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Examination of Blood for Hepatitis B Virus (HBV) and possible Transmission by Mosquito (*Aedesaegypti*)

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Abstract: *Hepatitis is an inflammation of the liver tissue and its presentations ranges from complete asymptomatic to severe liver failure. Among others, the causes include viruses and parasites. In 2005, chronic hepatitis B infected 343 million peopleworldwide. Blood tests and clinical picture are sufficient for diagnosis. Seroepidemiological survey of volunteers was conducted. Three volunteers out of 100 were found infected. Aedesaegyptiwere artificially fed with positive blood samples and were allowed to bite rabbits. The rabbits presented geophagy, loss of appetite, thinning, loss of fur and inflammation of liver. This study provides an evidence for transmission of HBV through mosquito. However, this study is limited to transmission to rabbit. A study of transmission to human is recommended.*

Keywords: *Blood, Examination, Hepatitis B, Transmission, Virus*

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I. Introduction

The ancient Sumerians believed that the liver was the home of the soul, and attributed the findings of jaundice to the attack of one's liver by a devil named Ahhazu (Trepo, 2014). Around 400 B.C., Hippocrates recorded the first documentation of an epidemic jaundice, in particular noting the uniquely fulminant course of a cohort of patients who all died within two weeks. He wrote, ''The bile contained in the liver is full of phlegm and blood, and erupts….A such an eruption, the patient soon raves, becomes angry, talks nonsense and barks like a dog'' (Oon, 2012).

Hepatitis B is the most common cause of viral hepatitis in the world with more than 240million chronic carriers of the virus, 1 million of whom are in the United States (Carroll, 2015). Inendemic regions, transmission is thought to be associated with exposure during birth and close contact between young infants (Dienstag, 2015). Risk of infection is highest among intravenous drug users, individuals with high-risk sexual behaviours, healthcare workers, individuals with a history of multiple transfusions, organ transplant patients, dialysis patients and newborns infected during the birthing process (Carroll, 2015). In approximately two-thirds of patients who develop acute hepatitis B infection, no identifiable exposure is evident (Dienstag, 2015). Close to 780,000 deaths in the world are attributed to hepatitis B. The most endemic regions are in sub-Saharan Africa and East Asia where as much as 10% of adults are chronic carriers (W.H.O., 2014).

Causes of hepatitis can be divided into the following major categories: infectious, metabolic, ischemic, autoimmune, genetic, and other. Infectious agents include viruses, bacteria, and parasites. Toxins, drugs, alcohol, and non-alcoholic fatty liver disease are metabolic causes of liver injury and inflammation. Autoimmune and genetic causes of hepatitis involve genetic predispositions and tend to affect characteristic populations. Parasites can affect liver and activate the immune response, resulting in symptoms of acute hepatitis with increased serum IgE (Harder, 2008). Of the protozoan, *Trypanosomacruzi*, Leishmania species, and the malaria-causing Plasmodium species all can cause liver inflammation (Harder, 2008).

Chronic hepatitis presents similarly, but can manifest signs and symptoms specific to liver dysfunction with long-standing inflammation and damage to organ (Khalili, 2013). Acute viral hepatitis follows pattern of infection that involves three distinct phases: 1.The initial prodromal phase; 2. Yellowing of the skin and whites of the eyes follow the prodrome after about 1 -2 weeks and can last for up to 4 week; and 3. The recovery phase, characterized by resolution of the clinical symptoms of hepatitis (Dienstag, 2014).

Screening for hepatitis is very important as it aimed at identifying people infected with the disease as early as possible (Chou, 2014). Screening consists of a blood test that detects hepatitis B surface antigen (HBsAg). If the (HBsAg) is present, a second test – usually done on the same blood sample (Chou, 2014).

For viral hepatitis and other acute causes of hepatitis, the patient's blood test and clinical picture are sufficient for diagnosis (Friedman, 2015). For other causes of hepatitis, especially chronic causes, blood tests

may not be useful (Friedman, 2015). In this case, liver biopsy is the gold standard for establishing the diagnosis as histopathologic analysis is able to reveal the precise extent and pattern of inflammation and fibrosis (Friedman, 2015). However, liver biopsy is typically not the initial diagnostic test because it is invasive and is associated with a small but significant risk of bleeding that is increased in patients with liver injury and cirrhosis (Grant, 1999).

Acute hepatitis B infections have a favourable course towards complete recovery in95- 99% of patients (Dienstag, 2014). However certain factors may portend a poorer outcome, such as co-morbid medical conditions or initial presenting symptoms of ascites, edema, or encephalopathy. Overall, the mortality rate for acute hepatitis is low: ~0.1% in total for cases of hepatitis A and B, but rates can be higher in certain populations (Dienstag, 2014).

For prevention of hepatitis B, the Center for Disease Control (CDC) recommends the routine vaccination of all children under the age of 19 with the hepatitis B vaccine (CDC, 1999). The first dose starts with the first dose administered as a shot into the muscle before the newborn is discharged from the hospital. An additional two doses should be administered before the child is 18 months (CDC, 2016).

Since mosquitoes can spread some diseases, such as malaria and yellow fever, it is tempting to think they can spread other blood-borne diseases like HIV AIDS and hepatitis (Charles, 2017). In 1974, George and Tzenny have reported on role of mosquitoes in transmission of hepatitis B Virus Infection and their study had involved school children; in 1995, Zheng, *etal*. reported on animal transmission of hepatitis B virus through three genera of mosquitoes and their study involved monkeys, *TupaiaBelangeri*;and in 2015, Hanan had reported that Hepatitis B as Vector-borne transmitted infection but has not ascertain the ability of the insect to be infected as a result of biting of human while we tried transmission of the virus by mosquito to rabbit in this study and this is the first of its kind.

I. Materials and Method

We collected 100 serum samples of volunteers. Chromatographic immunoassay for qualitative detection of HBsAg was used and positive samples were separated from the negative ones. In an experimental environment, a laboratory- raised species of mosquito, *Aedesaegypti* was artificially fed with the infected blood samples.The mosquitoes were allowed to bite rabbits for one week. The health status of rabbits was instantly subjected to a close physical observation, a month after the bite, their venous blood samples were taken to detect HBsAgand their livers were examined.

II. Results and Discussion

The result for volunteers' serum examination revealed 3% positive while a physical observation of the volunteers did not reveal any sign and symptom of hepatitis. This had agreed with what is known of this disease as reported in *MedilinePlus* (2016); and by WHO(2016) that some people have no symptoms whereas others develop yellow discolouration of the skin and whites of eyes, poor appetite, vomiting, tiredness, abdominal pain or diarrhea.Rutherford and Dienstag (2016) had further reported that hepatitis has a broad spectrum of presentations that range from a complete lack of symptoms to severe liver failure. This had further explained that the hepatitis status of those infected was acute as its symptoms were not noticed from the volunteers. Acute hepatitis can sometimes resolve on its own, progress to chronic hepatitis, or rarely result in acute liver failure (Bernal and Wendon, 2013). The acute form of hepatitis, generally caused by viral infection, is characterized by constitutional symptoms that are typically self-limiting (Dienstag, 2016). Viral hepatitis is the most common type of hepatitis worldwide (WHO, 2013).

Hepatitis B is the mostcommon cause of viral hepatitis in the world with more than 240 million chronic carriers of the virus, 1 million of whom are in the United States (WHO, 2014). In approximately two-thirds of patients who develop acute hepatitis B infection, no identifiable exposure is evident and most hepatitis B cases are also self-limiting and will resolve in 3 -4 months (Dienstag, 2016). Of those acutely infected, 25% become lifetime carriers of the virus (Carroll, 2016). Risk of infection is highest among intravenous drug users, individuals with high-risk sexual behaviours, healthcare workers, individuals with a history of multiple transfusions, organ transplantpatients, dialysis patients and newborns infected during the birthing process (Carroll, 2016). Close to 780,000 deaths in the world are attributed to hepatitis B (WHO, 2014). The most endemic regions are in sub-Saharan Africa and East Asia where as much as 10% of adults are chronic carriers (Dienstag, 2016). Carriers rate in developed nations are significantly lower, encompassing less than 1% of the population (WHO, 2014).

Moreover, for acute hepatitis B, in healthy patients, 95 – 99% recovers with no long-lasting effects, and antiviral treatment is not warranted (Dienstag, 2016). Age and comorbid conditions can result in a more prolonged and severe illness. Certain patients warrant hospitalization, especially those who present with clinical signs of ascites, peripheral edema, and hephatic encephalopathy, and laboratory signs of hypoglycemia, prolonged prothrombin time, low serum albumin, and very high serum bilurubin (Dienstag, 2016).

The close physical observation of health status of the rabbits revealed geophagy, loss of appetite, thinning, and lose of fur among them. These are suspected to be due to the viral infection and they slightly deviate different from what is observed in humans. The symptoms in human involved among others, yellow discolouration of the skin, and poor appetite (*MedilinePlus*, 2016). However, the detection of HBsAg from the serum of the rabbits may prove these symptoms are due to viral infection and considering that HBsAg and anti-HBs were conventional. In the use of test card to test card to check HBsAg, the sensitivity was 88.8% and the specificity was 100% (Fu-yu, *etal*., 2015).

In a different trial, using *Tupaiabelangeri*, by Zheng*etal*. (1995), a monthafter biting, venous blood samples were taken to detect serum HBsA, HBeAg, Anti-HBs and Anti-HBc, among the 29 monkeys, 9 were HBV infective serum markers positive. Moreover, there was evidence that certain species of mosquitoes could transmit hepatitis B virus to animalsleading to immunity (Hannan, 2015).

Inflammation of the liver was also observed among the rabbits. This had agreed with what is reported in humans and it is a clear definition of hepatitis as reported by *NIAID* (2016) that hepatitis is inflammation of the liver tissue. In 4 of 16 test monkeys liver biopsies, acute inflammatory changes were found with Haemotoxylin-Eosine staining and in the hepatic cells, HBsAg and HBcAg were detected by immunohistochemical double labeling assays, and HBV-DNA was also positive byin situ hybridization in monkey liver sections (Zheng, 1995).Generally, there is an initial insult that causes liver injury and activation of an inflammatory response, which can become chronic, leading to progressive fibrosis and cirrhosis (Dienstag, 2016). The specific mechanism variesand depends on the underlying cause of the hepatitis. The pathway by which hepatic viruses cause viral hepatitis is best understood in the case of hepatitis B and C (Dienstag, 2016). The viruses do notdirectly cause apoptosis (cell death). Rather, the infection of liver cells activates the innate and adaptive arms of the immune system leading to an inflammatory response which causes cellular damage and death (Dienstag, 2016).

The result of this study provided an evidence for the possibility of Hepatitis B Virus transmission through mosquito. The question of whether the hepatitis B could be a vector-borne transmitted infection was around in the scientific field since the 1949 (Newkirk, 1975). Towards this, over a period of 29 years a total of 12 researches and two review articles were done to investigate this question. In addition, around five non-English Language articles were published during the same period (Hannan, 2015). The majority of the studies agreed that Hepatitis B Virus could be found in bedbugs for a longer period of time than in the mosquitoes. Moreover, there was evidence that certain species of mosquitoes could transmit Hepatitis B Virus to animals leading to immunity; however, this did not reflect their ability to transmit the virus to humans because it was done under experimental conditions and on a small number of animals (Hannan, 2015).

The role of mosquitoes in transmission of Hepatitis B Virus infection was conducted by George and Tzenny in (1974). They conducted seroepidemiological surveys of schoolchildren in Arachova and Anthili, Greece to study the possible role of mosquitoes in transmission of Hepatititis B Virus infection and had reported the overall prevalence of hepatitis B antigen 2.7% in the Anthili population and 0.9% in the Arachova population. And further explained that antibody to Hepatitis B Virus wasdetected in a significantly higher frequency in the Anthili (20.5%) than in the Arachova (5.1%) populationand concluded that this finding gives further support to the previously suggested hypothesis that mosquitoes may playrole in the transmission of Hepatitis B Virus infection; In 1975 Newkirk and colleagues studied the fate of HBsAg following a blood meal in two species of mosquitoes (*Aedesaegypti* and *Culextsarsalis*). Both mosquito species were experimentally fed on HBsAg positive blood. In the two mosquito species, the highest concentrations of HBsAg were detectedfor a minimum of 20 hours after feeding an infected blood. They suggested that one possible explanation of this was the release of protease trypsin in mosquitoes after blood meal, which destructed the virus. As a result, for the mosquitoes to be effective mechanical transmitters, they would have to be killed during the second feeding within a few hours of complete HBsAg [positive blood mral, making this route of transmission of low significance; and in 1995 Zheng*etal*. (1995) conducted study on transmission of Hepatitis B Virus through mosquitoes they fed three genera of mosquitoes with human blood infectedwith Hepatitis B Virus artificially. The mosquitoes were used to bite monkeys and HBsAg and HBcAg were detected from the hepatic cells of the monkeys and concluded that these results provided an evidence for the possibility of transmission of HBV through mosquitoes and suggest its epidemiological significance in mosquito infested areas.

III. Conclusion

Overall, this study proves the role of mosquito in transmission of Hepatitis B Virus infection. This research is an advancement of researches on Hepatitis B Virus transmission by vectors and it filled the widen gap, 1995 - 2018of research in transmission of Hepatitis B Virus through vector. This study has also presents another dimension of the research, via rabbit.

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