2020

Predictors of the Health Effects of Marijuana Use on the Hepatic Function

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Walden University
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Abstract

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Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

Public Health

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Abstract

The quantity of marijuana use, the length of time it was used, and the age of initiation of the drug are at the core of the discussions about the potential health effects of marijuana use on the liver. Results of recent studies regarding how the drug affects human health have resulted in a number of conflicting conclusions. Nevertheless, based on these findings, marijuana users are being denied liver transplants. The objective of this study was to identify predictors of the health effects of marijuana on the liver and provide guidance in the care management of marijuana users. To address the inconsistencies in the research findings, this study was designed to investigate possible associations between the quantity of marijuana use, the duration of use, and the age of initiation as they relate changes in liver enzymes. Data from a random sample of 702 participants obtained from the National Health and Nutrition Examination Survey were analyzed in a least square linear regression model. The study found that the quantity of marijuana use has a significant effect on the serum total bilirubin (TB) level with an apparent detrimental effect on the serum level of TB, $R^2 = .106$, $F(3, 19) = 4.859$, $p < .05$, 95% CI [-.896, -.175]. The duration of use significantly affects the serum level of alkaline phosphatase, $R^2 = .074$, $F(4, 18) = 4.661$, $p < .05$, 95% CI [.00, .004] and total protein, $R^2 = .077$ $F(4, 18) = 3.401$, $p < .05$, 95% CI [-.013, .000]. The age of initiation failed to have a significant health effect on any liver enzymes. This study has the potential to improve care management for marijuana users by helping to accelerate the diagnosis process and by improving the policy of liver transplant denial for marijuana users.

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Dedication

I dedicate this document to my children, Giovanni, Orstin, Audrey and Ashely for the unconditional love and to my wife for the love, support and encouragement during this doctoral journey.

Acknowledgements

First, my deepest gratitude goes to my advisor Dr. Manoj Sharma for his guidance, time and support during this doctoral journey. I also, would like to thank members of my committee, Dr. Garland Brinkley and Dr. Aaron Mendelsohn for their constructive criticisms, suggestions and guidance during this process.

Above all, I'm grateful to the unlimited support of my family and friends. This dissertation would not be accomplished without you, my children who have been patient and have allowed me to take time away from you. A special thanks to my parents for their prayers and support. I also would like to thank my ASTOCI family, especially the board members for their support and encouragement during this doctoral journey.

A heartfelt gratitude goes to you, my wife for your unconditional love, prayers, encouragement and continuous support. Words would not express enough how much I appreciate you in my life. Much love to all my friends and family members who I have not listed here but have been there for me. I hope I made you all proud.

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Chapter 1: Introduction to the Study

In 2013, the National Institute of Drug Abuse (NIDA, 2015) reported a significant increase in the number of people who were using illicit drugs, which was primarily associated with the increase in marijuana use due to the new laws that decriminalized the drug (Maier, Mannes, & Koppenhofer, 2017). Drinking alcohol, smoking tobacco, and the use of cocaine decreased over the same time period (NIDA, 2015), while the rise in marijuana consumption resulted in an increase in cannabis-related emergency department visits (Zhu & Wu, 2016). Results of recent studies regarding how the drug affects human health have resulted in a number of conflicting conclusions. Some studies found a significant therapeutic effect of the drug on human health, including a positive effect on the liver, whereas other research warned of marijuana's adverse health effects (Adejumo et al., 2017, Volkow et al., 2014). In the medical field, many questions related to the actual impact of the drug on different organs of the human body, the impact of the drug on less frequent users, and the effect of the age of initiation and method of consumption of the drug remain unanswered (Sznitman & Room, 2018).

In recent years, more studies related to marijuana use have emerged (Maier, Mannes, & Koppenhofer, 2017). Researchers have begun to investigate the risk factors associated with marijuana and how it affects different human organs including the liver (Gudsoorkar & Perez Jr., 2015; Kazory & Aiyer, 2013). Although many findings are still in their early stages, they appear to point in many different directions, including reports of positive and negative health effects, as well as no significant health effect (Adejumo et al., 2017; T. Liu et al., 2014; Terry-McElrath et al., 2017). It is important to note that

several of these studies were conducted on groups of individuals with preexisting liver conditions, which has the potential to be a source of bias in the findings. In more than half of these studies, researchers did not consider the age of initiation of cannabis use, the duration of cannabis use, the quantity of cannabis use, or the method of consumption in their analysis (Sznitman & Room, 2018). The few studies conducted with healthy participants failed to distinguish marijuana users from those who were using or abusing other substances. For instance, several studies reported difficulties in isolating those individuals who only use cannabis, which has the potential to reduce the sample size and can negatively impact and skew study results (Quraishi, Jain, Chatterjee, & Verma, 2013). In addition, when evaluating the effect of marijuana on the liver, most studies failed to consider the difference in age, gender, and race. These unique sociodemographic factors are potentially important and may affect the results of these studies.

In 2015, more than 11 million Americans reportedly consumed marijuana (NIDA, 2015). The prevalence of marijuana use more than doubled from 2001-2002 to 2012-2013, which coincided with an increase in the number of cannabis-related emergency department visits and marijuana-related admissions in hospitals and residential service treatments (Substance Abuse and Mental Health Services Administration [SAMHSA], 2014). Interestingly, clear evidence of adverse health effects of marijuana use on the liver was inconsistent. Researchers then began questioning why liver transplants were being denied to marijuana users and whether marijuana should be classified as a Schedule I drug. More studies are essential to fully understanding the actual effects of marijuana on the liver.

In the laboratory, the health of the liver is assessed by identifying the serum levels of liver function parameters such as the serum level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), alkaline phosphate (ALP), total bilirubin (TB), total protein (TP) and the gamma-glutamyl transpeptidase (GGT) (Fumeaux, Scarpelli, Tettamanti, & Palmiere, 2018). These parameters are indicative of different functions of the liver and help to assess the health condition of the liver. Hence, the scope of this study was to analyze how marijuana affects them individually, which is valuable in the identification of specific effect of the drug on a healthy liver.

Accordingly, in this study, I analyzed the predictors of the health effects of marijuana on the liver function in different categories of users and evaluated whether the age of initiation, the length of time marijuana was used, and the amount of marijuana smoked daily have a significant effect on hepatic function.

Background

The core issues associated with the health effects of marijuana use on the liver have been found to be related to the user's age, the age of initiation, the amount of marijuana smoked, the method of consumption and the preexisting condition of the smokers (Sznitman & Room, 2018).

Age is a risk factor for many chronic diseases including liver diseases (H. Kim et al., 2015). Previous research has indicated that younger individuals who use marijuana are less at risk of developing nonalcoholic fatty liver disease (NAFLD) than older individuals are (D. Kim et al., 2017). The age-specific onset of marijuana use has varied throughout history. The percentage of adolescents who tried marijuana before the age of

13 years increased between 1991 and 1999, but a steady decrease has been observed from 1999 to 2015 (Youth Risk Behavior Surveillance System (YRBSS, 2017). For example, after using marijuana for more than 30.5 months with an average age of onset of 21.8 years, 34 patients presented no abnormal liver function tests (Kotan et al.,2017). By contrast, at the age of onset of 15.31 years with 9.53 years of smoking marijuana, 51% of cannabis dependent patients displayed abnormal liver patterns (Quraishi et al.,2013). These findings suggest that the duration and the amount of marijuana were critical factors in determining the detrimental health effects of the drug on the liver. The conclusions from the above findings were different from other findings that suggested marijuana use has a therapeutic effect on NAFLD and the severity of hepatitis C virus (HCV) infection (Adejumo et al., 2018; D. Kim et al., 2017). This contradictory conclusion suggested that the more marijuana is used and the longer it is used, the more positive effect it has on the NAFLD and HCV-infected patients. Terry-McElrath et al. (2017) and Quraishi et al. (2013) also found that the duration of marijuana use is a factor that should be taken into consideration when analyzing the health effects of marijuana. Terry-McElrath et al. (2017) associated increased risk of adverse health effects of 50-year-old marijuana users with moderate to heavy long-term use of marijuana. In addition, Quraishi et al. (2013) linked the long-term use of marijuana to abnormal liver patterns. The quantity of marijuana used has also been considered in several studies that evaluated the impact of marijuana on the liver. Adejumo et al. (2017) suggested that heavy cannabis use represents a positive contributing factor on the prevalence of NAFLD, whereas Terry-McElrath et al. (2017) concluded that the intensity of marijuana smoked is a strong

predictor of negative health effects for 50-years old users. Unlike previous studies, T. Liu et al. (2014) found no significant difference in the health of the liver of 21 patients infected with HCV who used marijuana compared to HCV-infected nonusers.

Several previous studies have assessed the impact of marijuana on specific liver enzymes and found conflicting results. Mohamed et al. (2015) concluded that chronic marijuana use was associated with the hepatic enzymatic alteration. Several other studies have reported results that indicated the detrimental health effect associated with marijuana on liver enzymes, including ALT, AST, ALP, TB, ALB, TP, and GGT. (Mohamed et al., 2015; Quraishi et al., 2013; Wani, Khan, & Singh, 2017). In contrast to the findings of the above authors, some studies have suggested that marijuana improves the activity of the liver enzymes (Adejumo et al., 2018; Kim et al., 2017) while another research group found no significant health impact of the drug on hepatic function (Kotan et al., 2017; Muniyappa et al., 2013; Rahmayanti et al., 2017).

Problem Statement

Marijuana is the most common illicit drug used in the U.S. (NIDA, 2015). Concerns about its use are reportedly associated with its adverse cognitive effect (Mandelbaum & de la Monte, 2017) and its likelihood to cause adverse cardiovascular events (Thomas, Kloner, & Rezkalla, 2014). With the recent change in social attitudes toward the use of marijuana and its continued decriminalization (Maier et al., 2017), there has been an increase in interest related to risk factors associated with use of the drug and how it affects several organs in the human body (Gudsoorkar & Perez Jr., 2015; Kazory & Aiyer, 2013). Predictors of the health effects of alcohol and tobacco on the liver are

well documented (Liu et al., 2017). However, except for a single study published in Brazil in 2004 (Borini, Guimarães, & Borini, 2004) that attempted to evaluate the predictors and health effects of marijuana use on the liver in a healthy population, studies of risk factors associated with the effects of marijuana use on the liver in healthy populations are relatively scarce in the literature. For example, when Kim et al. (2017) showed that marijuana use is associated with NAFLD and Tarantino, Citro, and Finelli (2014) found that marijuana use potentially creates health risk in patients with concomitant chronic liver diseases, neither study assessed how the difference in the amount of marijuana use, the length of time it was used, and how the age of initiation may have differently affected the study results. In addition, these studies did not assess the risk factors associated with individual liver function markers, as each of them may be indicative of other liver health issues.

To close the gap in the literature, the intent of this study was to identify potential predictors of the health effects of marijuana use on hepatic function while examining the association between the number of joints or pipes of marijuana smoked daily, the age of initiation, the length of time the drug was used, and the individual liver function markers using a large nationally representative sample.

Purpose of the Study

The purpose of this study was to identify potential predictors of the liver health effects associated with marijuana use on hepatic function, while also examining the effects of the number of joints or pipes use daily, the age of initiation, and the length of time marijuana was used. In a cross-sectional analysis, this study used the number of

joints or pipes smoked daily, the age of initiation, and the duration of use as the independent variables, and the biochemistry profile of the liver, including the serum level of ALT, AST, ALB, ALP, TB, TP, and GGT were used as the outcome variables. I categorized the population sample by the age of the study participants, the age of initiation, and the length of time the drug was regularly used. The study approach reduced bias or the possible confounding effects of liver risk factors by controlling for aging factors, gender, alcohol use and body mass index (BMI).

Research Question

The research question addressed in this study was as follows: Is there any significant association between the number of joints or pipes of marijuana smoked in a day, the age of initiation, the duration of use and each of the serum level of liver function markers including the serum levels of ALT, AST, ALB, ALP, TB, TP, and GGT while controlling for the age of participants, their gender, the average alcohol use and BMI?

$H_0$1: There is no significant association between the number of joints of marijuana smoked in a day, the age of initiation, the duration of use and each of the serum level of liver function markers including the serum levels of ALT, AST, ALB, ALP, TB, TP, and GGT while controlling for the age of participants, their gender, the average alcohol use and BMI.

$H_1$1: There is significant association between the number of joints of marijuana smoked in a day, the age of initiation, the duration of use and each of the serum level of liver function markers including the serum levels of ALT, AST, ALB, ALP, TB, TP, and

GGT while controlling for the age of participants, their gender, the average alcohol use and BMI.

Theoretical Framework

This study was grounded in the ecosocial theory introduced by Krieger in 1994 (Krieger, 2011). The key premise of this theory is that the pattern of health and disease can be explained by a complex web of interconnected risk and protective factors rather than by direct causative agents (Krieger, 2011). The theory explains how exposure to certain commodities, such as tobacco, alcohol, and illicit drugs are possible pathways that affect the physiology and gene expression in humans, which, in turn, affects their health outcomes (Krieger, 2011).

The major construct of ecosocial theory, embodiment, entails the claim that disease and health pattern are the results of what humans biologically embody during the course of their life and that disease can be explained not only by innate factors, but also by the effects of exogenous factors on the human body (Krieger, 2011). Alongside the core construct, the theory calls for attention to the joint interplay of exposure, susceptibility, and resistance at multiple levels across the course of a person's life (Krieger, 2011). Acknowledging the pathway of the joint interplay of exposure, susceptibility, and resistance helps to identify exposures, potential confounders, and effect modifiers concerning the social group, time, and place (Krieger, 2011). It is also a critical pathway for evaluating the likely impact of risk factors on disease burden (Krieger, 2011). The theory allows for better conceptualization to examine risk factors associated with diseases at the individual and population levels (see Figure 1).

Adapting ecosocial theory to this study reveals that understanding the health effects of marijuana use on the liver requires the examination of the social and biological risk factors that mediate the relationship between marijuana use and hepatic function (see Figure 1). The embodiment construct facilitates a better understanding of the major factors that are associated with marijuana use. Kalant (2004) reported that the early onset of marijuana use, especially weekly or daily use, are strong predictors of future adverse health effects, and Borini et al. (2004) found that the health effects of cannabis depend on the dose received. The interconnection of these risk factors and their roles in the physiology and gene expression in the human body are evidence of adverse health outcomes such as liver diseases. Hall (2009) observed that it is challenging to assess risk factors associated with adverse health outcomes of cannabis use only because users are also likely to use alcohol, tobacco, and other illicit drugs. This observation points to the potential presence of confounders and/or effect modifiers concerning risk-factor assessment and analysis in marijuana users, which Krieger (2011) proposed to elucidate or evaluate using the pathway of the joint interplay of exposure, susceptibility, and resistance (see Figure 1).

This theoretical framework provides the basis to understand the exposures to marijuana use and to evaluate associated risk factors and their impact on the hepatic function.

Considering the key premise of ecosocial theory that exposure to certain risk factors affects the physiology and gene expression in humans, which are the source of many diseases (Krieger, 2011), with the exposure pathway that called attention to the

joint interplay of exposure, susceptibility, and resistance, the results of this study are expected to provide insight into how the age of initiation, the daily dose of marijuana use, and the frequency at which marijuana is used affect liver function of users.

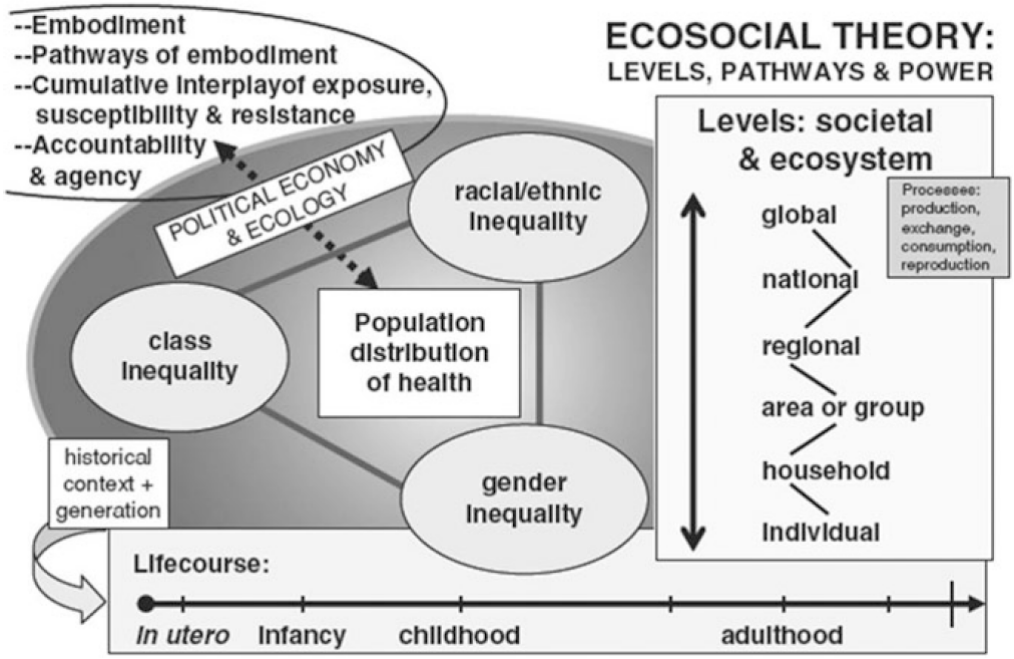


Figure 1. Ecosocial theory and embodying inequality: core constructs. (Krieger, 1994; Krieger, 2008). Reprinted from Krieger, N. (1994). Ecosocial Theory of Disease Distribution. In N. Krieger (Ed), Epidemiology and people's Health: Theory and Context (pp. 202-235). New York, NY: Oxford Press.

Nature of the Study

The study had a primarily quantitative cross-sectional design with a focus on secondary data collected from the National Health and Nutrition Examination Survey (NHANES, National Center for Health Statistics [NCHS], 2015b). Data analysis will take into consideration several parameters that assess the risk of developing liver diseases when the organ is exposed to marijuana use. The study will consider only sample

participants aged 20 to 59 years old. An approach of data analysis will use a complex sample general linear model (CSGLM) in SPSS where the outcome variables need to be continuous and the predictor variables can be categorical or continuous. The CSGLM takes into consideration the multistage sampling method use during the NHANES data collection. A deep analysis of the statistical tools and the effects of confounding variables will be considered and assessed later during the study.

Definition of Variables

In this section, I define the key variables in the context of this project.

Independent Variables

Number of joints or pipes smoked daily: In the context of this project, this variable is related to the number of joints or pipes of marijuana or hashish, an individual, male or female aged 20 to 59 years smoked in a day.

Age of initiation: This is the age at which an individual, male or female, aged 20 to 59 years, began regularly using marijuana or hashish.

Duration of marijuana use: This was the period in a lifetime when marijuana was used.

Dependent Variables

Alanine aminotransferase (ALT): ALT is a circulating transaminase in human serum and a specific marker for liver dysfunction (Huang et al., 2017). According to the NHANES data documentation and description, an elevated level of ALT can be indicative of hepatic disease, myocardial infarction, and/or muscular dystrophy and organ damage. In the context of this study, ALT will be assessed to diagnose liver disease.

Aspartate aminotransferase (AST): As a liver enzyme, AST increased during liver dysfunction. Apart from being elevated in liver diseases, the activity of the enzyme is also influenced by certain disease states, such as myocardial infarction, muscular dystrophy, pulmonary emboli and acute pancreatitis (Marshall, Lapsley, Day, & Ayling, 2014). In the context of this study, AST will be used uniquely for its ability to diagnose liver disease.

Alkaline phosphatase (ALP): Alkaline phosphatase is another enzyme used as part of liver function analysis to evaluate possible dysfunction of the liver (Bishop et al., 2018; Lowe & John, 2018). Because it is a nonspecific enzyme, 80% of ALP found in the serum originates from the liver, bones, and in small amounts from the intestine. The enzyme serves as a marker of extrahepatic cholestasis such as stones in the bile duct or intrahepatic cholestasis such as drug-induced cholestasis or biliary cirrhosis (Bishop et al., 2018; Lowe & John, 2018; Sharma, Pal, & Prasad, 2014). The enzyme will be used in the context of this study to evaluate liver dysfunction.

Total protein (TP): Synthesized in the liver, the evaluation of serum total protein is useful to assess the synthetic ability of the liver. Although the protein level is not a sensitive marker for liver damage, it is useful to quantify the severity of liver dysfunction (Bishop et al., 2018). In the context of this study, TP will be used to evaluate liver dysfunction.

Albumin (ALB): Synthesized by the liver, Albumin is the major form of protein in human serum, which is involved in maintaining proper osmotic pressure and the transport of various substances through the body (Bishop et al., 2018; Morman & Varacallo, 2018).

A low concentration of albumin is most commonly associated with possible liver disease (Morman & Varacallo, 2018). In the context of this study, the level of serum albumin will be employed as a liver disease marker.

Total bilirubin (TB): Bilirubin is a breakdown of old and damaged red cells collected in the liver. Sometimes the body can have an excessive amount of bilirubin, which is referred to as hyperbilirubinemia and recognized as jaundice (yellow discoloration of the skin, eyes and mucous membranes) when the excess is accumulated in the tissue (Bishop et al., 2018). According to the NHANES data documentation and description, elevated bilirubin is associated with hemolytic jaundice, internal hemorrhage, acute hemolytic anemia, while low bilirubin is associated with chronic nephritis and aplastic anemia. In the context of this study, variations in bilirubin levels will be exclusively measured to assess the presence of liver-related diseases.

Gamma-glutamyl transaminase (GGT): Elevated activity of GGT is found in liver dysfunction, hepatobiliary disorders and chronic alcohol consumption (Bishop, 2018; Koenig & Seneff, 2015). A drug such as warfarin, phenobarbital, and phenytoin are noted to increase the enzyme level. According to the NHANES data documentation and description, GGT is the most sensitive marker of liver disease.

Controlled Variables

Alcohol use: In the context of this study, alcohol consumption is categorized into light drinkers (less than 2 drinks per day), moderate drinkers (2 to 4 drinks per day), and heavy drinkers (4 or more drinks per day). One drink is defined as a 12-oz beer, a 5-oz

glass of wine, or 1.5 oz of liquor (National Institute on Alcohol Abuse and Alcoholism, n.d.)

Body mass index (BMI): In the context of this study, BMI refers to the weight in kilograms of any participant divided by his or her height in meters squared, rounded to one decimal place.

Age: In the context of this study, age refers to the age of the participants at the date of screening.

Gender: In this study, the gender of the participants can be male or female.

Definition of Terms

Marijuana: Also known as weed, herb, pot, grass, bud, ganja, and "Mary Jane," marijuana refers to the dried leaves, flowers, stems, and seeds from the hemp plant, *Cannabis sativa*. Marijuana used in a hand-rolled cigarette is referred to as a joint, and it is referred to as a pipe when used in pipes or in water pipes.

Delta-9-tetrahydrocannabinol (THC) is the main psychoactive chemical, responsible for the intoxicating effect found in marijuana. It is mainly found in the resin produced by the leaves and buds of the cannabis plant.

Hepatitis is an inflammation of the liver. There are many types of hepatitis depending on etiological factors. Viral hepatitis is caused by a viral infection, and depending on the type of virus, viral hepatitis can be subdivided into hepatitis A through E. Alcoholic hepatitis, caused by increased alcohol consumption is also common. Other medical conditions such as autoimmune diseases can also cause inflammation of the liver Snyder, 2016).

Assumptions

Although the different markers employed in this study are known in the diagnosis and treatment of other diseases, they were exclusively used in this study for their function in liver-related diseases.

The age of initiation or the age of onset usually refers to the age at which an individual had the first contact with marijuana or hashish. But in the context of this study, the age of initiation or the age of onset was assumed to be the age at which an individual had contact with marijuana or hashish and continued using it for at least one year.

Scope and Delimitations

This study was limited to participants aged 20-59 years old, ages at which marijuana use may not have a considerable effect on the liver. Younger or older members of the population may display a different pattern of the effect of marijuana on the liver. This study was also unable to account for the difference in the size of joints or pipes of marijuana and the variation in the daily amount used. Furthermore, this study does not consider any gender difference when assessing the data. Although the gender-related health effect of marijuana on the liver could be mentioned during analysis, it is not the fundamental objective of this study.

Participants who tested positive for hepatitis B and C were excluded from the study because such individuals may develop an increased level of liver enzymes that could interfere with the study results. It has been proven that these patients develop an increased level of ALT and AST (Bishop et al., 2018). Other liver diseases, such as NAFLD, liver cirrhosis and liver cancer also commonly increase the level of liver

enzymes (Bishop et al., 2018). Unfortunately, data on these diseases which could have improved the relationship between the different variables were not available to introduce them in the current study for analysis.

In brief, this study could not establish a cause-effect relationship, but it was designed to be capable of identifying associations between possible predictors and outcome variables (Asomoah, 2014).

Limitations

In any research study, it is important to acknowledge the limitations. These limitations should focus on the problems related to the research question(s) being studied (Connelly, 2013). The NHANES data have an important number of missing marijuana use data due to the sensitivity of the drug use questionnaires. For this study, I ignored any case with one or more missing data points, which evidently reduced the sample size. To avoid possible selection bias caused by such procedure, I considered and combined data collected over several years (2009 to 2016). To make the data representative of the general population and as recommended by the NHANES, I adjusted the dataset for the weight to indicate the combination of four years of data collection cycles.

It is important to note that the NHANES data were self-reported answers to a series of questions. Although subjected to many processes of verification to ensure accuracy, self-reported data are always prone to bias (Connelly, 2013).

Significance of the Study

An increase in the number of people consuming illicit drugs was reported in 2013 by the NIDA (2015). The increasing decriminalization of marijuana was identified as a

major contributing factor to the rise of the drug consumption (Maier et al., 2017). More than 11 million Americans used marijuana in 2015 alone (NIDA, 2015). While alcohol drinking, tobacco smoking, and cocaine use have decreased over the years (NIDA, 2015), marijuana use is on the rise, which has resulted in more cannabis-related emergency department visits (Ayangbayi et al., 2016). Not only was research on the health effects of marijuana on the liver scarce in the literature, but the findings in the studies that were available were also conflicting. Although some findings suggested a therapeutic effect of the drug on the liver (Adejumo et al., 2017; Kim et al., 2017), other findings warned of its adverse effects on human health (Wolkow et al., 2014). Researchers also lack approaches to assess possible predictors such as the age of initiation, the quantity of marijuana use, and the duration for which it was used in the analysis of the health effects of marijuana on hepatic function.

By analyzing the impact of the age of initiation, the quantity of marijuana use, and the duration for which marijuana was used on the individual liver enzymes, this study was intended to provide insight on the predictors of the health effects of marijuana on the liver. Liver enzymes were assessed individually because each of them indicates a different health status of the liver.

Summary

In Chapter 1, I presented the scope of the study, including the nature and the different assumptions of the study. In Chapter 2, the literature review, the state of marijuana use in the United States and its related issues are first discussed. Thereafter, findings from recent studies are explored, which are related to how the age of initiation of

marijuana, the amount of marijuana used, and the length of time during which marijuana was used appear to have an effect in the health effect of marijuana on the biological markers, including the serum level of ALT, AST, ALB, ALP, TB, TP and GGT.

Chapter 2: Literature Review

Introduction

The purpose of this literature review is to find articles related to the key variables of the study topic and use them to demonstrate that there is a significant gap in the literature regarding the predictors of the health effects of marijuana on hepatic function. In addition, this literature review serves as a broad overview of the new developments regarding the association between marijuana use and liver disease. This literature review also evaluates the role played by the key variables, including the age of participants, age of initiation, duration of marijuana use, and ALT, AST, ALP, TP, TB, ALB, and GGT in similar studies.

Organization of the Review

This literature review first presents an overview of the state of marijuana use and related issues in the United States. For a better understanding of the health effects of marijuana use on the liver, I also evaluate current studies on the possible association of marijuana use and liver diseases including NAFLD, hepatitis-C infection, liver fibrosis, and liver cirrhosis. I then provide an overview of liver diseases and risk factors. Additionally, after a brief review of the key variables, the literature review includes an evaluation of the role played by the age of participants, the age of initiation, the duration of marijuana use, and the dose of marijuana use in liver diseases. Finally, I examine the effect of marijuana use on the different outcome variables including ALT, AST, ALP, TP, TB, ALB, and GGT.

Literature Search Strategy

Articles on the health effects of marijuana on hepatic function were found by searching online databases such as MEDLINE, CINAHL, and Google Scholar. I selected only peer-reviewed articles using keywords such as *cannabis, marijuana, THC, pot or weed, liver, liver disease, liver function parameters, ALT, AST, ALP, TP, ALB, TB,* and *GGT*. Multiple combinations of the keywords were used to identify potential articles on the association between the independent and outcome variables. Recent peer-reviewed articles on possible covariables were also identified using a combination of keywords, including *alcohol, tobacco, liver, liver function parameters,* and *marijuana*. I also identified several articles from the U.S. National Library of Medicine (NLM) and the National Institute of Health (NIH).

A combined search of MEDLINE and CINHL limited to 2013-2019 using the keywords *marijuana, cannabis, pot, weed,* and *THC*, cross-searched with keywords *liver disease,* or *liver failure,* or *liver cirrhosis* yielded 78 peer-reviewed articles. The full-text articles identified narrowed the count down to 56 articles (42 articles in MEDLINE and 14 in CINAHL). Among the 56 articles, 45 were considered relevant to the study either by elucidating the variables in the study or serving as a ground of possible association between predictors and response variables. The articles also served to demonstrate the existing gap or to make a case for the significance of the study. Through cross-searches using the same keywords in Google Scholar for the same period, I obtained 49 results consisting of articles and books. A combination of approximately 20 articles and books were considered relevant and introduced into the literature review. A search of the term

marijuana and the individual outcome variables (ALT, AST, ALB, ALP, TP, TB, GGT) yielded less than 15 results for each variable in MEDLINE and CINHL, which was also the case with the Google Scholar search. Approximately five articles per outcome variable were found to be relevant to the study. The same keywords were cross-searched in the U.S. NLM and NIH (PubMed) websites, and although some of the articles found had already been identified in the MEDLINE, CINHL and Google Scholar searches, I identified approximately 30 new relevant articles. A few articles related to the confounding variables through multiple combinations of the keywords *alcohol, tobacco, marijuana and liver disease, liver failure,* or *liver cirrhosis* were selected.

Theoretical Foundation

This study was grounded in the ecosocial theory introduced in 1994 by Krieger. The theory explains the cause of diseases, disease patterns, and disease distributions as an interconnected web of social and economic exposure through the course of the lives of the individual or population (Krieger, 2011). Unlike other disease etiology theories that connected disease occurrence to the interrelation between host-agent and environment (Egger, Swinburn, & Rossner, 2003), the ecosocial theory depicts a new multidimensional and more dynamic perspective of the cause of diseases, disease patterns, and disease distributions. The theory was based on four major propositions.

The Core Propositions of the Theory

The core propositions of the ecosocial theory are based on a multidimensional and dynamic perspective in the inquiry and analysis of the changing pattern of population health (Krieger, 2001). The first core proposition, embodiment, posits the idea that

human beings embody their life experiences, which, in turn, affect the disease patterns. The idea of embodiment is a recognition of the continued interaction between the human body and its environment. This interaction along the course of life is manifested at different levels and affects disease patterns. This interaction has a primary consequence of changing both gene regulation and expression (Krieger, 2001). The proposition of embodiment is that the determinants of disease patterns and distributions are exogenous to people's bodies and cannot solely be limited to the biological characteristics but also to the societal context (Krieger, 2001). The second core proposition of the theory suggests that there are *multiple pathways of embodiment.* The pathway of embodiment brings attention to the idea that specific exposures, including, for instance, exposure to toxic substances, social and economic precarity, cause poor health. The third core proposition, the joint interplay of exposure, susceptibility, and resistance, calls attention to the time it takes for the body to respond to the embodied materials, including change in gene expression. The fourth core proposition, *accountability*, calls on how health disparities should be monitored and analyzed. It also calls attention to the political and economic driving force of health-related issues, which represent grounds for the changing patterns in health.

Ecosocial Theory and Marijuana Use

The ecosocial theory has been used as grounds for inquiries in several social sciences, medicine, and environmental studies. Developed to address the question of "who and what drives social inequalities in health," the theory has been central in facilitating inquiries about the changes in the population's health patterns (Krieger,

2011). The theory has been used across different population groups including different age, gender, and ethnicity groups. Inquiries into the distribution in infectious, acute, and chronic disease have also seen the use of the ecosocial model. Epidemiological factors, as well as environmental factors, have been evaluated across multiple health issues using the ecosocial theory as a model of inquiry. Gomaa et al. (2016) assessed the cause of oral health inequality using the core proposition of the ecosocial theory, making the case that inquiries based only on biomedical and behavioral approaches to understand oral health inequality were ineffective. The authors analyzed a pathway through which social factors affected oral health outcomes, became embodied, and altered biological factors, including the expression of genes (Gomaa et al., 2016). The theory has also been a driving force in the development of new perceptions during illicit drug use inquiries (Duff, 2007; Ettorre, 2004). For example, like Krieger (2005) who emphasized that the body tells stories, Duff (2007) studies drug use by the female gender and reported that the human body is a social identity and means of self-expression. Ettorre (2004) found that different factors were associated with drug use, including the cost of the drug, the availability of the drug, the level at which the body desires the drug, social exclusion, and the culture of the drug use which later, he stated, becomes embodied experiences. The first major proposition of the ecosocial theory, embodiment has been applied in different drug use, substance use, intoxication, and addiction studies, while also emphasizing the social and environmental context in which users live (Angus, 2013; Duff, 2007; Ettorre, 2004).

The Rationale of Choosing the Ecosocial Theory

Human bodies often tell stories that may not be consistent with their assertion, and these stories should be studied in the context of the individual's course of life (Krieger, 2005). The ecosocial theory explains disease patterns by emphasizing on social and biological interactions. In addition, several authors linked the predictors of drug use to social factors and the environment in which the users live (Angus, 2013; Duff, 2007; Ettorre, 2004). Consequently, understanding the predictors of marijuana use on the hepatic function required the examination of the user's adverse factors exposure. In her theory, Krieger (1994) also stipulated that, once embodied, the adverse exposures altered gene expression as the source of diseases. In this study, the predictors once embodied may affect gene expression and alter hepatic function. In brief, exposure to illicit drugs such as marijuana is a potential pathway that affects the physiology and gene expression in certain users, which in turn, affects the condition of their health. Like many recent studies (Adejumo et al., 2017; D. Kim et al., 2017), the current study broke with studying disease burden while relying only on the biological factors. The examination of social and biological interactions during disease pattern inquiries helped to identify risk factors or potential predictors of the health effects of marijuana use on hepatic function. The embodiment and the pathways of embodiment constructs helped to understand how once embodied these risk factors altered the biology of gene expression, which can be the source of adverse health outcomes. The third construct, the pathway of the interplay of exposure, susceptibility, and resistance helped the study to frame the presence of

potential confounders and/or effect modifiers concerning risk factors assessment and analysis in marijuana users.

The ecosocial theory not only provides a new perception in the study of potential effects of marijuana use on the liver but also guides to understand that during the human course of life, risk factors are embodied and altered the biology of gene expression, which are a source of diseases.

Marijuana Use: State of the Problem

Marijuana is the oldest and most widely used illicit drug in the world (NIDA, 2015). Historically, it was available in the U.S. around 1900 (Chasteen, 2016), and as of 2015, more than 11 million Americans used the drug (NIDA, 2015). With the increasing decriminalization of marijuana, it is imperative to reevaluate the health risks and health benefits associated with the drug.

In recent years, there has been a change in perceived risk associated with the use of marijuana, and people who believe that marijuana use is associated with health risk is decreasing (Okaneku et al., 2015). Okeneku, et al. (2015) analyzed the data from 2002 to 2012 from the National Survey on Drug Use and Health (NSDUH) and found a significant decrease in perceived risk in occasional and regular marijuana users. When the model was stratified by age, gender, and their past month of use, the study revealed that decreases in the perceived risk of marijuana use were associated with younger age, male gender, and past their month of use. The study associated such decreases in risk perception to the change in marijuana laws. There was a marginally significant increase in young adults' acceptance of marijuana use because of the implementation of medical

marijuana laws and a perception that marijuana use has no or low risk (Wen, Hockenberry, & Druss, 2018). In Washington and Colorado, where recreational marijuana has been legalized, Washington has seen an increase in adolescent marijuana use when Colorado pre- and post-legalization saw no change (Cerdá et al., 2017).

Marijuana use is on the rise compared to the other illicit drug use in the U.S. In 2011, the Drug Abuse Warning Network (DAWN) reported 455,668 emergency department visits related to marijuana use, representing a 52% increase between 2004 and 2011 and 36% of all non-alcohol illicit drug emergency department visits (SAMHSA, 2014). In 2015, the SAMHSA reported 157,733 marijuana/hashish-related admissions in hospitals or residential service treatments. Overall, the prevalence of marijuana consumption more than doubled from 2001-2002 to 2012-2013 (SAMHSA, 2014). This significant increase has been observed across all population subgroups (sex, age, race/ethnicity, education, marital status, income, urban/rural, and region) (SAMHSA, 2014). During that same period, there was a significant increase in marijuana use disorders (Hasin et al., 2015). Interestingly, between 1990-1991 and 2001-2002, the prevalence of marijuana was stable with an increase in marijuana use related disorders. This inverse result seen during the two-study periods suggested the presence of a new factor, which several scholars have associated with the increase in potency of marijuana. In fact, ElSohly et al. (2016) found that the THC potency in marijuana on the illicit market has consistently increased from approximately 4% in 1995 to 12% in 2014 .When assessing the issues related to such an increase in potency in illicit marijuana, Wolkow et

al. (2014) concluded that the health consequences of marijuana may be worse now than in the past.

Although multiple studies have reported adverse effects from the use of marijuana, many other studies have found positive health effects from the use of the drug. Wolkow et al. (2014) published a report that addressed the adverse effects of marijuana use, including its effects on the brain development, the risk of cognitive impairment, altered mental health, diminished life satisfaction, poor educational outcomes, and the development of symptoms of chronic bronchitis. The report also acknowledges the health benefit of marijuana use, including its ability to relieve the symptoms of glaucoma, nausea, chronic pain, inflammation, multiple sclerosis, and epilepsy. However, Volkow and his colleagues suggested making better use of the medical benefits of marijuana to avoid exposing people who are sick to the intrinsic negative effects of the drug.

Although some studies have reported beneficial health effects of marijuana, and other reported adverse health effects, the issues associated with the health effects of marijuana on the human body remain the subject of heated debates among scientist and policymakers.

An Overview of Liver Diseases and Risk Factors

The major causes of liver disease are related to a variety of factors, include an increase and continued consumption of alcohol, autoimmune disorders, viral infections, drug-related causes and non-alcoholic accumulation of fat in the liver cells. (Fumeaux et al., 2018; Wang, Fan, Zhang, Gao, & Wang, 2014). Common liver diseases are hepatitis B virus (HBV), HCV, alcoholic and nonalcoholic liver disease, cirrhosis and

hepatocellular carcinoma (Wang et al., 2014). The liver is the largest and the most important organ in the human body. It performs more than 500 vital functions that range from cleaning toxins from the blood and providing the body with nutrients to storing energy by participating in the metabolism of carbohydrate, lipid, and protein (Snyder, 2016; Woldin, 2014).

It is important to note that liver disease can be acute or chronic. Acute liver disease can develop into chronic. For example, viral hepatitis such as hepatitis A through E are considered acute viral diseases. However, Hepatitis B and C can progress into chronic hepatitis due to the continued and longtime inflammation of the liver (Snyder, 2016). In addition, heavy alcohol consumption can also progress from acute liver disease to a chronic liver disease which is manifested by cirrhosis of the liver (Snyder, 2016; Woldin, 2014). In brief, three different scenarios can occur when someone is diagnosed with acute liver disease. Either the liver recovers from the acute inflammation process within a few weeks or few months or gets worse very quickly (fulminant hepatitis). In certain other conditions, acute liver disease can elevate to a chronic disease such as chronic hepatitis-C or cirrhosis (Snyder, 2016) which can lead to liver cancer or liver failure.

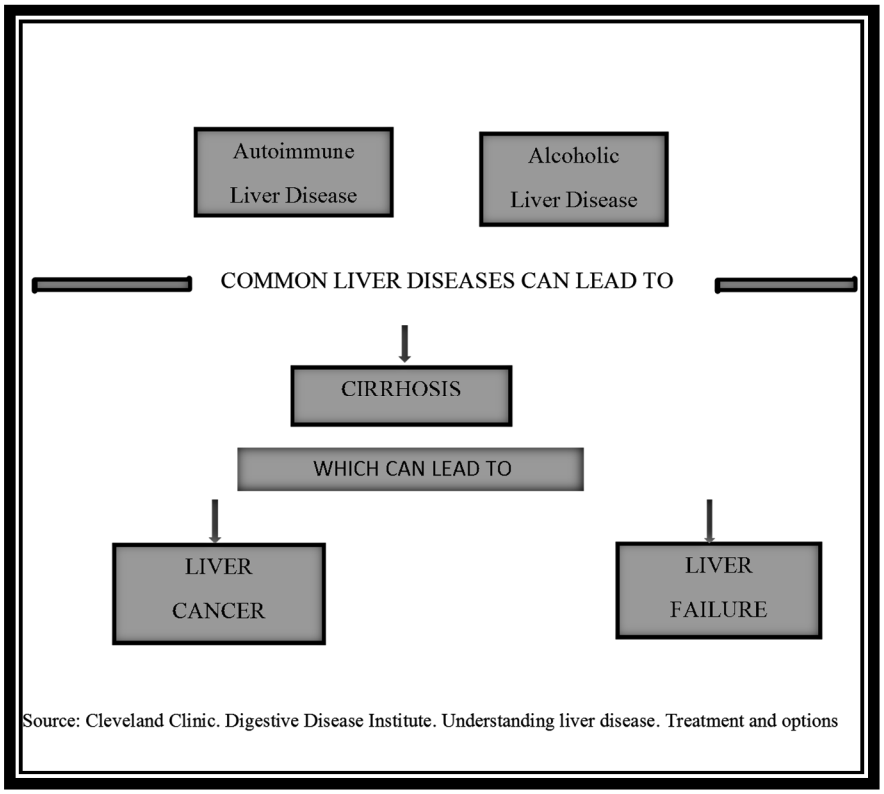


Figure 2. Liver diseases.

Current Studies on Marijuana Use and Liver Disease

Several studies in recent years are finding more about the positive health effects of marijuana (Adejumo et al., 2017; Kim et al., 2017). However, the question remains whether the health benefits of marijuana seen in recent years outweigh the negative health effects that have pushed U.S. health institutions and institutions around the world to consider marijuana as a Schedule I drug (SAMHSA, 2014). Findings of the health effects of marijuana on the liver vary from negative, positive, or no significant health effects, depending on preexisting liver conditions of the population studied (Kim et al., 2017; Pateria, de Boer, & MacQuillan, 2013). Most of the researchers evaluated the

effects of the marijuana use on individuals with preexisting liver conditions such as NAFLD, hepatitis C, and liver cirrhosis and have found different results.

The Effect of Marijuana on Nonalcoholic Fatty Liver Disease (NAFLD)

NAFLD is an inflammation of the liver that is not caused by alcohol but by the accumulation of fat in the liver (Bull, 2013). NAFLD can progress into nonalcoholic steatohepatitis (NASH) which is more prone to the development of cirrhosis (Pattnaik et al., 2018). Possible risk factors are diabetes mellitus, metabolic syndrome, high blood pressure and hyperlipidemia (Al-Dayyat, et al., 2018; Pattnaik et al., 2018). With the increasing level of obesity, NAFLD has emerged as the most common chronic liver disease in the developed world (Ajmera & Loomba, 2017). With the exception of a few clinical studies published before 2010 (Purohit et al., 2010) that found that smoking of marijuana may be detrimental to the liver and increase the risk of developing NAFLD, most recent epidemiological studies are finding that the consumption of marijuana has a therapeutic effect on the health of the liver (Adejumo et al., 2017; Kim et al., 2017). When analyzed the function of the endocannabinoid system that comprises the CB1 and CB2 receptors, a clinical study found that marijuana use may be detrimental and constitute a risk factor for NAFLD (Purohit et al., 2010). CB1 and CB2 are phytocannabinoids receptors of THC, the principal cannabinoid compound found in marijuana (Mallat & Lotersztajn, 2010). CB1 and CB2 receptors play a key role in hepatic steatosis, hepatic inflammation, and liver fibrogenesis (Alswat, 2013). No recent population-based studies, at least up to and included in this review, have found detrimental effects of marijuana on NAFLD. Instead, several recent population-based

studies have found therapeutic health effects associated with marijuana on the liver of NAFLD patients. In a cross-sectional study, when exploring the 2014 National Inpatient Sample (NIS) data to analyze the possible association between cannabis use and NAFLD, Adejumo et al. (2018) indicated that cannabis use is associated with a significantly lower prevalence of NAFLD with an adjusted odds ratio of 0.82. The findings suggest that cannabis has a suppression or reversal effect on the development of NAFLD. These findings are in line with the study results conducted by Donghee et al. (2017). Both studies used different data, though they found the same therapeutic effect of cannabis as a result of their investigations. When using the NHANES data, Donghee et al. (2017) found that marijuana users were less likely to be suspected of having NAFLD with an odds ratio (OR) of 0.90 (95% CI: [0.82-0.99]), and 0.68 (95% CI: [0.58-0.80]), respectively, for past and current users. The analysis of age, gender, and an ethnicity-adjusted model of ultrasonography-diagnosed data also revealed an inverse association between NAFLD and marijuana with an OR of 0.75 (95% CI: [0.57-0.98]) for current users. A similar trend was observed when current light and heavy users were compared to non-users. The results of the study also strongly suggested that the inverse association observed is independent of BMI value, educational level, economic status, smoking status, alcohol consumption, diabetes, hypertension and current use of cocaine. This independent association of marijuana with NAFLD was also the result of a population-based study conducted by Adeyinka et al. (2017), which reported a lower prevalence of NAFLD in cannabis users utilizing the Nationwide Inpatient Sample (NIS) of the Healthcare Cost and Utilization Project (HCUP). In addition to comparing cannabis users to nonusers, this

latter study has differentiated between dependent cannabis users and nondependent cannabis users and found a dose-response effect of cannabis on NAFLD. Dependent cannabis users are less likely to have NAFLD when compared to nondependent cannabis users. Because alcohol is known as a predictor of a higher prevalence of NAFLD (Adeyinka et al., 2017), the analysis of cannabis use on NAFLD in alcohol-dependent and nondependent patients has indicated that only nondependent alcohol users gain the benefit of the therapeutic effect of cannabis use.

The Effect of Marijuana use on Hepatitis C

Like in NAFLD, patients with hepatitis-C infection can also advance to the fibrosis stage or liver steatosis (Liu et al., 2014). With knowledge of the health effects of substance abuse on the liver, several researchers also examined the health effects of marijuana on the liver of hepatitis-C patients, and interestingly found conflicting results.

A recent Canadian study found that marijuana use is not a predictor of liver steatosis, inflammation or the advancement to the fibrosis stage in hepatitis-C patients using liver biopsy data of 550 patients with whom 159 individuals were self-reported marijuana users. Instead, the study found that the age of the patients, HIV seropositive status, and history of intravenous drug use were predictors to the advanced stage of fibrosis in hepatitis-C patients (Liu et al., 2014). Unlike the above study, while examining the NIS of U.S. adult patients infected with chronic hepatitis C virus, Adejumo et al. (2018) demonstrated that cannabis use reduced the incidence of cirrhosis caused by HCV infection. The study also demonstrated that cannabis use reduced the prevalence of cirrhosis due to HCV infection including a reduction in the prevalence of ascites, variceal

bleeding, hepatorenal syndrome, hepatic encephalopathy, portal hypertension, and jaundice. Overall, reduction in inpatient mortality, length of hospital stay, and total health care costs have all been associated with marijuana use in HCV infection patients (Adejumo et al., 2018).

Marijuana and the Key Independent Variables

This portion of the existing literature review examines the influence of the age of marijuana user, the age of initiation of marijuana use, the quantity and the duration of marijuana use on the health condition of the liver.

Marijuana, the Age Factor, and Liver Disease

The age of marijuana users and the age of onset of marijuana use are at the core of the discussions regarding the effects of marijuana on human health (Johnson et al., 2015). Age has been found to be a risk factor for many chronic diseases including liver diseases. For example, Cheng et al., (2013) found increased prevalence of metabolic syndrome and fatty liver disease among the elderly population. In the United States, there was a modest decrease in the prevalence of marijuana use from 1999 to 2009, yet there was a steady increase since 2009. During that same period, adolescent use of marijuana has also decreased (Johnson et al., 2015). However, the age-specific onset of marijuana use has varied throughout recent history. The percentage of adolescents who tried marijuana before the age of 13 years increased from 1991 to 1999 but has seen a steady decrease from 1999 to 2015 (YRBSS, 2017). When assessing how marijuana affects the prevalence of NAFLD in two different subgroups (< 40 vs. > 40), the study found that the younger population (less than 40 years old) who heavily used marijuana displayed a 35%

reduction in risk of prevalence of NAFLD compared to a 26% reduction in the older population (greater than 40 years old) (Kim et al., 2017). Contrary to the above findings, Kotan et al. (2017) presented the results of the effect of cannabis use on 34 cannabis users who used cannabis for the first time at age 21.8 years (*SD* 5.0); after an average of more than 30.5 months of use, the patients still displaying close to normal liver function parameters. In other hands, Quraishi et al. (2013) found that at the age of initiation of 15.31 years (SD 4.7), and after using cannabis for more than 9.53 years (*SD* 8.06), 51% of cannabis dependent patients showed abnormal liver-related parameters. These findings suggested that the age of marijuana users, the age of initiation and the duration of the substance use introduced some degree of variation in the effects of marijuana use on the health condition of the liver.

The Duration of Marijuana Use and Liver Disease

Addressing the marijuana-related health issues requires a better understanding of how much and how long people use marijuana during their lifetime and the degree to which it was used (NIDA, 2018). Terry-McElrath et al. (2017) associated increased risks of negative health outcomes in 50 years old marijuana users with moderate or heavy long-term use of marijuana. As previously mentioned, Quraishi et al. (2013) and Kotan et al. (2017) have shown that the duration of cannabis use is a predictor of the impact on liver health associated with the use of marijuana. Kotan et al. (2017) have found no negative health outcome at a mean duration of cannabis use of 30.5 months whereas Quraishi et al. (2013) found detrimental health effects at a longer duration of cannabis use of 9.53 years (*SD* 8.06). In addition, a Sudanese case-control study showed a strong

correlation between the duration of marijuana use and liver enzyme activity. The study found a significant difference in the activity of the enzymes of the control group compared to the subject group using 120 samples (60 samples of abusers and 60 samples of control cases) (Mohamed et al., 2015).

The Quantity of Marijuana Use and Liver Disease

The quantity of marijuana use is another factor at the core of the health effects of marijuana on users (Terry-McElrath et al., 2017). Out of 14,080 NHANES participants, Donghee et al. (2017) found that 56.1%, 36.9%, and 7% respectively reported never used, used in the past, and current users of marijuana. Of the 7 percent who reported currently using marijuana, light users represented 4.9 %, while heavy users represented 2.1%. When the study assessed the association between dose-dependent marijuana consumption and suspected NAFLD, the prevalence rates of suspected NAFLD were 30.5%, 38.0 %, and 40.7 %, respectively, in current light users, past users and those individuals who never used. The results were consistent when ultrasonography diagnosed NAFLD data, with 23.2%, 29.4%, and 35.0%, respectively, in current light users, past users and those individuals who never used. In brief, it has become apparent that current or past marijuana use has been significantly associated with a lower risk of the suspected NAFLD. In addition, when the study assessed only current users, light users were reportedly inversely associated with NAFLD, while there was no significant association with heavy users due to the small number of heavy users among the participants. The results of this study were consistent with the results of a study conducted by Adejumo et al. (2017), which analyzed the association with NAFLD in three categories of users (non-

cannabis users, nondependent cannabis users, and dependent cannabis users) using the 2014 HCUP data of 5,950,391 participants. After adjusting for possible covariates, the study found a 43 % lower prevalence (AOR: 0.57[0.42–0.77]; $p < 0.0001$) of NAFLD in cannabis-dependent users compared to nondependent users, suggesting that heavy cannabis use has a positive effect factor on the prevalence of NAFLD. In addition to NAFLD patients, a study conducted in Canada found that in HCV positive patients, cannabis users have the lowest frequencies of liver cirrhosis when compared to nonusers. In a dependent and nondependent category comparison, the prevalence of liver cirrhosis decreased by 15 % and 48% respectively among nondependent and dependent users (Adejumo et al., 2018). By contrast, Liu et al. (2014) found no significant difference in biopsy fibrosis, liver inflammation, and steatosis in 21 HCV positive patients classified as "high daily marijuana users" (> 1 g /day for marijuana use) compared to non-current marijuana users. Terry-McElrath et al. (2017) also demonstrated that the intensity of marijuana smoked is a strong predictor of negative health outcomes at 50-year-old individuals.

Marijuana and the key outcome variables

The liver function test consists of testing the level of ALT, AST, the level of ALT/AST ratio (LSR), TB, GGT, and LDH in a serum sample (Huang, et al., 2017). Several other studies also introduced ALB, TP and ALP as part of the liver function test (Fumeaux et al., 2018).

ALT and Marijuana

ALT is a circulating transaminase in the human body and a specific marker for liver dysfunction (Huang et al., 2017). The enzyme activity is influenced by clinical factors including hepatitis-related diseases, alcohol consumption, disease states such as NAFLD, and certain medications and physiological factors such as extreme physical exertion (Z. Liu, Que, Xu, & Peng, 2014). Socio-demographic factors such as age, gender, and ethnicity may also interfere with the enzyme activity (Kim et al., 2017; Ruhl & Everhart, 2012). Although the enzyme is measured to assess overall health (Z. Liu et al.,2014), the elevated level is often observed in liver dysfunction meaning that the enzyme is more specific to liver disease (Marshall et al., 2014). Per Mohamed et al. (2015) chronic marijuana use is associated with the hepatic enzymatic alteration. Mohamed et al. (2015) conducted a case-control study of 60 people with a history of cannabis use and 60 samples in a control group. Age and sex were matched with age ranging from 18 to 60 years old. The study excluded people with liver cirrhosis, hepatitis, jaundice, hepatomegaly, and liver carcinoma and found a significant statistical difference in ALT between the two groups. The study concluded that cannabinoids are possible hepatotoxic substances. By contrast, Kotan et al. (2017) found a normal ALT level (mean 28.4, *SD* 18.9, normal ALT < 45) in 118 Indians male cannabis users who used cannabis for more than 30 months. In cannabis-dependent users, Quraishi et al. (2013) also found that, although it is difficult to solely isolate cannabis-dependent subjects, cannabis-dependent with co-morbid substance use showed an elevated ALT level in 51 subjects with a 17.6% increase compared to the control group. Contrary to its toxicity effect

presented in non-liver disease patients, cannabis may have a therapeutic effect by normalizing the level of ALT in NAFLD (Kim et al., 2017). In a cross-sectional study, Kim et al. (2017) found a statistically significant difference in ALT levels between current cannabis users and past users and those individuals who never used cannabis who are suffering from NAFLD. Heavy cannabis users with NAFLD showed a normal low ALT level (28.0 *SD* 2.0, $p < 0.001$) compared to light users (30.5 *SD* 0.8, $p < 0.001$). A significant statistical difference has also been seen in the ALT levels across gender between current, and past users and those who never used presenting with NAFLD.

AST and Marijuana

Like ALT, the serum level of AST also increases during liver dysfunction. However, AST is less specific to the liver compared to ALT. Apart from being elevated in liver diseases, the activity of the enzyme is also influenced by the state of particular disease such as in myocardial infarction, muscular dystrophy, pulmonary emboli, and in acute pancreatitis (Marshall et al., 2014).

Demographic factors such as age, gender and ethnicity are also important when evaluating the activity of AST (Kim et al., 2017; Ruhl & Everhart, 2012). It is also known that marijuana alters the effect of AST (Mohamed et al., 2015). Mohamed et al. (2015) elucidated in a case-control study involving 60 subjects that the AST level in marijuana users is significantly different compared to the control group in a Sudanese population. The study then concluded that a possible alteration effect of marijuana on AST exists. Contrary to the above finding, Kotan et al. (2017) discovered a normal level of AST (31.2, *SD* 22.0, Normal AST < 37) in 118 Indians male cannabis users who used

cannabis for more than 30 months. In another case-control study, Quraishi et al. (2013) found that cannabis dependent users with comorbid substance use had elevated AST activity with 33.33% of the study subjects showing abnormal values of AST. By contrast, Rahmayanti et al. (2017) revealed that most of the patients who used cannabis and other drug exhibited normal AST levels. The study evaluated cannabis use with comorbid drug users in more than 823,810 cases with more than 50% using cannabis and found that most of the subjects (88.83%) displayed within normal range serum AST level. Only 1.12% of participants showed below normal range AST level, and 11.6% of the participants displayed AST levels that are above the normal range. Other studies like the one conducted by Muniyappa et al. (2013) found no effect of cannabis on AST. In a cross-sectional, case-control study, involving 30 cannabis smokers (12 women, 18 men, Average age = 27 years, *SD* 8) and 30 control subjects matched for age, sex, ethnicity and BMI, Muniyappa et al. (2013) found no statistical difference between the AST of cannabis smokers and nonsmokers (control group).

ALP and Marijuana

ALP is another enzyme used as part of liver function tests to evaluate possible dysfunction of the liver (Bishop et al., 2018; Lowe & John, 2018). A non-specific enzyme, 80% of ALP found in the serum originates from the liver and bones and in small amounts from the intestine. In most adult patients, an elevated ALP is an indicator of liver disease (Lowe & John, 2018). The clinical significance of ALP activity lies in the diagnostic of cholestatic liver disease (Bishop et al., 2018). The enzyme serves as a marker of extrahepatic cholestasis such as stone in the bile duct or intrahepatic

cholestasis such as drug-induced cholestasis or biliary cirrhosis (Bishop et al., 2018; Lowe & John, 2018; Sharma et al., 2014). However, the interpretation of ALP is difficult because variation in the enzyme activity can occur in different liver conditions including liver cirrhosis, chronic hepatitis, viral hepatitis and in the absence of liver damage such as congestive heart failure, related bone disorders and in primary and metastatic cancer (Bishop et al., 2018; Lowe & John, 2018). Age, gender, and ethnicity are demographic factors associated with variations in ALP activity. The ALP level is slightly higher in men compared to women, and it decreases in the 15 to 50 age group, then and increases again in the old age (Lowe & John, 2018). A positive association has also been found between ALP body weight and smoking (Bishop et al., 2018; Lowe & John, 2018). Marijuana has been shown to be strongly correlated with an increase in ALP level in a case-control study in the Sudanese population using 60 patients who used cannabis for more than ten years matched with 60 controls non-smoker subjects. Chronic smokers showed a significant increase in ALP compared to non-smokers (Mohamed et al., 2015). A study conducted in India in 34 cannabis users (mean duration of cannabis use was 30.5 months; *SD* 31.8) showed normal ALP serum level (Mean ALP = 73.4, *SD* 30.0; ALP normal < 136; Kotan et al., 2017). Contrary to the above finding, Quraishi et al. (2013) found a significant increase in ALP in cannabis-dependent subjects pointing to an abnormality in liver function due to cannabis use. A total of 51 substance-using subjects with a mean duration of cannabis use of 9.53 years (*SD* 8.06 years) and 30 control subjects were used during the study. Findings revealed an elevation of 37.25% in

substance-using subjects compared to the control group (control group 98.82, *SD* 26.46; subjects' group 217.53, *SD* 95.84., $p < 0.05$)

TB and Marijuana

Bilirubin is a breakdown of old and damaged red cells collected in the liver. Sometimes the body can have an excess of bilirubin, a condition called hyperbilirubinemia and recognized as jaundice (yellow discoloration of the skin, eyes and mucous membranes) when the excess is accumulated in the tissue (Bishop et al., 2018). Clinical conditions including liver disorders, liver infections such as hepatitis, cholestasis and Gilbert syndrome may increase the level of bilirubin in human serum. However, medications, such as phenobarbital and theophylline, contribute to lower levels of bilirubin in the human body (Bishop et al., 2018; VanWagner & Green, 2015).

Bilirubin is part of a liver function test to evaluate a possible disorder of the liver (Bishop, 2018). Factors other than liver disease may cause an increase in bilirubin. So, it is important to know the etiology of the Jaundice, (VanWagner & Green, 2015; Bishop, 2018). In prehepatic jaundice such as in acute and chronic hemolytic anemia and post-hepatic anemia including gallstones and tumors, the liver should be ruled out as the primary cause of the increased level of bilirubin (Bishop, 2018). In hepatic jaundice, the primary cause of elevated bilirubin is due to liver diseases or disorders related to bilirubin metabolism and transport which are functions intrinsic to the liver (Bishop, 2018). Among factors that increase the level of bilirubin, cannabis is a contributing factor. In India, a case-control study involving 250 male subjects (125 cannabis abusers and 125 control group) found a significant difference in the mean value of TB when the two

groups were compared. Cannabis abusers exhibited a TB value of 14.78 (*SD* 3.10) compared to 11.1 (*SD* 3.23) in the control group (Wani et al., 2017). However, in a cross-sectional study involving 34 cannabis users (Average age 21.8 years, *SD* 5.0; duration of cannabis use 30.5 months, *SD* 31.8), Kotan et al. (2017) showed that cannabis users presented a normal value TB levels (normal TB: 0.3- 1.2 mg/dl). By contrast, Quraishi et al. (2013) found that the consumption of cannabis increases the level of serum bilirubin by 13.72% compared to noncannabis users using a case-control study in 30 control subjects and 51 cannabis dependent subjects (Average age of initiation 15.31 years, *SD* 4.7; duration of cannabis use 9.53 years *SD* 8.06).

TP and Marijuana

The serum TP, which is mainly synthesized by the liver, is of great importance because it serves in the regulation of several physiological functions, maintaining the osmotic pressure, transport of various metabolites, and participation in the activity of the immune system (Bishop, 2018). The level of serum TP gradually decreases with age and varies across gender (Tian, Qian, Shen, Li, & Wen, 2014). The evaluation of serum TP is useful to assess the synthetic ability of the liver. Although the protein level is not a sensitive marker for liver damage, its useful in quantifying the severity of liver dysfunction (Bishop et al., 2018). Findings of the health effect of marijuana on the Total serum protein in adults varies across studies. A case-control study conducted in India which assessed the level of serum TP in 125 cannabis abusers, compared 125 non-cannabis smokers and found a decreased level of serum TP in cannabis abuser compared to non-smokers (Wani et al., 2017). Unlike the previous study, Quraishi et al. (2013)

found in another case-control study involving 51 cannabis-dependent participants and 30 control subjects that smoking cannabis increased the level of serum TP by 15.68%.

ALB and Marijuana

Synthesized by the liver, albumin is the major type of protein in human serum. It's involved in maintaining proper osmotic pressure and in the transport of various substances through the body (Bishop et al., 2018; Morman & Varacallo, 2018). A low concentration of albumin is most commonly associated with potential liver disease (Morman & Varacallo, 2018). Illegal drug use such as cannabis has been shown to decrease the level of albumin in human serum. In a recent case-control study, Quraishi et al. (2013) demonstrated the presence of a low albumin level in cannabis dependent patients compared to non-users with a mean duration of cannabis use being 9.53 years and the mean age of initiation being 15.31 years. Like the previous study, Wani et al. (2017) also found a lower score of albumin level in cannabis users compared to non-users. In a different cohort, with age of initiation 21.8 years and the duration of cannabis use being 30.5 months, Kotan et al. (2017) found a normal level of albumin in 34 participants with average ALB level of 4.20g/dl (SD 0.6, normal range 3.5-5.2g/dl).

GGT and Marijuana

Elevated activity of GGT is found in liver dysfunction, hepatobiliary disorders and chronic alcohol consumption (Koenig & Seneff, 2015; Bishop., 2018). Drugs such as warfarin, phenobarbital, and phenytoin are noted to increase the enzyme level. Marijuana consumption is also noted to affect the level of GGT. Wani et al. (2017) noted in a case-control study of 250 male participants (125 cannabis abusers and 125 control group,

mean age 25.32 years) that cannabis abusers exhibited a higher level of GGT compared to non-users. However, 34 participants with a mean age of 21.8 years who use cannabis for 30.5 months (SD 31.8), exhibited a normal level of GGT of an average 24.6 (SD 13.1, Normal range < 55 u/l; Kotan et al., 2017).

Summary of the Literature Review

Four major factors are at the core of the increasing health problems associated with marijuana, including: (1) increasing decriminalization of the drug, (2) decrease in perceived risks seen in young male subjects, (3) increase in potency of illegal marijuana from 4 to 12% in recent years, and (4) the increased prevalence of marijuana consumption, which has more than doubled in recent years. Despite these four core issues, epidemiological study findings of the health effects of marijuana are conflicting. Several studies reported the therapeutic effect of the drug with respect to certain diseases, while others are still warning about its adverse health effects and have recommended more epidemiological investigations.

It is important to point out that in this literature review, the health effect of marijuana on the liver has been more studied on individuals with preexisting liver conditions including NAFLD, NASH, and hepatitis-C. The available literature on the effect of marijuana on the liver of healthy participants is rare.

Finally, several studies have shown that the quantity of marijuana smoked, the age of initiation and the duration of marijuana use are critical factors in assessing the health effects of marijuana on the liver. These studies revealed that marijuana consumption causes some variations in the enzymatic activity of the liver.

Chapter 3: Research Method

This chapter provides explanation for the different steps in the data analysis process from the data collection to the testing of the above hypotheses. I explain the rationale of the epidemiological design chosen to guide the study, the sampling procedure, the data collection procedure, and how the variables were measured and coded. Finally, Chapter 3 presents the data analysis plan, which includes a procedure for how the dataset was cleaned to make it appropriate to analyze and how the hypotheses were tested. The data analysis plan gives an overview of all assumptions necessary to use Linear regression analysis to test the hypotheses

Study Design and Rationale

Study designs are key factors needed to appropriately address the study questions. Choosing an inappropriate study design has the potential to undermine the study validity, which is critical in determining the scientific value of any effective research study (Munnangi & Boktor, 2018). Thorough planning and accurate identification of study factors and study subjects are important in selecting an adequate study design (Szklo & Nieto, 2014). The goal of this section is to explain the ground on which a cross-sectional study design is chosen to elucidate the study questions and how it is to be applied to answer the study questions. In addition, the advantages and disadvantages of using a cross-sectional study design in the context of the project are also presented. To understand the relation between the disease factors and specific behavior in group of people, or the cause of diseases, or the disease patterns, epidemiologic scholars established specific epidemiologic study designs that helps to appropriately address

possible questions during epidemiological events (Friis & Sellers, 2009; Munnangi & Boktor, 2018; Szklo & Nieto, 2014).

As stated previously, I designed this study to evaluate the predictors of the health effects of marijuana on the hepatic function. The study addresses the question of whether the dose of marijuana use, the age of initiation of marijuana use, and the duration of use are associated with liver dysfunction. Three independents variables, seven dependent variables, and four controlled variables were manipulated to answer the study questions. The independent variables included the number of joints or pipes smoked daily, the age of initiation, and the duration of marijuana use. The dependent variables were the liver function parameters, which comprise the serum level of ALT, AST, ALB, ALP, TB, TP, and the GGT. The four controlled variables were the age of the participants, their gender, the average alcohol consumed daily and the BMI of the participants.

An effective study design depends on the intent of the investigator, the unit of analysis and the time dimension (Friis & Sellers, 2009; Szklo & Nieto, 2014). For instance, if the investigator is willing to control the exposure of interest, the best option to address study questions is to opt for experimental study design. Otherwise, the observational study design is more appropriate (Friis & Sellers, 2009). In an experimental study design, the investigator has control over the research setting and randomly assign subjects to the exposed and non-exposed group (Friis & Sellers, 2009). Contrary to the experimental study design, in an observational study, the investigator has no control over the circumstances in the research setting and does not control the exposure of interest or manipulate the study subjects (Friis & Sellers, 2009; Munnangi & Boktor, 2018; Szklo &

Nieto, 2014). The observational study design moves closer to the cross-sectional study design. Depending on the unit of analysis and the temporal dimension of the study, an observational study design can be subcategorized into a cohort study, case-control study and a cross-sectional study (Babby, 2017; Munnangi & Boktor, 2018). In all three study designs, the exposure is measured at the individual level. In cross-sectional study designs, the exposure and disease are measured at the individual level at a single point of time (Friis & Sellers, 2009; Munnangi & Boktor, 2018, Szklo & Nieto, 2014). Cross-sectional studies, used to assess the prevalence of the disease in a population, do not require a follow-up period and cannot provide a cause-and-effect relationship (Munnangi & Boktor, 2018).

The dataset used in this study was self-reported data, which includes exposure and outcome information at an individual level at a specific point of time. For that reason, it was more appropriate, in the case of these types of data to explore a cross-sectional study design to answer the study questions. Furthermore, the intent of this study was not to establish a cause-and-effect relationship but to assess a possible association between predictors of the health effects of marijuana and hepatic function. Correlation data analysis is an appropriate tool to evaluate such an association. According to Lau (2017), the correlational study can be used to determine the prevalence and to predict future events based on known data. A correlational study is more concerned about establishing a relationship between exposure and outcome variables without any attempt to influence them (Asamoah, 2014; Lau, 2017). In correlational studies, researchers need to identify the study variables, establish study questions and hypotheses, select appropriate sample

and data, calculate correlation, and finally report and interpret results (Asamoah, 2014; Lau, 2017). In this study, the variables, the study questions, the hypothesis, and the data source have already been mentioned. However, the statistical technique that allowed establishing possible correlation has not yet been adequately addressed.

This study, I used statistical techniques to test the hypotheses that answered the study questions. The choice of statistical techniques that adequately answered the study questions depended on the sample size, the type of research questions being asked, and the scale of measurement (Nayak & Hazra, 2011). The choice of linear regression to test the hypotheses in this study was based on two major factors. First, the goal was to establish a relationship between two or more variables, and secondly, the outcome variables were continuous variables. Assuming that all other assumptions were met, the two factors mentioned above were sufficient to adequately use linear regression. In this context, data were collected using multistage sampling techniques. So, instead of using a standard multiple regression, a complex sample general linear model (CSGLM) was used in SPSS to analyze the data. The CSGLM procedure performs linear regression analysis, as well as analysis of variance and covariance, for samples drawn by complex sampling methods (International Business Machines [IBM], 2017)

Stated simply, this study was an observational cross-sectional study by nature, which used a linear regression model to test the hypotheses. It is important to note that the study can only suggest that there is a relationship between two or more variables, it cannot imply causality.

Multiple Linear Regression

The regression model is important to describe the relationship between an outcome variable and one or more independent variables (Hosmer et al., 2013). Linear regression is used for that same purpose. It allows to prediction of variability in an outcome variable based on other variables (Kutner, 2004) However, linear regression differs from other regression models as it examines the association between one or more independent variable that are continuous or categorical and one continuous outcome variable (Kutner, 2004). The model follows the equation

$$Y = b_0 + b_1x_1 + b_2x_2 + \cdots + b_ix_i$$

Where: *Y is the expected value of the independent variable*

x_1 through x_i are i distinct independent or predictor variables

b_0 is the value of Y when all of the independent variables (x_1 through x_i) are equal to zero

b_1 through b_i are the estimated regression coefficients

Each estimate regression coefficient represents the change in the dependent variable for a one-unit change in the corresponding independent variable, holding all other independent variable constant (Kutner, 2004).

Methodology

The Study Population

The study population was drawn from the NHANES data from 2009 to 2016 data cycles. The NHANES contains health data from representative U.S. residents, or more specifically, the civilian noninstitutionalized U.S. population. The survey comprised

household sample screening, interviews, and physical examinations (NCHS, 2015b). Although the NHANES contains data on all health-related issues and all ages, I drew the sample for this study from participants aged 20 to 59 years old who have had contact with marijuana or hashish. I excluded the population sample with a history of HBV and HCV infections.

The NHANES relied on questionnaires and examination surveys to select and collect data on the target population. For the present study, questionnaires related to the age of the participants, the age at which the participants first tried marijuana or hashish , the time since the participants last used marijuana, the number of joints or pipes the participants use in a day, and how often the participants use marijuana were critical in compiling the study sample. Liver function test results performed in the laboratory on participant's blood samples during the physical examination process were also part of the study sample. These laboratory tests included the blood serum level of ALT, AST, ALB, TP, TB, ALP, and GGT.

Sampling and Sampling Procedures

The NHANES, conducted by NCHS at the Centers for Disease Control and Prevention (CDC) gathered health information on the U.S. noninstitutionalized population including the U.S. 50 states and the District of Columbia. The survey excluded people in custody in an institutional setting, all active-duty military personnel, active-duty family members living overseas, and all other U.S. citizens living outside of the U.S. (Johnson, Dohrmann, Burt & Mohadjer, 2014).

Established in 1970, the NHANES has evolved since 1999 to become a continuous health data collection program that collects data and releases data on a two-year cycle (NCHS, 2015a). The survey consists of a household screening, an interview process, and a physical examination at a medical examination center (MEC; Johnson, Dohrmann, Burt, & Mohadjer, 2014). The household screen determines if the household is eligible for an interview and a physical examination. The interview process collects person-level data on demographic factors, health, and nutrition information as well as household information. The third step that includes a physical examination helps to collect data on the participant's blood pressure, dental health status, and to collect blood samples for laboratory testing (Johnson et al., 2014). The NHANES gathered the health data through a complex multistage probability sampling design. The first stage is selecting the Primary Sampling Units (PSU) from all counties in the U.S. (NCHS, 2015b). In some cases, due to sample size requirements, some small counties or adjacent counties may be combined (Johnson et al., 2014). The second stage consists of selecting area segments comprising census blocks or combinations of census blocks containing cluster of households designed to produced equal sample size per PSU. (NCHS, 2015b). The third stage consists of selecting specific households and dormitories within each segment. The fourth stage consists of selecting persons in each sampled household. (Johnson et al., 2014). The NHANES oversampled some specific race, age, sex, and income subgroups to increase the precision rate (NCHS, 2015a; Johnson et al., 2014). For example, in the latest NHANES (2015-2016), Hispanic persons, non-Hispanic black persons, non-Hispanic Asian persons; non-Hispanic white and other persons who

reported race other than black, Asian or white aged 80 years and older and persons below 185% of the U.S. Department of Health and Human Services (HHS) guidelines were oversampled (NCHS, 2015a). Every year, the expected annual sample size is 6888 persons with 5000 participants expected to have the physical examination (Johnson et al., 2014). In the 2015-2016 survey cycle, the latest data cycle released, 15,327 persons were selected for NHANES from 30 different survey locations. Of those selected, 9,971 completed the interview and 9,544 were examined (NCHS, 2015b).

For the current study, the sample was compiled from the NHANES data from the 2009-2010 survey cycle to the 2015-2016 survey cycle which is a combination of 4 survey cycles over eight years of data collection. As part of this study, the sample selection was based on the participants who answered the drug questionnaire: "have you ever used marijuana or hashish." All participants who were 20 -59 years old, males or females, who answered "YES" to the drug-using questionnaires were included in the study. However, to reduce bias, I applied some exclusion criteria. Only the participants who used marijuana or hashish within the last 30 days of the survey were included and, people with severe hepatitis B and C disease were excluded from the study.

Sample Size Justification

Software such as G*Power, and SPSS sample power are available to evaluate the minimum sample size required to obtain acceptable power for a study validation. Although this software make the calculation of minimum sample size easy and less time consuming, multiple regression using the G*Power to determine the actual sample size required the R^2 (percentage of variability in the outcome variable that is explained by the

predictor variables). At this point in the study, these required data are not yet available. However, G*Power offers a priori calculation of the sample size base on the anticipated effect size, the desired statistical power and the number of predictors and an estimated R^2 of .5.

In this study, the calculation of the minimum sample size using the F-statistical test for multiple linear regression (Fixed model, R^2 deviation from zero) in G*Power yielded a sample size of 153. It's important to note that the calculation was performed following the established guideline of G*Power 3.1.7 (Faul et al., 2013) with alpha 0.05, a power of 0.95, and a medium effect size of 0.15 and R^2 of .5. for three predictor variables. A posthoc analysis of sample size was performed when data became available.

Data Collection Procedures

Started in 1960, the NHANES was designed to access the health status of the American people of all ages with various health conditions (NCHS, 2015a). From 1999, the survey started collecting data continuously and included nutritional risk factors as part of its inquiries (NCHS, 2015a). Each year, the survey sampled more than 5000 participants located in different counties across the United States (Johnson et al., 2014). Using the multistage sampling process to identify potential candidates, the survey followed three steps. The prescreening process helped to identify if a household qualifies for the interview process. The interviews were carried out at the participant's home by trained health professionals using the Computer-Assisted Personal Interview (CAPI) on questions related to sociodemographic, dietary health, and general health is a very delicate part of NHANES operations (NCHS, 2015b). The interviews were followed by a

physical examination at the MEC. Three MEC are often active in a given time in the U.S. which were designed to cover the 15 counties selected during any survey period (NCHS, 2015a). During the examination surveys, on top of conducting a dental, medical, physiological examinations and laboratory tests, the health professionals also gather data on the prevalence of chronic conditions on the participants. Data on risk factors associated with the participant's lifestyles, environmental factors, and hereditary conditions are also collected. Cigarette and tobacco smoking, alcohol consumption, drug use status, sexual practice, physical activities, reproductive health, contraception, breastfeeding practice, weight, and dietary intake data are also collected during the medical examinations (NCHS, 2015a).

The local health and government officials in an upcoming survey area are notified ahead of the survey. The participants receive letters from the director of the NCHS to introduce the survey (NCHS, 2015a). To increase and facilitate participation, the NHANES provide transportation to and from the MEC. Participants also receive compensation and a report of the medical examination findings. The Unique feature of the NHANES compared to other surveys in the US is the collection of the medical examination data on each participant. For that reason, the operation at the MEC is carefully designed (NCHS, 2015a). An average of approximately 450 persons are examined in each of the 15 locations during every survey cycle. To minimize cost and increase the response rate, the survey is set up to sample a larger number of persons within a selected household (NCHS, 2015a).

The NHANES data have been used in several instances to develop and monitor nutrition and health programs across the US. research organizations, universities, and health care providers. Health educators benefited from the NHANES data to study key health issues, monitor and develop health programs, implement awareness programs and reduce health-related risk factors (NCHS, 2015a). The NHANES data have been key factors in establishing a growth chart that was used nationally by pediatricians. The data have also been instrumental in the implementation of policy that contributed to the reduction of the level of lead in food and canned soft drinks (NCHS, 2015a). The trend in overweight prevalence, policy, and related programs and awareness were initially linked to NHANES data. National programs to reduce hypertension, cholesterol and undiagnosed diabetes have found their root in the NHANES data (Division of Nutrition, Physical Activity, and Obesity, 2014).

Gaining Access to the Data

The NHANES data were available at no cost on the CDC's website. All related documentations, including questionnaires, physical examinations, laboratory protocols, recommendations on analytical techniques were also available. The data were available in SAS format and need to be exported in the appropriate software format.

Variables Operationalization, Measurement, and Coding

Independent variables.

Number of joints or pipes of marijuana use daily was measured using the responses to the question "During the time you smoked marijuana or hashish, how many joints or pipes would you usually smoke in a day?" The answers were collected on a scale

of 1 per day, 2 per day, 3-5 per day, and 6 or more per day. The term marijuana included all forms of the drugs known as pot or grass either smoked as a cigarette (joints) or in a pipe or cooked. Hashish is a form of marijuana known as "hash" or hash oil. For analysis purposes, the categories 1 per day, 2 per day, 3-5 per day, and 6 or more per day were used as defined.

Age of initiation was measured using the responses to the question "How old were you when you started smoking marijuana or hashish at least once a month for one year? The answer to this question is known in NHANES as the age at which the participants started regularly smoking marijuana. In regard to this study, the age of initiation was the age at which someone started and continued regularly using marijuana. I relied on the recommended age categorization from the United Nations (UN) which was categorized into five groups, including under 15, 15-24, 25-44, 45-64, and 65+ (UN, 1982). For this study, I recoded the above categories into three categories, including teen (under 15 years old), youth (15 to 24 years old), and adult (25 to 59 years old).

Duration of marijuana use is the period in a lifetime when marijuana was used. The duration of marijuana used was calculated by subtracting the age since marijuana was last used from the current participant age. To account for only current users, I excluded any participants who had not used marijuana during the last 30 days from the analysis. The calculation of the duration of marijuana use also assumed that the participants continuously used marijuana since their time of the first contact. The age since marijuana was last used was measured in the NHANES by asking the question "How long has it been since you last smoked marijuana or hashish at least once a month

for one year?". For this study, I categorized the duration of marijuana use into five groups: under 10 years, 10 to 19 years, 20 to 29 years, 30 to 39 years, and greater than 40 years.

Dependent variables.

ALT levels were determined using laboratory standard testing methods. Collected plasma and serum samples were sent to the approved laboratory for testing. After appropriate storage and handling ALT levels were tested on approved calibrated instruments. The normal range for ALT expressed in international units per liter (IU/L) for males and females aged 20 years and older was 11 to 47 IU/L and 7- 30 IU/L respectively.

AST levels were determined using laboratory standard testing methods. Collected plasma and serum samples were sent to the approved laboratory for testing. After appropriate storage and handling, AST levels were tested on approved calibrated instruments. The normal range for AST expressed in international units per liter (IU/L) for males and females aged 20 years and older was 13 to 33.

ALB levels were determined using laboratory standard testing methods. Collected plasma and serum samples were sent to the approved laboratory for testing. After appropriate storage and handling, ALB levels were tested on approved calibrated instruments. The normal range for ALB expressed in gram per deciliter published by NHANES for males and females aged 18 years and older was 3.7 to 4.7 g/dL.

ALP levels were determined using laboratory standard testing methods. Collected plasma and serum samples were sent to the approved laboratory for testing. After

appropriate storage and handling ALP levels were tested on approved calibrated instruments. The normal range for ALP expressed in international units per liter published by NHANES for males and females aged 18 years and older was 36 to 113 IU/L.

TB levels were determined using laboratory standard testing methods. Collected plasma and serum samples were sent to the approved laboratory for testing. After appropriate storage and handling TB levels were tested on approved calibrated instruments. The normal range for TB expressed in milligrams per deciliter (mg/dL) published by NHANES for males and females aged 18 years and older was 0.2 to 1.3 mg/dL.

TP levels were determined using laboratory standard testing methods. Collected plasma and serum samples were sent to the approved laboratory for testing. After appropriate storage and handling, TP levels were tested on approved calibrated instruments. The normal range for TP expressed in grams per deciliter (g/dL) published by NHANES for males and females aged 18 years and older was 6.4 to 7.7 mg/dL.

GGT levels were determined using laboratory standard testing methods. Collected plasma and serum samples were sent to the approved laboratory for testing. After appropriate storage and handling, GGT levels were tested on approved calibrated instruments. The normal range for GGT expressed in International Units per Liter (IU/L) published by NHANES for males and females aged 18 years and older was 10 to 65 IU/L and 8 to 36 U/L respectively.

Control variables.

Alcohol use was measured through the question "In the past 12 months, on those days that you drank alcoholic beverages, on average, how many drinks did you have?" One drink was defined as 12 oz of beer, 5 oz of wine, or 1.5 oz of liquor with one alcoholic drink equivalent to any beverage containing 14 g of pure alcohol (Office of Disease Prevention and Health Promotion [ODPHP], n. d.). Following the dietary guidelines for the year 2015-2020, I categorized alcohol consumption into light drinkers (less than 2 drink per day), moderate drinkers (2 to 4 drinks per day) and heavy drinkers (4 or more drinks per day).

BMI is a calculation of the weight in kilograms divided by the height in meter squared. The weight and height were measured during the examination survey. The BMI was categorized following the guidelines recommended by the National Heart, Lung, and blood Institute (NHLBI, n.d.) where less than 18.5 was defined as underweight; between 18.5 and 24.9 as normal weight; 25 to 24.9 as overweight; and 30 or greater as obese.

Age: In the context of this study, age refers to the age of the participants at the date of screening.

Gender: In this study, the gender of the participants can be male or female.

Data Analysis Plan

I used the IBM SPPS statistic version 25 to analyze the data. The NHANES data was initially published in SAS software format. The SPSS 25 was used to import the data into the SPPS software format for analysis.

I based the interpretations of the results on the p value, the value of the R^2, the B coefficient (the slope coefficient) and the 95% confidence interval (95% CI). The B

coefficient represents the change in the dependent variable for a one-unit change in the independent variable while all other independent variables are kept constant (Laerd statistics, 2015). The *B* coefficient needs to be statistically significant to be included in the equation. *R*-squared (R^2) is the proportion of variance in the dependent variable that is accounted for by the independent variables (Laerd statistic, 2015).

Data Cleaning and Screening

Missing values. The presence of missing values in a dataset compromises the reliability of the study and leads to smaller sample size (Kwak & Kim, 2017). To obtain adequate data for analysis, and to avoid selection bias, I exclude all cases that had one or more missing values. I applied a listwise deletion technique in SPSS for a complete-case analysis.

Outliers. The presence of outliers in a sample data introduce bias that may lead to underestimation or overestimation of statistical results. Both univariate and multivariate outliers must be assessed and removed from the data sample to reduce possible bias (Kwak & Kim, 2017). I applied winsorization function and the Mahalanobis distance function in SPSS to identify, assess and reduce the effect of univariate outliers and multivariate outliers. The winsorization consists of replacing the value of the outlier that is being tested with an expected value, the largest of the second smallest value in the observation (Kwak & Kim, 2017). The Mahalanobis distance function is used to assess and reduce the effect of multivariate outliers.

In this study, I assessed the following research question and hypotheses and conducted a statistical analysis to evaluate the predictor of the health effects of marijuana on hepatic function:

Research question: Is there any significant association between the number of joints or pipes of marijuana smoked in a day, the age of initiation, the duration of use and each of the serum level of liver function markers including the serum levels of ALT, AST, ALB, ALP, TB, TP, and GGT while controlling for the age of participants, their gender, the average alcohol use and BMI?

$H_0$1: There is no significant association between the number of joints of marijuana smoked in a day, the age of initiation, the duration of use and each of the serum level of liver function markers including the serum levels of ALT, AST, ALB, ALP, TB, TP, and GGT while controlling for the age of participants, their gender, the average alcohol use and BMI.

$H_1$1: There is significant association between the number of joints of marijuana smoked in a day, the age of initiation, the duration of use and each of the serum level of liver function markers including the serum levels of ALT, AST, ALB, ALP, TB, TP, and GGT while controlling for the age of participants, their gender, the average alcohol use and BMI.

As stated earlier, I used CSGLM to analyze the data. For validity and inference purposes, linear regression requires that the dataset meets some basic assumptions. For instance, the outcome variable must be continuous, and one or more of the independent variables (s) must be measured either on a continuous or nominal scale. Furthermore, the

dependent variables and all independent variables must be mutually exclusive (Osborne, 2015). Other major assumptions that the dataset must meet to consider using linear regression as an appropriate statistical tool to test the hypotheses include the assumption of linearity, the assumption of normality, the assumption of no multicollinearity, the assumption of homoscedasticity and the assumption of no significant outlier, no high leverage points or no highly influential points (Osborne, 2015).

Major Assumptions of Linear Regression

Assumption of Linearity

In linear regression, the independent variables need to be linearly related to the dependent variable (Laerd statistic, 2015; Osborne, 2015). However, when in the presence of more than one independent variable, the independent variables collectively need to be linearly related to the dependent variable (Laerd statistic, 2015) . In this study, a scatterplot of the studentized residuals (SRE) against the (unstandardized) predicted values was used to evaluate the presence or lack of linearity. The assumption of linearity is met if the pattern of a scatterplot allows the presence of a straight line. Otherwise, the data is said to fail the assumption of linearity.

Assumption of Lack of Multicollinearity

Multicollinearity is basically due to the presence of a high correlation between one or more of the independent variables. Multicollinearity makes it difficult to identify which independent variable causes the variation in the dependent variable (Leard statistic, 2015). In this study, two values, the Tolerance (TOL) value and the variance inflation factor (VIF) value were used to assess the collinearity. A TOL of less than 0.10 or a VIF

greater than 10 implied the presence of collinearity (University of California, Los Angeles [UCLA], n. d).

Assumption of Normality

For inferential purposes, the error in prediction also knows as the residual needs to be normally distributed (Kutner et al., 2004). The normality was assessed in SPSS using Normal Q-Q Plot of the studentized residuals which plot two sets of quantiles again each other. The data is normally distributed if the scatterplot forms a straight line (Kutner et al., 2004). If the data is extremely skewed, the normality of the distribution may not be obtained. However, remediation techniques can be applied to obtain a normal distribution of the data (Kutner et al., 2004; Laerd statistic, 2015).

The Assumption of Homoscedasticity

Homoscedasticity is a condition required in a linear regression analysis where there are equal error variances for all values of the predicted independent variables. Homoscedasticity is evaluated in SPSS by plotting the studentized residuals (SRE) against the unstandardized predicted values (PRE). Homoscedasticity is present when the spread of the residual shows a particular pattern.

Assumption of Independence of Observations

One of the major assumptions when using the least square method for regression analysis is the lack of autocorrelation in the regression residuals (Kutner et al., 2004). The presence of autocorrelation generates regression estimates that may not be effective (Kutner et al., 2004). Autocorrelation in the residuals of the regression model has traditionally been estimated using the Durbin-Watson (DW) statistic (Bazilevsky, 2018).

I used SPSS command through the linear regression analysis to generate the DW values in this study.

Assumption of No Significant Outliers, High Leverage Points, or Highly Influential Points

As described earlier, I used the SPSS univariable outlier identification function to assess univariates outliers and I also applied Mahalanobis distance evaluation technique to identify and assess the effect of multivariate outliers. The leverage and the influential point were measured using Cook's distance. Cook's distance gives information on the residual and the influential point (UCLA, n. d). The lowest value of the Cook's distance is zero, and the higher the Cook's distance is, the more influential the point is. The cutoff point of Cook's distance is 4/n, where n is the number of observations (UCLA, n. d).

I conducted a descriptive statistical analysis to better understand of the dataset and its different variations. The statistical description included assessing the mean, median, mode, variance, the maximum and the minimum values, the skewness and the kurtosis of each variable of the study population. I assessed the adequacy of the model and evaluated the contribution of each independent variable to the model, and finally interpreted the study results. During this study, I set the significance level (p-value) to 0.05 meaning that the probability of rejecting a null hypothesis when it was true is 0.05.

Validity of the Study

Portino (2018) defined the validity of a research study as to how well the results of a study on a sample of a population represent a true finding of that population outside

the limit of the study. Portino distinguished two types of validity: internal and external validity

Internal Validity

Internal validity examines the extent to which a study was designed, conducted, analyzed to allow a true result (Andrade, 2018). In brief, the internal validity refers to a methodological error in a research study (Portino, 2018). For example, in the current study, the internal validity can be threatened by how the participants were selected, how the measurement scale was applied, error in recoding the data, consideration in minimum sample size, error in data collection, inappropriate analytical plan and statistical tool used, and how the results of the study were approached and interpreted. Many research techniques were used to improve the internal validity of the current study. These techniques included a careful selection of study participants, predetermine minimum sample size, appropriate data cleaning, and screening. Furthermore, this study ensured that an appropriate statistical tool was used with respect to all required assumptions. Analytical plan and results interpretation followed the guidance of several statistical and epidemiological manuals authored by Asamoah (2014), Lau (2017), Munnangi and Boktor (2018), and Osborne (2015).

External Validity

External validity examines if the results of a study apply to a similar population in a different setting or if the findings are generalizable to other study contexts (Portini, 2018; Andrade, 2018). For example, in this study, a good external validity means that the findings apply to all 20 to 59 years old marijuana users in the US. Another factor that

ensures validity in this research study is the weighting criteria initially applied to the NHANES data that I intend to use. Weighting the data produces an estimate representative of the general population parameters when the sampling population is chosen with unequal probability (NCHS, 2015a). In the context of this study, weighting criteria was applied during the analysis to take into consideration the multistage sampling technique used during the NHANES survey. To ensure that the study had a good external validity, participants were randomly selected from the population, and several statistical adjustments were applied for the sample to be representative of the population.

Ethical Procedure

Data were accessed from the NHANES website. The NHANES data are public data available to be downloaded online. Access to the data requires no permission from the NHANES staffs. Data were completely de-identified and anonymous. The NHANES protocol was developed and reviewed to comply with requirements for the protection of human subjects in research. The policy required ethical treatment of all research subjects including vulnerable populations (NCHS, 2015a). The protocol was continuously reviewed and amended by the CDC's Institutional Review Board (IRB). Every participant received an inform consent which detailed the survey process and their right as survey participants. Information on confidentiality and how their privacy is to be protected was also given to the participants in the form of a brochure. Data related to the current study were downloaded on an electronic storage device and kept in a safe place. Data were uniquely used for this study and will be safely discarded 5 years after the study.

Summary

The above chapter gives an overview of the different steps in the data analysis process from the data collection to the testing of the hypotheses. The rationale of the epidemiological design chosen to guide the study, the sampling procedure, the data collection procedure, and variables coding were all explained. The chapter also presents the different assumptions testing procedures, the data analysis plan, the minimum sample size identification procedure, and how the dataset was cleaned to make it appropriate to analyze and test the hypotheses. Finally, this chapter presents the scope in which the study is valid such as ensuring that an appropriate statistical tool is used, all required assumptions are met, analytical plan and results interpretation followed the guidance of statistical and epidemiological theories and that the results of the study apply to a similar population in a different setting or the findings are generalizable to other study contexts.

Chapter 4: Results

The purpose of this study was to use a cross-sectional analysis to identify the health effects of three potential marijuana use predictors on hepatic function. The health effects of the predictor variables associated with marijuana use such as the number of joints or pipes of marijuana smoked daily, the age of initiation and the length of time marijuana was used were examined. The study was designed to address one research question, which was to evaluate if any association exist between the three predictor variables and each of the biochemistry profile of the liver, including the serum level of ALT, AST, ALB, ALP, TB, TP, and the GGT. This chapter is organized into three sections. The first section addresses the data collection and manipulation for analysis, the second section evaluates the different assumptions, and the final section presents and discusses the study results.

Data Collection and Manipulation for Analysis

A combination of four survey cycles of data collected from 2009 to 2016 was obtained from NHANES. A total of 40,439 cases were obtained. The dataset included data from drug questionnaires and laboratory test results. Variables in the dataset included basic demographic variables, such as the age of the participants, their gender, and their race. The drug questionnaires included the age at which marijuana was first tried, the age at which participants started regularly using marijuana, the time since marijuana was last used, and the number of joints or pipes of marijuana used per day. The laboratory data included data regarding the biochemistry profile of the liver, such as the serum levels of ALT, AST, ALB, ALP, TB, TP, and GGT. The response rate for the

NHANES varied from 79.4 and 77.3 for the interview and examination survey, respectively, in the 2009-2010 survey cycle to 61.3 and 58.7 in the 2015-2016 survey cycle, also respectively. Those in the study dataset only included participants who attended both the interview and examination section of the survey and had used marijuana during the last 30 days of the survey.

For analysis purposes and to reduce the risk of biased results due to the presence of missing values, all cases with one or more missing values were removed from the initial dataset. The removal of the missing cases generated a final sample size of 702 participants. Figure 3 summarizes all exclusion criteria applied to the initial dataset and showed the sample size at each step.

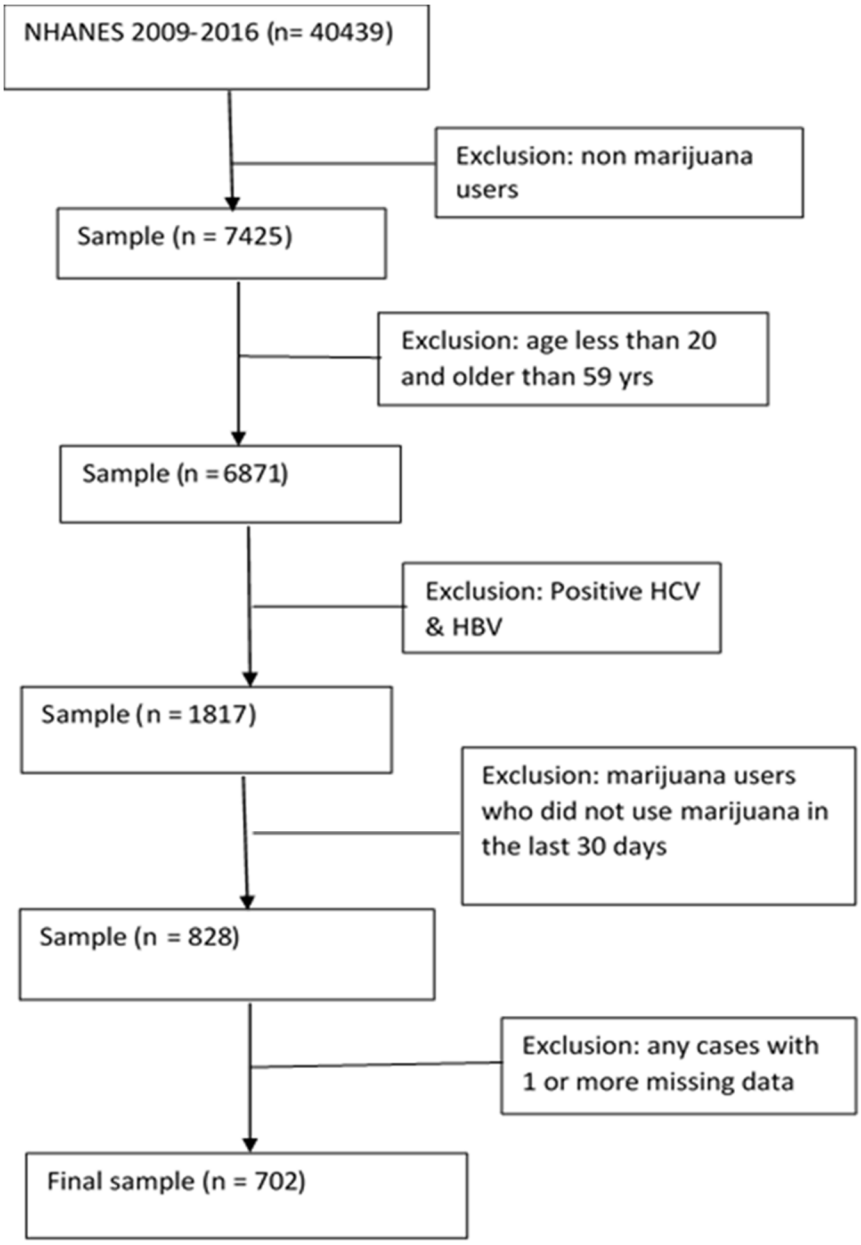


Figure 3. Sample size and screening criteria.

Data were transformed into different categories as indicated in Chapter 3. The number of joints or pipes of marijuana used daily was categorized as 1, 2, 3-5, and 6 or more per day. The age of initiation was categorized as teen (under 15 years old), youth (15 to 24 years old), and adult (25 to 59 years old). The duration of marijuana use was calculated and categorized into five groups: under 10 years, 10 to 19 years, 20 to 29 years, 30 to 39 years, and 40 and greater than 40 years. Alcohol consumption was categorized into light drinkers (less than 2 drinks per day), moderate drinkers (2 to 4 drinks per day) and heavy drinkers (4 or more drinks per day). BMI was categorized following the guidelines recommended by the NHLBI (n.d.) where less than 18.5 was defined as underweight, between 18.5 to 24.9 as normal weight, 25 to 29.9 as overweight, and 30 or greater as obese. The outcome variables, which comprised the biochemistry profile of the liver were continuous variables and were taken as reported.

Results

Descriptive Statistics

The mean age for the participant sample was 37 years with 75% of them being 47 years old. Approximately, 63% of the participants were male, and 37% were female. Non-Hispanic White participants accounted for 67.4% of the sample, while non-Hispanic Black represented 14.2%, and, the remaining 18.4% was represented by American Mexican, other Hispanic, and multiracial groups. On average, participants tried marijuana for the first time at 16 years old, while they started using it regularly at 18 years old. The number of joints or pipes used in a day was around two, with 75% of the participants smoking three or fewer joints or pipes per day. Among marijuana users, 75% of the

participants used marijuana within 13 days of the survey. Approximately, 29.3% of the participants used marijuana for less than 10 years of their life, 26.3% used it for 10 to 19 years, 16.8% used it between 20 to 29 years, 23.8% used it for 30 to 39 years and 3.8% used it for more than 40 years, which demonstrated that the length of use was age-dependent.

Table 1

Descriptive Statistics: Categorical Variables

Variables	Percent (%)	Population size
Gender		
Male	63	5,255,757
Female	37	3,083,015
Race		
Mexican American	7.1	588,479
Other Hispanic	5.1	429,147
Non-Hispanic White	67.4	5,620,662
Non-Hispanic Black	14.2	1,188,212
Other race	6.1	512,272
Number of joints or pipes smoked/day		
1 per day	42.5	3,540,280
2 per day	31.2	260,8815
3-5 per day	20.8	1,735,156
Six or more per day	5.5	454,521
Age of initiation		
Teen (<15 yrs.)	26.7	2,228,084
Youth (15 - 24 yrs.)	64.9	5,413,327
Adult (25 - 59 yrs.)	8.4	697,361
Duration of use		
Under 10 yrs.	29.3	2,446,015
10 to 19 yrs.	26.3	2,190,251
20 to 29 yrs.	16.8	1,403,711
30 to 40 yrs.	23.8	1,985,461
40 yrs. and greater	3.8	313,331
BMI		
Underweight	1.6	133,126
Normal weight	31.9	2,656,910
Overweight	35.9	2,990,771
Obese	30.7	2,557,965
Alcohol/day		
Light drinker	39.1	3,257,070
Moderate drinker	35.1	2,927,236
Moderate drinker	25.8	2,154,466

Table 2

Descriptive Statistics: Continuous Variables

			95% CI		
	Mean	Std Error	Lower	Upper	Population size
Age of participant	37.2	.63	35.9	38.5	8,338,772
Age of initiation	17.94	.23	17.46	18.41	8,338,772
ALB	4.36	.02	4.37	4.39	8,338,772
ALP	63.39	.90	61.53	65.26	8,338,772
AST	25.07	.55	23.92	26.22	8,338,772
ALT	25.58	.58	24.39	26.78	8,338,772
GGT	27.68	1.10	25.40	29.97	8,338,772
TB	.65	.02	.61	.68	8,338,772
TP	7.05	.030	6.99	7.11	8,338,772
Avg # alcoholic /day	3.80	.15	3.49	4.12	8,338,772
BMI	28.13	.22	27.67	28.60	8,338,772
Duration of use	19.29	.53	18.19	20.38	8,338,772

Preparing for Analysis

For analysis purposes, I evaluated the dataset for adequate sample size, missing data and the normality of the distribution. I also examined the dataset for all required assumptions to employ general linear regression analysis.

Post hoc analysis of sample size. Seven CSGLMs were employed in this study corresponding to each of the outcome variables. Each of the CSGLMs yielded a different R^2 value used to calculate the required sample size. The R^2 values obtained from the CSGLM analysis ranged from .074 to .287 and achieved a power of 1.00. In short, with a sample size of 702 participants and a medium effect size of .15 at alpha .05, a power greater than 99% was achieved, which was enough to detect possible associations between the variables.

Assessing and addressing missing data. The missing data in the NHANES survey are data that are completely unavailable due to participants non-response and component non-response. The participants non-response occurred both at the interview and the medical examination phases of the survey. Not all those who were interviewed were selected to participate in the examination phase of the survey, thus creating missing data in the examination phase of the survey. The component non-response was related to situation where persons who were selected to participant in the medical examination phase of the survey did not fully participate in a component of the examination. For example, there were individuals who agreed to have their blood drawn but did not get their blood pressure taken, thus creating a missing value in the data set. A third situation that added to the number of the missing values was when the participants refused to answer a particular question or answered 'unknown'. These values were coded in the NHANES as 7, 77, 777 or 9, 99, 999 depending on the number of digits in the variable value range. In this study, I also coded these values as missing.

I used the missing values pattern under the multiple imputations function in SPSS to assess the pattern of missing data. Overall, the dataset had eight cases of missing data with 99% of complete cases.

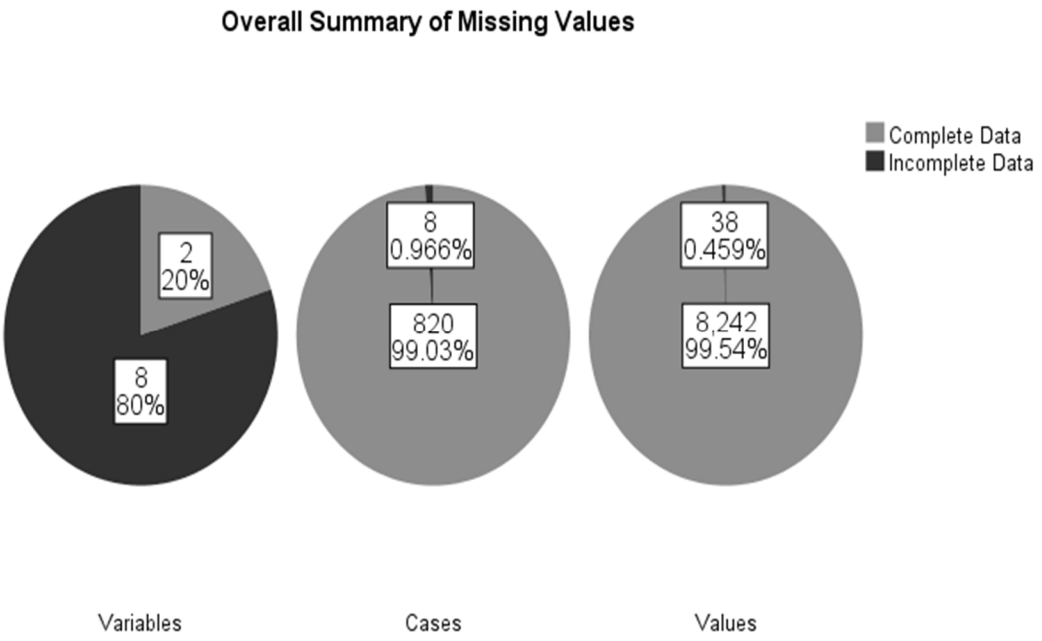


Figure 4. Summary of missing data.

According to the analytical guideline provided by the NHANES, if 10% or less of data for the main outcome variable for a specific component is missing it is acceptable to continue analysis without further evaluation or adjustment. In this study, there was less than 1% of missing cases, and consequently, a listwise deletion technique was applied to the dataset and all cases with one or more missing values were removed.

Analysis of Assumptions

Assumption of independence of observations.

One of the major assumptions when using the least square method for regression analysis is the lack of autocorrelation in the regression residuals (Kutner et al., 2004). The presence of autocorrelation generates regression estimates that may not be effective. Autocorrelation in the residuals of the regression model has traditionally been estimated

using the Durbin-Watson (DW) statistic (Bazilevsky, 2018). In this study, I used the Durbin-Watson test to evaluate possible autocorrelation between the independent variables. The test command is available as an option through the multiple linear regression procedure in SPSS. The following table presents the values of DW obtained when each of the multiple regression analysis was performed for each outcome variable.

Table 3

Durbin-Watson Test Results Per Regression Analysis

	ALT	AST	ALB	ALP	TB	TP	GGT
Durbin-Watson	1.899	2.070	2.005	1.948	2.022	1.984	1.996

The DW statistic ranged in values from 0 to 4 (Kutner et al., 2004). However, as a rule of thumb, a value of 2 indicates that there is no correlation between residuals (Laerd Statistics, 2015). In conclusion, after contrasting the values in the above table and the rule of thumb, the independence of observations as required for linear regression analysis was met.

Assumption of multicollinearity Evaluation.

In multiple regression, there must be no correlation between the predictor variables (Kutner et al., 2004). Multicollinearity was evaluated through the linear regression analysis in SPSS which offers collinearity diagnostic. I obtained two values to test for the collinearity, the Tolerance (TOL) and the variance inflation factor (VIF) values. The TOL is an indication of the percent of the variance in the predictor that cannot be accounted for by the other predictors (UCLA, n. d). The VIF is the reciprocal

of the TOL value. A TOL of less than 0.10 (i.e. a VIF greater than 10) implied the presence of collinearity (UCLA, n.d.).

Table 4

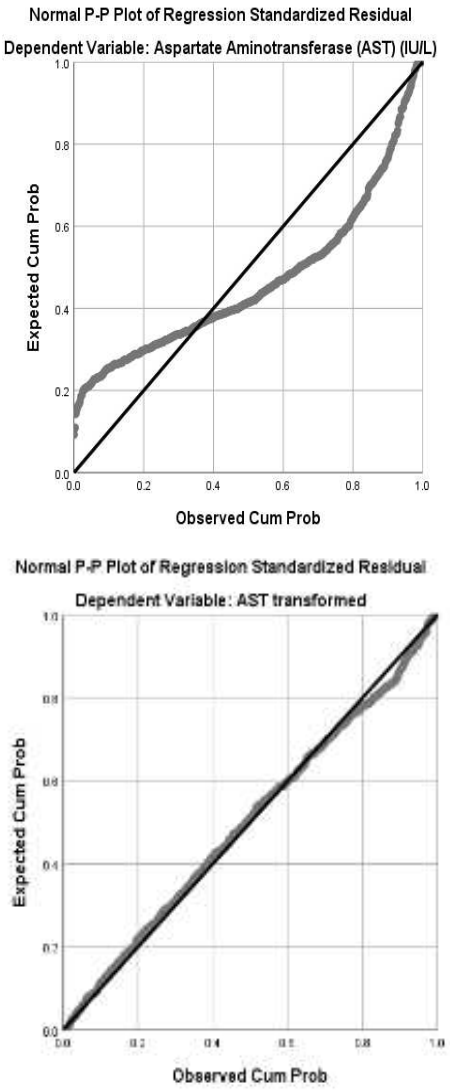
TOL &VIF Values Per Regression Analysis

	ALT		AST		ALB		ALP		TB		TP		GGT	
	Tol	VIF	TOL	VIF	TOL	VIF	TOL	VIF	TOL	VIF	TOL	VIF	TOL	VIF
Gender	.934	1.071	.933	1.072	.934	1.071	.932	1.074	.932	1.074	.932	1.074	.934	1.071
Age in years	.103	9.716	.056	17.935	.103	9.716	.053	18.961	.053	18.961	.053	18.961	.103	9.716
# Joints/day	.909	1.100	.913	1.096	.909	1.100	.907	1.102	.907	1.102	.907	1.102	.909	1.100
Age of initiation	.521	1.919	.055	18.220	.101	9.922	.953	1.049	.953	1.049	.953	1.049	.953	1.049
Duration of use	.101	9.922	.954	1.048	.953	1.049	.924	1.082	.924	1.082	.924	1.082	.924	1.083
BMI	.953	1.049	.923	1.083	.924	1.083	.343	2.918	.343	2.918	.343	2.918	.521	1.919
Alcohol/day	.924	1.083	.279	3.584	.521	1.919	.051	19.493	.051	19.493	.051	19.493	.101	9.922

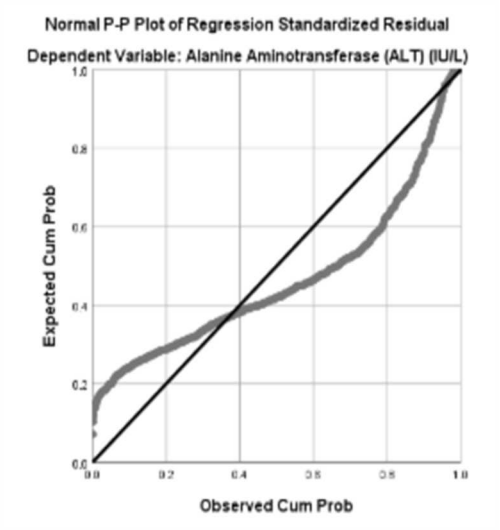
After conducting an analysis, it appeared that multicollinearity existed in the dataset. The control variable age of the participants was correlated to the predictor variable “age of initiation” as well as to the variable “alcohol consumed” while the regression was performed with respective outcome variable AST, ALP, TB and TP. Kutner et al. (2004) advised that the simplest way to remedy the problem of multicollinearity is to drop one of the offending variables, although it might affect the regression estimates and the R^2 values. As a result, the age of participant variable was dropped from the analysis where multicollinearities were observed.

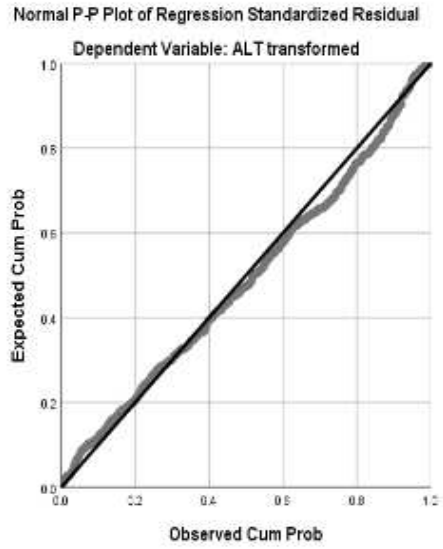
Assumption of normality Evaluation.

An initial evaluation of the adequacy of the data for linear regression showed histograms of the standardized residuals that appeared to be extremely positively skewed, thus generating normality curves that significantly departed from the expected normality. According to Laerd Statistic (2015), possible remediation of a variable that is not showing normality is to transform the variable. In this study, all outcome variables were transformed except for ALB and TP by computing the reciprocal of their data. The following figures showed the normality curves before and after the transformations were conducted.

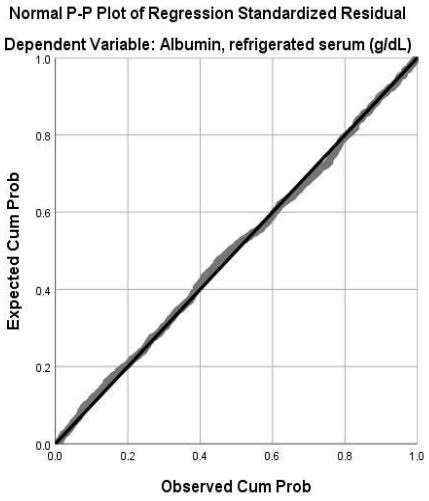


Normality curve for AST before and after transformation

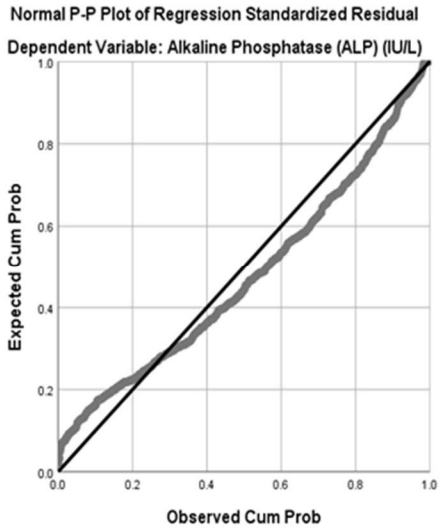


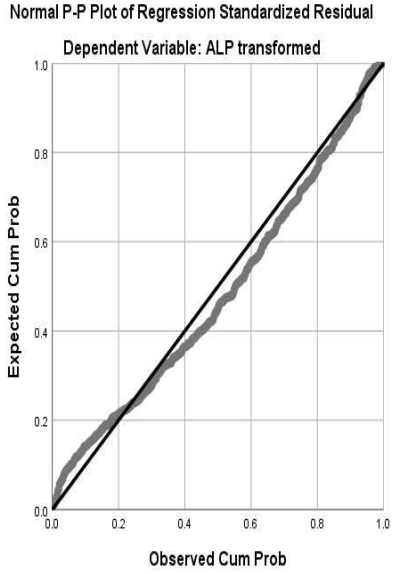


Normality curves for ALT before and after transformation

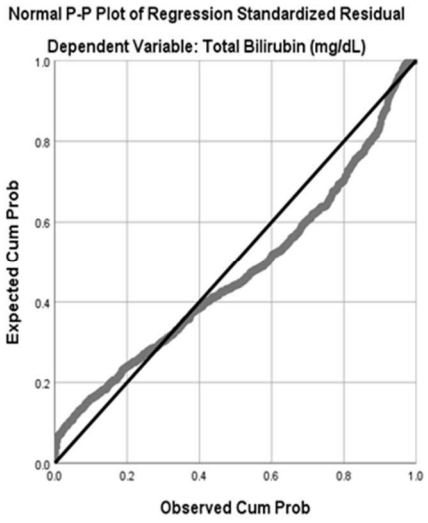


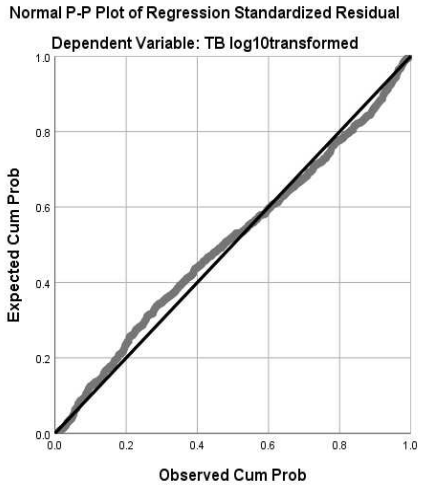
Normality curve for ALB. No transformation needed



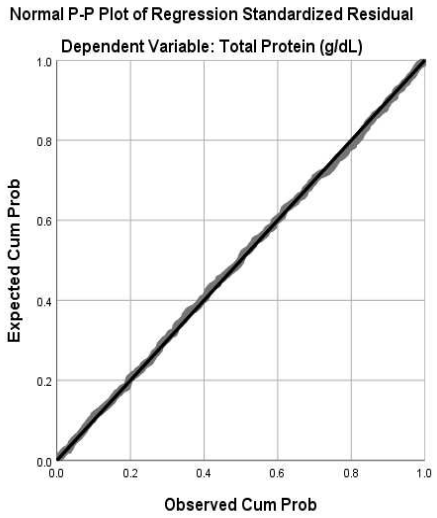


Normality curves for ALP before and after transformation

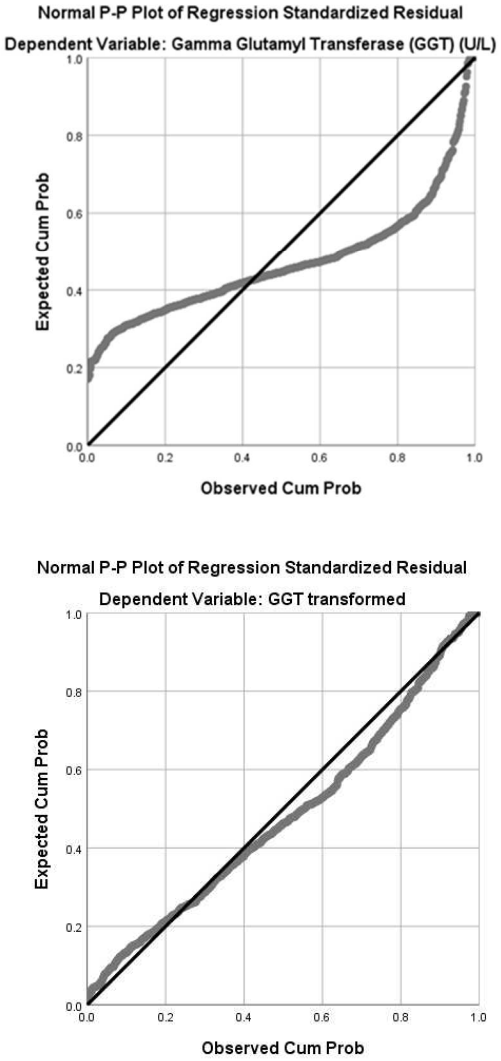




Normality curves for TB before and after transformation



Normality curve for TP. No transformation needed



Normality curves for GGT before and after transformation

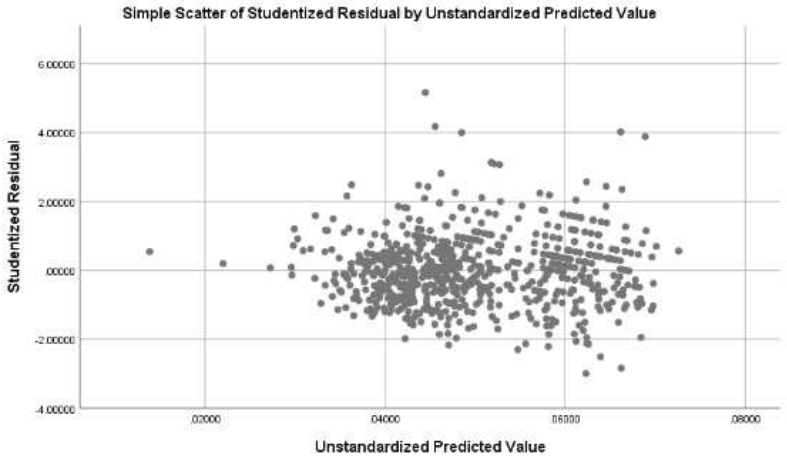
Figure 5. Normality curves.

The assumption of normality has then been satisfied after the outcome variables were transformed.

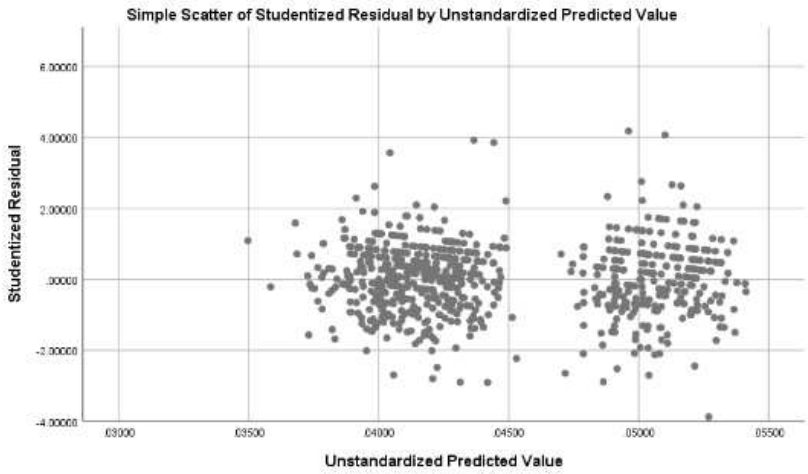
Assumption of homoscedasticity evaluation.

A major assumption required to apply the least squares regression model is that the variance is equal for all values of the predicted dependent variable (Kutner et al.,

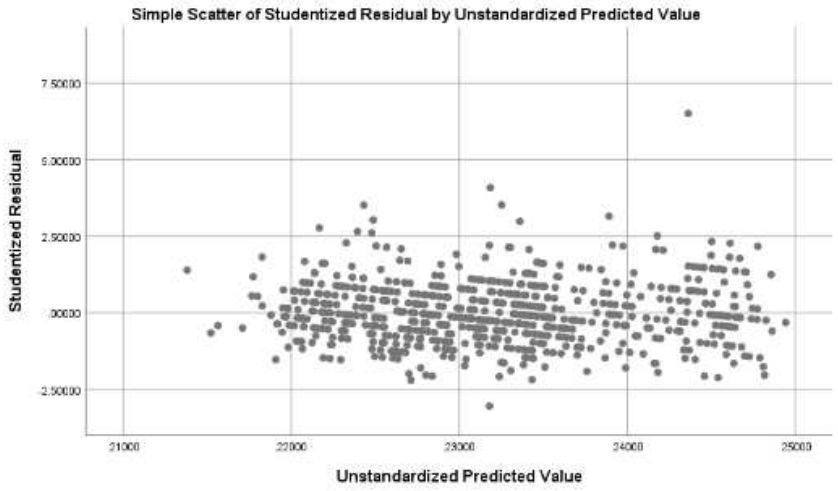
2004). I evaluated the assumption of homoscedasticity by plotting the studentized residuals against the unstandardized predicted values. The scatterplots of the different multiple regression analyses were shown below. Homoscedasticity is present when the spread of the residual shows a particular pattern. In each of the scatterplot below, a pattern was not observed. The assumption of homoscedasticity has been met.



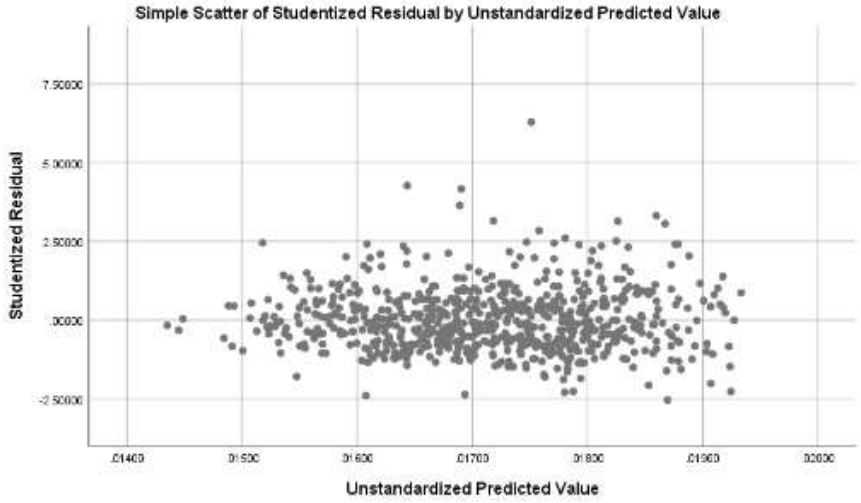
Homoscedasticity scatterplot with ALT as the dependent variable



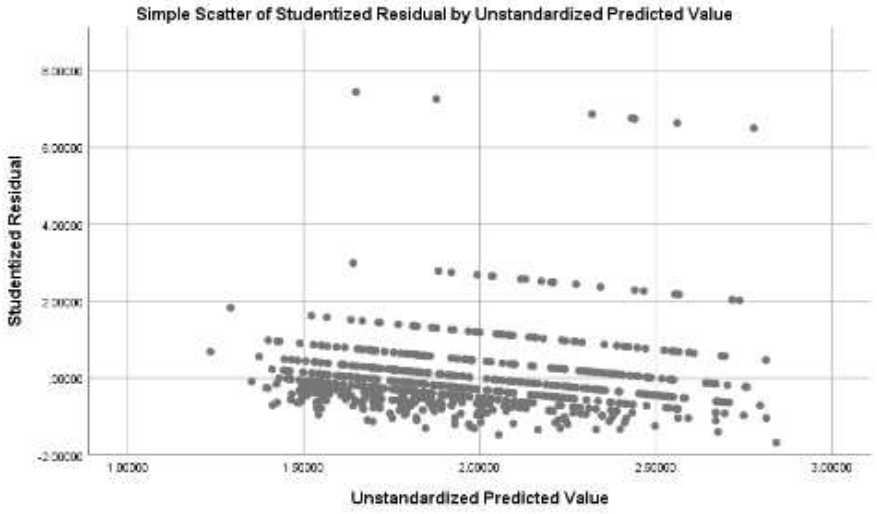
Homoscedasticity scatterplot with AST as the dependent variable



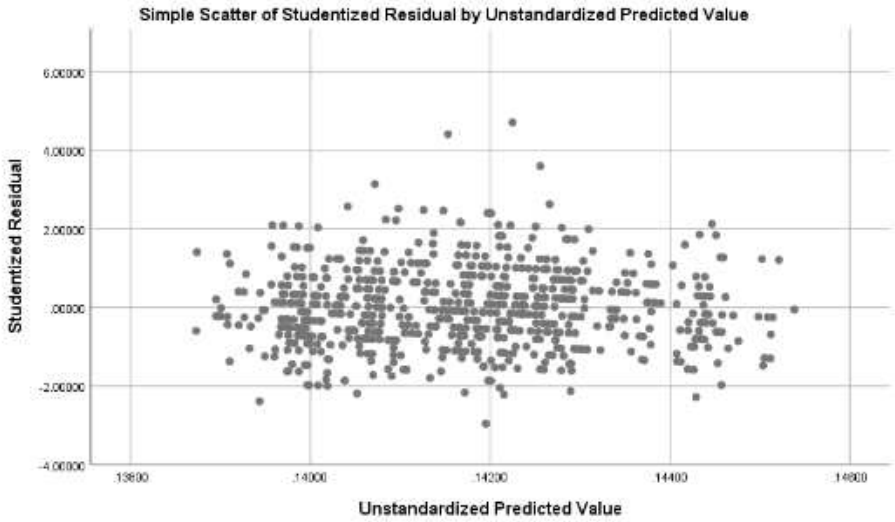
Homoscedasticity scatterplot with ALB as the dependent variable



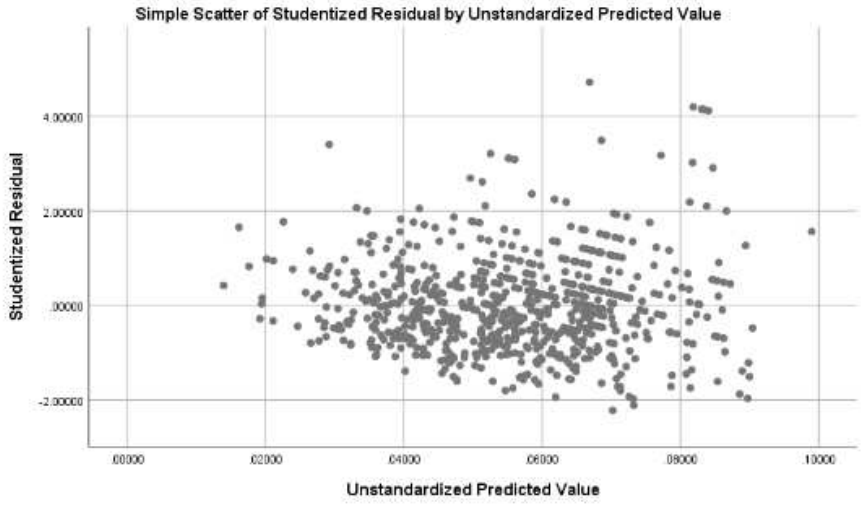
Homoscedasticity scatterplot with ALP as the dependent variable



Homoscedasticity scatterplot with TB as the dependent variable



Homoscedasticity scatterplot with TP as the dependent variable



Homoscedasticity scatterplot with GGT as dependent variable

Figure 6. Homoscedasticity scatterplots.

Assumption of linearity Evaluation.

A least squares regression analysis required a linear relationship between the dependent and independent variables collectively and between the dependent and each of the independent variables (Kutner, et al., 2004; Laerd Statistics, 2015). The scatterplots of the studentized residuals against the unstandardized predicted values obtained above for the homoscedasticity evaluation were also used to evaluate the collective linearity between the predictors and outcome variables. The observation of the scatterplots showed that there was a linear relationship between the predictors and the outcome variables. The linearity was also evaluated for the relationship between each independent variable and the outcome variable. The patterns of scatterplots obtained appeared to be showing linearity. As a conclusion, the assumption of linearity has been met.

Outlier's Assessment.

Outliers are extreme observations. According to Kutner et al. (2004), in the regression model, it is difficult to identify outliers by simple graphical means. When in the presence of more than one or two predictors variables, a multivariable outlier's analysis may be necessary. In multiple regression analysis, some univariate outliers may not be extreme, and, conversely, some multivariable outliers may not be detectable in single-variable or two-variable analysis (Kutner et al., 2004). In this study, I used the Mahalanobis distance function in SPSS to detect multivariate outliers. I identify three outlying cases. To ascertain whether the identified outlier cases were influential, I estimated Cook's distance value for each of them. Cook's distance combines information on residual and Leverage (Kutner et al., 2004). The higher the Cook's distance, the more influential is the outlying point. The conventional cut-off for the Cook's distance is (k +1)/n where k is the number of independent variables in the model, and n is the number of observations.

In the context of this study, the three outliers that were identified had Cook's distance values that were less than .005 (4/702), which was the cut-off for Cook's distance in the study. Although the three cases were identified as outliers, they were not influential in the regression analysis. Consequently, these three cases were kept in the dataset for analysis.

Research Question Analysis

I analyzed the research question by performing seven different CSGLM analyses, with each different liver enzyme as the outcome variable. I based the interpretations of the results on the *p* value, the value of the R^2, the *B* coefficient (the slope coefficient) and the 95% confidence interval (95% CI). A parameter was said to contribute to the model,

and the null hypothesis rejected if the *p* value is less than .05 and the 95% CI does not include 0. In any other case, a failure to reject the null hypothesis meant that the parameter failed to contribute to the model with a *p* value greater than .05 and a 95% CI includes 0.

During the presentation of the results, the "mean model" was often used. The mean model is the model without any predictor variable, it is simply the mean of the dependent variable (Laerd Statistics, 2015).

Part 1 of the research question. Is there a significant association between the duration of marijuana use, the age of initiation of marijuana use, the number of joints or pipes of marijuana use and the serum levels of ALT while controlling for age, gender, alcohol use, and BMI?

To analyze the effect of the three predictor variables on ALT, I performed a complex linear regression analysis in SPSS with the duration of marijuana use, the age of initiation of marijuana use, the number of joints of marijuana use as predictor variables, while the control variables were age, gender, alcohol consumption, and the BMI. ALT was used in the regression analysis as the outcome variable. The model revealed a *p* value less than .0005 which satisfied $p < .05$ with an R^2 value of .237, suggesting that the presence of all the independent variables produced a model that statistically significantly predicted the dependent variable and provided a better fit to the data than the mean model. An R^2 value of .237 means that the presence of all predictor and control variables in the regression model explained 23.7% of the variability observed in ALT.

Table 5

Test of Model Effects with ALT as the Outcome Variable

Source	df1	df2	Wald F	Sig.
(Corrected Model)	16.000	6.000	142.117	.000
(Intercept)	1.000	21.000	32.055	.000
Gender	1.000	21.000	60.170	.000
# of joints/day	3.000	19.000	.424	.738
Avg # Alcohol	2.000	20.000	4.107	.032
BMI	3.000	19.000	27.482	.000
Age of initiation	2.000	20.000	1.075	.360
Duration of use	4.000	18.000	.536	.711
Age of participant	1.000	21.000	.158	.695

a. Model: ALT = (Intercept) + Gender + # of joints /day + Avg # Alcohol + *BMI* + Age of initiation + Duration of use + Age at screening

Based on the analysis of results presented in the above table, the effect of the age of initiation of marijuana on ALT revealed a p value greater than the cut off value of .05, $F(2, 20) = 1.075$, $p > .05$, and the duration of use also showed a p value greater than .05, $F(4, 18) = .536$, $p > .05$,. Finally, the effect of the number of joints of marijuana also showed a p value greater than .05, $F(3, 19) = .424$, $p > .05$. These results suggest that the three predictors failed to be statistically significant predictors of the effect of marijuana use on ALT. In this case, the null hypothesis, which stated that there is no significant association between the age of initiation, the dose of marijuana consumed, and the duration of use of marijuana, and ALT failed to be rejected.

Table 6

Parameter Estimates with ALT as the Outcome Variable

			95% CI		Hypothesis Test		
Parameter	Estimate	Std. Error	Lower	Upper	t	df	Sig
(Intercept)	.041	.017	.005	.076	2.391	21.000	.026
Male	-.014	.002	-.018	-.010	-7.757	21.000	.000
Female	.000[b]	.	.	.	.	.	.
1 joints/day	-.003	.003	-.008	.003	-1.093	21.000	.287
2 joints/day	-.001	.002	-.006	.003	-.625	21.000	.539
3-5 joints/day	-.002	.003	-.008	.004	-.590	21.000	.562
6 or more joints/day	.000[b]	.	.	.	.	.	.
Light drink (< 2 drink / day)	.003	.002	-.001	.007	1.736	21.000	.097
Moderate drink (2-4 drinks / day)	-.002	.003	-.007	.004	-.584	21.000	.566
Heavy drink (>= 4 drinks per day)	.000[b]	.	.	.	.	.	.
Underweight (< 18.5 kg/ m^2)	.012	.007	-.002	.026	1.831	21.000	.081
Normal weight (18.5 – 24.9 kg/ m^2)	.014	.002	.010	.017	7.479	21.000	.000
Overweight (25 – 29.9 kg/ m^2)	.002	.002	-.002	.006	1.075	21.000	.294
Obese (30 kg/ m^2 or greater)	.000[b]	.	.	.	.	.	.
Initiation as Teen (< 15 yrs old)	.004	.004	-.004	.011	.935	21.000	.360
Initiation as Youth (15 – 24 yrs old)	.001	.004	-.008	.010	.303	21.000	.765
Initiation as Adult (25 – 59 years old)	.000[b]	.	.	.	.	.	.
< 10 yrs. of use	.013	.009	-.006	.032	1.411	21.000	.173
10 to 19 yrs. of use	.011	.008	-.005	.027	1.420	21.000	.170
20 to 29 of use	.007	.006	-.006	.021	1.129	21.000	.271
30 to 39 yrs. of use	.006	.006	-.006	.018	1.062	21.000	.301
>40 yrs. of use	.000[b]	.	.	.	.	.	.
Age of participants	6.791E-5	.000	.000	.000	.397	21.000	.695

a. *Model: ALT = (Intercept) + Gender + # of joints /day* + Avg # Alcohol + *BMI* + Age of initiation + Duration of use
b. Reference category

The analysis of the results presented in the above table revealed that the coefficient estimates of the different categories of each of the three predictor variables, the age of initiation, the duration of use and the quantity of use failed to be statistically

significant. This result was normal considering that the null hypothesis failed to be rejected.

Part 2 of the research question. Is there a significant association between the duration of marijuana use, the age of initiation of marijuana use, the number of joints or pipes of marijuana use and the serum levels of AST while controlling for gender, alcohol use and BMI?

To analyze of the effects of the three predictor variables on AST, I performed a complex linear regression analysis in SPSS with the duration of marijuana use, the age of initiation of marijuana use, the quantity of marijuana use as predictor variables, while the control variables were, gender, alcohol consumption, and the BMI. AST was used in the regression analysis as the outcome variable. In this model, the age of the participants as a control variable was dropped due to its correlation with another independent variable in the model. The model revealed a significant $p < .0005$ which satisfied $p < .05$ with R^2 of .142, suggesting that the presence of all the independent variables produced a model that statistically significantly predicted the dependent variable and provided a better fit to the model than the mean model. An R^2 value of .142 means that the presence of all predictor and control variables in the regression model explained 14.2% of the variability observed in AST.

Table 7

Test of Model Effects with AST as the Outcome Variable

Source	df1	df2	Wald F	Sig.
(Corrected Model)	15.000	7.000	34.080	.000
(Intercept)	1.000	21.000	1079.146	.000
Gender	1.000	21.000	46.490	.000
# of joints/day	3.000	19.000	1.189	.341
Avg # Alcohol	2.000	20.000	2.793	.085
BMI	3.000	19.000	4.023	.023
Age of initiation	2.000	20.000	.176	.840
Duration of use	4.000	18.000	2.781	.058

a. *Model: AST transformed = (Intercept) + Gender + # of joints /day +* Avg # Alcohol *+ BMI +* Age of initiation *+* Duration of use

Based on the analysis of the results presented in the above table, the three predictor variables failed to be statistically significant predictors of the effects of marijuana use on AST. Consequently, the three predictor variables did not contribute to the variation in AST observed above. Here, the statistical significance level of the effects of the age of initiation on AST exhibited a value of .840, $F(2, 20) = .176$, $p > .05$, while the significance level of the dose of marijuana consumed on AST was at .341, $F(3\ 19) = 1.189$, $p > .05$. Finally, the third predictor variable, the duration of use, showed a statistical significance level of .058, $F(4, 18) = 2.781$, $p > .05$. The independent effects of all three predictor variables showed non-statistically significant *p* values suggesting that the null hypothesis failed to be rejected.

Table 8

Parameter Estimates with AST as the Outcome Variable

		Std.	95% CI		Hypothesis Test		
Parameter	Estimate	Error	Lower	Upper	t	df	Sig
(Intercept)	.043	.004	.034	.052	10.264	21.000	.000
Male	-.008	.001	-.010	-.006	-6.818	21.000	.000
Female	.000[b]	.	.	.	.	.	.
1 joints/day	-.002	.002	-.006	.002	-1.056	21.000	.303
2 joints/day	-.001	.002	-.006	.003	-.625	21.000	.539
3-5 joints/day	.000	.002	-.004	.004	-.132	21.000	.896
6 or more joints/day	.000[b]	.	.	.	.	.	.
Light drink (< 2 drink / day)	.001	.001	-.001	.003	1.101	21.000	.283
Moderate drink (2-4 drinks / day)	-.002	.001	-.003	.000	-1.942	21.000	.066
Heavy drink (>= 4 drinks per day)	.000[b]	.	.	.	.	.	.
Underweight (< 18.5 kg/ m^2)	-.002	.004	-.010	.007	-.393	21.000	.698
Normal weight (18.5 – 24.9 kg/ m^2)	.002	.001	-.001	.005	1.301	21.000	.207
Overweight (25 – 29.9 kg/ m^2)	-.001	.001	-.004	.002	-.861	21.000	.399
Obese (30 kg/ m^2 or greater)	.000[b]	.	.	.	.	.	.
Initiation as Teen (< 15 yrs. old)	.001	.002	-.003	.004	.555	21.000	.585
Initiation as Youth (15 – 24 yrs. old)	.001	.002	-.003	.004	.394	21.000	.697
Initiation as Adult (25 – 59 yrs. old)	.000[b]	.	.	.	.	.	.
< 10 yrs. of use	.010	.004	.001	.018	2.462	21.000	.023
10 to 19 yrs. of use	.007	.004	-.001	.015	1.865	21.000	.076
20 to 29 yrs. of use	.007	.003	.000	.014	2.228	21.000	.037
30 to 39 yrs. of use	.006	.003	-.001	.013	1.722	21.000	.100
>40 yrs. of use	.000[b]	.	.	.	.	.	.

a. *Model: AST transformed = (Intercept) + Gender + # of joints /day* + Avg # Alcohol + *BMI* + Age of initiation + Duration of use

b. Reference category

The analysis of the above table showed that the coefficient estimates of the different categories of the three predictor variables failed to be statistically significant, which is normal when the null hypothesis failed to be rejected.

Part 3 of the research question. Is there a significant association between the duration of marijuana use, the age of initiation of marijuana use, the number of joints or

pipes of marijuana use and the serum levels of ALB while controlling for age, gender, alcohol use and BMI?

To analyze the effects of the three predictor variables on ALB, I performed a complex linear regression analysis in SPSS with the duration of marijuana use, the age of initiation of marijuana use, the quantity of marijuana use as predictor variables while the control variables were, age, gender, alcohol consumption, and the BMI. ALB was used in the regression as the outcome variable. The model revealed a significant $p < .0005$ which satisfied $p < .05$ with R^2 of .194, suggesting that the presence of all the independent variables produced a model that statistically significantly predicted the dependent variable and provided a better fit for the data than the mean model. An R^2 value of .194 means that the presence of all predictor and control variables in the regression model explained 19.4% of the variability observed in ALB.

Table 9

Test of Model Effects with ALB as the Outcome Variable

Source	df1	df2	Wald F	Sig.
(Corrected Model)	16.000	6.000	20.489	.001
(Intercept)	1.000	21.000	388.836	.000
Gender	1.000	21.000	42.133	.000
# of joints/day	3.000	19.000	2.250	.116
Avg # Alcohol	2.000	20.000	1.927	.172
BMI	3.000	19.000	9.130	.001
Age of initiation	2.000	20.000	.040	.961
Duration of use	4.000	18.000	.507	.731
Age of participants	1.000	21.000	.053	.820

a. *Model: ALB = (Intercept) + Gender + # of joints /day +* Avg # Alcohol *+ BMI +* Age of initiation *+* Duration of use *+ age of participants*

Based on the results of the analysis presented in Table 8, the effect of the age of initiation on the ALB revealed a p value greater than .05, $F(2, 20) = .040$, $p > .05$ and the duration of use also showed a p value than was greater than .05, $F(4, 18) = .507$, $p > .05$. Finally, the effect of the number of joints of marijuana use also showed a p value that was higher than .05, $F(3, 19) = 2.250$, $p > .05$. These results suggest that the three predictors failed to be statistically significant predictors of the effect of marijuana use on ALB. In this case, the null hypothesis which stated that there is no significant association between the age of initiation, the dose of marijuana consumed, and the duration of use on ALB failed to be rejected.

Table 10

Parameter Estimates with ALB as the Outcome Variable

		Std.	95% CI		Hypothesis Test		
Parameter	Estimate	Error	Lower	Upper	t	df	Sig
(Intercept)	.245	.017	.210	.281	14.265	21.000	.000
Male	-.010	.002	-.014	-.007	-6.491	21.000	.000
Female	.000[b]	.	.	.	.	.	.
1 joints/day	.001	.003	-.005	.006	.221	21.000	.827
2 joints/day	.001	.003	-.005	.006	.239	21.000	.813
3-5 joints/day	.004	.002	-.001	.009	1.635	21.000	.117
6 or more joints/day	.000[b]	.	.	.	.	.	.
Light drink (< 2 drink / day)	.002	.002	-.002	.006	.959	21.000	.348
Moderate drink (2-4 drinks / day)	-.003	.002	-.007	.001	-1.493	21.000	.150
Heavy drink (>= 4 drinks per day)	.000[b]	.	.	.	.	.	.
Underweight (< 18.5 kg/ m^2)	-.024	.007	-.039	-.009	-3.381	21.000	.003
Normal weight (18.5 – 24.9 kg/ m^2)	-.009	.002	-.013	-.005	-4.739	21.000	.000
Overweight (25 – 29.9 kg/ m^2)	-.008	.002	-.013	-.004	-3.944	21.000	.001
Obese (30 kg/ m^2 or greater)	.000[b]	.	.	.	.	.	.
Initiation as Teen (< 15 yrs. old)	.000	.005	-.010	.010	-.088	21.000	.931
Initiation as Youth (15 – 24 yrs. old)	-.001	.004	-.009	.008	-.170	21.000	.867
Initiation as Adult (25 – 59 years old)	.000[b]	.	.	.	.	.	.
< 10 yrs. of use	-.006	.008	-.023	.011	-.698	21.000	.493
10 to 19 yrs. of use	-.005	.006	-.018	.008	-.790	21.000	.438
20 to 29 of use	-.004	.005	-.014	.006	-.784	21.000	.442
30 to 39 yrs. of use	-.004	.003	-.011	.003	-1.232	21.000	.232
>40 yrs. of use	.000[b]	.	.	.	.	.	.
Age of participants	6.347E-5	.000	-.001	.001	.230	21.000	.820

a. *Model: ALB = (Intercept) + Gender + # of joints /day +* Avg # Alcohol *+ BMI +* Age of initiation *+* Duration of use *+ age of participants*

b. Reference category

As shown in Table 9, the coefficient estimates of the different categories of the three predictor variables failed to be statistically significant with each category showing a *p* value greater than .05.

Part 4 of the research question. Is there a significant association between the duration of marijuana use, the age of initiation of marijuana use, the number of joints of marijuana use and the serum levels of ALP while controlling for gender, alcohol use and BMI?

To analyze the effect of the three predictor variables on ALP, I performed a complex linear regression analysis in SPSS with the duration of marijuana use, the age of initiation of marijuana use, the quantity of marijuana use as predictor variables while the control variables were, gender, alcohol consumption, and the BMI. ALP was used in the regression analysis as the outcome variable. The model revealed a significant $p < .0005$ which satisfied $p < .05$ with R^2 of .074, suggesting that the presence of all the independent variables produced a model that statistically significantly predicted the dependent variable and that the data was a better fit to the model than the mean model. An R^2 value of .074 means that the presence of all predictor and control variables into the regression model explained 7.4% of the variability observed in ALP.

Table 11

Test of Model Effects with ALP as the Outcome Variable

Source	df1	df2	Wald F	Sig.
(Corrected Model)	15.000	7.000	5.262	.017
(Intercept)	1.000	21.000	1516.742	.000
Gender	1.000	21.000	4.161	.054
# of joints/day	3.000	19.000	1.715	.198
Avg # Alcohol	2.000	20.000	.236	.792
BMI	3.000	19.000	3.354	.041
Age of initiation	2.000	20.000	2.572	.101
Duration of use	4.000	18.000	4.661	.009

Model: ALB = (Intercept) + Gender + # of joints /day + Avg # Alcohol *+ BMI +* Age of initiation *+* Duration of use

Based on the results of the analysis presented in of Table 10, the age of initiation and the quantity of marijuana use failed to be statistically significant predictors of the effect of marijuana use on ALP with respective p values of .101, $F(2, 20) = 2.572$, $p >$ .05, and .198, $F(3, 19) = 1.715$, $p > 0.05$. However, the duration of use showed a statistically significant p value level of .009, $F(4, 18) = 4.661$, $p < .05$, suggesting that the duration of use significantly contributed to the variation in ALP observed above. The analysis of the coefficient estimates in the next table shows coefficient estimates values for each category of the duration of use that were not statistically significant except for the category where participants used marijuana for 10 to 19 years. So, the duration of use of marijuana was a statistically significant predictor of the effect of marijuana use on ALP. In this case, the null hypothesis partially failed to be rejected for the age of initiation of marijuana use and for the quantity of marijuana use, and the alternate hypothesis involving the effect of duration of use on ALP was accepted, as the duration of use significantly predicted variation on ALP.

Table 12

Parameter Estimates with ALP as the Outcome Variable

		Std.	95% CI		Hypothesis Test		
Parameter	Estimate	Error	Lower	Upper	t	df	Sig
(Intercept)	.014	.001	.012	.016	13.427	21.000	.000
Male	-.001	.000	-.002	1.811E-5	-2.040	21.000	.054
Female	.000[b]	.	.	.	.	.	.
1 joints/day	.002	.001	.000	.003	2.382	21.000	.027
2 joints/day	.002	.001	-9.796E-5	.003	1.960	21.000	.063
3-5 joints/day	.001	.001	.000	.003	1.889	21.000	.073
6 or more joints/day	.000[b]	.	.	.	.	.	.
Light drink (< 2 drink / day)	.000	.001	-.001	.002	.659	21.000	.517
Moderate drink (2-4 drinks / day)	.000	.001	-.001	.002	.571	21.000	.574
Heavy drink (>= 4 drinks per day)	.000[b]	.	.	.	.	.	.
Underweight (< 18.5 kg/ m^2)	-.002	.002	-.005	.002	-.958	21.000	.349
Normal weight (18.5 – 24.9 kg/ m^2)	.001	.000	.000	.002	1.667	21.000	.110
Overweight (25 – 29.9 kg/ m^2)	.001	.000	.000	.002	2.766	21.000	.012
Obese (30 kg/ m^2 or greater)	.000[b]	.	.	.	.	.	.
Initiation as Teen (< 15 yrs. old)	.000	.001	-.002	.001	-.512	21.000	.614
Initiation as Youth (15 – 24 yrs. old)	.001	.001	-.001	.002	1.159	21.000	.259
Initiation as Adult (25 – 59 yrs. old)	.000[b]	.	.	.	.	.	.
< 10 yrs. of use	.001	.001	-.001	.003	.891	21.000	.383
10 to 19 yrs. of use	.002	.001	.000	.004	2.574	21.000	.018
20 to 29 of use	.000	.001	-.001	.002	.337	21.000	.740
30 to 39 yrs. of use	.000	.001	-.001	.002	.266	21.000	.793
>40 yrs. of use	.000[b]	.	.	.	.	.	.

a. *Model: ALP = (Intercept) + Gender + # of joints /day +* Avg # Alcohol *+ BMI +* Age of initiation *+* Duration of use *+ age of participants*

b. Reference category

In the above table, except for the estimates of the duration of use of marijuana ranging from 10 to 19 years, estimates for other categories of the predictor variables were not statistically significant. The estimate of the duration of use for people who used marijuana for 10 to 19 years was 0.002, B= .002, $p < 0.05$, 95%CI [.000 - .004]. This result meant that people who used marijuana for 10 to 19 years had a serum ALP value that was .002 IU/dl greater those who used marijuana for more than 40 years. Clinically, the difference observed was too close to zero, meaning that there was no difference in the serum level of ALP between people who used marijuana for 10 to 19 years compared to people who used it for more than 40 years.

Part 5 of the research question. Is there a significant association between the duration of marijuana use, the age of initiation of marijuana use, the number of joints of marijuana use and the serum levels of TB while controlling for gender, alcohol use and BMI?

To analyze the effect of the three predictor variables on TB, I performed a complex linear regression analysis in SPSS with the duration of marijuana use, the age of initiation of marijuana use, the quantity of marijuana use as predictor variables, while the control variables were gender, alcohol consumption, and the BMI. TB was used in the regression analysis as the outcome variable. The model revealed a significant $p < .0005$ which satisfied $p < .05$ with an R^2 *value* of .106, suggesting that the presence of all the independent variables produced a model that statistically significantly predicted the dependent variable and is a better fit to the data than the mean model. An R^2 value of .106

means that the presence of all predictor and control variables in the regression model explained 10.6 % of the variability observed in TB.

Table 13

Test of Model Effects with TB as the Outcome Variable

Source	df1	df2	Wald F	Sig.
(Corrected Model)	15.000	7.000	20.980	.000
(Intercept)	1.000	21.000	152.623	.000
Gender	1.000	21.000	25.738	.000
# of joints/day	3.000	19.000	4.859	.011
Avg # Alcohol	2.000	20.000	.374	.692
BMI	3.000	19.000	3.007	.056
Age of initiation	2.000	20.000	1.462	.256
Duration of use	4.000	18.000	1.794	.174

Model: ALB = (Intercept) + Gender + # of joints /day + Avg # Alcohol + BMI + Age of initiation + Duration of use

As shown in Table 12, the statistical significance level of the quantity of marijuana use was .011, $F(3, 19) = 4.859$, $p < .05$, the duration of use was .174, $F(4, 18) = 1.794$, $p > .05$, and the age of initiation was .256, $F(2, 20) = 1.462$, $p > .05$. The p values for the quantity of marijuana use was less than the cut-off value of .05. In conclusion, the quantity of marijuana use was found to have a statistically significant effect on TB. However, the age of initiation and the duration of use failed to have a statistical effect on TB. Consequently, the null hypothesis was partially accepted for the age of initiation and the duration of use, which means that there is no statistically significant association between the age of initiation, the duration of use and TB. For the

quantity of marijuana use, the null hypothesis was rejected, meaning that the study found a statistically significant association between the quantity of marijuana use and TB.

Table 14

Parameter Estimates with TB as the Outcome Variable

		Std.	95% CI		Hypothesis Test		
Parameter	Estimate	Error	Lower	Upper	t	df	sig
(Intercept)	2.912	.394	2.093	3.732	7.388	21.000	.000
Male	-.544	.107	-.767	-.321	-5.073	21.000	.000
Female	.000[b]	.	.	.	.	.	.
1 joints/day	-.536	.173	-.896	-.175	-3.091	21.000	.006
2 joints/day	-.332	.187	-.722	.057	-1.773	21.000	.091
3-5 joints/day	-.274	.213	-.716	.168	-1.289	21.000	.211
6 or more joints/day	.000[b]	.	.	.	.	.	.
Light drink (< 2 drink / day)	-.025	.107	-.247	.198	-.231	21.000	.819
Moderate drink (2-4 drinks / day)	-.104	.117	-.348	.140	-.887	21.000	.385
Heavy drink (>= 4 drinks per day)	.000[b]	.	.	.	.	.	.
Underweight (< 18.5 kg/ m^2)	-.577	.368	-1.342	.188	-1.568	21.000	.132
Normal weight (18.5 – 24.9 kg/ m^2)	-.310	.128	-.575	-.045	-2.431	21.000	.024
Overweight (25 – 29.9 kg/ m^2)	-.256	.111	-.486	-.026	-2.313	21.000	.031
Obese (30 kg/ m^2 or greater)	.000[b]	.	.	.	.	.	.
Initiation as Teen (< 15 yrs old)	.034	.170	-.320	.387	.199	21.000	.844
Initiation as Youth (15 – 24 yrs old)	-.184	.150	-.496	.128	-1.225	21.000	.234
Initiation as Adult (25 – 59 years old)	.000[b]	.	.	.	.	.	.
< 10 yrs. of use	.122	.218	-.331	.574	.558	21.000	.583
10 to 19 yrs. of use	.260	.141	-.034	.554	1.840	21.000	.080
20 to 29 of use	.048	.148	-.259	.355	.323	21.000	.750
30 to 39 yrs. of use	-.025	.186	-.412	.363	-.133	21.000	.896
>40 yrs. of use	.000[b]	.	.	.	.	.	.

a. Model: TB = (Intercept) + Gender + # of joints /day + Avg # Alcohol + BMI + Age of initiation + Duration of use

b. Reference category

The results of analysis presented in Table 13, showed that for the variable quantity of use, only the category of participants who used 1 joint of marijuana per day was statistically significant with a coefficient estimate value of -.536, B= -.536, $p < 0.05$, 95%CI [-.896, - .175]. This result meant that people who used 1 joint of marijuana per day had a TB level that was .536 mg/dl less than that for those who smoked more than 6 joints per day, with 95% confident that the difference in TB was between .175 mg/dl and .896 mg/dl.

Part 6 of the research question. Is there a significant association between the duration of marijuana use, the age of initiation of marijuana use, the quantity of marijuana use and the serum levels of TP while controlling for gender, alcohol use and BMI?

To analyze the effect of the three predictor variables on TP, I performed a complex linear regression analysis in SPSS with the duration of marijuana use, the age of initiation of marijuana use, the quantity of marijuana use as predictor variables while the control variables were, gender, alcohol consumption, and the BMI. TP was used in the regression analysis as the outcome variable. The model revealed a significant $p < .0005$ which satisfied $p < .05$ with an R^2 *value* of .078, suggesting that the presence of all the independent variables produced a model that statistically significantly predicted the dependent variable better than the mean model and was a better fit for the data. An R^2value of .077 meant that the presence of all predictor and control variables in the regression model explained 7.7% of the variability in TP.

Table 15

Test of Model Effects with TP as Outcome Variable

Source	df1	df2	Wald F	Sig.
(Corrected Model)	15.000	7.000	16.975	.000
(Intercept)	1.000	21.000	23338.468	.000
Gender	1.000	21.000	3.861	.063
# of joints/day	3.000	19.000	1.308	.301
Avg # Alcohol	2.000	20.000	1.276	.301
BMI	3.000	19.000	1.945	.157
Age of initiation	2.000	20.000	.050	.952
Duration of use	4.000	18.000	3.401	.031

Model: TP = (Intercept) + Gender + # of joints /day + Avg # Alcohol + BMI + Age of initiation + Duration of use

From the analysis of the results of Table 13, the effect of the age of initiation on the TP revealed *p* value greater than .05, $F(2, 20) = .050, p > .05$, and that of the quantity of use also showed a *p* value that was greater than 0.05, $F(3, 19) = 1.308$, $p > .05$. Finally, the effect of the duration of use of marijuana showed a *p* value of less than .05, $F(4, 18) = 3.401, p < .05$. The preceding results suggest that two of the three predictor variables, the age of initiation and the quantity of marijuana use failed to be statistically significant predictors of the effect of marijuana use on TP. In this case, the null hypothesis partially failed to be rejected for the age of initiation and the quantity of marijuana use while it was rejected for the duration of use.

Table 16

Parameter Estimates with TP as the Outcome Variable

		Std.	95% CI		Hypothesis Test		
Parameter	Estimate	Error	Lower	Upper	t	df	sig
(Intercept)	.150	.004	.141	.159	36.150	21.000	.000
Male	-.002	.001	-.004	9.952E-5	-1.965	21.000	.063
Female	.000[b]	.	.	.	.	.	.
1 joints/day	-9.854E-5	.002	-.004	.004	-.049	21.000	.962
2 joints/day	-.002	.002	-.005	.002	-1.008	21.000	.325
3-5 joints/day	.000	.002	-.004	.005	.082	21.000	.935
6 or more joints/day	.000[b]	.	.	.	.	.	.
Light drink (< 2 drink / day)	.001	.001	-.001	.002	1.057	21.000	.303
Moderate drink (2-4 drinks / day)	-.001	.001	-.003	.001	-1.089	21.000	.289
Heavy drink (>= 4 drinks per day)	.000[b]	.	.	.	.	.	.
Underweight (< 18.5 kg/ m^2)	-.006	.003	-.013	.000	-1.937	21.000	.066
Normal weight (18.5 – 24.9 kg/ m^2)	.000	.001	-.002	.003	.383	21.000	.706
Overweight (25 – 29.9 kg/ m^2)	.000	.001	-.002	.002	-.200	21.000	.843
Obese (30 kg/ m^2 or greater)	.000[b]	.	.	.	.	.	.
Initiation as Teen (< 15 yrs. old)	.000	.002	-.004	.003	-.286	21.000	.778
Initiation as Youth (15 – 24 yrs. old)	.000	.002	-.004	.003	-.121	21.000	.905
Initiation as Adult (25 – 59 years old)	.000[b]	.	.	.	.	.	.
< 10 yrs. of use	-.008	.003	-.013	-.003	-3.058	21.000	.006
10 to 19 yrs. of use	-.005	.002	-.010	-.001	-2.331	21.000	.030
20 to 29 of use	-.005	.002	-.009	.000	-2.192	21.000	.040
30 to 39 yrs. of use	-.005	.003	-.010	.000	-1.915	21.000	.069
>40 yrs. of use	.000[b]	.	.	.	.	.	.

a. Model: TP = (Intercept) + Gender + # of joints /day + Avg # Alcohol + BMI + Age of initiation + Duration of use

b. Reference category

As shown in Table 16, the coefficient estimates of each category of the predictor variables were not statistically significant except for the duration of use, where the coefficient estimates of the categories of people who use marijuana for less than 10 years, 10 to 19 years and 20 to 29 years were all statistically significant with respective coefficient estimates of -.008, $B = -.008, p < .05, 95\%CI[-.013, -.003]$, -.005, $B = -.005, p < .05, 95\%CI[-.010, -.001]$ and -.005, $B = -.005, p < .05, 95\%CI[-.009, .000]$. These result indicate that people who used marijuana for less than 10 years had a TP level that was .008 mg/dl less than that of people who used marijuana for more than 40 years, and people who used marijuana for 10 to 19 years had a TP level that was .005 mg/dl less than that of those who used marijuana for more than 40 years. There was no difference in the decreased amount of TP between people who used marijuana for 10 to 19 years and 20 to 29 years compared to those who used it for more than 40 years. Concisely, the difference in TP levels observed for each category of duration of use was too close to zero, indicating that the differences were not clinically significant. As a result, there was no difference in the serum level of TP for someone who used marijuana for less than 10 years, 10 to 19 years, and 20 to 29 years compared to those who used the drug for more than 40 years.

Part 7 of the research question. Is there a significant association between the duration of marijuana use, the age of initiation of marijuana use, the quantity of marijuana use and the serum levels of GGT while controlling for age, gender, alcohol use, and BMI?

To analyze the effect of the three predictor variables on GGT, I performed a complex linear regression analysis in SPSS with the duration of marijuana use, the age of initiation of marijuana use, the quantity of marijuana use as predictor variables while the control variables were age, gender, alcohol consumption, and the BMI. TP was used in the regression analysis as the outcome variable. The model revealed a significance with $p < .0005$ which satisfied $p < .05$ with an R^2 *value* of .287, suggesting that the presence of all the independent variables produced a model that statistically significantly predicted the dependent variable and provided a better fit for the data than the mean model. The R^2 value of .287 meant that the presence of all predictors and control variables in the regression model explained 28.7 of the variability in GGT.

Table 17

Test of Model Effects with GGT as the Outcome Variable

Source	df1	df2	Wald F	Sig.
(Corrected Model)	16.000	6.000	49.921	.000
(Intercept)	1.000	21.000	22.745	.000
Gender	1.000	21.000	117.066	.000
# of joints/day	3.000	19.000	.931	.445
Avg # Alcohol	2.000	20.000	19.181	.000
BMI	3.000	19.000	10.702	.000
Age of initiation	2.000	20.000	.409	.670
Duration of use	4.000	18.000	.989	.438
Age of participants	1.000	21.000	.586	.452

Model: GGT = (Intercept) + Gender + # of joints /day + Avg # Alcohol + BMI + Age of initiation + Duration of use + Age of participants

As shown in Table 16, the effect of the age of initiation on the GGT revealed a *p* value greater than .05, $F(2, 20) = .409, p > .05$, and the duration of use also showed a *p* value that was greater than .05, $F(4, 18) = .989, p > .05$. Finally, the effect of the

quantity of use also showed a *p* value greater than .05, $F(3, 19) = .931, p > .05$. The preceding results suggest that the three predictor variables failed to be statistically significant predictors of the effect of marijuana used on GGT. In this case, the null hypothesis which stated that there is no significant association between the age of initiation, the dose of marijuana consumed, and the duration of use on GGT failed to be rejected.

Table 18

Parameter Estimates with GGT as Outcome Variable

		Std.	95% CI		Hypothesis Test		
Parameter	Estimate	Error	Lower	Upper	t	df	sig
(Intercept)	.061	.024	.011	.111	2.556	21.000	.018
Male	-.021	.002	-.024	-.017	-10.820	21.000	.000
Female	.000[b]	.	.	.	.	.	.
1 joints/day	-.006	.007	-.019	.008	-.869	21.000	.395
2 joints/day	-.005	.006	-.017	.007	-.894	21.000	.382
3-5 joints/day	-.003	.006	-.016	.010	-.503	21.000	.620
6 or more joints/day	.000[b]	.	.	.	.	.	.
Light drink (< 2 drink / day)	.013	.003	.006	.019	4.214	21.000	.000
Moderate drink (2-4 drinks / day)	1.673E-5	.002	-.005	.005	.007	21.000	.995
Heavy drink (>= 4 drinks per day)	.000[b]	.	.	.	.	.	.
Underweight (< 18.5 kg/ m^2)	.016	.013	-.012	.044	1.186	21.000	.249
Normal weight (18.5 – 24.9 kg/ m^2)	.020	.003	.013	.027	5.849	21.000	.000
Overweight (25 – 29.9 kg/ m^2)	.009	.002	.004	.014	3.778	21.000	.001
Obese (30 kg/ m^2 or greater)	.000[b]	.	.	.	.	.	.
Initiation as Teen (< 15 yrs. old)	-.002	.009	-.020	.015	-.283	21.000	.780
Initiation as Youth (15 – 24 yrs. old)	-.003	.007	-.019	.012	-.438	21.000	.666
Initiation as Adult (25 – 59 years old)	.000[b]	.	.	.	.	.	.
< 10 yrs. of use	.017	.012	-.008	.043	1.413	21.000	.172
10 to 19 yrs. of use	.008	.010	-.013	.029	.785	21.000	.441
20 to 29 of use	.007	.009	-.013	.026	.699	21.000	.492
30 to 39 yrs. of use	.006	.008	-.010	.022	.744	21.000	.465
>40 yrs. of use	.000[b]	.	.	.	.	.	.
Age of participants	.000	.000	-.001	.000	-.766	21.000	.452

a. Model: GGT = (Intercept) + Gender + # of joints /day + Avg # Alcohol + BMI + Age of initiation + Duration of use
b. Reference category

Based on the results presented Table 18, the estimates of each category of the three predictor variables were not statistically significant.

Summary

The study was designed to address one research question, which was to evaluate if any association exist between the three predictor variables and each of the biochemistry profile of the liver, including the serum level of ALT, AST, ALB, ALP, TB, TP, and the GGT. After ensuring that the dataset was adequate and met all required assumptions to use linear regression to test the hypotheses, the results of the analysis revealed that the age of initiation failed to be a significant predictor of the health effect of marijuana on all the liver function markers, while the duration of use significantly predicted variations in ALP and TP and the quantity of marijuana use was a predictors of the variation in TB with an apparent detrimental effect on the serum level of TB level.

Chapter 5: Discussion, Conclusions, and Recommendations

The scope of this study was to identify possible predictors of the health effects of marijuana on hepatic function. The primary objective was to assess potential associations between the number of joints or pipes of marijuana use, the age of initiation and the duration for which marijuana was used with each of the serum levels of liver enzymes including the serum level of ALT, AST, ALB, ALP, TB, TP and GGT using a large nationally representative sample from the NHANES. Results of the analysis, revealed that the age of initiation failed to be a significant predictor of the health effect of marijuana on all the liver function markers, while the duration of use significantly predicted variations in ALP and TP and the quantity of marijuana use was a predictors of the variation in TB.

Interpretation of the Findings

Findings related to the health effect of marijuana on the liver are not consistent. Studies revealed toxicological and therapeutic health effects on the liver associated with marijuana use (Adejumo et al., 2018; Kim et al., 2017; McElrath et al., 2017). The findings from prior studies were obtained in many cases from patients with preexisting liver conditions where there was a possible lack of assessment of the health effects of the predictors on individual liver enzymes. It is important to note that abnormal levels of the individual liver enzymes are characteristic of different liver diseases, and thus investigating the variation of these enzymes individually is very crucial. Furthermore, many scholars, such as Wolkow et al. (2014), have reported that long-term use of

marijuana may have detrimental health effects on the liver in a manner similar to the use of alcohol and tobacco.

The Quantity of Marijuana Smoked and the Serum Level of Liver Enzymes

The findings in this study revealed that the amount of marijuana use is not a statistically significant predictor of the health effects of marijuana on the hepatic enzymes except for TB. According to this study, people who used 1 joint (or pipe) of marijuana per day had a serum TB level that was .536 mg/dl less than that in people who used 6 or more joints (or pipes) per day. In other words, heavy marijuana smokers tended to have a higher level of serum TB compared to light smokers. This result is consistent with a case-control study in India that involved 250 male subjects (125 cannabis abusers and 125 in the control group), where a significant difference was found in the mean value of TB when the two groups were compared. Cannabis abusers exhibited a total bilirubin value of 14.78 (*SD* = 3.10) compared to 11.1 (*SD* = 3.23) in the control group (Wani et al., 2017). Similarly, Quraishi et al. (2013) found that the consumption of cannabis increases the level of serum bilirubin by 13.72% compared to noncannabis users in a case-control study of 30 control subjects and 51 cannabis dependent subjects (mean age of initiation = 15.31 yrs., *SD* = 4.7 yrs., duration of cannabis use = 9.53 yrs., *SD* 8.06 yrs.). In addition, Borini et al. (2004) observed that there was no correlation between the amount of marijuana use and the serum level of ALT, AST, ALP and GGT, which is also consistent with this study's results. No speculation was made regarding the association between the quantity of marijuana use and the serum level of ALB, TB and TP in Borini et al.'s study.

Contrary to the findings in this study, other researchers found that the use of marijuana has no detrimental effect on TB (Kotan et al., 2017).

The quantity of marijuana used is at the core of the discussions regarding the predictors of the effects of marijuana on human health. Several studies associated the quantity of marijuana use with a lower prevalence of disease and even therapeutic effect to the drug (Adejumo et al., 2017; Donghee et al., 2017). The results of the current study appeared to contradict these findings. It is apparent that elevated consumption of the drug is associated with increased level of TB, which is known to be associated with hemolytic jaundice, internal hemorrhage, and acute hemolytic anemia (Bishop et al., 2018).

Duration of Use and the Serum Levels of Liver Enzymes

The findings in the current study revealed that the duration of marijuana use is a statistically significant predictor of the serum level of ALP and TP in marijuana users but failed to be a predictor of the variation in the serum levels of ALT, AST, ALB, TP, and GGT. Although there was a significant effect of the duration of use on ALP and TP, there was no clinically significant difference in the serum level of ALP and TP when someone who had used the drug for less than 10 years was compared to someone who had used it for more than 40 years. Several studies have shown that the duration of marijuana use is a possible predictor of the health effect of marijuana use of the liver. For example, Quraishi et al. (2013) found that using cannabis for more than 9.53 years (SD = 8.06) had detrimental health effects on the liver, while Kotan et al. (2017) found that there were no health effects when cannabis was used for 30.5 months. It is apparent that the longer marijuana is used, the more significant health effects it appears to have. In the current

study, the average duration of marijuana use was 19.20 years (*SD* = 11.68). Still, the effect of the duration of use was only significant on ALP and TP levels with no difference in the level of ALP and TP among the users was found. The above results suggested the need to introduce other epidemiological factors that may improve the association between the duration of marijuana use and the level of liver enzymes. It has been well known that aging factors, gender, and ethnicity have possible effects on the activity of the liver enzymes (Bishop, 2018; Kim et al., 2017; Ruhl & Everhart, 2012; Tian et al., 2014). In the present study, the impact of the age of the participants has not been adjusted due to its correlation with other independent variables in the regression equation. However, the regression equation in this study has been adjusted for gender, for ALP, and for TP. The introduction of gender into the regression slightly modified the R^2 value but had no significant effect on the coefficient estimates. Although the results of the current study have not been consistent with respect to whether the duration of use has a detrimental or therapeutic effect on the liver, it is essential to note that any imbalance caused by the consumption of marijuana on TP and ALP is significant. Serum total protein, which is mainly synthesized by the liver, is particularly important because it serves in the regulation of several physiological functions, maintaining the osmotic pressure, transport of various metabolites, and participation in the activity of the immune system (Bishop, 2018). As for ALP, variation in the enzyme activity can occur in different liver conditions, including liver cirrhosis, chronic hepatitis, and viral hepatitis, and in the absence of liver damage such as congestive heart failure, related bone disorders and in primary and metastatic cancer (Bishop et al., 2018; Lowe & John, 2018)

The Age of Initiation and the Serum Levels of Liver Enzymes

The findings of the current study revealed that the age of initiation is not a statistically significant predictor of the health effect of marijuana on liver enzymes. These findings contradicted results from other studies (Quraishi et al., 2013) that indicated the age of initiation is a possible predictor of the variations observed in the liver-related health parameters. Kotan et al. (2017), for example, found that the age of initiation was a significant predictor of the variations in liver enzymes.

The discrepancy observed in this study compared to other epidemiological studies may be evidence of the variations in the age and gender of participants across study samples. As much as the effects of age and gender factors were reduced in this study, the impact of other potential confounders such as growth factors, nutrition factors, hormone, injury factors, and diseases related factors could not be reduced. These factors were all known to be contributing factors associated with the variations in levels of liver enzymes.

Limitations of the Study

The major strength of this study was that a large, nationally representative sample was used. A combination of four survey cycles collected from 2009 to 2015 increased the sample size and made it more appropriate for inference purposes. I excluded patients with HBV and HCV infection, which have been found to increase liver enzyme levels. The participants' ages were limited to 20 to 59 years old to reduce the effect of aging factors known to have a possible effect on observed variations in liver enzymes. Although, the inclusion of a control group which in this case should be the non-marijuana users may greatly strengthen the ability to draw conclusions from a study (Godby, 2018), I did not

consider including control group in this study. Self-reported data are known to be a source to bias. In the data sample used for this study, several steps including oversampling of the minority population were taken to reduce selection bias. Although the study was adjusted for some epidemiological factors such as age and gender, many other epidemiological factors, including for example, income level, education level, and lifestyle, would have contributed to the variations in liver enzymes and improved the relationship between the variables. The study was only adjusted for the effect of BMI. However, the effect of several metabolic factors including glucose and cholesterol, which had been found to affect the function of the liver, could have been adjusted for a more specific relationship between the predictors and outcome variables. Although excluding HBV and HCV infected participants from the study may have contributed to reducing the selection bias, other disease states, such as liver cirrhosis and liver cancer are known to affect the level of liver enzymes (Bishop, 2018). Participants with these diseases have not been excluded or the effect of these diseases have not been controlled due to the unavailability of pertinent data. Several therapeutic drugs, such as phenobarbital and theophylline, have also been found to affect the enzymatic level of the liver (Bishop et al., 2018; VanWagner & Green, 2015). Data on these therapeutic drugs were not also available to analyze their impact on the level of liver enzymes in the current study.

It would be interesting to investigate those who use other illicit drugs separately from those who use only marijuana to ascertain whether there is a specific relationship between the variables. Separating marijuana users from users of other illicit drugs has not been investigated in this study, and it is also important to note that several authors have

reported on the difficulties in doing so. The statistical methodology applied in this study relied on linear regression analysis, which used the least square technique to analyze the data. Many other statistical techniques exist, and their application to the current study might be more appropriate to enhance the findings to the study. The results of the current study were obtained based on hypotheses generated during the study using self-reported data and interpretations of the results are limited to the context of this study. However, alternative interpretations could not be completely ruled out.

Recommendations

This study used a large nationally representative sample to evaluate the health effects of three predictor variables, including the quantity of marijuana use, the length of time it was used, and the age of initiation, on the level of individual liver enzymes in a healthy population. The findings revealed a significant effect of the quantity of marijuana smoked on TB, while the duration of use was a statistically significant predictor for levels of ALP and TP. The age of initiation failed to predict variations in any of the liver enzymes. The study could be improved if marijuana users can be isolated from those individuals who use other illicit drugs. It is well known that risk factors associated with marijuana abuse include the concurrent use of other illicit drugs (Palamar et al., 2015). In addition, illicit use of drugs such as cocaine can lead to several liver abnormalities, ranging from mild asymptomatic elevation in liver enzymes to severe liver injury (Pateria et al., 2013). Consequently, other illicit drugs are potential confounders in the association of marijuana use with the liver, thus reducing the effects of these potential confounders to obtain a clear association between marijuana uses and the liver become very important.

There is evidence that the pattern and the effects of marijuana use are related to race and ethnicity. For example, Pacek et al. (2012) found that marijuana use disorder was greatest among African Americans compared to other race/ethnicities. In this study sample, 67.4% of participants were non-Hispanic white. As a result, the study did not consider a large proportion of the population who use marijuana. More studies with different proportions of racial/ethnicities groups as well as studies in the minorities black and Hispanic groups are needed to identify if the pattern of marijuana use in relation to race and ethnicity is a defining factor in the relationship between marijuana use and liver enzymes. The activities of the enzymes in the human body are complex and affected by disease stages, lifestyle, nutritional status, and metabolic factors. Thus, they are potential confounders in the relationship between marijuana use and the liver. Designing studies that can adjust for these factors in a preexisting liver disease population and in a healthy population sample is also needed. As observed in this study, the quantity of marijuana use has effect on the activity of TB. However, this study is observational by design. There is a need for experimental studies to quantify the amount of marijuana that is clinically significant to affect the activity of the TB.

Implications for Social Change

The results of this study can be used for social change on two significant levels, both to improve care management for marijuana users and to improve liver transplant denial policy for marijuana users.

It is not uncommon in the medical diagnostic process to eliminate possible illnesses or causes of disease one at a time using clinical information, history and lifestyle

of the patient (Committee on Diagnostic Error in Health Care, 2015). Knowing exactly the effect of marijuana on the liver enzymes will probably accelerate the process in marijuana users and help in accurate diagnosis.

Despite the inconsistencies in the findings regarding the health effects of marijuana on the liver, it remains a schedule I drug in the United States. Furthermore, marijuana users are routinely denied liver transplants. Like other studies, the current study also revealed that marijuana does not affect all liver enzymes. Accordingly, this study adds to the existing literature to help review, or if necessary, to improve the liver transplant denial policy for marijuana users.

Conclusions

Several studies have evaluated the effects of marijuana use on the liver by comparing the prevalence of liver diseases in marijuana users and nonusers or by comparing the quantity and the duration of marijuana use in people with preexisting liver conditions. In this study, the effects of the drug on the individual liver enzymes was evaluated in a healthy population. The study revealed that the length of time marijuana was used is a possible predictor of variations in serum levels of ALP and TP although, no significant difference has been observed in the level of ALP and TP in relation to whether marijuana was used for 10, 20, 30 years compared to those who use it for more than 40 years. The quantity of marijuana used has also been revealed as a significant predictor of variations in the serum level of TB with an apparent detrimental effect on the serum level of TB level. Finally, the age of initiation has been found to have no significant effect on the variations in the serum levels of liver enzymes.

The current study used a nationally representative sample and applied linear regression to evaluate hypotheses generated during the study. However, the data on marijuana utilization were self-reported, and therefore it may include bias. Several epidemiological, physiological and metabolic factors that are known to affect the activities of the liver enzymes could have been controlled during analysis to reduce potential bias and enhance the relationship between the variables.

The current study adds to the existing literature to help improve the health of marijuana users by first, enhancing the care management of the drug users by accelerating the medical diagnostic process, and secondly, by helping to improve liver transplant denial policies for marijuana users.

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