Hepatocellular Carcinoma Preventive Vaccines and Clinical Trials

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DOI: 10.29322/IJSRP.9.04.2019.p8859
http://dx.doi.org/10.29322/IJSRP.9.04.2019.p8859

Abstract - Hepatocellular Carcinoma is the most common liver malignancy in the world and accounts for the most number of cancer related deaths worldwide. Larger incidences of HCC cases are reported from developing countries and much lesser in developed countries. According to many studies men are at more risk of getting HCC than females. Hepatitis B virus remains as the most important risk factor while Hepatitis C virus, alcoholism, diabetes mellitus and obesity play a major role in HCC. Cirrhosis remains as an additional important risk factor specially in the United States. Although HCC is a very aggressive malignancy, prevention of HCC can be achieved by carefully examined measurements. Hepatitis B vaccination, anti viral therapy, immunotherapy and DNA analogs are used to prevent and control the tumor aggression. Continued improvements in vaccines have demonstrated positive outlooks in overall survival. Researches on molecular targeted vaccines are being conducted and are on the rising. With the advancements of these vaccines, the future of preventing HCC seems brighter.

Key Words – Hepatocellular Carcinoma, Vaccine, Clinical Trial, Prevention

1. Introduction

Primary liver cancer is the third major reason in cancer mortality in the world (Altekruse, Henley, Cucinelli, & McGlynn, 2014). Among them hepatocellular carcinoma (HCC) is the fifth most recurrent malignancy in the world and is estimated to result approximately half a million deaths yearly. Because of its excessive fatality rates, the incidence and mortality rates are almost equal (Hashem B El-Serag, 2002). Hepatocellular carcinoma (HCC) also accounts for more than 90% of liver cancers in the world and there has been a significant increase in HCC related annual morality rates during the past two decades (Omata et al., 2017). Despite all the advances in prevention techniques, screening, and recent technologies in both diagnosis and treatment, mortality continues to rise (Omata et al., 2017). In order to prevent hepatocellular carcinoma, vaccines against the risk factors have been produced and thus far

they have been playing an important role worldwide in preventing hepatocellular carcinoma. The aim of this literature review to assess these vaccines and their clinical significance in terms of preventing hepatocellular carcinoma.

2. Epidemiology

HCC occurs more often in males than females with a larger incidence in Southern and Eastern Asia, Western and Middle Africa, Melanesia, Micronesia and Polynesia. The age adjusted incidence of HCC has increased from 1.6 per 100,000 to 4.6 per 100,000 individuals among Alaskan natives and American Indians followed by Whites, blacks and Hispanics (Balogh et al., 2016) The biggest age adjusted incidence rates (>20/100,000) are reported in East Asia which includes North and South Korea, Vietnam, and China and sub Saharan Africa. Approximately 75% of liver cancers occur in Asia, with China being responsible for more than 50% of the world's cases. The incidence of HCC is probably to increase over the next 10 to 20 years and to reach around 2030 (Manuscript, 2014a) In the contrary, the incidence of HCC is much lesser in developed countries in North America resulting in 6.8 cases per 100,000 person per year for men and 2.2 cases per 100,000 person per year for women, Europe (except the southern Europe), South and Central America, Australia and New Zealand (Manuscript, 2014a) Although, the overall 5 year survival rate is less than 12%, making HCC the most rapid rising cause of cancer related death in United States. Both male and females showed a 3 fold increase in incidence of HCC from 1975 to 2007 (Manuscript, 2014b) Intermediate rate HCC locations, where the incidence rates are normally between 10/100,000 and 20/100,000, are ideally located in central Europe which includes Italy, France, Switzerland, Greece. Regardless of the magnitude of the incidence rate, almost all areas report rates in males that are two to three fold higher than rates in females. Significant exceptions to this gender relation are the relatively equal incidence rates reported by registries in Harare, Zimbabwe, Costa Rica, Cali, Colombia and South Karachi, Pakistan (McGlynn & London, 2011)

In addition to variability by gender, many areas record incidence rate varies by race and ethnicity. As for an example in the United States HCC incidence is the largest among Asians and Pacific islanders resulting 11.7/100,000 and lowest among white people resulting 3.9/100,000. In between to these rates are those of Hispanics resulting 8.0/100,000, black people resulting 7.0/100,000 and American Indians and Alaska Natives resulting 6.6/100,000. Just as different as the rates among various ethnic groups inhabiting in one area are the rates among members of a single ethnic group living in various areas. For example, incidence rates among Chinese people are significantly lower in the U.S. than they are in either China or in Singapore. As with gender divergent and race and ethnic differences in risk are likely to be related to the prevalence of major risk factors in each groups (McGlynn & London, 2011)

3. Risk factors

3.1 Hepatitis B Virus

HBV is the major leading risk factor for HCC globally and is responsible for at least 50% cases of HCC (Parkin, n.d.) and virtually all child cases (Bedossa & Paradis, 2011) The role of chronic infection with hepatitis B virus in the etiology of hepatocellular carcinoma is well identified (Parkin, n.d.) In a population based cohort study which carried out in 1980s involved 22,708 Taiwanese men who were followed up for 8-9 years, the occurrence of HCC was 98.4 times higher in HBV carriers than non-carriers (Manuscript, 2014a) Presently, about 5% of the world's population (350 million people) is chronically infected with HBV. The evidence supporting the causal association of HBV with HCC is rather common (McGlynn & London, 2011) In endemic areas in Asia and Africa, where HBV infection is transmitted from mother to infants, up to 90% of infected persons have a chronic course, with recurrent integration of HBV into host DNA. Even though HBV can end up with hepatocellular carcinoma in the absence of cirrhosis, the majority (70 to 80%) of patients with HBV related hepatocellular carcinoma end up having cirrhosis. The risk of hepatocellular carcinoma among people with chronic HBV infection (those who are positive for hepatitis B surface antigen [HBsAg]) (T. Tseng et al., 2012) is further expanded if they are male or elderly, have been infected for a long period of time, have a family record of hepatocellular carcinoma, have been introduced to the mycotoxin aflatoxin, have consumed alcohol or tobacco, are co infected with HCV, have increased levels of HBV hepatocellular replication (Chen, Yang, & Iloeje, 2009) or are infected with HBV. HBV DNA can also be detected in persons who are HBsAg negative, but the association with risk of hepatocellular carcinoma is unclear in these cases (Bedossa & Paradis, 2011) The lifetime risk of HCC among chronic HBV-infected patients is known to be around 10–25%. Risks of HCC among HBV infected patients vary by several factors, the major one being serum HBV-DNA levels although there is no exact cutoff level (Chen, Iloeje, & Yang, 2007)

The risk and the presence of HCC in hepatitis B carriers seems to be related to ethnicity. White hepatitis B carriers are most likely to develop HCC at a later age after a period of progressive liver cirrhosis, whereas African and Asian individuals are likely to develop HCC in the young adulthood and in the middle age and might show fewer signs of cirrhotic liver disease than white hepatitis B carriers. Genetic variation might be the reason for the differences between these ethnic groups. Alternatively, these differences might be explained by disparities in the age at which HBV infection is acquired in different ethnicity group populations. Vertical transmission is the major mode of acquisition of HBV in Asia, whereas horizontal transmission in early life is the most dominant mode of transmission in Africa. By contrast, in Western countries HBV is mostly transmitted in adolescence and adulthood through high risk behaviors, such as intravenous drug use, sexual exposure or iatrogenic causes including blood transfusion, unsafe needle practices, invasive procedures, hemodialysis or organ transplantation (Manuscript, 2014a)

3.2 Hepatitis C Virus

Chronic HCV infection is one of the well-known causes of chronic liver diseases and hepatocellular carcinoma both in patients with histories of exposure to viral diseases which are transmitted from parents and in patients who have no noticeable source of infection. (Fattovich et al., 1997) More importantly, HCCs developing after HCV infection have occurred in elderly patients with more severe liver diseases than tumors developing after HBV infection.(Fasani et al., 1999) It is reported that Hepatitis C is the leading cause for both chronic liver disease and HCC in most Western countries, including the USA (Manuscript, 2014b) In a community based prospective study in Taiwan which focused on effects of hepatitis C virus (HCV) infection on the incidence of hepatocellular carcinoma (HCC), it was suggested that HCV plays a significant role in hepatocarcinogenesis in an area endemic for chronic HBV infection resulting a 20 fold increased risk of developing HCC with HCV than people who didn't have HCV (Sun et al., 2003) Hepatitis C virus (HCV) is normally transmitted from mother to child during pregnancy and childbirth although the timing and the precise biological mechanisms that are involved in this process are not utterly understood yet (Utero, 2017) A meta-analysis of case control in USA showed that from 178 HCC patients 163 patients were diagnosed for liver diseases with HCC being the dominant one (Fasani et al., 1999) Many meta analyses and case control studies have shown that patients with both HBV and HCC infections have more risk of getting HCC(Cho et al., 2011)

3.3 Alcohol and tobacco

Most of the HCC cases occur in cirrhotic livers but not all. Cirrhosis is a pathogenic step in liver carcinogenesis which results in hepatocellular carcinoma and in alcoholics, prolonged and excessive alcohol consumption ends up in alcoholic cirrhosis. Moreover, alcohol may intensify and/or speed up hepatocarcinogenesis in patients with HBV and/or HCV infection, genome alterations, or non-alcoholic fatty liver disease (Turati et al., 2018) A meta-analysis carried out in Taiwan showed that risk of HCC was higher in users of alcohol than non-users (Loomba et al., 2010) Women are more prone to liver injury than men from alcohol intake and women are more likely to develop cirrhosis at equivalent alcohol intakes than men mostly because of the sex differences in alcohol metabolism (Frezza et al., 1990) A meta-analysis which consisted of 19 cohorts, including a total of 5550 deaths and 4445 incident cases from liver cancer, noted a significant 16% increased risk of hepatocellular carcinoma among alcohol drinkers of 3 or more drinks per day, compared with non-drinkers. Proof for a positive connection between heavy alcohol drinking and hepatocellular carcinoma derived mainly from embedded case control studies. The increased risk for those who drink 6 or more drinks per day, compared with non-drinkers, was 22%. Moderate drinkers who take less than 3 drinks per day, were not at risk of liver cancer. The dose risk curve provided evidence that a linear relationship with rising alcohol intake in drinkers with approximate potential risk of 46% per 50g of ethanol per day and 66% for 100g per day (Turati et al., 2018) Effects of alcohol use and extreme obesity (BMI, >30) together showed that the risk of HCC was accelerated synergistically in alcohol users who had excessive obesity compared to those without extreme

obesity and nonusers of alcohol (Loomba et al., 2010) Although there are sufficient evidence that prove tobacco smoking increased the risk of hepatocellular carcinoma, not persistent findings in studies of the same populations and the inter relation of smoking with other risk factors, such as HBV, HCV infection, and alcohol consumption, have made the relationship between tobacco and HCC difficult to identify and understand (Omata et al., 2017)

3.4 Aflatoxin

Aflatoxin is a mycotoxin produced by molds of the Aspergillus species which are Aspergillus flavus and Aspergillus parasiticus. These molds contaminates maize, groundnuts and tree nuts in warm, high moisture environments and is a well-known hepatic carcinogen (McGlynn & London, 2011) Aflatoxins contaminate dietary staple foods and are sound animal hepatocarcinogens and are cancerous in humans with exceptional high risks in individuals who have developed a concomitant infection with HBV. Exposure can be reduced at community levels by either before or after the harvest by limiting fungal contamination of crops. Technical approaches may suggest low technology post-harvest measures to terminate fungal growth or genetic engineering of plants to be resistant to fungal infection or toxin production (Wild & Hall, 2000) A meta-analysis carried out in China provided sufficient evidence that Aflatoxin is one of the major carcinogens in HCC by a follow up study of urinary markers of aflatoxin exposure (Qian et al., 1994) There are four major Aflatoxins as B_1, B_2, G_1 and G_2 (McGlynn & London, 2011) and evidence shows that there is a synergistic between aflatoxin B_1 and HCV towards HCC (Kuang et al., 2005)

3.5 Diabetes Mellitus and Obesity

In the past couple of years, many evidence has suggested a strong link between diabetes mellitus (DM) and hepatocellular carcinoma. Many cohort studies and meta-analysis have been carried through out to statistically quantify the association of diabetes mellitus with hepatocellular carcinoma. A meta-analysis which involved 823 patients suggested that DM increased the risk of hepatocellular carcinoma only in the presence of other risk factors such as hepatitis B or C or alcoholic cirrhosis. Hepatitis C infection and alcoholic cirrhosis is known to be responsible for most of HCC among veterans (H B El-Serag, Richardson, & Everhart, 2001) Another quantitative assessment of this association between DM and HCC has provided string evidences that there is a positive relationship between DM and increased risk of HCC in both males and females (C. Wang et al., 2012) Obesity also has been identified as one of the risk factors for several malignancies, including hepatocellular carcinoma (Larsson & Wolk, 2007) A meta-analysis on overweight and obesity in relation to liver cancer risk provided enough evidences that excess body weight can potentially increase the risk of HCC and the results indicated that the risk was 89% higher among people who were overweight and obese, respectively, compared with those of weight considered normal. (Larsson & Wolk, 2007)

3.6 Genetic Factors

The host genetic makeup known to be an important factor but to what extent it effects is still not fully discovered. A very small amount of HCC cases are associated with genetic disorders of mendelian inheritance, such as hereditary hemochromatosis or alpha 1 antitrypsin deficiency. Currently, the most widely studied inherited genetic risk factor in HCC are forms of glutathione S-transferases. They are responsible for detoxifying a variety of electrophilic molecules, including oxidized lipid and DNA compounds generated by reactive oxygen species damage to intracellular molecules (White, Li, Nurgalieva, & El-Serag, 2008) Also tumor necrosis factor α (TNF-α) has been identified to play a significantly important role in the progression and development of hepatocellular carcinoma. A meta-analysis proved that the TNF α polymorphism is closely associated with increased susceptibility to HCC (Q. Hu, Lou, Liu, Qian, & Lv, 2014)

4. Materials and methods

This study includes information from published review articles from 1990 to 2018 and Medline database from 2000 to 2018 to identify cases regarding hepatocellular carcinoma and the clinical trials of the vaccines to understand how management and prevention of hepatocellular carcinoma is done by administering vaccines against the risk factors. This study also includes a full manual search from bibliographies of selected papers to have a better idea how each individual has responded to these vaccines.

4.1 Study criteria

1) Each case study was necessitated to have published information of the number of clinical participants and age-adjusted details. Also it was taken in to consideration that each and every case study possesses 95% confidentiality.
2) This literature includes only the studies that used preventive vaccines for following risk factors of hepatocellular carcinoma: Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV). And following vaccines which play a major role in preventing hepatocellular carcinoma: interferon vaccines, nucleotide analog treatment and immunotherapy.
3) Studies with less than 100 individuals were disbarred as it was not enough to determine positive or negative results.
4) When more than one volume from the same study was accessible only the final publication was utilized.

4.2 Data extraction

Data were individually procured from each study using predetermined forms. The information for this literature was extracted in terms of: the number of cases, mean age of the individuals, the area where the studies were carried out, the number of cases for each category. After collecting all the data and information from each study they were carefully examined and analyzed to determine the actions of these vaccines inside the body

5. Prevention

Cancer prevention can be attempted in three steps (Kew, 2010)

1. Primary prevention is preventing the etiological agent from commencing the carcinogenic process. This is the first strategy and is achieved by eliminating, avoiding, or neutralizing the carcinogenic agent, or by disturbing the in vivo conversion of a precarcinogen into a carcinogen.
2. Secondary prevention is dealing with the metabolism of a carcinogen, or stopping it from reaching its target or interacting with nucleophiles, especially DNA.
3. Tertiary prevention is preventing precarcinogenic lesions from progressing to cancer.

5.1 Prevention of HBV related HCC and clinical trials

HBV is responsible for 80% of the HCC cases worldwide (Parkin, n.d.) The first ever HBV vaccine was developed from human serum in 1981 in the united states (Wong & Chan, 2012) The goal and strategy of universal administering of hepatitis B vaccination is to prevent HBV related HCC to reduce new infection as primary prevention (Omata et al., 2017) HBV vaccination is carried out globally to prevent new HBV infection in healthy individuals. There are more than 250 million people chronically infected with HBV and these chronic bearers are at high risk of developing adverse liver diseases and hepatocellular carcinoma (T.-C. Tseng & Huang, 2017) There are multiple etiologies of HCC, the most critical one is chronic viral hepatitis B which account for 80% to 90% of all HCC cases globally (Xie, Ma, Feng, & Wei, 2017). Despite the accessibility of highly effective vaccines over 20 years, hepatitis B virus (HBV) infection is still the major cause of liver-related morbidity and mortality being hepatocellular carcinoma one of them (Xie et al., 2017). Efficacy trials have proved that antiviral therapy improves the outcomes of patients with chronic hepatitis B virus (HBV) infection regarding hepatocellular carcinoma. However, potential data regarding the effect of antiviral therapy on the incidence of hepatocellular carcinoma (HCC) are limited(Kim et al., 2015). A safe and effective HBV vaccine is available and should be given to all newborns and persons without immunity who are at high risk for infection. National HBV vaccination programs have dramatically reduced the prevalence of HBV infection, and there has been a concomitant decrease in the incidence of hepatocellular carcinoma (Bedossa & Paradis, 2011)

Many clinical trials have been carried out to determine the results of HBV vaccination. In Taiwan the first universal HBV vaccination program in newborns started 20 years ago, with infants of mothers who were at high risk for HBV infection (HBsAg-positive) administering both the injection of hepatitis B immune globulin and the vaccine. Since the program started, the incidence of hepatocellular carcinoma in children between 6 and 14 years of age has drastically fallen by 65 to 75% (Bedossa & Paradis, 2011) A

reduction in the average annual as follow in the incidence of HCC from 0.70 per 100,000 children between 1981 and 1986 to 0.57 and 0.36 for the time periods of 1986 to 1990 and 1990 to 1994 was shown (Chang et al., 1997) A randomized controlled clinical trial was carried out in Qidong China administering HBV vaccine to 75000 infants with the purpose of making them immunized. After an average of about 25 years of follow-up, the incidence ratio of primary liver cancer in the vaccination at birth group to the control group (68% of whom received catch-up vaccinations at ages 10–14 years) was 0.16 (Qu et al., 2014) Another interventional randomized clinical trial was carried out in symptomatic adult men which a total of 549 subjects were collected and treated with highly purified formalin inactivated virus particles derived from the plasma of chronic carriers of hepatitis B. A total of 534 were gathered to the placebo group. Both groups were given vaccinations at 0, 1 month, and 6 months to determine the efficacy of a hepatitis vaccine in preventing hepatitis B. Within two months, 77% of the vaccinated persons had higher levels of antibody against the hepatitis B surface antigen. This rate increased to 96% after the booster dose and remained essentially unchanged for the duration of the trial. For the first 18 months of follow-up, hepatitis B or subclinical infection developed in only 1.4 to 3.4% of the vaccine recipients as compared with 18 to 27% of placebo recipients. The reduction of incidence in the vaccines was as high as 92.3. A significant reduction of incidence was already seen within 75 days after randomization this observation suggests that the vaccine may be efficacious even when given after exposure (Szmuness et al., 1980)

5.2 Prevention of HCV related HCC

The incidence of hepatocellular carcinoma (HCC) due to HCV infection is increasing in several Western countries, being responsible for approximately one-third of HCC cases in the USA (Armstrong et al., 2006) Despite of the long years of experiments still there is no HCV vaccine that can be used clinically (Naderi et al., 2014) Despite the number of research over many years into the development of a vaccine against HCV, there seems to be little likelihood of such a vaccine being available in the near future. Difficulties hindering the development of this vaccine include the excessive variabilities of the genomic structure of the virus, mostly in the hypervariable region, the huge number of quasispecies (which is a group of viruses linked by the similar mutation or mutations, competing against each other within a highly mutagenic environment in the blood of infected people, and the lack of proof for an effective neutralizing antibody against the virus (Kew, 2010) But in order to prevent complications after being infected with Hepatitis C Virus following vaccinations are administered by medical practitioners; Hepatitis A, Hepatitis B, Haemophilus influenza type b, Human papillomavirus vaccine, Influenza, Pneumococcal, Tetanus, diphtheria, whooping cough (“Vaccinations for Adults with Hepatitis C Infection,” n.d.) Although there is no exact vaccine that has been developed to prevent HCV related HCC following vaccines are used as substitutes; recombinant viral vaccine vectors to trigger cell mediated and humoral responses against multiple epitopes. Types of viruses that are used as vectors include adenovirus, vaccinia virus and canarypox (Siler et al., 2002) Recombinant protein subunit vaccines are also used which are subunit vaccines containing recombinant HCV protein molecules. These different

proteins act against different HCV genotypes to prevent the harmful effects by the infection or chronic infection (Naderi et al., 2014) Peptide vaccine is another type of vaccination that is used against HCV, Small peptides are incorporated which they can bind directly to MHC class I or II molecules without affecting the antigen processing pathways (C.-T. Hu, 2005)

5.3 Anti-viral therapy and clinical trials

Antiviral treatment is known to decrease the risk of HCC in patients with viral hepatitis. Considering the extraordinary progress made in antibacterial therapy in the past couple of decades, the advances made in specific treatment for viral disease have been disappointingly low. Until now there are only a very small number of antiviral agents which are utilized in a restricted number of clinical situations. Furthermore, most of the antiviral drugs do not selectively inhibit virus replication without injuring and damaging the host cell simultaneously, and therefore these agents are often accompanied by serious side effects. Thus, potential beneficial effects of antiviral drugs must be balanced against possible immunosuppressive and other unpredictable side effects (Stalder, 1977) A clinical trial which investigated whether perioperative antiviral therapy is guaranteed for resection of HBV infections related hepatocellular carcinoma. Patients with large HBV-related HCC from January 2012 to December 2012, were retroactively allocated to one of two groups based on the fact that they received perioperative antiviral therapy (antiviral group) or did not receive (control group). Patients were consecutively recruited to address potential source of influence. Eliminations from these criteria included: other concurrent hepatitis (e.g. Hepatitis A Virus (HAV), Hepatitis C Virus (HCV), Hepatitis D Virus (HDV), Hepatitis E Virus (HEV), and autoimmune hepatitis) patients with ongoing major surgical procedures, such as bowel resection, bile duct resection etc. The findings of this study suggested that perioperative antiviral treatment enhances patient safety by decreasing morbidity and speeding up the recovery of postoperative liver function for HBV-related major HCC resection (Zhang et al., 2015) Another meta-analysis which was aimed to characterize HCC recurrence patterns after anti-viral therapy suggested that HCC recurrence rates after anti-viral therapy was rather promising and improving (Yopp, Odewole, & Singal, 2018) A clinical trial which was aimed to scrutinize the reactivation hepatitis B virus in primary hepatocellular carcinoma and to determine the effects of trans arterial chemoembolization (TACE) together with antiviral therapy. This prospective study involved 98 patients with HBV related HCC underwent TACE procedures with serial HBV DNA tests. Patients were separated into the antiviral treatment group and the no-antiviral group. The antiviral group was treated entecavir antiviral therapy and the other group received no antiviral therapy. Two groups of patients were compared in levels of HBV reactivation and liver function before and after only 1 session of TACE in average 1 month follow up after operation. The clinical trial concluded that antiviral therapy can minimize the risk of reactivation and help improve liver function after TACE (K. Wang, Jiang, Jia, Zhu, & Ni, 2018)

5.4 Interferon therapy and clinical trials

Interferons are cytokines which possess a variety of biologic properties such as antiviral, immunomodulatory, anti proliferative, and antiangiogenic effects (von Marschall et al., 2003) Interferon is very effective in suppressing the replication of HBV and is the first method approved for the treatment of chronic HBV infection which can ultimately result in HCC. Response to interferon treatment is associated with improved clinical outcome and less cirrhosis-related complications and long-term studies suggested that it may decrease the rate of HCC in patients with HBV-related cirrhosis (Q. Hu et al., 2014) A clinical trial was carried out to explain the influence of long term interferon administration on the level of occurrence of hepatocellular carcinoma (HCC) in patients with hepatitis B virus (HBV) related cirrhosis. 313 patients with HBV related HCC were gathered and 94 of them were treated with interferon for a little over 6 months and the remaining 219 individuals were administered with no interferon or any other antiviral drug. The results showed that interferon therapy for individuals with HBV related cirrhosis remarkably decreased the HCC rate, especially in patients with a bigger amount of serum HBV DNA. If interferon is administered properly for a selected group of individuals, a successful strategy of cancer prevention can be accomplished, even in patients with cirrhosis (Saitoh et al., n.d.) Another randomized controlled trial was conducted to investigate whether the prognosis after hepatic resection could be improved in patients with predominantly hepatitis B related hepatocellular carcinoma (HCC) with adjuvant interferon therapy. Since February 1999, patients with no post-surgical disease after hepatic resection for HCC were randomly allocated and administered with interferon (IFN-I group and IFN-II group) three times weekly for 16 weeks. Enrollment to the IFN II group was discontinued from January 2000 because adverse effects resulted in treatment termination in the first 6 patients. By June 2002, 40 patients each had been assigned into the control group and IFN I group. The baseline clinical, laboratory, and tumor characteristics of both groups were comparable. The results sophisticated that in a group of patients with predominantly hepatitis B related HCC complement interferon therapy showed a trend for survival benefit, primarily in tumors (Lo et al., 2007)A randomized clinical trial from Hong Kong suggested that IFN was associated with better overall survival in HCC patients (von Marschall et al., 2003) Another clinical study provided evidences that IFN inhibit the tumor growth and reduced micro vessel density (Zhuang, Zeng, Yang, & Meng, 2013)

5.5 DNA vaccine and DNA analogues

DNA based immunization is one of the latest version of vaccination methods and now is on the rise. Nucleotide analogs may be potent in minimizing the risk of recurrent HBV related HCC after curative treatment (Omata et al., 2017) Administration of massively expressed HCV core gene, as one huge dose or repeated injections of lesser doses, may suppress core specific immune response. Then, the latter is induced by a heterologous DNA prime or protein boost particle that eludes the negative effects of intracellular core expression (Alekseeva et al., 2009) Given the structural proteins of HCV-like particles (HCV-LPs) are presented in a native, virion

like conformation, the HCV LP is superior in eliciting a protective immune response compared with the recombinant subunit-based vaccine (Naderi et al., 2014) A DNA vaccine consisting AFP and HSP70 could generate antitumor immunity. Such effective AFP specific T cells response and explicit antitumor effects on AFP producing tumors induce clinical testing of this approach as a therapeutic vaccine for HCC (Rinaldi, Iurescia, Fioretti, Ponzetto, & Carloni, 2009) Alpha-fetoprotein (AFP) is generated principally in fetal liver, gastrointestinal tract and the yolk sac which temporarily exists during embryonic development. AFP is overexpressed in almost all of hepatocellular carcinoma (HCC) cases and thus offers an appealing target for immunotherapy against this neoplasm. Anti HCC effects were created in a therapeutic setting with a DNA vaccine encoding mouse AFP and co-expressing heat shock protein 70 (HSP70) gene. The vaccine prompted a marked and massively effectual AFP specific CTL reaction against AFP positive target cells. This vaccine also induced the elongation of life span in mice bearing the tumor and the HCC eradication. It is forecasted that vaccine strategies such as this may largely contribute to the future treatment of hepatocellular carcinoma effectively in clinical practice (Lan et al., 2007)

6. Overview of HCC vaccination strategies

The main strategy of vaccination in HBV related HCC is to make individuals immunized. Especially in newborns whose mothers carry the HBV infection are at high risk of getting the virus. Administrating the HBV vaccine at birth trigger the acquired immunity passively and make the individuals fight against the virus if they encounter, preventing them from getting the virus and ultimately lessening the chances of acquiring HBV related HCC. Although there is no exact vaccine against HCC, many other vaccines are administered to minimize the side effects and avoid complications. Interferon therapy is mainly used to boost the immune system and potentially decrease the mutagenic environment inside the body and the effectiveness of interferon along with anti-viral therapy have showed promising results through many clinical trials.

Specific response for HCC was activated by strategies using tumor associated self-antigens. Gene array and proteomics studies have joined to the list of HCC specific gene outcomes that can be aimed (Vaccines, Bioscience, & Surgery, 2014) A DNA vaccine encoding AFP and HSP70 could trigger anti-tumor immunity. Very recently, the scientist Lan and his colleagues found out anti HCC effects in a therapeutic environment with a DNA vaccine encoding mouse AFP plus HSP70 genes. Therefore this vaccination strategy may contribute to successful treatments for human HCC in near future (Lan et al., 2007) Molecular chaperones, as for an example, heat shock protein 70 combined with other antigens can strongly elicit immunogenicity presumably through improved processing and presenting of antigens to their APCs (Lan et al., 2007) DNA vaccines act to an especially promising methodology against allergens, pathogens and cancer. Indeed, DNA based immunization induces very strong and powerful cellular and humoral immune response

against a variety of antigens, including tumor derived ones such as melanoma, breast cancer, ovarian carcinoma, prostate carcinoma, neuroblastoma, small cell lung cancer, etc. Gene based vaccines were evaluated either as preventative or therapeutic treatment for infectious illnesses, allergies and cancer including HCC (Ulmer, Wahren, & Liu, 2006) Uncharacterized, non-specific and mutated antigens also can be targeted with complete tumor cell or tumor lysate based immunization strategies, as well as using vectors carrying genes making tumor cells immunogenic. The immune system in these instances shall develop specificity against these new immunogenic target antigens (Rinaldi et al., 2009)

7. Future of HCC preventive vaccines

There is a continuing need for innovative, alternative therapies for hepatocellular carcinoma (HCC). Molecular targeted vaccines and Immunotherapy of cancer is attractive because of the exquisite specificity of the immune responses.

7.1 Molecular targeted vaccines

A number of molecular targeted agents have been methodically investigated including angiogenesis inhibitors such as ramucirumab and mTOR inhibitors such as everolimus. All of these molecular targeted vaccine trials were conducted in biomarker unselected HCC patients. However most of the ongoing trials have not shown survival benefits, many of the results are yet to be revealed.

7.2 Immunotherapy

The concept of immunotherapy is the systemic and specific elimination of tumor, based on the expression of certain proteins by the tumor. To date, few different strategies of immune activation studies have demonstrated that biological activity in HCC individuals and a subset of those have demonstrated clinical efficacy. The published data provides evidence that adoptive transfer of activated effector cells and complex tumor derived vaccines can make an impact on the recurrence and survival of HCC subjects. Novel vaccines and combinations of vaccines with many more standard therapies are just initiating and hold a promising future for improvement in impact of immunotherapy for HCC (Vaccines et al., 2014)

8. Conclusion

HCC is a very antagonistic cancer which affects majority of the people around the world. While Hepatitis B and Hepatitis C virus being the major two risk factors, chronic liver disease and cirrhosis also play a major role in hepatocellular carcinoma. HCC, of all liver cancers can be, prevented by appropriate methods such as HBV vaccination, anti-viral therapies, immunotherapies and advanced molecular methods. Universal screening and public education on alcoholism and intravenous drug using can also aid in preventing HCC.

Significant improvement in vaccines that are administered to prevent HCC, is shown throughout the time. DNA vaccines and immunotherapy are the main two fields scientists are working on in preventing vaccines and the future seems brighter. A vaccine against hepatitis C virus also can be expected within the next couple of years thanks to the major findings advances in clinical research. But more studies and research should be conducted in understanding how bio markers contribute to these instances. In order to prevent tumor regression and to modify the vaccines, studies should be carried out nonetheless.

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