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Serum Aspartate Transaminase and γ-Glutamyl Transferase as Surrogate Markers of Liver Function in Alcohol, Opioid and Methamphetamine Abuse

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Glossary of Abbreviations

ALT	Alanine Transaminase
ADH	Alcohol Dehydrogenase
AUD	Alcohol Use Disorder
ALDH	Aldehyde Dehydrogenase
ALP	Alkaline Phosphate
AST	Aspartate Transaminase
ADHA	Attention Deficit Hyperactivity Disorder
CNS	Central Nervous System
CYP450	Cytochrome P450
Δ	Delta Receptors
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders
GABA	Gamma-Aminobutyric Acid
γGT	Gamma-Glutamyl Transferase
GCC	Gulf Cooperation Council
HDL	High-density Lipoprotein
ICD-10	International Classification of Diseases
IV	Intravenous
K	Kappa Receptors
LFT	Liver Function Test
LDL	Low-density Lipoprotein
μ	Mu Receptors
NMDA	N-methyl-D-aspartate
NIAAA	National Institute on Alcohol Abuse and Alcoholism
NADH	Nicotinamide Adenine Dinucleotide + Hydrogen
NS	Not Significant
OUD	Opioid Use Disorder
PRISMA	Preferred Reporting Items for Systematic Review and Meta-Analysis
SPSS	Statistical Package for Social Sciences
SUD	Substance Use Disorder

UAE	United Arab Emirates
UK	United Kingdom
UNODC	United Nations Office on Drugs and Crime
US	United States
WHO	World Health Organization

Abstract

Introduction: Substance use disorder (SUD) is considered as a mental disorder that alters the brain and impacts the behavior. SUD ranges from moderate to severe, and addiction is the most severe form of the condition. SUD imposes a great challenge to the patients as well as to the community. However, the impact of psychoactive substances on somatic health are often overlooked or not fully explored by institutions dealing with addicts. This emphasizes the need to carefully analyze and properly interpret the routine laboratory tests in order to prevent and treat any unforeseen comorbidities.

The liver plays a pivotal role in metabolism of xenobiotics like all drugs of abuse through phase I of mainly hydroxylation to increase hydrophilicity and phase II of mainly conjugation to facilitate excretion. However, the effects of different psychoactive substances on different blood markers and especially the liver function tests are different and incompletely explored. Specific non-functional plasma enzymes continue to be understudied and poorly utilized. This study addresses this gap by throwing light on some of the liver function plasma enzymes in relation to selected cases of SUD.

Aim: To explore the relationship between alcohol, opioid and methamphetamine use and selected classical liver function plasma enzyme markers; namely aspartate transaminase (AST) and γ-glutamyl transferase (γGT) which are routinely used to assess patients with liver damage.

Methodology: A retrospective exploratory cross-sectional study was implemented using secondary data obtained from St. George's University of London, UK, through personal

communications. The study involved 329 subjects (207 males and 122 females), with the age of the participants ranging from 18 to 89 years old. The patients were divided into three groups: alcohol, opioid and methamphetamine dependents, and were then investigated regarding the substance of abuse impact on the selected liver function plasma enzyme markers (AST and γGT). A comparison of mean values of (AST and γGT) in each subgroup were investigated using independent sample t-test and correlations between variables were also explored using appropriate regression analysis.

Results: The Mean ± SD of age among male and female patients were (50 ± 20.3) and (45 ± 20) years, respectively, a statistically significant difference between the mean age of males compared to females (P value= 0.03) was reported. Both male and female opioid dependent subjects had the highest average in age, Mean ± SD of age (51.3 ± 20.1) years. However, the difference from mean age of ethanol group was not statistically significant. The age difference between opioid and methamphetamine group was statistically significant, with a (P value of 0.001), as the mean age group in methamphetamine users was Mean ± SD (44.2 ± 19) years. In relation to age, no statistically significant correlation was observed between age of patients and each of AST or γGT level in any of the three groups (P value= 0.34 and P value= 0.57, NS) respectively. In addition, direct correlation between the levels of AST and γGT was observed in all study population, with (R=0.7, P value= 0.001). Chi-square test revealed that men had statistically significant association with the use of ethanol, (Chi^2= 13.2, P value= 0.001). Mean serum AST level in patients who abused alcohol, Mean ± SD (38 ± 13.1) is statistically significantly higher than in patients who abused opioids, Mean ± SD (21.2 ± 9.0), (P value= 0.001); and in patients who abused methamphetamine, Mean ± SD (24 ± 6.3), (P value= 0.001). Moreover, mean serum γGT level in patients who abused alcohol, Mean ± SD (57 ±

34.4) is statistically significantly higher than in patients who abused opioids, Mean ± SD (25 ± 9.0), (P value= 0.001); and in patients who abused methamphetamine, Mean ± SD (26.3 ± 10.2), (P value= 0.001).

Conclusions: The findings demonstrated a pattern of deviated values of AST and γGT in patients who abuse ethanol, and also in the other two groups, albeit in a different and less remarkable way . Ethanol use had the most dramatic effect on AST and γGT among the three tested groups in this study. Although the level of the plasma enzymes was also higher in opioid or methamphetamine users, the levels remained in the upper reference range and did not cross into the abnormal level outside the reference range. This might indicate the need for special reference ranges for these enzyme markers when used to assess SUD subjects. The results raise the alarm about liver cell damage with alcohol abuse, and also with opioid and methamphetamine abuse, and the possible use of these markers as indicators for alcohol abuse and assessment of abstinence. Other enzyme markers, e.g., creatine kinase, might be similarly suggested for future research for other drugs of abuse, e.g., cocaine or methamphetamines.

Keywords: Liver Enzymes, Aspartate Transaminase (AST), γ-Glutamyl Transferase (γGT), Opioid, Alcohol, Methamphetamine, Susbatnce Use Disorder.

Chapter 1

Introduction

This chapter sets the scene for this thesis by initially providing a background information concerning substance use disorder, psychoactive substances and surrogate markers of liver function. It then discusses the epidemiology, and it moves to outline the aims, objectives, and hypothesis. Then it moves on to discuss the rationale, purpose and relevance of the current research topic and its importance within the broad clinical practice. Finally, it will move to discuss the gaps in human knowledge.

1.1 Background Information

1.1.1 Substance Use Disorder

Substance use disorder (SUD), as per the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), belongs to the class of disorders that is related to action of ingesting or abusing drugs. It is sub-classified based on the severity into mild, moderate and severe. The main characteristics associated with SUD includes: risky drug use, loss of self-control that manifests in compulsive drug seeking despite the desire to discontinue using, social consequences and pharmacological changes (i.e., tolerance and withdrawal). In fact, Addiction specifically which is defined as a chronic relapsing disease, is the most severe form of a full spectrum of SUD (Doran, 2016; National Institute on Drug Abuse, 2018; National Institute on Drug Abuse, 2019).

1.1.2 Psychoactive Substances

Psychoactive substances or psychotropic drugs such as stimulants, depressants, and hallucinogen, as seen in (Table 1), are the substances that affect the mental processes such as cognition, perception, mood, and consciousness. Yet, these are not necessarily going to induce dependence (World Health Organization, 2021). Many of them have originally therapeutic actions such as analgesics, however, they are associated with a high potentiality to being addictive (Sanli et al., 2015). The use of psychoactive substance is usually prohibited or controlled when used outside legally sanctioned channeles by law. In the same vein, the term illicit drugs are commonly referred to the psychoactive drugs which should not be consumed or owned by public as per the legal framework in a specific geographical area (World Health Organization, 2021).

Table 1: Classes of psychoactive substances and their effect

Depressants	Stimulants	Hallucinogen
Substances that inhibit the activity of the CNS	Substances that induce alertness, agitation, and impaired judgment, alter heart rate, increase blood pressure and increase sweating.	Substances that alter the perception, feeling and thinking
Examples: opioids, alcohol, sedatives, hypnotics and barbiturates.	Examples: nicotine, caffeine, amphetamine and cocaine	Examples: LSD, psilocybin, mescaline and phencyclidine.

(A Glossary of Terms, 2000)

1.1.3 Aspartate Transaminase and γ-Glutamyl Transferase

Aspartate Transaminase (AST) and γ-Glutamyl Transferase (γGT) are liver enzymes that are used to index liver injury. γGT is a glycoprotein composed of both carbohydrates and proteins, they help in digestion and is mainly found in vital liver cells and in other cells responsible for the production of bile including the biliary epithelial cells. This enzyme is a sensitive marker of hepatobiliary diseases and is known as an oxidative stress marker. Studies

have shown that elevated levels of γGT is an early indicator of liver diseases and chronic heavy alcohol intake, however, it is not an exclusive marker for heavy alcohol consumption (Peterson, 2005; Van Beek et al., 2013).

Aspartate Transaminase (AST) is an enzyme that metabolizes amino acids, and is found in many body organs including the liver, heart, muscles, kidney and brain (Peterson, 2005). An increased level of AST indicates a damaged cell membrane of the liver, thus mark hepatocellular injury. Both increased levels of γGT and AST are proposed to be surrogate marker of fatty liver (Van Beek et al., 2013). One could argue that substance use disorder is concerned about taking the drugs in excess, over a long period of time and multiple times a day, which could put the liver at a higher risk of injuries and abnormalities.

2.1 Epidemiological Aspect

Based on the recent report released on 24 June 2021, by the United Nations Office on Drugs and Crime (UNODC), the number of illicit drug users is estimated to be 275 million people in 2020. The report demonstrated that between 2010 and 2019 the number of drug users have increased by 22 per cent around the world, and it was suggested that a further increase is expected by 11 per cent in 2030 (United Nations Office on Drugs and Crime, 2021).

Cannabis is the most widely used illicit drugs with 192 million users around the world (World Health Organization, 2019). Among all drugs of abuse, opioid continues to have the highest link between its use and the burden of disease (World Health Organization, 2021). In 2019, UNODC has estimated the number of people who have used opioid globally to be 61,650.

In the Americas opioid abusers were estimated to be 12,580, while in Europe 3,610. Asia had the highest estimated users of opioid of 35,750. Amphetamine and methamphetamine abusers were estimated to be (8,710), (12,670), (2510) in Americas, Asia and Europe, respectively (United Nations Office on Drugs and Crime, 2019).

Alcohol is the most commonly abused substance in the United States (Addiction Center, 2017). According to the National Institute on Alcohol Abuse and Alcoholism (NIAAA), in 2019 the number of people who suffered from alcohol use disorder in the US was nearly 15 million. Furthermore, although alcohol is a legal substance, its global burden has been on the rise, with 3 million deaths reported in 2016 attributable to alcohol consumption. NIAAA, has also reported that in 2019, 43.1 per cent of all the liver diseases deaths in the US were related to alcohol consumption (National Institute on Alcohol Abuse and Alcoholism, 2021).

The novel coronavirus, which is now termed as SARS-CoV-2, was first identified in 2019 and was announced as pandemic on 11 March 2020 by the World Health Organization (WHO). Since then, many countries around the world responded with lockdown regulations and physical distancing measures, which resulted in economic recession, unemployment and emotional distress. This have created an international public health concern and crisis(United Nations Office on Drugs and Crime, 2020). In fact, all the aforementioned factors are known to impact negatively and tend to increase drug use (Lopez-Pelayo et al., 2020). In May 2020, UNODC has stated that the pandemic is disrupting drug trafficking routes due to air, land and sea travel restrictions. This has created shortage at the retail level reflecting on a decrease on some substances of abuse such as heroin in Europe, North America and South West Asia. Unfortunately, this shortage has led to a more harmful use and domestically produced

substances with low quality. In fact, it was found that heroin dependents have switched to fentanyl and its derivatives which is approximately 50-100 times more potent than morphine, also an increase in pharmaceutical products such as benzodiazepine was reported. UNODC has also reported that the risk of drug overdose is expected to increase among people infected with Covid-19 (United Nations Office on Drugs and Crime, 2020; World Health Organization, 2021).

3.1 The Three Most Common Illicit Drugs

As mentioned earlier, opioids, alcohol and methamphetamine seem to be the most common illicit drugs, and they will be the main focus in our study in relation to their effect on AST and γGT. To provide a better understansing of these three substances, this section will present the history, the pharmacology and the pathophysiology of each of these substances.

3.1.1 Opioids

History and Definitions

Opioids use has a long history, as it was used for medical reasons or recreational purposes. Opium has been cultivated as early as 3400 BC, and the term opium indicates a mix-true of alkaloids from the poppy plant *Papaver somniferum*. On the other hand, the term opiates refers to the naturally occurring alkaloids, while semisynthetic and fully synthetic opioids are more chemically distinct and require some manipulation in manufacturing (Dadiomov, 2020). Examples of opioids are listed in (Table 2).

Table 2: Classification of opioids

Natural opiates:	Semi-synthetic opioids:	Synthetic opioids:
Morphine	Heroin	Fentanyl
Codeine	Hydromorphone	Pethidine
	Hydrocodone	Methadone
	Oxycodone	Tramadol
		Dextropropoxyphene

(National Institute on Drug Abuse, 2013)

Opioid is the term that has been used broadly and identifies all compounds that act on the opioid receptors. Narcotics, which is a Greek term for stupor, was originally used to describe sleep medication, then it was used to describe opioids only, and recently it has been used as a legal term for drugs of abuse (Trescot et al., 2008).

Opioid use disorder (OUD) is defined as a chronic condition associated with opioid use, and results in significant distress, impairment, dependence and addiction. Based on the American Psychiatric Association DSM-IV, OUD diagnostic criteria includes a strong desire to obtain the drug despite harmful consequences, tolerance, and withdrawal. OUD brings suffering to those drug-dependent individuals and people around them, which is a clear indication that its impact goes beyond the user to people surrounding him/her. Accordingly, illicit opioids and its related consequences impose a great challenge on the community from a wide range of aspects including: health, social and economic aspects (Saunders et al., 2016).

Pharmacology and Pathophysiology

Pharmacology

Although the chemical structures of opioid drugs differ, they all share the same mechanism of action which is binding to the μ opioid receptor in the central nervous system (CNS). Accordingly, they are capable of producing analgesia and varying degrees of euphoria and sedation as seen in (Table 3). Furthermore, they are able to stimulate μ and possibly δ receptors found in the dopaminergic pathway or the reward systemin the brain, which manifest in addiction. Opioid receptors are distributed within the CNS as well as throughout the peripheral tissues and are normally stimulated by the endogenous peptides (endorphins, enkephalins, and dynorphins) (Trescot et al., 2008).

Table 3: Opioids receptors

mu (μ) receptors:	kappa (κ) receptors:	delta (δ) receptors:
Found primarily in the medial thalamus and the brainstem and activating them can cause respiratory depression, sedation, euphoria, physical dependence, and decreased gastrointestinal motility.	Principally found within the spinal cord, limbic, brainstem, and activating them can cause drowsiness, dysphoria, spinal analgesia and respiratory depression.	Found largely in the brain but not well studies but believed to produce analgesia, cardiovascular effects (hypotension, bradycardia).

(Saunders et al., 2016; Trescot et al., 2008)

The vast majority of opioids have high gastrointestinal permeability, so they get completely absorbed from the gastrointestinal tract (GIT). However, they have a low bioavailability due to the hepatic first pass effect, and it gets slowly releasesd into the bloodstream, thus it takes longer to show its effects but once it starts it stays longer. On the other hand, when opium is inhaled, it is heated until the active alkaloids evaporate and are smoked. This process causes the loss of a great amount of the active ingredients. Yet, smoking opium has an intense and fast onset of action as it avoids the first pass effect, but its duration is considerably short. Opioids then get distributed throughout the whole body tissues, and cross the blood-brain barrier to reach the central nervous system, which is the main site of action (Drewes et al., 2012; Najafipour & Beik, 2016).

Metabolism of opioids might be the most important part of the pharmacokinetics of opioids (Pruskowski & Arnold, 2015). Metabolism is basically a biotransformation process to render the drug more polar and hence, gets eliminated easily. However, metabolism also produces substances that are both clinically significant and toxic. Many of the side effects as well as the pharmacological effect of opioids are related to their metabolites (Smith, 2009). Some drugs remain intact through the whole process from performing their functions to getting excreted from the body, while many other drugs will require metabolism to enable them to perform their actions in an appropriate time and then get eliminated from the body. Opioids are a class of drugs that require metabolism which produces active and inactive metabolites. These metabolites can be more potent than the parent compound. Opioids are metabolized mainly via cytochrome P450 (CYP450), in the liver, kidneys and the gastrointestinal tract (Atici et al., 2005; Trescot et al., 2008).

Opioids undergo 2 phases of metabolism: Phase I imainly subjects the drugs to oxidation or hydrolysis, and are carried by CYP450, CYP3A and CYP2D6 enzymes which are primarily responsible for opioid metabolism. It is vital to mention that opioids getting metabolized by CYP3A have a higher risk of drug-drug interactions, posing serious side effects and health problems (Smith, 2009; Overholser & Foster, 2011). With regards to Phase II, opioids get conjugated to hydrophilic substances, such as glucuronic acid, which helps the drug get excreted through the kidneys. (Smith, 2009; Overholser & Foster, 2011). For instance, morphine is biotransformed in the liver by glucuronidation to its inactive metabolite morphine-3-glucuronide as well as to the biologically active morphine-6-glucuronide. These active and inactive metabolites tend to increase liver enzymes secretion. This is particularly concerning in case of opioid use disorder, since continuous and larger doses of opioids could have a negative impact on the liver causing liver function impairment (Pawan et al., 2011).

Studies have shown that opioid overdoses have been linked to acute liver injury and elevated levels in serum aminotransferases as well as to signs of hepatic failure. However, it is important to note that studies have not confirmed a causal relationship between opioid and liver toxicity. It was noted that opioids causing ischemic liver is usually due to respiratory failure, shock, anoxia and cardiovascular collapse as a result of opioid overdose (U.S National Library of Medicine, 2020).

Pathophysiology

The dopaminergic neurons in the ventral tegmental area of the brain is known to be implicated in addiction; and opioids activate this circuit which consequently produce a strong

central reinforcement (Pergolizzi et al., 2017). In addition, continuous administration of opioid for 10 – 14 days might result in tolerance, dependance and withdrawal. Tolerance mainly occur due to the downregulation of opioid receptors, while dependence is usually associated with withdrawal syndrome that occurs due to cessation of opioid exposure (Dydyk, Jain & Gupta, 2021; Elnebrisi lecture, Pharmacology of Opioids, 2021).

3.1.2 Alcohol

History and Definitions

Alcohol consumption goes back to 1700 B.C. Ethanol is the active ingredient found in alcoholic beverages such as beer, wine and liquor. Alcohol use is popularly accepted and legally used in various regions of the world, and for many years alcohol was perceived as a source of food energy (Ifeany et al., 2014; Drugs.com, 2021). Despite its popularity, the harmful effects of alcohol have significantly burdened the world, as it ranked among the five risk factors that are associated with death and disabilities worldwide (Saunders et al., 2016).

The DSM-IV have integrated the terms ‘alcohol abuse’ and ‘alcohol dependence’ into ‘alcohol use disorder’ (AUD), and added subclassification of mild, moderate and severe AUD. The criteria for diagnosis of AUD are, impaired control over use, cravings, developing tolerance, withdrawal, continuing use despite negative consequences. Anyone who meets two of the previously mentioned diagnosis in the past 12 months, will be diagnosed with AUD (Saunders et al., 2016).

Pharmacology and Pathophysiology

Pharmacology

Ethyl alcohol or ethanol is a water-soluble substance and it is effectively absorbed in the small intestine and the stomach. It reaches its peak blood concentration within 30-60 minutes from ingestion, and it gets widely distributed throughout the body. Metabolism of ethanol occurs mainly in the liver, and a small amount of ethanol gets metabolized in the stomach through gastric first-pass metabolism (Saunders et al., 2016). Two to ten percent of ingested alcohol escapes the metabolic activity and gets excreted directly though lungs and in urine. The excreted alcohol from the lungs is of a practical use of forming the basis of breath testing especially in the intoxicated persons and motor car offences.

In the liver hepatocytes, there are two enzymes that are responsible for the oxidation of ethanol: alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). Ethanol is oxidized by the enzyme (ADH) into acetaldehyde, a very reactive molecule that gets quickly converted into acetate by the enzyme (ALDH). In addition, acetaldehyde has the ability to bind to certain proteins forming modifications that are harmful causing damage to liver and brain cells (Beecroft et al., 2010).

1. $CH_3CH_2OH + NAD^+ \xrightarrow{ADH} CH_3CHO + NADH + H^+$
2. $CH_3CHO + NAD^+ \xrightarrow{ALDH} CH_3CO_2 + NADH + H^+$

The liver is susceptible to the harmful effects of alcohol mainly due to its vital role as a main site of alcohol metabolism. The oxidation reaction of the metabolic breakdown of alcohol to acetaldehyde tend to change the chemical state of the hepatocyte, causing NADH to

accumulate and consequently affecting the other elements of liver metabolism. The accumulation of fatty acids which results from the increasing synthesis of fatty acids leads to the inhibition of gluconeogenesis, which refer to the metabolic production of glucose from non-carbohydrate sources. As a result, the increased deposited fat will eventually lead to what is known as fatty liver or steatohepatosis (Beecroft et al., 2010).

Alcohol elimination is another important aspect of its pharmacokinetics, since it follows the zero order kinetics. This means that a fixed amount of 7-10 grams of ethanol is metabolized per hour, regardless of its concentration in the boold. This is particulary concerning in cases of overdose (Elnebrisi lecture, Pharmacology of Alcohol, 2021).

Pathophysiology

Alcohol tends to have a strong reinforcing and rewarding effects that includes euphoria and anxiolysis. It is believed that the mesolimbic system or reward system in the brain is involved in these effects. Alcohol is a CNS depressant, and tends to potentiate the GABA receptor-mediated inhibitory function in the brain, and at higher concentrations it tends to inhibit the excitatory function of the NMDA. Science has proved the release of endogenous opioids when ingesting alcohol, which binds to opioid receptors and triggers the release of dopamine in the reward system, which manifests in dependence (Saunders et al., 2016).

Alcohol use disorder usually develops as a result of the chronic and excessive use of alcohol, which leads to various adaptive neurochemical and physiological changes in the brain. With the increased consumption over a long period of time, the brain tries to compensate the function of NMDA by decreasing the number of GABA receptors and increasing the number of

NMDA receptors, consequently this process leads to the development of tolerance, one of the signs of alcohol dependence (Saunders et al., 2016).

3.1.3 Methamphetamine

History and Definitions

Methamphetamine is a powerful synthetical psychostimulant, which is highly addictive and affects mainly the CNS. It takes the form of a crystalline usually white odorless powder, which can easily be dissolved in water and alcohol (National Institute on Drug Abuse, 2019). Methamphetamine is a derivative from its parent drug, amphetamine, and was developed in the early 20th century. Originally, it used to be added in nasal decongestant and bronchial inhalers. However, the main characteristics of methamphetamine such as, euphoria, talkativeness, alertness, decreased appetite, pleasurable sense of well-being, make it a drug with increased potential for misuse. Currently, methamphetamine is classified as schedule II stimulant in the US, a legal medication that is used medically to treat attention deficit hyperactivity disorder (ADHD) (National Institute on Drug Abuse, 2019).

Methamphetamine use disorder is considered to be a complex brain disease that can increase dopamine level in the brain manifesting in reward-seeking behavior and dependence. Long term use of methamphetamine can eventually lead to memory loss, impulsivity, altered mood and damage the CNS (National Center on Substance Abuse and Child Welfare, 2021).

Pharmacology and Pathophysiology

Pharmacology

Neurochemical mechanisms are known to be affected by methamphetamine due to its potent stimulant effect on the central nervous system. These mechanisms are mainly responsible for regulating body temperature, attention, appetite, blood pressure, heart rate and responses that are associated with alertness. Furthermore, the acute effect of methamphetamine mimics the physiological and psychological effect of the neurotransmitter epinephrine including elevated blood pressure and heart rate, bronchodilation, vasoconstriction and hyperthermia (National Library of Medicine, 2015).

Methamphetamine can be injected, sniffed, ingested, but the most common route of administration is smoking. Smoked or injected methamphetamine results in an intense quick sense of euphoria which usually lasts several minutes. On the other hand, oral and intranasal route of administration take generally 5 to 20 minutes to reach peak euphoric state, but the effect tend to last longer than smoked or injected methamphetamine (Courtney & Ray, 2014). Moreover, Methamphetamine is widely absorbed from the GIT, and it reaches its peak concentration within 3 to 6 hours. Additionally, a high degree of absorption occurs when methamphetamine is administered intranasally or through inhalation. Due to the lipophilicity of methamphetamine, it gets distributed across most parts of the body as it easily crosses the blood brain barrier and the placenta (National Library of Medicine, 2015; The University of Arizona, 2021).

The liver is the main site of methamphetamine metabolism, and it occurs primarily through three processes:

1- *N*- demethylation via CYP450

2- Aromatic hydroxylation via cytochrome CYP450
3- B-hydroxylation producing amphetamine, 4-hydroxymethamphetamine and norephedrine. (Matsumoto et al., 2014)

Pathophysiology

Methamphetamine acts mainly by facilitating the release of catecholamines: dopamine and noradrenaline as well as serotonin (which is not a catecholamine). It inhibits their reuptake which leads to an increase of these neurotransmitter in the synapse and consequently increasing the stimulation of postsynaptic receptors (National Library of Medicine, 2015).

4.1 Research Objectives and Hypothesis:

4.1.1 Aims and Objectives

Aim of the study is to explore the relationship between each of opioids, alcohol and methamphetamine use and selected classical liver function markers, namely aspartate transaminase and γ-glutamyl transferase routinely used to assess patients with liver damage. To achieve this aim, a set of five objectives were formulated as follows:

1. To explore the trends of change in AST with each of ethanol, opioid or methamphetamine use, even if they remain within the reference range.
2. To explore the trends of change in γGT with each of ethanol, opioid or methamphetamine use, even if they remain within the reference range.
3. To explore whether age affect the levels of the alteration in AST or γGT in opioid, alcohol or methamphetamine use disorder.

4. To explore if these patterns can be used for checking the successful abstinence, recurrence, continuity of abuse.

4.1.2 Research Hypothesis (H_1) and Null Hypothesis (H_0)

(H_1): There is a relationship between each of opioid, alcohol and methamphetamine abuse and changes in AST and γGT activities in the plasma.

(H_0): There is no relationship between each of opioid, alcohol and methamphetamine abuse and changes in AST and γGT activities in the plasma.

4.1.3 Study Rational and Significance

Despite the importance of laboratory values in guiding treatment and preventing any unforeseen consequences, only few studies in literature have explored the effect of consuming opioid, acohol and methamphetamine on liver function markers (AST, γGT). As delineated earlier, the liver plays a pivotal role in metabolism (phase I, II) to convert the drug or substances into products (metabolites) that can be easily excreted by the kidney.

Being able to identify health problems or complications among substance abusers through these laboratory values will provide an ample opportunity not to just optimize healthcare provided to patients or drug abusers, but also create prevention programs and campaigns and raise awareness about the potential diseases related to abusing these substances (Oduola et al., 2005). Furthermore, it is believed that the outcome of this research will help stakeholders with both theoretical and practical implications to improve the

substance abusers' treatment journey. In addition, this research and the outcomes of it can be used to inform other studies conducted in different settings and geographical areas, hence further flourish the understanding of clinicians related to this topic specifically.

5.1 Gaps in Human Knowledge

A literature search brought to light that even though studies around this area shed light on the influence of psychoactive substances on biochemical and hematological markers in humans (Dennis, 2020), the main focus of literature was the effect of these substances on the CNS. Checking routine laboratory tests such as blood chemistry in patients diagnosed with SUD and properly interpreting the results is cornerstone for good management (Langowska-Grodzka, Ziólkowski & Czarneck, 2016). By doing so, clinicians are able to obtain baseline laboratory values, which can be used to provide guidance to create a comprehensive assessment and treatment plan for patients with SUD. This practice ensures that patients with substance use disorder do not suffer from contradicting conditions that might interfere or interact with a particular therapy, as well as assessing any abnormalities that could be caused either by the previously abused drugs or by the prescribed medication for their future treatment.

To illustrate, analyzing the changes in total blood counts, blood glucose, aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyl transferase (γGT), and total cholesterol, HDL, LDL, triglycerides, may have an indication of the occurrence of different diseases that might necessitate the need of further diagnosis and management. However, as per the literature search and as further discussed in depth in chapter two, the impact of these

substances on the somatic health is yet to be fully understood and explored by institutions dealing with addicts such as rehabilitation centers. And studies in literature were not tackling this specific aspect. Moreover, controversial results were detected in recent studies regarding the effect of opium on biochemical markers (Oduola et al., 2005), and the effect of different psychoactive substances on different blood markers and specifically liver function tests continue to be understudied, (Langowska-Grodzka, Ziólkowski & Czarnecki, 2016; Sanli et al., 2015), and this study addresses this gap. And this further confirms the need to carefully analyze and properly interpret the routine laboratory tests. Additionally, research have suggested that hospitalization of drug-dependent persons is a huge opportunity that health care providers have to take in order to perform these laboratory tests and utilize them in future studies and research (Langowska-Grodzka, Ziólkowski & Czarneck, 2016).

Chapter 2

Systematic Literature Review

The aim of developing this chapter is mainly to provide a comprehensive overview of the previously published studies on various segment of the current research's topic. In order to identify the factors that have influenced the research question, this review will demonstrate and scholarly critique the existing literature on the dynamics of the selected psychoactive substances (opioids, alcohol and methamphetamine) and their possible effect on surrogate liver markers, specifically plasma aspartate transaminase (AST) and γ-glutamyl transferase (γGT).

2.1 Review Questions and Objectives

1- To explore the possible relationship between (ethanol, opioid, methamphetamine) and elevated liver function markers (AST and γGT) in literature.

2- To explore the trends in deranged liver function markers (AST and γGT) between (ethanol, opioid and methamphetamine) and compare them.

2.2 Review Process

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) was used in reporting this review as seen in (Figure 1).

2.3 Selection Criteria

2.3.1 Study Selection

The following inclusion criteria was set to retrieve studies:

1. Study population: Patients diagnosed with either opioid, alcohol or methamphetamine use disorder, or were referred to as having addiction.
2. Study aim and content: to evaluate the nature of the association between the selected substance of abuse and liver function marker enzymes (AST and γGT).
3. Publication language, data, and status: published in English, between the year 2010 and 2020, and is available as full text.

Papers were excluded according to the following:

1. Patients were not diagnosed with either opioid, alcohol or methamphetamine use disorder, or were not referred to as having an addiction.
2. The study content was not to evaluate the nature of the association between the selected substance of abuse and liver enzymes (AST and γGT).
3. Outdated and unavailable as full text articles.

2.4 Search Strategy

The search strategy was shaped by the review objectives/ question, hence ensuring purposeful and selective searching. Additionally, the search process involved using manual and electronic searching strategies. For the electronic search strategies, the following key terms were used while searching the four electronic databases Google Scholar, PubMed, ScienceDirect and ProQuest Health & Medical Complete (opioid use disorder AND liver function) OR (opioid use disorder AND liver enzymes) OR (opioid use disorder AND LFT's) AND (alcohol use disorder AND liver function) OR (alcohol use disorder AND liver enzymes) OR (alcohol use disorder AMD LFT's) AND (methamphetamine use disorder AND liver function) OR (methamphetamine AND liver enzymes) OR (methamphetamine use disorder AND LFT's). The key terms were mainly stemming from the review questions and objectives, the scoping search, and thesaurus and subject headings search.

With regards to the additional search, the reference lists of the included papers were cross-checked, and relevant journals were hand-searched. Moreover, citation tracking and an author search, through Google Scholar, SCOPUS and the Web of Science, were performed to identify the relevant papers (Gough, Oliver & Thomas, 2012).

2.5 Results

All stages of the search are presented in (Figure 1). Four databases were searched using the combination of key terms. This have resulted in identifying a total number of 3930 records or papers. The search was limited to studies that were published in English, and done on human. After screening the papers using the inclusion criteria, 8 studies were found to be suitable for inclusion in this systematic review, and was possible to source them as a full text. All details regarding the studies and the results of the studies are presented in (Table 4).

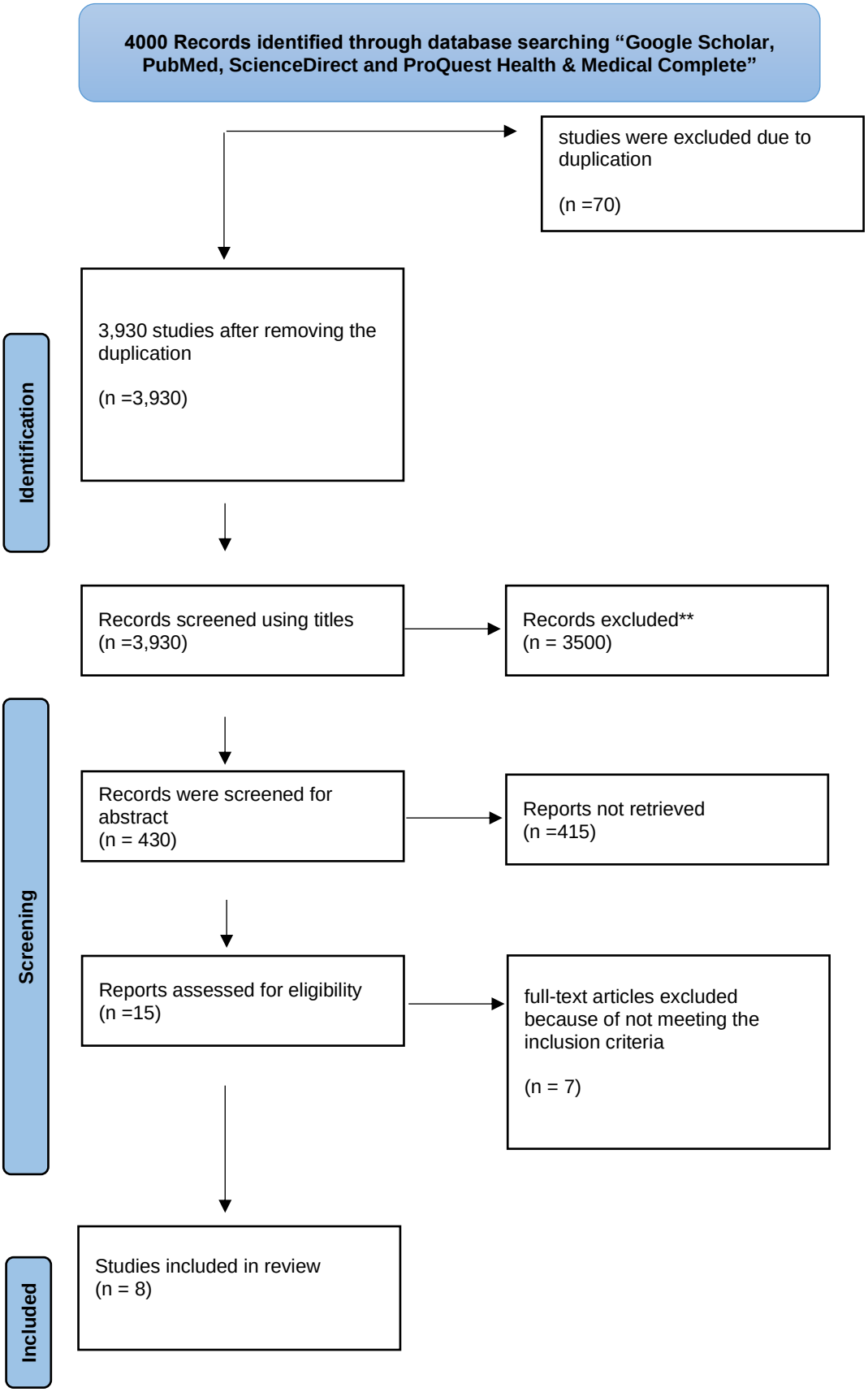


Figure 1. *Preferred Reporting Items for Systematic review (PRISMA) flow chart.*

2.5.1 The Impact of Alcohol on Liver Function Markers (AST, γGT) in Literature:

All studies that have been included in this systematic review have confirmed the increased LFT's due to alcohol abuse. Moreover, two out of three studies in this systematic review, stated that they have clearly diagnosed the patients with alcohol dependence based on the ICD-10 or DSM-IV, the last study has referred to the patients as alcoholics. These studies targeted different demography and population in terms of age, gender and nationality.

Langowska-Grodzka and his collegues (2016), conducted a study in Poland, to evaluate the effect of different psychoactive substances on serum biochemical parameters compared to a controlled group. Results brought to light that alcohol and opioid dependents had the highest γGT, AST levels, and had the highest ALT levels among all groups (Table 4). Roscoff and collegues (2019), has conducted a study in Maryland, US to examine the association between high-intensity binge drinking and lipid and LFT levels. This cross-sectional study targeted a total of 2065 participants of which 1519 were alcoholics. Data were collected between 2005 and 2017 from the NIAAA clinic. The main findings of this study were that increased levels of ALT, AST and γGT detected in high-intensity binge drinking participants. γGT was found to have the largest increase associated with high-intensity binge drinking patients which indicate that γGT might be the most sensitive marker (Table 4).

A cross-sectional observational study was done in India. This study was conducted over a period of 6 months among 150 participants, the main objective was to correlate alcohol intake and its impact on LFT. This study main results found that out of the 150 participants, 120 were found to have abnormal liver tests, most of them belong to the age group of 21-30

years, they explained that by the fact that young Indian tend to consume alcohol more than other age groups. Also, they examined the socioeconomic status along with poor nutrition, and they found that it linked with altered liver function, more accurately, they found that alcohol combined with poor nutrition led to having an effect on elevated LFT (Gogoi et al., 2017) (Table 4).

2.5.2 The Impact of Opioids on Liver Function Markers (AST, γGT) in Literature:

The studies that have been included in this systematic review shared exploring the effect of opioid on liver function markers despite having different approach in doing so. The main parameters explored among all studies in this systematic review are liver function markers, specifically (AST and γGT). Some studies focused mainly on the effect of opioids on these parameters as well as other LFT's, others studied the effect of opioids on different biochemical and hematological markers. Moreover, some studies compared the results of opioid's impact on liver functions with other substances of abuse. Patients in all studies have been selected upon meeting the criteria of dependency either according to the ICD-10 or the DSM-IV, however, one study referred to the participants as "addicts" and did not mention how were they diagnosed.

Langowska-Grodzka and collegues (2016), conducted a study in Poland, on 93 participants of both genders, that were hospitalized at least for two weeks for the treatment of alcohol, amphetamine and opioids. The study's main results were that opioids and alcohol dependent patients had the highest average markers of liver injury (AST, γGT and ALT) to amphetamine dependent-persons (Table 4). Moreover, Pawan et al., (2011), conducted a

study in district hospital of Barmer city in Bangladesh, to assess the harmful effect of opium on liver and lungs. The sample size consisted of 25 opioid dependents and 25 apparently healthy adults (controlled group) and their age ranged from 30 to 50. Findings unraveled that AST and ALT were significantly higher (P value= 0.05) among opium addicts than controlled group, and as per the study this could be due to the fact that opioid gets metabolized by the liver causing an increase in the liver markers over a long period of use (Table 4). In Istanbul, Turkey, a similar study was conducted by Sanli et al., (2015), that aimed at determining the effect of several psychoactive substances on serum biochemical parameters. 324 drug dependents were included and 69 controls, 46 out of 324 were opioid dependents, those patients were admitted to the Erenkoy Mental Health and Neurology Training and Research Hospital between 2013 and 2014. The study found that the use of more than 2 years of psychoactive substances resulted in more profound impact on the serum biochemical markers. Moreover, AST and γGT were found to be increased among opioid dependent compared to the control group, however, this increase was not significant (Table 4).

2.5.3 The Impact of Methamphetamine on Liver Function Markers (AST, γGT) in Literature:

The main parameters explored among all studies in this systematic review are AST, γGT and ALT, however, γGT was not examined in all methamphetamine related studies. All the studies focused mainly on the effect of methamphetamine on these parameters, others included other biochemical and heamatological parameters. Searching for studies in this specific domain was particularly challenging, very few studies were allocated that were

interested in exploring the effect of methamphetamine on liver function markers, despite being linked to a change in these markers in published researches.

A retrospective case-control study was conducted in Ibn Sina hospital in Shiraz, Iran, studied the impact of chronic use of methamphetamine on different hematological and biochemical markers. A total of 120 participants were included in this study, of which 60 addicts to methamphetamine were allocated and were chosen based on using methamphetamine for at least 3 months and at least 5 days a week. However, the tool that has been used to diagnose these patients was not fully demonstrated. The study has found that liver enzymes such as AST, ALT and ALP were noticed to be significantly higher in methamphetamine users compared to healthy individuals (P values= 0.001) (Tavasolian et al., 2015) (Table 4). Another study conducted in Taiwan, aimed at identifying the potential laboratory tests and clinical characteristic associated with natural death among methamphetamine dependents. A total of 1,254 Patient were diagnosed based on DSM-IV and were admitted to the psychiatric center in Taiwan between 1990 and 2007, 48 subjects died of natural causes and were defined in this study as case group. The study was composed of phase 1 nested case-control study and phase 2 cohort study. Various potential factors were linked with natural death in phase 1 study, including AST, ALT, comorbid alcohol use and antipsychotic drugs. While in phase 2, the previously mentioned factors were confirmed using survival analysis in the whole cohort. The main findings of this study indicated that deceased patients had significantly higher levels of AST and ALT, moreover, of these case patients a high proportion had alcohol use disorder besides being methamphetamine dependents, and found to have the highest levels of AST and ALT than did the control group. For validating these findings, phase 2 was conducted to further understand the association between AST, ALT and

natural death. They found that, elevated AST whether mildly or markedly increased the risk of natural death, whereas only markedly elevated ALT increased the risk of natural death. Since the comorbidity of alcohol significantly associated with natural death, the study was then restricted to examine the patients who did not have alcohol comorbidity, and they yielded similar results (Kuo et al., 2012) (Table 4).

Table 4: Summary of the Systematic Review of Selected Studies that Address the Impact of Alcohol Abuse on Liver Function Tests

Author	Objective	Population (Sample size, type of participants, recruitment technique)	Location	Methodology/ Methods (Design)	Results
*Langowska-Grodzka, Ziólkowski & Czarneck (2016)	The aim of this study was to evaluate the frequency of reference value deviations in blood chemistry in patients treated for substance abuse and to demonstrate the relationship between the type of dependence and abnormalities in biochemistry studies.	Sample size: 93 hospitalized patients Characteristics: 1. Participants hospitalized for the treatment of opioid, alcohol and amphetamine dependence. 2. Age ranged between 19 to 42, in both men and women Recruitment technique: Not mentioned but as per the description it is convenience sampling.	Poland	Design: Observational cross-sectional design Data collection method: 1) Socio-demographic interviews 2) Laboratory tests (venous blood samples – serum – taken from an ulnar vein on an empty stomach), such as AST, ALT and GTP, total cholesterol, LDL cholesterol. The evaluation occurred in the first two weeks of stay. The selected subgroups of patients were compared in terms of averages and deviations from the reference value in the research activity of GTP, total cholesterol, AST and ALT Ethics: Written consents obtained and approved by ethics committee	1- The highest average markers of liver injury (GTP, AST and ALT) occurred among the patients with alcohol (respec- tively 42, 30 and 44 U/l) and opiate dependence (respectively 95, 42 and 37 U/l), the lowest among those with amphetamine dependence (respectively 17, 19 and 21U/l). 2- These results might indicate liver disease among patients with elevated AST, ALT, and GTP. Elevated AST, ALT and GTP occur in the case of organ damage

· This study was reported twice as it touches on the two drugs of abuse (alcohol and opioids)

Author	Objective	Population (Sample size, type of participants, recruitment technique)	Location	Methodology/ Methods (Design)	Results
Roscoff et al., (2019)	To examine associations of high intensity binge drinking with lipid and LFT levels in a cross-sectional sample enriched with participants who engage in HIBD.	Sample size: 1519 alcoholics, where participants recruited for either the NIAAA screening protocols or inpatient alcohol treatment program Characteristics: 1. Healthy volunteers and Alcohol-dependent participants (diagnosed using the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) 2. Both gender 3. 18 years of above treatment-seeking participants were detoxified from alcohol consumption and participated in the 28-day NIAAA alcohol treatment program, during which extensive clinical and physical examinations were performed Recruitment technique: Convenience sampling through local advertisements, word of mouth, or the NIAAA alcohol treatment program.	Bethesda, Maryland. US	Design: Cross-sectional study using data from the NIAAA clinical sample collected from March 3, 2005, to August 21, 2017 Data collection method: 1. Blood and plasma samples collected post 10 hours of fasting after inpatient admission day, or during a screening visit 2. TLFB information related to alcohol consumption for the 90 days prior to screening or admission Ethics: Written consents obtained and approved by ethics committees.	1- Serum levels of high-density was associated with increased levels of HDL-C, TC, TRG, ALT, AST, and GGT. 2- GGT showed the largest increases associated with HIBD (high impact binge drinking), which suggests that GGT may be most sensitive to HIBD

Author	Objective	Population (Sample size, type of participants, recruitment technique)	Location	Methodology/ Methods (Design)	Results
Gogoi et al., (2017)	To correlate alcohol intake and its effect on liver function test	Sample size:150 study participants screened Characteristics: - Above the age of 10 - Both gender - Coming to the clinical biochemistry laboratory Recruitment technique: Not mentioned but as per the description it is convenience sampling.	India	Design: A cross sectional observational study Data collection method: - Detailed history obtained through a questionnaire which inquired about alcohol consumption. - Blood sample to look at the following: serum bilirubin and fraction, ALT, AST, ALP, total serum protein, serum albumin and GGT. Ethics: Written consents obtained and approved by ethics committees.	1- 120 (80%) were detected to have abnormal liver function tests (LFT) (65 females and 55 males). 2- Maximum numbers of participants with abnormal LFT belong to age group of 21- 30 yrs.

Table 4: Summary of the Systematic Review of Selected Studies that Adress the Impact of Opioids Abuse on Liver Function Tests (Continued)

Author	Objective	Population (Sample size, type of participants, recruitment technique)	Location	Methodology/ Methods (Design)	Results
*Langowska-Grodzka, Ziólkowski & Czarneck (2016)	The aim of this study was to evaluate the frequency of reference value deviations in blood chemistry in patients treated for substance abuse and to demonstrate the relationship between the type of dependence and abnormalities in biochemistry studies.	Sample size: 93 hospitalized patients Characteristics: 1-Participants hospitalized for the treatment of opioid, alcohol and amphetamine dependence. 2-Age ranged between 19 to 42, in both men and women Recruitment technique: Not mentioned but as per the description it is convenience sampling.	Poland	Design: Observational cross-sectional design Data collection method: 1-Socio-demographic interviews 2-Laboratory tests (venous blood samples – serum – taken from an ulnar vein on an empty stomach), such as AST, ALT and GTP, total cholesterol, LDL cholesterol. 3-The evaluation occurred in the first two weeks of stay. The selected subgroups of patients were compared in terms of averages and deviations from the reference value in the research activity of GTP, total cholesterol, AST and ALT Ethics: Written consents obtained and approved by ethics committees.	1-The highest average markers of liver injury (GTP, AST and ALT) occurred among the patients with alcohol (respec- tively 42, 30 and 44 U/l) and opiate dependence (respectively 95, 42 and 37 U/l), the lowest among those with amphetamine dependence (respectively 17, 19 and 21U/l). 2-These results might indicate liver disease among patients with elevated AST, ALT, and GTP. Elevated AST, ALT and GTP occur in the case of organ damage

· This study was reported twice as it touches on the two drugs of abuse (alcohol and opioids)

Author	Objective	Population (Sample size, type of participants, recruitment technique)	Location	Methodology/ Methods (Design)	Results
Pawan et al., (2011)	To observe the changes in some liver and lung function parameters in opium addicted subjects	Sample size: 50 adults, 25 were opium addicts, and 25 apparently healthy subjects. Characteristics: 1-Males 2-Age ranged from 30 to 50 years 3-Opium addicts consuming about 5-11 gm/day for > 2 years. Recruitment technique: Not mentioned but as per the description it is convenience sampling.	Bangladesh	Design: Observational cross- sectional design Data collection method: 1.Detailed medical and family history. 2.Clinical examination. 3.After an overnight fast (5ml), blood samples were taken. This included: blood sugar level AST, ALT, total bilirubin, Alkaline phosphatase were estimated in both the groups. 4.Various pulmonary parameters were measured using vitalograph. Ethics: 1-Written consents obtained and approved by ethics committees. 2-Written informed consent was obtained from each subject	1-AST, ALT and alkaline phosphatase levels were found significantly ($p<.05$) higher in opioid dependents as compared to healthy group. 2-FVC, FEV1, FEV1/ FVC% were significantly ($p<.05$) lower in healthy group than opioid dependents group. Again, PEF and FEF were also significantly ($p<.001$) lower in group B as compared to those of group A

Author	Objective	Population (Sample size, type of participants, recruitment technique)	Location	Methodology/ Methods (Design)	Results
Sanli et al., (2015)	To determine the effects of different psychoactive substances on serum biochemical markers	Sample size: 324 drug dependent and 69 controls. 46 out of 324 were opioid dependent Characteristics: 1-Both gender 2-The average age was 26.5 between drug dependent 3-Admitted to Erenkoy Mental Health and Neurology Training and Research Hospital between January 2013 and January 2014.	Istanbul, turkey	Design: Observational cross- sectional design Data collection method: -Urine and blood sample. Urine samples were tested simultaneously for heroin, cannabinoids, cocain, ben-zodiazepins, opiates, buprenorfine, amphetamines, extacy and ethyl glucuronide. Ethics: Written consents obtained and approved by ethics committees	1-Statistically significant difference in (GGT), uric acid, creatinine, urea, albumin, (AST) medians between the dependent and control groups ($P < 0.05$). 2-Statistically significant difference in sodium and albumin levels between the opium-dependent and control groups ($P < 0.05$).

Table 4: Summary of the Systematic Review of Selected Studies that Address the Impact of Methamphetamine Abuse on Liver Function Tests (Continued)

Author	Objective	Population (Sample size, type of participants, recruitment technique)	Location	Methodology/ Methods (Design)	Results
Tavasolian et al., (2015)	The aim of this study was to evaluate the hematological and biochemical parameters in methamphetamine addicts and to compare them with healthy-individuals	Sample size: 120 participants (60 individuals addicted to methamphetamine and 60 healthy subjects as a control group). Characteristics: 1- Adult males 2- Age: 20 – 50 3- Methamphetamine users, using methamphetamine on a regular basis for at least 3 months and at least 5 days a week Recruitment technique: Not mentioned but as per the description it is convenience sampling.	Shiraz, Iran	Design: retrospective case-control study Data collection method: - Blood sample (fasting 12 hours) to determine the biochemical variables (albumin, blood urea nitrogen (BUN), creatinine, total cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and uric acid). Ethics: Written consents obtained and approved by ethics committees.	1- Alanine aminotransferase, aspartate amino transferase, alkaline phosphatase, WBCs and platelet count and serum creatinine levels in methamphetamine addicts were significantly higher than the control group (p-value <0.001), while hemoglobin, hematocrit and albumin levels were lower in these patients (p-value <0.001). 2- Increased levels of creatinine and liver enzymes such as AST, ALT and ALP was observed among drug abusers compared to the healthy subjects (P value <0.001).

Author	Objective	Population (Sample size, type of participants, recruitment technique)	Location	Methodology/ Methods (Design)	Results
Kuo et al., (2012)	The authors aimed to identify potential laboratory indices and clinical characteristics associated with natural death through a two-phase study.	Sample size: Methamphetamine-dependent patients (n = 1,254). Forty-eight subjects died of natural causes, and were defined as the case subjects Characteristics: 1- Patients admitted to a psychiatric center between 1990 and 2007 2- Male gender 3- Mean age is between 29 and 31 Recruitment technique: Not mentioned but as per the description it is convenience sampling.	Taiwan	Design: 1- Phase I: Nested case-control study 2- Phase II: Cohort study Data collection method: Chart review Ethics: Written consents obtained and approved by ethics committees	1- Deceased patients had significantly higher mean levels of AST and ALT. 2- Mildly elevated AST increased the risk of natural death (odds ratio = 4.37, p,0.01) relative to normal AST levels; the risk was even higher when AST was markedly elevated (odds ratio = 53.35, p,0.001). Mildly and markedly elevated ALT levels also raised the risks of natural death (odds ratio = 5.11, p,0.05; odds ratio = 7.65, p,0.001).

2.6 Discussion

2.6.1 The Impact of Alcohol on Liver Function Markers (AST, γGT) in Literature

Langowska-Grodzka, Ziólkowski and collegues (2016), discussed that the increased levels of γGT, AST and ALT among alcohol consumers may be due to the use of other psychoactive substances. The study has also stated that these elevated liver enzymes are suggestive of liver damage, however, they argued that increased activity of these enzymes might indicate a co-use of opioid or other substances that might have resulted in this increase. Yet, these confounding variables have not been stated clearly nor how they were controlled and assessed. One could argue that confounding variables might not be sufficiently controlled and stated in some studies and this appeared in both Langowska-Grodzka, Ziólkowski & Czarneck, (2016) and Roscoff et al., (2019) studies, which might have affected some of their results. Especially that Rsocoff and collegues have mentioned that some variables such as smoking, dietary, the use of other medications or substances were not adjusted in their study.

Roscoff et al., (2019), included a large sample size in their study which added strength to the study, since larger sample size are more likely to be presentative of the whole population, providing more reliable results with precision and power. Also, the study has clearly stated the limitation they faced in conducting the research, which showed their transparency.

2.6.2 The Impact of Opioids on Liver Function Markers (AST, γGT) in Literature

The findings of Langowska-Grodzka and his collegues (2016), indicated the presence of liver disease and injury due to increased levels of liver function markers, and they supported their findings with the results of other studies, which confirmed the increase in liver enzymes among people suffering from opioid use disorder. Moreover, they have stated that other studies have mentioned that the increase was found to be associated with the use of other psychoactive substances taken through IV, and infected with hepatitis C, or as a harmful effect of the chronic use of opioids on the liver function. However, the study has not mentioned if these confounders factors have affected their results or how they have been controlled. Moreover, it was stated that these results were bothering giving the average young age of the participants.

Some of the studies included in this systematic review had relatively small sample size as seen in Pawan et al., (2011) & Langowska-Grodzka, Ziólkowski & Czarneck, (2016) studies. Generally, the main limitation associated with small size sample is that it could produce false-positive results, in a sense of over-estimating the association or the magnitude (Hackshaw, 2008).

Sanli and collegues (2015), stated that most patients with SUD suffer from malnutrition, since they prioritize their spending on buying drugs. In addition, the study has argued that poor living conditions and nutritional factors are likely to affect changes in biochemical parameters in patients with SUD. However, the study did not mention if these factors were assessed in their study or whether they have been controlled or not. Moreover, the study had notable

variation in age, therefore comparison between young dependents and older dependents might be beneficial in exploring whether age has or has not contributed in elevated levels of these markers.

2.6.3 The Impact of Methamphetamine on Liver Function Markers (AST, γGT) in Literature

According to the Taiwanese study, their findings could indicate that methamphetamine has a direct impact on the liver as well as on other organ systems leading to death, since AST is not only present in the liver but also in the cardiac muscle, kidneys, brains tissues and skeletal muscles. Additionally, the study recommended a routine clinical examination and follow-up of those surrogate markers in patients with methamphetamine use disorder and especially when it's combined with alcohol use disorder (Kuo et al., 2012). This study showed strength in including a large sample size, and in providing adequate details on the methods and tools they have used in conducting their study.

2.7 Limitations:

While evaluating the quality of the studies included in this systematic review, three major items were observed in most of them, which are generalizability of findings in the discussion, pointing out potential bias as well as information about the funding. Additionally, it was noticed that some studies had variation in the duration of data collection as well as the sample size of the studies, which might have influenced the results.

Another limitation observed was publication bias in systematic reviews, which might have led to false-positive overall conclusion. In fact, several studies might be still in the publication process and yet to be available as a full-text to be incorporated in this review. In addition, most of the included studies in this systematic review followed the cross-sectional design, which might have limited their assessment of causal relationship.

2.8 Conclusion

In conclusion, the findings of this systematic review proved the association between the abuse of (ethanol, opioid and methamphetamine) and elevated levels of both (AST and γGT). However, some variation in the results were detected between different studies, indicating the need for further research in this domain of substance use disorder.

Chapter 3

Research Methodology

This chapter is discussing the research methods and strategies used to meet the study objectives and answer the research questions. It also discusses the rationale and justification regarding the selected study design. Finally, ethical consideration is presented.

3.1 Study Design

The quantitative research design was used to help in answering the research questions and achieve the study objectives, especially that the laboratory values of liver function tests are numerical figures, besides all the relevant studies in literature were conducted using a qualitative methodology. The current study is a retrospective exploratory cross-sectional study having an exploratory nature. Secondary data was used to account for the aforementioned study topic provided by St. George's University of London Medical School, UK, through personal communication. The researcher's role in the current study was limited to reviewing existing literature on the research topic, selecting some of the data, conducting analysis, interpreting the results and presenting the discussion.

The researcher was not involved in the data collection process. The demographic data on age, gender, trends on the primary drug of abuse; opioids, alcohol and methamphetamine and selected liver function markers (AST, γGT) were statistically analyzed. Precisely, the current study employed both descriptive and inferential statistics to account for the trends of the

aforementioned pair of selected substances of abuse (opioid, alcohol and methamphetamine) in relation to their possible effect on selected surrogate markers of liver functions.

Exploratory studies are important to lay the foundation for future investigation in the relevant areas. Exploratory investigations provide a strong and in-depth detail regarding the study problem and ensure a broader picture and generalizability of the research findings (Žukauskas, Vveinhardt, and Andriukaitienė, 2018). The exploratory studies will help in exploring the nature of the relationship of the substances of abuse and the abnormality in biochemical parameters. Furthermore, exploratory research helps to assess the study results through simple yet comprehensive analysis techniques (Given, 2012). Similarly, in 2016, Brown described exploratory research as helpful in analyzing the problems, that are not well defined. It helps to provide us with a well understanding of an existing research problem as mostly exploratory studies are conducted when a problem is in its preliminary stage. However, this research has some limitations, that will be discussed later (Brown, 2016).

3.2 Study Sampling & Sample Size

The sampling of the current study involved all the individuals who were using the selected drugs (ethanol, opioid and methamphetamine). The current study involved the random selection of n= 329 cases, and they were further divided based on the particular drug of abuse.

3.3 Inclusion and Exclusion Criteria

The sample selection in the current study was also based on a designated inclusion and exclusion criterion. Both criteria are summarized in (Table 5).

Table 5: Inclusion and exclusion criteria of cases

Inclusion Criteria	Exclusion Criteria
• Male and female gender • Age between 18 – 89 years • Suffer from ethanol, opioid or methamphetamine use disorder	• Under age 18 or above 89 • Patients with diagnosed chronic health conditions that might impact the study results, e.g., cancer, chronic illnesses such as chronic liver and kidney disease, diabetes mellitus, patients on long term therapy for hypertension or hyperlipidemia or hyperuricemia etc.

3.4 Data Collection

The data used for the study is secondary data obtained through personal communication from St. George's University of London. Only a selection of data has been used for this study.

3.5 Study Variables

3.5.1 Independent Variables

Substances of abuse that were involved in the study and were considered as independents are:

1. Ethanol
2. Opioid
3. Methamphetamine

3.5.2 Dependent Variables

Table 6: The dependent variables of the study involved plasma enzyme liver markers

	γ-Glutamyl transferase (γGT) IU/L	Aspartate transaminase (AST) IU/L
Reference range	8 – 61	8 – 48

3.6 Data Analysis

Statistical Package for Social Sciences (SPSS), version 27, was used for data analysis purposes, and results are represented in tables and graphs. First, descriptive statistics are utilized to report the findings of demographical characteristics such as age and gender, further

measures of central tendency i.e., mean, mode and median were calculated. Also, inferential statistics are used to test the relationship and compare the different variables. Chi-square test and t-test are used according to the type and requirement of the study assessment. Using the Chi-square test was suitable as the data was parametric with normal distribution, to test the association between two categorical data. T-test was used to examine the significant difference between the means of two groups. Moreover, Mann-Whitney U-test was used to compare the differences in the median values between our study and other published studies. The significance level was set up at $P \leq 0.05$.

3.7 Compliance with the Ethical Considerations

Ethical approval was obtained from Dubai Medical College to conduct this study (Appendix 1). As previously stated, these were anonymous secondary data and were obtained and used only for research purposes, consent forms were used by the original data collector who also obtained ethical approval from their organization, anonymity and confidentiality were all maintained all the time. A password protected personal laptop has been used while working on the data. All data have been kept confidential and secured in a file locked by another password. Data will be kept for up to 5 years after finalizing this dissertation, by using a password that only the researcher has access to, also the laptop will be locked in a safe place outside business hours.

3.8 Data Protection

Data protection will be kept under strict consideration to avoid any ethical infringement of clinical research principles. **Table 7** below, summarizes the principles of the Data Protection Act (1998), and their applicability in the current research.

Table 7: Compliance to Data Protection Act (1998) in this Study

Principles	Application in the Current Research
Data should be lawfully and fairly treated/evaluated	Data was collected by the original researcher and evaluated based on available results
Data should be obtained for the specified purposes	Data were obtained for the clinical research purposes
Data should not involve the researcher's personal bias	Data was not contaminated by the researcher's own bias
Data should not be kept longer than the necessary period	Data will not be kept for more than 5 years after the finalizing the dissertation
Data should be kept private	Data privacy and confidentiality is strictly obligated
Data should not be revealed to a third party without permission	Data will not be revealed or shared with any third party

(Legislation.gov.uk, 1998)

3.9 Results Dissemination

Dissemination and generalizability of research findings are important aspects of conducting clinical research. According to Strech et al., (2018), the instant and effective dissemination of clinical research results helps to develop new ideas and techniques in medical sciences that further facilitates the clinical research with even more in-depth findings. Thus, the dissemination plan of the current study involves summarizing the full project into an empirical research article, that could be sent for publication in the relevant medical sciences journal. Depending on the nature of the study and results, the article could also be represented in national and international conferences as this exploratory research will help to usher in a new era of research in drugs abuse and its impacts.

Chapter 4

Results

This chapter is demonstrating the results of this study utilizing the methodology presented previously. Baseline demographic is demonstrated, liver function tests (AST and γGT) are presented in groups according to the substance of abuse (opioid, ethanol and methamphetamine) for comparison. Finally, the outcomes of this study are explored using inferential statistics and linear regression models.

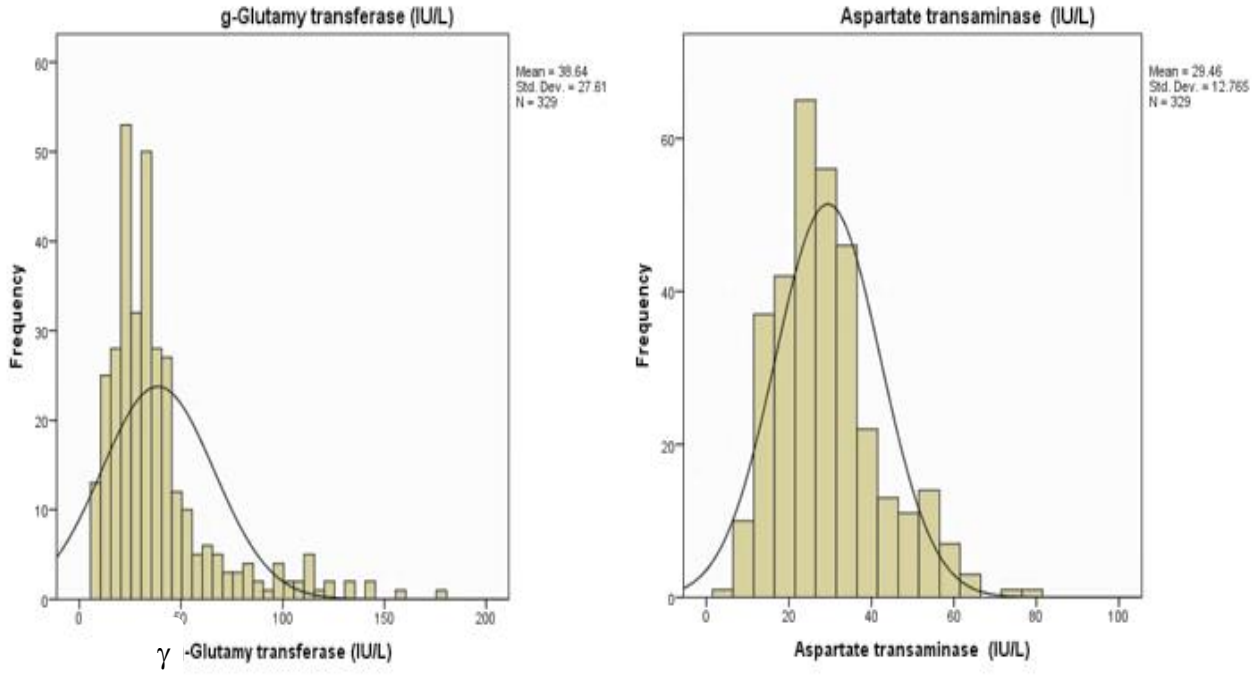
4.1 Baseline Characteristics

Three subgroups of baseline characteristics were presented, these included ethanol dependents, opioid dependents and methamphetamine dependents, each group was then investigated regarding their substance of abuse effect on selected liver function markers (AST and γGT). Within this section, all data were considered parametric in nature and were normally distributed, assessed through the use of histogram (**Figure 2, 3**), hence mean with 95% confidence interval of the mean, standard deviation, median and range are documented. Due to the nature of the data being parametric, independent sample t-test was used to determine the mean difference between variables. Also, chi-square test was used for categorical data to test the association between two variables.

Figure 2: The Age distribution of both men and women

In this study population, the age distribution of both men and women is considered normal

Figure 3: The liver enzymes (AST and γGT) distribution of all three groups



In this study, the Liver enzymes (AST and γGT) distribution is considered normal, although a small skew is observed but numerically it was negligible and parametric statistics could be safely applied.

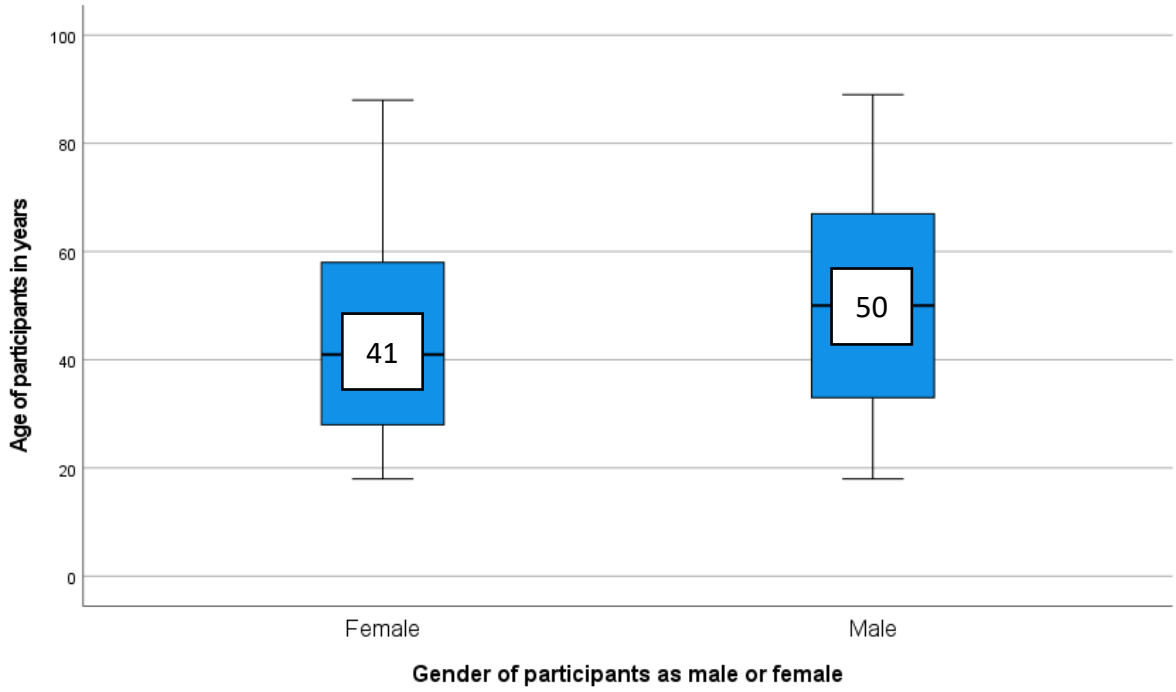
A total of 329 patients (207 males and 122 females) were recruited for this study, and were diagnosed with either ethanol, opioid or methamphetamine use disorder. The age of the participants varied from 18 years old to 89 years old. The Mean ± SD of age among male and female patients were (50 ± 20.3) and (45 ± 20) years, respectively (Table 8) (Figure 4), and statistically significant difference was reported between the mean age of men compared to women (P value= 0.03).

Table 8: Demographic data of subjects involved in the study

Age, Years	Study population		
	Total N=329	Male N=207	Female N=122
Mean	47	50	45
95% Confidence interval of the mean	44.3-49	47-53	41.2-48.2
Median	45	50	41
Std. Deviation	20.1	20.3	20
Range	71	71	70

In the study population, men (mean= 50, 95% CI=47-53) were statistically significantly older than women (mean= 45, 95% CI= 41.2-48.2), (P value= 0.03).

Figure 4: Median values comparison of age between males and females



The median age of men (median= 50 years) was higher than the median value of age in women (median= 41 years), (P value= 0.03).

167 out of 329 were ethanol dependents and the Mean ± SD of age was (48.1 ± 21) years. While opioid dependents consisted of 74 subjects, and it had the highest mean of age, Mean ± SD of age (51.3 ± 20.1) years. Methamphetamine dependents were 88 subjects with a Mean ± SD of age (44.2 ± 19) years (Table 9).

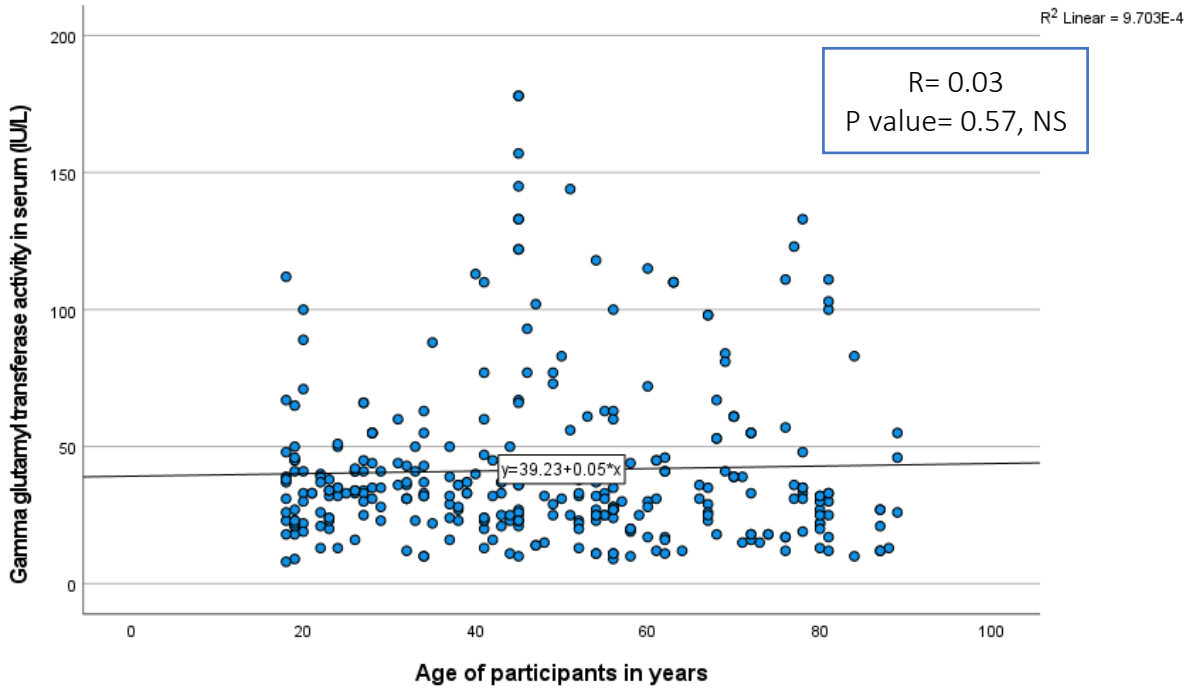
Table 9: Characteristics of the involved population according to the substance of abuse

Age, years	Substance of abuse		
	Ethanol	Opioid	Methamphetamine
Mean	48.1	51.3	44.2
95% Confidence interval of the mean	45-51.3	47-56	40.2-48.3
Median	45	53	43
Std. Deviation	21	20.1	19
Range	71	71	69

In the study population, opioid dependents were found to be older in age than in ethanol and methamphetamine dependents. The difference from ethanol was not statistically significant, but the difference from methamphetamine was statistically significant, with a P value of 0.001.

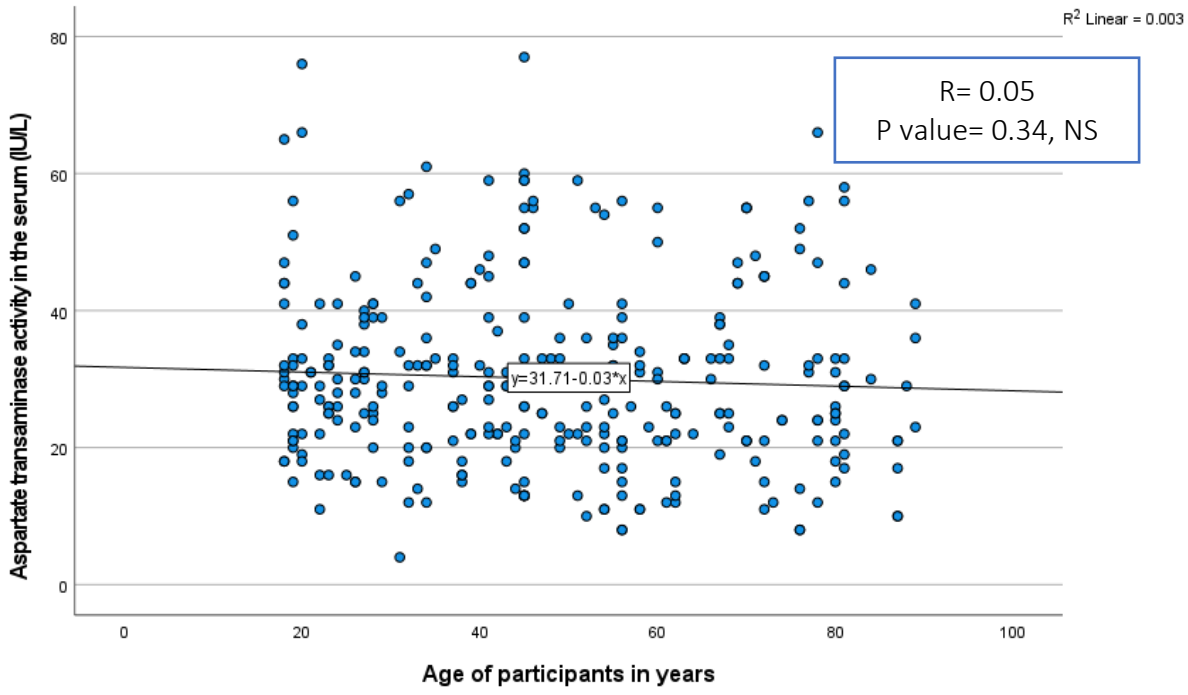
Another area of interest in this study was to explore whether age has influenced levels of AST and γGT among dependent patients. Analysis of correlation coefficients showed that there is a lack of statistical significance between the variation in age and the levels of AST and γGT (P value= 0.34 and P value= 0.57, NS), respectively. Moreover, analysis revealed that the age of participants in relation to AST and γGT levels was considered very weak (R= 0.05) (R= 0.03) respectively (**Figure 5, 6**).

Figure 5: Scatterplot of γGT (IU/L) vs Age of participants in years



There is no correlation between the level of γGT activity and age of patients in the study population, (R= 0.03), (P value= 0.57, NS).

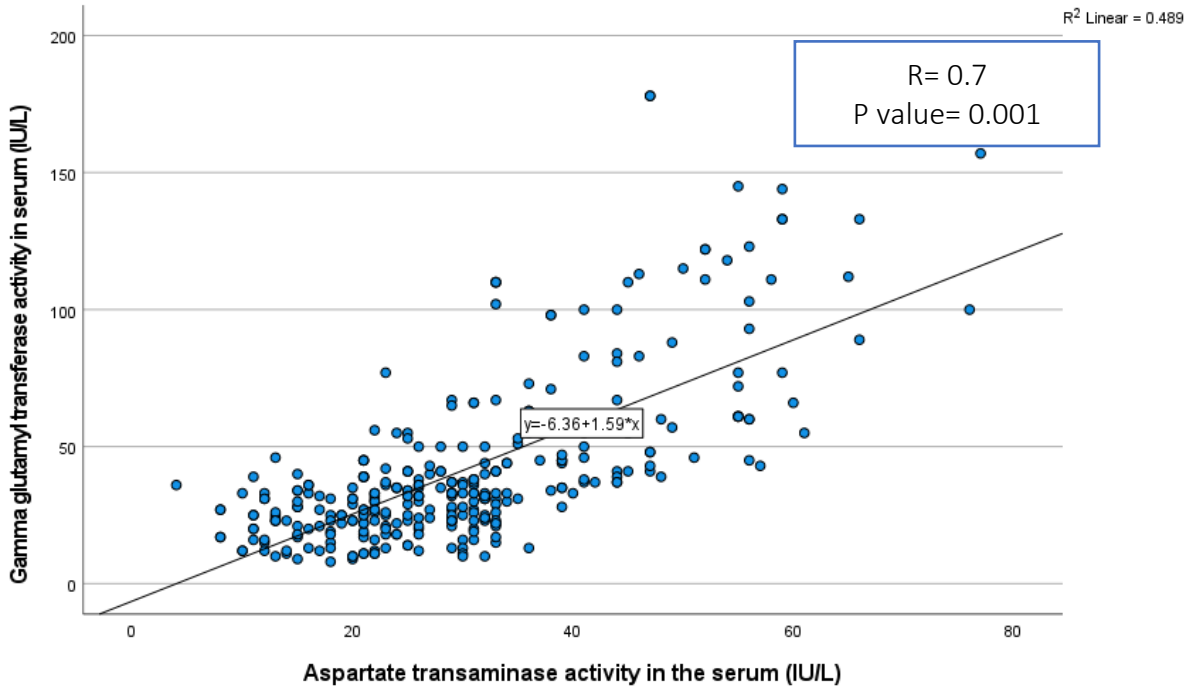
Figure 6: Scatterplot of AST (IU/L) vs age of participants in years



There is no correlation between the level between AST activity and age of patients in the study population (R= 0.05), (P value= 0.34, NS)

The significant direct correlation between the levels of AST and γGT indicated a strong positive linear relationship between the two variables (R=0.7) (P value= 0.001) (figure 7).

Figure 7: Scatterplot of γGT (IU/L) vs AST (IU/L)



A direct correlation was observed between the activity between AST and γGT, (R= 0.7) (P value= 0.001)

Chi-square test was performed to test the possible association between gender and substances of abuse. It was found that men had statistically significant association with the use of ethanol, (Chi^2= 13.2, P value= 0.001). On the other hand, no association was detected between gender and other substances of abuse studied.

4.2 Main Study Findings

The concentration of biochemical parameters, specifically liver function markers (AST and γGT) in the serum were measured and compared among the substance-dependents (ethanol, opioid and methamphetamine dependents). Investigation established that among

ethanol dependents the level of AST was the highest, Mean ± SD (38 ± 13.1) IU/L, while in opioid and methamphetamine dependents, the levels of AST were, Mean ± SD (21.1 ± 9) and (24±6.3) IU/L, respectively (Table 10) (figure 8). Furthermore, the highest average marker of γGT levels in serum was among ethanol dependents, Mean ± SD (57 ± 34.4) IU/L, while in opioid and methamphetamine dependents the Mean ± SD were (25 ± 9) and (26.3 ± 10.2) IU/L, respectively (Table 11) (Figure 9).

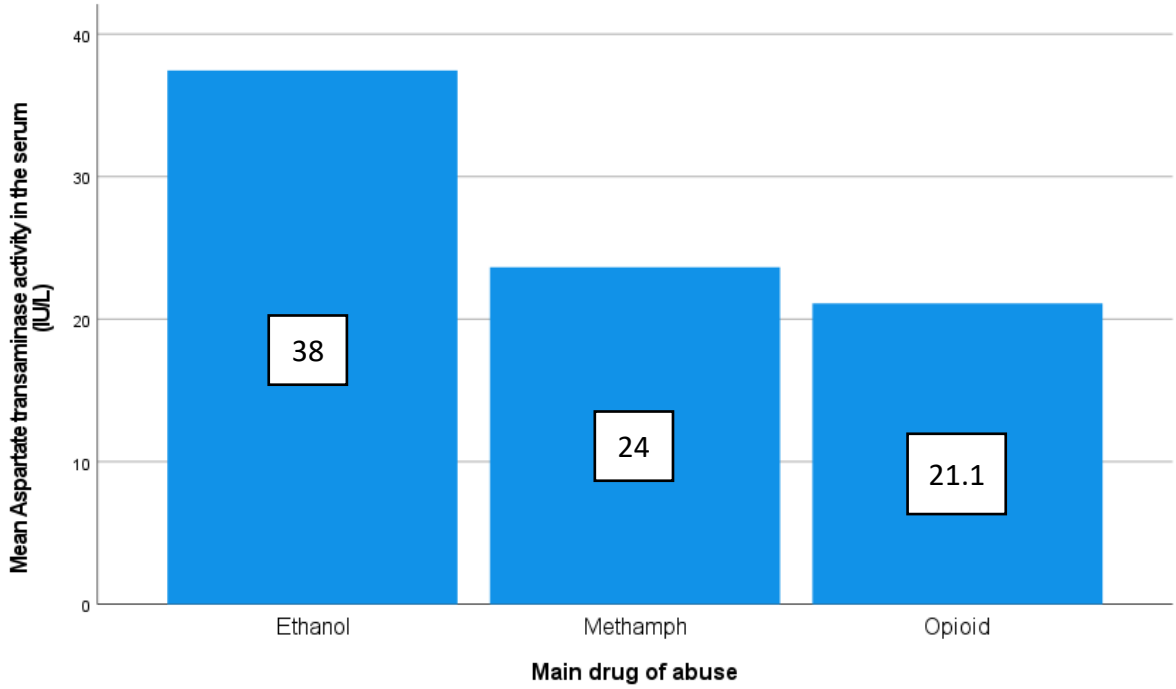
Moreover, there was a statistically significant difference in the serum level of AST between ethanol and opioid dependents, and ethanol and methamphetamine dependents (P value= 0.001) in both cases. Further to this, γGT levels had statistically significant difference in ethanol compared to opioid dependents, and in ethanol compared to methamphetamine dependents (P value= 0.001) in both cases. On the other hand, no statistical significance difference was reported between the levels of both AST and γGT between opioids and methamphetamine dependents (P value= 0.37, NS)

Table 10: Results of Selected Liver Function Marker (AST) in Ethanol, Opioid and Methamphetamine

Selected liver function marker; Aspartate transaminase activity in the serum (IU/L)	Substance of abuse		
	Ethanol	Opioid	Methamphetamine
Mean	38	21.1	24
95% Confidence interval of the mean	36-40	19.1-23.2	22.3-25
Median	35	21	24
Std. Deviation	13.1	9	6.3
Range	62	35	24

In the study population, the levels of AST were statistically higher in ethanol dependents (mean=38, 95% CI=36-40), than in opioid dependents (mean= 21.1, 95% CI=19.1-23.2) (P value= 0.001), and in methamphetamine dependents (mean= 24, 95% CI=22.3-25), (P value= 0.001).

Figure 8: Mean values of AST (IU/L) among ethanol, opioid and methamphetamine dependents



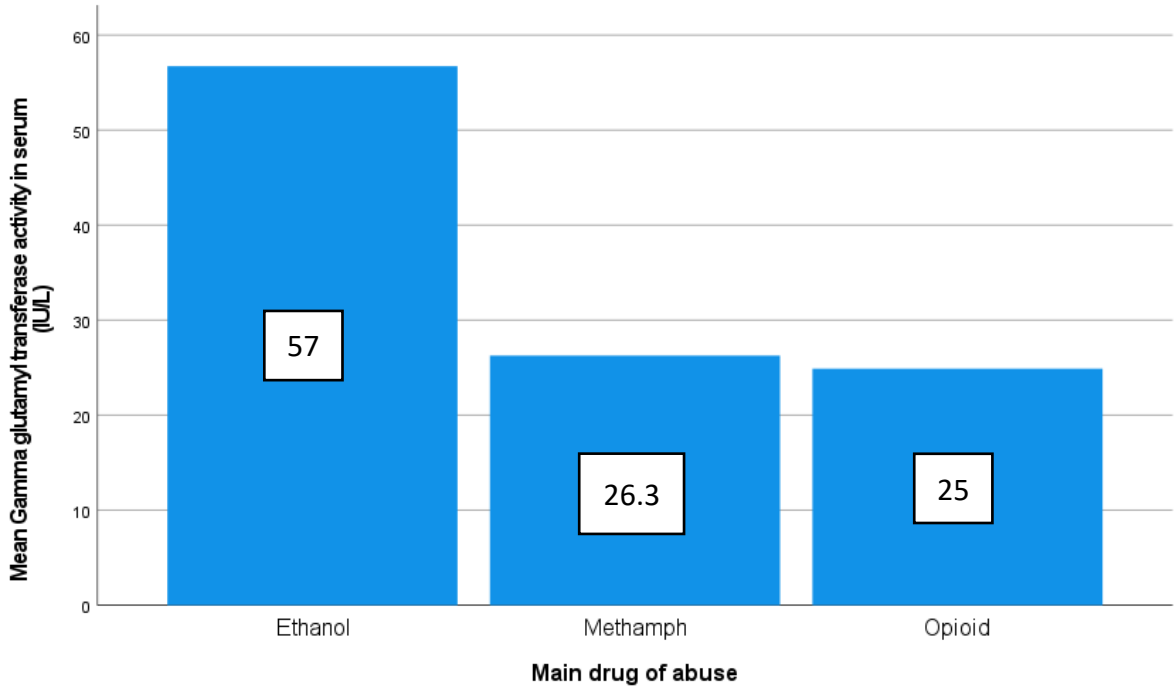
The mean value of AST in ethanol dependents (38 IU/L) was higher than the mean value of AST in opioid dependents (21.1 IU/L) and in methamphetamine dependents (24 IU/L), these differences were statistically significant, with P values of 0.001 in both cases.

Table 11: Results of selected liver function marker (γGT) in ethanol, opioid and methamphetamine

Selected liver function marker; γ-glutamyl transferase activity in serum (IU/L)	Substance of abuse		
	Ethanol	Opioid	Methamphetamine
Mean	57	25	26.3
95% Confidence interval of the mean	52-62	23-27	24.1-29
Median	45	24	25.5
Std. Deviation	34.4	9	10.2
Range	166	35	47

In the study population, the levels of γGT were statistically higher in ethanol dependents (mean=57, 95% CI=52-62), than in opioid dependents (mean= 25, 95% CI= 23-27) and in methamphetamine dependents (mean= 26.3, 95% CI=24.1-29), with a P value of 0.001 in both cases.

Figure 9: Mean values of γGT (IU/L) among ethanol, opioid and methamphetamine dependents



The mean value of γGT in ethanol dependents (mean=57 IU/L) was higher than the mean value of γGT in opioid dependents (mean=25 IU/L) and in methamphetamine dependents (mean=26.3 IU/L), these differences were statistically significant, with a P value of 0.001 in both cases.

Chapter 5

Discussion

This study is aiming at exploring the possible impact of selected substances of abuse (ethanol, opioid and methamphetamine) on selected liver function markers (AST and γGT). This chapter is discussing the findings of the study and comparing them with current literature. The research objectives' achievements are assessed. Lastly, this chapter will present the study limitations and implications.

5.1 Sample Characteristics

In the United Kingdom, drug abuse has remarkably increased during the past few years and more than four million people are abusing drugs (Black, 2020). The local healthcare providers consider it as a potential healthcare concern for the near future. In 2020, Holland reported that alcohol, methamphetamine, hashish, opioids, and heroin are recorded as the most preferred drugs in British society (Holland, 2020)

In the current study, the age of the participants varied from 18 to 89 years old, men (mean= 50 years) were significantly older than women (mean= 45 years) (P value= 0.03). Opioid dependents had the highest mean age among all groups, this was consistent with Langowska-Grodzka, Ziólkowski & Czarneck (2016) study, where opioid dependents were the oldest among other groups of addicts. One could speculate that the increased age of opioid dependents could be in part due to the prescriptive use of opioid analgesics. In fact, West and colleagues (2015), have conducted a study in this regard which determined that the rates of misuse of prescription opioid among older adults were higher than in younger adults. Methamphetamine

dependents were the youngest in this study (mean= 44.2 years); according to Lin and his colleagues, (2021), methamphetamine use is most prevalent and preferred among the young adults. Age and comparison of the levels of AST and γGT among different drug of abuse was examined and revealed a lack of statiscital significance (P value= 0.34,NS and P value= 0.57, NS) respectively, this was in line with Langowska-Grodzka, Ziólkowski & Czarneck (2016) study.

Furthermore, gender was examined in relation to the preferred substance of abuse, and it was reported that men had statistically significant association with the use of ethanol (Chi^2= 13.3, P= 0.001). According to the National Survey of Drug Use and Health in 2019, alcohol is most prevalent among males than in females (Substance Abuse and Mental Health Services Administration, 2019).

5.2 Main Findings of the Current Study

5.2.1 Aspartate Transaminase and γ-Glutamyl Transferase

A direct correlation was observed between the activity of AST and γGT, (R= 0.7) (P value= 0.001). That might be of importance in practice, since it could suggest testing one of the enzymes without the need of the other, as they can predicit each other. It also might be suggested that the enzyme test of a lower cost is routinely tested, while the other enzyme is tested when clinically indicated.

5.2.2 The Impact of Alcohol on Liver Function Markers (AST and γGT) in the Current Study

The selected subgroups of participants were compared in relation to averages and deviations from the reference value of AST and γGT. Invistigation has revealed that the mean values of all three groups had AST and γGT within the reference ranges. However, ethanol dependents had levels of AST and γGT that were closer to the higher end values of reference ranges.

Ethanol dependents had statistically significant higher mean values of both AST (mean= 38IU/L) and γGT (mean= 57 IU/L) than among opioid (mean= 21.1 IU/L, P value= 0.001) (mean= 25 IU/L, P value= 0.001) and methamphetamine dependents (mean= 24 IU/L, P value= 0.001) (mean= 26.3 IU/L, P value= 0.001). This finding is consistent with current literature. In addition, it was confirmed by research that for years liver function markers such as AST, ALT and γGT have been considered as specific markers of alcohol abuse and dependence (Langowska-Grodzka, Ziólkowski & Czarneck, 2016). Other studies have reported that alcohol has in fact a direct effect on the physiological functioning of the liver and that it is linked to alteration in liver function markers. Studies have also shown that several factors could contribute to these elevated markers such as the duration and the pattern of alcohol consumption, socioeconomic status and poor nutritional habits (Gogoi et al., 2017). All these factors might also be encountered in drug use disorder and interpretation must consider these factors. Unfortunately, the aforementioned factors that could have been affected the results in our study could not be obtained and interpreted. Studies in this area are needed in order to determine risk factors that could contribute to the development and progression of liver damage. It is believed that the results of these investigations will help in identifying patients

at higher risk of developing liver diseases, hence, intense follow up and management will be needed (Gogoi et al., 2017).

5.2.3 The Impact of Opioid on Liver Function Markers (AST and γGT) in the Current Study

On the other hand, the results of the levels of AST and γGT in opioid dependents were in partial contradicting with Langowska-Grodzka and colleagues (2016), who reported increased average markers of AST and γGT in both ethanol and opioid dependents equally. The contradiction in the results of opioid dependents with the results of our study, might have risen from the fact that most opiate addicts have been associated with the use of other psychoactive substances through IV or were infected with hepatitis, according to Langowska-Grodzka and his colleagues (2016). To further understand the impact of opioid on liver function markers, these confounding factors have to be addressed and carefully controlled in future research.

The nature of our study compared liver function markers between groups addicted to three different substances of abuse, and not against a controlled healthy group. Many previous studies as we have indicated in our systematic review above have compared the use of substances of abuse such as opioid with a controlled group of healthy subjects, and they revealed a significant differences in AST and γGT between the groups. The levels of serum enzymes of AST and γGT were higher in opioid dependents as compared to control subjects. In order to further understaned the differneces between our study and the aforementioned study, we compared the median of AST and γGT levels in opioid dependents in our study with the median of AST and γGT levels in the study conducted by Sanli et al., (2015). It was noticed

that the results were close and had similar pattern (AST= 20.5 IU/L in our study and 19.0 IU/L in Sanli's study, NS) and (γGT= 24 IU/L in our study and 25 IU/L in Sanli's study, NS), using the Mann-Whitney U test for the comparison in both cases. Although the levels of AST and γGT remained within the reference range in opioid dependents group, they were nearer the upper limit of the range, this might indicate the need for special reference ranges for these enzyme markers when used to assess OUD subjects. In fact, research reports have suggested that increased levels of AST and γGT in opioid dependents might indicate liver disease and assess the extent of hepatic damage (Pawan et al., 2011; Langowska-Grodzka, Ziólkowski & Czarneck, 2016). These findings suggest the need for routine assessment of these plasma enzymes in opioid.

5.2.4 The Impact of Methamphetamine on Liver Function Markers (AST and γGT) in the Current Study

Similarly, the findings of our study regarding the levels of AST and γGT in methamphetamine dependents were contradicting with current literature. In literature, the mean values of AST among methamphetamine dependents were higher compared to our study, as well as they were significantly higher than healthy subjects in the same study (AST= 24 in our study, and 31.2 In Tavasolians's study), using the Mann-Whitney U test for the comparison in both cases between our study and Tavasolians's study, it was noticed that the difference between both means is significant (P value= 0.001). It could be suggested that these differences might be as a result of conducting the study in different population where they might have other causes of liver function diseases. Moreover, Tavasolian et al., (2015), suggested that increased levels of liver enzymes such as AST and ALT in methamphetamine

dependents were found to be linked to liver damage. More investigations in a larger study are expected to sort out these apparent controversies.

Literature that discussed the effect of methamphetamine abuse and its effect on the liver was limited, and it has been explained in that literature that the mechanism of methamphetamine toxicity is unclear (Tavasolian et al., 2015). Comorbid substances or illnesses might have an impact on these results, and findings in animal studies could help in shedding the light on the possible harm of methamphetamine on various organs including the liver (Zhang et al., 2018). A study was carried out on rats concluded that methamphetamine in high doses caused significant structural damage to liver and functional hepatotoxicity. Increased levels of AST and ALT were observed and confirmed that the cellular damage was concurrent with these structural changes (Halpin, Gunning-III & Yamamoto, 2013). Further research is necessary and routine laboratory tests are needed in order to monitor the pattern of liver function surrogate markers, in order to detect any possible drug abuse damage to the liver in the future.

5.3 Research Objectives

The findings provide evidence to reject the null hypothesis and conclude a difference within the outcomes in favor of the ethanol dependents group. However, no absolute conclusions can be drawn due to some limitations that will be discussed later in this paper. All other objectives were extensively discussed earlier in this chapter and were successfully achieved.

5.4 Limitations

5.4.1 Secondary Data

In the current study, the major disadvantage that was faced is that data were secondary, especially in regard to the geographic region and population desired. Data were initially going to be gathered within the UAE population, However, due to availability of subjects being difficult to obtain, the use of secondary data and extensive systematic review were appropriate. Moreover, since the researcher did not participate in collecting the data, there was no control over content and quality of data gathered (Rosenberg, Greenfield & Dimick, 2007). For example, some liver function tests were meant to be included such as alanine transaminase (ALT) and creatine kinase (CK), especially that these enzymes are usually analyzed alongside with AST and γGT in patients with SUD (Kuo et al., 2012). Another limitation to secondary data is that confounding variables and accuracy of comorbidities were not addressed (Rosenberg, Greenfield & Dimick, 2007). In further details, factors that are known to impact liver function markers (e.g., medication, health conditions, etc.) were not provided and fully stated, therefore residual confounding might have affected the results of this study (Agarwal, Fulgoni-III & Lieberman, 2016). Selection bias is another area of concern in secondary data, since it could be subjected to the original researchers' own bias (Pannucci & Wilkins, 2011). The method of data collection, sample selection, tools of diagnosis and other related information were unknown.

5.4.2 Study Design

The main limitation of this study is the inability to determine cause-effect relationship due to cross-sectional study design. Moreover, due to the nature of the study being a retrospective as well as data were secondary, some data were inevitably missing (Talari & Goyal, 2020). Retrospective studies are also known to be subject to confounding, which is explained by the existence of other risk factors not being measured or controlled.

5.5 Possible Implications

Research in the domain of exploring and analyzing the effect of substances of abuse on biochemical and hematological markers is noticeably lacking. These studies are very crucial in shedding the light on the importance of obtaining clinical tests at the time of diagnosis to provide the patients with the best managements plans. The importance of these studies resides in notifying the healthcare providers of the possible trends in deranged biochemical and hematological markers related to the selected substances of abuse. This will possibly provide them with an insight of the need for intensive monitoring and treatment programs. The need for this research will allow the health and social care authorities to implement prevention and management programs to raise awareness about the potential adverse effects of substances of abuse especially among the first-time consumers (Sanli et al., 2015)

.

The main application might be highlighting the need for further research, tackling different psychoactive substances, geographical regions and populations. A large definitive study should target the UAE population who suffers from SUD, in order to explore routine

biochemical markers that might be significant for proper management. However, we believe that the current study will set the foundation for future research tackling this issue in the UAE.

The United Arab Emirates (UAE) was established on 2 December 1971 (Embassy of the United Arab Emirates, 2021). Since the birth of UAE, it experienced an exponential transformation in every aspect of life in such a short period of time (Al Ghaferi et al. 2017). In UAE addiction was first identified in the early 1980s, and since then the number of individuals suffering from SUD is increasing (Alsuwaidi, 2019), and Emirati nationals are experiencing the highest rates among other nationalities within the UAE. It is called the 'silent epidemic of the gulf', as it is one of the least explored health conditions or issues in the gulf cooperation council (GCC) (Alsuwaidi, 2019). Recent and accurate data about the prevalence of the problem were lacking and were difficult to retrieve and throughout the literature search conducted while developing the current study, it was noted that the country's specific data were untraceable. However, a study done in the UAE in 2016 estimated that 14077 (0.2%) of the general population used alcohol in a harmful way; and 1408 (0.02%) used opiates, all of which aged between 15-64 years (Doran, 2016). Moreover, according to the United Nations Office on Drugs and Crime, cannabis use in the UAE was last estimated in 2006 by (5.4%) of the population. Also, they estimated the drug related deaths and mortality by 60 deaths in 2018, opioids and amphetamines ranked as primary cause of deaths (United Nations Office on Drugs and Crimes, 2019).

It is true that published epidemiological data regarding the problem of addiction in the UAE is limited, however, it is almost impossible to neglect the fact that addiction is a growing problem and its harmful consequences have resulted in personal distress, health problems,

and family disruption (Alsuwaidi, 2019). This Indicates the need to conduct studies in the field of addiction in the UAE to better understand the problem, and explore the impact of psychoactive substances on biochemical and hematological markers is no exception.

It was confirmed by studies that metabolism of drugs such as opioid and alcohol differ according to age, sex and ethnicity. For instance, those of Japanese and Chinese origins experience high levels of acetaldehyde due to having a sub-functioning form of the aldehyde dehydrogenase enzyme, this leads to experiencing adverse effects in lower doses of alcohol compared to people from other origins (Saunders et al., 2016). One could argue that all these factors should be taken into account when conducting these types of studies, since the effect of these substances might differ according to ethnicity. To the best of our knowledge, no study has been conducted in the UAE and the results of other studies might not be the best to generalize on this population. Pressuring the need to conduct these studies on different ethnic groups and populations.

Chapter 6

Conclusions

6.1 Conclusions

The main focus of this study was to explore the impact of selected substances of abuse (ethanol, opioid and methamphetamine) on liver function markers particularly aspartate transaminase (IU/L) and γ-glutamyl transferase (IU/L). The overall results revealed that ethanol is the most influential substance on liver function markers, among all other substances in this study. Although the level of the plasma enzymes were also higher in opioid and methamphetamine users, the levels remained in the upper reference range and did not cross into the abnormal level. This might indicate the need for special reference ranges for these enzyme markers when used to assess SUD subjects. The results of our study along with current literature raise the alarm about liver cell damage specifically with alcohol abuse and the possible use of these markers as indicators for alcohol abuse and assessment of abstinence. Other enzyme markers, e.g., creatine kinase, might be suggested for future research for other drugs of abuse, e.g., cocaine or methamphetamines. The results of our study along with current literature propose that increased levels of AST and γGT could be resulted from the abuse of the selected substances, and that could result in liver injury and disease.

The exploratory nature of this study enabled in investigating changes in AST and γGT in relation to the selected substances of abuse. However, generalization might be minimum at the moment, yet it is expected to provide a foundation for further research in the future.

It is crucial that in any study to interpret the results with caution, this was particularly challenging due to the small sample size of each subgroup and methodological limitation discussed in chapter 5. However, using the SPSS in creating tables, graphs and tests of associations, strengthened the study and helped in understanding and interpreting the results. Moreover, causality could not be solely attributed to only the selected group of drugs due to the retrospective design and the potential influence of undetected confounding bias.

6.2 Recommendations

Drug-induced toxicity is one of the most common causes of liver damage and injury. Excessive drugs' usage accounts for almost one-half of the cases regarding acute liver diseases and liver cancer (Kaplowitz, 2011). Consequently, long-term use increases one's vulnerability to not only liver injury but also other diseases. This type of research will hopefully provide health care providers with a clearer pattern of elevated liver function markers in relation to the selected substances of abuse, as well as recommend selected lab tests to obtain from patients with SUD according to their drug of abuse at the time of diagnosis, this approach will help in minimzing the costs, time and efforts, since unnecessary tests will be avoided.

Furthermore, in order to be able to conduct these types of research, it is recommended to advise health care providers to obtain all the necessary laboratory tests and for the stakeholders to cooperate in sharing data so further investigations can be done.

Recommendation of current research might also indicate the need to conduct prospective study design while minding the ethical issues that might arise from this study design. Besides, a larger sample size is recommended to ensure generalizability of the findings of future studies.

Chapter 7

Personal Reflection

The process of completing my dissertation and conducting this study "Serum Aspartate Transaminase and γ-Glutamyl Transferase as Surrogate Markers of Liver Function in Alcohol, Opioid and Methamphetamine Absue" enriched my knowledge and provided me with a valuable opportunity to have a contribution of that topic. I had a spark of interest in this topic, especially that it was somehow neglected and did not receive much attention, despite its major consequences on patients as well as on societies. The challenges that were faced helped in developing my skills both as a researcher and as a health care provider. It enhanced my skills in regard to critical thinking, utilizing the SPSS, statistical analysis and interpretation, and conducting a systematic literature review. Moreover, through completing my dissertation I realized I had to become well-versed in all the current literature surrounding this topic.

Initial planning and time management were two main goals to achieve in completing this dissertation. Conducting my first study was challenging and I might have set unrealistic deadlines and goals, which may have resulted in times where I felt less encouraged and less motivated. However, I soon gained back my enthusiasm in moving on reminding myself of why I chose this topic and what value it will add to me on a personal and professional levels as well as to the patients' care.

The initial goal of this topic was supposed to target the UAE population, to fill the gap in research, especially that to my best of knowledge, no study was conducted in this domain in this specific geographic region. However, in mid-semester I came to realize that obtaining data within the UAE population was not an option. These challenges, although were discouraging they did not stop me from navigating different paths in achieving my goal in exploring the topic. Secondary data was then provided by my academic supervisor which I was grateful for. This allowed me to carry on with my dissertation and provided me with a foundation for my hopefully next thesis in the UAE. In fact, this journey has helped in increasing the awareness of the challenges and obstacles that I might encounter in the future, I believe I will be able to anticipate them and set more realistic goals and deadlines.

References

1- A Glossary of Terms (2000), United Nations Office for Drug Control and Crime Prevention, viewed 14, April 2021, <https://www.unodc.org/pdf/report_2000-11-30_1.pdf>

2- Addiction Center (2017), *Addiction Statistics - Facts on Drug and Alcohol Use*, Addiction Center, viewed 20 May 2021, <https://www.addictioncenter.com/addiction/addiction-statistics/>

3- Agarwal, S, Fulgoni-III, V & Lieberman, H. (2016), 'Assessing alcohol intake & its dose-dependent effects on liver enzymes by 24- h recall and questionnaire using NHANES 2001-2010 data', *Nutrition Journal*, 15(62), pp. 1-12.

4- Al Ghaferi, H, Ali, A, Gawad, T & Wanigaratne, S. (2017), 'Developing substance misuse services in United Arab Emirates: The National Rehabilitation Centre experience', *BJPsych International*, 14(4), pp. 92-96.

5- Alsuwaidi, H (2019),' Understanding the Barriers to Integration to Society, Recovering Patient from Addiction Face: Qualitative Study in UAE', Harvard Library: Office for Scholarly Communication.

6- Atici, S, Cinel, I, Cinel, L, Doruk, N, Eskandari, G & Oral, U. (2005), 'Liver and kidney toxicity in chronic use of opioids: An experimental long term treatment model', *Indian Academy of Science*, 30(2), pp. 245-252.

7- Beecroft, J, Kemp, A, Lassila, S, Sheedy, D & Ward, E. (2010), 'The Biochemical and Pathophysiological Effects of Alcohol Consumption', *Christian Spirituality and Science*, 8(1), pp. 25-43.

8- Black, D (2020), *Review of Drugs: Executive Summary,* HRB National Drugs Library , viewed 20 August 2021, <https://www.drugsandalcohol.ie/31655/>

9- Brown, A. (2016), 'An Exploratory Study Investigating the Impact of a University Module That Aims to Challenge Students' Perspectives on Ageing and Older Adults.', *Practitioner Research in Higher Education*, 10(2), pp. 25–39.

10- Courtney, K & Ray, L. (2014), 'Methamphetamine: An Update on Epidemiology, Pharmacology, Clinical Phenomenology, and Treatment Literature', *Drug and Alcohol Dependence*, 143, pp. 11-21.

11- Dadiomov, D. (2020), 'Laboratory Testing for Substance Use Disorders', *Absolute Addiction Psychiatry Review*, pp. 17-30.

12- Doran, CM (2016),' Preliminary Estimates of the Economic Implications of Addiction in the United Arab Emirates', *Eastern Mediterranean Health Journal*, 22(10), pp. 749-755.

13- Drewes, A, Jensen, R, Nielsen, L, Droney, J, Christrup, L, Arendt-Nielsen, L, Riley, J & Dahan, A. (2012), 'Differences between opioids: pharmacological, experimental, clinical and economical perspectives', *British Journal of Clinical Pharmacology*, 75(1), pp. 60-78.

14- Drugs.com (2021), *Alcohol*, Drugs.com, viewed 1 June 2021, <https://www.drugs.com/alcohol.html>

15- Dydyk, A, Jain, N & Gupta, M. (2021), 'Opioid Use Disorder', *StatPearls Publishing*, viewed 4 May 2021, <https://www.ncbi.nlm.nih.gov/books/NBK553166/?report=printable>

16- Elnebrasi, E. (2012), ' Pharmacology of Alcohol', PowerPoint presentation, Dubai Medical College

17- Elnebrisi, E. (2021), 'Pharmacology of Opioids', PowerPoint presentation, Dubai Medical College

18- Embassy of the United Arab Emirates (2021), *History*, Embassy of the United Arab Emirates, viewed 16 July 2021, < https://www.uae-embassy.org/about-uae/history>

19- Given, L. (2012) 'Exploratory Research', *The SAGE Encyclopedia of Qualitative Research Methods*. doi: 10.4135/9781412963909.n166.

20- Gogoi, J, Bhasin, M, Behera, J, Gairola, K & Baruah, H. (2017), 'A study of effect of alcohol on liver function tests (LFT) in Garhwal hills, India', *International Journal of Research in Medical Sciences*, 6(1), pp. 94-98.

21- Gough, D, Thomas, J & Oliver, S. (2012), 'Clarifying Differences Between Review Designs and Methods', *Systematic Review*, 1(28), pp. 1-9.

22- Hackshaw, A. (2008), 'Small studies: strengths and limitations', *European Respiratory Society*, 32, pp. 1141-1143.

23- Halpin, L, Gunning-III, W & Yamamoto, B. (2013), 'Methamphetamine causes acute hyperthermia-dependent liver damage', *Pharmacology Research & Perspectives*, 1(1), pp. 1-11.

24- Holland, A. (2020) 'An ethical analysis of UK drug policy as an example of a criminal justice approach to drugs: a commentary on the short film Putting UK Drug Policy into Focus', *Harm Reduction Journal*, 17(1), pp. 1–9.

25- Ifeanyi, O, Ndukaku, O, Ndubuisi, T, Kalu, O & Obioma BL, E. (2014), 'Some Haematological and Biochemical Parameters of Chronic Alcoholics in Umuahia, Abia State, Nigeria', *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 5(2), pp. 831-836.

26- Kaplowitz, N (2004) 'Drug-Induced Liver Injury', *Clinical infecious diseases*, 1(38), pp. 44-48.

27- Kuo, C, Tsai, S, Liao, Y, Conwell, Y, Lee, W, Huang, M, Lin, S, Chen, C & Chen, W. (2012), 'Elevated Aspartate and Alanine Aminotransferase Levels and Natural Death among Patients with Methamphetamine Dependence', *Plos One Journals*, 7(1), pp. 1-7.

28- Kwako, L, Bickel. W & Goldman, D. (2018), 'Addiction Biomarkers: Dimensional Approaches to Understanding Addiction', *Trends in Molecular Medicine*, 24(2), pp. 121-128.

29- Langowska-Grodzka, B, Ziólkowski, M & Czarnecki, D. (2016),'Evaluation of drug dependent persons' health on the basis of routine laboratory test results', *Alcoholism and Drug Addiction,* 29(1), pp. 9-15.

30- Legislation.gov.uk 1998, *Data Protection Act*, Legislation.gov.uk, viewed 18 August 2021, < https://www.legislation.gov.uk/ukpga/1998/29/schedule/1>

31- Lin, Y, Chen, P, Liu, H, Chen, Y, Chou, W & Huang, M. (2021), 'Shortened Leukocyte Telomere Length in Young Adults Who Use Methamphetamine', *Translational Psychiatry*, 11(519), pp. 1-7.

32- Lopez-Pelayo, H, Aubin, H, Drummond, C, Dom, G, Pascual, F, Rehm, J, Saitz, R, Scafato, E, & Gual, A. (2020), 'The post-COVID era": challenges in the treatment of substance use disorder (SUD) after the pandemic', *BMC Medicine*, 18(241), pp. 2-8.

33- Matsumoto, R, Seminerio, M, Turner, R, Robsom, M, Nguyen, L, Miller, D & O'Callaghan, J. (2014), 'Methamphetamine-induced toxicity: an updated review on issues related to hyperthermia', *Pharmacology & Therapeutics*, 144(1), pp. 28-40.

34- Najafipour, H & Beik, A. (2016), 'The Impact of Opium Consumption on Blood Glucose, Serum Lipids and Blood Pressure, and Related Mechanisms', *Frontiers in Physiology*, 7(436), pp. 1-13.

35- National Center on Substance Abuse and Child Welfare (2021), *Methamphetamine*, National Center on substance Abuse and Child Welfare, viewed 15 June 2021, <https://ncsacw.samhsa.gov/topics/methamphetamine.aspx>

36- National Institute on Alcohol Abuse and Alcoholism (2021), *Alcohol Use in the United States*, National abuse on Alcohol Abuse and Alcoholism, viewed 25 May 2021, < https://www.niaaa.nih.gov/publications/brochures-and-fact-sheets/alcohol-facts-and-statistics>

37- National Institute on Alcohol Abuse and Alcoholism (2021), *Alcohol Use in the United States*, National Institute on Alcohol Abuse and Alcoholism, viewed 2 April 2021, < https://www.niaaa.nih.gov/publications/brochures-and-fact-sheets/alcohol-facts-and-statistics>

38- National Institute on Drug Abuse (2013), What Are the Different Types of Opioids?, National Institute on Drug Abuse, viewed 17 April 2021, <https://archives.drugabuse.gov/blog/post/real-teens-ask-what-are-different-types-opioids>

39- National Institute on Drug Abuse (2018), *The Science of Drug Use and Addiction: The Basics*, National Institute on Drug Abuse, viewed 1 May 2021, viewed 1 May 2021, < https://www.drugabuse.gov/publications/media-guide/science-drug-use-addiction-basics>

40- National Institute on Drug Abuse (2019), *Methamphetamine Research Report*, National Institute on Drug Abuse, viewed 5 June 2021,

<https://www.drugabuse.gov/publications/research-reports/methamphetamine/overview>

41- National Institute on Drug Abuse (2019), *The Science of Drug Use and Addiction: The Basics*, National Institute on Drug Abuse, viewed 1 May 2021, < https://www.drugabuse.gov/publications/media-guide/science-drug-use-addiction-basics>

42- National Library of Medicine (2015), *Methamphetamine*, National Library of Medicine, viewed 5 June 2021, <https://pubchem.ncbi.nlm.nih.gov/compound/Methamphetamine>

43- Niemelä, O. (2015), 'Biomarker-Based Approaches for Assessing Alcohol Use Disorders', *International Journal of Environmental Research and Public Health*, 13(166), pp. 1-19.

44- Oduola, T, Adeosun, O, Oduola, T, Agbaje, N & Raheem, Z. (2005), 'Drinking patterns: biochemical and haematological findings in alcohol consumers in Ile-Ife, Nigeria**',** *African journal of Biotechnology,* 4(11), pp. 1304-1308.

45- Overholser, B & Foster, D. (2011), 'Opioid Pharmacokinetic Drug-Drug Interactions', *The American Journal of Managed Care*, 17(11), pp. 276-287.

46- Overholser, B & Foster, D. (2011), 'Opioid Pharmacokinetic Drug-Drug Interaction', *American Journal of Managed Care*, 17(11), pp. 276-287.

47- Pannucci, C & Wilkins, E. (2011), 'Identifying and Avoiding Bias in Research', *Plastic and Reconstructive Surgery*, 126(2), pp. 1-11.

48- Pawan, Choudhary, R, Mathur, R & Kamla, M.R. (2011), 'Study on Harmful Effects of Opium on Liver and Lungs in Chronic Opium Addicts of Western Rajasthan', *Journal of Bangladesh Society of Physiologist*, 6(2), pp. 122-126.

49- Pergolizzi Jr, J, LeQuang, J, Berger, G & Raffa, R. (2017), 'The Basic Pharmacology of Opioids Informs the Opioid Discourse about Misuse and Abuse: A Review', *Pain and Therapy*, 6, pp. 1-16.

50- Peterson, K. (2005), 'Biomarkers for Alcohol Use and Abuse', *Alcohol Research and Health*, 28(1), pp. 30-37.

51- Pruskowski, J & Arnold, R. (2015), *Fast Facts and Concepts #307 Opioid Pharmacokinetics*, Palliative Care Network of Wisconsin, viewed 1 May 2021, <https://www.mypcnow.org/wp-content/uploads/2019/03/FF-307-Opioid-pharmacokinetics1.pdf>

52- Roscoff, D, Charlet, K, Jung, J, Lee, J, Muench, C, Luo, A, Longley, M, Mauro, K & Lohoff, F. (2019), 'Association of High-Intensity Binge Drinking with Lipid and Liver Function Enzyme Levels', *Jama Network*, 2(6), pp. 1-12.

53- Rosenberg, A, Greenfield, M & Dimick, J. (2007), 'Secondary Data Analyses' *Clinical Research for Surgeons*, pp. 175-200, DOI: 10.1007/978-1-59745-230-4_11.

54- Sanli, D, Bilici, R, Suner, O, Citak, S, Kartkaya, K & Mutlu, F (2015), 'Effect of Different Psychoactive Substances on Serum Biochemical Parameters', *International Journal of High Risk Behaviors and Addiction,* 4(2).

55- Saunders, J, Conigrave, K, Latt, N, Nutt, D, Marshall, E, Ling, W & Higuchi, S. (2016), *Addiction Medicine*, Oxford University Press, United Kingdom.

56- Smith, H. (2009), 'Opioid Metabolism', *Mayo Clinic Proceedings,* 84(7), pp. 613-624.

57- Strech, D, Sievers, S, Märschenz, S, Riedel, N, Wieschowski, S, Meerphl, J, Langhof, H, Müller-Ohlraun, S & Dirnagl, U. (2018) 'Tracking the timely dissemination of clinical studies. Characteristics and impact of 10 tracking variables', *F1000Research*, 7, pp. 1-14.

58- Substance Abuse and Mental Health Services Administration (2019), *2019 National Survey of Drug Use and Health (NSDUH) Releases*, Substance Abuse and Mental Health Services Administration, viewed 7 July 2021, < https://www.samhsa.gov/data/release/2019-national-survey-drug-use-and-health-nsduh-releases>

59- Talari, K & Goyal, M. (2020), 'Retrospective studies – utility and caveats', *Journal of the Royal College of Physicians of Edinburgh*, 50(4), pp. 398-402.

60- Tavasolian, F, Abdollahi, E, Samadi, M & Vakili, M. (2015), 'Analysis of Hematological and Biochemical Parameters in Methamphetamine Addicts Compared with Healthy Individuals', *Medical Laboratory Journal*, 9(4), pp. 38-42.

61- The University of Arizona (2021), *Pharmacology*, Methamphetamine and Other Illicit Drug Education, viewed 2 June 2021, <https://methoide.fcm.arizona.edu/infocenter/index.cfm?stid=166>

62- Trescot, A, Datta, S, Lee, M & Hansen, H. (2008), 'Opioid Pharmacology', *Pain Physician Journal*, 11, pp. S133-S153.

63- U.S National Library of Medicine (2020), *Opioids*, U.s National Library of Medicine, viewed 20 April 2021, < https://www.ncbi.nlm.nih.gov/books/NBK547864/>

64- United Nations Office on Drugs and Crime (2019), *DATAUNODC; United Arab Emirates,* United Nations Office on Drugs and Crime, viewed 2 July 2021, <https://dataunodc.un.org/content/Countryprofile?country=United%20Arab%20Emirates>

65- United Nations Office on Drugs and Crime (2019), *Statistical Annex*, United Nations Office on Drugs and Crime, viewed 20 May 2021, < https://www.unodc.org/unodc/en/data-and-analysis/wdr2021_annex.html>

66- United Nations Office on Drugs and Crime (2020), *COVID-19 causes some illegal drug prices to surge, as supplies are disrupted worldwide*, United nations Office on Drugs and Crime, viewed 20 June 2021, < https://news.un.org/en/story/2020/05/1063512>

67- United Nations Office on Drugs and Crime (2021), *UNODC World Drug Report 2021: pandemic effects ramp up drug risks, as youth underestimate cannabis dangers*, United Nations Office on Drugs and Crime, viewed 5 May 2021, < https://www.unodc.org/unodc/press/releases/2021/June/unodc-world-drug-report-2021_-pandemic-effects-ramp-up-drug-risks--as-youth-underestimate-cannabis-dangers.html>

68- United Nations Office on Drugs and Crime; World Drug Report (2020), *EXECUTIVE SUMMARY | IMPACT OF COVID-19 | POLICY IMPLICATIONS*, United Nations Office on Drugs and Crime, viewed 20 May 2021, < https://wdr.unodc.org/wdr2020/en/exsum.html>

69- Van Beek, J, De Moor, M, De Geus, E, Lubke, G, Vink, J, Willemsen, G, Boomsma, D. (2013), 'The Genetic Architecture of Liver Enzyme Levels: GGT, ALT and AST', *Behavior Genetics*, 43(4), pp. 1-18.

70- West, N & Dart, R. (2015), 'Prescription opioid exposures and adverse outcomes among older adults', *Pharmacoepidemiology & Drug Safety*, 25(5), pp. 539-544.

71- World Health Organization (2021), *Drug (psychoactive)*, World Health Organization, viewed 19 April 2021, < https://www.who.int/health-topics/drugs-psychoactive#tab=tab_1>

72- World Health Organization (2021), *Opioid Overdose*, World Health Organization, viewed 5 July 2021, <https://www.who.int/news-room/fact-sheets/detail/opioid-overdose>

73- Zhang, Y, Shu, G, Bai, Y, Chao, J, Chen, X & Yao, H (2018), 'Effect of Methamphetamine on the Fasting Blood Glucose in Methamphetamine Abusers', *Metabolic Brain Disease*, vol. 33, pp. 1585-1597.

Appendix 1: Faculty Research Ethics Committee Approval Letter

February 17th, 2021

Mira Al Shamsi,

MSc Addiction Sciences Batch 1,

Dubai Medical College,

Dear Mira

Re: Exploring the Effect of Different Illicit Drugs on Classical Biochemical Markers in Patients with Substance Use Disorder at Erada Center.

I am writing this letter to confirm that the Ethics Committee at Dubai Medical College has approved your submitted proposal entitled above, having accommodated the feed-back given in the previous response, including the rephrasing of aims and objectives, and properly dealing with the other requested details.

We take this opportunity to wish you all the best for the future with the study. Please inform the Ethics Committee at DMC of publications and oral presentations that might be achieved as a result of this research project. Let us know of any intended changes to the submitted protocol before implementation.

Sincerely,

Dr. Hafez Ahmed,

PhD, MSc, MB BCh, Dip-RCPath (London, Chemical Pathology),

HEA Member (UK), PG-Cert (HED., Kingston University, UK).

Professor of Biochemistry,

Director of Research Ethics Committee,

DMC, Dubai, UAE.