

Hepatitis B: The view from West Africa

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ABSTRACT

The Prevention of Liver Fibrosis and Cancer in Africa (PROLIFICA) study began in 2011 in The Gambia, Sénégal and Nigeria. The study aims to reduce the risk of hepatocellular carcinoma (HCC) in West Africa through the suppression of the Hepatitis B virus (HBV). The biological samples collected allow for the detection of novel liver cancer biomarkers in the hope of improving the diagnostic ability of early disease states. The PROLIFICA platform hopes to improve cancer diagnostics whilst simultaneously providing the training, skills and infrastructure necessary to develop the quality of liver cancer care in West Africa.

Introduction

The hepatitis B virus (HBV) is a parenterally transmitted DNA virus causing chronic infection in more than 350 million people worldwide [1]. Over 600,000 people die annually from the acute or chronic consequences of HBV, with 15-25% of those infected during childhood dying as a result of advanced cirrhosis or hepatocellular carcinoma (HCC) [2].

HBV Virology and Epidemiology

HBV is an immunopathic virus of the *Hepadnaviridae* family. Virions consist of an icosahedral nucleocapsid core surrounded by an outer lipoprotein envelope, containing surface antigen glycoproteins (HBsAg) – see Figure. 1. Circular partially double-stranded viral DNA (dsDNA) is covalently linked to a HBV polymerase molecule and situated within the core [3].

There is significant global heterogeneity regarding the prevalence of HBV, with the highest rates observed in Sub-Saharan Africa (SSA) and East Asia [4]. Globally, HBV is responsible for over half the adult cases of HCC as well as nearly all childhood cases [5].

Natural History

HBV transmission can occur parenterally as well as from vertical transmission at the time of birth. Acute HBV infection can prove self-limiting, with the elimination of virus and the development of persistent immunity preventing reinfection. The persistence of HBsAg for more than 6 months is defined as chronic infection [6].

A major determinant of the risk of chronic infection is the age at which exposure to the virus occurs. Unless vaccinated, infection within the first 6 months of life is associated with an 80-90% chance of chronic viral

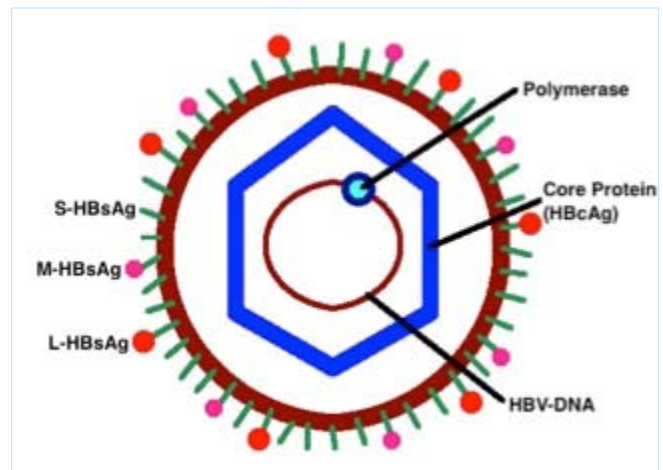


Figure 1. The Hepatitis B Virus

carriage. The risk of chronic carriage decreases to 30% if infection is acquired before 6 years of age. The incidence of chronicity in adulthood is less than 5% [7].

Few studies have investigated the natural history of chronic HBV within an African setting, with the majority of published literature deriving from American, European and Asian groups.

HBV in The Gambia

The Gambia is the smallest country of mainland Africa, located on the West coast, with a population of 1.8 million – see Figure 2. It is a narrow country and other than an 80km stretch of Atlantic coastline, is completely surrounded by Senegal.

The rate of chronic HBV carriage in the adult Gambian population is 8.2% [8]. HCC is the most common cancer type amongst males in The Gambia, with at least 60% of cases being directly attributable to HBV [9].

Genotype E is the predominant genotype found within



Figure 2. Map of The Gambia - Reproduced by kind permission of Globe Media, www.worldtravels.com

West Africa and in particular, The Gambia, where it is present in over 90% of chronic carriers [10]. The clinical impact of this is yet to be elucidated.

The PROLIFICA Platform

The Prevention of Liver Fibrosis and Cancer in Africa (PROLIFICA) study began in 2011 and consists of two main research platforms: the West African Treatment Cohort for Hepatitis B (WATCH) study and the Hepatocellular Carcinoma Case-Control (HC4) study. This international, multi-centre study is coordinated by Mark Thursz, Professor of Hepatology at Imperial College, and brings together experts from The Gambia, Senegal, Nigeria, France, Italy and the UK.

The WATCH study aims to establish the effect of anti-viral therapy on HCC incidence. Individuals found positive for HBsAg from population-based screening programmes using a point-of-care test (Alere, Determine, USA) are invited to the MRC, The Gambia Unit Liver Clinic in Fajara for further assessment including clinical examination, abdominal ultrasound, transient elastography and blood tests. Those eligible for treatment, in accordance with the European Association for the Study of the Liver (EASL) 2012 guidelines [11], are offered anti-viral therapy with Tenofovir.

The HC4 case-control platform aims to evaluate the importance of currently recognised risk factors in this SSA population and generate serum, urine and DNA samples for analysis by proteomics, metabonomics and genome wide association studies.

HBV-DNA Quantification

The quantification of HBV-DNA is important for patient monitoring and helps determine the necessity of treatment, with elevated viraemia being a surrogate marker for poor disease outcomes such as cirrhosis and HCC [12].

There remain no established local guidelines on the management of HBV in SSA. The EASL guidelines state that pharmacological intervention should be considered in patients with HBV-DNA levels of 2,000 IU/ml or greater, with elevated serum alanine aminotransferase (ALT) levels and the severity of liver fibrosis also guiding management [11]. Anti-viral therapy is required in order to reduce long-term morbidity and mortality risk through viral suppression. For those not fulfilling treatment guidelines, bi-annual follow up is recommended due to the unpredictable nature of viral replication.

The Challenge

Within the PROLIFICA platform, the current method of HBV-DNA quantification involves venous blood sampling during participant assessment at the MRC Fajara liver clinic (Figure 3). Plasma, serum and buffy coat samples are stored at -80°C with the quantification of viral load performed using real-time PCR (qPCR) from cryopreserved samples. On occasion, venepuncture sampling is performed at field locations followed by the transport of samples to central laboratories. This process is expensive, as samples require cold-chain shipping. Samples in transit are a biohazard and need processing within 8 hours to ensure optimal sample quality.

Dried Blood Spots (DBS)

Filter paper analysis of blood is used today for the detection of metabolic and genetic conditions in neonates [13]. Capillary blood from a finger or heel prick, dried onto filter paper, not only minimises the volume of blood required for analysis but also reduces patient distress (Figure 4). Dried samples stored with desiccant can be safely transported as non-hazardous material to central laboratories for elution without time restraints. There are no laboratory or energy requirements at the point of collection, making the method ideal for remote locations

with minimal resources. The method requires fewer physical supplies, whilst eliminating the risk of needle stick injuries.

The quantification of HBV-DNA is important for patient monitoring and helps determine the necessity of treatment. Work is currently underway in order to develop a DBS sampling system for the quantification of HBV-DNA in order to make the process of sample acquisition simpler and more cost efficient.



Figure 3. A venepuncture sample being obtained from a clinic attendee



Figure 4. A filled Dried Blood Spot card

Conclusion

Working in resource-poor settings highlights the need for efficient and financially viable research methods and techniques. The development of an optimised DBS sampling system for the quantification of HBV-DNA could prove beneficial in countries such as The Gambia, helping to reduce costs while improving healthcare access to a greater proportion of the population. The continued work on identifying novel liver cancer biomarkers to assist with the earlier diagnosis of liver cancer in resource-poor settings remains a hopeful target, whilst the continued investment in local skills and infrastructure is helping to improve the quality of liver cancer healthcare in West Africa.

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