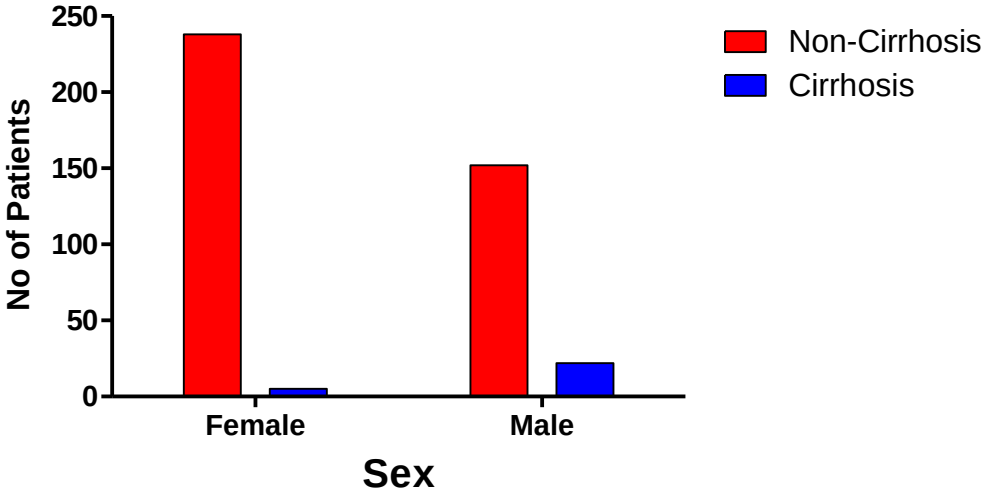

Figure 5-29 Sex vs cirrhosis in first generation immigrant Africans

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Sex	Female	238/417	5/417	<.001	6.89	2.55 to 18.59
	Male	152/417	22/417			

Table 5-23 Univariate statistics for sex vs cirrhosis in first generation immigrant African patients

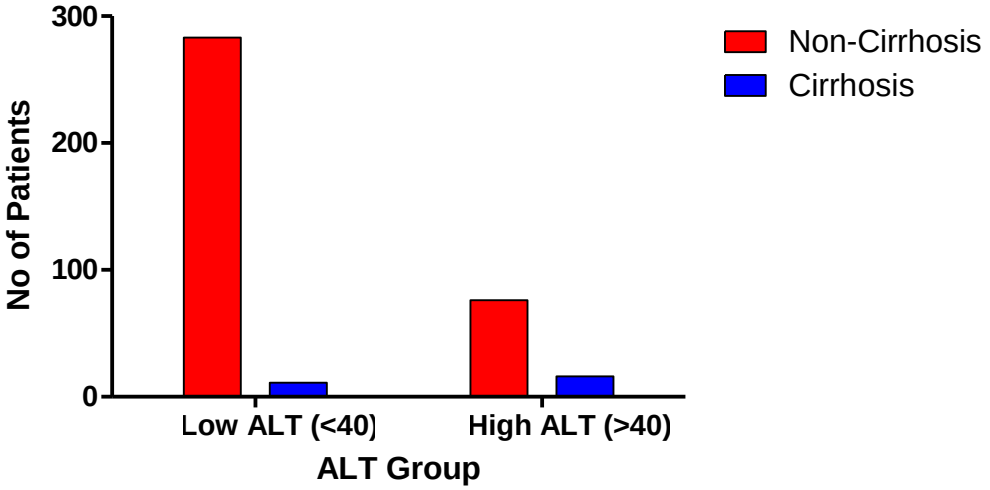

Figure 5-30 ALT vs cirrhosis in first generation immigrant African patients

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
ALT at diagnosis	Low (≤40)	283/386	11/386	<.001	5.42	2.41 to 12.16
	High (>40)	76/386	16/386			

Table 5-24 Univariate statistics for ALT vs cirrhosis in first generation immigrant African patients

As age group, sex, and ALT group, were significant from univariate analyses, they were used for multivariate analysis. As mentioned before, treatment, either currently or in the past, was not utilised because of its uncertain clinical significance. Binary logistic regression using the forced enter method was used.

Table 5-25 shows that being both age over 40 and having elevated ALT are significant predictors for developing cirrhosis in chronic HBV in first generation immigrant African patients when the other variables are controlled for.

	Sig.	Odds Ratio Exp(B)	95% Confidence Interval for EXP(B)	
			Lower	Upper
Age group	<.001	5.53	2.06	14.87
Sex	.111	.41	.14	1.23
ALT	<.011	3.32	1.32	8.35

Table 5-25 Logistic regression showing significant variables for predicting cirrhosis with odds ratio and confidence intervals in African patients.

5.4.2.3 Risk factors for decompensated disease

Below are the 2x2 contingency tables, p values, odds ratio and 95% confidence intervals of various significant risk factors associated with decompensated disease in the first generation immigrant African patients. Risk factors such as age (Figure 5-31 and Table 5-26); sex (Figure 5-32 and Table 5-27); ALT at diagnosis (Figure 5-33 and Table 5-28); smoking (Figure 5-34 and Table 5-29); and alcohol (Figure 5-35 and Table 5-30) were significant using univariate analysis. Being on anti-HBV treatment at the present time and in the past were also significant independent factors for decompensated disease, but for the aforementioned reasons these were not displayed graphically. Other independent risk factors such as diabetes, e-antigen status at diagnosis and HBV DNA viral load at diagnosis were not statistically significant.

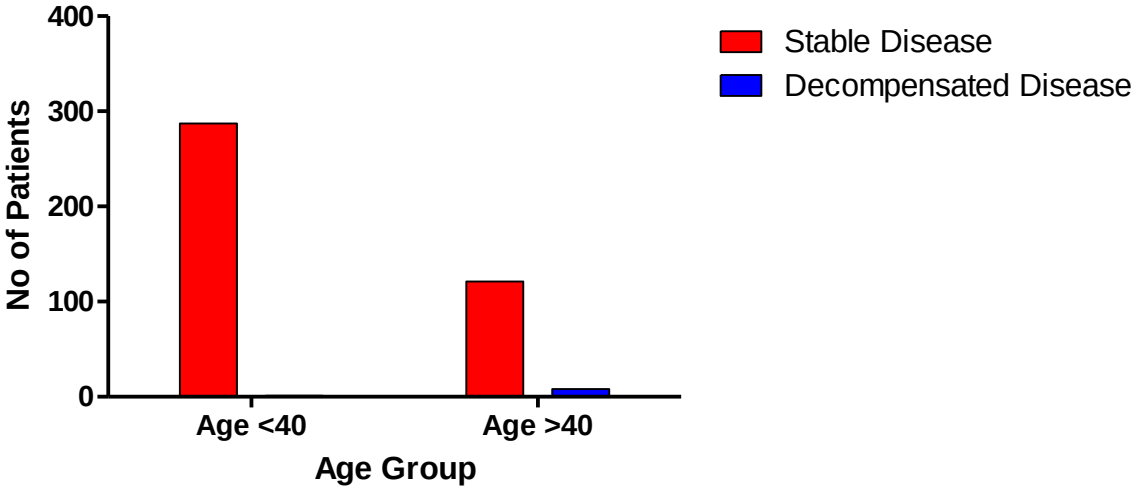

Figure 5-31 Age vs decompensated disease in first generation immigrant African patients

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Age	<40	287/417	1/417	<.001	18.98	2.35 to 153.4
	≥40	121/417	8/417			

Table 5-26 Univariate statistics for age vs decompensated disease in first generation immigrant African patients

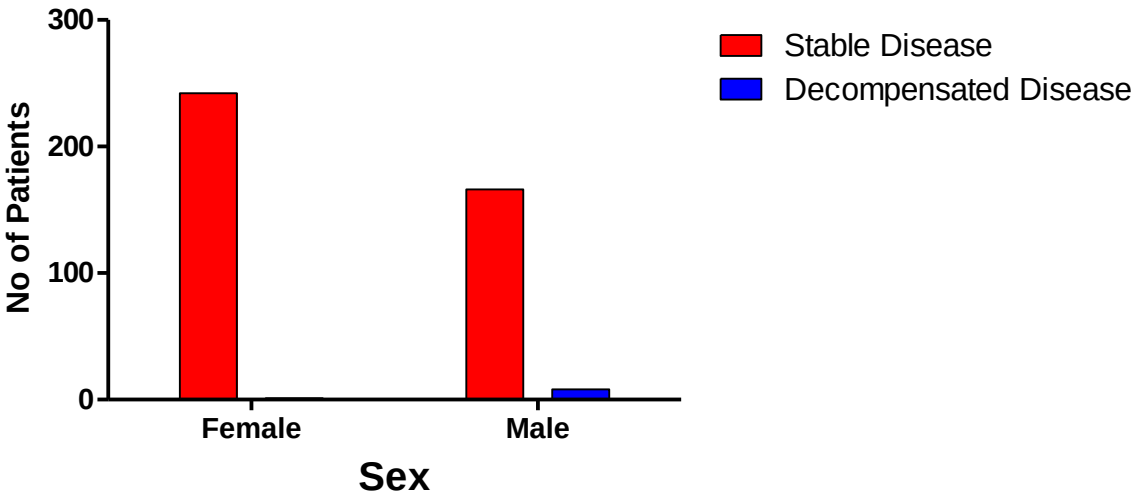

Figure 5-32 Sex vs decompensated disease in first generation immigrant African patients

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Sex	Female	242/417	1/417	<0.01	11.66	1.44 to 94.17
	Male	166/417	8/417			

Table 5-27 Univariate statistics for sex vs decompensated disease in first generation immigrant African patients

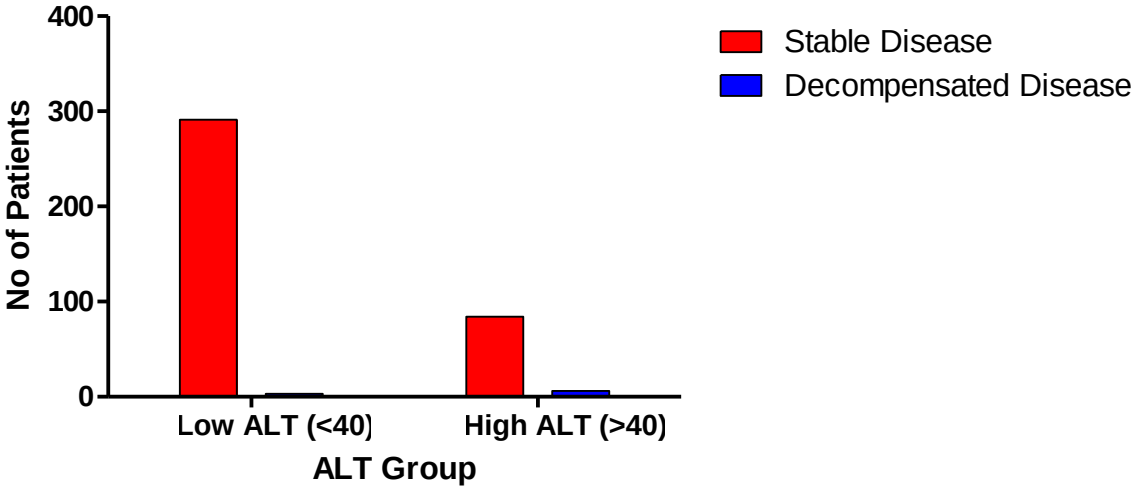

Figure 5-33 ALT vs decompensated disease in first generation immigrant African patients

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
ALT at Diagnosis	Low (≤40)	291/386	3/386	<0.01	6.93	1.70 to 28.30
	High (>40)	84/386	6/386			

Table 5-28 Univariate statistics for ALT vs decompensated disease in 1st degree immigrant African patients

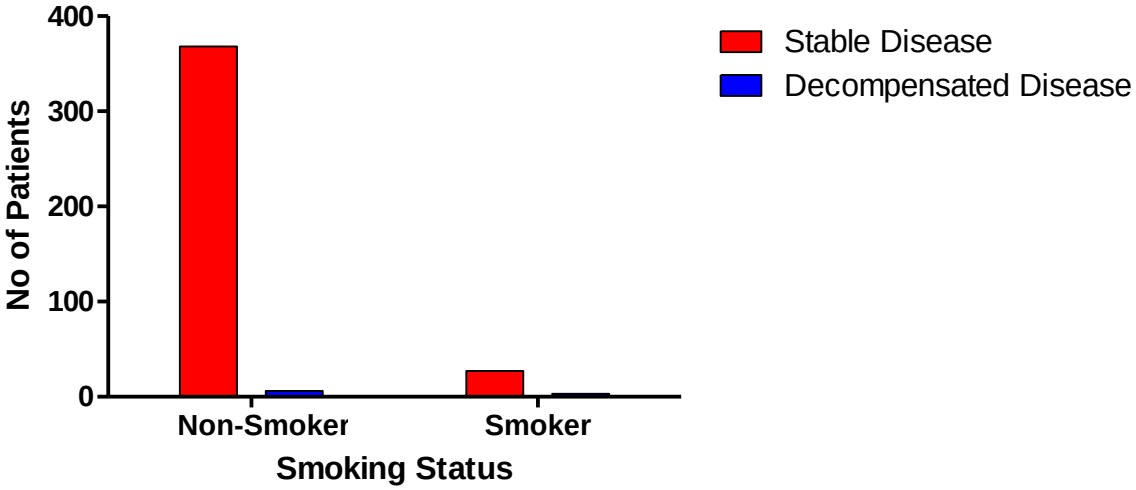

Figure 5-34 Smoking vs decompensated disease in first generation immigrant African patients

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Smoking	Never smoked	368/404	6/404	0.02	6.82	1.61 to 28.77
	Smoker	27/404	3/404			

Table 5-29 Univariate statistics for smoking vs decompensated disease in first generation immigrant African patients

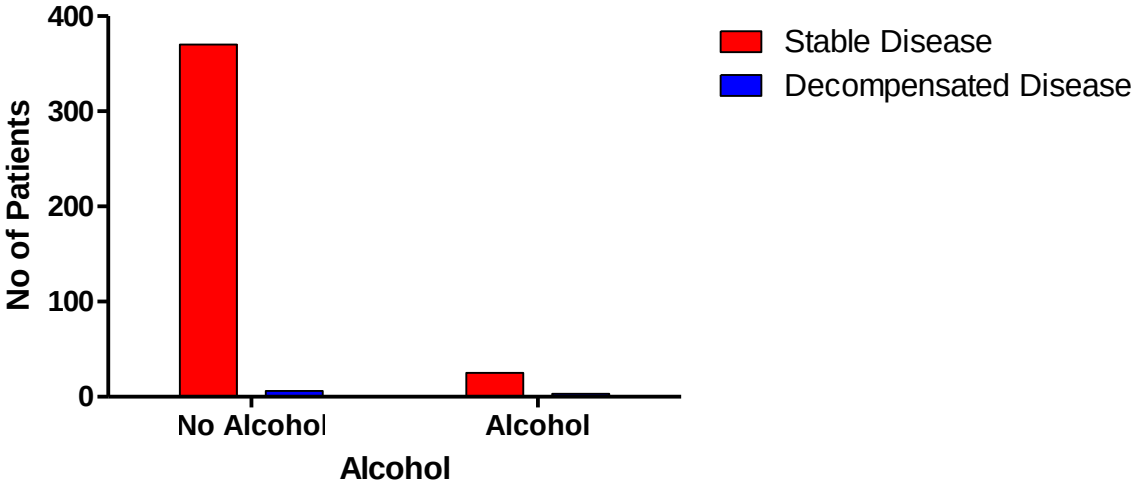

Figure 5-35 Alcohol vs decompensated disease in first generation immigrant African patients

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Alcohol drinker	No alcohol	370/404	6/404	0.02	7.40	1.75 to 31.37
	Alcohol	25/404	3/404			

Table 5-30 Univariate statistics for alcohol vs decompensated disease in first generation immigrant African patients

The significant univariate predictors of decompensated disease: age group, sex, ALT group, smoking, and alcohol, were analysed using multivariate analysis. Again, binary logistic regression using the forced enter method was adopted (see Table 5-31). Only age >40 was statistically significant but with a very wide 95% confidence interval suggesting that it is not very accurate at estimating the true population odds.

	Sig.	Odds Ratio Exp(B)	95% Confidence Interval for EXP(B)	
			Lower	Upper
Age group	<.03	12.50	1.47	106.37
Sex	.42	2.55	.26	25.12
ALT	.10	3.65	.80	16.65
Smoking	.46	1.93	.34	11.10
Alcohol	.18	3.30	.57	19.15

Table 5-31 Logistic regression showing significant variables for predicting decompensated disease with odds ratio and confidence intervals in African patients.

5.4.3 Far Eastern chronic HBV patients

5.4.3.1 Demographics

For the purposes of this study, the Far East was deemed to include all Asian countries North and East of the Indian Subcontinent (see Figure 5-36). Chinese patients made up half of this cohort of chronic HBV patients who attended outpatients. Along with Africa, this region has been classified as being of high HBV prevalence.

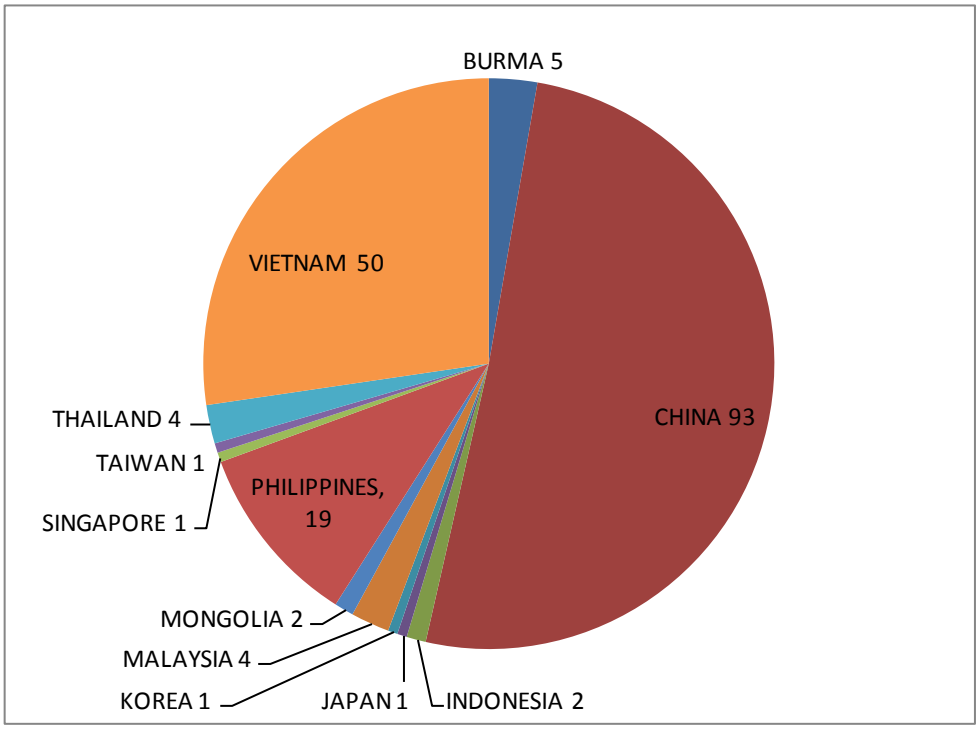

Figure 5-36 Distribution of first generation Far Eastern immigrant patients with chronic HBV attending clinics

5.4.3.2 Risk factors for cirrhosis

This section shows the 2x2 contingency tables, p values, odds ratio and 95% confidence intervals of the various significant risk factors associated with cirrhosis in the first generation immigrant Far Eastern chronic HBV patients. Unlike the African patients the only significant risk factors found using univariate analysis were age (Figure 5-37 and Table 5-32) and sex (Figure 5-38 and Table 5-33). Being on anti-HBV treatment at the present time and in the past were also statistically significant but as mentioned before the clinical significance of this is unclear. Smoking, alcohol, diabetes, ALT group, e-antigen status and HBV DNA viral load at diagnosis were not statistically significant

Four patients with liver cancer did not have underlying cirrhosis and for the purposes of this analysis they were not included in this section.

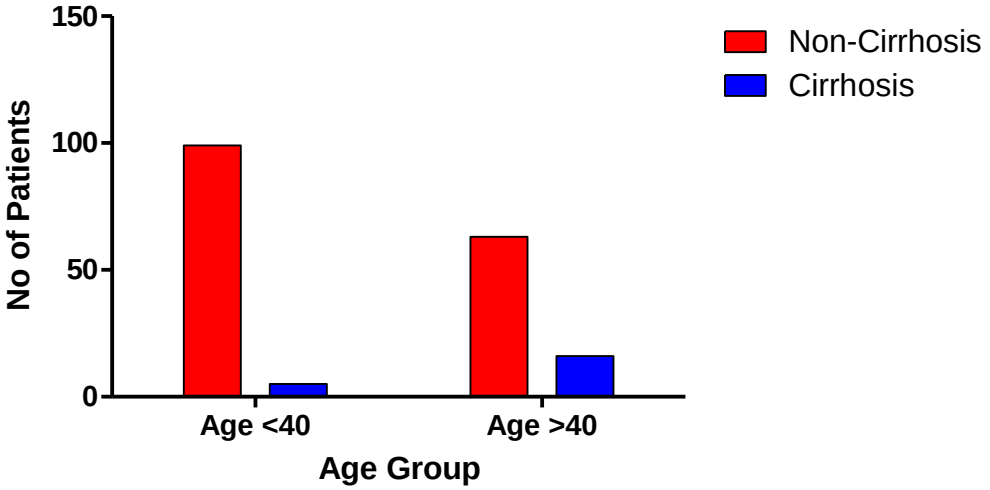

Figure 5-37 Age vs cirrhosis in first generation immigrant Far Eastern patients

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Age	<40	99/183	5/183	<0.01	5.03	1.75 to 14.41
	≥40	63/183	16/183			

Table 5-32 Univariate statistics for age vs cirrhosis in first generation immigrant Far Eastern patients

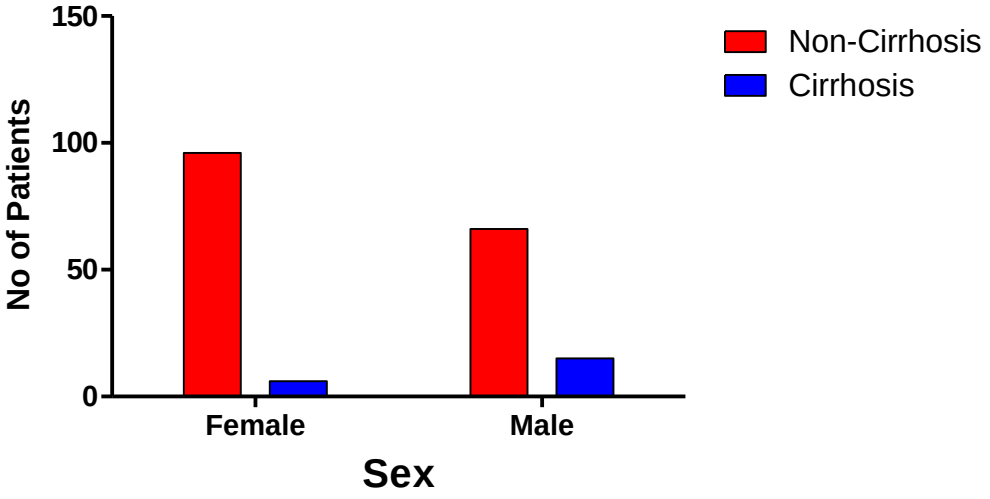

Figure 5-38 Sex vs cirrhosis in first generation immigrant Far Eastern patients

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Sex	Female	96/183	6/183	<0.001	3.64	1.34 to 9.86
	Male	66/183	15/183			

Table 5-33 Univariate statistics for sex vs cirrhosis in first generation immigrant Far Eastern patients

As before, multivariate analysis using logistic regression with certain factors was performed to see if a model for developing cirrhosis could be developed. Unlike African patients, only age group and sex were significant from univariate analyses, and were used for multivariate analysis. A forced enter binary logistic regression was performed.

Table 5-34 shows that both being male and age over 40 are significant predictors for developing cirrhosis in chronic HBV in the 1st degree immigrant Far Eastern patients. The lower limits of the confidence intervals are greater than 1 which gives us confidence that the direction of the relationship we have observed is true in the population.

	Sig.	Odds Ratio Exp(B)	95% Confidence Interval for EXP(B)	
			Lower	Upper
Age group	<.0001	5.26	3.06	9.04
Sex	<.0001	4.74	2.56	8.80

Table 5-34 Logistic regression showing significant variables for predicting cirrhosis with odds ratio and confidence intervals in Far Eastern patients.

5.4.3.3 Risk factors for decompensated disease

This section shows the 2x2 contingency tables, p values, odds ratio and 95% confidence intervals of the various risk factors significantly associated with decompensated disease in the first generation immigrant Far Eastern chronic HBV patients. Unlike the analysis of cirrhosis, sex was not a significant risk factor for decompensated disease, but age (Figure 5-39 and Table 5-35), smoking (Figure 5-40 and Table 5-36) and diabetes (Figure 5-41 and Table 5-37) were. In addition to these, being on anti-HBV treatment, either at the present time or at any time in the past were also significant risk factors. ALT, alcohol, e-antigen status and HBV DNA viral load were not statistically significant.

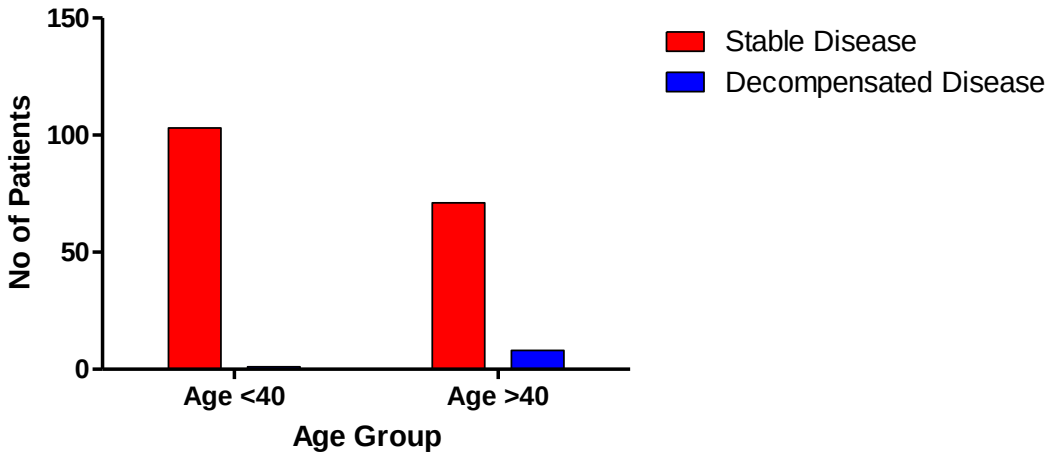

Figure 5-39 Age vs decompensated disease in first generation immigrant Far Eastern patients

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Age	<40	103/183	1/183	<0.01	11.61	1.42 to 94.89
	≥40	71/183	8/183			

Table 5-35 Univariate statistics for age vs decompensated disease in first generation immigrant Far Eastern patients

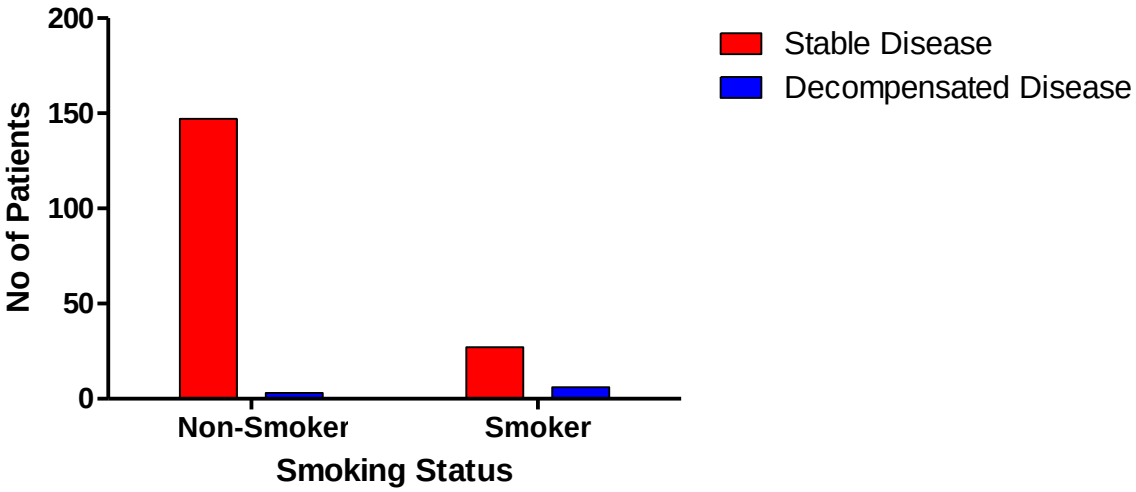

Figure 5-40 Smoking vs decompensated disease in first generation immigrant Far Eastern patients

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Smoking	Never smoked	147/183	3/183	<0.001	10.89	2.57 to 46.22
	Smoker	27/183	6/183			

Table 5-36 Univariate statistics for smoking vs decompensated disease in first generation immigrant Far Eastern patients

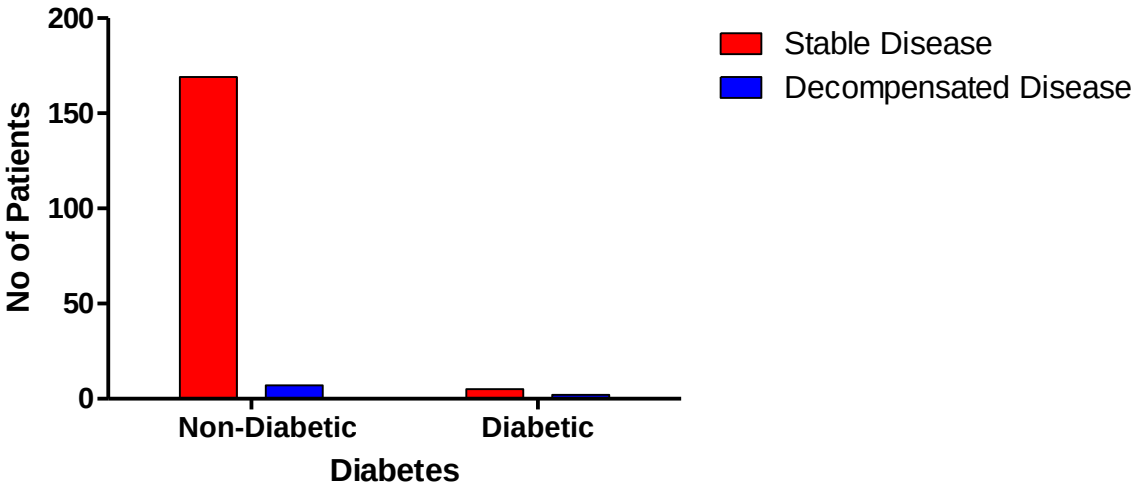

Figure 5-41 Diabetes vs decompensated disease in first generation immigrant Far Eastern patients

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Diabetes	Non-diabetic	169/183	7/183	0.04	9.66	1.59 to 58.78
	Diabetic	5/183	2/183			

Table 5-37 Univariate statistics for diabetes vs decompensated disease in first generation immigrant Far Eastern patients

Unlike Africans and Indian Subcontinent patients, where male sex and alcohol were significant risk factors, now only age group, smoking and diabetes were found to be significant and were used for multivariate analysis. A forced enter binary logistic regression was performed.

Table 5-38 shows that all 3 factors were statistically significant predictors for developing decompensated disease. However in each of the 3 cases (age >40, smoking and the presence of diabetes), there were large 95% confidence intervals meaning that they are not very accurate in estimating the true population odds.

	Sig.	Odds Ratio Exp(B)	95% Confidence Interval for EXP(B)	
			Lower	Upper
Age group	<.0001	19.61	4.54	84.68
Smoking	<.0001	6.49	3.08	13.68
Diabetes	<.01	2.87	1.29	6.41

Table 5-38 Logistic regression showing significant variables for predicting decompensated disease with odds ratio and confidence intervals in Far Eastern patients

5.4.4 Eastern European chronic HBV patients

5.4.4.1 Demographics

Although not as large as Africa, Europe and especially Eastern Europe has recently undergone political upheaval that has resulted in the formation of a number of new independent states. For the purposes of clarity in describing where these patients originated from, patients from the former Soviet states (Azerbaijan, Moldova, Ukraine and Uzbekistan) and the former Yugoslav states (Bosnia, Kosovo and Macedonia) were grouped together (see Figure 5-42). In 16 of the 87 cases, the exact country of origin could not be gleaned from the notes.

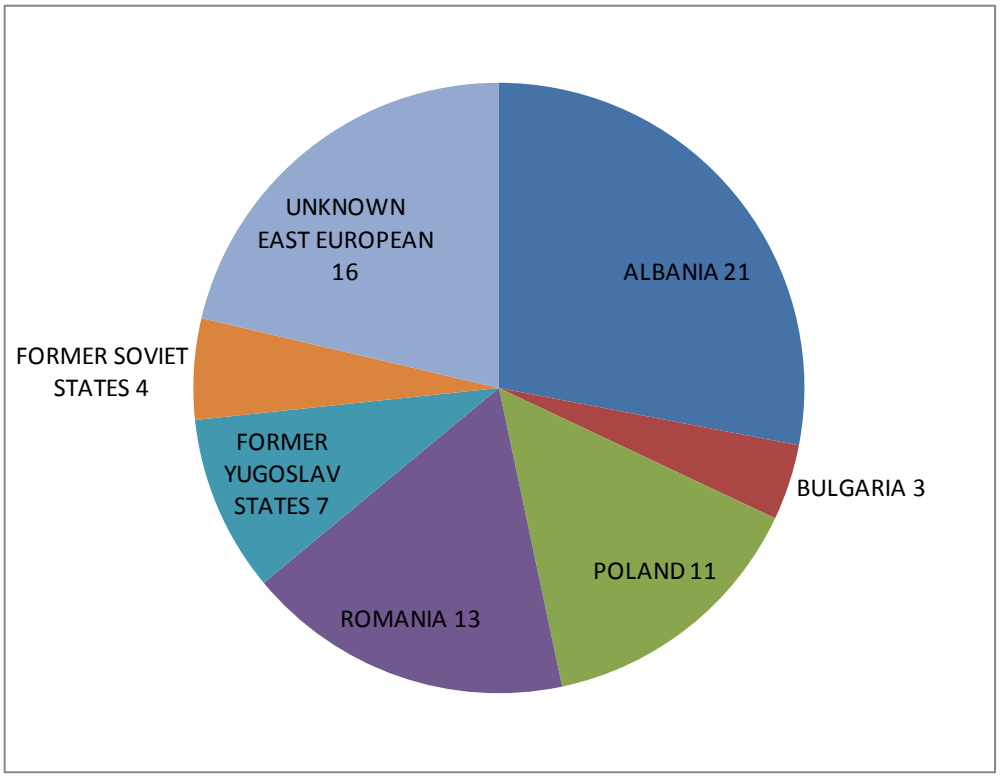

Figure 5-42 Distribution of first generation Eastern European immigrant patients with chronic HBV attending clinics

5.4.4.2 Risk factors for cirrhosis

The 2x2 contingency tables, p values, odds ratio and 95% confidence intervals of the various risk factors significantly associated with cirrhosis in first generation immigrant Eastern European chronic HBV patients are shown below. The only significant risk factors are age (Figure 5-43 and Table 5-39) and smoking (Figure 5-44 and Table 5-40). Being on anti-HBV treatment in the past was also statistically significant but not currently being on treatment. Other independent factors like sex, ALT, alcohol, diabetes, e-antigen status and HBV DNA viral load at diagnosis were not statistically significant. Only 1 patient developed liver cancer and that was on a background of underlying cirrhosis; he was included in the analysis of cirrhosis here.

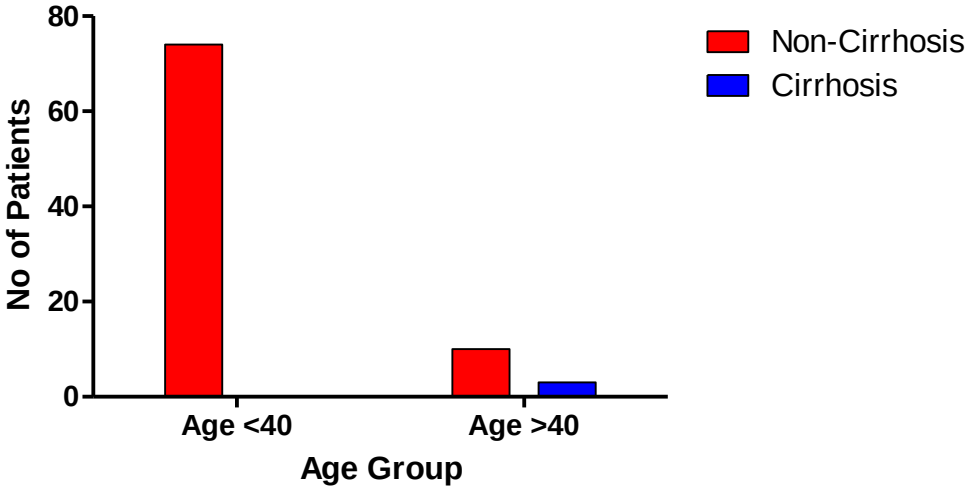

Figure 5-43 Age vs cirrhosis in first generation immigrant Eastern European patients

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Age	<40	74/87	0/87	<0.01	49.67	2.39 to 1032
	≥40	10/87	3/87			

Table 5-39 Univariate statistics for age vs cirrhosis in first generation immigrant Eastern European patients

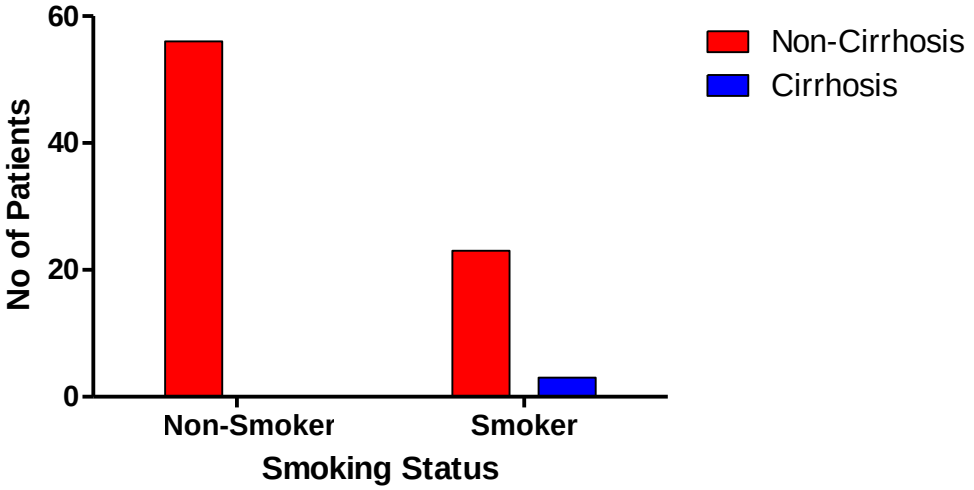

Figure 5-44 Smoking vs cirrhosis in first generation immigrant Eastern European patients

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Smoking	Never smoked	56/82	0/82	0.03	16.83	0.84 to 339.0
	Smoker	23/82	3/82			

Table 5-40 Univariate statistics for smoking vs cirrhosis in first generation immigrant Eastern European patients

The 2 risk factors associated with cirrhosis, age and smoking, were analysed further using logistic regression to see if a model for developing cirrhosis could be predicted.

A force enter binary logistic regression was performed but it showed that neither variable was statistically significant when analysed using multivariate statistical tests.

5.4.4.3 Risk factors for decompensated disease

In the first generation immigrant Eastern European chronic HBV patient group, only one patient had decompensated disease and that was due to liver carcinoma. Therefore it is unlikely to be very meaningful to display contingency table of these risk factors with this condition. Through univariate analysis, only treatment ever and diabetes were statistically significant. Since only 2 patients were diabetic and one of those had cancer it would appear that this is not clinically significant.

5.4.5 British chronic HBV patients

5.4.5.1 Risk factors for cirrhosis

The only significant risk factor for developing cirrhosis in White-British chronic HBV patients was smoking (see Figure 5-45 and Table 5-41). Treatment at present was also statistically significant but this has already been discussed previously. The other risk factors of age, sex, ALT at diagnosis, alcohol, diabetes, eAg status and HBV DNA viral load at diagnosis were not significant. Because of the lack of other significant risk factors, no further analysis was possible.

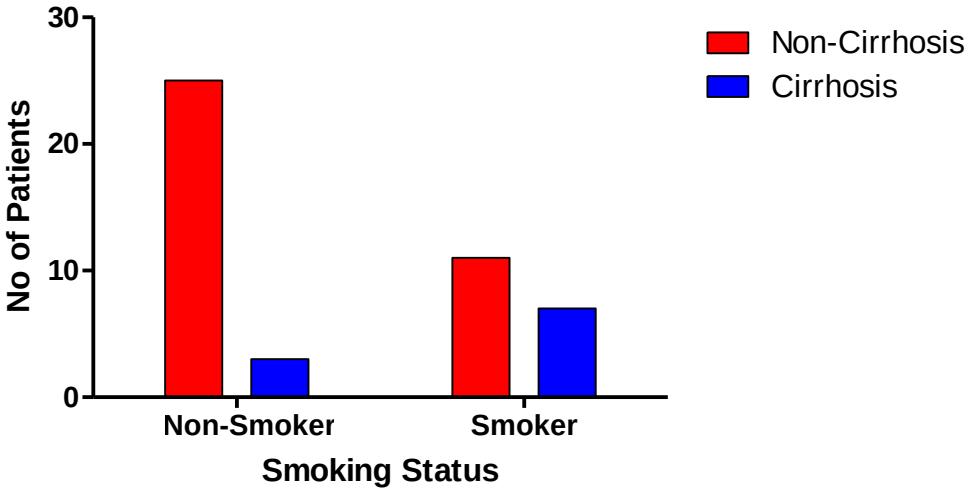

Figure 5-45 Smoking vs cirrhosis in British patients

Risk Factor	Category	Non-Cirrhosis	Cirrhosis	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Smoking	Never smoked	25/46	3/46	0.033	5.30	1.15 to 24.43
	Smoker	11/46	7/46			

Table 5-41 Univariate statistics for smoking vs cirrhosis in British patients

5.4.5.2 Risk factors for decompensated disease

As with the risk factors for cirrhosis in British patients, the same risk factor, smoking, was associated with the development of decompensated disease (see Figure 5-46 and Table 5-42). Treatment ever was also associated with the development of decompensated disease. The other risk factors of age, sex, ALT at diagnosis, alcohol, diabetes, e antigen status at diagnosis, and HBV DNA viral load at diagnosis were not significant. The lack of other significant risk factors meant that no further analysis was possible.

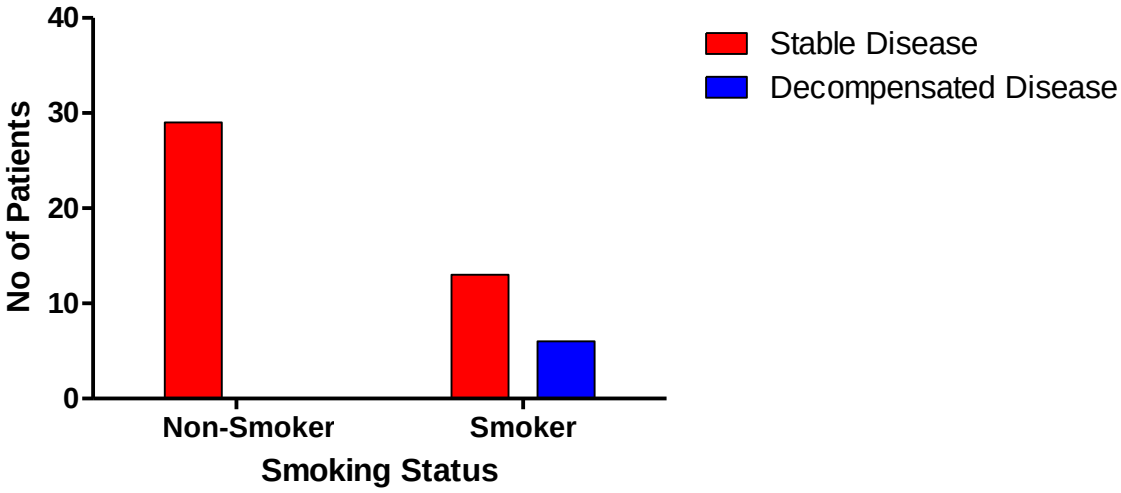

Figure 5-46 Smoking vs decompensated disease in British patients

Risk Factor	Category	Stable Disease	Decompensated Disease	Fisher's Exact P value	Odds Ratio	95% Confidence Interval
Smoking	Never smoked	28/46	0/46	0.002	28.41	1.49 to 541.90
	Smoker	12/46	6/46			

Table 5-42 Univariate statistics for smoking vs decompensated disease in British patients

5.5 Discussion

5.5.1 Prevalence of HBV in all ethnic groups

This study has given estimates of the prevalence of diagnosed HBV being seen in the two local, secondary care hospitals. Table 5-1 shows the disparity between the numbers of patients being reviewed in hospital compared to the potential numbers who are in the local community unaware that they are carrying HBV. Of the 1209 CHB patients being followed up in hospital, we estimate there are another 6220 (84%) who are undiagnosed in the community and not having outpatient care. A proportion of these will have cirrhosis, because stable disease doesn't cause any specific symptoms. However, very few of the undiagnosed HBV individuals are likely to have decompensated liver disease because this would manifest itself as ascites, variceal bleeding, encephalopathy, jaundice or HCC and would likely cause most patients to seek medical attention.

Determining HBV prevalence from clinic attendance is bound to under-estimate the true figure because the values are dependent on clinic and local population numbers. In the Central Asian group, 37 patients from a relatively small population of 1098 gave a prevalence of 3.4% which is much higher than the high endemic regions of the Far East and Africa, which we calculated as having 2.1% and 1.5% respectively. Whilst 3.4% may be a relative accurate calculated prevalence for this region, the calculated values for the Far Eastern and African patients are very low when compared to the derived infection prevalence back home. Clinic patients are a self-selecting group in that they choose to attend their hospital appointment for investigation and management of their condition. GP referrals would give a more accurate result but it still is dependent on the patient seeing their doctor to have the necessary blood tests. Pregnant women and blood donors have their blood screened automatically for HBV but only represent part of the population and are still "volunteering" to have the blood test. Only by screening everybody could you get the true prevalence of HBV. With time the prevalence of CHB in other countries will decrease because of the

universal perinatal HBV vaccination programme, which should reduce the pool of HBV available to infect others.

At the time of this study I did not record the patient's source of the referral or whether they already knew they had underlying HBV at the time of their first clinic appointment. In a number of cases this information was not recorded and so it is not possible to comment on why they presented to hospital services when they did. However from clinical experience referrals typically come from 3 sources: GPs, antenatal service and genito-urinary clinics. Most patients who get referred from GPs are asymptomatic and HBV is diagnosed either because another family member or partner has been diagnosed with HBV, or because the patient has some derangement of the liver function tests and the GP has followed this up by performing hepatitis serology testing. All pregnant women are automatically tested for HBV, HIV and rubella unless they specifically object to having these tests done. A proportion of these may be having their 2nd or more child and so HBV would (hopefully) have been detected in their 1st pregnancy. Only patients with HBV mono-infection would have been referred from genito-urinary clinics as they tend to manage the HIV-HBV co-infected patients. A considerable number of patients are referred but did not attend hospital. In this study we only included those patients who attended as then we could determine their socio-demographic and disease status.

There is speculation as to whether the health of migrants is the same as the population that they have emigrated from. It may well be that migrants are "healthier" than non-migrants and so the prevalence of HBV might be less in this population than back home. The healthy migrant effect and our population based study confirm that this applied to people from the Indian sub-continent and is likely to apply to the ethnic groups described here.[172] However, individuals from Eastern Europe typically have a lower standard of living, including worse healthcare and job prospects, back in their home country compared to the UK. In this case, economic reasons may mean that poorer, and possibly less healthy, people migrate and bring their underlying conditions with them. Because their countries are part of the European Union, they are entitled to free NHS healthcare and so maybe keener to

engage with health services than other ethnicities. This could help explain why more of a proportion of this group is seen in the clinics. Cultural beliefs such as stigma and understanding of what the infection is and its sequelae can be huge obstacles to being screened, getting referred, having treatment and vaccinating the rest of the family.[209;231]

Eighty-eight out of 1209 patients had cirrhosis confirmed by histology or radiology. The ethnic groups with the highest numbers of cirrhotics were patients from Africa (27), Indian Subcontinent (31), and the Far East (21). Of note, around a quarter of Caucasian British patients being followed up in the clinic had cirrhosis (10/47). The Caribbean and British groups had relatively high proportions of cirrhosis although small total numbers of patients attending the clinics. Given the size and population of the respective countries compared to densely populated regions such as Africa and Asia, this would suggest that these patients tend to seek medical help sooner rather than being associated with worse disease. Univariate analyses looking at immigrants of all ethnicities showed that age, sex, ALT, smoking and diabetes were all significantly associated with the development of cirrhosis. When multivariate analyses were employed age over 40, male sex and raised ALT appeared to predict cirrhosis even when controlling for the other variables.

Thirty-four out of 1209 patients had decompensated disease as shown by cirrhosis with complications or HCC; the latter need not necessarily occur in cirrhotic patients. Again the ethnic groups with the highest numbers of decompensated disease came from Africa (9), the Indian Subcontinent (14) and the Far East (9). Six British patients had decompensated disease. As in chapter 4, the statistically significant variables for decompensated disease were the same: age, sex, ALT, smoking, alcohol and diabetes. Using multivariate statistics in the form of binary logistic regression age over 40, smoking and presence of diabetes were predictors of decompensated disease.

The presence of diabetes was significantly associated with cirrhosis and decompensated disease on univariate analysis. HBV is not a commonly recognised risk factor for the development of diabetes unlike the association between hepatitis C

and diabetes. A recent meta-analysis has shown that diabetes that develops after HBV has been diagnosed is linked with cirrhosis and decompensated disease.[232] Unfortunately our study could not determine the temporality of the diabetes to the infection.

5.5.2 Cirrhosis and decompensated disease in African immigrants

There were 417 first generation African immigrants seen in hospital. Although 24 countries were represented, individuals from Ghana, Nigeria and Somalia made up over half of this population. Collectively they have the highest proportion of hepatitis B patients seen in the outpatient clinics. Most of the countries represented have high endemicity rates greater than 8% but our study suggested that the prevalence of HBV was 1.5%. As has been proposed before, it may be that the more affluent persons are the type to leave the original country and that these people may be relatively healthier which is why the prevalence is less than back in their home country.

Of this cohort 6.5% had cirrhosis. The risk factors for cirrhosis in this group were age over 40, male sex and high ALT from univariate statistics. Using multivariate statistics, age and ALT were significant but with low odds ratios and wide 95% confidence intervals.

Decompensated disease occurred in 2.2%. There are different risk factors for developing decompensated disease: male sex, age >40, high ALT, smoking, and alcohol. Only age >40 was statistically significant when the other variables were controlled for.

5.5.3 Cirrhosis and decompensated disease in Far Eastern immigrants

There were 183 first generation Far Eastern immigrants seen in the clinics. As with individuals from Africa, although a number of countries are included in this region,

certain countries are more represented than others in the outpatient clinics. China makes up more than half of the patients, Vietnam about a quarter and the Philippines about 10%. Countries such as Indonesia and Japan have larger populations than Vietnam and the Philippines but have only about 1% of the outpatient attendance. In this way outpatient attendance is more of a reflection of net immigration to the UK than population back in the home country. As with other ethnicities, the estimated prevalence is dependent on those patients who have been identified and attending clinic. As mentioned before, language difficulties and cultural differences may mean that individuals from an ethnic Chinese background may prefer to attend another centre further afield if they can bypass the usual referral routes and can speak to someone in their language rather than via an interpreter[224].

In this group 11.5% had cirrhosis. The only risk factors for cirrhosis were age and sex, which were also statistically significant at multivariate level. Although the odds ratios are fairly low, the corresponding 95% confidence intervals are not too wide.

Decompensated disease was found in 4.9% of this cohort. Unlike the risk factors for cirrhosis, sex was not a significant risk factor but, smoking and diabetes were along with age. All of these were also statistically significant at multivariate level.

5.5.4 Cirrhosis and decompensated disease in Eastern European immigrants

Approximately 8.5% (87) of the total number of patients seen were first generation Eastern European immigrants. Albanians, Romanians and Poles make up the predominant ethnicities identified. The smaller numbers than expected to come to the clinic are the same as for the other ethnic groups and due to language difficulties predominantly.

Cirrhosis was seen in 3.4% of this cohort. Only age and smoking were significant risk factors using univariate analyses. Neither risk factor was significant using multivariate statistics.

Only 1 patient developed decompensated disease, in this case HCC, and so univariate statistics were not used as they would be unlikely to be statistically or clinically significant.

5.5.5 Cirrhosis and decompensated disease in British patients

Forty-seven Caucasian, British patients with chronic, mono HBV infection were being followed up in the hepatology clinic. Unlike the other ethnic groups, the UK is regarded as a country of low prevalence. It may be that infection in this group is more likely to be due to horizontal transmission through sex or via blood (illicit drug use, operations and needlestick injuries) and less likely through maternal or childhood transmission.

Ten (21.2%) patients had cirrhosis and 6 (12.7%) had decompensated disease. In both cohorts smoking was the only significant risk factor. Unlike other cohorts neither age nor sex had any bearing on the development of advanced disease (cirrhosis or decompensation). If the disease was transmitted horizontally rather than vertically then it may be that there has been less time for the disease to progress compared to individuals from more endemic countries. Significantly more patients with cirrhosis and decompensated disease are on treatment and this is most likely an attempt to prevent further sequelae occurring.

5.5.6 Bias and confounding

Determining the prevalence of HBV in these ethnic groups was reliant on primary care referring infected patients to the hospital. Case finding is very difficult in patients who are asymptomatic[206]. Patient reluctance to be seen or primary care perceptions that this is a disease that is either benign or incurable may have had an impact on referral patterns, and ultimately numbers being seen in clinic. Other sources of referral include maternity and attendees of genito-urinary clinics, where all mono HBV infections should be referred for specialist follow up. These latter patients can bypass

their general practitioners and can self-refer themselves; they may not necessarily be seen in the hepatology clinic unless the patient and consulting physician agree to do so.

Figure 5-6 shows that 135 patients, including all the White British patients, were born in the UK. As mentioned in Chapter 4, routine screening of mothers for HBV was not fully implemented until April 2000[207] and so it cannot be said with total certainty whether the second generation immigrants and British patients were born with the disease or got it transmitted to them horizontally. Also it is not possible to say whether they acquired their disease because of vaccine failure unless they were specifically vaccinated as a baby.

Figure 5-8 shows a preponderance of young females of all ethnicities (age 16-39) being seen in the clinic. This is probably a reflection of them being detected through antenatal screening which is automatically done by 20 weeks gestation. All women who screen positive for HBV are referred to hepatology services. It makes sense that if more women are screened, then there will be a higher detection and referral rate, and by inference, increased clinic attendance rate.

The accuracy of all retrospective studies is dependent on the results of patient notes and records. This will have a subsequent impact on the significance of proposed risk factors. ALT, HBV DNA and e-antigen status were all taken from the earliest recorded visit that the patient made to the hospital but the recorded results may have been taken from a much later visit because the test was not requested at first visitation. Similarly, the effects of the amount and duration of smoking and alcohol or the severity of diabetes were not possible to elucidate from some of the notes.

Again the role of anti-viral medications on disease progression is hard to estimate. Treatment could have been initiated to halt disease that was already progressing or as a preventative measure against acquiring HCC or cirrhosis.

5.5.7 Comparison with other studies

Along with other studies, we have used clinic attendance as a surrogate marker for determining the prevalence of the disease. The problem with this is that it relies up on the patient seeking medical help in order to calculate the scale of the problem. A European report suggests that 90% of carriers don't know that they have viral hepatitis and that only 21% know what that means.[231] Our study estimated the prevalence in the Far Eastern population (including Chinese) to be 2.1% but other studies have quoted much higher values in Chinese immigrants. A study looking at patients attending genito-urinary clinics in London, where no external referral is necessary, estimated the prevalence to be 12.8%.[224] A Central London health centre that has Chinese speaking doctors and purely deals with patients of Chinese origin quotes similar numbers (personal communication). Even though healthcare in the UK is free, patients' perception of their disease and their ability to relate to someone who understands their language can affect their attendance. However such studies may give an indication of numbers of patients who are HBsAg positive but don't report any more about the prevalence of cirrhosis and decompensated disease.

A recent study by McPherson et al did look at HBV screening and disease severity in Chinese immigrants in North East England using DBS from volunteers attending health centres and churches. They found 53/606 tested positive for HBsAg but only 31 accepted the invitation for outpatient review and investigation. Three patients were found to have active disease and started on treatment, but none had cirrhosis or decompensated disease.[173]

In the SIMIT cross-sectional study, all the HBV outpatient attendances over a 5 month period to 74 centres in Italy were analysed. Of 3760 patients included, about a quarter were immigrants. Most of the immigrants were from Central Italian centres, which would have included the capital Rome, whilst the Italian cases were distributed more in the South and North. Most studies looking at immigrants typically show that immigrants seem to prefer to live in the metropolitan cities and capitals. Asia and Eastern Europe were the predominant regions of origin. The immigrants were

younger, predominantly female, less likely to be co-infected with HCV, HDV or HIV, less likely to be HBeAg positive, and were less likely to have cirrhosis or HCC.[226] However, the study period was short, significantly more immigrants presented on only 1 occasion and fewer of this group had had liver biopsies, meaning that it would be difficult to establish the true stage of the disease if the patients did not continue their follow up. The same applies to treatment as it is very unlikely treatment would have been started on the first outpatient visit unless the patient was known to have active HBV infection. At the time of our study the routine testing of HDV was not common place and so it is possible that we may have had more HDV co-infections had we tested for it.

The CUSHI-B study involved analysing 698 demographics, clinical details and blood samples of CHB patients referred from 15 centres over a 2 year period. Patients were born in 61 countries, with most coming from the Commonwealth countries of Asia and Africa, which generally reflects the ethnic mix of UK. Sixty percent were men and aged between38-50. Like the SIMIT study, genotype analysis was performed and showed good correlation between ethnicity and genotype. At the time of our study genotype analysis was not routinely done on all patients with HBV infection. The study does suffer from referral bias as immigrants may well be over-represented. Our study looked at all attendances to two hospitals in East London (neither of which were included in the CUSHI-B study), rather than look at a selection of cases of varying severity referred from multiple centres, each of which have differing rates of prevalence.

5.5.8 Conclusion

The results of this study show that there is massive under-diagnosis of CHB in immigrants in East London and as such we are probably under treating this disease. As shown in chapter 4, various factors are associated with progression to decompensated disease (decompensated cirrhosis and HCC). Allowing us to predict

disease progression will help us plan and develop adequate services for the future as well as trying to reduce morbidity and mortality.

Further studies need to examine the strength of the correlates of this association. For example, the role of different genotypes was not explored and this may well have a role to play; different ethnicities are associated with different genotypes which are also associated with varying rates of HCC incidence.[233] Similarly the roles of smoking and diabetes have not been explored very much in the past and yet are significant here. The possible reasons for why they may have an effect are discussed in Chapter 6.

6 Discussion

6.1 Summary of findings

The purpose of this thesis was to investigate chronic hepatitis B infection in the immigrant communities of East London. Chapters 3, 4 and 5 deal with prevalence and the risk factors for cirrhosis and decompensated liver disease (decompensated cirrhosis and HCC) in the Bangladeshi & Pakistani and other immigrant groups. Chapter 2 details the preliminary work done by myself in collaboration with others in validating an OMT method for diagnosing CHB infection. This was necessary in order to calculate the prevalence of HBV in the Bangladeshi and Pakistani populations as described in chapter 3.

The Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus (REVEAL-HBV) study is the landmark study looking at the natural history of CHB infection. Approximately 90,000 residents from 7 townships in Taiwan were offered screening for HBV, of which about 24,000 accepted. Of these, 4155 were HBsAg+ but 3653 individuals were included in the study. Over an 11 year period and without being given antiviral treatment, they showed that CHB infection was associated with increased mortality from all causes of liver disease, HCC, chronic liver disease and cirrhosis. The study authors proposed that elevated HBV DNA ($>10^4$ copies) at baseline was the most important predictor of developing HCC and was independent of HBeAg, ALT, genotype status and even the presence of cirrhosis (on ultrasound).[50;234] This is a ground breaking study that, for obvious ethical reasons, cannot be repeated. However, the study does provide an insight into the factors that should be incorporated into future models and we have used these factors in a cross sectional analysis of patients with chronic hepatitis B living in East London.

The REVEAL study examined only patients from SE Asia. The UK as a whole has low prevalence for HBV, but in East London there are multiple, diverse ethnic communities with a high prevalence of CHB. Since patients of different ethnicities living in a Western society have not been studied in detail and as our clinical

impression was of slow disease progression in patients of Bangladeshi and Pakistani origin, we studied the epidemiology and natural history of CHB in our local community.[172] As many immigrants originate from Bangladesh and Pakistan we conducted a detailed analysis of this population and also studied other communities where data was available. A number of assumptions were made in our study. We assumed that representative people from Bangladesh and Pakistan would attend local mosques (allowing the community prevalence to be estimated from studies in local religious centres) and because healthcare is provided free, we assumed that those with severe disease would attend the local hospitals. Although these assumptions could be challenged we believe that they are reasonable and an on-going study involving testing in primary care will allow some of these assumptions to be examined in further detail. Our study targeted this immigrant group to see if the same risk factors found in the REVEAL study also applied here. We looked at hospital attendance and the development of advanced disease (cirrhosis and decompensated liver disease) over a 2.5 year period whilst analysing socio-demographic, biochemical and virological factors.

The major findings of this study are summarised below:

1. In the Bangladeshi and Pakistani populations, cirrhosis is significantly associated with older age (≥40), male sex, elevated ALT (>40) at diagnosis, smoking and diabetes. The same factors and alcohol are associated with decompensated liver disease at a univariate level. At multivariate level, age and sex are statistically significant predictors of cirrhosis and smoking is a predictor of decompensated disease. HBeAg status, HBV DNA and genotype are not associated with either condition. Only men over the age of 40 were admitted to hospital with decompensated liver disease. The calculated annual admission rates were 1.4% for Bangladeshis and 2.7% for Pakistanis.
2. Looking at all immigrant groups together, the same variables, at univariate level, for developing cirrhosis and decompensated liver disease were noted. With multivariate analysis, older age, male sex and elevated ALT are

predictors for cirrhosis, and older age, smoking and presence of diabetes are predictors for decompensated disease.

Age and smoking were significant predictors in all the ethnic sub-groups at univariate level.

6.2 Unexpected findings

The most surprising finding of this thesis was that baseline HBV DNA and HBeAg status had no predictive value for the development of cirrhosis or decompensated liver disease in this population or any of the ethnic sub-groups. The original REVEAL-HBV study showed that high viral loads (>10^6 copies/ml) were significantly associated with HCC and cirrhosis over time, but that low viral loads (<10^4 copies/ml) could also be linked with these same complications.[50;65] The study authors imply that high levels of circulating virus are responsible, to a degree, for disease progression. This idea is endorsed by international consensus guidelines for initiating antiviral treatment.[47;157] The problem is that the REVEAL-HBV authors and others do not explain what happens to viral load once these complications have occurred. It may be that with cirrhosis and HCC, the ensuing advanced hepatocyte damage leads to clearance of the virus from the liver and a corresponding drop in circulating virus. As the viral load decreases it would typically be associated with HBeAg negativity. The liver damage associated with HBV infection is immune-mediated because HBV is not directly cytopathic as shown by the immune tolerant phase, where there are high viral loads, HBeAg positivity and normal ALT levels. This is followed by an immune active/clearance phase, where there is liver inflammation associated with elevations to ALT, reduction in viral load and HBeAg loss. The duration and extent of this phase varies on an individual basis but in some cases may lead to end-stage liver disease even with the absence of symptoms. The REVEAL authors do contend that significant damage may occur many years after HBeAg seroconversion and this could explain why HBeAg is less important in predicting HCC. The REVEAL-HBV study does not

mention what happened to HBV DNA levels (or HBeAg) during follow up and it may be that with the onset of cirrhosis and HCC these amounts decreased.

The role of HBV DNA in predicting cirrhosis and HCC is complicated. It is assumed that cccDNA transcription correlates with intrahepatic HBV DNA which in turn is reflected by serum HBV DNA but this may not necessarily be the case.[235] One of the problems about using baseline HBV DNA levels is who to screen. In the original REVEAL-HBV study, the subjects were aged 30 or older when an elevated HBV DNA would probably have been associated with the immune active phase. In our study, the youngest patients were age 16 and their elevated viral loads most likely reflected the underlying immune tolerant phase. It is impossible to say from the duration of our study whether those individuals would have gone on to develop advanced liver disease. Viral load is also associated with HBV genotype. Genotype B has been shown to cause HCC in younger patients, that do not necessarily have to develop cirrhosis, and this can occur with lower viral loads and ALT.[236;237] Genotypes and associated mutations (such as the basal core promoter mutation A1762T/G1764A) were not tested in our study as it was not routinely available and so this could have made a difference to our results. In the REVEAL-HBV study, those patients who developed HCC with low viral loads tended to be older and drank alcohol. Alcoholic liver disease can cause cirrhosis HCC in its own right and the extent to which this played a part cannot fully be assessed. It maybe there are different genotypes of HCC and cirrhosis to which the role of viral load is still emerging.

In our study we differentiated HBV DNA into 2 groups: those with viral loads ≤4.0 versus >4.0 log IU/ml. Young patients with immune tolerant disease typically have very high viral loads, are HBeAg positive and have normal ALT. These patients could therefore have been put in a separate group with very high viral loads >8.0 log IU/ml as they may have been contributing to HBV DNA being statistically insignificant as a risk factor for cirrhosis and decompensated disease. However on scrutiny of the data, it is not straightforward determining who is in this immune tolerant group. Forty-five patients had viral loads >8.0 log IU/ml and were HBeAg positive. Of these only 14 patients had normal ALT (<40): 9 were Far Eastern, 2 African, 1 Bangladeshi and 2

were Europeans. All of these patients were aged between 22 and 41. Five of these patients had liver biopsy of which 4 were 0/6 fibrosis but 1 had cirrhosis (6/6). This result would appear to justify our decision to keep HBV DNA as 2 groups (high and low).

Our study showed that smoking (or previous smoking) was statistically significant at univariate level for cirrhosis and multivariate level for decompensated disease. The original REVEAL-HBV study also found smoking to be a significant predictor of cirrhosis and HCC but only at univariate level. The same authors have constructed a REVEAL nomogram with a REACH-B (risk estimation for HCC in chronic hepatitis B) to help predict 3, 5 and 10 year risk of developing HCC, but whilst alcohol consumption and ALT, amongst others are included, smoking history isn't.[204;238] Up until recently, smoking was regarded as "controversial" in the development of HCC due to HBV.[239] This is in spite of the International Agency for Research on Cancer stating that HCC was a smoking-related cancer.[240] A recent meta-analysis of 9 studies claims that there is an additive interaction between CHB and HCC although this link is not as strong as the relationship between HCV and HCC. How this happens is not fully understood but there are a number of possibilities: tobacco smoke contains a number of toxins and carcinogens (such as benzo[a]pyrene, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, and N'- nitrosonornicotine) which are metabolised in the liver; liver fibrosis may occur due to increased oxidative stress, systemic and tissue inflammation, tissue hypoxia, iron accumulation, hepatocellular injury and insulin resistance; or it may be due to the additive (and confounding) effects of alcohol which is often taken at the same time.[179;241] It is possible that smoking acts as an initiator of carcinogenesis and HBV contributes as a promoter through liver inflammation and cellular inflammation. Given the results of our findings it suggests that more effort should be spent trying to persuade HBV patients to stop smoking and see whether this improves their chances of not getting HCC.

One of the unique features about studying HBV in a predominantly Muslim population of Bangladeshis and Pakistanis is that because of their religious beliefs, the

vast majority (>95%) don't drink alcohol, and so this removes alcohol as a potential confounding variable in the development of end stage liver disease. However, we know from the treatment of the same population of patients who have been infected with HCV, that genotype 3 is their predominant genotype, and this is associated with steatosis and diabetes. Diabetes is associated with the metabolic syndrome, hyperlipidaemia, excess body weight and obesity and all of these have been implicated with the development of cirrhosis and HCC secondary to NAFLD/NASH. Another peculiar finding is that this phenomenon occurs when applied to the total immigrant population, of whom almost 90% claimed not to drink alcohol. This leaves the question as to whether the liver complications are due to diabetes/NASH rather than HBV or whether HBV, cirrhosis and HCC cause diabetes. Up until now there has been a dearth of studies showing a link between HBV and diabetes in the progression of liver disease. A recent, retrospective, nationwide, cohort study from Taiwan looked at the incidence of diabetes in patients who had already been diagnosed with CHB to see whether they developed cirrhosis or decompensated liver disease over a 9 year period. Of 14,523 patients identified with HBV from a national insurance registry, 351 developed diabetes over the study period and 7886 were followed up as non-diabetic HBV controls. The authors found that those patients who developed diabetes tended to be older and have more co-morbidities than the controls. In the diabetic cohort there was a statistically significant difference in the development of cirrhosis and decompensated liver disease, and that the latter complication always followed the development of cirrhosis. This work relied on the review of insurance registries and the accuracy of the people recording the information, but the authors cite this as evidence for diabetes causing cirrhosis.[232] Unfortunately our study cannot determine the temporality of diabetes to HBV infection and the subsequent development of complications. However, if diabetes is the predominant contributor to disease advancement it could explain why viral load had no effect in our study. Future studies would need to examine the role of diabetes in greater detail: duration, level of diabetic control, development of diabetic complications, type of treatment, along with other socio-demographic (body mass index, weight, presence of central obesity) and biochemical markers (serum lipids). The liver biopsies would need to be

examined for the presence of steatosis as well as fibrosis. Until then, we should emphasise to CHB patients the importance of good diabetic control and encourage developing a healthier lifestyle (by eating less and doing more exercise, reduce alcohol and stop smoking), as there is evidence that this can impact on the long-term progress of their disease.

6.3 Limitations of this work

With all retrospective studies, the data generated is heavily reliant on the quality of source material. The problem is that patient's may not have the correct blood tests either at their initial appointment or their subsequent appointments for a variety of reasons: patient refused blood tests; doctor did not order blood tests; blood test was not done i.e. due to difficulty of phlebotomy; laboratory did not process blood result; or patient had blood test performed at another laboratory and the result was not available in the hospital notes. Whilst every effort was made to obtain the hospital notes, this was not always possible. Even if the notes were found then in a number of cases the quality of the details may have been too sparse to make assumptions. For instance in 24 cases it was not possible to establish the patient's ethnicity. These patients were seen in general hepatology or gastroenterology outpatient clinics, sometimes by junior doctors who, due to lack of experience, may not have known what tests to order or the correct questions to ask. A number of patients, especially those referred following antenatal screening, presented to the clinic once or twice and then did not attend again. If the correct blood tests and demographic details were not recorded then it was not possible to get this information. We had not sought ethical approval to contact patients by phone and this defect should be rectified in future studies of this kind. Because the vast majority of patients were not born in the UK, their ability to speak English may have prevented them from coming for follow up even if an interpreter was available.

This prevalence and disease burden modelling of Bangladeshis and Pakistanis relied on the data derived from the Mosque study. As with all screening studies we depend

on volunteers agreeing to take part and this is affected by their aptitude, knowledge of the condition and also their availability to take part in screening. However other UK-based studies have indicated that our data is accurate and a very small scale study in primary care suggested that the prevalence in primary care was similar to that seen in our study indicating that our data is likely to be correct.[206;242] We noted that the prevalence of HBV in women was less than that seen in men, which is in line with previous studies in HBV. However, the prevalence in Bangladeshi women was 5 times less than that of Bangladeshi men (0.4% versus 2.0%), whereas the prevalence in Pakistani females was 1.6% and was close to the male prevalence of 2.0%. It is unclear whether this is a true finding or reflects the relatively few Bangladeshi women who agreed to be tested. The stigma attached to viral hepatitis may have been too great for some individuals to want to be tested i.e. for an individual (especially female) to seek testing in a public place, such as a mosque, may have led to aspersions being cast about why that person would consider being tested. This point probably applies to all ethnicities but seems to be more of an issue with South Asian Muslims.

The prevalence estimates for patients from the rest of the world depended on published data and clinic attendance. Given the wide geographic areas considered and the local differences in prevalence our estimates of disease burden can only be rough estimates. Nevertheless these data do provide the first insights into the possible burden of undiagnosed infection in immigrant communities in the UK. Studies to estimate the true prevalence of infection will require community based screening projects in these communities and a trial (based in a large part on the work presented here) is currently under way in East London.

Our prevalence calculations are based on data from the ONS 2001 census looking at age, sex, ethnicity and country of birth. At the time of the study we asked for detailed statistics on the Bangladeshi, Pakistani and Indian populations of London at borough level. It is not possible to look at every ethnicity and country of birth for all age groups in London, let alone the UK. However, because these 3 ethnicities are quite common in the UK, accurate numbers were attainable. Although completion of the census is

required by law, it is not legally enforced. It is clear that not everybody completes the census accurately or at all, especially if you are not living in the UK legally. The ONS collects a plethora of data including the ten yearly census, national insurance number (NINo) allocations to foreign individuals, labour force surveys (LFS), international passenger survey (IPS) and total international migration (TIM). Each of these gives different pieces of data, which are not mutually exclusive; some give total figures from one point in time, whilst others are derived from representative samples done throughout the year. Pendleton et al in their report on the prevalence of HBV in the UK chose not to use the 2001 census because they felt it was out of date.[225] They instead preferred to use the LFS and data commissioned from the Institute of Public Policy Research.[243] The LFS is a quarterly survey of 60,000 private addresses in Great Britain which will give data including country of birth. However private addresses don't include communal establishments like hostels, local authority houses or residential and nursing homes. At the time of our study, we felt that the census data was the most accurate and widely used but would suffer the same level of inaccuracies that other population surveys typically face.

In the REVEAL study cirrhosis was diagnosed on ultrasound. Ultrasound is a non-ionising radiation modality that is cheap, safe, effective screening tool but which is highly operator-dependent. Even in the hands of experienced radiologists, the diagnosis can be over-estimated if there is "coarse echotexture" or "irregular liver outline". In their study it was performed by gastroenterologists qualified in ultrasound. In our study the diagnosis of cirrhosis was made on liver biopsy (either percutaneous or transjugular) or there were confirmatory findings on triphasic CT liver or MRI liver and the patient had clinical and endoscopic features of portal hypertension (low albumin, low platelets, splenomegaly, and presence of varices or ascites). Because the criteria we used to diagnose cirrhosis was more robust than theirs, it is possible that both studies (ours and theirs) underestimated subclinical cirrhosis. This is because the ultrasound changes may have been too subtle to be picked up. AFP is routinely tested but at least 10% of patients with HCC do not secrete AFP so there may have been patients with undiagnosed HCC who had normal AFP and

relatively normal ultrasounds. If these patients did not attend further outpatient appointments or they went elsewhere for their treatment (although there was no financial incentive for them to do so) then their condition could have deteriorated without us knowing.

At the time of this study, a number of tests were not routinely available which could have affected the outcome of the results. Genotype testing was done on small number of patients. Delta virus screening was also not routinely done then. This small RNA virus, which only occurs in patients with HBV infection, can suppress HBV DNA, cause rapid progression of liver damage to cirrhosis and decompensated liver disease.[244] It is possible that there were more patients with HDV co-infection and at the time of the study this test either wasn't available or the patient had ceased to be followed up for the test to be done. However we now routinely test all patients for delta virus and, of note, no infected patients have been identified who were born in the Indian Subcontinent. Quantitative measurement of HBsAg wasn't routinely available. There is growing evidence that this variable may give more insight into who will progress to HCC. It has been proposed that quantitative HBsAg levels give a more accurate marker of cccDNA transcription than HBV DNA and that it gives a marker of response to interferon treatment. Those patients with low HBsAg levels <1000 IU/ml probably have inactive disease and levels <100 IU/ml may have a better chance of spontaneous HBsAg seroconversion.[245;246]

6.4 Future studies

This work has provided as many questions as it has tried to answer. We are still trying to find the most effective and efficient way of determining the prevalence of CHB. Our HIV colleagues are getting their disease under more control. There is better screening, testing is done quicker and the result relayed to the patient faster. If the test is positive then assessment and treatment can be offered quickly. As a result the development of AIDS in the UK is declining. A similar approach needs to be adopted with HBV (and HCV). We need to develop a quick, reliable, valid test for HBsAg and

HBcAb that does not need to be administered in hospitals. Once patients are identified then the disease needs to be confirmed. We then need to find ways of making sure patients are followed up.

The next step is to identify those markers which will determine who will progress to cirrhosis and/or decompensated liver disease. Genotype, delta and quantification of HBsAg are variables that should be measured in future to see if this will improve diagnosis of current disease but improve prediction of disease advancement. The original REVEAL-HBV authors have already proposed a nomogram that will give 3, 5 and 10 year predictive scores but this has only been tested in Taiwan and Korea with very homogenous populations.[247] Patients with acute HBV may not be truly "acute" and instead may be having a reactivation of their CHB, in which case they may benefit from treatment. It has been proposed that one way of differentiating between the two conditions is HBcAb avidity but a test for this is not routinely available.[248] If this were to be developed it would help our understanding and management of these patients.

6.5 Conclusion

Our study, the first to evaluate the burden of disease due to HBV in immigrant communities living in the UK clearly shows that there is a very large burden of undiagnosed disease in the immigrant communities living in East London. Although the retrospective approach used here and the difficulties in accessing all patient records may reduce the confidence in the magnitude of the burden there can be no doubt that this work has highlighted a significant diagnostic shortfall. Our initial hypothesis that different ethnic groups would have different risk factors for disease progression has not been confirmed by our study. The previously identified risk factors for disease progression were confirmed in our analysis. However we did not find an association between viral load and advanced liver disease that has been reported by others but we believe this is due to the well described decline in viraemia with advanced disease. Of note our work highlights the importance of smoking and

diabetes in disease progression in patients with chronic HBV and more attention should be paid to these easily remediable factors.

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7 APPENDIX

7.1 R&D Approval for validation of OMT for HBV testing

Queen Mary University of London

Barts and The London NHS Trust

FINAL R&D APPROVAL

Joint Research and Development Office
24-26 Walden Street
Whitechapel
London
E1 2AN

Tel: 0207 882 7272
Fax: 0207 882 7277
Email: david.jackson@bartsandthelondon.nhs.uk

Dr Aruna Dias
The Wingate Institute
26 Ashfield Street
London
E1 2AJ

13 July 2007

Dear Dr Dias,

Re: Validation of oral mucosal transudate for hepatitis B testing

ReDA Reference: 005084

Thank you for sending confirmation of your approval from the ethics committee. I am now happy to inform you that the Joint R&D Office of Barts and The London NHS Trust and Queen Mary, University of London has arranged full indemnity cover for your study against any negligence that might occur during the course of your project.

Please note that all research with an NHS element is subject to the Research Governance Framework for Health and Social Care 2005. If you are unfamiliar with the standards contained in this document, or the BLT and QMUL policies that reinforce them, you can obtain details from the Joint R&D Office, tel 0207 882 7250 or go to http://www.dh.gov.uk/PolicyAndGuidance/ResearchAndDevelopment/ResearchAndDevelopmentAZ/ResearchGovernance/fs/en.

You must stay in touch with the Joint R&D Office during the course of the research project, particularly if/ when:

- There is a change of Principal Investigator;
- The project finishes;
- Amendments are made, whether minor or substantial;
- Serious Adverse Events have occurred (must be reported within 24 hours of becoming aware of the event).

This is necessary to ensure that your indemnity cover is valid. Should any untoward events occur it is **essential** that you contact the Joint R&D Office immediately. If patients or staff are involved in an incident, you should also contact the Clinical Risk Manager on 0207 480 4132.

I hope the project goes well, and if you need any help or assistance during its course, please do not hesitate to contact the Office.

Yours sincerely,

Gerry Leonard
Head of Research Resources

7.2 Cluster of differentiation antigens study

Introduction

The World Health Organization estimates that there are about 350 million people with chronic HBV and about 180 million with chronic HCV. Of these, there are about 600,000 deaths due to HBV annually and 350,000 deaths due to HCV. It has been suggested that only about 10% of patients infected with *Mycobacterium tuberculosis* actually develop clinical tuberculosis and less than 1% of those infected with malaria die of the disease.[249] 2 fundamental questions that form the cornerstones of the management of chronic disease are who will get "bad disease" and who will respond to treatment. Currently we cannot predict either and we know that genotypic disease does not necessarily predict phenotypic disease.

There are many proteins that have been implicated as biomarkers of disease. For example, alpha fetoprotein (AFP) has been used in the diagnosis of HCC. However it has only moderate sensitivity and specificity in screening for this disease.[250] Another option is to look at the components of the immune system as this plays an important, but not altogether fully understood, role in the pathogenesis of disease. One of the components of this is the cluster of differentiation (CD) antigens that exist on the surface of leukocytes and other cells. CD antigens are glycoproteins that were named because they act as receptors and ligands for various monoclonal antibodies. At the 9th International Workshop and Conference on Human Leukocyte Differentiation Antigens in 2010, there were 360 known CD antigens.[251] The CD antigens provide a number of functions such as providing a receptor for various antibodies, such as CD4 with HIV and CD35 for complement, cell signalling and cell adhesion.

Immunophenotyping using CD antigens provides the basis of differentiating between types of acute leukaemias and diagnosing subgroups (i.e. M0-M7 subgroups of acute

myeloid leukaemia).[252] In the past this has been done using flow cytometry of cells to identify the CD antigens involved which was very time consuming. However, Woolfson et al, have developed a new approach involving direct measurement of serum CD antigens that are presumably released by cells of the immune system. The detection system involves multiple testing for multiple antigens whereby a number of monoclonal antibodies are arranged in a grid-like array. In each site there is a specific antibody, delivered there robotically, that will react with specific CD antigens. The amount of capture for each antibody gives an indication of the CD antigen expression in the patient's peripheral blood. This technique was used in a number of patients with different types of leukaemia and lymphoma and the consensus rate with established criteria was 94% with peripheral blood and 98% with bone marrow samples. It has also been studied with patients with different types of HIV infection: long term non-progressive patients not on treatment, those being maintained on first line treatment, and patients requiring salvage treatment. Compared to healthy controls, the HIV positive patients had decreased expression of CD60, CD102 and CD126 and increased expression of HLA-DR and CD20. Hence this new technique offers a rapid, serum based assessment of immune system function and we speculate that this approach may be diagnostically useful.

There is evidence that CD antigens change in viral hepatitis. It has been suggested that HBV may protect hepatoma cells from complement dependent cytotoxicity by up-regulating CD46.[253] Whilst with HCV, interferon appears to down-regulate CD81 and those patients that ultimately go on to have SVR had lower circulating levels of CD81.[254;255]

By identifying the amount of expression of the different types of CD proteins in the blood it may be possible to determine who will develop progressive viral hepatitis and who will develop stable disease. Furthermore it is possible that these assays may predict treatment response.

Hypotheses

1) Different viral infections of the liver are associated with different CD antigen profiles in serum – thus patients with acute HBV infection will have a different profile from patients with chronic HBV infection that will differ from that seen in acute and chronic HCV infection.

2) The CD profiles of patients with different phases of chronic HBV infection will differ.

3) The CD antigen profile in patients with HBV and HCV infection differs in those who respond to interferon based therapies.

Methods

Design

This was a retrospective study using blood samples from patients with HBV and HCV. The samples were taken from HBV, HCV and a control group that consisted of non-viral hepatitis patients with varying stages of disease as shown in Table 1. Numbers shown are the actual number of samples collected and sent to the reference laboratory.

HBV		**HCV**		**Controls**	
HBeAg- patients with low viral load & normal LFTs	25	Rapid virological response patients	17	Patients with normal LFTs	22
HBeAg- patients with high viral load & raised LFTs	9	Early virological response patients	19	Patients with raised LFTs	17
HBeAg+ patients with high viral load but normal LFTs	11	Patients who relapsed on treatment	11		
HBeAg+ patients with high viral load but raised LFTs	12	Patients who did not respond to treatment	8		
Patients with HBeAg seroconversion with PEG-IFN	2				
HBeAg+ patients who failed to respond to PEG-IFN (5)					
Patients who cleared HBsAg spontaneously (7)					
Patients who cleared HBsAg with treatment (2)					

Table 1 Patient samples collected for CD antigen study. Numbers are samples collected and sent to the reference laboratory.

In the HBV cohort, the first four rows correspond to the different stages of HBV carriage described in chapter 1. HBeAg positive patients with high viral load and normal LFTs correspond to the immunotolerant phase. HBeAg negative patients with low viral loads and normal LFTs correspond to the immunosurveillance phase. HBeAg positive patients with high viral loads and deranged LFTs represent the immunoactive phase. HBeAg negative patients with high viral loads and elevated LFTs signify the immunoescape phase. The other four HBV rows correspond to patients who have either had treatment or spontaneously lost circulating HBsAg.

In the HCV cohort, the top two rows correspond to patients who were cured of their HCV. Rapid virological response (RVR) means that their viral load was undetectable following 4 weeks of treatment. Early virological response (EVR) means that they had a ≥2 $\log_{10}$ reduction from baseline HCV RNA by 12 weeks of treatment. Both of these groups went on to achieve sustained virological response (SVR) whereby HCV RNA was not detected six months after the end of treatment. Relapser means that there was undetectable viraemia during and/or at the end of treatment but the virus was detectable once the treatment stopped. Non-responders are patients who had detectable HCV RNA throughout their treatment.

In the control group, none of these patients were infected with HBV or HCV. Patients with normal LFTs are patients who had no history of chronic liver disease and also happened to have normal LFTs. Patients with deranged LFTs are patients with non-alcoholic fatty liver disease (NAFLD) but had elevated ALT.

Once samples from the patients had been obtained they were sent to a commercial laboratory in Cambridge, ProteinLogic Ltd, for processing and analysis. This study was approved by the local ethics committee (See Appendix 2).

Identification and collection of samples

Identifying suitable HBV and HCV patients was a labour intensive task as no patient databases existed at BLT. Using the electronic patient record at the Royal London Hospital it was possible to view all patients who were attending the hepatology clinics as well as those who had attended previously. Only data on clinic attendees was readily available and no diagnosis was available in the out-patient listing. After discovering who was attending the clinics, I checked the pathology results to determine which patients had HBV and HCV. Following this I consulted patient notes and clinic letters and cross checked these with the pathology results to determine at what stage of the disease each and every patient was. This took a number of months to accomplish. Whilst identifying the patients, I also recorded their socio-demographic data (such as ethnicity, gender, age and medical co-morbidities) along with their biochemical and virological parameters. Particular note was made of those patients who had been given treatment for their condition and when this was started. As treatment either with oral anti-viral agents or pegylated interferon could have an effect on the CD antigen profile it was essential to get samples from patients before they started treatment. The dates when treatment was started were recorded in the notes and were confirmed with the hepatitis nurse, who also kept details of the outcomes of treatment for HCV patients.

The next step was identifying the correct sample. The RLH pathology system records all the sample numbers of all the patients. All virology samples are kept for two years in the departmental freezers. Because of the volume of samples, there is not enough physical space to keep samples beyond two years. It is for this reason that I had to identify HBV and HCV patients as soon as possible. The serum samples were stored at -30°C, whilst the plasma samples were stored in a separate freezer at -80°C. Using appropriate handling equipment, I located and retrieved as many suitable samples as possible. Ideally all the samples would have been either plasma or serum but because of the difficulty finding suitable patients and then tracking down their sample, it was necessary to acquire a mixture of plasma and serum samples. Ethical approval had been given for 300 samples to be studied but in the end 167 samples were included in the study and their proportions are shown in Table 1.

No supplementary consent from the patients was required. Patients attending the hepatology clinics had already given consent to having their blood taken for routine analysis and under the Human Tissue Act samples surplus to diagnostic purposes could be used in research projects as long as they were anonymous to the researcher. The samples were to be coded before being sent to the reference laboratory. Each of the samples stored in the virology freezers could hold 2ml, however in many cases there was often less than 500µl and so not enough for analysis. For the study, 500µl was extracted from the original virology sample. This was then put in another vial, sealed with parafilm, coded and stored in another freezer at -80°C.

Before the samples could be sent, a material transfer agreement was agreed. The co-investigator, Dr Adrian Woolfson, had originally planned to examine the samples at the ProteinLogic laboratories in Cambridge. However, for operational reasons the laboratory service transferred to the USA (Gentel Biosciences Inc, Finchberg, Wisconsin). As the samples were now being sent to the USA a substantial amendment was required to ensure that it was ethical to send samples outside the EU and approval was needed from CDC for the transportation of hepatitis samples. Once these had been granted, the samples were sent by courier and transported on dry ice to keep them at -80°C. The costs of transportation were borne by the hepatology research group whilst the costs of analysis were borne by Dr Woolfson.

Procedure

This was carried out by Dr Woolfson's team at Gentel Biosciences. The procedure used was the same as the method described in his previous study with HIV samples.[256]

In order to "train" their computer, the research team requested that 98 of the 167 samples be unblinded: 41 HBV, 34 HCV and 23 controls. These unblinded samples

were a mixture of plasma and serum samples. Only the underlying condition was revealed and not the disease stage. When the analysis was finished then all the samples with their disease stage were unblinded to the research team.

Results

Of the 167 samples sent for analysis, 96 were analysed: 46 HBV, 35 HCV and 15 controls.

Figure 1 shows the scatter plots for the different CD antigens comparing controls to HBV. There appears to be down regulation of CD antigen expression with the HBV samples.

Table 2 shows the sensitivity and specificity these CD antigen profiles comparing controls and all other samples to HBV. The table shows that there is very low specificity comparing HBV with controls suggesting that CD antigens are very poor at differentiating between those that have the disease and those that don't.

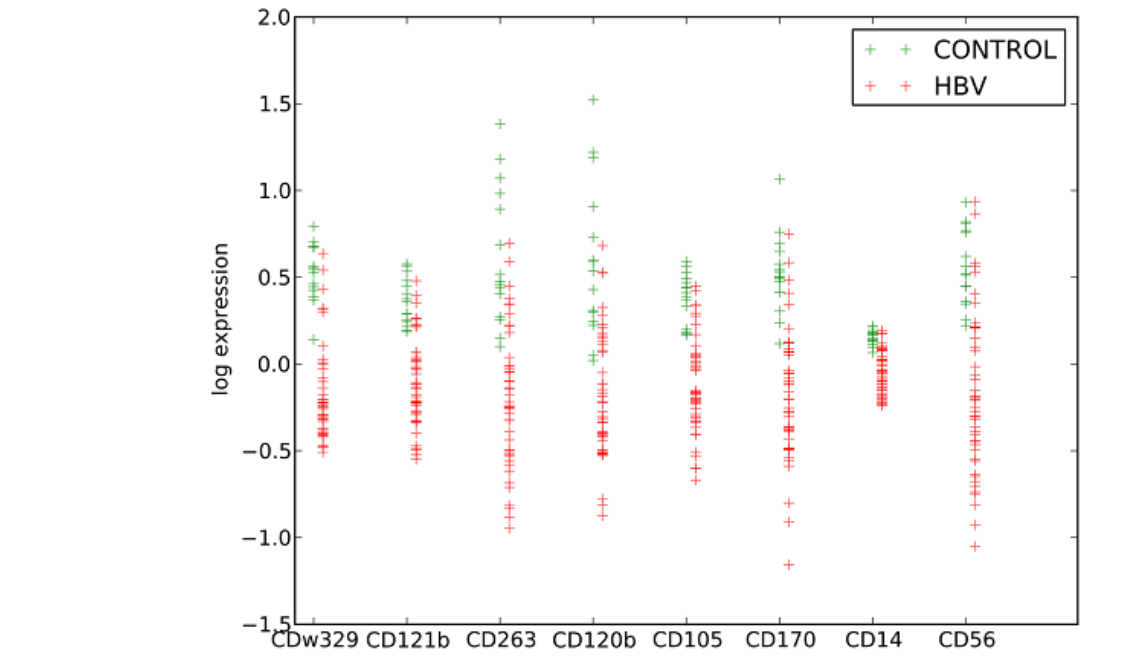

Figure 1 Scatter plot comparing control and HBV samples for various CD antigens and their cell surface expression

	Sensitivity	Specificity
Control/HBV	93%	0.78%
All/HBV	85%	90%

Table 2 Predictive accuracy of CD antigens in differentiating between controls and HBV, and between all other samples and HBV

Figure 2 shows the scatter plots for the different CD antigens comparing controls to HCV. Unlike with HBV, the scatter plot for HCV CD antigens is very similar to that for the controls.

Table 3 shows the sensitivity and specificity these CD antigen profiles comparing controls and all other samples to HCV. Whilst there is good sensitivity, the specificity of the test is only about 60%.

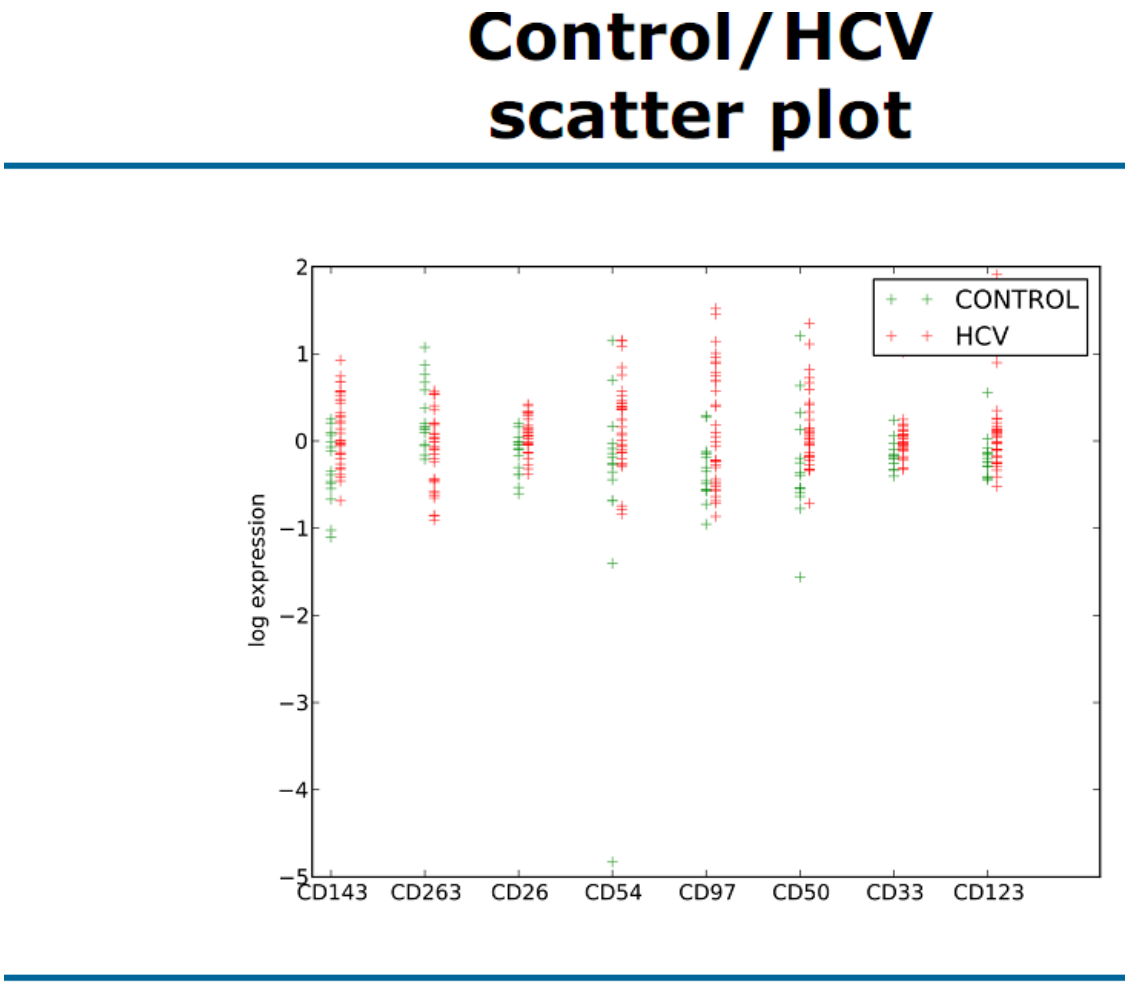

Figure 2 Scatter plot comparing control and HCV samples for various CD antigens and their cell surface expression

	Sensitivity	Specificity
Control/HCV	92%	63%
All/HCV	65%	91%

Table 3 Predictive accuracy of CD antigens in differentiating between controls and HCV, and between all other samples and HCV

Discussion

Although the data is not shown, the researchers at ProteinLogic doing the analysis compared the best CD antigens at distinguishing controls with HBV and HCV samples and analysed them by disease phase. Unfortunately there were no differences seen. The results of this study show that there are no statistically significant specific CD antigen profiles associated with HBV or HCV, and that none of these can discriminate between the different phases of the infections.

The procedures done in this study were similar to that adopted in other studies using HIV and leukaemia patients as it involved the same researcher (Adrian Woolfson).[256;257] In the HIV study, although there was up and down-regulation of certain CD antigens between HIV patients and normal controls, there were no significant CD antigen patterns between the 3 HIV subgroups (long term non-progressors, first line treatment and salvage treatment patients).[256] Compared to that study, we used far higher numbers of patients for the test groups and for the subgroups. In that study they had 4 controls and between 6 and 10 patients in each of the 3 groups. We used 73 HBV, 55 HCV and 39 control samples. Although there are at least 360 known CD antigens, at the time of the previous study, the array could only test for 84. There is always the possibility that HBV and HCV samples, in varying stages of their disease, may be associated with different CD antigens that were not tested for in this study.

Although this pilot study did not reveal any major diagnostic markers of clinical value only a proportion of the known CD antigens were tested and the data does indicate subtle differences between HBV and HCV infections providing encouragement that a diagnostic/prognostic CD profile may be detected and further studies in this area are currently in progress.

Queen Mary
University of London

Barts and The London NHS
NHS Trust

FINAL R&D APPROVAL

Dr Aruna Dias
The Wingate Institute
26 Ashfield Street
London
E1 2AJ

Joint Research and Development Office
24-26 Walden Street
Whitechapel
London
E1 2AN

Tel: 0207 882 7272
Fax: 0207 882 7277
Email: david.jackson@bartsandthelondon.nhs.uk

07 September 2007

Dear Dr Dias,

Re: CD antigen expression in patients with chronic viral hepatitis – are different infections associated with different CD antigen profiles? Viral Hepatitis and CD Markers

ReDA Reference: 005112

Thank you for sending confirmation of your approval from the ethics committee. I am now happy to inform you that the Joint R&D Office of Barts and The London NHS Trust and Queen Mary, University of London has arranged full indemnity cover for your study against any negligence that might occur during the course of your project.

Please note that all research with an NHS element is subject to the Research Governance Framework for Health and Social Care 2005. If you are unfamiliar with the standards contained in this document, or the BLT and QMUL policies that reinforce them, you can obtain details from the Joint R&D Office, tel 0207 882 7250 or go to http://www.dh.gov.uk/PolicyAndGuidance/ResearchAndDevelopment/ResearchAndDevelopmentAZ/ResearchGovernance/fs/en.

You must stay in touch with the Joint R&D Office during the course of the research project, particularly if/ when:

- There is a change of Principal Investigator;
- The project finishes;
- Amendments are made, whether minor or substantial;
- Serious Adverse Events have occurred (must be reported within 24 hours of becoming aware of the event).

This is necessary to ensure that your indemnity cover is valid. Should any untoward events occur it is **essential** that you contact the Joint R&D Office immediately. If patients or staff are involved in an incident, you should also contact the Clinical Risk Manager on 0207 480 4132.

I hope the project goes well, and if you need any help or assistance during its course, please do not hesitate to contact the Office.

Yours sincerely,

Gerry Leonard
Head of Research Resources

7.3 Novel vaccine treatment for chronic hepatitis B

Introduction

In a significant number of patients, chronic HBV is associated with progressive liver inflammation that leads, in some, to cirrhosis. The principles of treatment are to prevent transmission of the virus to others, stop further disease progression and avert the development of HCC. Current treatments are with immunomodulators (PEG-IFN) or with oral nucleosides (i.e. Entecavir) or oral nucleotides (i.e. Tenofovir). They target the viral polymerase and viral replication but cannot eradicate the infection due to cccDNA remaining in the hepatocytes. The treatments may be life-long and are prone to the development of drug toxicity and resistance. There is a pressing need for a more effective short duration antiviral therapy that will eliminate HBV from the majority of patients.

HBV is not directly cytopathic to hepatocytes and whilst virtually all these cells can demonstrate actively replicating virus, there is not necessarily histological or biochemical damage.[30;258] Instead this damage is immune-mediated and in particular due to the adaptive response through a complex interplay between virus-specific CD4+ and CD8+ T cells. Those individuals who clear the virus mount a forceful, polyclonal response with cytotoxic T cell lymphocytes (CTLs) that enter the liver, identify the antigen, destroy infected hepatocytes and lead to the secretion of IFNγ to inhibit HBV gene expression. Where HBV infection persists, the CD4 response is not triggered early enough and there is induction of functionally impaired, narrowly focused CD8 cells which can lead to tissue damage but are not vigorous enough to clear the virus.[259;260] These T cell defects are thought to be related to continued, large scale exposure of viral antigens such as HBsAg and HBeAg over time and can lead to long term "exhaustion", which results in loss of functions like cytokine production (TNFα and IL-2), cytotoxicity and cell propagation. How this occurs is not fully understood but may be due to the over-expression of receptors such as programmed death-1 (PD-1) on HBV-specific T cells which, inhibit the production of IFN and

prevent the production of memory cells.[57;261-263] Manipulation of the immune system appears to be a viable strategy to achieve sustained resolution of this chronic infection.

The principle of vaccination is to introduce an inactivated pathogen that will stimulate the immune system to mount a response should it encounter that pathogen again. The current HBV vaccine is a recombinant HBsAg that is given on at least 3 occasions via an intramuscular (im) injection. However, over the past 3 decades, there have been attempts to use the standard HBsAg vaccine as treatment of CHB. One of the first attempts was carried out by Pol *et al*. Patients were given 3 standard recombinant HBV vaccination injections im and the HBV DNA load was noted to be undetectable in 31% of patients at the end of 6 months follow up. However, in this small study, the authors do not mention whether HBsAg loss also occurred.[264] Other pilot studies using vaccines with HBsAg also failed to show any significant improvement in viral load, HBeAg or HBsAg seroconversion. A large multi-centre study looking at patients given im HBsAg recombinant vaccine in combination with lamivudine also failed to show any significant clinical treatments outcomes when compared to patients treated with lamivudine alone.[265] The rationale being that reducing the viral load would also stimulate T cell responsiveness, but this was not maintained either in this or other studies.[266] One of the reasons for varying degrees of "success" of using HBsAg as a therapeutic vaccine may be because of the variability of HBV disease progression. Spontaneous clearance of the virus does occur, albeit not very often, but is more likely to happen during the active phase when there is raised ALT and elevated HBV DNA rather than in the inactive carrier state due to inaction by the immune system. If the study used patients in the active phase, then in order to detect a clear difference there would need to be larger numbers in the control arms.

It now appears that it is HBcAg rather than HBsAg or HBeAg which is associated with stimulating the immune response into clearing the virus.[267-269] Patients who clear acute HBV infection or whose CHB disease progression is kept in check, have more HBcAg-specific cytotoxic T lymphocytes (CTL) than those patients who have uncontrolled viral loads and progressive liver damage. An early multicentre HBcAg-

based epitope vaccine study was performed by Heathcote *et al* in which they administered various im doses of the vaccine into patients on a number of occasions. Unfortunately there were no significant treatment responses and the CTL responses were weaker in HBV patients than in healthy volunteers.[270]

HBV vaccine is a recombinant DNA vaccine produced by inserting the gene for the S envelope protein into yeast or mammalian cells where they are grown and purified. The problem with subunit and recombinant vaccines is that they require an adjuvant to enhance the specific immune response against the antigen. There are a number of different adjuvants, but the most common used ones are aluminium based (either aluminium phophate or hydroxide). Aluminium adjuvants have been used in vaccine treatments in humans against CHB but with only limited success and no long term loss of virus.[271] There are other possible adjuvant systems such as other mineral salt adjuvants (salts of calcium, iron and zirconium), adjuvant emulsions, carbohydrate adjuvants (inulin), bacterial adjuvants, and cytokine adjuvants (granulocyte colony stimulating factor, PD-1 antagonists and IL-2) but most have not been tested against hepatitis B and even then not with human subjects *in vivo*. [272-275]

DNA vaccines derived from plasmids are another alternative to recombinant vaccines and that can induce both cytotoxic and Th1 responses.[276;277] There have been some promising results, albeit in trials with small numbers. One study showed that even with patients who had experienced virological breakthrough with lamivudine some patients displayed HBeAg seroconversion, there was induction of IFNγ-secreting T cells and increased production of specific NK but that this effect was only temporary.[278] Another study showed that a DNA vaccine encoding for multiple HBV antigens combined with IL-12 adjuvant and lamivudine treatment over 12 months reduced viral load, and in some cases suppressed it completely up to 3 years post cessation of treatment, but still didn't eradicate HBsAg.[279]

Bacteria could potentially be used as an adjuvant to carry the vaccine and so stimulate the immune system. *Salmonella typhi* (*S.typhi*) is a serovar of *Salmonella enterica*, a gram negative bacterium, which can lead to the systemic disease in

humans called typhoid fever. When ingested it can cross the gastrointestinal mucosa via the M-cells and can colonize in the gut-associated lymphoid tissue.[280;281] *S.typhi* specifically targets immune cells and triggers a variety of cellular, humoral, and mucosal immune responses. It is this broad immune response which makes *S.typhi* attractive as a possible vector for antigens. For *S.typhi* to work as a vaccine it needs to retain its immunogenicity but not cause bacteraemia. Various studies have shown that at least 2 deletions are necessary for this to happen: aroC$^-$ (which limits bacterial growth) and ssaV$^-$ (which prevents bacteriaemia and toxicity).[282-284] The authors of these studies have carried out clinical trials using various, single doses of this particular avirulent *S.typhi* in healthy volunteers. They found that the oral preparation was well tolerated, there were very few adverse events (and none of these were serious) but that the vector was highly immunogenic generating profound Th1 responses.[285;286]

In the present study, the *S.typhi* strain described before (S. *typhi (Ty2 aroC$^-$ ssaV$^-$*) M01ZH09, Emergent Biosolutions, Wokingham, UK) was modified to have ssaG-HBcAg promoter-gene fused to it and to generate HBcAg; the modified vaccine was named M04NM11. A phase 1 study utilised healthy volunteers who were given 2 doses of M04NM11 and showed it to be safe and well tolerated. It also showed that subjects mounted a Th1 response as evidenced by a rise in IFNγ in T cells removed and challenged with HBcAg. The question is whether M04NM11 would be safe and tolerable in patients with CHB as well as show an increase in Th1 T cells.

Aims

The principal aim of this study was to assess and compare the safety profile of a new investigational medicinal product (IMP), called M04NM11, to a placebo drug in CHB patients both during treatment and 6 months after completion of vaccination. The secondary aims were to assess the effects of the treatment on the patient's immune response and HBV viral load after 6 doses of vaccine and, in particular to monitor

conversion of HBeAg positive to negative; development of anti-HBe; drop of HBV DNA by 2 $\log_{10}$ or to <10^4 copies/ml; or a reduction of ALT.

Methods

Design

This was a multi-centre, randomised, double-blind, placebo-controlled, dose-escalation trial. It was sponsored by Emergent Biosolutions (Wokingham, UK) and involved one centre in the UK (Royal London Hospital, RLH) and 4 centres in Serbia. The study had been given REC and Medicines and Healthcare products Regulatory Agency (MHRA) approval for using the IMP (see Appendix 3).

Each patient would receive 6 doses of either M04NM11 or placebo. M04NM11 consisted of live-attenuated *Salmonella enterica serovar Typhi* (*S.typhi*) bacteria (*S. typhi (Ty2 aroC⁻ ssaV⁻ ssaG-HBcAg) RSC5*) which had been genetically modified to carry HBcAg to stimulate the immune system but not induce Typhoid fever. The first dose was given within 28 days of screening and consisted of 10^8 colony forming units (CFU). The second dose was 10^9 CFU and the third 10^10 CFU. If the dose was safely tolerated then the patient would be given the next higher dose at the next visit according to the schedule (see Table 1) with a maximum of 4 doses of 10^10 CFU in a 6 month treatment trial.

There were 3 study groups of chronic HBV patients each consisting of a maximum of 15 patients:

Group 1: HBeAg positive with low ALT (≤2 x ULN) and HBV DNA load ≥ 10^6 copies/ml

Group 2: HBeAg positive with high ALT (>2 x ULN and ≤5 x ULN) and HBV DNA load ≥ 10^6 copies/ml

Group 3: HBeAg negative with high ALT (>2 x ULN and ≤5 x ULN) and HBV DNA >10^5 copies/ml

For men the normal range for ALT was defined by the reference laboratory (Simbec laboratories, Merthyr Tydfil) as 13-67 U/L and for women this was 9-33 U/L.

Once 9 patients in group 1 had reached dose 3 a safety review committee would meet and assuming there were no significant safety issues, recruitment of patients into groups 2 and 3 could begin. As raised ALT is a marker of liver dysfunction and as there were concerns that the vaccine might induce a severe, liver threatening, 'hepatic flare', the trial was designed so that patients with less inflamed livers (group 1) and who would not normally undergo anti-HBV treatment should be tried first with the test drug.

Safety and tolerability were assessed clinically to see if patients had experienced any adverse events and by analysis of blood, urine and stool tests at an initial screening visit and then 19 other dosing and safety visits as per the schedule in the table below. At each visit routine blood tests were performed for full blood count, urea & electrolytes, LFTs and clotting. Urine analysis was also undertaken and if there was significant proteinuria or the presence of nitrites this was to be sent for microscopy, sensitivity and culture. Virological tests such as HBeAg and HBeAb status and HBV DNA were done on selected visits (see table). All analytical tests were undertaken by an independent central laboratory, Simbec laboratories, Merthyr Tydfil, and samples were sent appropriately packaged by courier to them.

Visit No	Screening	1	2	3	4	5	6	7	8	9
Visit type	Safety (S)	Dose 1	S	S	S	Dose 2	S	S	Dose 3	S
Day	-	0	3	7	14	28	35	42	56	70
Virology	x					x		x	x	x

Visit No	10	11	12	13	14	15	16	17	18	19
Visit type	Dose 4	S	Dose 5	S	Dose 6	S	S	S	S	S
Day	84	98	112	126	140	154	168	196	252	308
Virology	x	x	x	x	x	x	x	x	x	x

Table 1 Schedule of trial visits and dates of virology testing. x denotes when virology testing occurred.

Because the study used a genetically modified organism (GMO) that could potentially cause Typhoid fever, the Department of the Environment, Food and Rural Affairs (DEFRA), on the advice of the Health and Safety Executive (HSE), maintained that all testing had to be done in an isolated unit. Even though a phase 1 study using GMO *S.typhi* had shown that there was no shedding of the bacteria in faeces by day 7, subjects had to remain within England for 14 days post dosing.

All visits occurred in a separate building called the clinical research centre (CRC), which is a dedicated building for running clinical trials and walking distance from the Royal London Hospital (RLH). Prior to giving the 1st dose, the study investigator rang

Clinphone, an independent organisation, who assigned the subject a randomisation number. This number was then taken to the pharmacy department where the medication (either M04NM11 or placebo) was reconstituted, prepared and then dispensed. After fasting, the subject was given a solution containing 150ml water and an effervescent tablet that was allowed to dissolve and containing 2.6g sodium bicarbonate, 1.65g ascorbic acid and 30mg aspartame (to act as a buffer against gastric acid). The study medication was then added to this solution. This was drunk within 30 minutes of preparation. On the dosing visits, the patients had to arrive fasted for at least 90 minutes and be kept in a separate, enclosed room with the study investigator for at least 60 minutes post ingestion to see if there were any immediate adverse events like anaphylaxis. If there were any adverse events post dosing or there were abnormal laboratory results then unscheduled visits and further analysis and investigations could be initiated after discussion with the principal investigator, medical monitor and trial sponsor.

By the time of start of the trial I had been trained in good clinical practice methods. My role was to identify and recruit suitable patients; conduct all the screening, dosing and safety visits, including taking history, physical examination and carrying out the blood tests; design and implement the standard operating procedure for out of hours medical cover and un-blinding of patients; and to assess, record and report all adverse events accurately and to the relevant authorities appropriately in a timely manner.

Patients

Trial candidates were selected from the patients attending the hepatology clinics in RLH and could be included if they had: chronic HBV infection (shown by 2 positive HBsAg samples at least 6 months apart); were aged over 18; had stable LFTs and PT; had a liver biopsy within 2 years; and were able to attend all scheduled visit days.

Because of the use of GMO and the possible implications to public health there were a number of stringent exclusion criteria: they must not work as commercial food handlers (i.e. butchers and the catering trade) because of the risk of spreading *S.typhi*; work with or be in regular contact with immuno-compromised individuals (HIV/HCV/HDV co-infected patients); have cirrhosis or decompensated liver disease; be pregnant or breast-feeding women; or be with children aged under 2 or adults aged over 70; have hypersensitivity to trimethoprim-sulfamethoxazole and ciprofloxacin (in case they needed to be treated with antibiotics if they developed a typhoid like illness) or anaphylaxis to the ingredients M04NM11; have a recent infection that necessitated antibiotic use within 7 days of dosing; have significant derangement of liver function shown by ALT >5.1 x ULN, PT >1.25 x ULN or bilirubin >1.5 x ULN; or be having current or previous treatment of their HBV within the last 12 months.

Results

Patient recruitment occurred between December 2006 and September 2008. A total of 10 patients were screened: 9 patients for group 1 and 1 for group 2. There were 4 patients who failed screening and all of these were for group 1: one because of elevated ALT, one because of newly diagnosed HIV co-infection, and two because their viral loads were too low.

The demographics of the 6 patients involved in the trial at RLH are shown in Table 2. Patients 1-5 were in group 1 and patient 6 was in group 2. Three patients were given the actual test drug (patients 2, 3 and 6) and three were given placebos (patients 1, 4 and 5). The identities of the test drug were not revealed until the whole trial had finished and the results were analysed. None of the patients withdrew from the study before the scheduled end of the trial nor did any adverse events necessitate the need for premature un-blinding.

Patient	Age	Sex	Ethnicity	Genotype	Screening	Final	Trial drug
1	41	M	Ghana	E	19/12/06	13/11/07	M04NM11
2	47	M	Bangladesh	D	20/12/06	14/11/07	Placebo
3	19	M	Philippines	A	19/12/07	21/11/08	M04NM11
4	20	M	Somalia	D	12/01/08	08/12/08	Placebo
5	36	F	China	B	23/01/08	19/12/08	Placebo
6	24	F	Poland	D	22/05/08	20/04/09	M04NM11

Table 2 Demographic details, initial screening and end visit dates, and test drug given

Figure 1 and Figure 2 show the variation of ALT and HBV DNA throughout the 19 visits. Two patients (patient 1 and patient 5) had fairly stable ALT values throughout the trial. Both of these patients had the placebo medication. The other 4 patients all suffered flares in their HBV, in that the ALT went up many fold but the patients themselves were clinically well. Again one of these patients (patient 4) had the placebo medication but his flare appeared right at the end of the trial and so was unlikely to be associated with the test medication.

Patient 2 had a flare soon after dose 1 and as result had extra unscheduled visits. He was not dose-escalated at visit 5 and instead was given another 10^8 CFU (and not 10^9) dose as his second dose.

Patient 3 had two big peaks in his ALT - 343 U/L occurring at visit 8 when he had dose 3 and 665 at visit 17. However both "flares" had a fairly long duration period: 56 and 84 days respectively, but only with the second flare was there a corresponding drop in HBV DNA from more than 8 log IU/ml to 5.84 log IU/ml. In the trial, analysis of HBV

DNA was recorded in copies/ml but throughout this research period (and clinical practice) I have used IU/ml with a conversion rate of 1 IU/ml = 5.5 copies/ml.

None of the other patients had a significant drop of HBV DNA from baseline by the end of the trial period. Patient 1 did have a drop in his viral load to 5.24 log IU/ml at week 14 but this was not sustained. Unfortunately due to problems with transportation and analysis of his samples there were no virology results at visits 13 and 15 so it is not possible to establish the full extent of this viral load drop. He was given a placebo so any changes in viral DNA amounts were unlikely to be drug related.

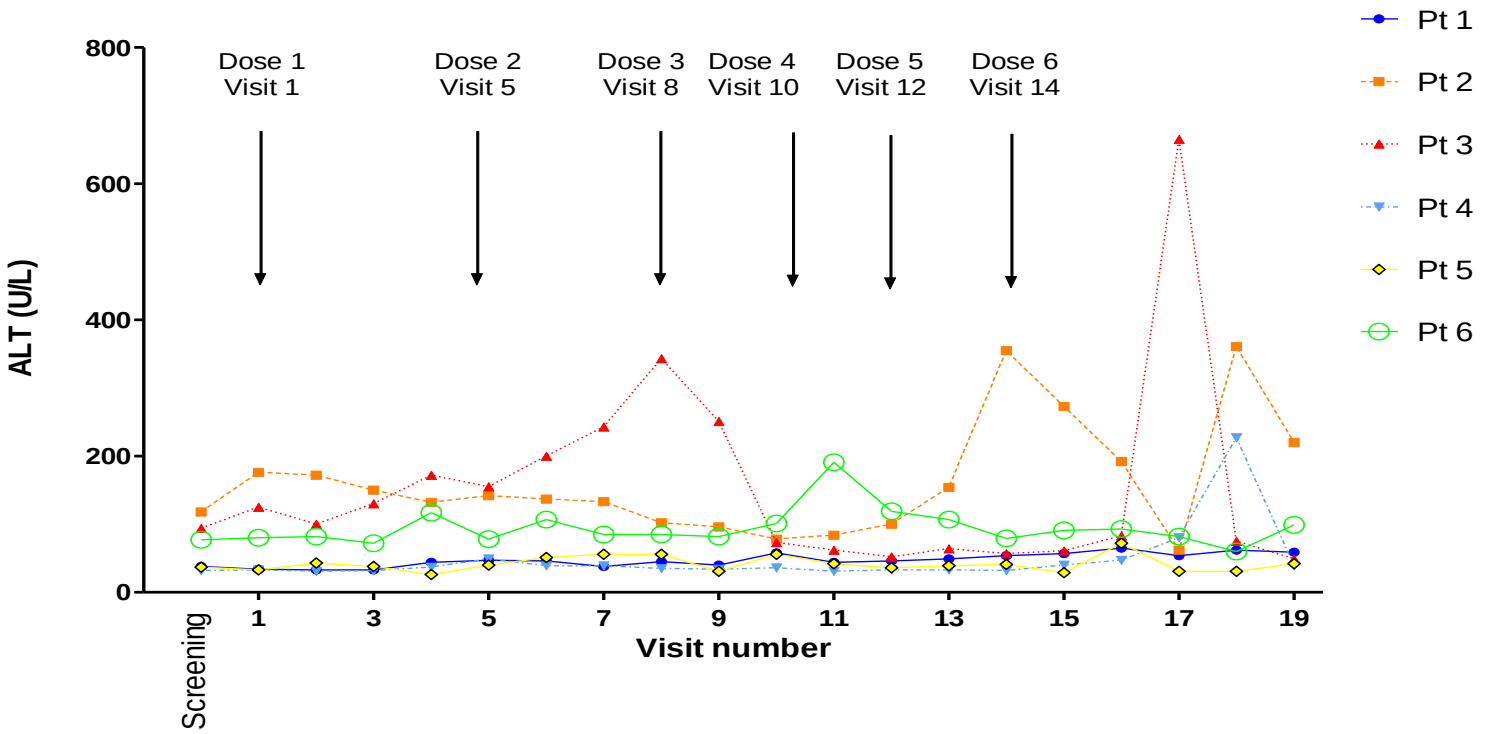

Figure 1 Graph showing variation of ALT with each of the trial visits and in relation to dosing for each of the trial patients

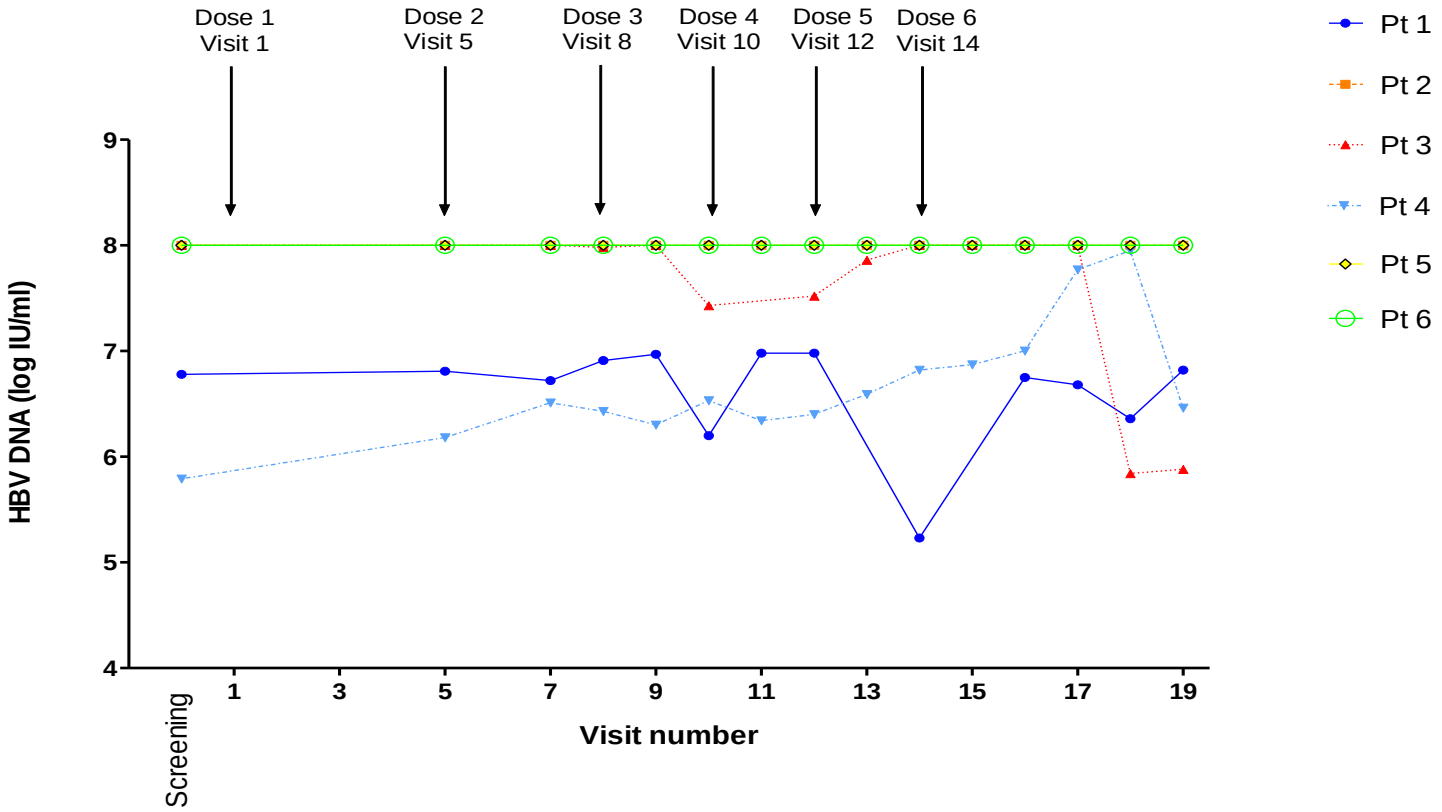

Figure 2 Graph showing variation of HBV DNA with each of the trial visits and in relation to dosing for each of the trial patients

As with all treatment trials, patients can suffer adverse events. In this study, some patients did experience these and they are shown in Table 3. Adverse events are defined as any unexpected events that occur in a subject who has been given a medicinal product and include occurrences that are not necessarily caused or related to the original product. The event can be classed as serious or not serious. According to the MHRA, serious adverse events (SAE) are associated with death, or life-threatening injury, or with the subject requiring hospitalisation, or can result in persistent or significant disability, clinical anomaly or birth defect. Based on this definition, the post-tonsillectomy bleed that occurred with patient 2 was classed as a SAE. This is because the patient attended the A&E department of RLH at 2200 three days after his tonsillectomy, and was seen by a doctor at 2315 but subsequently discharged at 0040 of the next day. As the event had transcended beyond midnight it was classed as an overnight hospital admission even though the actual admission had lasted less than a few hours. The bleed *per se* was most likely due to the patient not adhering to the post-operative instructions given to him rather than due to the study drug. As per the protocol, this SAE was reported immediately to the sponsor and the details carefully documented. No specific treatment was necessary and the patient was given appropriate advice.

The enlarged tonsil of patient 2 and the haematuria of patient 4 (due to pre-existing kidney stones) were deemed unlikely to be associated with the study drug as they were both noted at the time of screening.

Patient 3 was trekking in the Pyrenees Mountains of Spain and was bitten multiple times by mosquitoes. Although insect bites were the most likely cause of his rash, as it happened whilst he was still enrolled in the trial, albeit after he had finished all his doses, it was still counted as an adverse event possibly related to the study drug.

Patient 5 was working on a market stall when she sustained a laceration to her head from a falling overhead pole. This accident was unlikely to be due to the study drug and

she only required sutures to the wound but not antibiotics (thereby invalidating the study).

None of the patients developed a fever or diarrhoeal symptoms suggestive of Typhoid fever and there were no positive bacteraemias or positive urine cultures.

Patient	Adverse event	Action plan	Relation to trial drug
2	Enlarged right tonsil	ENT referral & tonsillectomy	Unlikely to be related
2	Post-tonsillectomy bleed	Observation	Possibly related
3	Skin rash following insect bites	Observation	Possibly related
4	Painless haematuria	Urology referral & Ultrasound	Unlikely to be related
5	Laceration to head	Sutures to wound	Unlikely to be related

Table 3 Adverse events, action plan and relationship to trial drug.

Discussion

This study achieved its primary aim by showing that both M04NM11 and the placebo are safe and well tolerated. Unfortunately none of the secondary aims were achieved. None of the patients seroconverted from HBeAg positive to HBeAg negative, nor did the HBV DNA drop significantly after the sixth dose. The viral load of patient 3 did drop by 2 $\log_{10}$ by the end of the trial from baseline but it is unclear whether this was related to medication or to the natural fluctuations of chronic HBV infection. One aim was to see

what effect M04NM11 had on the immune system by looking at IFNγ production from Th1 cells. The (unpublished) phase 1 study had shown that M04NM11 did cause increased production of IFNγ. Unfortunately the samples were taken and were due to be analysed by University College London but to the best of our knowledge this analysis has not been completed and the company involved in the study has now ceased trading suggesting that further data is unlikely to be available.

The study was supposed to have 15 patients in each of the three groups. Within the 2 year time period, 16 patients had been recruited into group 1, 3 into group 2 and 5 into group 3. Of the 1161 mono-infected HBV patients that I found to being followed up in the RLH and NUH clinics, only a fraction were known at the time of recruitment. The strict trial inclusion criteria made it very difficult to even find patients for group 1: have high viral loads (>6 $\log_{10}$ copies/ml), normal ALT values, HBeAg positive, mono-infected, liver biopsy within 2 years of screening and not have been on any form of treatment within the last year. Most of these patients would typically be young patients in the immuno-tolerant phase of the disease. Because they are generally asymptomatic, a lot of potential subjects declined screening because of the fear that the test drug would make their condition worse. Immuno-tolerant patients typically don't get started on treatment because the immune system is not thought to be very active, which is highlighted by their normal ALT values. This might explain why none of the secondary aims were achieved. Although it is interesting to see that in the three young patients (3, 4 and 6) all experienced transient rises in their ALT despite being asymptomatic. A recent study by Kennedy et al has suggested that the immunotolerant phase seen in young adults and children may be more complicated than first thought. Their T cell profile shows some characteristics of "exhaustion" through expression of PD-1, although less than in adult patients. Also a proportion of patients with persistently normal ALT showed evidence of fibrosis on liver biopsy.[49] This would suggest careful follow up of such patients may identify more active disease than is currently believed.

Recruitment for groups 2 and 3 were possibly lower because patients in these two groups (high ALT, high viral load and HBeAg positive and negative respectively) are candidates for treatment. The rationale would have been to start them on treatment rather than wait until recruitment of these groups began in early 2008.

All the patients we recruited were non-British immigrants and had a multitude of genotypes, which is probably different to the patients enrolled in Serbia where there is medium endemicity (UK is low) and possibly more uniformity of genotype. From the overall results only 1 patient had a significant, sustained drop in HBV DNA during the trial and he/she was in group 3 and from Serbia. Without the immunology results it is not possible to say what the Th1 and IFNγ responses are. Before the study began we were told that the trial would be published but as the company no longer exists we have not been able to insist on the data being entered into the public domain. This study was carried out before the creation of the clinical trials database (www.clinicaltrials.gov) which was designed to prevent this from happening in the future.

To date there are no other similar trials where a bacterial vaccine has been used to stimulate the immune system to treat HBV in CHB patients. Most of the studies using *Salmonella sp* vaccine have been about the development of a single dose, oral HBV vaccine. They have used modifications to either a *Salmonella typhimurium* serovar in mice or *S. typhi* in humans and have looked at the CTL response. They have found that a variety of routes can be utilised such as oral, rectal and nasal with varying degrees of success.[280;282-284;287] None have attempted to treat patients who already have CHB.

By giving an oral vaccine, the rationale is to enhance the mucosa-associated lymphoid tissue (MALT) humoral and cellular response against infection as that is where 80% of immune cells are located.[288] However enterally delivered vaccines have to negotiate the same obstacles faced by microbes: gastric secretions and acid, mucus gel and epithelial barriers.[289] All these may have contributed to the failure of M04NM11 to treat CHB. There is also the possibility of mucosal tolerance whereby the MALT does not respond to

the perceived threat of the vaccine. Because there are no immunology results from this study it is impossible to know what the Th1 response was. Possibly increasing the dose may have resulted in different results.

In conclusion, the study drug was shown to be safe but at the doses delivered it was not shown to be effective at treating CHB. The closure of the company involved in the study sadly precluded further meaningful information being derived from this trial.

NIHR CRN
NIHR Clinical Research Network Coordinating Centre

NHS
National Institute for Health Research

NIHR Clinical Research Network
Coordinating Centre
Fairbairn House
71-75 Clarendon Road
Leeds LS2 9PH

Tel: 0113 343 2314
Fax: 0113 343 2300
Email: info@ukcrn.org.uk
www.crncc.nihr.ac.uk

19th February 2009

Professor Graham Foster
ICMS Adult & Paediatric Gastroenterology
Barts and the London
School of Medicine and Dentistry
Turner Street
London
E1 2AD

Dear Professor Foster

Re: Enhancing the immune response in patients chronically infected with Hepatitis B: Pilot study to examine the efficacy of a novel antigen presentation system. (NIHR CRN ID: 6481)

Thank you for completing the minimum dataset for the above study. I can confirm that the study is eligible for, and has therefore been included on, the National Institute for Health Research (NIHR) Clinical Research Portfolio. The record for this study can be viewed on the Portfolio Database at http://www.ukcrn.org.uk/index/clinical/portfolio_new.html.

Benefit of inclusion in the NIHR Portfolio
Inclusion in the NIHR Portfolio of studies **ensures your study can access NHS service support and research infrastructure support in England** (i.e. support to help with study promotion, approval, identification of eligible patients, recruitment, and follow up etc). This support is now flowing through the Comprehensive Clinical Research Network to the 25 Comprehensive Local Research Networks (CLRNs) across England. Funding allocations to the CLRNs include an activity-based component driven by the data which are held on the UKCRN Portfolio Database and it is therefore essential that your study record is kept up-to-date. Please contact us as soon as possible via email (portfolio@ukcrn.org.uk) if any changes are required.

Collecting your accrual data
In order to ensure that your study remains on the NIHR Portfolio and receives appropriate support through the relevant Comprehensive Local Research Network(s), the UKCRN Coordinating Centre must collect accrual data for the above study from April 2008 and then each month on an ongoing basis.

If you haven't already had the opportunity to send this data to us, we would be grateful if you could do so as soon as possible. Accrual data should be supplied via the UKCRN Accrual Upload System and we will be contacting you in the near future to talk you through this process. Further information and data templates for uploading accrual data can be found on the UKCRN website at: http://www.ukcrn.org.uk/index/clinical/portfolio_new/P_accrual.html. Please contact us (accrual@ukcrn.org.uk) if you have any queries about the process.

We would also encourage you to provide data on accrual prior to April 2008 in order to contribute to the CCRN "baseline" and to provide information on the overall level of recruitment into this study. This can be submitted in a simplified format, simply stating the total number of patients recruited prior to April 2008.

Additional and new studies

Please note that some new studies funded by NIHR Partners (as defined in the Eligibility Criteria) might need to undergo a further adoption process prior to inclusion onto the Portfolio (e.g. if individual studies are part of a programme grant). All new "non-automatic" studies (those funded by non-UK governments, e.g. EU, NIH, and industry-supported, non-industry sponsored - IITs) will also need to undergo a full adoption process.

UKCRN is keen to ensure that all studies which are eligible for inclusion into the NIHR Portfolio are identified so that they can be supported through the Comprehensive Clinical Research Network. If you are aware of any other potentially eligible studies which are recruiting or actively following up patients from April 2008, and which have not yet been confirmed as being on the Portfolio, we would be very grateful if you would let us know. Further details are available at http://www.ukcrn.org.uk/index/clinical/portfolio_new.html.

Thank you for your support in this exercise which will be critical to the successful development of the national Comprehensive Clinical Research Network. Our aim is to ensure the provision of high quality infrastructure to support clinical research in the NHS and support the delivery of your study.

Please do not hesitate to contact me if you require any further information.

Best wishes

Dr Sam Taylor
Portfolio Lead
NIHR Clinical Research Coordinating Centre (NIHR CRN CC)
Fairbairn House
71-75 Clarendon Road
Leeds
LS2 9PH

Tel: 0113 343 0403
Fax: 0113 343 2300
Email: s.taylor@ukcrn.org.uk
www.crncc.nihr.ac.uk