

The S-gene mutations in the circulating HBV genotypes/sub-genotypes associated with hepatitis B infection in Uganda and their effects on cytokines expression in liver disease progression.

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Abstract – Hepatitis B virus is one of the major causes of chronic liver diseases, including hepatocellular carcinoma. Liver damage as a result of HBV infection is due to host immune response and is modulate by cytokines. The HBV is classified into 10 genotype denoted as A, B, C, D, E, F, H, I, and J together with several sub-genotypes which have diverse geographical distribution. Selection pressures such as vaccination, antiviral therapy especially in HIV co- infection, as well as host immune responses have resulted into emergency of viral variants. These are associated with severity of the liver diseases. In addition, viral mutations have been implicated in influencing the clinical outcome of HBV infection. Lack of advanced clinical laboratory for routine and accurate patient testing and the limited knowledge about the circulating genotypes in the developing world has escalated the spread of the disease. This concept paper posits that knowledge about the circulating HBV genotypes and mutations particularly in the S- gene is paramount in providing evidence based clinical management of HBV in Uganda. The paper also underpins the need for more elucidation of cytokine profiles in HBV virus infection since liver disease progression is cytokine modulated especially in the scenario where mutations are common yet they influence cytokine profiles. The concept paper also emphasizes the need for rapid and accurate diagnosis of HBV in our country since management of any infectious disease is contingent upon rapid and accurate diagnosis. The paper also justifies the need for routine genotyping to match vaccine strains with field strains because of the multi-genotype nature of the virus and yet new ones are continuously emerging due to diverse selection pressures emanating from the host immunity and the use of highly active anti-retroviral therapy (HAART) in HBV-HIV co-infection.

Keywords: : Hepatitis B virus, Genotypes, Cytokines, Mutations

Section one: Background

Hepatitis B virus (HBV), a member of the *Hepadnaviridae*, is a circular, partially double-stranded DNA virus and is one of the major causes of chronic liver diseases, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (Matsuura et al., 2009, Khamduang et al., 2013). The HBV genomic structure has been exclusively reviewed by Coleman (2006), Suppiah, Mohd Zain, Bahari, Haji Nawi, & Saat (2014), Ding, Miao, Li, Dai, & Yu (2015) as a partially double stranded DNA of genomic size of approximately 3.2kb with four open-reading frames (ORF). The ORFs encode four genes including the polymerase gene designated as P gene, core (C) gene, large, medium and small surface antigen proteins (S gene) and the X protein (Coleman, 2006, Kahila Bar-Gal et al., 2012). The HBV genome S gene is paramount importance in the molecular genetics of the virus since it is concerned with the expression of the surface antigens and classification of the viral strains (Suppiah et al., 2014) as well as the antigenic variation of the virus. Most importantly, mutations in the S-gene have been reported to affect pathogenicity and oncogenic potential which in turn affects cytokine profile in HBV infection (Svicher, Cento, & Salpin, 2011). Cytokine are critical molecules in progression of the liver disease as reported earlier by Akpolat, Yahsi, Godekmerdan, Demirbag, & Yalniz (2005) and Frodsham et al (2006). Damage to the liver as a result of HBV infection is due to immune response as reported in earlier studies by Racanelli & Rehermann (2006) and in recent studies by Wang & Zhang (2009) and is cytokine modulated but cytokine profiles in HBV virus infection need more elucidation especially in the scenario where mutations are common.

Arauz-Ruiz, Norder, Robertson, & Magnius, (2002) previously classified HBV into 8 genotype identified as A-H based on an intergroup divergence of 8% or more in complete nucleotide sequence whose geographical distributions was previously extensively studied by Sanchez-Tapias, Costa, Mas, Bruguera, & Rodes, (2002) who documented that genotype A is pandemic, B and C are predominant in Asia, D in southern Europe, E in Africa, F in United States of America, G in France while H in Central America. However recent studies by McMahon (2009), Cao (2009), Kurbanov, Tanaka, & Mizokami, (2010) have introduced two new genotypes designated as I and J giving a total of 10 genotypes together with several sub-genotypes.

In a recent study by Dunford et.al., (2012), genotype variations in HBV together with specific viral mutations have been implicated in influencing the clinical outcome of HBV infection. A similar study by Singhatiraj, Suri, & Goulston, (2012) has documented that genotype A is the most common in co-infection, genotypes B and C are associated with higher viral loads when compared to A and D. Selection such as vaccination, antiviral therapy especially in HIV co-infection, as well as host immune responses have resulted into emergency of viral variants associated with severity of the liver diseases (Dunford et al., 2012). Multiple studies by Thio,

(2009) and Matthews et al., (2013) have shown that the use of highly active anti-retroviral therapy (HAART) have resulted into hepatotoxicity and has been implicated as the major cause of mortality in HBV-HIV co-infection.

According to the world health organization (WHO), countries of Africa, Asia and South America have carrier rates as high as greater than 8% (Franco et al., 2012) with Sub-Saharan Africa accounting for 20% of global burden (Khamduang et al., 2013) . In Uganda, recent media reports show that 10% (3.5million) of the population are chronic carriers and are either already suffering from liver disease or at a risk of developing liver disease. This challenge is precipitated by lack of advanced clinical laboratory for routine and accurate patient testing (Franco et al., 2012) as well as the limited knowledge about the circulating genotypes and sub genotypes in the developing world (Singhatiraj et al., 2012). Previously, a novel field deployable, rapid, simpler, single temperature, nucleic acid amplification method, termed loop-mediated isothermal amplification (LAMP), has been developed for laboratory diagnosis of many infections (Notomi *et al.*, 2000). However no study has been reported to evaluate the use of LAMP in the diagnosis of HBV in Uganda. It has been used for the timely diagnosis of hepatitis B virus (Nyan et al., 2014), malaria (Hopkins et al., 2013), African Swine fever Virus (Atuhaire et al., 2014), foot-and-mouth disease virus (Kafeero et al., 2016) Human African Trypanosomiasis (Matovu, Enock, Kuepfer, Boobo, Kibona, & Burri, 2010). This concept paper underpins the urgent need to use rapid diagnostic assays such as LAMP and comparing its diagnostic sensitivity and specificity with the commonly used assays of ELISA and polymerase chain reaction in order to come up with recommendations to the policy makers and the Ministry of Health about the potential benefits of the assay that has received a lot of attention in the recent times.

This concept paper is divided into four sections. After introducing the key conceptual issues in section one, there is section two which provides the conceptual objectives and hypothesis that underpin the development of the whole concept. Section three explicates the conceptual problem which this paper is trying to address. Section four summarizes the literature that informed the design of the concept. The objective of this paper is to elicit Ugandan scientists, physicians and policy makers to appreciate the magnitude of the current and future effect of HBV in our country and think outside the box, using evidence based practice to manage HBV. This is in line with the recently ushered in sustainable development goals as stipulated in goal 3 target 3.3 which is aimed at eliminating hepatitis B by 2030.

Section two: Conceptual objectives and hypothesis

I hypothesize that mutations have occurred not only in the S-gene implicated in the antigenicity of the virus but also in the entire genome of the hepatitis B virus resulting into evolution and emergence of several HBV genotypes which influence cytokine profiles. Immigrants into our country from all other parts of Africa in particular and World over in general have

profound effect on the HBV circulating genotypes yet some are issued with immigration documents without valid HBV vaccination documents. This re-affirms my hypothesis of novel genotypes in our region. Liver disease progression, vaccination and antiviral therapy are genotype specific. Unfortunately there is no clear documentation on the circulating genotypes in Uganda underscoring the burden of the disease.

General objective

To major aim of the study is to establish the S-gene mutations in the circulating HBV genotypes/ sub-genotypes associated with hepatitis B infection and the role of cytokines in liver disease progression.

Specific objectives

1. To determine the prevalence of HBV DNA and antibodies in blood/serum of the study subjects.
2. To establish the circulating genotypes and sub-genotypes of HBV in Uganda.
3. To determine the S-gene mutations in the ORF of HBV genome.
4. To profile the panel of cytokines in patients infected with HBV.

Section three: Conceptual problem

Management of HBV requires the knowledge of mutations particularly the S-gene mutations as well the genotypes and the sub-genotypes because they affect antiviral therapy (Kurbanov, F., Y. Tanaka, K. Fujiwara, F. Sugauchi, D. Mbanya, N. Ndembu, C. Ngansop, L. Kaptue, T. Miura, E. Ido, M. Hayami, & Ichimura, 2005). According to CY Wai, Chu, Hussain, & Lok, (2002) Kobayashi, Arase, & Ikeda, (2002) Kao, Chen, Lai, & Chen, (2002), Yuen et al., (2003), Tanaka et al (2004), Kurbanov, F. et al (2005), Cooksley, (2010), Ferreira & Tenore, (2010), Lin & Kao, (2011), Singhatiraj et al., (2012), Dunford et al., (2012), Matthews et al., (2013) there have been increasing lines of evidence to indicate influences of HBV sub-genotypes on the outcome of liver disease, HBeAg seroconversion, severity of the disease and the response to antiviral therapies.

To date there is no curative treatment to eradicate the HBV but the commonly used therapies such as interferon, pegylated interferon as well as nucleoside / nucleotide analogs prolong the suppression of viral replication and establish host immune control over the virus (Alazawi & Foster, 2008). The success of these therapies is contingent upon the specific viral genotype infecting the host (Kao et al., 2002, Janssen, van Zonneveld, & Senturk, 2005) as well as There is paucity of information about HBV genotypes, S-gene mutations, cytokine profiles in HBV infections elsewhere (Arauz-Ruiz et al., 2002, Sanchez-Tapias et al., 2002, McMahon, 2009, Coleman, 2006, Kahila Bar-Gal et al., 2012, and Zhang et al., 2016). However no study in Uganda to date on the molecular genomics of the circulating genotypes/ subtypes,

mutations and their effects on disease progression in terms of cytokine profiles in pathological conditions. Since migrations and variations in selection pressures affect the circulating genotypes and the subsequent mutations (Kahila Bar-Gal et al., 2012), these affect the efficacy of the antiviral drugs. These challenges underscore the burden of the disease which is a public health concern. The recently ushered in sustainable development goals (SDGs) have emphasized the need to eliminate hepatitis B virus infection by 2030. Therefore this concept paper retaliates for an urgent need for studies on molecular biology of the HBV in order to provide physicians and other health workers with evidence based information particularly in areas of molecular genetics of the virus required in the management of HBV in Uganda.

Section four; Literature review

Hepatitis B genotypes and subtypes

The HBV genome is composed of approximately 3,200 nucleotides (Matsuura et al., 2009). Arauz-Ruiz, Norder, Robertson, & Magnius, (2002) previously classified HBV into 8 genotype identified as A-H based on an intergroup divergence of 8% or more in complete nucleotide sequence whose geographical distributions was previously extensively studied by Sanchez-Tapias, Costa, Mas, Bruguera, & Rodes, (2002) who documented that genotype A is pandemic, B and C are predominant in Asia, D in southern Europe, E in Africa, F in United States of America, G in France while H in Central America. However recent studies by McMahon (2009), Cao (2009), Kurbanov, Tanaka, & Mizokami, (2010) have introduced two new genotypes designated as I and J giving a total of 10 genotypes together with several sub-genotypes. With the exception of the newly identified genotypes, the other genotypes and sub-genotypes have well characterized ethnic and geographical distribution (Lin & Kao, 2011). According to the study by Lin & Kao, (2011) genotype A has three sub-genotypes (A1-3) with A1 having prevalence in Sub-Saharan Africa whereas A3 located in West Africa. Genotype B has six sub-genotypes B1-6 with none of them isolated in Africa. Genotype, C with five sub-genotypes C1-5 and none of them in Africa. Genotype D, with sub-genotypes D1-5, and none of them in Africa. Genotype E has no reported sub-genotype up to date and it is restricted in West Africa. Genotype F has four sub-genotypes and none of them is distributed in Africa. The other genotypes G- J have no sub-genotypes and none of them has been reported in Africa.

According to Tanaka et al (2004), Kurbanov, F. et al (2005), there have been increasing lines of evidence to indicate influences of HBV sub-genotypes on the outcome of liver disease and the response to antiviral therapies. From previous studies by Jia-horng Kao, Chen, Lai, & Chen (2002), hepatitis B virus genotypes have been linked to staging of the disease progression. According to their study, young patients with hepatocellular carcinoma are likely to be infected with HBV genotype B than genotype C whereas older patients with more

advanced liver disease are more likely to be infected with genotype C than genotype B. In an earlier study by Yuen et al., (2003), it was established that patients with HBV genotype B had more severe presentation of the disease and at a more risk of hepatic decompensation as compared to HBV/C infection. In a related study by Kobayashi et al., (2002) it has shown that patients with HBV/B have more serious liver disease than patients with HBV/C. The HBV genotypes have also been implicated in variations in seroconversion to hepatitis B e antigen (HBeAg) antigen. Studies by Chu, Hussain, & Lok, (2002) have shown that patients with genotype B achieve HBeAg seroconversion 10 years earlier than patients with genotype C. Variations in response to treatment are also affected by genotypes. Evidence from studies by Wai, Chu, Hussain, & Lok, (2002) have shown that patients with genotype B respond better to IFN- γ as compared to patients with genotype C.

The HBV S gene mutations

The HBV genomic structure has been exclusively reviewed by Coleman (2006), Suppiah, Mohd Zain, Bahari, Haji Naw, & Saat (2014), Ding, Miao, Li, Dai, & Yu (2015) as a partially double stranded DNA of genomic size of approximately 3.2kb with four open-reading frames (ORF). The ORFs encode four genes including the polymerase gene designated as P gene, core (C) gene, large, medium and small surface antigen proteins (S gene) and the X protein (Coleman, 2006, Kahila Bar-Gal et al., 2012). Studies on the full genome analysis of hepatitis B genome have given a paucity of information including identification of mutations reported world over in all the four ORFs.(Quer et al., 2008).

The HBV genome S gene is paramount importance in the molecular genetics of the virus since it is concerned with the expression of the surface antigens and classification of the viral strains (Suppiah et al., 2014) as well as the antigenic variation of the virus. These genetic mutations in the S-gene enable the virus to escape the host's immune system as well other selection pressures such as antiviral drugs and vaccines. The challenge in giving health care services to chronic HBV infections as a results of the antigenic variations of the virus is reduced sensitivity and specificity of the assays used in the diagnosis of the virus giving false negatives, failure of medication, and vaccination if the mutations are not timely identified.

Cytokine profile in HBV infection

Cytokine are critical molecules in progression of the liver disease as reported earlier by Akpolat, Yahsi, Godekmerdan, Demirbag, & Yalniz (2005), Frodsham et al (2006) and it common knowledge that damage to the liver as a result of HBV infection is due to immune response as reported in earlier studies by Racanelli & Rehermann (2006) and in recent studies by Wang & Zhang (2009) and is cytokine modulated but cytokine profiles in HBV virus infection need more elucidation especially in the scenario where mutations are common.

Rapid detection of HBV

The hepatitis B infection is a global public health concern. This is aggravated in countries where health care facilities are poor due to the shrinking resource allocation to the health care services in the national budgets (Nyan et al., 2014). This problem is worsened by the natural coincidence of the disease being endemic in these poor countries (Nyan et al., 2014). According to the world health organization (WHO), countries of Africa, Asia and South America have carrier rates as high as greater than 8% (Franco et al., 2012). This challenge is precipitated by lack of advanced clinical laboratory for routine and accurate patient testing (Franco et al., 2012) as well as the limited knowledge about the circulating genotypes and sub genotypes in the developing world (Singhatiraj, Suri, & Goulston, 2012). In many countries, HBV diagnosis is based on screening for HBV surface antigen, antibodies to the core HBV, and HBV DNA (Nyan et al., 2014). These tests are performed with enzyme-linked immunosorbent assay (ELISA) and real time polymerase chain reaction (rRT-PCR). These tests are slow and require expensive laboratory equipment such as the ELISA reader, real time PCR machine in addition to specially trained laboratory staff (Caliendo, Valsamakis, & Bremer, 2011, Kao, JH 2008). A recent study in Uganda by Mullis et al., (2013) revealed a high frequency of false-positive hepatitis C virus in Rakai. In their study, the high prevalence of false positive was due to clearance of HCV RNA but not the antibody. However this explanation is invalid since in their study, there was no single sample that was positive by both the HCV RNA Abbot real time HCV assay and ELISA assay suggesting that the positives by ELISA are most likely to be false positive. These studies provide a basis for adopting the use of alternative assays in the detection of HBV with rapidity, high sensitivity, specificity and at lower cost without the need of sophisticated laboratory equipment and trained staff in Uganda.

Conclusion

The concept paper has underlined the need to investigate the S-gene mutations in the circulating hepatitis B viral strains in Uganda. The S-gene encodes for the surface protein coat which has been implicated in antigenicity of the virus which in turn influences the effectiveness of antiviral therapy. It has also highlighted the need to for routine HBV genotyping in order to match vaccine strains with field strains for effective immunization programs in our country. The concept paper has underpinned the need to screen immigrants using HBV genotype specific assays in order to inform the physicians so as to adopt evidence based HBV management.

The paper has outlined the need for rapid and accurate detection of HBV which is paramount in management of the disease. The paper has quoted studies which left several questions unanswered hence leaving knowledge gaps.

Competing interests

We declare that we have no any competing interests

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