

## A Comparative Study on the Prevalence of HCC Seromarkers among Tribal and Coastal Population

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**Abstract:** An extensive study on the prevalence of seromarkers (HBsAg and AFP) of hepatocellular carcinoma among two low socio-economic groups of Karumanthurai tribes and Kanyakumari coastal populations were carried out. The results obtained suggest that the mean HBsAg value of tribal was more than that of the coastal population. The mean AFP of elevated and normal cases was found to be invariably significant ( $P < 0.01$ ) over the two areas. Coastal patients tended to be significantly older than the tribal patients ( $P < 0.005$ , Wilcoxon rank sum test). All two racial/geographic regions showed a preponderance of male patients but this tendency was significantly more marked ( $P < 0.05$ ) in the tribal group than in the coastal populations. Liquor habit and lack of personal hygienic practices were associated with the prevalence of HBsAg and AFP in both the areas. Surprisingly, a positive association has been found between the consumption of marine foods and reduced risk of HCC. Thus, the reduced risk of HCC among the coastal populations probably the development of immunity against HBV due to the dietary pattern of marine food consumption by coastal population.

**Key words:** Hepatocellular carcinoma · HbsAg · AFP · HBV

### INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common internal malignancies worldwide causing approximately half a million deaths annually. Its high fatality rates results in almost equal incidence and mortality rates. In some countries of high incidence, HCC is the leading form of cancer and overall it rates as the seventh most common malignancy in males and the ninth in females [1-4]. South-east Asian countries (Taiwan, Korea, Thailand, Hong Kong, Singapore, Malaysia, southern China) and tropical Africa show the highest incidence, with 10-20 percent in 100 000 population. The lowest rates of 1-3 percent in 100 000 population for HCC have been reported in western countries including Australia, South America and India [5]. The epidemiology of HCC is characterized by marked demographic (age, gender, race/ethnicity) and geographic variations. The major risk factors for HCC are chronic infection followed by aflatoxins and alcoholic cirrhosis. There occurs an association between HBV and HCC which has

been demonstrated by the presence of HBsAg in more than 50% of patients with HCC and by the integration of HBV DNA transformation [6]. Hepatitis B virus infection, with and without aflatoxin exposure, is responsible for most cases in developing countries; better control of these risk factors has resulted in a recent decline in HCC in some places like Taiwan and China. In India, the epidemiology of HCC is quite enigmatic and also 2 to 11% of populations are HBsAg carriers. The risk of HBV infection is enhanced through contacts with HBV infected communities, sexual promiscuity, blood transfusion and low socio-economic status [7]. Most HCC occurs in patients with liver cirrhosis caused by chronic HBV infection, hepatitis C virus infection or alcohol [8]. In addition contamination of food items, grains and oilseeds with aflatoxins is widespread in India. Paradoxically the incidence of HCC has been low in all population based registries and the data from hospital registries and autopsy studies from India. In contrast, Indian immigrants to USA and mortality rates of HCC among Indians in Canada are similar to the rates reported in the native

Caucasian population [8-10]. In India, particularly in Tamil Nadu the HBV seroprevalance among tribal population have been reported by Rajendran *et al.* [11]. In this present study an attempt was made to study the prevalence of HBsAg and associated HCC among two low socio-economic group including tribes and coastal population of Karumanthurai and Kanyakumarai respectively.

## MATERIALS AND METHODS

### Collection of Samples

**Area of Sample Collection:** The study area, the Karumanthurai hills is situated in Salem district of TamilNadu, 54 kilometers away from Attur. It has a maximum elevation of 1000 feet above the mean sea level. The people living in this area are called as 'Malayalis' and coming under 'scheduled tribes' with their occupation as agriculture. The people have the custom of close community marriage, socio-economically backward and have poor healthcare awareness facilities. The second area is Kanyakumari, a south coastal area of Tamilnadu. Most of the people come under fishermen community. They are socio-economically backward with less health care awareness.

**Blood Sample Collection:** In Karumanthurai and Kanyakumari, a medical camp was organized and conducted by District Siddha Medical Department, Salem and Department of Microbiology, Vivekanandha College of Arts and Science for Women. Blood samples were collected aseptically from the subjects, those who had been examined and instructed through physicians after obtaining the case history through standard questionnaire. Serum samples were separated by centrifugation and transported in dry ice to the laboratory for further processing.

**Detection of Hepatitis B surface antigen (HBsAg) in Human Serum by-EIA:** The kit (PATHOZYME-HBsAg) used for the detection of HBsAg was manufactured by OMEGA DIAGNOSTICS (UK). All the kit components and the test sera were kept at room temperature prior to start the assay. The diagnosis was carried out according to the manufacturer's protocol. Briefly 50 $\mu$ l of test samples both negative and positive control serum were dispensed into the appropriate wells. 50 $\mu$ l of conjugate was dispensed into each well. The plate was gently shaken for 30s and incubated at 37 $^{\circ}$  C for 60 min. At the end of the incubation period the plate was washed five

times with the diluted wash buffer and excess fluid was removed. 50 $\mu$ l of TMB substrate was dispensed into each well and gently shaken for 5 seconds. The plate was kept at room temperature in the dark for 30 min to develop color reaction. 100 $\mu$ l of stop solution was added into each well and the color change was noted. The change from blue to yellow in wells containing enzyme indicates the presence of HBsAg. The absorbance of each well was measured at 450nm using ELISA reader (Labnics, USA).

**Detection of alpha-fetoprotein (AFP)-ELISA:** AFP is the most useful marker for the diagnosis and management of hepatocellular carcinoma. MONOBIND (USA) kit was used for the quantitative determination of AFP. The diagnosis was carried out according to the manufacturer's protocol. Briefly 25 $\mu$ l samples were added to a streptavidin coated well, followed by 100 $\mu$ l of biotinylated monoclonal and enzyme labeled antibodies. The contents mixed by swirling the micro plate gently for 20-30 seconds and incubated at room temperature for 60 min. The wells were washed three times by adding 300 $\mu$ l of washing buffer followed by 100 $\mu$ l of working substrate solution was added to each well and incubated at room temperature for 15 min. 50 $\mu$ l of stop solution was added to each well and mixed gently for 15-20 seconds. The color intensity was measured at 450nm and also 630nm as reference wavelength in a micro plate reader. It has been approximated that 97-98% of the normal healthy population has AFP levels less than 8.5ng/ml. Therefore, AFP levels of <10ng/ml was taken as normal and >10ng/ml as elevated.

## RESULTS AND DISCUSSION

**HBV Infection and AFP Levels:** HBV (hepatitis-B virus) appears to be a most important factor in the etiology of primary hepatocellular carcinoma in various parts of the world. The AFP level at a cut value of 30ng/ml was more useful in detecting non-viral HCC. Therefore, considering the usefulness of AFP for non-viral etiology and HBsAg for viral etiology, the diagnosis for HBsAg and AFP were carried out in both the population.

Table 1 presents the mean and standard error of HBsAg positive and negative cases over the coastal and tribal area. The assessment of statistical significance by independent t-test revealed a significant gradation in mean HBsAg between the positive and negative cases. The mean HBsAg of positive and negative cases was found to be invariably significant ( $P < 0.01$ ) over the two areas. It was also observed that the mean HBsAg value of

Table 1: The mean values of positive and negative cases of HBsAg and AFP among coastal and tribal population

Population	HbsAg		AFP	
	Positive	Negative	Elevated	Normal
Coastal	1.191*±0.389	0.0387±0.002	15.30*±5.00	0.98±0.11
Tribal	1.187*±0.278	0.050±0.004	20.55*±5.23	3.39±0.28

\* Significant at 0.01 level

tribal is 0.05, which is more than the mean HBsAg value 0.0387 of coastal area. Eighty percent of cases of HCC worldwide are estimated to be etiologically associated with HBV infection [12] and the incidence of HCC parallels carrier rates of HBV infection.

Table 1 presents the mean and standard error of AFP values of elevated and normal over the coastal and tribal area. The assessment of statistical significance by independent t-test revealed a significant gradation in mean AFP between the elevated and normal cases. The mean AFP of elevated and normal cases was found to be invariably significant ( $P < 0.01$ ) over the two areas.

At present no data are available regarding the burden of HCC in tribal and coastal population. This is a pioneer study regarding the relationship between the two populations. The highest elevation of HCC seromarkers have been observed in tribals than in coastal population. Blumberg and London [13] stated that HCC occurs commonly in the same regions where chronic HBV carriers are prevalent. Thus the Karumanthurai tribes are HBV carriers and more prone to HCC than the coastal population. In India the HBsAg carrier rates are Arunachal Pradesh (5.5%-8.4%), Madhyapradesh (6.8%-11.8%), Nicobarese tribes (23.3%), shompens (37.8%), onges (31%) and Andamanese (3.7%). In particular on consideration of Tamilnadu only two tribal populations (Nilgris and Kolli hills) have been studied extensively for HBsAg carrier rates. In Nilgris tribal population, kurumpas group showed highest positivity (24.5%) and other groups such as Totas and Irulas showed 3.4 and 10.3% respectively. But the HBsAg prevalence was very low in Kolli hills (1.86%) [14]. From this it can be inferred that Karumanthurai population is the second most prevalent tribal population in Tamilnadu.

**Factors in the Etiology of HCC:** Since a difference in the occurrence of HCC among tribal and coastal population was observed some etiological factors were considered in order to study the risk factors that makes the difference between the HCC incidence rates.

**Age:** In 200 cases the age of HBsAg positive patient was recorded. In both the population, most of the patients were between 45 and 75 years. Coastal patients tended to be significantly older than the tribal patients ( $P < 0.005$ , Wilcoxon rank sum test). The median ages of the two groups were 70 and 50 years respectively. HCC may occur from as early as 2 years of age in areas of high incidence [15]. Since the incidence of HCC in India is at the lowest rate [5] the appearance of HCC at such an early age was not observed. In general, the incidence increases with age in all populations and shows a slight decrease in the elderly. The age peak in a given region tends to be inversely related to the frequency of the tumour, i.e. the age peak is in younger patients in areas of high incidence and in elderly patients in areas of low incidence. Thus, a relation between the age and incidence of HCC among tribal population was observed indicating high incidence and predominance among tribal population compared to coastal population.

**Sex:** All two racial/geographic regions showed a preponderance of male patients but this tendency was significantly more marked ( $P < 0.05$ ) in the tribal group than in the coastal sample. Of the patients with elevated AFP levels (58%) were men and (42%) were women. It has been estimated that men are two to three times more often affected by HCC than women. Overall, the distribution of HCC between men and women varies between ethnic groups and geographic regions. In most reports, the ratio of men to women is more pronounced in high incidence areas. But in the present study the same predominance ratio has been observed and indicating more prevalence among male when compared to that of females. Supportively McGlynn *et al.* [16] indicated the increased prevalence of HCC among males. This can be due to men with HCV are at higher risk (2:1 to 9:1) for the development of HCC than are women [17,18].

**Other Etiological Factors:** Liquor habit and lack of personal hygienic practices were associated with the prevalence of HbsAg and AFP in both the areas.

Alcoholics showed highest HBsAg prevalence in both coastal (33.3%) and tribal (11.1%) population. AFP elevation is also correlated with alcoholism, 50% and 30% cases of AFP elevation was noted in alcoholic fisheries and tribes respectively. There is no evidence of alcoholism causing HCC as it leads to micronodular cirrhosis in which there is a slight increased risk of HCC. Often patients with alcoholic liver disease have higher sero prevalence of HCV, but not of HBV disease. Patients with chronic hepatitis C drinking 60 gm of ethanol or more daily for five years have higher titers of serum HCV RNA and lower levels of serum neopterin, a marker of activation of cell-mediated immunity, linking impaired cellular immunity in chronic alcoholics to severity of viral infection. In contrast, the risk of HCC following HBV related macronodular cirrhosis is very high. This can be explained from the results of Brechot *et al.* [19] where the HBV DNA sequences were usually integrated into the host genome of the tumor, even in cases in which serum HBV marker were absent.

Among the HBsAg positive cases, poor hygienic practices play a role in both the populations. 13.3% in coastal and 18.5% in tribes. Oral ingestion has been documented as a potential route of exposure of non-percutaneous modes of HBV transmission.

**Social and Clinical Factors:** Irrespective of the area, the prevalence of HBsAg and AFP was more among the married than unmarried. Moreover sexually transmitted disease symptoms such as sudden onset of fever, weight loss, continuous diarrhea, swelling on back and armpits, wound in and around mouth and white patches over the body were high in tribes than fisheries. The high rate of sexual transmission among tribal can be correlated to their poor civic conditions. Risk habits of tattooing is high (23.8%) in tribal, unlike in coastal. As tattooing is very popular among tribals, the use of contaminated needles without proper sterilization could be a source of infection. The proportion of the positive cases of HBsAg and AFP among the subjects undergone previous surgery, previous blood transfusion and pregnancy were not significantly associated with the onset or the progress of HCC.

**Food Habits:** The food habit of the two study area was compared. Traditional habits of marine food intake by coastal was found to be predominant than that compared with tribals. Even within the two populations there was a difference between the prevalence of HCC among the consumers and non-consumers of marine food. It has

been observed that people taking marine foods are less prone to HCC. But with respect to liver cancer, several studies have reported an inverse association between food consumption and risk of hepatocellular carcinoma (HCC) [20-24], although other studies, two of which from Greece, did not support this association [25-27]. Surprisingly, an association has been found between the consumption of marine foods and reduced the risk of HCC. Thus, a semi-quantitative marine food frequency intake over a control population has to be carried out in order to study the dietary effect on HCC.

## CONCLUSION

From the present study it can be emphasized that the probable risk factors of HCC among the two study population includes sexual practices, poor sanitary conditions, tattooing and lack of proper awareness. On considering the risk factors among the two low socio-economic groups, both are in the same verge of developing the disease but the reduced risk of HCC among coastal population probably the development of immunity HBV due to their dietary pattern of marine food consumption.

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