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DIABETES AND OBESITY AS RISK FACTORS FOR THE DEVELOPMENT OF HEPATOCELLULAR CARCINOMA IN THE HISPANIC POPULATION

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DIABETES AND OBESITY AS RISK FACTORS FOR THE DEVELOPMENT OF HEPATOCELLULAR CARCINOMA IN THE HISPANIC POPULATION

by

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Dissertation

Presented to the Faculty of the Graduate School of

The University of Texas Medical Branch

in Partial Fulfillment

of the Requirements

for the Degree of

Masters of Science, Clinical Sciences

The University of Texas Medical Branch

November 2008

Dedication

I dedicate this work to my wife Anuja who has endured and tolerated the countless hours I have spent gazing into a computer monitor during my fellowship and graduate school.

Acknowledgements

I would like to acknowledge my mentor Dr. Gagan Sood and my supervisors Dr. Don Powell and Dr. Karen Szauter for their valuable comments and advices that have been incorporated into this study. I want convey my thanks to Dr. Grady and Ms. Han for assistance with statistical analysis. This study would not have been possible without the help of Suneal Agarwal M. D. who assisted me in every aspect of this project.

DIABETES AND OBESITY AS RISK FACTORS FOR THE DEVELOPMENT OF HEPATOCELLULAR CARCINOMA IN THE HISPANIC POPULATION

Publication No._____

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The University of Texas Medical Branch, 2008

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Hepatocellular carcinoma is the fifth most prevalent cancer in the world with an overall 5-year survival rate of 6.9%. The well known etiological factors for HCC are infection with hepatitis B virus (HBV) and hepatitis C virus (HCV), alcohol abuse and environmental exposure to aflatoxins. The prevalence and mortality from HCC is rising in the U.S. In the U.S, Hispanics have a 2.7 times higher risk for HCC development than non-Hispanic whites and the highest mortality rate next to Asian population. The reason for this increased HCC risk in Hispanics is unknown. While it is conceivable that this disparity could be secondary to the incidence of hepatitis or alcohol abuse in the Hispanic population, the primary objective of this study was to evaluate other non-conventional risk factors in Hispanics such as diabetes and obesity. Diabetes and obesity are widespread health problems in the Hispanic population. Diabetes and obesity are known

to predispose to the development of fatty liver disease resulting in Non Alcoholic Steatohepatitis (NASH) and cirrhosis. Primary liver cancer is about 4 times more likely in diabetic patients than non diabetics. Increasing BMI has also been shown to increase cancer risk and HCC risk. There is recent evidence suggesting diabetes and obesity not only accelerate the development of fibrosis in patients with chronic hepatitis, but also promote the molecular carcinogenesis of HCC.

My hypothesis is that, after controlling for traditionally recognized risk factors, an increased incidence of diabetes and obesity in the Hispanic population plays a causative role in the development of HCC. Using local inpatient and outpatient hospital data, a retrospective case control study was conducted with Hispanic patients afflicted with HCC as cases and patients with cirrhosis grouped as controls. A total of 63 cases and 98 controls were identified. The mean age of controls 52.28 and cases was 57.34. The mean BMI was 29.66 for controls and 28.78 for cases. 20 patients with HCC and 33 patients in the control group had been diagnosed with diabetes.

Univariate analysis did not show an increase in the odds of HCC development in patients with diabetes or obesity. Multivariate logistic regression analysis was then performed to control for various confounding factors. The adjusted odds ratio for diabetes as predictor for HCC development was 0.74 with CI (0.34-1.61) and was 0.80 for obesity with CI (0.35- 1.77). Neither diabetes nor obesity was a statistically significant factor in predicting the development of HCC in the Hispanic population. The results are subject to usual limitations of a retrospective study. Large prospective cohort studies are required to accurately determine the effect of diabetes and obesity on HCC risk in the Hispanic population.

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Chapter 1: Abstract

Hepatocellular carcinoma is the fifth most prevalent cancer in the world with an overall 5-year survival rate of 6.9%. The well known etiological factors for HCC are infection with hepatitis B virus (HBV) and hepatitis C virus (HCV), alcohol abuse and environmental exposure to aflatoxins. The prevalence and mortality from HCC is rising in the U.S. In the U.S, Hispanics have a 2.7 times higher risk for HCC development than non-Hispanic whites and the highest mortality rate next to Asian population. The reason for this increased HCC risk in Hispanics is unknown. While it is conceivable that this disparity could be secondary to the incidence of hepatitis or alcohol abuse in the Hispanic population, the primary objective of this study was to evaluate other non-conventional risk factors in Hispanics such as diabetes and obesity. Diabetes and obesity are widespread health problems in the Hispanic population. Diabetes and obesity are known to predispose to the development of fatty liver disease resulting in Non Alcoholic Steatohepatitis (NASH) and cirrhosis. Primary liver cancer is about 4 times more likely in diabetic patients than non diabetics. Increasing BMI has also been shown to increase cancer risk and HCC risk. There is recent evidence suggesting diabetes and obesity not only accelerate the development of fibrosis in patients with chronic hepatitis, but also promote the molecular carcinogenesis of HCC.

My hypothesis is that, after controlling for traditionally recognized risk factors, an increased incidence of diabetes and obesity in the Hispanic population plays a causative role in the development of HCC. Using local inpatient and outpatient hospital data, a retrospective case control study was conducted with Hispanic patients afflicted with HCC as cases and patients with cirrhosis grouped as controls. A total of 63 cases and 98

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Chapter 2: Background and Significance

HCC Epidemiology and Global Burden

Hepatocellular carcinoma is the fifth leading cancer accounts for about 6% of all cancers worldwide(Parkin, Bray et al. 2001). The reported worldwide incidence of HCC is 1 million cases per year.(WHO 1983) HCC affects more than 500,000 people annually with a five year mortality rate exceeding 95%. The mortality rate from HCC is equal to its incidence rate which makes HCC the third highest cause of cancer death in the world(Parkin 2001). More than 50% of HCC worldwide is due to chronic hepatitis B and 25% due to chronic HCV infection. The distribution of HCC varies worldwide due to the predominant etiological factors unique to different regions. Eighty percent (80%) of the patients with HCC are in the sub-Saharan Africa and parts of the Far East such as China, Taiwan, Korea and Vietnam due to high incidence of HBV infection, except in Japan where HCV infection is more prevalent. HBV infection still constitutes the most important risk factor for HCC and infection rates correlate closely with HCC incidence rates all over the world. Infection with hepatitis B virus (HBV), hepatitis C virus (HCV), cirrhosis due to alcohol abuse or environmental toxin exposures are considered major risk factors for HCC(Sherman 2004). Environmental factors include exposure to aflatoxin and polycyclic aromatic hydrocarbons which have also been shown to have a definitive role in the HCC development (Li-Yu Wang 1996; Wu, Wang et al. 2007). The global trend in HCC is however undergoing a major shift. The incidence of HCC is rapidly decreasing in the Far East due to universal vaccination against HBV, however, the incidence in the

western world and Japan is increasing due to the surge in HCV prevalence along with other risk factors such as obesity and alcohol abuse.

Race:

HCC incidence rates are not uniformly distributed among various ethnic groups living in the same geographic region. In a population based study from Singapore, the age-adjusted rates for HCC ranged from 21.21/100,000 in the Chinese males to 7.8/100,000 among ethnic Indian males living in the same country(Parkin 2002). In the U.S, the incidence and the mortality rates for HCC are 2-3 times higher in African Americans than in Caucasians. African Americans are younger in age at presentation, and are more likely to have regional and distant metastasis at presentation. (El-Serag and Mason 1999; El-Serag, Davila et al. 2003) African Americans are also less likely to undergo resection and have an overall poorer survival when compared to Caucasians.(Sloane, Chen et al. 2006). In most administrative databases, Hispanics were reported as whites until recently. This has made health epidemiological data for Hispanics very scarce. A study from Florida from 1985 -95 reported an average annual incidence rates for Hispanics to be on par with African Americans which is approximately twice the rate in the white population(Shea, Fleming et al. 2001). A recent study based on the SEER database shows the age-adjusted incidence rates in Hispanics to be 6.3 per 100,000 person-years while for African Americans, it was 5.0 per 100,000 population. However, the highest incidence rates were found in the Asians/Pacific Islanders (10.8 per 100,000 person years) and the lowest rates were found in the non-Hispanic white population (2.4 per 100,000 person-years).(El-Serag, Lau et al. 2007)

Sex:

Throughout the world, males have higher HCC rates than females. The proportion of male to female HCC cases ranges between 2:1 in the African subcontinent and South America to about 4:1 in Europe and Japan. The increased HCC rates in men are probably related to higher rates of infection with HCV and HBV, increased alcohol consumption, cigarette use and higher iron stores. Higher BMI and higher androgen levels have also been proposed as contributing factors to HCC development in men. Studies from Taiwan have shown an increased incidence of HCC in HBV infected males with higher testosterone levels.(Yu and Chen 1993; Yu, Yang et al. 2001)

Age

The age distribution of HCC in different parts of the world is related to the region, incidence rate, sex and etiology.(Parkin 2002) HCC is very uncommon below the age of 40 except in populations where HBV infection is hyperendemic as in some parts of China. The incidence of HCC slowly increases with increasing age. In high incidence countries such as China, Vietnam and Japan, the mean age of HCC diagnosis is about 10-20 years earlier than in low incidence countries such as those in Europe and in the U.S.(El-Serag 2001) Throughout the world, the peak age of HCC occurrence in females is approximately 5 years older than for corresponding rates in males. In the western countries and in Asia, the age specific rates are highest in people aged 75 and older. In Africa, however, the HCC rates peak between 60-70 years and then declines, probably due to early age of infection and the dominance of HBV infection. Recently, there has been a trend towards HCC occurrence at younger ages in the U.S, corresponding to increasing rates of diagnosis of HCC.

HCC RISK FACTORS

Viral hepatitis

HBV infection with an estimated 300 million people infected worldwide is the most important cause of HCC in the world. There is a very strong correlation between incidence of HCC and incidence of HBV infection in a population. Patients with chronic HBV infection are at about 20 fold increased risk of developing HCC when compared to the general population(Parkin 2001). Though HBV commonly induces chronic hepatitis and cirrhosis leading to the development of HCC, HCC can develop in HBV infected patients in the absence of cirrhosis due to the carcinogenic nature of the HBV virus. The increased risk of HCC is especially evident in populations where chronic HBV infection is prevalent due to maternal-fetal transmission (vertical transmission). Such a transmission results in a chronic course of the disease in about 90% of the patients. In comparison, the other modes of transmission such as sexual or blood borne modes about 90% of infections result in spontaneous clearance (El-Serag 2002). Of all the patients with HCC due to HBV infection about 70-80% of patients have cirrhosis as a result of chronic HBV infection. A study from Taiwan determined increased severity of liver damage in HBV infection with genotype C than the other genotypes.(Kao, Chen et al. 2002) The risk of HCC is also substantially lower in patients who develop immunity to HBV either after infection or after vaccination. Interferon treatment for HBV infection has also been suggested to reduce HCC risk than in untreated patients with HBV. A meta analysis that included 12 studies identified a lower incidence of HCC in treated patients which was however not statistically significant.(Camma, Giunta et al. 2001)

The association between HCC and HCV infection has been proven in numerous studies(Di Bisceglie 1997). The modes of HCV transmission are predominantly through parenteral routes such as sharing needles, tattoos, blood transfusion and possibly sexual activity. Eighty percent (80%) of HCV infection results in chronic hepatitis which leads to cirrhosis if not treated. Hepatocellular cancer develops in the background of cirrhosis and is thought to be due to fibrosis and hepatocyte transformation. Among patients infected with HCV, risk factors for HCC development are older age at HCV infection, male sex, high BMI, diabetes, heavy alcohol use and simultaneous infection with either HBV or Human Immunodeficiency Virus (HIV).(Di Bisceglie 1997; Poynard, Bedossa et al. 1997; Colombo 1999) A meta-analysis of 32 studies showed a 17 fold increased risk of development of HCC in patients with HCV infection and a 23 fold increased risk for HCC development with HBV infection than uninfected population. The same study also showed that concomitant infection with hepatitis B and hepatitis C had a synergistic effect and can increase the odds of development of HCC 167 times when compared to the unexposed groups.(Francesco Donato 1998)

Alcohol abuse

Alcohol abuse resulting in cirrhosis is a well known risk factor for HCC occurrence. There is no evidence to show a direct carcinogenic effect of alcohol in inducing HCC. Heavy alcohol use defined as 50-70 grams/day for men and about 20-30 grams/day for prolonged periods is a well established risk factor for development of cirrhosis. Alcohol has been shown to increase HCC risk in a linear fashion when the daily intake was more than 60 g. A synergistic effect of alcohol use in the presence of HCV infection has also been demonstrated. When compared to alcohol use alone, concomitant

HCV infection increased the risk of HCC two fold suggesting a synergistic effect.(Donato, Tagger et al. 2002)

Toxin exposure

Aflatoxins are common dietary mold contaminants produced by the fungus *aspergillus fumigatus*. These fungal toxins commonly contaminate maize, groundnuts and other crops. Aflatoxins exposure is reported to be higher in the tropical areas of the world and is also nations with low or middle income countries. Multiple studies have proved beyond doubt that aflatoxins have an etiological role in hepatocarcinogenesis.(Ross, Yuan et al. 1992; Zhang, Rossner et al. 2006) Once ingested, aflatoxins are metabolized to an active intermediate, AFB_1 -*exo*-8, 9-epoxide, which can bind to DNA and cause damage, including producing a characteristic mutation in the p53 tumor-suppressor gene (p53 249^{ser}). These genetic alterations are accentuated in the presence of previous genetic damage caused by cirrhosis and viral hepatitis. A recent cohort study from Taiwan demonstrated the risk of development of HCC to increase from 7-60 fold with concomitant exposure to aflatoxins in the diet, also suggestive of two independent mechanisms (Li-Yu Wang 1996)

Hemochromatosis

Hepatic iron overload has been shown both in experimental and clinical studies to increase the risk of HCC even in the absence of Hereditary Hemochromatosis (HH)(Turlin, Juguet et al. 1995; Deugnier, Turlin et al. 1998). Patients with cirrhosis due to HH have a 45% reported incidence of HCC development. (Schafer and Sorrell 1999) A Swedish population-based study indicated a 1.7 fold increase in HCC incidence among individuals with HH who were homozygous for C282Y mutation. Two studies evaluated

the incidence of HFE heterozygosity in patients with HCC reported a 3 and 5% prevalence in two small case series.(Blanc, De Ledinghen et al. 2000; Willis, Bardsley et al. 2005) In case- control studies, HFE heterozygozity was identified more commonly in patients with HCC than controls. The incidence of H63D mutations was not different between HCC cases and controls. Whether the coexistence of viral hepatitis increased HCC risk in patients with HFE mutations is largely unknown.

Diabetes

Numerous studies have shown an association between increased cancer risks in diabetes. Several case-control studies from the U.S, Italy, Taiwan and Japan have studied the association between diabetes and HCC. While the majority of the studies have shown a positive association, at least a few studies showed a weak association or negative association. These studies are however limited by a potential bias caused by lack of temporal association between HCC and diabetes, as cirrhosis can cause impaired glucose tolerance resulting in the diagnosis of diabetes. Data from cohort studies are not subject to this bias and have predominantly shown a positive association between HCC risk and diabetes.(Adami, Chow et al. 1996; El-Serag, Tran et al. 2004) A recent meta-analysis of 26 studies evaluating the association between diabetes and HCC. Twenty-two (22) out of the 26 studies revealed a positive association between diabetes and HCC. The meta analysis also identified a 2.5 times increased risk of HCC development in patients with diabetes when compared to non diabetics, however the results could have suffered some confounding effects due to obesity and diet. (El-Serag, Hampel et al. 2006). Diabetes is a risk factor for the development of Non Alcoholic Fatty Liver Disease (NAFLD) which

leads to a more severe form of fatty liver disease- Non Alcoholic Steatohepatitis (NASH). Diabetes and obesity are known risk factors for fat deposition in the liver cells leading to non-alcoholic fatty liver disease (NAFLD). NAFLD can lead to chronic inflammation and development of fibrosis and cirrhosis, which is a well known risk factor for the development of HCC. (Figure 1) Diabetes has been identified as an independent risk factor for both disease progression in NAFLD and increased mortality rate. (Adams, Lymp et al. 2005; Adams, Sanderson et al. 2005) Both NAFLD and NASH lead to HCC in about 5% of the patients. Diabetes is a known risk factor for the development of cirrhosis in patients with NASH. Obesity is associated with insulin resistance and is an independent risk factor for the development of NASH. In a prospective study of 900,000 U.S adults who were followed for 16 years, the risk of HCC development was found to be 4.5 times in obese subjects compared to the general population. The increased in HCC risk was also the highest among all other cancers.(Calle, Rodriguez et al. 2003)

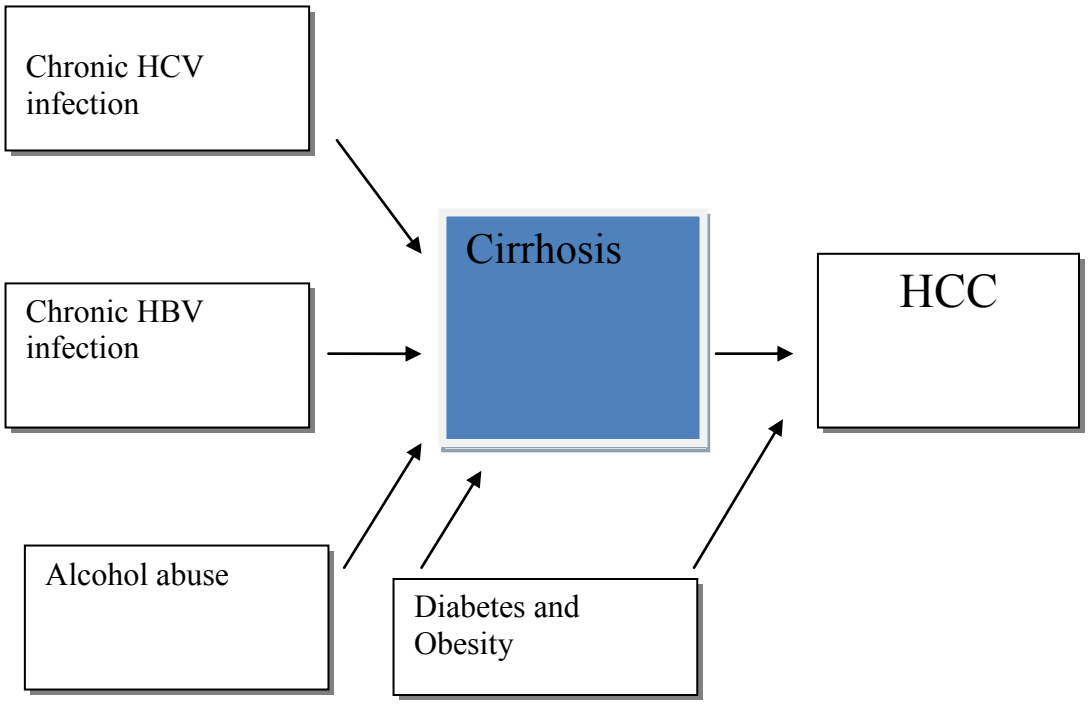


Figure 1: Risk Factors for cirrhosis and HCC development

Obesity

Increasing BMI has been shown to be associated with increasing fibrosis in patients with no other risk factors. In a prospective cohort study in France, obesity and diabetes were determined to be independent risk factors for HCC development in patients with viral or alcohol related cirrhosis.(N'Kontchou, Paries et al. 2006) Higher BMI was also observed in patients undergoing liver transplant for HCC when compared to patients without HCC.(Satheesh Nair 2002). Multiple case control studies have also shown increasing BMI has a synergistic effect on HCC development in addition to other well known risk factors for HCC.(Hashem B. El-Serag 2001; Tazawa, Maeda et al. 2002). A

large prospective cohort study by the American Cancer Society that prospectively followed about 1.1 million participants found a 52% and 62% higher cancer related death rates for men and women with BMI 35-40. However with respect to liver cancer, the rates were about 450% higher in men in the corresponding BMI group and about 70% higher for women.(Calle, Rodriguez et al. 2003)

Two population based cohort studies from Sweden and Denmark found about 2-3 fold increased risk of HCC in obese men and women compared to subjects with normal BMI.(Moller, Mellemgaard et al. 1994; Wolk, Gridley et al. 2001) Obesity has been associated with increased insulin resistance thereby leading to hepatic steatosis. Higher levels of hepatic steatosis contribute to increased fibrosis, necroinflammatory activity and development of cirrhosis, which is a well documented risk factor for HCC.

A recent Japanese study evaluated the risk of obesity as a cofactor in HCC development in patients with chronic HCV. Obese patients with a BMI greater than 30 had a 3 fold increased risk for HCC development when compared to patients with normal weights. The study has also shown a stepwise increase in the incidence of HCC with increasing BMI.(Ohki, Tateishi et al. 2008).

HCC IN THE UNITED STATES

HCC related mortality is the fastest growing cause of cancer related death in men in the U.S. Though in the U.S, HCC occurs most commonly among Asian immigrants and their descendents, the incidence is rapidly increasing in the white

population (Hispanic and non Hispanic). In the U.S, about 48% of the cancers occur in Caucasians, 15% in Hispanics, 13% in African American and 24% in other races which constitutes predominantly Asians. The age-adjusted incidence of HCC in the United States has doubled from 1.4 per 100,000 individuals in 1975-77 to 3.3 per 100,000 in 1996-98 and is predominantly due to HCV and alcohol related cirrhosis (El-Serag and Mason 1999; El-Serag 2001; El-Serag 2002). In a population based study from Los Angeles county, the proportions of HCV positive HCC cases for the periods 1984-90, 1990-1995, and 1996-2001 were 33%, 36% and 48% respectively. In the same study, the corresponding incidence of HBV-related HCC cases for the same period was 40%, 41% and 27%. (Yuan, Govindarajan et al. 2004) HCV related HCC has contributed the most to the proportional increase in HCC when HBV related HCC rates have decreased or remained stable.(El-Serag, Lau et al. 2007)

Hispanics and HCC

Until recently, data has been lacking about the epidemiology of HCC in Hispanic population. In the U.S, Hispanics constitute about 14% of the population which is expected to grow to 25% by 2050. Hispanics constitute about 15% of the total HCC cases detected in the U.S every year. Between the periods of 1992-95 and 2000-2002, a 31% and 63% increase in the incidence of HCC has been reported in Hispanic men and women respectively.(El-Serag, Lau et al. 2007) A recent analysis of the publicly available SEER database to estimate the incidence and mortality rate from HCC in Hispanics showed that Hispanics had the highest incidence rate of HCC second to Asian/Pacific islanders and Hispanics have a 2.7 times greater risk for HCC development

than non-Hispanic whites. Among Hispanics, the age adjusted incidence rates for HCC in males was 3.2 times higher than that of females(El-Serag, Lau et al. 2007). Recent studies have also suggested that Hispanics present with more advanced tumor and have a higher mortality rate from HCC when compared to other ethnic groups(Davila and El-Serag 2006). The etiology of this increased incidence of HCC in Hispanics is largely unknown, although it has been attributed higher prevalence of obesity and diabetes in the Hispanics in addition to conventional risk factors. Only one population based study had estimated the incidence of HCC in Hispanic population in Florida to be at par with the African American population which was about two times that of other races.(Shea, Fleming et al. 2001) A recent population based study in the Rio Grande valley in South Texas also identified an increasing incidence of end stage liver disease and HCC in the Hispanic population(Perez, Anzaldua et al. 2004)

PATHOPHYSIOLOGY OF HEPATOCELLULAR CARCINOMA

HBV, HCV infection and alcohol abuse are major risk factors leading to chronic hepatic inflammation (hepatitis) which progresses over time to development of cirrhosis. Cirrhosis is characterized by liver cell destruction and fibrosis. Metabolic abnormalities such as diabetes, and further alcohol use contribute to progression to HCC. The vast majority of HCC develop in the background of cirrhosis, though about 10% of patients with chronic HBV infection can develop HCC without the development of cirrhosis. Hepatocarcinogenesis is believed to be a multi-step process. HCC development is strongly linked to chromosomal changes, genetic mutations, epigenetic alterations and

alterations in molecular cellular pathways that results in carcinogenesis(Wong and Ng 2008). There is an activation of various growth factors such as IGF-I, IGF-II, TGF-α and TGF-β and a simultaneous inactivation of the various tumor suppressor genes. It is still unclear if the initial viral infection or the subsequent fibrosis and regeneration promote carcinogenesis. HBV however is known to produce an antigen HBx that can directly integrate into the liver cell and is involved in hepatocarcinogenesis(Tang, Oishi et al. 2006). HBx also has been shown to bind to p53 and attenuate DNA repair and apoptosis. Chromosomal instability (CIN) and telomere shortening, which limits the number of cell divisions of cells and may affect the regenerative capacity of organ systems during ageing and chronic disease, have also been implicated in HCC development(Tang, Oishi et al. 2006). In geographical areas of aflatoxin exposure and chronic viral hepatitis, such as China and Africa, a point mutation at the third position of p53 codon 249^{ser} resulting in a G:C to T:A transversion is well described in HCC(Bressac, Kew et al. 1991). This mutant p53 inhibits wild p53 mediated apoptosis and confers a survival advantage to the tumor cells. This mutation is thought to be specific to aflatoxin induced HCC and is not seen with HBV or HCV associated HCC.

Genetic variation has long been suspected to play a role in the development of HCC. This is largely due to the observation that a vast majority of the patients with cirrhosis and risk factors do not develop HCC, but in a minority of cases, HCC develops in patients without risk factors. A recent meta-analysis had concluded that two polymorphisms involving the Glutathione-S-transferase were commonly seen in patients with HCC(El-Serag and Rudolph 2007).

Chapter 3: Specific Aims

1. **Using Hispanic patients with cirrhosis as controls, determine the influence of BMI on the development of HCC in Hispanic patients using multivariate analysis to control for confounding factors.**

Patients with HCC and age matched controls with cirrhosis will be identified from the UTMB database using an International Classification of Diseases-9 (ICD-9) code search for patients who have identified themselves as Hispanic ethnicity. The cases and the controls will be tabulated and using a combination of chart and electronic medical record review, information about BMI, age, alcohol use, HIV status, and diagnosis of diabetes, HCV infection, HBV infection, Model for End-stage Liver Disease (MELD) score (discussed in detail in later sections) and hemochromatosis will be obtained.

2. **Using Hispanic patients with cirrhosis as controls, determine the influence of diabetes on the development of HCC in Hispanic patients using multivariate analysis to control for confounding factors**.

Patients with HCC and age matched controls with cirrhosis will be identified from the UTMB database using an ICD-9 code search for patients who have identified themselves as Hispanic ethnicity. The cases and the controls will be tabulated and using a combination of chart and electronic medical record review, information about BMI, age, alcohol use, HIV status, and diagnosis of diabetes, HCV infection, HBV infection. MELD score and hemochromatosis will be obtained.

Chapter 4: Methods

STUDY POPULATION

This is a hospital based study from the clinical patient database of The University of Texas Medical Branch (UTMB) in Galveston, Texas. UTMB is oldest medical institution in the state of Texas and includes an 84 acre campus including 4 schools, three institutes for advanced study, a major medical library and a network of hospitals and clinics that provide a full range of primary and specialized medical care and numerous research facilities. This tertiary referral center boasts of more than 1000 faculty and about 2500 students.

UTMB's patient population includes patients from Galveston County and eight other surrounding counties including a large area covering East Texas. UTMB also provides care to prisoners under the care of Texas Department of Criminal Justice (TDCJ) and to Federal prisoners. This combination constitutes people of various races, age, ethnicity, religion, socioeconomic status and various languages. Due to the geographic location of the university, it serves a substantial number of Hispanic patients, who form the basis of this study.

CHART REVIEW AND DATA COLLECTION

The original institutional review board approval for completion of the study was obtained in 2007. The required training to handle human research participants and data was also completed as a part of IRB requirements.

Using the electronic coding and billing database at UTMB which includes TDCJ, patients with the International Classification of Diseases, 9th Revision (ICD-9) codes 155.0 for primary hepatocellular cancer diagnosed between Jan 2005 and Jun 2007 were identified. This group would constitute the "cases" in my study. A second group of patients "controls" with ICD-9 code 571.x for diagnosis of cirrhosis were selected from coding and billing database. Using electronic medical records accessed through the internet at http://my.utmb.edu and using the hospital based electronic medical record database system EPIC EMR (EPIC Systems, Wisconsin, USA); patient demographics were verified to select patients who had identified themselves as of Hispanic ethnicity. The identities of these patients were stored in a password protected Excel database with restricted access. This list was used to obtain medical charts from the UTMB medical records department. The charts were stored in a protected room with key access at the UTMB hospital. The chart review was performed carefully over a period of 6 months as access to all the charts was not possible at the same time and charts were obtained in batches for review. This chart review was supplemented by electronic medical record review from the inpatient and outpatient electronic medical record database. The laboratory data was also reviewed and initial lab data- serum creatinine, total bilirubin and INR (International Normalized Ratio) was obtained to determine the MELD score.

The data from the chart review was stored in a data collection sheet (Table 1) without patient identification information. These data sheets were stored in a file with restricted access and kept locked in our clinical offices. After completion of data collection, the data from the sheets were carefully entered into an electronic database. This database was stored in a password protected format in a computer with restricted password access.

Table 1: Data Collection Form:

Sex:
Age
Weight
Height
COMORBID CONDITIONS
Diabetes (Yes/No)
HIV (Yes/No)
Alcohol use Yes/No:
Hepatitis C (Yes/No)
Hepatitis B (Yes/No)
Hemochromatosis(Yes/No)
Serum Total bilirubin at diagnosis
INR at diagnosis
Serum Creatinine at diagnosis
Calculated MELD Score

STUDY DESIGN

The study design is a retrospective case-control study using the cases and controls identified as described above. The objective is to identify if obesity and diabetes are risk factors for development of HCC in the Hispanic population after adjustment for other covariables.

Using the protocol for data collection as described above, cases and controls were identified, charts reviewed and data extracted. An initial 1:2 matching of cases and controls were planned but due to limited number of cases and controls within the study timeframe between 2005 and 2007, the case: control ratio was limited to about 1: 1.5.

Cases were patients diagnosed with HCC during this time period using ICD-9 code search. The diagnosis of HCC was made by a gastroenterologist/hepatologist after review of one of more of the following criteria as noted in the chart.

1. Liver lesion(s) with typical features of early arterial enhancement and rapid washout on a triple-phase CT scan.
2. Biopsy of a liver lesion that was interpreted by a pathologist as HCC
3. Either (1) or (2) with an elevated Alpha- Fetoprotein level.

Patients were included as cases if the date of initial diagnosis was in the study period. The control group was identified using the database search with ICD-9 codes of 571.x for cirrhosis diagnosed over the same time period between 2005 and 2007..

Chapter 5: Data compilation and Statistical Methods

DESCRIPTIVE DATA ANALYSIS

Statistical analysis of the data was performed using JMP statistical discovery software (JMP, Version 7. SAS Institute Inc., Cary, NC, 1989-2007) and StatsDirect statistical software (StatsDirect Ltd., www.statsdirect.com, England) was used for complex data analysis such as multivariate analysis. The data was compiled into a single Excel sheet with all the variables tabulated as columns and each row representing data from a single patient. The columns represented various variables such as age, HCV positivity, HIV positivity, MELD score, history of alcohol use etc. For statistical purposes the data was entered as categorical variables 0 or1 for absence or presence of a particular variable. For example, history of HCV infection would be reported as categorical variable 1 under the HCV column. An absence of alcohol use would be reported as 0 under the alcohol column for a particular individual. This nomenclature was used for all the risk factors.

Age:

The mean age was calculated for the cases and the controls from the age at diagnosis of HCC in the cases and initial diagnosis of cirrhosis in the controls using the average function of the Excel software. Excel was used to represent the data in graphical form.

Sex:

The number of males and number of females in the cases and controls were calculated and the respective proportions determined in each of the two groups.

BMI

BMI (Basic Metabolic Index) was calculated using the data from height and weight obtained on initial diagnosis during the chart review using excel. The formula BMI = Weight (in Kgs)/Height (in meters) ^{2} was used and the data was represented in graphical format using excel software for both the cases and the control group.

The patients were divided to two categories for data analysis- Obese BMI >30 and normal weight/overweight BMI ≤30. Using the standard nomenclature described earlier, patients with BMI> 30 were denoted with 1 and ≤ 30 were denoted with 0 for obesity.

HCV Infection:

HCV positivity was defined as positive antibody serology to Hepatitis C virus identified in the laboratory data. Patients were classified as HCV positive or negative and the data was entered as either 0 or 1 as described earlier.

HIV:

HIV positivity was defined as a diagnosis of HIV as reported in the chart or a serological test (Western Blot) assay in the laboratory data confirming the diagnosis of HIV infection. HIV positive individuals were marked

MELD Score:

Using laboratory data from chart review including INR, Creatinine and Total Bilirubin at the time of diagnosis, MELD Score was calculated in the cases and the controls using the formula:

MELD = 3.78[Ln serum bilirubin (mg/dL)] + 11.2[Ln INR] + 9.57[Ln serum creatinine (mg/dL)] + 6.43

MELD score was treated as a continuous variable for the data analysis.

Diabetes:

Patients were diagnosed to have diabetes based on chart review and reporting by a physician of elevated blood sugar and or treatment with diabetic medications. Patients were classified as positive or negative for diagnosis of diabetes and denoted as either 0 or 1.

UNIVARIATE ANALYSIS

Univariate Analysis was performed using JMP software. Univariate analysis helps in determining the individual risk conferred by each predictor to increase the risk of the outcome, which in this study is the risk of cancer.

Using JMP statistical software, the data was tabulated and represented graphically in the form of a mosaic table for each of the risk factors. The univariate analysis is shown in the results section for diabetes and obesity. The univariate analyses of the other risk factors are shown in Appendix 1.

Univariate analysis is used as a step in data analysis to evaluate significant predictors of an outcome. It provides the unadjusted odds ratio which may be skewed due to modification of the outcome by a cofactor. So univariate analysis is always followed up by multivariate analysis if multiple cofactors are present to affect an outcome.

MULTIVARIATE ANALYSIS

The multivariate analysis was performed using logistic regression analysis. Logistic regression model fits and analyses models for binary or dichotomous outcome or response data with one or more predictors. Unlike other binomial models, logistic models

can be used for both prospective and retrospective data analysis and is commonly used in case control and cohort studies.

Multivariate analysis was done using StatsDirect statistical software. I used logistic regression analysis to determine the odds ratio for each predictor after adjusting for various cofactors. The odds ratio was determined along with the 95% confidence interval to determine the margins of error.

Chapter 6: Results

BASELINE CHARACTERISTICS AND DEMOGRAPHICS:

The patient baseline characteristics are summarized in Table 2. A total of 63 patients with HCC (cases) and 98 patients with cirrhosis (controls) were included in the study. The mean age for the cases was 57.3 years and 52.3 years for the controls. (Figure 2) There were more males in both the groups when compared to females. The male to female ratio in the control group was 89:9 and the control group was 57:6. The mean BMI for the cases was 28.8 and the control group was 29.8. (Figure 3) Subjects were divided into obese and non obese based on a BMI >30. 41.05% of subjects in the control group and 29.03% of cases were obese. The patients' individual MELD score was calculated as described earlier. The mean MELD score for the cases and controls was 13.3. The percentage of patients with diabetes in the control group was 33.67% while in the cases it was 31.75%. Three patients in the control group and 2 patients in the HCC group were diagnosed with HIV. No patients during the chart review were identified with HBV and so HBV infection was excluded as a predictor of HCC development in this study.

HCV was the predominant risk factor for cirrhosis in both the groups. Seventy-seven (77) patients were diagnosed with HCV in the controls (78.5%) and 45 patients with HCV in the cases (70.3%). Seventy-one (71) controls (72.45%) and 53 cases (84.13%) of the patients reported a history of alcohol use. (Figure 3)

Table 2: Demographics and baseline characteristics

	Hispanic - Controls	Hispanics- HCC
Total Number	98	63
Female (% of total)	9 (8.8)	6 (9.5)
Male (% of total)	89 (90.8)	57 (90.4)
Age (mean)	52.3	57.3
Standard Deviation	9.4	9.0
Age (Female)	59.0	66.7
Age (Male)	52.2	56.4
Average BMI (Kg/m^2)	29.8	28.8
Median BMI (Kg/m^2)	28.7	27.9
Diabetes	33	20
% with DM	33.67%	31.75%
Diagnosis of HIV	3	2
Alcohol use	71	53
% with Alcohol use	72.45%	84.13%
Hepatitis C	77/98	45/64
% with Hepatitis C	78.5	70.3
Obesity(BMI> 30)	39/95	18/62
MELD Score (Mean)	13.3	13.3

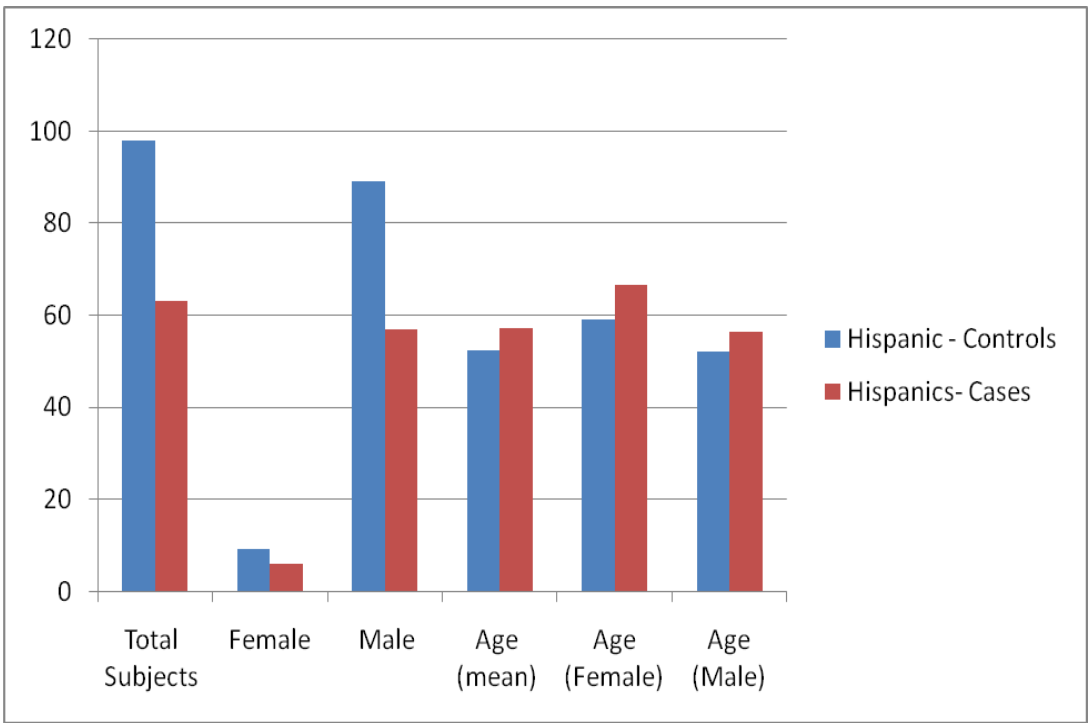


Figure 2: Age and gender distribution: Cases and Controls.

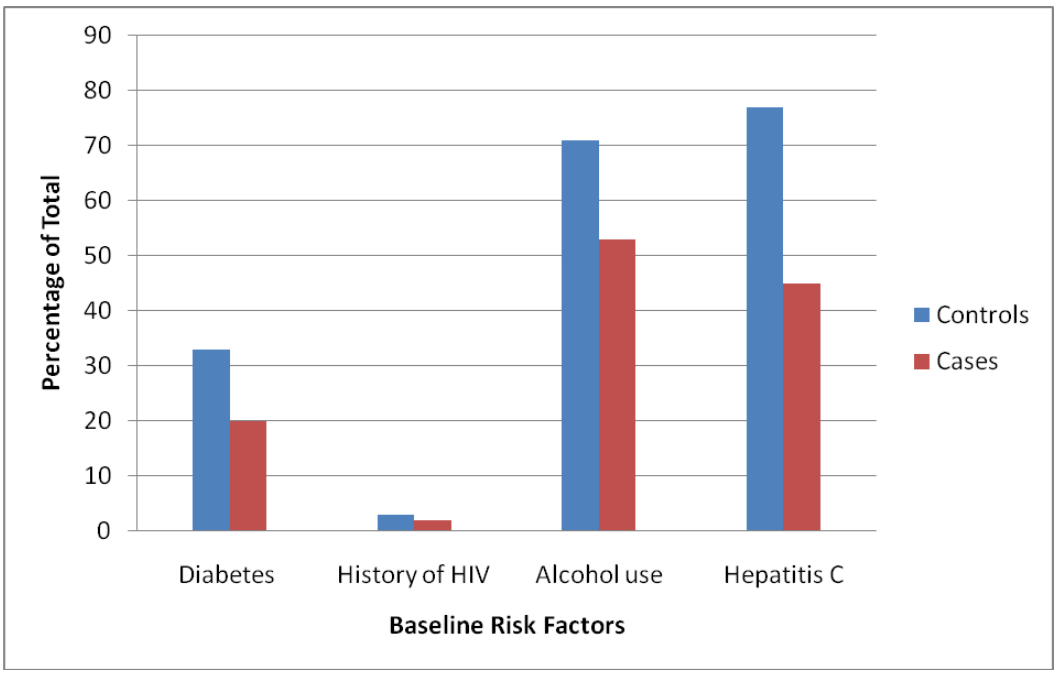


Figure 3: Risk Factor distribution: Cases and Controls.

UNIVARIATE ANALYSIS OF RISK FACTORS

Univariate analysis of Cases/Controls by Diabetes

Univariate analysis was performed to estimate the increased risk of HCC development due to diabetes. The mosaic plot is shown in Figure 4 depicts similar proportions of diabetic patients in both the case and the control group. The contingency table is a 2X2 table that depicts the presence or absence of the predictor and the outcome. Univariate odds ratio was calculated from the table as shown in Table 3.

The unadjusted odds ratio of HCC development in patients with diabetes is 0.91 with CI (0.46- 1.8) which is not statistically significant.

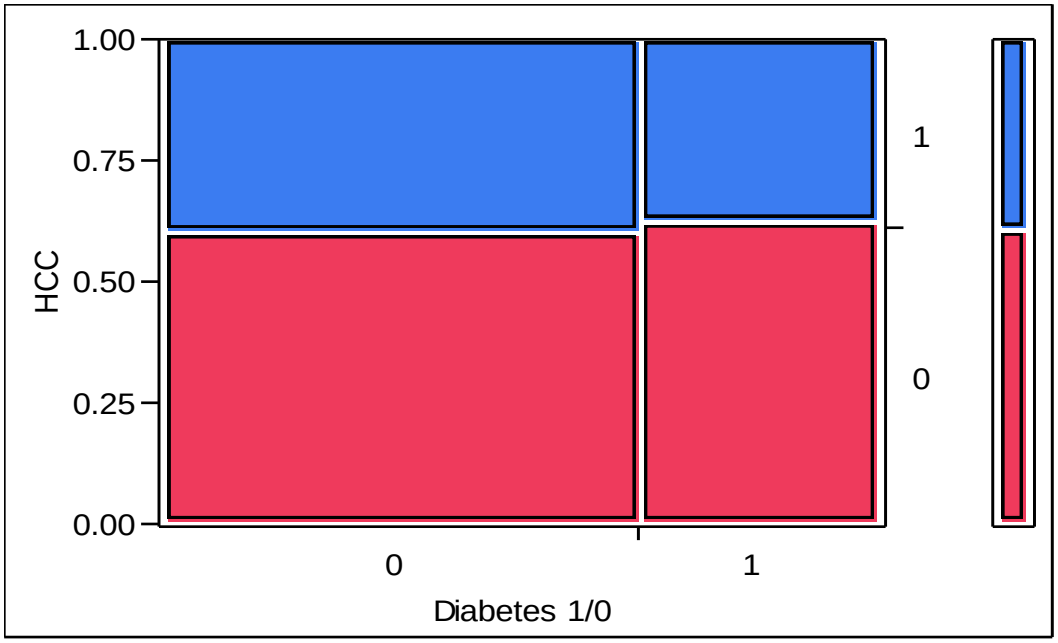


Figure 4: Mosaic Plot- Cases/Controls by Diabetes

Cases/Controls

Count Total %	0 (Controls)	1 (Cases)	
0 (Non Diabetic)	65 40.37	43 26.71	108 67.08
1 (Diabetic)	33 20.50	20 12.42	53 32.92
	98 60.87	63 39.13	161

Odds Ratio	Lower 95%	Upper 95%
0.916138	0.466012	1.801046

Table 3: Contingency Table: Cases/Controls by Diabetes

Univariate analysis of Cases/Controls by Obesity (BMI =>30)

Similar to the method described above for diabetes, Univariate analysis was performed to estimate the increased risk of HCC development due to obesity (BMI>30). The mosaic plot is shown in Figure 5 shows the proportion of obese patients is greater in the controls when compared to the cases. Univariate odds ratio was calculated from the contingency table as shown in Table 3.

The unadjusted odds ratio of HCC development in patients with obesity is 0.58 which shows a possible protective effect of obesity but with CI (0.29- 1.16) which is not statistically significant. Also, univariate analysis is subject to confounding due to cofactors and necessitates adjustment with multivariate analysis.

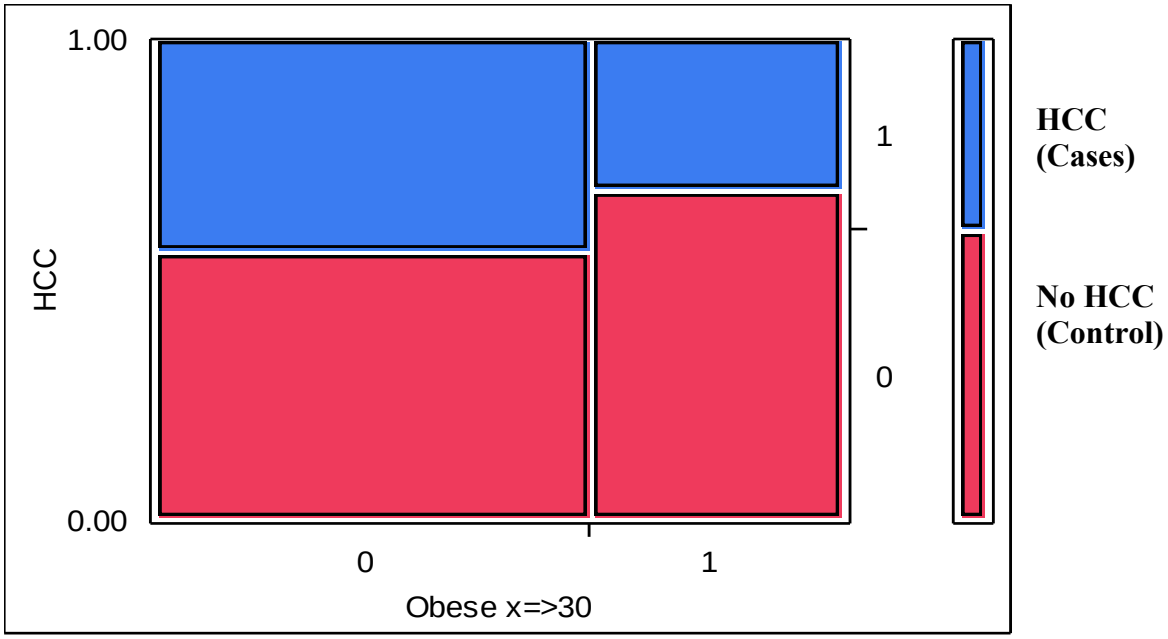


Figure 5: Mosaic Plot: Cases/Controls by Obesity

Count Total %	0 (Controls)	1 (Cases)	Total
0 (Non-Obese)	56 35.67	44 28.03	100 63.69
1 (Obese)	39 24.84	18 11.46	57 36.31
	95 60.51	62 39.49	157

Odds Ratio	**Lower 95%**	**Upper 95%**
0.587413	0.296414	1.164094

Table 4: Contingency Table: Cases/Controls by Obesity

MULTIVARIATE LOGISTIC REGRESSION- ADJUSTMENT FOR CONFOUNDING FACTORS:

The multivariate analysis was performed using logistic regression function in the StatsDirect software. The results are shown in Table 5. The complete logistic regression analysis and the equations are appended in appendix 2.

The adjusted odds ratio for diabetes as predictor for HCC development was 0.74 with CI (0.34-1.61) which is not statistically significant. The adjusted odds ratio for obesity as risk for HCC is 0.80 with CI (0.35- 1.77).

Neither obesity nor diabetes was associated with increased odds of development of hepatocellular cancer when compared to patients with cirrhosis in this retrospective case control study.

Parameter	Estimate	Odds Ratio	95% CI
MELD	0.002824	1.002827	0.960063 to 1.047496
Age	0.06863	1.07104	1.026282 to 1.117751
Alcohol Use	0.846667	2.331861	0.877828 to 6.194353
Hepatitis C	-0.044215	0.956748	0.40722 to 2.247844
HIV	-0.161371	0.850976	0.075086 to 9.644386
Diabetes	-0.296124	0.743695	0.341457 to 1.61977
Obese BMI=>30	-0.211847	0.809089	0.368955 to 1.774268

Table 5: Logistic regression - odds ratios

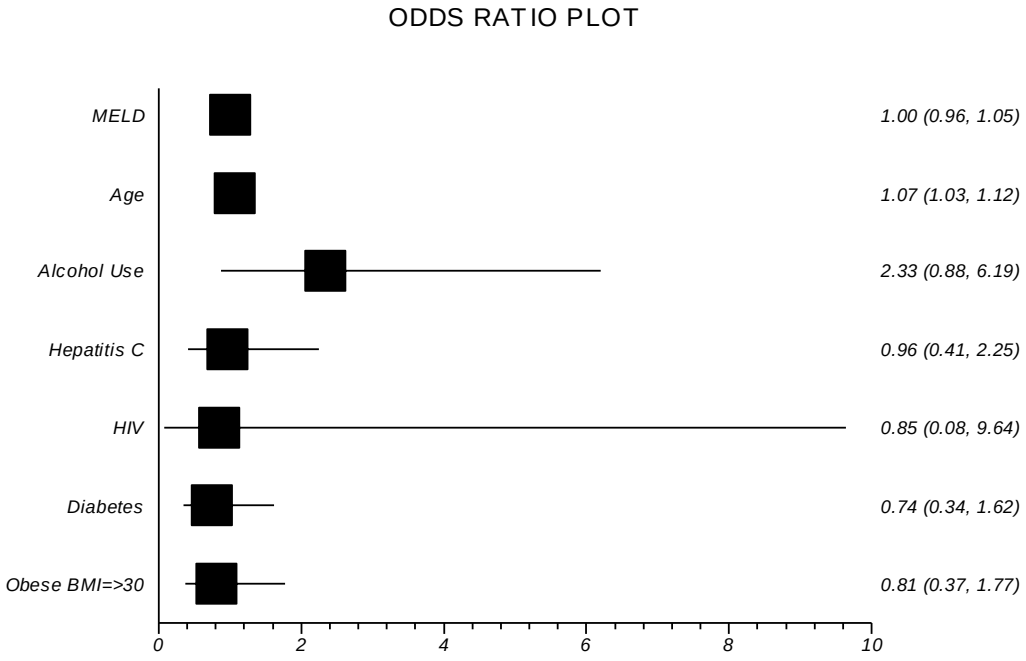


Figure 6: Odds ratio plot

Chapter 7: Discussion

This is a pilot study evaluating the risk conferred by diabetes and obesity, two common but least studied risk factors for HCC development in the Hispanic population. HCC is a major public health problem. The mortality rate from HCC is equal to its incidence rate which makes HCC the third highest cause of cancer death in the world(Parkin 2001). It is one of the deadliest cancers with limited treatment options such as local ablation, resection or liver transplantation, of which liver transplantation is the most definitive treatment with least mortality rates. Due to the limited availability of liver transplant donors, and the expenses involved in transplantation care, managing patients with HCC constitutes a huge burden to the growing health care crisis in the U.S.(El-Serag, Siegel et al. 2006) A recent study based on the SEER database shows the age-adjusted incidence rates in Hispanics was second only to the Asian population.(El-Serag, Lau et al. 2007) With the number of Hispanics in the U.S set to rise significantly in the next decade, the prevalence of HCC will increase proportionally. Increased emphasis on identifying risk factors to direct preventive efforts can potentially limit morbidity and mortality associated with HCC.

The inconclusive results from this study should be viewed with the knowledge of the numerous limitations of this study. First and foremost is the possibility of a Type II error due to a small sample size and the task of identifying a possibly small effect. Since

this was a pilot study with limited number of patients and no preliminary data, it was not feasible to calculate a sample size accurately.

Studies similar to the current study have shown that diabetes (Hassan, Hwang et al. 2002) and obesity (N'Kontchou, Paries et al. 2006; Ohki, Tateishi et al. 2008) have an association with development of hepatocellular cancer. However, it should be noted that the few studies that have shown diabetes to have a positive association with HCC risk, the control group constituted normal subjects without cirrhosis. In these studies, the cases are exposed to numerous risk factors for HCC in addition to diabetes and obesity. The majority of these confounding factors act through the common pathway of cirrhosis leading to HCC. This leads to significant amount of confounding errors in the results. Our strategy was that by using controls that had similar risk factors and baseline cirrhosis, the impact of diabetes and obesity on HCC development can be studied accurately. This is very important as most of the patients with HCC in our study have baseline cirrhosis. The recent French study that evaluated diabetes and obesity used cirrhotic patients as controls and did show a positive association between HCC risk and diabetes and obesity. (N'Kontchou, Paries et al. 2006) However, the major difference between our study and the 2006 French study is that theirs was a prospective study with about 4 times the number of cases and controls. Since our study was looking at a particular ethnic group, the number of patients was limited. A prospective cohort study is also superior to a retrospective study in quality and reliability of the data.

In addition to the factors described, there are numerous inherent limitations of a retrospective study. Though extensive chart review was done, the data was not collected first hand and was dependent on physician documentation. Many of the patients had died and it was not possible to obtain additional information or laboratory information if

necessary. Recall bias and observer bias could have affected primary data recording. Since the initial patient selection was based on ICD-9 code based search of billing and coding database, if patients did not report a Hispanic ethnicity, they would not have been identified leading to a potential selection bias. Other factors such as socioeconomic status could play a potential role in seeking treatment, which could not be addressed due to limitations in data.

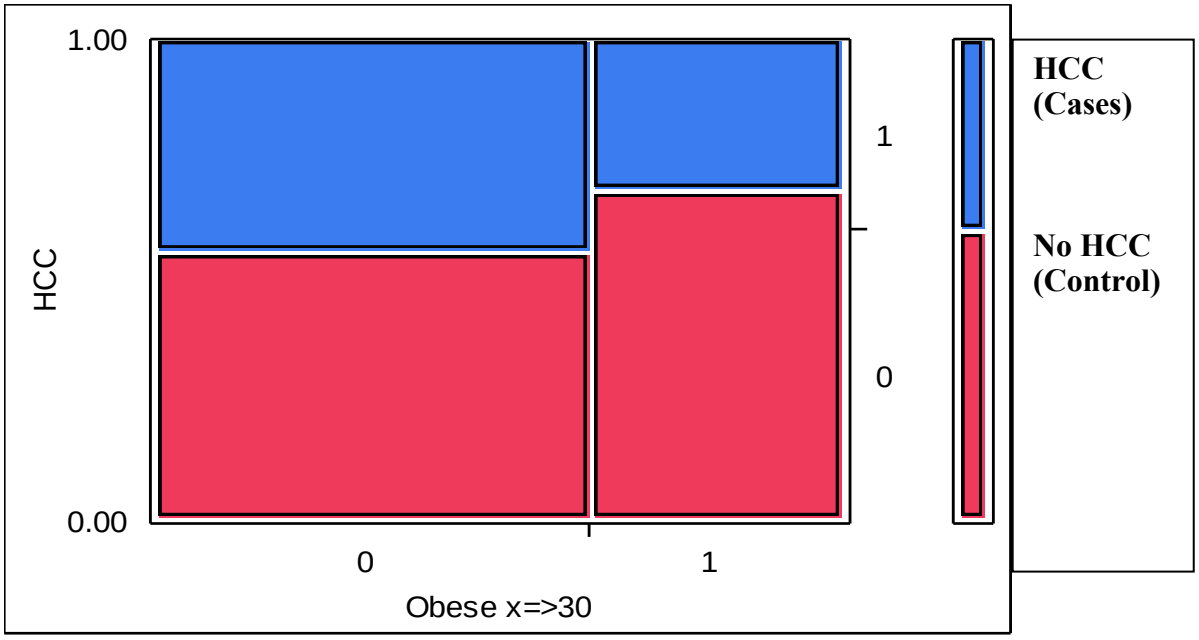
Large prospective cohort studies in Hispanic patients with cirrhosis could potentially unravel the significance of diabetes and obesity in the pathogenesis of cirrhosis and HCC. Though data from other populations suggest that this association is very likely, this has not been studied in the U.S Hispanic population. To what extent diabetes and obesity influences HCC development also needs to be explored.

Appendices

APPENDIX 1

Univariate analysis of Cases/Controls by Obesity (BMI =>30)

Mosaic Plot: Cases/Controls by Obesity



Contingency Table: Cases/Controls by Obesity

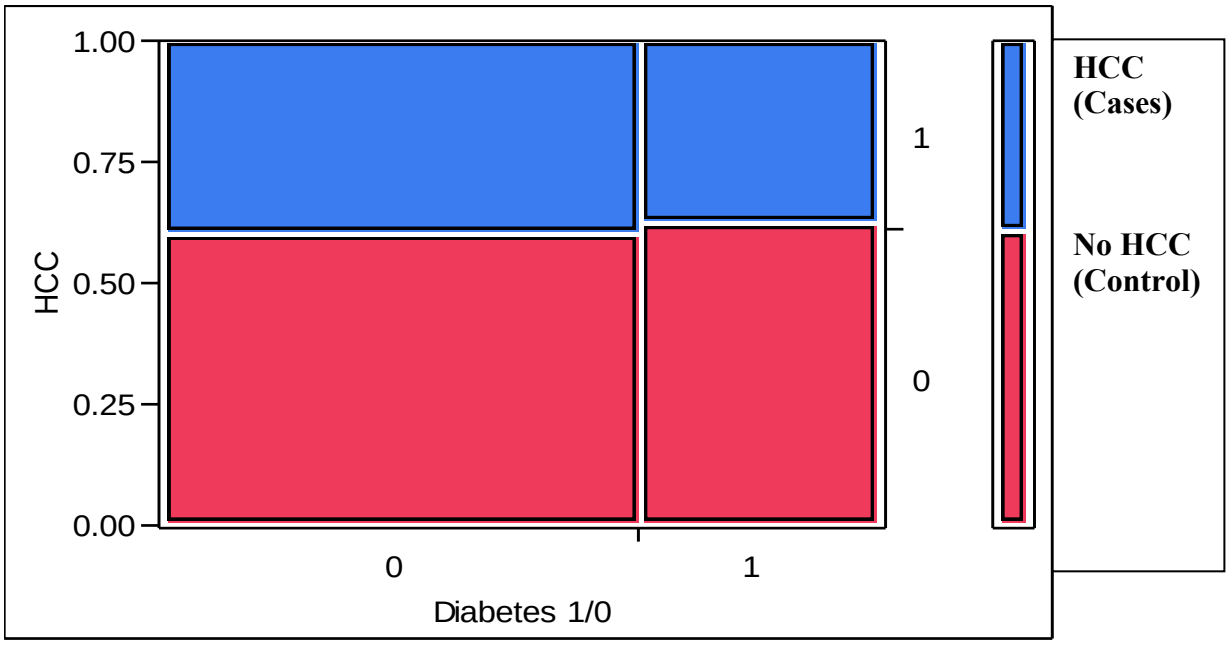
Cases/Controls

Count Total %	0 (Controls)	1 (Cases)	
0 (Non-Obese)	56 35.67	44 28.03	100 63.69
1 (Obese)	39 24.84	18 11.46	57 36.31
	95 60.51	62 39.49	157

Odds Ratio	**Lower 95%**	**Upper 95%**
0.587413	0.296414	1.164094

Univariate analysis of Cases/Controls by Diabetes

Mosaic Plot: Cases/Controls by Diabetes



Contingency Table: Cases/Controls by Diabetes

Cases/Controls

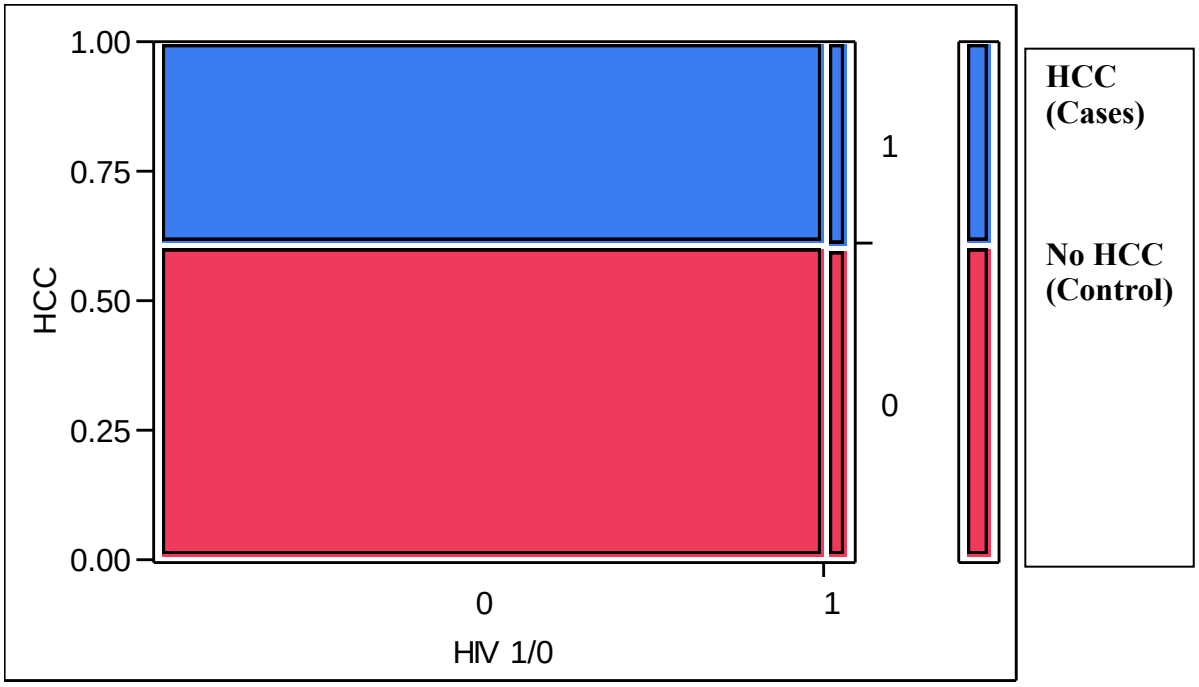
Count Total %	0 (Controls)	1 (Cases)	
0 (Non Diabetic)	65 40.37	43 26.71	108 67.08
1 (Diabetic)	33 20.50	20 12.42	53 32.92
	98 60.87	63 39.13	161

Odds Ratio

Odds Ratio	**Lower 95%**	**Upper 95%**
0.916138	0.466012	1.801046

Univariate analysis of Cases/Controls by HIV 1/0

Mosaic Plot



Contingency Table

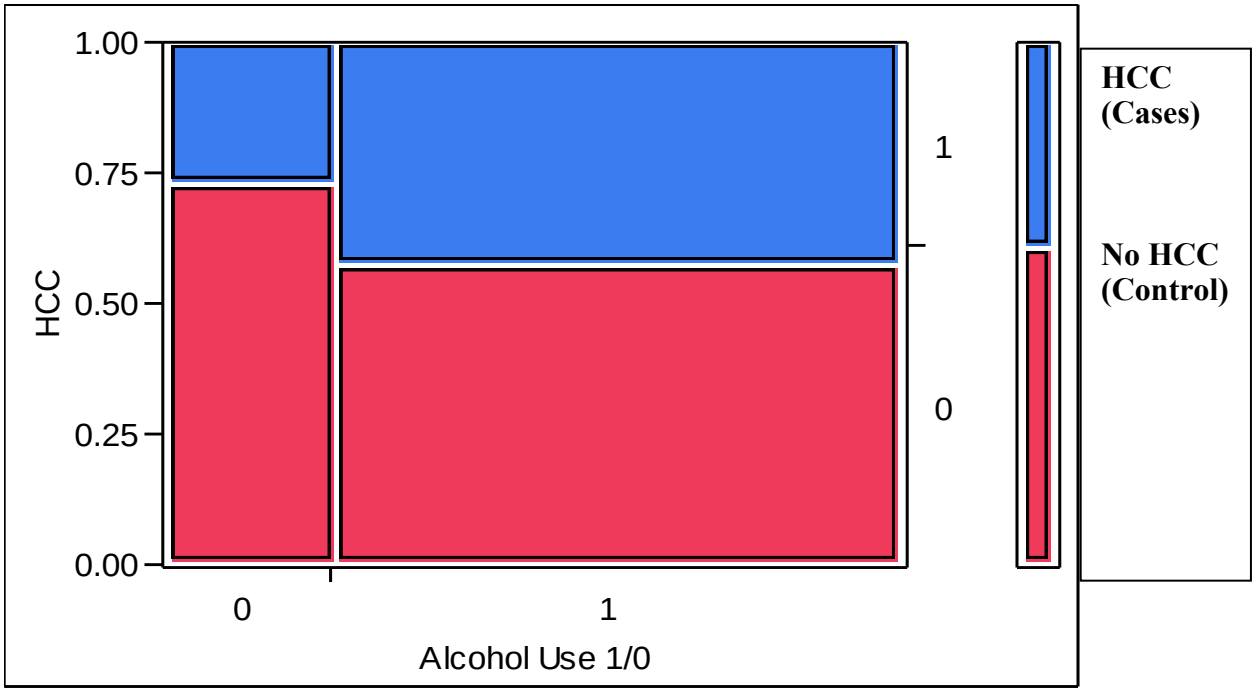
HIV 1/0 by HCC Cases/Controls

Count Total %	0	1	
0 (HIV Negative)	95 59.01	61 37.89	156 96.89
1 (HIV Positive)	3 1.86	2 1.24	5 3.11
	98 60.87	63 39.13	161

Odds Ratio

Odds Ratio	**Lower 95%**	**Upper 95%**
1.038251	0.168583	6.394263

Univariate analysis of Cases/Controls by Alcohol Use 1/0
Mosaic Plot



Contingency Table

Alcohol Use 1/0 by HCC

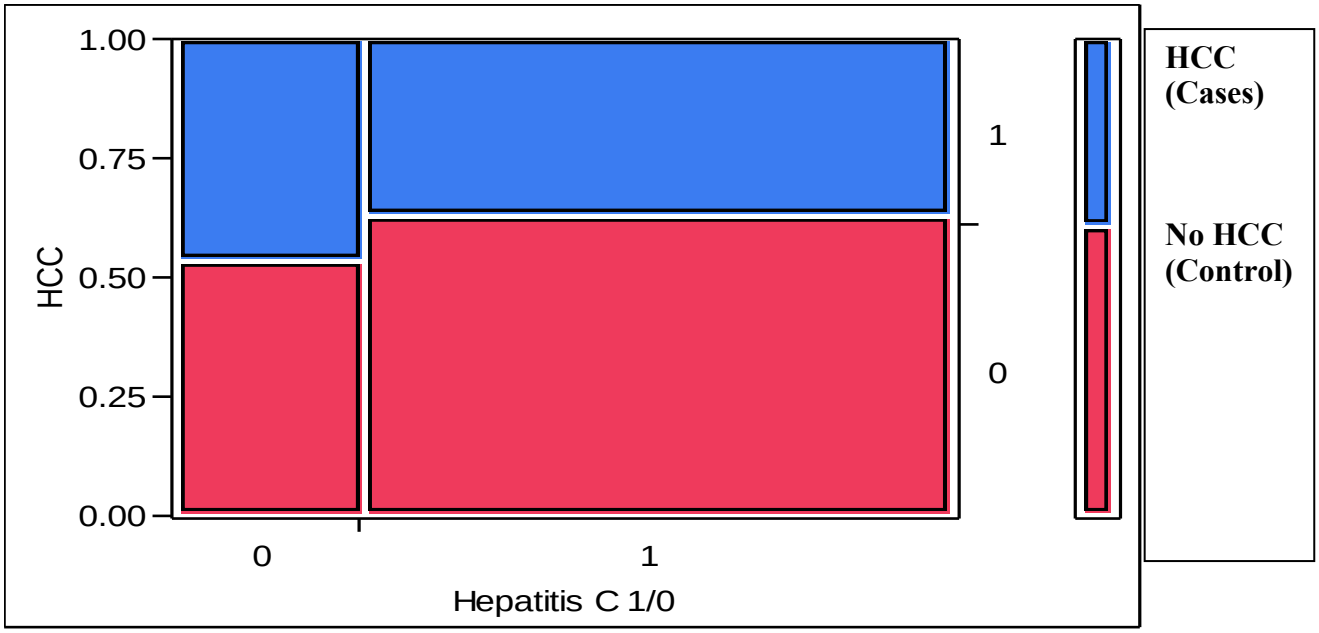
Count Total % Col % Row %	0	1	
0 (No alcohol use)	27 16.77 27.55 72.97	10 6.21 15.87 27.03	37 22.98
1 (Alcohol use)	71 44.10 72.45 57.26	53 32.92 84.13 42.74	124 77.02
	98 60.87	63 39.13	161

Odds Ratio

Odds Ratio	**Lower 95%**	**Upper 95%**
2.015493	0.898324	4.521992

Univariate analysis of Cases/Controls by Hepatitis C 1/0

Mosaic Plot



Contingency Table

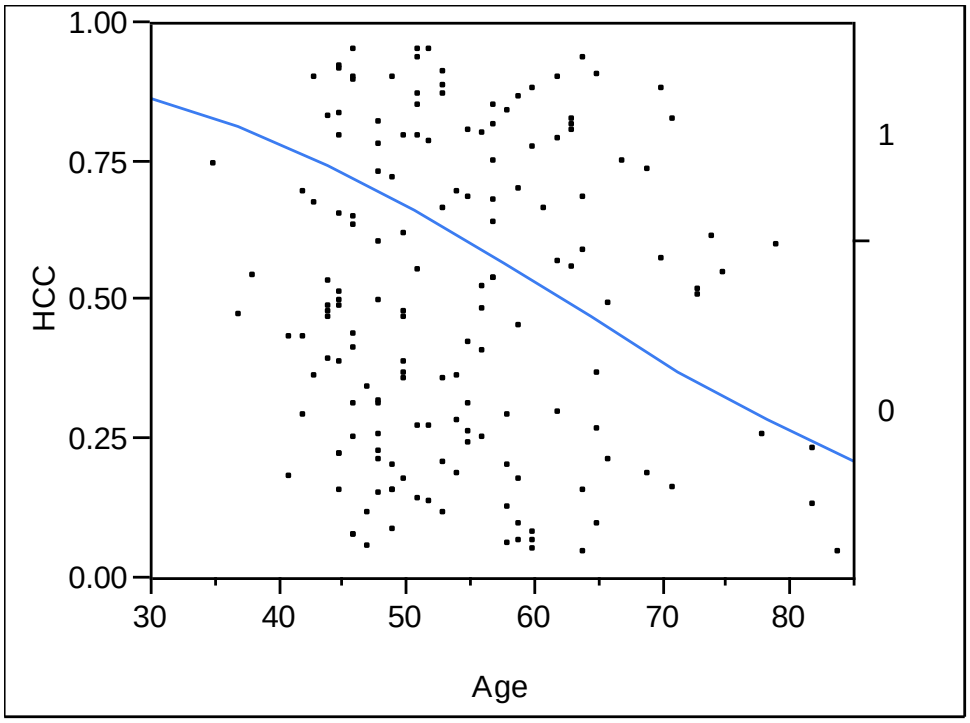
Hepatitis C 1/0 by HCC Cases/Controls

Count Total % Col % Row %	0 (Controls)	1 (Cases)	
0 (No HCV infection)	21 13.04 21.43 53.85	18 11.18 28.57 46.15	39 24.22
1 (HCV infection)	77 47.83 78.57 63.11	45 27.95 71.43 36.89	122 75.78
	98 60.87	63 39.13	161

Odds Ratio

Odds Ratio	**Lower 95%**	**Upper 95%**
0.681818	0.328868	1.413564

Univariate analysis of Cases/Controls by Age

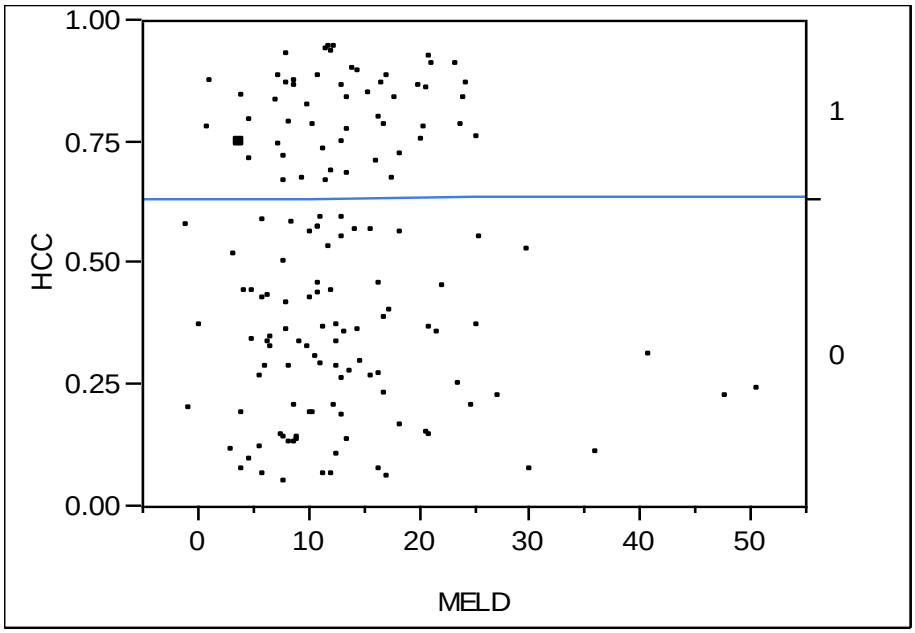


Parameter Estimates

Term	Estimate	Std Error	Chi-square	Prob>ChiSq	Unit Odds Ratio	Odds Ratio
Intercept	3.59305182	1.0144114	12.55	0.0004	.	.
Age	-0.0576751	0.0182478	9.99	0.0016	0.94395655	0.05924451

For log odds of 0/1

Univariate Analysis of cases/controls by MELD



Parameter Estimates

Term	Estimate	Std Error	Chi-square	Prob>ChiSq	Unit Odds Ratio	Odds Ratio
Intercept	0.5467675	0.3280223	2.78	0.0955	.	.
MELD	0.00056497	0.0209848	0.00	0.9785	1.00056513	1.02955672

For log odds of 0/1

APPENDIX 2

Logistic regression

Deviance goodness of fit chi-square = 173.648858 df = 137 P = 0.0187 *
Deviance (likelihood ratio) chi-square = 16.743886 df = 7 P = 0.0191

Intercept	b0 = -4.849859	z = -3.126759	P = 0.0018
MELD	b1 = 0.002824	z = 0.126985	P = 0.899
Age	b2 = 0.06863	z = 3.151087	P = 0.0016
Alcohol Use 1/0	b3 = 0.846667	z = 1.698551	P = 0.0894
Hepatitis C 1/0	b4 = -0.044215	z = -0.101453	P = 0.9192
HIV 1/0	b5 = -0.161371	z = -0.130278	P = 0.8963
Diabetes 1/0	b6 = -0.296124	z = -0.745615	P = 0.4559
Obese x=>30	b7 = -0.211847	z = -0.528775	P = 0.597

Logistic Regression Equation:

logit Y = -4.849859 +0.002824 MELD +0.06863 Age +0.846667 Alcohol Use 1/0 -0.044215 Hepatitis C 1/0 -0.161371 HIV 1/0 -0.296124 Diabetes 1/0 -0.211847 Obese x=>30

Logistic regression - odds ratios

Parameter	Estimate	Odds Ratio	95% CI
Constant	-4.849859		
MELD	0.002824	1.002827	0.960063 to 1.047496
Age	0.06863	1.07104	1.026282 to 1.117751
Alcohol Use 1/0	0.846667	2.331861	0.877828 to 6.194353
Hepatitis C 1/0	-0.044215	0.956748	0.40722 to 2.247844
HIV 1/0	-0.161371	0.850976	0.075086 to 9.644386
Diabetes 1/0	-0.296124	0.743695	0.341457 to 1.61977
Obese x=>30	-0.211847	0.809089	0.368955 to 1.774268

References

Adami, H. O., W. H. Chow, et al. (1996). "Excess risk of primary liver cancer in patients with diabetes mellitus." J Natl Cancer Inst **88**(20): 1472-7.

Adams, L. A., J. F. Lymp, et al. (2005). "The natural history of nonalcoholic fatty liver disease: a population-based cohort study." Gastroenterology **129**(1): 113-21.

Adams, L. A., S. Sanderson, et al. (2005). "The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies." J Hepatol **42**(1): 132-8.

Blanc, J. F., V. De Ledinghen, et al. (2000). "Increased incidence of HFE C282Y mutations in patients with iron overload and hepatocellular carcinoma developed in non-cirrhotic liver." J Hepatol **32**: 805 - 811.

Bressac, B., M. Kew, et al. (1991). "Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa." Nature **350**(6317): 429-431.

Calle, E. E., C. Rodriguez, et al. (2003). "Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults." N Engl J Med **348**(17): 1625-38.

Camma, C., M. Giunta, et al. (2001). "Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach." J Hepatol **34**(4): 593-602.

Colombo, M. (1999). "Hepatitis C virus and hepatocellular carcinoma." Semin Liver Dis **19**(3): 263-9.

Davila, J. A. and H. B. El-Serag (2006). "Racial differences in survival of hepatocellular carcinoma in the United States: a population-based study." Clin Gastroenterol Hepatol **4**(1): 104-10; quiz 4-5.

Deugnier, Y., B. Turlin, et al. (1998). "Iron and neoplasia." Journal of Hepatology **28**(Supplement 1): 21-25.

Di Bisceglie, A. M. (1997). "Hepatitis C and hepatocellular carcinoma." Hepatology **26**(3 Suppl 1): 34S-38S.

Donato, F., A. Tagger, et al. (2002). "Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women." Am J Epidemiol **155**(4): 323-31.

El-Serag, H. B. (2001). "Epidemiology of hepatocellular carcinoma." Clin Liver Dis **5**(1): 87-107, vi.

El-Serag, H. B. (2002). "Hepatocellular carcinoma: an epidemiologic view." J Clin Gastroenterol **35**(5 Suppl 2): S72-8.

El-Serag, H. B., J. A. Davila, et al. (2003). "The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update." Ann Intern Med **139**(10): 817-23.

El-Serag, H. B., H. Hampel, et al. (2006). "The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence." Clin Gastroenterol Hepatol **4**(3): 369-80.

El-Serag, H. B., M. Lau, et al. (2007). "Epidemiology of hepatocellular carcinoma in Hispanics in the United States." Arch Intern Med **167**(18): 1983-9.

El-Serag, H. B. and A. C. Mason (1999). "Rising incidence of hepatocellular carcinoma in the United States." N Engl J Med **340**(10): 745-50.

El-Serag, H. B. and K. L. Rudolph (2007). "Hepatocellular carcinoma: epidemiology and molecular carcinogenesis." Gastroenterology **132**(7): 2557-76.

El-Serag, H. B., A. B. Siegel, et al. (2006). "Treatment and outcomes of treating of hepatocellular carcinoma among Medicare recipients in the United States: A population-based study." Journal of Hepatology **44**(1): 158-166.

El-Serag, H. B., T. Tran, et al. (2004). "Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma." Gastroenterology **126**(2): 460-8.

Francesco Donato, P. B. M. P. (1998). "A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma." International Journal of Cancer **75**(3): 347-354.

Hashem B. El-Serag, P. A. R. J. E. E. (2001). "The role of diabetes in hepatocellular carcinoma: a case-control study among United States veterans." American Journal of Gastroenterology **96**(8): 2462-2467.

Hassan, M. M., L. Y. Hwang, et al. (2002). "Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus." Hepatology **36**(5): 1206-13.

Kao, J. H., P. J. Chen, et al. (2002). "Genotypes and clinical phenotypes of hepatitis B virus in patients with chronic hepatitis B virus infection." J Clin Microbiol **40**(4): 1207-9.

Li-Yu Wang, M. H. C.-J. C. B. L. S.-L. Y. S.-N. L. M.-H. W. W.-P. W. L.-W. W. Q. W. G.-T. H. P.-. (1996). "Aflatoxin exposure and risk of hepatocellular carcinoma in Taiwan." International Journal of Cancer **67**(5): 620-625.

Moller, H., A. Mellemgaard, et al. (1994). "Obesity and cancer risk: a Danish record-linkage study." Eur J Cancer **30A**(3): 344-50.

N'Kontchou, G., J. Paries, et al. (2006). "Risk Factors for Hepatocellular Carcinoma in Patients With Alcoholic or Viral C Cirrhosis." Clinical Gastroenterology and Hepatology **4**(8): 1062-1068.

Ohki, T., R. Tateishi, et al. (2008). "Obesity is an independent risk factor for hepatocellular carcinoma development in chronic hepatitis C patients." Clin Gastroenterol Hepatol **6**(4): 459-64.

Parkin, D. M. (2001). "Global cancer statistics in the year 2000." The Lancet Oncology **2**(9): 533-543.

Parkin, D. M. (2002). "Cancer Incidence in five continents." IARCPress, Lyon (2002) IARC scientific publications I **volume VII**([No. 155]).

Parkin, D. M., F. Bray, et al. (2001). "Estimating the world cancer burden: Globocan 2000." Int J Cancer: 153-156.

Perez, A., M. Anzaldua, et al. (2004). "High frequency of chronic end-stage liver disease and hepatocellular carcinoma in a Hispanic population." J Gastroenterol Hepatol **19**(3): 289-95.

Poynard, T., P. Bedossa, et al. (1997). "Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups." Lancet **349**(9055): 825-32.

Ross, R. K., J. M. Yuan, et al. (1992). "Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma." Lancet **339**(8799): 943-6.

Satheesh Nair, A. M. J. E. G. L. R. P. P. (2002). "Is obesity an independent risk factor for hepatocellular carcinoma in cirrhosis?" Hepatology **36**(1): 150-155.

Schafer, D. F. and M. F. Sorrell (1999). "Hepatocellular carcinoma." The Lancet **353**(9160): 1253-1257.

Shea, K. A., L. E. Fleming, et al. (2001). "Hepatocellular carcinoma incidence in Florida. Ethnic and racial distribution." Cancer **91**(5): 1046-51.

Sherman, M. (2004). "Pathogenesis and screening for hepatocellular carcinoma." Clin Liver Dis **8**(2): 419-43, viii.

Sloane, D., H. Chen, et al. (2006). "Racial disparity in primary hepatocellular carcinoma: tumor stage at presentation, surgical treatment and survival." J Natl Med Assoc **98**(12): 1934-9.

Tang, H., N. Oishi, et al. (2006). "Molecular functions and biological roles of hepatitis B virus x protein." Cancer Science **97**(10): 977-983.

Tazawa, J., M. Maeda, et al. (2002). "Diabetes Mellitus May Be Associated with Hepatocarcinogenesis in Patients with Chronic Hepatitis C." Digestive Diseases and Sciences **47**(4): 710-715.

Turlin, B., F. Juguet, et al. (1995). "Increased liver iron stores in patients with hepatocellular carcinoma developed on a noncirrhotic liver." Hepatology **22**(2): 446-450.

WHO (1983). "Prevention of liver cancer." World Health Organ Tech Rep Ser **691**: 1-30.

Willis, G., V. Bardsley, et al. (2005). "Hepatocellular carcinoma and the penetrance of HFE C282Y mutations: a cross sectional study." BMC Gastroenterology **5**(1): 17.

Wolk, A., G. Gridley, et al. (2001). "A prospective study of obesity and cancer risk (Sweden)." Cancer Causes Control **12**(1): 13-21.

Wong, C.-M. and I. O. L. Ng (2008). "Molecular pathogenesis of hepatocellular carcinoma." Liver International **28**(2): 160-174.

Wu, H.-C., Q. Wang, et al. (2007). "Polycyclic aromatic hydrocarbon- and aflatoxin-albumin adducts, hepatitis B virus infection and hepatocellular carcinoma in Taiwan." Cancer Letters **252**(1): 104-114.

Yu, M. W. and C. J. Chen (1993). "Elevated serum testosterone levels and risk of hepatocellular carcinoma." Cancer Res **53**(4): 790-4.

Yu, M. W., Y. C. Yang, et al. (2001). "Hormonal markers and hepatitis B virus-related hepatocellular carcinoma risk: a nested case-control study among men." J Natl Cancer Inst **93**(21): 1644-51.

Yuan, J. M., S. Govindarajan, et al. (2004). "Synergism of alcohol, diabetes, and viral hepatitis on the risk of hepatocellular carcinoma in blacks and whites in the U.S." Cancer **101**(5): 1009-17.

Zhang, Y. J., P. Rossner, Jr., et al. (2006). "Aflatoxin B1 and polycyclic aromatic hydrocarbon adducts, p53 mutations and p16 methylation in liver tissue and plasma of hepatocellular carcinoma patients." Int J Cancer **119**(5): 985-91.

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This dissertation was typed by Sathya Jaganmohan M.D.