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Impact of miRNA (146a, 149a, 196a) Polymorphism of Antibody Response to Hepatitis B Vaccine

A Thesis

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1.1 Introduction.

Hepatitis is an inflammation of the liver parenchyma. It is caused by different infectious and non-infectious agents. Among the most common infectious causes are hepatitis viruses. These are a family of heterogenous viruses including A, B, C, D, E and G, where type A, B and C are the most common (CDC, 2016). Hepatitis B virus (HBV) is an acute infection of the liver parenchymal cells caused by the hepatitis B virus (Ferri, 2017).

According to recent estimates, about 2 billion people worldwide are infected with HBV; among whom are about 400 million with chronic infection (Jensen and Balistrei, 2016). The death figure from direct or in direct complications related to this infection was found to be 1.2 million people every year (Lavanchy, 2004). The most important global impact of HBV infection is related to chronic infection. Approximately 20% of acute infection develops to chronic. On person in every 4 with chronic infection develop serious complications mainly chronic liver disease and hepatocellular carcinoma(Wong and Gish, 2016).

Chronic hepatitis B patients are generally treated either with interferon, which facilitates the immune in achieving seroconversion, or with nucleotide analogues (NAs), which suppress viral replication and prevent the progression of liver disease by combating inflammation (Lee *et al*., 2006).

A part from health problem, the burden economical loss due to HBV is very high. Surprisingly, the highest incidence rate of HBV infection was recorded among the most productive age class 20-39 years old. Hence, it can be imagined how many work hour are lost due to such infection. In one of the neighboring countries, it was estimated that annual total cost for chronic hepatitis B, HBV-related liver cirrhosis and hepatocellular

carcinoma was 3094.5, 17483 and 32958 US dollars respectively for each patient(Kavosi *et al*., 2014).

Preventive measure such as safe blood transfusion and safe injection are very effective in reducing the disease prevalence, but vaccination is considered the cornerstone for effective control. Using full recommended doses of the vaccine induces protective antibody level in 95% of infants, children and young adult (WHO, 2017a).

Approximately 5-10% of healthy vaccinated individuals fail to produce protective levels of antibodies following standard vaccination protocols. Subsequently, these individuals are still susceptible to HBV infection (SjIgren, 2005). The precise mechanisms leading to non-responsiveness to hepatitis B vaccine are not yet clearly defined. Genetic background is thought to play a critical role in modulating responsiveness to the vaccine. Among the genes investigated and associated with nonresponse to hepatitis B vaccine are those encoding human leukocyte antigen (HLA) (Png *et al.*, 2011). Toll-like receptors (TLRs), cytokines and cytokine receptors (Macedo *et al.*, 2010; Chen *et al.*, 2011).

Micro RNAs (miRNA) are group of small non-coding (≃22 nucleotides) RNAs. They have very important role in regulation of gene expression, through binding to their target mRNAs and then promoting or inhibiting its translation (Davidson- Moncada *et al.*, 2010). It was found that miRNAs are able to modulate the expression of 30-60% of all protein coding human genes which reflects the crucial role of these non-coding oligonucleotides in different biological body processes (He and Hannon, 2004).

Among the huge number of discovered miRNAs, studies showed that particular miRNAs are effective in regulation of immune response. Of these are miR-146a, miR-149 and miR-196a2(Roy and Sen, 2011). Some single nucleotide polymorphisms (SNPs) in the genes encoding for these miRNAs influence the ability of the individual miRNA to bind and regulate the target mRNA. Three SNPs (miR-146a G>C, miR-149 C>T and miR-196a2 C>T) have been widely investigated and found to be significantly associated with different pathologies such as lung cancer, gastric cancer, hepatocellular carcinoma and coronary artery diseases (Jiang *et al*., 2016, Huang *et al*., 2017, Wang *et al*., 2017, Yin *et al*., 2017) . Therefore, it is reasonable to assume that such SNPs may have an impact on immune response to HBV vaccine. In Iraq, there is no previous study which addressed the role of miRNA gene polymorphisms in immune response to HBV vaccine. Therefore this study was conducted.

1.2 Aims of study.

1- To study the association of some demographic characteristics such as age, gender and body mass index with the antibody response to hepatitis B vaccine.

2- To evaluate the role of three polymorphism in miRNA genes (miRNA -146 G>C, miRNA-149 C>T and miRNA-196 a2 C>T) in the antibody response to hepatitis B vaccine

Summary

Infection with hepatitis B virus (HBV) is prevalent worldwide causing a high mortality rate and a significant potential for hepatocarcinoma. Prophylaxis is mainly based on vaccination which protects the vast majority of population from this infection. However, a considerable percentage of vaccinated individuals cannot develop the protective level of anti-HBAg antibodies, which indicates a genetic role in the antibody response to this vaccine. Micro RNAs have been shown to regulate the gene expression of huge numbers of genes. A single nucleotide polymorphism in miRNA genes may influence the activity of the encoded miRNA.

This study aims to determine the activity of hepatitis B vaccine to induce the antibody response and relate it with some demographic characteristics such as age, gender and body mass index as well as to evaluate the role of three polymorphism in miRNA genes (miRNA -146 G>C, miRNA-149 C>T and miRNA-196 a2 C>T) in the immune response to hepatitis B vaccine.

A total of 86 healthy children and adolescents who received three doses of HBV were collected from Al-Zahraa Health Center in Baghdad during the period between October 2016 to January 2017. Blood samples(whole blood and serum) were obtained from each participant. Enzyme linked immune sorbant assay (ELISA) was used to estimate the serum levels of anti-HBsAg IgG titer. DNA was extracted from the whole blood, and the gene fragments corresponding the three singe nucleotide polymorphism (SNPs) were amplified with specific primers using a conventional polymerase chain reaction(PCR). Genotyping was performed with a direct sequencing.

According to the instructions of the ELISA kit, only 58 (67.44%) had a good antibody response while other 28(32.55%) had a weak antibody response. Anti-HBs Ab positivity rate was non-significantly regarding age, gender and BMI among studied population.

Both miR-146a G>C and miR196a2 C>T were found to have a significant association with a antibody response to HBV vaccine. In recessive model, the combine genotype GC+CC of miR-149a G>C polymorphism was more frequent in responders than non-responders with a significant difference of (OR=2.598,95% Cl=1.008-6.698, P=0.046). The minor allele (C allele) was more frequent in responders than non-responders with a significant difference of (OR= 2.16, 95% Cl= 1.029- 4.53, P=0.039). For miR-196a2 C>T polymorphism there were significantly more subjects carrying the wild homozygous genotype (CC) in responders than non-responders (OR=2.88,95% Cl=1.044-7.95, P=0.04). The prevalence of the major allele (allele C) was significantly higher in responders than non-responders (OR=2.72, 95% Cl=1.25-5.92, P=0.013). The statistical analysis of the association between different genotypes and antibody response revealed a significantly higher antibody titer associated with CC genotype of miR-146a G>C. None of demographic characteristics (age, gender and body mass index) has any significant impact on the antibody response to Hepatitis B vaccine.

These data indicate the role of miR-146a G>C and miR-196 a2 C>T in antibody response to Hepatitis B vaccine.