I, Kathleen T Ryan M.D., hereby submit this original work as part of the requirements for the degree of Master of Public Health in Epidemiology.

It is entitled:
Occult Hepatitis B in HIV Positive Botswana

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Occult Hepatitis B in HIV positive Batswana

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Abstract:

Hepatitis B infection is the leading cause of cirrhosis and hepatocellular carcinoma (HCC) worldwide. Diagnosis of chronic hepatitis B virus (HBV) occurs through detection of the hepatitis B surface antigen (HBsAg). In contrast, occult hepatitis B infection (OBI) is defined as the presence of HBV DNA in the absence of HBsAg. A literature review of OBI in Africa demonstrated that OBI was present throughout much of the African continent. Most cases of OBI in Africa are positive for HBV core antibody with low (less than 200 IU/mL) HBV viral loads. Persons with HIV or hepatitis C co-infection have higher rates of OBI. In HIV positive cohorts, the rate of OBI is only slightly lower (80%) than the chronic HBV infection rate. There is no consistent information available regarding risk factors associated with OBI in Africa.

Botswana is hyperendemic for chronic HBV (greater than 8%) and has a HIV prevalence of 25%. Despite this, OBI had never been studied in Botswana. This study discovered an OBI prevalence of 24% in HIV positive adults of Botswana. Detection of hepatitis B core antibody was more common in chronic HBV/HIV co-infected subjects than HIV/OBI subjects. However, both groups had significantly increased rates of HBV core antibody positivity compared to HIV mono-infected individuals. More than 30% of OBI cases had undetectable core antibody, demonstrating that core antibody positivity is not an appropriate screening tool for OBI in this population. No sociodemographic factors were associated with OBI in univariate or multivariate analysis. Tenofovir/emtricitabine-containing HIV therapy suppressed OBI DNA detection in almost all (65 of 66) individuals evaluated one year after start of therapy.

*Keywords: Occult Hepatitis B, OBI, Botswana, HIV*
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Hepatitis B virus (HBV) is responsible for more than two billion infections worldwide. Acute HBV infections are considered to represent the majority of infections and resolve spontaneously. However, there are an estimated 240 million chronic HBV infections [1]. Chronic HBV infection is the leading cause of hepatocellular carcinoma (HCC) and liver failure worldwide and is defined as the presence of hepatitis B surface antigen (HBsAg) for more than 6 months. Treatment options include nucleoside analogs that suppress but do not eliminate the infection. As a result, the recommendation to initiate treatment is dependent on the HBV viral load.

With the advent of nucleic acid amplification techniques (NAT) to detect and follow HBV DNA viral load, a new entity, occult hepatitis B infection (OBI), was first identified in the 1970s [2]. OBI is defined as the presence of HBV DNA in liver tissue (with or without serum detection) but the absence of detectable HBsAg [3]. Due to increasing recognition of this entity, a European Association for the Study of Liver (EASL) conference was held in Taormina, Italy, in March 2008 to create this definition and better describe OBI. The consensus statement acknowledged that although OBI requires detection of HBV DNA from the liver, there are no standardized or validated tests to detect OBI from liver tissue. Additionally, liver samples are often not available. Highly sensitive nucleic acid detection from serum is often used as a proxy for OBI detection, false negative results do occur since these assays do not always reflect the presence of virus in the liver. Conditions associated with an increased incidence of OBI include co-infection with human immunodeficiency virus (HIV) or hepatitis C virus (HCV), recurrent blood
exposures (both through hemodialysis and/or repeated blood transfusions), and an immunocompromised state [4, 5].

HBV is endemic throughout Africa. Different regions of Africa have distinct genotypes of HBV and different risk factors for OBI. Until recently, however, there was very limited research on OBI within Africa. Due to the need for very sensitive NAT to detect OBI, research has been limited by cost and technical capacity in much of Africa. Although OBI has been demonstrated to lead to hepatocellular carcinoma and liver cirrhosis, understanding the pathogenesis of OBI is limited, particularly in Africa.

This review article summarizes the currently published literature on OBI throughout the African continent. PubMed was utilized to search for articles about occult hepatitis using terms “OBI”, “occult HBV” and “occult hep* B” with separate searches for each country name, as well as “Africa”. A brief review of titles eliminated any spurious results. The bibliographies of primary articles were reviewed resulting in the inclusion of additional articles for this review. A total of 17 of 54 African countries with OBI data including 57 articles are included in this review (Figure 1).

**Viral Load:**

Only one study has evaluated OBI within liver tissues in Africa (The Gambia) [6]. The remaining studies have evaluated OBI via testing of blood samples. The Taormina consensus statement defines OBI as having a viral load of less than 200 IU/mL while anything above 200 IU/mL is
considered “false OBI” [3]. False OBI is attributed to mutations in the surface protein resulting in either lack of circulating HBsAg in the serum or failure of tests to detect the HBsAg (i.e. diagnostic failure rather than lack of HBsAg synthesis) [3]. This definition is questioned by several studies in Africa. Most studies in Africa have found that the majority of the OBI cases have viral loads close to or below 200 IU/mL irrespective of geographical region or patient population [7-13]. Although the majority of OBI cases fall within the expected OBI range, many studies have detected occasional cases of HBV viral loads greater than 2000 IU/mL [14-18].

There are also several studies in which the majority of individuals have higher HBV viral loads (between $10^2$ to $10^6$ IU/mL), including HCV co-infected cohorts in Egypt [19-22], as well as HIV cohorts both in Western [23] and Southern Africa [24, 25]. In some studies, “false OBI” samples are excluded from the study. In other studies, “false OBI” samples with the high HBV DNA viral load and HBsAg negative are called “reactivation” or “covert HBsAg” HBV infections rather than OBI. This lack of consistency in defining and reporting cases is challenging when comparing study results.

Most cases of OBI detected have qualitatively positive samples with viral loads lower than the level of quantitative detection. In many studies, this results in the majority of OBI having viral loads less than 20 IU/mL. If the lower limit of detection in a study is higher than 20 IU/mL many cases of OBI may be missed. The lower limit of quantification of studies within Africa have ranged from 1 copy/mL (less than 6 IU/mL) up to 800 IU/mL [8, 9, 13, 16, 25-32].
Antibody status of OBI:

The Taormina consensus statement defines two separate groups (seropositive OBI and seronegative OBI) within OBI. The consensus further suggests that seropositive OBI (OBI with HBV core antibody positive, HBV surface antibody positive, or dual HBV core and surface antibody positive) differs from seronegative OBI (HBV core and surface antibody negative OBI) in human T cell immune responses to the HBV virus between these two groups [3]. Despite this, HBV core antibody positivity is often utilized as a less than ideal surrogate for identifying OBI. In many resource-limited areas, including much of Africa, highly sensitive HBV DNA detection by NAT is not feasible. Antibody status of OBI and correlation of positive HBV core antibody with OBI has been evaluated in multiple studies (Table 1).

Within Africa, there are four distinct populations of OBI subjects in which HBV antibody status has been assessed. These populations include healthy controls, HCV-positive individuals, HIV-positive individuals and other high risk populations. In healthy individuals, most are core antibody positive. There is minimal difference between the prevalence of isolated core antibody positive OBI and the prevalence of dual core/surface positive OBI. Seronegative OBI accounts for the third most frequent category with few individuals having isolated HBV surface antibody positive.

HCV is a known risk factor for OBI. The underlying etiology for OBI in HCV infected individuals is thought to be HCV-induced host immune suppression of HBV [33]. In HCV-infected OBI within Africa, the majority of cases are isolated HBV core antibody positive. There is a decreased relative rate of HBV core/surface dual antibody-positive OBI compared to isolated HBV core
antibody positive, although there is significant variation between studies. Seronegative OBI is rare with even less occurrences of isolated HBV surface antibody positive OBI. When HCV/OBI dual-infected cohorts are also immunosuppressed (e.g. malignancy), the antibody status is more variable.

HIV-infected subjects and others with immunosuppression (e.g. with malignancy on chemotherapy) have a wide range of antibody status. There appears to be an approximately equal prevalence of isolated HBV core antibody positive, dual HBV core/surface antibody positive and seronegative OBI. Isolated HBV surface antibody OBI is observed only rarely.

The fourth category of OBI studies is loosely defined as “other increased risk of OBI”.

Individuals who were acutely ill, required frequent blood transfusions or repeated blood exposure via hemodialysis, as well as children born to mothers with chronic HBV infection, were included in this category. The varied nature of these studies make their interpretation difficult, but it appears that the HBV antibody profile is similar to that of immunocompromised cohorts with fairly equal rates of isolated HBV core antibody, dual HBV core/surface antibody and seronegative OBI.

The “e” antigen of HBV infection is a variant of the core antigen and is a marker for active replication of the virus. It is not observed in all infections but is associated with increased infectivity and disease progression in chronic HBV disease. In Egypt (where HBV genotype D predominates) two studies have demonstrated rates of “e” antigen positive OBI to vary greatly between 2.6% to 61.5% in pediatric patients [34, 35]. These studies were both small, which can somewhat explain the variability of this value. In the study where 2.6% of OBI was “e” antigen
positive, an additional 19% of patients were positive for “e” antibody. The “e” antibody only develops in individuals who had been previously positive for “e” antigen. This suggests that the age of the patients may also be important when evaluating “e” antigen or antibody status. In Ghana, where genotype E is predominant, 3 of 9 (33.3%) OBI patients are “e” antigen positive, with an additional 2 of 9 (22.2%) “e” antibody positive OBI [14]. No studies have reported an evaluation of “e” antigen or antibody status in southern or eastern Africa where genotype A predominates.

Prevalence of OBI:

The prevalence of reported OBI varies widely across Africa from 0.3% to 62.6% (Tables 2 - 4). This variability is also present within individual countries in Africa. For instance, Egypt has reported OBI prevalence between 1.2% and 60.3%, while South Africa has reported rates between 1% and 62.6% (Tables 2-4). There are several factors that can impact prevalence rates. As mentioned previously, the lower limit threshold of HBV DNA detection differs significantly between studies. Studies utilizing less sensitive assays will detect fewer cases of OBI. Secondly, several risks factors have been identified which are associated with increased prevalence of OBI. For example, studies involving the high risk populations of HCV or HIV-infected individuals have higher prevalence of OBI in comparison to studies involving “healthy controls”.
A major cause of variability within OBI prevalence is variability of exposure to the hepatitis B virus. Contact with chronically HBV infected individuals is the most common source of HBV exposure. Populations with different chronic HBV infection rates will have different HBV exposure rates, which results in variable rates of OBI. Within Africa, the endemicity of chronic HBV infections varies from intermediate (2%-7%) to hyper-endemic regions (greater than 15% prevalence). Describing the OBI prevalence as a ratio to the chronic HBV infection rate within the same cohort can account for the variable rates of exposure to HBV among different cohorts. The OBI-to-chronic HBV infection (OTC) ratio can therefore allow better comparisons between studies. In healthy adults, this OTC ratio varies widely from 0.038 to 1. Thus, one to 27 cases of chronic HBV infection occur for every one case of OBI. On average, chronic HBV infection is 4 to 6 times as likely of OBI in healthy adults. Cohorts with participants at lower risk for chronic HBV, including post HBV vaccination cohorts or cohorts that exclude subjects with high risks of exposure to HBV, were noted to have a higher OTC ratio. In other “at risk” subjects (those hospitalized, found to have hepatitis, or known to have repeated blood exposures), the OTC ratio increases significantly to more than 8 cases of OBI to every 10 cases of chronic HBV infection.

Specific high-risk populations:

**HCV**

Hepatitis C / OBI co-infections have been studied most frequently in northern Africa, particularly Egypt, where a high baseline prevalence of HCV occurs [4]. Table 3 demonstrates the prevalence data on OBI/HCV co-infected individuals which ranges from 1.8% to 53.8% with
a median prevalence of 33.3%. In symptomatic OBI/HCV co-infected cohorts (i.e. in HCC and cirrhosis), the median prevalence of OBI was significantly higher (45.7%) in comparison to a 10.4% rate among all OBI/HCV individuals. Within HCV-positive cohorts, the OTC ratio ranged from 0.7 to 14 with an average rate of 2, demonstrating that OBI was twice as common as chronic HBV infection.

**HIV**

HIV co-infection is also associated with increased prevalence of OBI. OBI/HIV co-infection has been evaluated in both Western and Eastern/Southern Africa, where high rates of both infections occur. OBI rates vary from 1.6% to 62.5% (see Table 4). However, the rates of chronic HBV infection also vary widely in these studies. The OTC ratio ranges from 0.2 to 6 with a median value of 1. People who are antiretroviral (ART) naïve or with symptomatic AIDS have more OBI and higher OBI-to-chronic HBV infection ratios. The highest rates of OBI (62.5%) were observed in HIV/OBI co-infected individuals undergoing treatment for malignancy, while the lowest rates occurred in pregnant women (of which 75% were on HBV-active highly active antiretroviral therapy (HAART) therapy). When hepatitis B core antibody positivity was not used as a marker for OBI infection, the prevalence was predominantly between 15%-20% (median 15.1%). However, when using core antibody as a “pre-screen” for OBI, the prevalence was typically lower (between 5-15%) with a median of 8.1%. 
**Genotypes of OBI:**

A variety of HBV genotypes circulate throughout Africa. The most common genotypes associated with chronic infection within each country are shown in Figure 1. Genotype is important because the different genotypes have different natural histories. For instance, genotype D has been associated with a lack of “e” antigen, and is often associated with pre-core variant chronic “e” antigen negative hepatitis [36]. Genotype E has a narrow range of diversity and is thought to represent a fairly new genotype of HBV [37]. Genotype A is associated with lower viral loads and earlier transition from “e” antigen positive to “e” antigen negative. Genotype A appears to have a better response rate to interferon therapy in comparison to genotype D [36]. However, genotype A1 is associated with increased incidence of hepatocellular carcinoma in young males with low level viremia, even in the absence of cirrhosis or liver failure [36]. Although hepatocellular carcinoma has been observed in older individuals with genotype A2, genotype D is associated with a higher risk of HCC than genotype A2 [36].

The countries with OBI data were divided into three sections based on the common genotypes circulating in those countries. Northern Africa is predominantly genotype D. Western African countries have a significant burden of genotype E, with less genotype A3, and even less genotype D present in the populations. Genotype A1 is the predominant genotype in Eastern and Southern Africa [38].

Within Egypt, where genotype D is responsible for greater than 80% of chronic HBV infections, genotype D also predominates during OBI [19, 21, 27, 39]. One study in Egypt with a cohort of
subjects with hepatocellular carcinoma demonstrated a more heterogeneous genotype distribution within OBI including 32% D, 24% B, 4% A, 8% C and 20% representing dual infections. This is in comparison to 10 of 10 chronic HBV-infected subjects demonstrating mixed A/D HBV infections in this same study [40]. An additional study in Egypt reported 4 of 5 cases of OBI were infected by genotype C [41].

In a similar fashion, genotype E is the predominant (greater than 85%) genotype in both OBI and chronic HBV infections in many Western African countries [8, 14, 42, 43]. Although E is still the major genotype associated with OBI in Cameroon, an increase in genotype A3 has been observed in OBI with HBV viral loads greater than 200 IU/mL (termed covert HBsAg infection) [10]. In Southern Africa, most OBI is associated with HBV genotype A [13, 44, 45]. Genotype A was also present in the majority of OBI cases within Uganda. This was consistent with predominance of genotype A in chronic HBV infections in neighboring Tanzania [46]. Throughout most of Eastern and Southern Africa, the most common sub-genotype is A1 with one study of HIV positive adults in South Africa finding primarily sub-genotype A2 [44]. In this study, A2 was more prevalent in OBI than chronic HBV infections. In a study of blood donors in South Africa (where genotype A1 was predominant), genotype D was found frequently (seven of 30) in individuals with isolated surface antibody positive OBI. A second study of blood donors in South Africa demonstrated two of four cases of OBI were due to genotype A while the other two were genotype D.

Among countries in Africa, Sudan is unique because of its heterogeneity of HBV genotypes. One study in Sudan demonstrated relatively equal frequency of genotypes D (including
subtypes D1, D2 and D6), genotype E and D/E dual infections in subjects with either chronic HBV or OBI [18]. Rare cases of genotype A infections occurred in this study as well. However, when genotype A was found, the subtype A2 predominated in OBI while A1 was present in most chronic HBV infection. HIV status was correlated with increased A2, as well as mixed infection with D/E in this study (in both OBI and chronic HBV infections). Chronic HBV infections in blood donors were due to genotypes E and A2. A second study in Sudan demonstrated five of six OBI infections were due to D genotype while one was due to B [47].

**Mutations Associated with OBI**

Genetic mutations of HBV are known which can result in the absence of detectable HBsAg. These mutations typically occur within the “a” determinant region of the surface gene (at amino acid numbers 137-147). Specific mutations identified include P120T, Q128H, G130N, S143L, and D144A, as well as several mutations within the pre-S region [38]. Some mutations (including G145R) within this region also result in vaccine escape mutants and therefore not prevented by HBV vaccine. The expert consensus at Taormina had labeled these mutant infections as “false OBI” [3]. Other mutations associated with “e” antigen negative chronic infection occur at amino acids numbers 1653, 1753-1757, 1762, 1764, 1766, and 1788, which are primarily in the core promoter (BCP) region. Drug resistance mutations are discussed later. Mutational analysis of OBI has been studied in Africa in the countries of Egypt, Sudan, the Gambia, Ghana, Cameroon, Nigeria, Gabon and South Africa [8, 10, 14, 15, 18, 19, 21, 23, 27, 39, 45, 48-50].
As mentioned previously, OBI with HBV viral loads greater than 200 IU/mL are often attributed to mutations of the surface antigen. However, several cohorts have reported OBI cases without any detectable “a” determinate region mutation [19]. This suggests that there are likely additional etiologies responsible for the observed high viral load OBI in addition to the lack of ability to synthesize or secrete surface antigen.

In acutely ill children in Egypt, the Y134F mutation of the “a” determinate region was found in all chronic (seven) and OBI (seven) infections [27]. The core/pre core mutation G1896A was present in three of seven OBI infections but only one of nine chronic infections.

There was no difference in core/pre-core promoter region noted in a small study in Egypt. Two of 10 OBI and three of seven chronic HBV infections had both A1762T and G1764A, while one of seven chronic and one of 10 OBI had the G1896A mutation [51].

While evaluating HIV positive patients in South Africa, 235 mutations were identified in OBI only (not chronic HBV-infected) patients [44]. Eighteen had previously been reported, while 27 new mutations were identified in this analysis. Another study from South Africa demonstrated three mutations S45P, P70H and V168A+P217L that only occurred during OBI [52].
Transmission

Transmission of OBI is presumed to be through the same routes as chronic HBV infection – occasional vertical transmission in Africa, and horizontal transmission through common childhood household contact exposure, blood product exposure and sexual contact. Current studies demonstrate a higher prevalence of OBI in blood exposed individuals (see Table 2). Additionally, HCV (which can be acquired from blood products or previous parental schistosomiasis therapy) is correlated with OBI prevalence (see Table 3). Mother infant studies suggest some degree of maternal to infant transmission which is described in the prevention of OBI with vaccination section below. However, very few studies have evaluated OBI transmission in Africa. Due to the difficulty in detection of OBI and costs involved with long term follow up of OBI, additional reliable data regarding transmission may be very difficult to obtain.

Risk Factors Associated with OBI

In young children with OBI and thalassemia, an increasing HBV viral load was correlated with increased age (greater than 6 years old) [35]. These children with OBI were more likely to be male, live in rural areas, and have ANA positive autoimmune phenomenon. In hemodialysis patients, OBI was associated with longer length of dialysis [22]. In HIV positive South Africans, OBI co-infection was correlated with increased ALT in males and an increased number of lifetime sexual partners in females [24]. HIV/OBI occurred more often in older males with
lower CD4 count when compared to chronic HBV/HIV co-infections. However, OBI/HIV co-infected patients in Sudan were less likely to have a history of jaundice compared to chronic HBV/HIV co-infected patients [17].

**Progression of Disease with OBI**

OBI has been associated with worse disease status and more rapid progression of HIV and HCV in co-infected patients. Additionally, OBI is believed to cause HCC and cirrhosis [53, 54]. Finally, patients with OBI may reactivate to acute or chronic HBsAg-positive disease. In patients undergoing chemotherapy in Egypt, one patient was found to have OBI prior to onset of immunosuppression. This patient had reactivated HBsAg positive HBV infection during chemotherapy. Three other patients developed OBI during chemotherapy [39]. In contradiction to the belief that HBV surface antibody positivity protects against reactivation of HBV (in addition to belief that it protects from initial infection), the patient who developed acute reactivation from OBI was positive for HBV surface antibody. At least one of the others to develop OBI during chemotherapy was also positive for HBV surface antibody.

In HCV-infected cohorts, there is a correlation between OBI positivity and worsening liver disease (cirrhosis and HCC) [20]. Kishk [19] found that in individuals with chronic HCV infection, those with OBI co-infection had higher HCV viral loads. El-Sherif [20] demonstrated an increase in the number of patients with cirrhosis (16 of 16 with OBI/HCV compared to 52 of 55 HCV infected, HBV core antibody positive cases and 23 of 29 HCV non-HBV infected. There was also
a statistically significant difference in severity of disease in OBI co-infected individuals, and a statistically significant increase in Child’s C classification of liver disease. This cohort of 100 patients had a high burden of cirrhosis. In acutely ill adults with a high rate of OBI/hepatitis C co-infection, those with OBI had higher ALT levels than those with chronic HBV infection [51].

**Treatment of OBI:**

Treatment of chronic HBV infection typically involves nucleoside analogs that inhibit the HBV reverse transcriptase. These drugs include lamivudine, emtricitabine and telbivudine, as well as adefovir and tenofovir. Clinically, a transient rise in liver enzymes is observed in OBI/HIV co-infected patients above that of chronic HBV/HIV co-infected patients. However, this appears to resolve within the first six months of therapy [15]. After one year of therapy with a HBV active HIV medication, 18 of 18 (100%) patients in South Africa who were treated with a tenofovir-containing regiment had an undetectable HBV viral load, while 19 of 21 (90.5%) on those receiving lamivudine as sole HBV active HAART therapy had an undetectable HBV viral load [13]. In contrast, a cohort of HIV patients in Gabon demonstrated no difference in HBV viral loads between those patients who had been on treatment with HBV active HIV therapy versus those who had not received HBV active therapy. A significant number of patients in this study had mutations that confer drug resistance [23].
Drug Resistance

Multiple mutations within the HBV genome have been discovered that are associated with drug resistance. The rtM204I (YMDD mutation) in the polymerase gene has been associated with telbivudine, emtricitabine, and lamivudine resistance. In contrast, the N236T mutation is associated with adefovir and possibly tenofovir resistance.

In treatment-naïve patients with OBI in Sudan, no drug resistance mutations were found [18]. In contrast, lamivudine naïve HIV/OBI positive patients in South Africa had four of 13 individuals (30.8%) with the rtM204I mutation resulting in lamivudine resistance. This same mutation was present in 85% of HIV/HBV and 20% of chronic HBV mono-infections within the same study [55].

In a second study of treatment naïve HIV positive individuals in South Africa, one of 30 (3%) individuals with OBI had the lamivudine resistant mutation rtM204V, while two of 19 (10%) chronic HBV infections had rtM204I mutation resulting in lamivudine and telbivudine resistance [44]. An additional study in South Africa found the rtM204I mutation in three of 15 (20%) chronic mono-infected HBV individuals, six of seven (86%) HIV/chronic HBV-infected individuals, and four of 13 (31%) OBI/HIV infected individuals [55].

In the HIV/OBI cohort previously mentioned from Gabon in which there was no detectible difference in HBV viral load with therapy, two of five (40%) on lamivudine had the M204V/I mutation, three of four (75%) of those who were drug naïve had the mutation, while the one OBI patient on tenofovir did not demonstrate this mutation [23].
Prevention of OBI with Vaccination:

Vaccination at birth is known to decrease incidence of chronic hepatitis B infection with or without administration of hepatitis B immunoglobulin (HBIG). The Gambia was the first country in Africa to start mass infant HBV vaccinations in 1986 [56]. In the early 1990s, The Gambia screened 358 previously vaccinated children. Using hepatitis core antibody as a pre-screen for OBI, one child (3% of core antibody positive, or 0.3% of total cohort) had OBI in comparison to two having chronic HBV infections [49]. Mphahlele [57] evaluated 162 children after receiving HBV vaccine in South Africa and found no detectable chronic or occult hepatitis B infections. In an Egyptian cohort of vaccinated children, no OBI was found in 63 children with insulin dependent diabetes mellitus or in 107 healthy controls [58].

In cohorts of people with increased risk of exposure to HBV, there are variable rates of OBI after vaccination. In post-vaccinated infants born to mothers with chronic hepatitis B infection, the HBV vaccine and HBIG immunoglobulin has been shown to be beneficial but not one hundred percent protective. In Mayotte, two of 100 infants born to mothers with chronic hepatitis B developed OBI (2%) [26]. This is in comparison to one of 100 (1%) who developed chronic HBV infection. One of the two infants who developed OBI received the full prophylactic regiment (HBIG and HBV vaccine within 12 hours of birth, with completion of HBV vaccine at one and six months). The other child was given HBIG and HBV vaccine within 12 hours of birth but did not complete either the one or six month booster vaccinations.

Two separate studies evaluated children who received frequent blood products. Both studies demonstrated no difference in the rate of OBI between the children who were vaccinated and
those who were not [34, 35]. In children who were vaccinated, fewer were positive for positive HBV core antibody [34]. In cancer patients with HCV infection who were presumed to be vaccinated against HBV, 32% were found to have OBI compared to none of the 50 matched cancer patients without HCV infection [21].

Infants born to mothers with HIV/HBV co-infection (either chronic or occult) were evaluated in South Africa, where HBV vaccination starts at 6 weeks of age instead of being given at birth. Of three infants born to mothers with OBI, one of the three infants (33%) developed OBI on follow up testing, while two additional infants born to a mother with chronic HBV infection also developed OBI. Only one infant (born to mother with chronic HBV/HIV co-infection) developed chronic HBV infection. Two infants born with OBI had mothers on anti-HIV medication containing an HBV active drug [30]. An additional study in adults with HIV demonstrated that two of 33 (11%) HBV-vaccinated adults had OBI, while six of 33 (18%) HBV-vaccinated adults had chronic HBV infection [59].

When evaluating presumed hepatitis B exposed people (either core antibody or HBsAg positive) in South Africa, there was only a slight decrease in prevalence of OBI from 56.4% in the younger vaccinated cohort compared to 58.3% in the older non vaccinated cohort. The chronic HBV infection had similar changes (14.5% in vaccinated youth, 17.3% in older non vaccinated adults). The average viral loads were similar in both pre- and post-vaccine groups. However, there was a larger OBI to chronic HBV infection ratio in the post vaccine group [52].
**Conclusion**

Overall, OBI genotypes are similar to the chronic HBV genotypes within the same geographical area. However, there may be an increase in either dual infections or infections with less common genotypes in OBI compared to infections with chronic HBV.

Seventy to 80% of OBI cases are HBV core antibody positive (either alone or in combination with surface antibody positive). However, this suggests that 20-30% of cases of OBI do not have prior “exposure” to hepatitis B as defined by HBV core antibody positivity. The prevalence of HBV core antibody negative OBI is nearly 40% in immunocompromised subjects. The majority of HBV core antibody negative OBI is “seronegative” OBI, although some do have isolated surface antibody positivity (appearance of protection from HBV). The hepatitis “e” antigen can be present in OBI, which is associated with increased viral replication, increased risk of transmission and faster progression of disease. However, more studies on “e” antigen positive OBI should be conducted.

Variable HBV viral loads have been detected in OBI within Africa. However, most studies demonstrate OBI HBV viral loads are less than 200 IU/mL. In fact, most HBV viral loads are below the lowest limit of detection used in these studies. However, higher viral loads have been well documented in OBI even in the absence of identifiable mutations associated with decreased surface antigen expression (i.e. “false OBI”).

In otherwise healthy individuals, there appears to be a four to six fold rate of chronic to occult HBV infection. In HIV positive individuals, OBI prevalence increased to nearly the same rate as
chronic HBV infection. HCV infection further increases the prevalence of OBI resulting in twice the prevalence of OBI than chronic HBV infection.

Genetic mutations have been identified in OBI but not chronic HBV infections. Additional research is needed to determine which of these mutations have significant phenotypic changes.

The hepatitis B vaccine does appear to reduce the incidence of both chronic HBV infection and OBI. However, vaccine may reduce the rate of chronic HBV infection more than OBI. With increased exposure to HBV, the rate of OBI in vaccinated individuals can be significant, especially in immunocompromised hosts.

Evidence does suggest that OBI can be transmitted from mother to infant, as well as OBI developing in infants born to mothers with chronic HBV infections. Frequent blood exposure also appears to have risk of transmission. There is very little known about other transmission and risk factors for OBI or about the progression of OBI disease. More research into these elements of OBI is needed. Treatment with nucleoside analogs for OBI demonstrates good response in some studies. Although drug resistance is less frequent in OBI than in chronic HBV infection, an overall decreased in effectiveness of treatment does occur due to drug resistance in OBI. Very little is known about the role of OBI in hepatocellular carcinoma in Africa despite Africa having high rates of both hepatocellular carcinoma and OBI (both with “e” antigen positive and “e” antigen negative disease). More research is needed to determine risk modification of hepatocellular carcinoma from OBI.
Figure 1: Countries with Published Studies on Occult Hepatitis B used for this Review

The predominant genotype of chronic hepatitis B infection within each country is illustrated by the color shading. Only countries with published data on OBI are included. Red arrow indicates The Gambia.
Table 1 – Antibody status of OBI patients

Study results represented as percentage of total (number of subjects). * indicates study data appears in multiple places within this table. When only incomplete data available, ranges represent possible ranges based on this data. Total number recorded excludes data which was not reported (DNR) within the original study. ___ were not evaluated within the reported study, and may result in underestimated pooled rates within the core negative OBI antibody status.

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Cohort</th>
<th>Core Positive</th>
<th>Surface Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Surface -</td>
<td>Both +</td>
</tr>
<tr>
<td>Allain, 2009 [45]</td>
<td>South Africa</td>
<td>Blood Donors (54)</td>
<td>44.4% (24)</td>
<td>44.4% (24)</td>
</tr>
<tr>
<td>Oluyinka, 2015 [8]</td>
<td>Nigeria</td>
<td>Blood Donors (72)</td>
<td>37.5% (27)</td>
<td>29.2% (21)</td>
</tr>
<tr>
<td>Mahgoub, 2011 [47]</td>
<td>Sudan</td>
<td>Blood Donors (6)</td>
<td>0% (0)</td>
<td>100% (6)</td>
</tr>
<tr>
<td>Cable, 2012 [60]</td>
<td>Egypt</td>
<td>Blood Donors (13)</td>
<td>DNR</td>
<td>46.2% (6)</td>
</tr>
<tr>
<td>Zahn, 2008(A) [14]</td>
<td>Ghana</td>
<td>Blood Donors &amp; Pregnant Women (9)</td>
<td>44.4% (4)</td>
<td>33.3% (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total Healthy</td>
<td>154 (143 recorded)</td>
<td>36% (55)</td>
</tr>
<tr>
<td>Raouf, 2015 [21]</td>
<td>Egypt</td>
<td>Ped HCV &amp; Cancer (16)</td>
<td>18.8% (3)</td>
<td>12.5% (2)</td>
</tr>
<tr>
<td>Elkady, 2013 [39]</td>
<td>Egypt</td>
<td>Ped Cancer (4)</td>
<td>25.0% (1)</td>
<td>50.0% (2)</td>
</tr>
<tr>
<td>Musyoki 2015 [61]</td>
<td>South Africa</td>
<td>HIV, HCV &amp; Cancer (10)</td>
<td>0% (0)</td>
<td>40.0% (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIV &amp; Cancer (20)</td>
<td>0% (0)</td>
<td>35.0% (7)</td>
</tr>
<tr>
<td>Salpini, 2016 [10]</td>
<td>Cameroon</td>
<td>HIV (12)</td>
<td>75.0% (9)</td>
<td>16.7% (2)</td>
</tr>
<tr>
<td>Lukhwareni, 2009 [62]</td>
<td>South Africa</td>
<td>HIV (34)</td>
<td>32.4% (11)</td>
<td>29.4% (10)</td>
</tr>
<tr>
<td>Bell, 2012 [24]</td>
<td>South Africa</td>
<td>HIV (45)</td>
<td>35.6% (16)</td>
<td>37.8% (17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total Immunosuppressed</td>
<td>141 (140 recorded)</td>
<td>28% (40)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mandour, 2015 [63]</td>
<td>Egypt HCV co-infection (18)</td>
<td>44.4% (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>El-Sherif, 2009 [20]</td>
<td>Egypt HCV co-infection (16)</td>
<td>100% (16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kitab, 2014 [12]</td>
<td>Morocco HCV co-infection (42)</td>
<td>88.1% (37)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Musyoki 2015 [61]</td>
<td>South Africa HIV, HCV &amp; Cancer (10)</td>
<td>0% (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Raouf, 2015 [21]</td>
<td>Egypt Ped HCV &amp; Cancer (16)</td>
<td>18.8% (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total HCV</td>
<td>102 (102 recorded)</td>
<td>47-63% (48-64)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chakvetadze, 2011 [26]</td>
<td>Mayotte Infants of chronic HBV moms (2)</td>
<td>0% (0)</td>
</tr>
<tr>
<td></td>
<td>Shaker, 2012 [35]</td>
<td>Egypt Ped Transfused (26)</td>
<td>92.3% (24)</td>
<td>7.7% (2)</td>
</tr>
<tr>
<td></td>
<td>Said, 2009 [34]</td>
<td>Egypt Ped Transfused (21)</td>
<td>19.0% (4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Elgohry, 2012 [22]</td>
<td>Egypt Hemodialysis (25)</td>
<td>60.0% (15)</td>
<td>12.0% (3)</td>
</tr>
<tr>
<td></td>
<td>Mandouer, 2015 [63]</td>
<td>Egypt Hemodialysis (3)</td>
<td>66.7% (2)</td>
<td>0% (0)</td>
</tr>
<tr>
<td></td>
<td>Mphahlele, 2002 [64]</td>
<td>South Africa Hepatitis tested (2)</td>
<td>0 % (0)</td>
<td>0% (0)</td>
</tr>
<tr>
<td></td>
<td>Kitab, 2014 [12]</td>
<td>Morocco Liver disease (17)</td>
<td>47.1% (8)</td>
<td>0% (0)</td>
</tr>
<tr>
<td></td>
<td>Apica, 2016 [46]</td>
<td>Uganda Emergency room (94)</td>
<td>57.4% (54)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total Other</td>
<td>190 (173 recorded)</td>
<td>13-56% (25-107)</td>
</tr>
</tbody>
</table>
Table 2 – Prevalence of OBI in various healthy and “at risk” individuals

“At risk” group include acutely or chronically ill or at risk for HBV exposure (i.e. maternal history of chronic HBV, dialysis patients). ^ Includes false OBI data. °Subgroup not included in total calculations (not double counted) * Study used for multiple tables.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>Location</th>
<th>Occult (Total)</th>
<th>Anti-HBc Positive OBI</th>
<th>Chronic</th>
<th>OBI-to-Chronic Ratio</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halima, 2010 [65]</td>
<td>Tunisia</td>
<td>2.5% (9/361)</td>
<td>39.1% (9/23)</td>
<td>9.1% (33/361)</td>
<td>0.273</td>
<td>Adults</td>
</tr>
<tr>
<td>El-Zayadi, 2008 [66]</td>
<td>Egypt</td>
<td>1.2% (9/760)</td>
<td>11.5% (9/78)</td>
<td>1.2% (9/760)</td>
<td>1.000</td>
<td>Blood Donors</td>
</tr>
<tr>
<td>Mayaphi, 2012 [31]</td>
<td>South Africa</td>
<td>1.0% (2/200)</td>
<td></td>
<td>2.0% (4/200)</td>
<td>0.500</td>
<td>Adult controls</td>
</tr>
<tr>
<td>Mphahlele, 2006 [32]</td>
<td>South Africa</td>
<td>1.6% 2/128</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forbi, 2010 [37]</td>
<td>Nigeria</td>
<td>0.4% (2/500)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nna, 2014 [7] ^</td>
<td>Nigeria</td>
<td>10.6% (12/113)</td>
<td></td>
<td>11.5% (13/113)</td>
<td>0.615</td>
<td>Blood Donors</td>
</tr>
<tr>
<td>Owusu-Ofori, 2005 [67]</td>
<td>Ghana</td>
<td>2.5% (21/834)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fortuin, 1994 [49]</td>
<td>The Gambia</td>
<td>0.3% (1/358)</td>
<td></td>
<td>0.6% (2/358)</td>
<td>0.500</td>
<td>Infants post vaccination</td>
</tr>
<tr>
<td>Gouas, 2012 [6]</td>
<td>The Gambia</td>
<td>2.5% (8/317)</td>
<td></td>
<td>13.6% (43/317)</td>
<td>0.186</td>
<td>Hospital based controls</td>
</tr>
<tr>
<td>Oluyinka 2015 [8]</td>
<td>Nigeria</td>
<td>16.8% (72/429)</td>
<td></td>
<td></td>
<td></td>
<td>Blood Donors</td>
</tr>
<tr>
<td>Compston, 2009 [68]</td>
<td>Ghana</td>
<td>1.6% (2/123)</td>
<td>2.3% (2/88)</td>
<td></td>
<td></td>
<td>Blood donors</td>
</tr>
<tr>
<td>Total Healthy</td>
<td></td>
<td>3.4% (140/4129)</td>
<td>10.6% (20/189)</td>
<td>12.0% (1459/12109)</td>
<td>0.209</td>
<td></td>
</tr>
<tr>
<td>OBI with comparison chronic HBV data only</td>
<td>1.9% (66/3541)</td>
<td>12.0% (1459/12109)</td>
<td>0.155</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meschi, 2010 [69]</td>
<td>Tanzania</td>
<td>0.4% (1/277)</td>
<td>7.7% 1/13</td>
<td>4.3% (12/277)</td>
<td>0.083</td>
<td>Ill kids – post HBV vaccine era</td>
</tr>
<tr>
<td>Elkady, 2013 [39]</td>
<td>Egypt</td>
<td>6.8% (4/59)</td>
<td>18.8% 3/16</td>
<td>20.3% (12/59)</td>
<td>0.333</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td>Youssef, 2009 [51] *</td>
<td>Egypt</td>
<td>60.3% (35/58)</td>
<td>6.9% (4/58)</td>
<td>8.750</td>
<td>acute ill adults</td>
<td></td>
</tr>
<tr>
<td>Youssef, 2013 [27]</td>
<td>Egypt</td>
<td>21.2% (7/33)</td>
<td>27.3% (9/33)</td>
<td>0.778</td>
<td>acute ill children</td>
<td></td>
</tr>
<tr>
<td>Mandour, 2015 [63]</td>
<td>Egypt</td>
<td>1.8% (3/165)</td>
<td></td>
<td></td>
<td></td>
<td>Dialysis adults</td>
</tr>
<tr>
<td>Elgohory, 2012 [22]</td>
<td>Egypt</td>
<td>26.8% (25/93)</td>
<td></td>
<td></td>
<td></td>
<td>Dialysis</td>
</tr>
<tr>
<td>Shaker, 2012 [35]</td>
<td>Egypt</td>
<td>32.5% (26/80)</td>
<td></td>
<td></td>
<td></td>
<td>Transfused kids</td>
</tr>
<tr>
<td>Said, 2009 [34]</td>
<td>Egypt</td>
<td>21.0% (21/100)</td>
<td>45.0% (45/100)</td>
<td>0.467</td>
<td>Transfused kids</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.1% (9/64)</td>
<td>43.8% 28/64</td>
<td>0.321</td>
<td>No HCV subgroup °</td>
<td></td>
</tr>
<tr>
<td>Total Other</td>
<td></td>
<td>11.8% (125/1061)</td>
<td>13.8% (4/29)</td>
<td>14.4% (104/723)</td>
<td>0.819</td>
<td></td>
</tr>
</tbody>
</table>
Table 3 – Prevalence of OBI in chronic HCV infected cohorts

<table>
<thead>
<tr>
<th>STUDY</th>
<th>Location</th>
<th>Occult (Total)</th>
<th>OBI (anti-HBc only)</th>
<th>Chronic</th>
<th>OBI : Chronic Ratio</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kishk, 2014 [19]</td>
<td>Egypt</td>
<td>1.8% (3/162)</td>
<td>7.5% (3/40)</td>
<td></td>
<td></td>
<td>HCV</td>
</tr>
<tr>
<td>Halima, 2010 [65]</td>
<td>Tunisia</td>
<td>12.2% (44/361)</td>
<td>67.7% (44/65)</td>
<td>5.0%</td>
<td>2.444</td>
<td>HCV</td>
</tr>
<tr>
<td>Said, 2009 [34]</td>
<td>Egypt</td>
<td>33.3% (12/36)</td>
<td></td>
<td>47.2%</td>
<td>0.706</td>
<td>transfused kids (60% post vaccination)</td>
</tr>
<tr>
<td>Youssef, 2009 [41]*</td>
<td>Egypt</td>
<td>53.8% (84/156)</td>
<td></td>
<td>3.8%</td>
<td>14.000</td>
<td>Acutely Ill</td>
</tr>
<tr>
<td>Mandour, 2015 [63]</td>
<td>Egypt</td>
<td>8.6% (18/210)</td>
<td></td>
<td></td>
<td></td>
<td>HCV</td>
</tr>
<tr>
<td>El-Sherif, 2009 [20]</td>
<td>Egypt</td>
<td>22.0% (22/100)</td>
<td>31.0% (22/71)</td>
<td></td>
<td></td>
<td>most cirrhotic</td>
</tr>
<tr>
<td>Kitab, 2014 [12]</td>
<td>Morocco</td>
<td>45.6% (42/92)</td>
<td></td>
<td></td>
<td></td>
<td>Symptomatic HCV</td>
</tr>
<tr>
<td>Raouf, 2015 [21]</td>
<td>Egypt</td>
<td>38.0% (19/50)</td>
<td></td>
<td></td>
<td></td>
<td>HCV/Cancer</td>
</tr>
<tr>
<td>Hassan, 2011 [40]</td>
<td>Egypt</td>
<td>47.4% (9/19)</td>
<td></td>
<td></td>
<td></td>
<td>HCC/HCV</td>
</tr>
<tr>
<td>Total HCV</td>
<td></td>
<td>21.3% (253/1186)</td>
<td>39.2% (69/176)</td>
<td>9.9%</td>
<td>2.149</td>
<td></td>
</tr>
</tbody>
</table>
Table 4 – Prevalence of OBI in HIV positive cohorts

DNR = Did not report.  ^ total cohort consisting of core antibody positive subjects only

<table>
<thead>
<tr>
<th>STUDY</th>
<th>Location</th>
<th>Occult (Total)</th>
<th>Occult (antiHBc only)</th>
<th>Chronic</th>
<th>Occult: Chronic Ratio</th>
<th>Drug Naïve?</th>
<th>CD4 Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamers, 2013 [13]</td>
<td>Zambia &amp; SA</td>
<td>5.1% (55/1087)</td>
<td>13.3% (55/414)</td>
<td>8.5%</td>
<td>0.598</td>
<td>Y</td>
<td>&lt;200</td>
</tr>
<tr>
<td>Compston, 2009 [68]</td>
<td>Ghana</td>
<td>13.3% (26/236)</td>
<td>13.3% (26/196)</td>
<td>3.6%</td>
<td>DNR</td>
<td>AIDS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DNR</td>
<td></td>
</tr>
<tr>
<td>Attia, 2012 [9]</td>
<td>Cote d'Ivoire</td>
<td>8.1% (40/491)</td>
<td>21.3% (40/188)</td>
<td>13.4%</td>
<td>0.606</td>
<td>Y</td>
<td>&lt;500</td>
</tr>
<tr>
<td>N'Dri-Yoman, 2010 [59]</td>
<td>Cote d'Ivoire</td>
<td>10.3% (51/495)</td>
<td>24.4% (51/209)</td>
<td>12.7%</td>
<td>0.809</td>
<td>Y</td>
<td>&lt;500</td>
</tr>
<tr>
<td>Barth, 2011 [16]</td>
<td>South Africa</td>
<td>2.5% (6/242)</td>
<td>9.7% (6/62)</td>
<td>4.1%</td>
<td>6.000</td>
<td>Y</td>
<td>DNR</td>
</tr>
<tr>
<td>Amponsah-Dacosata, 2015</td>
<td>South Africa</td>
<td>NA/pre-vaccine</td>
<td>58.3% (81/139)</td>
<td>17.3%</td>
<td>3.375</td>
<td>DNR</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NA/post-vaccine</td>
<td>56.6% (35/62)</td>
<td>14.5%</td>
<td>3.889</td>
<td>DNR</td>
<td></td>
</tr>
<tr>
<td>Bivigou-Mboumba, 2016</td>
<td>Gabon</td>
<td>8.0% (61/762)</td>
<td>26.8% (61/228)</td>
<td>9.3%</td>
<td>N (90%)</td>
<td>DNR</td>
<td></td>
</tr>
<tr>
<td>Ayuk, 2013 [28]</td>
<td>South Africa</td>
<td>16.0% (61/380)</td>
<td>33.7% (61/181)</td>
<td>20.0%</td>
<td>DNR</td>
<td>DNR</td>
<td></td>
</tr>
<tr>
<td>Opaley '14 [70]</td>
<td>Nigeria</td>
<td>11.2% (21/188)</td>
<td>28.6% (8/28)</td>
<td>DNR</td>
<td>DNR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Firnhaber, 2009 [25]</td>
<td>South Africa</td>
<td>7.6% (38/502)</td>
<td>88.4% (38/43)</td>
<td>4.8%</td>
<td>0.1583</td>
<td>Y</td>
<td>&lt;200</td>
</tr>
<tr>
<td>Core Ab+ HIV</td>
<td></td>
<td>8.1% (361/4439)</td>
<td>25.9% (464/1792)</td>
<td>10.2%</td>
<td>0.784</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chadwick, 2013 [15]</td>
<td>Ghana (ART Naïve)</td>
<td>9.9% (83/838)</td>
<td>16.7% (140/838)</td>
<td>25.8%</td>
<td></td>
<td>DNR</td>
<td></td>
</tr>
<tr>
<td>Mudawi, 2014 [17]</td>
<td>Sudan</td>
<td>15.1% (54/358)</td>
<td>11.7% (42/358)</td>
<td>1.544</td>
<td>Y</td>
<td>DNR</td>
<td></td>
</tr>
<tr>
<td>Lukhwareni, 2009 [62]</td>
<td>South Africa</td>
<td>17.7% (34/192)</td>
<td>22.9% (44/192)</td>
<td>0.773</td>
<td>Y</td>
<td>2-1069</td>
<td></td>
</tr>
<tr>
<td>Bell, 2012 [24]</td>
<td>South Africa</td>
<td>15.1% (45/298)</td>
<td>8.7% (26/298)</td>
<td>1.731</td>
<td>Y</td>
<td>&lt;200</td>
<td></td>
</tr>
<tr>
<td>Mayaphi, 2012 [31]</td>
<td>South Africa</td>
<td>3.5% (7/200)</td>
<td>6.5% (13/200)</td>
<td>0.538</td>
<td>DNR</td>
<td>&lt;200</td>
<td></td>
</tr>
<tr>
<td>Mphalele</td>
<td>South Africa</td>
<td>18.6% (31/167)</td>
<td>15.6% (26/167)</td>
<td>1.192</td>
<td>DNR</td>
<td>DNR</td>
<td></td>
</tr>
<tr>
<td>Musyoki, 2015 [61]</td>
<td>South Africa</td>
<td>62.6% (20/32)</td>
<td>3.1% (1/32)</td>
<td>20.000</td>
<td>DNR</td>
<td>11-757</td>
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<tr>
<td>Hoffmann, 2014[30]</td>
<td>South Africa</td>
<td>1.6% (3/189)</td>
<td>7.4% (14/189)</td>
<td>0.214</td>
<td>N</td>
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<tr>
<td>Non prescreen HIV</td>
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<td>13.4% (306/2274)</td>
<td>0.905</td>
<td></td>
<td></td>
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<tr>
<td>All HIV</td>
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<td>9.5% (638/6713)</td>
<td>11.4% (732/6434)</td>
<td>0.835</td>
<td></td>
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</table>
Bibliography:


Occult Hepatitis B in HIV Positive Batswana

Introduction

The Hepatitis B virus (HBV), a hepadnavirus responsible for both acute and chronic liver disease, has infected over two billion people worldwide and resulted in more than 240 million chronic active infections [1]. Although morbidity from acute HBV infection is fairly low, chronic HBV is the leading cause of end stage liver failure and hepatocellular carcinoma (HCC) worldwide [1]. Historically, HBV infections have been diagnosed by the detection of hepatitis B surface antigen (HBsAg). More recently, nucleic acid amplification testing (NAT) has been used to follow HBV viral loads of chronically infected individuals over time to determine treatment options and risk of disease progression. However, with the advent of highly sensitive NAT techniques, cases of HBsAg-negative but HBV DNA-positive infection (known as occult hepatitis B infection or OBI) have been discovered [2, 3]. OBI is defined as the existence of HBV DNA (typically less than 200 IU/mL) in the blood and/or hepatic tissue with the absence of serum HBsAg [4].

Although chronic HBV is considered the primary cause of liver failure and HCC, various studies also demonstrate that OBI is an important risk factor in the progression to end stage liver disease and HCC [3-6]. Transmission of OBI has been demonstrated through blood transfusion, organ donation, vertical transmission (from mothers to infants), and via household contact of chronic HBV-infected individuals [2, 3, 7, 8]. Without appropriate screening, occult HBV infection is undiagnosed, resulting in HBV transmission to others, as well as long-term sequelae of the HBV infection.
Multiple risk factors associated with OBI have been discovered. HIV co-infection is associated with increased risk of OBI but not with chronic HBV infection [9]. The prevalence of OBI varies between 1% of HIV positive individuals in the U.S. to greater than 15% of HIV positive individuals in countries such as South Africa [10, 11]. The eight HBV genotypes play different roles in the pathogenesis of chronic HBV infection and vary widely throughout the world. The role of HBV genotype in OBI is not understood. This study allowed for the opportunity to evaluate OBI in Botswana which is known to be hyper-endemic for chronic HBV infection and have a significant HIV burden. However, no data regarding OBI in Botswana currently exists.

The prevalence of OBI in HIV positive Batswana (the people of Botswana) was hypothesized to be similar to the prevalence of OBI previously observed in South Africa (between 10-15%). Additional hypothesis include complete suppression of detectable OBI viral load by one year after the initiation of tenofovir/emtricitabine-containing therapy. It was further hypothesized that markers of liver disease will be higher in the occult HBV group in comparison to those without HBV, but lower than those with chronic HBV.

Methods

Study Participants and Samples

This study was a secondary analysis of “The Botswana National Evaluation Models of HIV Care (Bomolemo Study): A Study Evaluating the Efficacy and Tolerability of Tenofovir and Emtricitabine (Truvada™) as the Nucleoside Reverse Transcriptase Inhibitor (NRTI) backbone as
first-line HAART therapy for adults in Botswana”. For the current study, de-identified plasma samples were obtained from 300 HIV-positive adults (18 years of age and older) prior to initiation of HAART therapy. When available, follow-up plasma samples after one year of HBV-active HAART were also included. At the onset of the study, all participants had WHO Stage III or IV HIV (CD4 less than 250 or AIDS-defining illness) and no previous antiretroviral (ART) exposure. An exception was made that allowed previous ART therapy to prevent mother to child transmission (PMTCT) which was completed more than six months prior to the enrollment. Laboratory results and questionnaire data collected for the original study were available for further analysis.

Sample Analysis / Screening

Two hundred seventy-two samples of persons who were previously determined to be HBsAg negative (without chronic HBV infection), were tested for HBV DNA using COBAS® AmpliPrep / TaqMan® HBV Test, version 2.0 (Roche Diagnostic, Mannheim, Germany). Quantitative levels were recorded when ≥ 20 IU/mL. Samples with HBV DNA detectable but below this quantitative threshold were reported as less than 20 IU/mL. During statistical analysis, 10 IU/mL was assigned to all values less than 20 IU/mL samples. Antibody screenings for hepatitis B core antibody (Monolisa™ Anti-HBC PLUS, Biorad, France) and for hepatitis B surface antibody (Monolisa™ Anti-HBS PLUS, Biorad, France) were performed in triplicate per manufacturer’s instruction. Individuals with OBI at baseline and available follow-up plasma samples obtained one year after initiation of HAART therapy were evaluated for HBV DNA at one year using the COBAS AmpliPrep/TaqMan® version 2.0 system.
Assessment of Livery Injury

Aspartate aminotransferase (AST) to platelet ratio index (APRI) and FIB-4 are two different markers for liver damage. Initially validated for hepatitis C virus, these scoring systems have been evaluated in other diseases of the liver [12, 13]. APRI score is equal to \( 100 \times \frac{\text{AST}}{40} / \text{platelet} \) and FIB-4 is equal to age \( \times \frac{\text{AST}}{\sqrt{\text{alanine aminotransferase (ALT)}}} \). Based on data from Tahiri et al, persons with HIV have an average FIB-4 score of 0.82 [12]. Another study demonstrated persons with chronic hepatitis B infection have an average FIB-4 score of 1.51 with interquartile range of 0.6 to 2.13 [13]. Both FIB-4 and APRI were calculated for all subjects with available data.

Statistical Analysis

Sociodemographic and clinical data from baseline and 12 month follow up visits were obtained from the original Bomolemo data set. The Chi-squared test was used to evaluate the difference in proportions for dichotomous variables. Kruskall-Wallis testing on Wilcoxon rank sum test was used for all continuous and ordinal non-parametric data. ANOVA with contrasting groups was utilized to compare antibody status among the HIV/OBI, HIV/chronic HBV-infected, and HIV mono-infected groups. Multinomial regression models were used to evaluate baseline sociodemographic data as potential risk factors for OBI. All statistical analyses were performed using SAS 9.4.
Results

OBI was detected in 72 of 300 (24%) subjects. As shown in Table 1, baseline demographics were similar among the OBI/HIV, chronic HBV/HIV and HIV mono-infected groups. HBV DNA levels were below 20 IU/mL (lower limit of quantification) in 49 of 72 (68%). Only six individuals (8%) had HBV DNA levels greater than 200 IU/mL and the highest viral load was 3280 IU/mL.

There were significant differences in surface and core antibody status among all three groups. Core antibody was detected in 82% of chronic HBV-infected individuals, 65% of individuals with OBI, and 45% of HIV mono-infected individuals (Table 2).

Multiple social and demographic data were evaluated for correlation with OBI status (Table 3). In univariate analyses, no variables were significantly associated with the presence of OBI. A multinomial regression model was utilized to evaluate multiple factors’ association with OBI status (Table 4). Differences in the HBV antibody status continued to remain significance in the regression model (p < 0.001). On further analysis, isolated HBV core antibody positivity was significantly different between both OBI and chronic HBV infected individuals, as well as OBI and HIV mono-infected individuals (p = 0.0143, p = 0.0346). Multiple multinomial models demonstrated that FIB-4 scores were more associated with OBI status than APRI score.

Sixty-five of the 72 subjects with OBI had adequate samples to evaluate HBV viral load after completion of 12 months of HIV therapy containing Truvada™ (emtricitabine/tenofovir) containing antiretroviral therapy. Only one of 65 individuals with OBI (1.5%) had detectible virus at one year at less than 20 IU/mL (Table 5). There was no difference in mortality or medication adjustments among the three study groups during the 2 year duration of the study.
Discussion

Chronic HBV rates have been reported between 5% and 7% in HIV positive individuals in Botswana [14, 15]. However, this study is the first to recognize OBI as a serious concern among HIV positive Batswana. A rate of 24% is higher than anticipated, although this may be in part due to better techniques allowing increased detection at very low viral loads. As 49 of 72 (68%) OBI samples identified had HBV DNA levels less than 20 IU/mL, a minor change in the lowest limit of detection may greatly change the detection rate of this disease.

The antibody status of HIV/OBI co-infected individuals varied widely in regards to HBV core and surface antibody status. Although core antibody positivity (either alone or in combination with surface antibody positivity) is a marker of previous exposure to HBV, this criteria is inappropriate in HIV positive individuals in areas of high endemicity due to the significant (almost 30%) rate of core negative OBI. The results of this study are consistent with at least two other studies in South Africa [10, 16].

Previous studies have suggested that there is a trend towards increased ALT and AST in OBI / HIV infected individuals [17]. One study found a transient rise in ALT and AST in OBI/HIV co-infected individuals when initiating therapy [18]. Other have suggested a correlation between OBI and age or OBI and increased CD4 in HIV co-infected individuals [16, 18]. However, none of these factors were found to have any significant correlation with OBI/HIV co-infection in the current study.

Using multiple regression modeling, OBI was significantly associated with core antibody status. There were unique differences in isolated core antibody positive subjects between HIV/OBI and
HIV mono-infected subjects as well as HIV/OBI and chronic HIV/HBV-infected subjects. However, this is not a perfect correlation and cannot distinguish among OBI, chronic HBV or non-infected individuals. Thus, core antibody status is not an appropriate screening testing for OBI. As NAT is cost-prohibitive in many parts of the world as a screening technique, more studies will need to be conducted to find better ways to identify individuals with occult hepatitis B.

There is a trend towards significance in subjects with communal water sources at home to be associated with OBI when compared to HIV mono-infection. This may represent poor hygiene conditions with subsequent increased horizontal transmission between household members.

No differences in mortality or drug failure were noted within the OBI cohort. Antiretroviral therapy containing Truvada demonstrated excellent OBI viral suppression at one year. This is consistent with other reports of complete HBV virological suppression while on tenofovir containing regiments [19].

Interestingly, the one subject with persistent HBV viral load started at a low HBV DNA level (less than 20 IU/mL). This subject’s HIV viral load at one year in the patient was less than 400 with good improvement of the CD4 count (482.6) which would indicate good compliance with the medication. This suggests that the persistent HBV is not due to a lack of medication. This subject had no evidence of change of therapy or other indication to explain the persistent HBV viral load. Due to the low viral load of this sample, the genome of this virus was not evaluated further. It is uncertain if persistence of virus is related to the virus itself (i.e. tenofovir resistance), host immune system factors, or other etiology.
Conclusion

Occult hepatitis B is a significant public health concern in Botswana as it is in most of Africa. Currently, methods to reduce chronic HBV (including vaccination) are also effective at reducing the incidence of OBI in healthy populations. Risk factors for OBI include concomitant HCV, HIV or other immunosuppression in addition to blood product exposure. This study, consistent with other previous studies, does not identify other epidemiological factors which could predict the risk of OBI. Without any additional modifiable risk factors, improved screening on blood products is of critical importance. Future research should focus on factors which contribute to the development of OBI in previously vaccinated individuals. Risk reduction strategies targeted at slowing the progression of liver disease to cirrhosis or preventing hepatocellular carcinoma will also be important moving forward.
Table 1: Baseline laboratory data from HIV infected patients.

Data represents median (interquartile range) except at noted. As all data was non-parametric, Kruskal-Wallis test from Wilcoxon Score was used with the exception of male gender (chi squared analysis) due to dichotomous nature of this variable. Total column includes chronic HBV, no HBV, and all unknowns (inadequate samples). ^Absolute number (percentage) represented.

<table>
<thead>
<tr>
<th></th>
<th>Occult HBV</th>
<th>Non occult HBV</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Chronic HBV</td>
<td>No HBV</td>
</tr>
<tr>
<td>Age</td>
<td>39.5 (35.75 – 45)</td>
<td>39.5 (35.75 – 46.25)</td>
<td>41.0 (36 – 47)</td>
</tr>
<tr>
<td>Male Gender ^</td>
<td>28 (38.9%)</td>
<td>10 (35.7%)</td>
<td>70 (35.2%)</td>
</tr>
</tbody>
</table>

|                                | BMI        | 21.6 (19.5 – 24.7) | 20.9 (18.9 – 23.6) | 21.4 (19.0 – 25.0) | 0.87 |
|                                | HIV Viral Load | 145,500 (48,900 – 427,500) | 80,800 (30,050 – 537,500) | 131,000 (43,100 – 382,000) | 0.63 |
|                                | HIV Viral Load (Log) | 5.17 (4.69 – 5.64) | 4.91 (4.48 – 5.73) | 5.12 (4.63 – 5.58) | 0.62 |
|                                | CD4 Count  | 157 (71 – 226)    | 172 (93 – 239)     | 160 (80 – 229)     | 0.84 |
|                                | Platelet   | 262 (204 – 327)   | 245 (204 – 300)    | 256 (205 – 314)    | 0.84 |
|                                | Hemoglobin | 11.2 (9.5 – 12.5) | 11.7 (9.6 – 13.1)  | 11.4 (10.1 – 13.0) | 0.26 |
|                                | ALT        | 19.1 (14.7 – 25.7) | 23.3 (16.3 – 36.7) | 21.4 (15.0 – 29.1) | 0.22 |
|                                | AST        | 28.2 (24.5 – 37.9) | 33.5 (24.4 – 43.7) | 28.5 (22.9 – 36.4) | 0.31 |
|                                | Alkaline Phosphatase | 73.6 (61.2 – 92.6) | 78.1 (62.7 – 104.6) | 69.9 (58.7 – 89.1) | 0.56 |
|                                | Total bilirubin | 6.14 (4.23 – 7.89) | 4.96 (4.26 – 6.07) | 5.81 (4.25 – 7.73) | 0.41 |
|                                | FIB-4      | 1.02 (0.84 – 1.50) | 1.08 (0.86 – 1.44) | 1.04 (0.75 – 1.38) | 0.47 |
|                                | APRI       | 0.29 (0.19 – 0.44) | 0.32 (0.28 – 0.46) | 0.28 (0.21 – 0.38) | 0.41 |
Table 2: Antibody Status

Fisher’s exact test analysis for antibody status between occult, chronic and non-infected HBV subjects. Total values (core positive or surface positive) represent percentage of OBI cases with specific antibody status positive.

<table>
<thead>
<tr>
<th></th>
<th>OBI (n=72)</th>
<th>No HBV (n=200)</th>
<th>Chronic (n=28)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core + Surface +</td>
<td>24 (33.3%)</td>
<td>59 (29.5%)</td>
<td>3 (10.7%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Core + Surface -</td>
<td>23 (31.9%)</td>
<td>29 (14.5%)</td>
<td>19 (67.9%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Core - Surface +</td>
<td>2 (2.8%)</td>
<td>10 (5.0%)</td>
<td>0 (0%)</td>
<td>0.59</td>
</tr>
<tr>
<td>Core - Surface -</td>
<td>21 (29.2%)</td>
<td>93 (48.0%)</td>
<td>5 (17.9%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (2.8%)</td>
<td>6 (3.0%)</td>
<td>1 (3.6%)</td>
<td></td>
</tr>
<tr>
<td>Total Core +</td>
<td>47/70 (65.3%)</td>
<td>88/195 (45.1%)</td>
<td>22/27 (81.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total Surface +</td>
<td>26/70 (36.1%)</td>
<td>69/194 (35.6%)</td>
<td>3/27 (11.1%)</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Table 3: Social Demographic Information

Univariate analysis on non-parametric data chi-squared * or via Kruskall-Wallis testing. Sample sizes varied for different variable. ¹ n=68, ² n = 189, ³ n= 193, ⁴ n = 192, ⁵ n = 194

<table>
<thead>
<tr>
<th></th>
<th>Occult HBV (n=70, n=68¹)</th>
<th>Non occult HBV (n = 189², 193³, 192⁴, 194⁵)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Church Attendance</td>
<td>31 (44.3%)</td>
<td>12 (44.4%) 86 (44.3%)</td>
<td>0.99*</td>
</tr>
<tr>
<td>Average Income</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤600 Pula</td>
<td>19 (27.9%)</td>
<td>7 (25.9%) 56 (29.6%)</td>
<td>0.93</td>
</tr>
<tr>
<td>601-6000 Pula</td>
<td>44 (64.7%)</td>
<td>19 (70.4%) 121 (64.0%)</td>
<td></td>
</tr>
<tr>
<td>&gt;6000 Pula</td>
<td>5 (7.4%)</td>
<td>1 (3.7%) 12 (6.3%)</td>
<td></td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>30 (42.8%) ¹</td>
<td>11 (40.7%) 83 (42.8%)</td>
<td>0.86</td>
</tr>
<tr>
<td>Married/ cohabitating</td>
<td>35 (50%) ¹</td>
<td>13 (48.1%) 100 (51.5%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>5 (7.1%) ¹</td>
<td>3 (11.1%) 11 (5.7%)</td>
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<td>Toilet Type</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Indoor</td>
<td>22 (31.4%)</td>
<td>11 (40.7%) 57 (29.5%)</td>
<td>0.72</td>
</tr>
<tr>
<td>Private Latrine</td>
<td>34 (48.6%)</td>
<td>12 (44.4%) 90 (46.6%)</td>
<td></td>
</tr>
<tr>
<td>Shared Latrine</td>
<td>14 (20.0%)</td>
<td>4 (14.8%) 46 (23.8%)</td>
<td></td>
</tr>
<tr>
<td>School Level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤Primary</td>
<td>29 (41.4%)</td>
<td>13 (48.1%) 74 (38.1%)</td>
<td>0.59</td>
</tr>
<tr>
<td>Jr Secondary</td>
<td>23 (32.8%)</td>
<td>8 (29.6%) 66 (34.0%)</td>
<td></td>
</tr>
<tr>
<td>&gt;Jr Secondary</td>
<td>18 (25.7%)</td>
<td>6 (22.2%) 54 (27.8%)</td>
<td></td>
</tr>
<tr>
<td>Household TV</td>
<td>43 (61.4%)</td>
<td>20 (74.1%) 129 (66.5%)</td>
<td>0.48*</td>
</tr>
<tr>
<td>Household Refrigeration</td>
<td>37 (52.9%)</td>
<td>18 (66.7%) 106 (54.6%)</td>
<td>0.45*</td>
</tr>
<tr>
<td>Household Car</td>
<td>16 (22.9%)</td>
<td>10 (37.0%) 56 (28.9%)</td>
<td>0.35*</td>
</tr>
<tr>
<td>Water Source</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>In Home</td>
<td>17 (24.3%)</td>
<td>10 (37.0%) 57 (29.7%)</td>
<td>0.31</td>
</tr>
<tr>
<td>Private tap</td>
<td>43 (61.4%)</td>
<td>15 (55.6%) 122 (63.5%)</td>
<td></td>
</tr>
<tr>
<td>Communal</td>
<td>10 (14.3%)</td>
<td>2 (7.4%) 13 (6.8%)</td>
<td></td>
</tr>
<tr>
<td>Household Electricity</td>
<td>34 (50.9%)</td>
<td>18 (66.7%) 96 (49.5%)</td>
<td>0.22*</td>
</tr>
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<td>Household Phone</td>
<td>53 (75.7%)</td>
<td>23 (85.2%) 165 (85.1%)</td>
<td>0.20*</td>
</tr>
</tbody>
</table>
Table 4 Multinomial Regression model for OBI status

* Statistically significant result.

<table>
<thead>
<tr>
<th>EFFECT</th>
<th>HBV comparison</th>
<th>Point Estimate</th>
<th>95% Wald Confidence Limits</th>
</tr>
</thead>
<tbody>
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<td>OBI to no HBV</td>
<td>0.43 *</td>
<td>0.20</td>
</tr>
<tr>
<td>*Dual core/surface Ab + to isolated core Ab +</td>
<td>OBI to chronic HBV</td>
<td>5.86 *</td>
<td>1.43</td>
</tr>
<tr>
<td>Isolated HBV Surface Ab + to isolated core Ab +</td>
<td>OBI to no HBV</td>
<td>1.99</td>
<td>0.39</td>
</tr>
<tr>
<td>Isolated HBV Surface Ab + to isolated core Ab +</td>
<td>OBI to chronic HBV</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Seronegative to Isolated HBV core Ab +</td>
<td>OBI to no HBV</td>
<td>1.76</td>
<td>0.86</td>
</tr>
<tr>
<td>Seronegative to Isolated HBV core Ab +</td>
<td>OBI to chronic HBV</td>
<td>1.51</td>
<td>0.31</td>
</tr>
<tr>
<td>FIB 4</td>
<td>OBI to no HBV</td>
<td>0.71</td>
<td>0.46</td>
</tr>
<tr>
<td>FIB 4</td>
<td>OBI to chronic HBV</td>
<td>0.80</td>
<td>0.42</td>
</tr>
<tr>
<td>CD4</td>
<td>OBI to no HBV</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>CD4</td>
<td>OBI to chronic HBV</td>
<td>1.00</td>
<td>0.99</td>
</tr>
<tr>
<td>&lt;Jr secondary to finished Jr Secondary</td>
<td>OBI to no HBV</td>
<td>0.99</td>
<td>0.49</td>
</tr>
<tr>
<td>&lt;Jr secondary to finished Jr Secondary</td>
<td>OBI to chronic HBV</td>
<td>1.34</td>
<td>0.43</td>
</tr>
<tr>
<td>Finished Jr Secondary to &gt; Jr Secondary</td>
<td>OBI to no HBV</td>
<td>0.84</td>
<td>0.36</td>
</tr>
<tr>
<td>Finished Jr Secondary to &gt; Jr Secondary</td>
<td>OBI to chronic HBV</td>
<td>0.33</td>
<td>0.06</td>
</tr>
<tr>
<td>No phone to having phone</td>
<td>OBI to no HBV</td>
<td>0.56</td>
<td>0.26</td>
</tr>
<tr>
<td>No phone to having phone</td>
<td>OBI to chronic HBV</td>
<td>0.64</td>
<td>0.16</td>
</tr>
<tr>
<td>No electricity to having electricity</td>
<td>OBI to no HBV</td>
<td>1.09</td>
<td>0.55</td>
</tr>
<tr>
<td>No electricity to having electricity</td>
<td>OBI to chronic HBV</td>
<td>0.57</td>
<td>0.18</td>
</tr>
<tr>
<td>Moderate sanitation to good sanitation</td>
<td>OBI to no HBV</td>
<td>0.85</td>
<td>0.37</td>
</tr>
<tr>
<td>Moderate sanitation to good sanitation</td>
<td>OBI to chronic HBV</td>
<td>0.48</td>
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<tr>
<td>Poor sanitation to good sanitation</td>
<td>OBI to no HBV</td>
<td>1.12</td>
<td>0.40</td>
</tr>
<tr>
<td>Poor sanitation to good sanitation</td>
<td>OBI to chronic HBV</td>
<td>0.59</td>
<td>0.10</td>
</tr>
<tr>
<td>3-5 in household to 1-2 in household</td>
<td>OBI to no HBV</td>
<td>1.02</td>
<td>0.51</td>
</tr>
<tr>
<td>3-5 in household to 1-2 in household</td>
<td>OBI to chronic HBV</td>
<td>1.57</td>
<td>0.47</td>
</tr>
<tr>
<td>&gt;5 in household to 1-2 in household</td>
<td>OBI to no HBV</td>
<td>0.80</td>
<td>0.36</td>
</tr>
<tr>
<td>&gt;5 in household to 1-2 in household</td>
<td>OBI to chronic HBV</td>
<td>1.18</td>
<td>0.29</td>
</tr>
</tbody>
</table>
Table 5: Post Treatment HBV Data

One year post treatment HBV viral load results. Two year (completion of study) drug changes and mortality.

<table>
<thead>
<tr>
<th>One Year Follow up Outcomes</th>
<th>OBI (n=65)</th>
<th>Chronic (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Viral Load Median (Total Range)</td>
<td>10 (10 – 3930)</td>
<td>59 (0 – &gt;1.7x10⁸)</td>
</tr>
<tr>
<td>Positive 12 Month Follow Up</td>
<td>1 (1.5%)</td>
<td>15 (65.2%) HBsAg + 8 (30.4%) DNA+</td>
</tr>
<tr>
<td>12 Month Viral Load – Median (Total Range)</td>
<td>10 (10)</td>
<td>10 (0-475)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Two Year Follow up Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>OBI (n=72)</td>
</tr>
<tr>
<td>Drug Changes</td>
</tr>
<tr>
<td>Mortality</td>
</tr>
</tbody>
</table>
Bibliography:


Appendices A

SAS code: (Example only, not complete)

/* Occult HBV Analysis from BHP
All files stored on the external hard drive (passport)

Datafiles used in this program:
baseline (from clean bomolomo...csv and clean bolomemo...xls)
alkptbili (from AlPTbiliBase.csv from bomolemo baseline additional data version 2...xls - baseline labs sheet)
hbvbaseline
ts005 (from ts005.csv from bomolemo baseline additional data version 2 (ts005CLEAN)...xls - ts005 sheet)

Datafiles created in this program:
baseline_Labs (combination of baseline, alkptbili, hbvbaseline)

Others still needed?
demo (demographic - ts005, eventually ca012 etc),
viral (viral loads)
Not needed (info in viral? - HBC (CAb), HBS (SAb)

Variables created in this program:
ID (numerical form of PID) STANDARD CONNECTING VALUE FOR EVERYTHING
*/

*Libname files 'F:\Occult HBV Data Files';
*Libname sasfiles 'F:\Occult HBV Data Files\SAS';
Libname files 'G:\Occult HBV Data Files';
Libname sasfiles 'G:\Occult HBV Data Files\SAS';
Libname files 'E:\Occult HBV Data Files';
Libname sasfiles 'E:\Occult HBV Data Files\SAS';
Libname files 'D:\Occult HBV Data Files';
Libname sasfiles 'D:\Occult HBV Data Files\SAS';
run;

/ baseline lab values from visit 1000, change PID from character into number
May still need to export to permanent file */

DATA baseline1; set sasfiles.baseline;
Label APRI = "APRI";
APRI = 100 * (astl_clean/40)/(plt_clean);
pid1=compress(pid,'-');
pid2=compress(pid1,'B');
id=input(pid2,7.);
drop pid1 pid2;
run;

*Arrange by ID number for later merging;
proc sort data=baseline1;
   by id;
run;

/*Evaluate if commands working properly;
/* Alk Phos and T bili data - 4 values with atypical PID number M112-002, M121-708, M123-008, M135-001 - not sure what these are... deleted them - If needed, will need to add back in to excel clean file, change csv file, reimport and rerun script here.... */

DATA baseline2; set sasfiles.AlkPTbili;
pid1=compress(PAT_ID,'-');
pid2=compress(pid1,'B');
id=input(pid2,7.);
keep id PAT_ID pid sample_visitid UTESTID result;
run;
*Arrange by ID number and lab value (alk p or t bili) for later merging;
proc sort data=baseline2;
   by id UTESTID;
run;
/*gets last lab from visit 1000, not first lab */

Data baseline2b;Set baseline2;by id;
retail AlkP Tbili;
if first.id then do;alkp=.;tbili=.;end;
if utestid='ALPL' then AlkP = result;
if utestid='BIL-' then tbili = result;
if last.id then output;
run;
/*Evaluate if commands working properly;
proc contents data=baseline2; run;
proc print data=baseline2; run;
proc contents data=baseline2b; run;
proc print data=baseline2b; run;
*/

/* HBV data from baseline, also change PID into number, change YES/NO into numbers
Yes = 1; No = 0 */

DATA hbv; set sasfiles.hbvbaseline;
pid1=compress(pid,'-');
pid2=compress(pid1,'B');
id=input(pid2,7.);
run;

*make all data the same (capitalized NEG and POS);
DATA hbvUp; set hbv;
ARRAY ALL_C[*] _CHARACTER_;
DO I = 1 to DIM(ALL_C);
ALL_C[I] = UPCASE(ALL_C[I]);
end;
drop I;
run;
/*test to see if this is correct;
proc print data=hbvUp; run;
*/

*Changing POS/NEG to 1/0 (POS = 1, NEG = 0)
Drop unnessary data
NEED TO DECIDE WHAT TO DO WITH <20 values - drop < or change to 10?;
DATA hbvbase; set hbvUp;
Label AbStatus = "Core and Surface Antibody Status, CAB+/SAB+ = 1; CAB+/SAB- = 2; CAB-/SAB+ = 3, CAB-/SAB- = 4";
ARRAY ALL_C[5] HBSAG HBSAB HBCAB HBEAG HBV_DNA;
ARRAY ALL_N[5] SAG SAB CAB EAG DNA;
DO I = 1 to 5;
  IF ALL_C[I] = 'POS'
    THEN ALL_N[I]= 1; /*NEED TO CHANGE FROM CHAR TO NUMBER input(1,1.);1; THEN
  ALL_[C]=INPUT(1,1.)*/
    IF ALL_C[I] = 'NEG'
      THEN ALL_N[I] = 0;
    IF ALL_C[I] = ' '
      THEN ALL_N[I] = .;
end;
drop I; *redundant with line below but keep incase using as template for programing later;
IF SAB = 0 AND CAB = 0
  THEN AbStatus = 4;
IF SAB = 1 AND CAB = 0
  THEN AbStatus = 3;
IF SAB = 0 AND CAB = 1
  THEN AbStatus = 2;
IF SAB = 1 AND CAB = 1
  THEN AbStatus = 1;
keep LID PID SAG SAB CAB EAG DNA HBV_VIRAL_LOAD ID AbStatus;
Run;

proc sort data=hbvbase;
  by ID;
run;

/* testing to ensure this works
proc print data=hbvbase; run;
proc contents data=hbvbase; run;
proc print data=hbvbase2; run;
proc contents data=hbvbase2; run;
*/
*Merging hbvbase with baseline1 - merges HBV antibody/antigen/viral load with baseline lab values;

DATA Visit1000; *attrib id would like to include in figure out how so that id variable is first listed;
Merge hbvbase baseline1 baseline2b;
   by ID;
drop pid1 pid2;
hbv_result = result_hbv_clean;
drop result_hbv_clean;
run;

proc contents data=visit1000; run;
proc print data=visit1000; run;

* Create new variable to categorize chronic HBV (=1), OBI (=2), no HBV (=0);
DATA All1000; set Visit1000; by ID;
retain HBV_result;
If DNA= . OR SAG = . then HBV_result = .;
   else if DNA = 0 then HBV_result=0;
if DNA= 1 then HBV_result=2;
if SAG=1 then HBV_result=1;
run;

proc print data = All1000; run;

Data cat; set All1000; by ID;
retain HBV_result id;
keep HBV_result id;
run;
proc print data=cat; run;

/*Test to verify it’s correct:
proc print data=Visit1000; run;
proc contents data=Visit1000; run;
proc print data=All1000; run;
proc contents data=All1000; run;
*/

/* EXPORT / PERMANENTLY SAVE BASELINE DATA FILE */
DATA sasfiles.baseline_Labs;
set Visit1000;
run;
DATA sasfiles.baseline_Labs2;
set All1000;
run;
DATA sasfiles.HBVCategory;
set cat;
run;

proc print data=cat; run;
proc print data=sasfiles.baseline_Lab2; run;

/* CATEGORIES IN SOCIAL / sasfile.ts005 :
DATA social; set sasfiles.ts005;
pid1=compress(pid,'-');
pid2=compress(pid1,'B');
id=input(pid2,7.);
drop pid1 pid2;

ARRAY ALL_N[*] _NUMERIC_;
DO I = 1 to dim(ALL_N);
   IF ALL_N[I] = -9 THEN ALL_N[I]=.;
   IF ALL_N[I] = -2 THEN ALL_N[I] = .;
   IF ALL_N[I] = -3 THEN ALL_N[I] = .;
   IF ALL_N[I] = 9 THEN ALL_N[I] = .;
   IF ALL_N[I] = 99 THEN ALL_N[I] = .;
end;
drop I cinitials HEADERDATE PInitials ProtocolNumber;
run;
proc sort data=social; by id visitid;
run;

DATA base_social; set social;
retain visitid;
If visitid = 1000 then output;
run;
proc sort data=base_social; by id;
run;

proc print data=base_social; run;

/* add HBV category 0= no HBV, 1 = chronic HBV, 2 = occult HBV to social info*/
DATA baseline_social;
merge base_social cat; by id;
run;

proc print data=baseline_social; run;

/* EXPORT / PERMANENTLY SAVE BASELINE DATA FILE */
DATA sasfiles.social_baseline;
set baseline_social;
run;

/* testing to ensure this works
proc print data=social; run;
proc contents data=social; run; */
SAS Code Part 2 –
*Find location of external hard drive with saved files;
Libname files 'F:\Occult HBV Data Files';
Libname sasfiles 'F:\Occult HBV Data Files\SAS';
Libname files 'G:\Occult HBV Data Files';
Libname sasfiles 'G:\Occult HBV Data Files\SAS';
Libname files 'E:\Occult HBV Data Files';
Libname sasfiles 'E:\Occult HBV Data Files\SAS';
Libname files 'D:\Occult HBV Data Files';
Libname sasfiles 'D:\Occult HBV Data Files\SAS';
run;

/* Social baseline demographic information from dataset */
DATA BaseSocial;
set sasfiles.social_baseline;
drop PID visitID;
run;

/* Just ID number linked to hep B status
0 = No, 1 = chronic 2 = occult
ID = B00 + recorded #
*/
DATA BaseCategorical;
Set sasfiles.HBVCategory;
run;

/* All HBV and labs from baseline (AST, ALT, T bili, Alk Phos, HIV, CBC, AlkP etc */
DATA BaseLabs;
set sasfiles.baseline_Labs2;
drop LID PID UTESTID result PAT_ID sample_visitid
run;

DATA FinalBaseline;
merge BaseSocial BaseLabs;
    by id;
run;

proc print data=FinalBaseline; run;

DATA sasfiles.FinalBaseline;
set FinalBaseline;
run;
To start here for additional analysis
Import the FinalBaseline File from external hard drive:

DATA FinalBaseline;
set sasfiles.FinalBaseline;
run;

/*
To obtain medians and interquartile ranges for continuous data
Proc univariate data=FinalBaseline;
   Class hbv_result;
   Var APRI fib4 bmi altl_clean astl_clean hgb_clean AlkP Tbili plt_clean;
Run;
Proc univariate data=FinalBaseline;
   Class hbv_result;
   Var cd4_clean viral_load viral_load_clean;
Run;

/* ANOVA for post hoc analysis of antibody status */

/* Chi Square Analysis */
Proc freq data=FinalBaseline;
   Table hbv_result*(CAB DNA EAG SAB SAG)/chisq; /*instead of using chisq I could write in fisher */
Run;
Proc freq data=FinalBaseline;
   Table hbv_result*AbStatus/chisq;
run;

/* This does not treat HBV_Result as a class variable???
Error on line for LSMeans - Tukey states expecting an =. */
proc glm data=FinalBaseline;
   Class AbStatus hbv_result;
   Model hbv_result = AbStatus;
   LSMEANS AbStatus / ADJUST Tukey PDIFF;
   Contrast 'Compare Antibody Status C+/S+ & isolated C+ status' AbStatus 1 -1 0 0;
   Contrast 'Compare Antibody Status C+/S+ & isolated S+ status' AbStatus 1 0 -1 0;
   Contrast 'Compare Antibody Status C+/S+ & C-/S- status' AbStatus 1 0 0 -1;
   Contrast 'Compare Antibody Status isolated C+ & isolated S+ status' AbStatus 0 1 -1 0;
   Contrast 'Compare Antibody Status isolated C+ & C-/S- status' AbStatus 0 1 0 -1;
   Contrast 'Compare Antibody Status isolated S+ & C-/S- status' AbStatus 0 0 1 -1;
Run;
quit;

proc glm data=FinalBaseline;
   Class AbStatus hbv_result;
   Model AbStatus = hbv_result;

LSMEANS AbStatus / ADJUST Tukey PDIFF;
Contrast 'Compare OBI to chronic HBV' hbv_result 0 -1 1;
Contrast 'Compare OBI to HIV mono-infected' AbStatus -1 0 1;
Contrast 'Compare OBI to Non OBI' AbStatus -1 -1 2;
Run;
Quit;

Proc freq data=FinalBaseline;
Table hbv_result*(merital_status howmanydependants averageincome electricity car phone tV Fridge toilet_type water_source church age gender_clean) / chisq;
Run;

Proc freq data=FinalBaseline;
Table hbv_result*(howmanyinhouseshold howmanylivechild education_level children headofhouse) / chisq;
Run;

/ * Non parametric testing (Wilcoxon Score - Kruskal-Wallis testing) */
Proc NPAR1WAY data=FinalBaseline;
Class hbv_result;
Var gender_clean viral_load_clean age cd4_clean ;
Run;

Proc NPAR1WAY data=FinalBaseline;
Class hbv_result;
Var viral_load ;
Run;

Proc NPAR1WAY data=FinalBaseline;
Class hbv_result;
var altl_clean astl_clean plt_clean hgb_clean bmi AlkP Tbili fib4 APRI; /*multiple continuous variables in this line after var just leave a space between them*/
Run;

Proc NPAR1WAY data=FinalBaseline;
Class hbv_result;
Var AbStatus ;
Run;

Proc NPAR1WAY data=FinalBaseline;
Class AbStatus;
Var hbv_result ;
Run;

/ *Multinomial Code for Regression Modeling */
Proc logistic data=FinalBaseline;
Class hbv_result (ref="2") AbStatus (ref="1") education_level (ref="1") phone (ref="1") electricity (ref="1")
water_source (ref="1") toilet_type (ref="1") / param=ref; /*put all categorical variables in the class statement*/
Model hbv_result=AbStatus APRI fib4 astl_clean astl_clean viral_load cd4_clean education_level phone electricity water_source toilet_type howmanydependants / link=glogit;
Run;

*Location:
http://www.ats.ucla.edu/stat/sas/dae/mlogit.htm
;

proc contents data=FinalBaseline;
run;

proc freq data=FinalBaseline;
table hbv_result*AbStatus / chisq nocol nofreq;
label hbv_result = "0= No HBV, 1= chronic HBV, 2 = OBI";
run;