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Serotonin Transport Gene Polymorphisms Effect in Alcoholic Criminal Behaviors

A Research

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Degree of Higher Diploma in Sciences Forensic Evidences**

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1443 AH

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿قالو سبحانك لا علم لنا إلا ما علمتنا إنك أنت العليم
الحكيم﴾

صدق الله العلي العظيم

سورة البقرة (آية 32)

Dedication

To my Lord, my supporter

*To Prophet Muhammad and the pure
infallible*

Imams, my ultimate guide...

*To my dear father, The kind heart my
mother, the secret of my existence...*

To my husband

To my brother and sisters...

To my country, my home and pride...

I dedicate this work.

Sahar

Certification

I certify that this Research was prepared under my supervision at the Department of Biology, College of science, University of Babylon, as a partial requirement for the Degree of Higher Diploma of in Forensic Evidence.

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In view of available recommendation, I forward this thesis for debate
By the Examination Committee.

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Summary:

The study was carried out on (40) individuals of Alcoholic addicts and 30 sample as a control group, The study lasted for three months for the period 23/5/2020 - 22/7/2021. This study was carried out at University of Babylon - Faculty of Science- Biology department-DNA laboratory, The aim of this study to estimation the level of serotonin and detected the polymorphisms of serotonin transporter gene and effect on the criminal behaviors, the physiological study was carried by estimation the level of serotonin hormone level in both alcohol addicts and control and compares between them ,polymerase chain reaction was applied to amplified serotonin transport gene and detected the polymorphemic in this gene and associated with serotonin concentration.

The result of current study show that the addicts was higher in age group (less than 30) years old (55%) while 45% in age group (more than 30), The drinker and control group registered in the present study were classified into five groups according to their BMI into under-weight 18.5, normal , over-weight ,obese and extremely obese , show that the majority of the drinker were overweight between (25-29.9) kg/m² (42.5%), while (40%) of them were normal and (17.7%) were obese, while majority of control were obese (3.33%), (33.3%) pre-obese and (63.33%) were normal.

The distribution of Adducted patients by Home (82.5%) of Adducted were from urban area while (17.5%) was from rural areas while majority of control were (53%) from urban area and (46.6%) from rural areas , the majority of drink duration percent was 65% to category less than 5 years and 30% between 5-10 years and the lesser was more than 10 year was 5% .

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Chapter One

Introduction

Introduction.....

1. Introduction

Serotonin is a neurotransmitter monoamine that is important to many complicated biological functions. (Samuels *et al.*,2016; Sarkar *et al.*, 2020) Its usual shorthand for this chemical name is 5-HT: 5-hydroxytryptamine. The reason behind Vittorio Ersparmer's discovery of serotonin is that in 1935, he tried to purify extract from enterochromaffin cells serendipitously (Fowler *et al.*, 2006).

Serotonin present largely in the enteric nervous system in GI tract (GI tract). But Merkel cells in the skin and the taste receptor cells in the tongue are also formed in the centralized nervous system (CNS), especially in the raphe nuclei in the brain stem. Serotonin is also stored in the blood platelets and released during restlessness and vasoconstriction, which then functions as a platelet agonist (schlienger and Lal, 2003).

In the cells enterochromaffin in the GI tract, approximately 90% of the total body amount of serotonin in the intestine, About 8% is found in platelets and 1–2% in the CNS, several studies have suggested that there may be linkages to a decline in bone density, however there is no significant evidence of this relationship. The early study assessed the alterations in mice that lacked brain serotonin This correlation was theorized (Kitson ,2007).

Increased blood serotonin levels have been associated to higher or more consistent bone density in humans. The 5-HT_{1B} receptor is thought to be the relationship between serotonin levels in the blood and bone density (Sadeh *et al.*,2010). There are 15 different types of serotonin receptors, with six G-protein coupled receptor families that mediate physiological responses (5-HT₁, 5-HT₂, 5-HT₄, 5-HT₅, 5-HT₆, 5-HT₇) and one ligand-gated ion channel family (5-HT₃)

Introduction.....

that depolarizes plasma membranes. Serotonin is classified as an inhibitory neurotransmitter because it has inhibitory effects on neurons, making it less likely for them to trigger action potentials (Katsuragi *et al.*,1999).

SLC6A4

The SLC6A4, the gene encoding the serotonin transporter protein (5-HTT), has been extensively examined as a risk factor for alcohol dependence (AD) and aggressive behavior. There is an insertion-deletion polymorphism in the promoter region (5-HTTLPR) of the SLC6A4, with the most common alleles being a 14-repeat short (S) allele and a 16-repeat long (L) allele. So, this study aims to estimation the level of serotonin and gene polymorphism and effect on crime behavers in alcoholic drinker.

1.2 Alcohol use disorder

Alcohol Use Disorder (AUD) is a relapsing chronic disorder that causes around 88,000 fatalities in the United States each year (Stahre *et al.*,2014). Neuroadaptations in certain brain circuits caused by alcohol addiction are associated to AUD behavioral indicators, including increased alcohol consumption, tolerance, dependence, and a proclivity to relapse after abstinence (Banerjee, 2014).

One of the main goals of AUD research is to uncover new or repurposed pharmacological treatments by identifying the underlying neuroadaptations and molecular targets. Important brain areas and neural circuits implicated in the development of AUD have been uncovered in recent decades, the ventral tegmental area (VTA) and nucleus accumbent are two examples of the mesolimbic reward system (NAc) (Roche *et al.*, 2016).

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This mechanism plays a crucial role in the alcohol's self-reinforcing effects (Banerjee, 2014). The inability to self-regulate alcohol consumption may result from alcohol-induced neuroadaptations in this circuit and other brain regions.

Advances in our understanding of addiction's neurocircuitry and its impact on the brain's reward, stress, and executive function systems have resulted in one of the most beneficial models for conceptualizing AUD and drug addiction. As a result, addiction has been described as a three-stage cycle with unique neurocircuitry and functional domains: (1) binge/intoxication, (2) withdrawal/negative affect, and (3) preoccupation/anticipation (Almaça *et al.*,2016).

1.3 Aims of this study

The aim of this study to estimation the level of serotonin and detected the polymorphisms of serotonin transpose gene and effect on the criminal behaviors.

This performed by following objective:

1. Sample collection
2. Estimation the level of serotonin hormone by ELISA
3. DNA extraction
4. Polymerase chain reaction to amplified serotonin transport gene
5. Comperes and analyze results.
6. Sequencing X analysis .

Chapter Two

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2.Literature Reviews

2.1 The Serotonin

membrane (SERT or 5HTT) expressed in several human tissues such as the brain, the pulmonary, gastrointestinal, blood, platelets and cardiovascular systems (Jeffrey Stehouwer, PhD and Arvind Chopra, PhD). In the brain SERT occurs largely in presynaptic neurons and the midbrain, caudate, putamen, thalamus, hypothalamus, midbrain, pons, medulla, and amygdala have been Serotonin (5-hydroxytryptamine (5HT) is a neurotransmitter transported via a serotonin carrier via the cell found to a large density of these transporter's; by comparison, the cortex has a lower carrier density. Changes in serotonergic neurotransmissions and brain SERT density have been involved in depression and schizophrenia pathophysiology and can contribute to suicide (Nemeroff and Owens, 2009).

2.2 biological function of serotonin

plays several roles in the human body, such as influencing learning, memory, happiness, and reward as well as physiological processes such as regulation of sleep, behavior, and appetite. The role of serotonin in neuroimmune circuits in inter-organ communication and in inflammation and immunity is a new emerging area Serotonin plays an important role in various cancers (Karmakar and Lal, 2021). Serotonin plays a significant role in the pharmacodynamics activities of antidepressants, such as SSRIs and SNRIS. This compound is not only found in mammals but is also present in insects, plants, and fungi. Play important role in maintaining energy homeostasis. By increasing sympathetic drive to brown adipose tissue, serotonin produced in the central

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nervous system regulates behavior, suppresses appetite, and promotes energy expenditure(Karmakar and Lal, 2020) .

Peripheral serotonin plays an important role in nutrient absorption and storage. Specifically, glucose and fatty acids stimulate the duodenum's release of serotonin, promoting gut peristalsis and nutrient absorption. Serotonin also enters the bloodstream and interacts with a variety of organs, priming the body for energy storage by increasing insulin secretion and de novo lipogenesis in the liver and white adipose tissue while decreasing lipolysis and metabolic activity in brown and beige adipose tissue. Peripheral serotonin acts as an endocrine factor to promote efficient energy storage by upregulating lipid anabolism. Potential drug targets for obesity, type 2 diabetes, and nonalcoholic fatty liver disease include pharmacological inhibition of serotonin synthesis or signaling in key metabolic tissues (NAFLD) (Yabut *et al.*,2019).

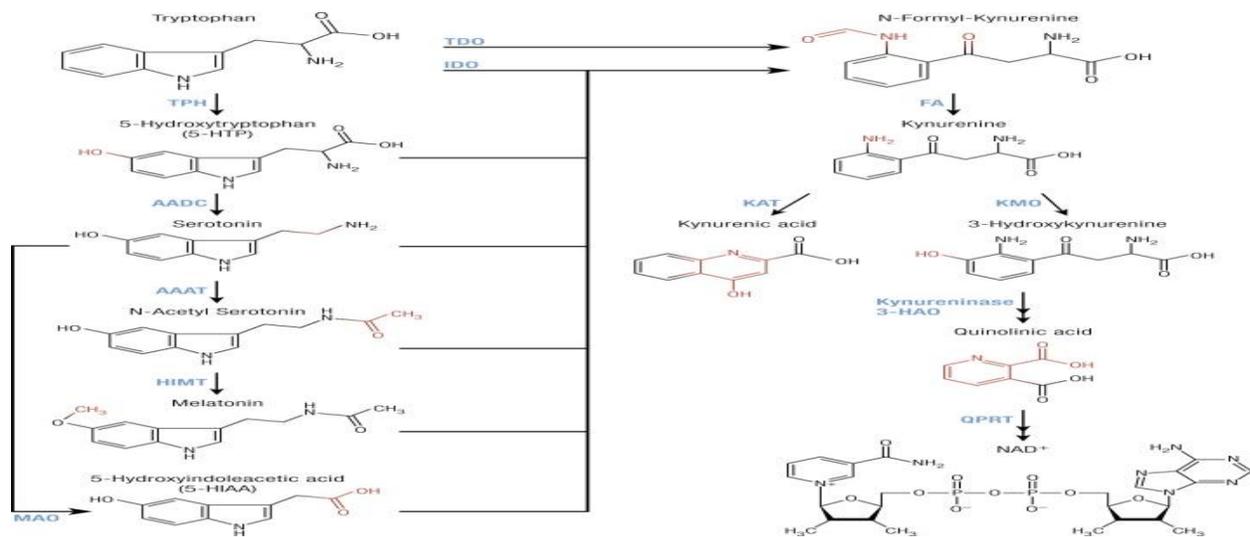
2.3 Serotonin Transporter

In alcoholics, the availability of brain stem serotonin transport (I-123) and computational tomography (Single Photon Emissions) were shown to be dramatically reduced and linked with depression and anxiety ratings following withdrawal (Heinz *et al.*,2004). A functional polymorphism, 5SLC6A4, was related with anxiety and dysphoria in the serotonin transporter, several association studies have revealed that the s-allele, which reduces transcriptional efficiency, is more common in French alcoholics (Hammoumi *et al.*,1996),

Severely affected German alcoholics (Sander *et al.*,1997), and early-onset, violent Finnish alcoholics with ASPD (Hallikainen *et al.*, 1997). In a family-based TDT analysis of U.S. residents, however, no linkage or association for the s-allele was discovered. Some alcoholics experiencing withdrawal symptoms from the

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COGA (Collaborative Study on the Genetics of Alcoholism) data collection (Edenberg et al 1998). In a study of alcoholics with withdrawal seizures in Japan (Ishiguro et al 1999), the long allele was found to be related with lower sensitivity to alcohol, i.e., with persons who may be more predisposed to developing alcoholism (Schuckit et al., 1999). Stratification of populations may be a difficulty for these association studies, because allele frequencies have been found to differ among the population of Europe, the Americas and Japan (Gelernter, et al 1997).



Key enzymes regulating tryptophan metabolism. Left panel: Tryptophan is metabolized by Tph to 5-HTP and subsequently metabolized to serotonin by amino acid decarboxylase (AADC). Serotonin can be metabolized into either 5-HIAA by MAO or N-acetyl-serotonin by aryl alkylamine N-acetyltransferase (AAAT). N-acetyl-serotonin is subsequently metabolized into melatonin by hydroxy indole-O-methyl transferase (HIMT). Right panel: Tryptophan is also a substrate for TDO to produce N-formyl kynurenine, which can be made into kynurenine by formamidase (FA). IDO can also metabolize tryptophan into Nformyl-kynurenine alongside any other molecules that contain an indole moiety.

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Kynurenine aminotransferase (KAT) and kynurenine 3-monooxygenase (KMO) form kynurenic acid and 3-hydroxykynurenine, respectively, from kynurenine. Kynurenine is broken down by kynureninase and 3-hydroxyanthranilic acid dioxygenase (3-HAO) to form quinolinic acid, which can be further metabolized by quinolinic acid phosphoribosyltransferase (QPRT) to form precursors for NAD. Atoms in red are the structural changes of the previous enzymatic reaction. MarvinSketch (from ChemAxon) was used for drawing and displaying chemical structures in this figure (Yabut *et al.*, 2019).

2.4 Serotonin, depression and mood disorder

2.4.1 Evidence for an association between serotonin and mood disorder

The connections between serotonins and recently discovered lysergic dioxide (LSD) began to be identified by scientists. Hit by LSD's considerable power (psychoactive as 20 mg) and by profound modulatory mood and cognitive effects certain mental diseases could be attributable to aberrant serotonergic activity (Herbst *et al.*, 1987), Although in the 1950s and 60s (Isbell *et al.*, 1959), as they are now (Carhart-Harris and Nutt, 2017).), the 'psychotomimetic' characteristics and related psychedelics were recognized as being extensively used as psychotherapeutic agents in the treatment of a range of conditions including depression and anxiety (Polovinkin *et al.*, 2018).

However, it has been noted that reserpine, that depletes 5-HT and noradrenaline in the brain (Pletscher *et al.*, 1955), also promotes depravedness in some people - see also the first and most direct evidence for the participation of monoamines in mood regulation (Antkiewicz Michaluk *et al.*, 2014). This observation was closely

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followed with the discovery of the antidepressant characteristics of the monoamine oxidase (MAOI) inhibitors (Udenfriend *et al.*, 1957) and then of the tricyclic antidepressants (TCAs) (Gur *et al.*, 1999; Matos, 1990). Studies showing a combined antidepressant effect with an MAOI plus tryptophan, the biochemical precursor to 5-HT, provided more specific evidence for 5-HT's involvement in depression (Young, and Leyton, 2002).

The idea that serotonergic mechanisms are involved in the pathogenesis and treatment of depression was controversial in the 1960s (Baumeister and Hawkins, 2004.); however, it gradually gained traction in the 1980s and into the 1990s with the development and licensing of SSRIs, particularly fluoxetine (Capitão *et al.*, 2005). Chronically administered, SSRIs increase synaptic 5-HT concentrations by blocking its reuptake (Carlsson, 1981), outperform placebo in depression (Matsunaga, *et al.*, 2011; Hieronymus *et al.*, 2016; Barth *et al.*, 2016), and are safer than MAOIs and TCAs.

Another important finding supporting the role of serotonin in depression is that acute tryptophan deficiency can cause a (transient) relapse in symptoms in previously depressed patients (Smith *et al.*, 2000), and plasma tryptophan levels have been found to be low in patients with severe depression (Leventhal *et al.*, 1990), possibly due to inflammation-related mechanisms (Kang *et al.*, 2005).

The fact that the potent mood-enhancing agent, MDMA, has marked 5-HT releasing properties adds to the evidence that serotonin is involved in mood regulation (Dill *et al.*, 2013). MDMA is a noradrenaline (NA) and dopamine (DA) releaser in rodents (Kankaanpaa *et al.*, 1998), but its 5-HT releasing properties are much stronger (Chaki *et al.*, 2013; Golembiowska *et al.*, 2016). Pre-treatment with the SSRI citalopram blocked the serotonin transporter, which greatly reduced

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MDMA's distinctive pleasant mood effects (Liechti *et al.*, 2001) — likely by preventing MDMA from interacting with the 5-HT transporter. Pre-treatment with the D2 antagonist haloperidol reduced MDMA's pleasant mood effects, suggesting that coupled DA and 5-HT functioning may have a synergistic effect on mood. In a separate study, combining the DA reuptake blocker methylphenidate with MDMA had no additional effect on positive mood and stimulants with greater DA than 5-HT releasing properties (such as amphetamine, cocaine, and methylphenidate) do not produce the same pro-empathy and pro-social sentiments as MDMA (Hysek *et al.*, 2014).

2.5 Serotonin Levels in Alcoholics

A serotonin decrease may result from a number of mechanisms. The brain cells can for example produce less serotonin, release less serotonin to the synapse, or return more serotonin to the cells. Serotonin metabolites can also be decreased in alcoholics, to present, it is still not known exactly the mechanisms behind changes in serotonin metabolite levels. The discovery of a link between alcoholism and the levels of serotonin metabolites in human alcoholics' urine and CSF, there was evidence that serotonin has a role in the development of alcoholism. For example, alcoholics' CSF concentrations of the first serotonin degradation product, 5-hydroxyindoleacetic acid, were lower than nonalcoholic of the same age and general health status, implying that alcoholics' brain serotonin levels are lower. (LeMarquand *et al.*, 1994a; Prudic, 1996; Virkunen *et al.* 1995)

Researchers are currently trying to determine whether alcoholics with an abnormal quantity of serotonin have certain gene variants coding for the Tryptophan Hydroxylase enzyme that creates serotonin from other cell components, the tryptophan hydroxylase gene has several variations, one of which appears to be

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more prevalent in alcoholics with a history of violence and suicidal tendencies (Virkkunen *et al.*,1995).

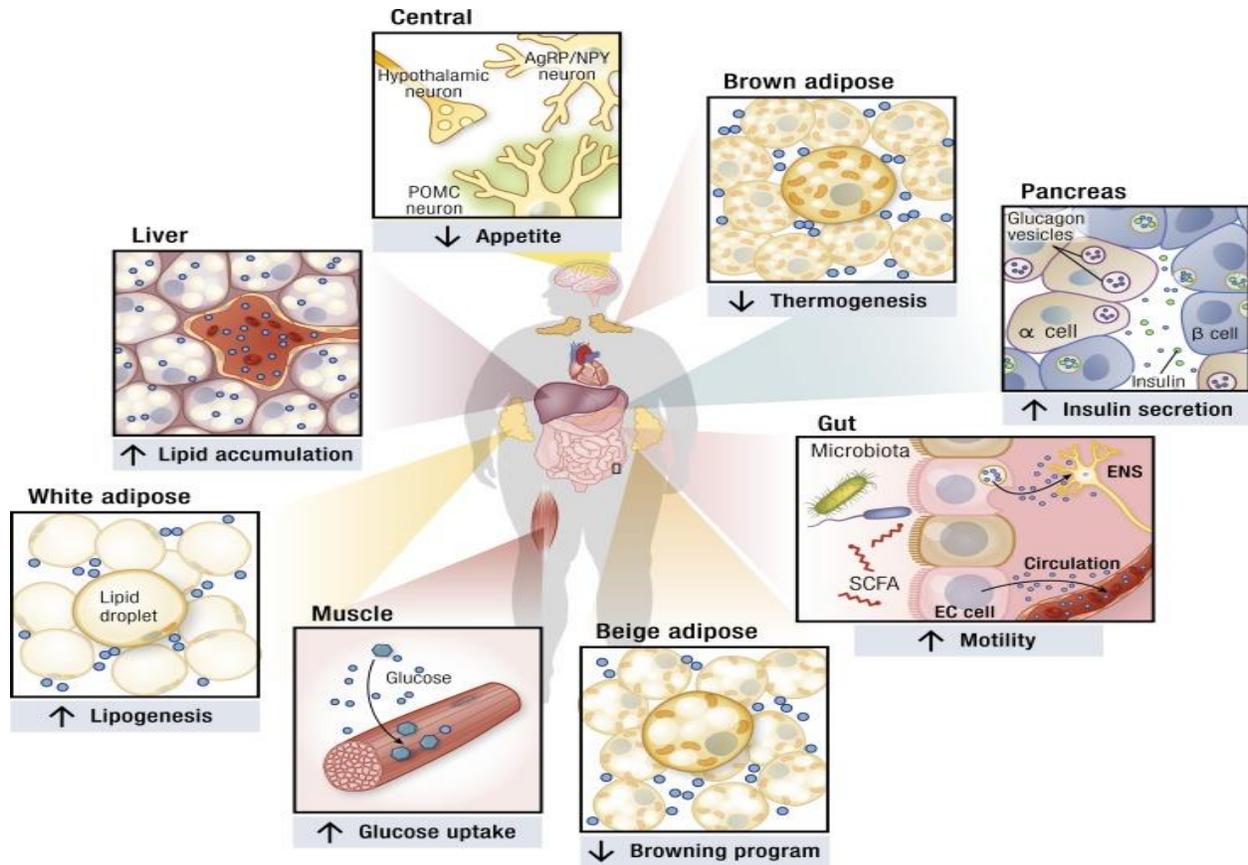


Figure (2-1) Metabolic functions of serotonin in different tissues

(Julian M Yabut and et al. ,2019)

2.6 Alcohol

Alcohol affects the central nervous system as a drug, like all other drugs (CNS). Ethanol with different alcoholic drinks containing various proportions is the type of alcohol that is frequently used. Ethanol acts in the style of anesthesia to depress the brain's function. Ethanol at low blood levels emits otherwise inhibited behaviors that generate feelings of relaxation and good mood. Can make socialization easier. (Gilpin and Koob ,2008).

The person is slowly searching for the drug over a period to reach the same height as before. He therefore begins to consume more and more alcohol until a point is reached when normal brain chemistry simply cannot work without alcohol (Walter *et al.*,2003). However, caution is advised because even little amounts of alcohol inhibit the hippocampus' ability to process information, which in turn hinders memory formation. Higher amounts of alcohol have a stronger effect on the brain, causing drunkenness and a momentary loss of coordination and judgment (Walter *et al.*,2003).

2.7 Alcohol's damaging effects on the brain

Alcohol clearly affects the brain, as evidenced by difficulty walking, blurred vision, slurred speech, slower reaction times, and poor memory. Some of these limitations can be detected after just one or two drinks and disappear fast if drinking is stopped. A person who drinks heavily over a long length of time, on the other hand, may have brain abnormalities that last long after achieving sobriety. The exact effects of alcohol on the brain, as well as the possibility of reversing the effects of heavy drinking on the brain, are still hot subjects in alcohol research today. we do know that heavy drinking can have a wide range of effects on the brain, ranging from minor memory lapses to permanent and debilitating conditions

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requiring lifelong care. And, as considerable research on the influence of alcohol on driving has demonstrated, even moderate drinking causes short-term impairment (Pine et al.,1996).

2.8 Genetics and alcoholism

Alcohol is commonly consumed, but excessive consumption generates severe physical, mental and social issues and contributes to several diseases. The pattern of excessive drinking that leads to major issues, is a maladaptive one (alcohol dependency, alcohol use disorders). Alcoholism is a complicated genetic illness, with changes in a large number of genes increasing risk, according to a substantial body of research. Some of these genes have been discovered, including two alcohol metabolism genes, ADH1B and ALDH2, which have the strongest known effects on alcoholism risk. GABRA2, CHRM2, KCNJ6, and AUTS2 are among the genes whose variations influence the risk of alcoholism or related features, according to research. A more complete picture of the various genes and pathways which can affect risk is discovered by collecting larger samples and analyzing more variants (Potts *et al.*,2013).

2.9 alcohol-associated diseases

An enormous range of ailments are affected by alcohol. Recent meta-analysis has found that the drinking of alcohol has been causally related to a wide range of infectious disorders, ranging from tuberculosis and pneumonia, heart disease, cirrhosis and numerous cancers, in particular the aerosol tract, colon, rectum and liver (Rehm ,2011) Genes affecting the use of alcohol, especially those indicated above that affect very excessive AUD use, may affect the likelihood of an alcohol-related disease in part , they may increase the overall risk of drinking or decrease the danger of drinking. Some genotypes that lower the risk of heavy drinking can

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also raise the risk of disease in the subset of people who drink extensively despite having them. The GI tract is particularly exposed to alcohol while passing through the mouth, esophagus, stomach, and intestine, with the majority of ethanol passing through the liver before the circulation (Wang *et al.*,2012)

The mouth cavity and the esophagus are exposed directly to these levels and the liver to high concentrations of the portal circulation. As a result, it is not surprising that alcohol consumption affects GI diseases such as cirrhosis, pancreatitis, and upper GI tract cancers. As a result, it's no surprise that alcohol usage affects GI disorders like cirrhosis, pancreatitis, and malignancies of the upper GI tract (Lewis SJ, Smith GD., 2005.; Kirpalani,. 2017). Heavy episodic (binge) drinking, which exposes tissues to high quantities of alcohol, has been shown to be particularly damaging (Edenberg and Foroud, 2013; Graff-Iversen S, *et al.* ,2012).

Binge drinking is usually described as a human who uses five standard drinks in 2 hours; women are often smaller and have a lower percentage of body water .a standard drink is defined as having approximately 14 g of pure ethanol .Because of their obvious connection to the disposition of alcohol in the body, researchers have looked into the link between genes encoding alcohol and Variants in the ADH and ALDH genes that increase acetaldehyde levels at least transiently reduce heavy drinking and the risk of alcoholism. aldehyde dehydrogenases and a variety of GI diseases. the same genes may increase cancer risk by increasing acetaldehyde levels in tissues (Agrawal *et al.*,2012; Lewis and Smith, 2005; Yokoyama *et al.*,2006).

2.10 Acute Alcohol Effects on the Brain's Serotonin System

Alcohol affects serotonergic synaptic transmission in the brain in a variety of ways. Even single-episode (acute) alcohol exposure alters several aspects of serotonin's

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synaptic functions. In humans, for example, after a single drinking session, the levels of serotonin metabolites in the urine and blood rise, indicating increased serotonin release in the nervous system (LeMarquand *et al.* 1994a) This increase could be due to improved signal transmission at serotonergic synapses. Animal studies have also found that acute alcohol exposure raises serotonin levels in the brain (LeMarquand *et al.* 1994b; McBride *et al.* 1998), implying that either more serotonin is released from serotonergic axons or the neurotransmitter is cleared more slowly from synapses. Increased serotonin release, for example, has been observed after acute alcohol exposure in brain regions that control the consumption or use of a variety of substances, including many drugs of abuse (McBride *et al.* 1998).

Researchers are currently attempting to identify the precise mechanisms underlying the alcohol-induced changes. They are looking into whether the net increase in synaptic serotonin levels is due to alcohol's direct actions on molecules involved in serotonin release and uptake or to more indirect alcohol effects. Alcohol also has an effect on the function of serotonin receptors. There are several types of these receptors, including the 5-HT₁, 5-HT₂, 5-HT₃, and 5-HT₄ receptors (see table). When activated by serotonin binding, the 5-HT₃ receptor generates electrical signals that rapidly increase neuron activity (Lovinger , 1993).

The electrical signals generated by the 5-HT₃ receptor are amplified by acute alcohol exposure. This change in receptor function is most likely the result of alcohol's direct action on the receptor protein or on molecules in the cell membrane that are closely associated with the receptor (Lovinger ,1993; Lovinger and Zhou 1994).

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Excessive stimulation of neurons in brain regions receiving information from serotonergic neurons is most likely caused by increased 5-HT receptor function. As a result of this stimulation, other neurotransmitters that play important roles in alcohol intoxication may be increased, the effects of acute alcohol consumption on serotonin receptors have also been studied in so-called knockout mice, in which specific genes (e.g., those coding for different serotonin receptors) have been experimentally inactivated, resulting in the animals being unable to produce the protein encoded by those genes (Crabbe *et al.*,1996).

Researchers can assess the role of a specific receptor in specific aspects of brain functioning and behavior by studying knockout mice that lack that receptor, such as responses to alcohol and alcohol consummator behavior. For example, scientists investigated the effects of acute alcohol exposure on a strain of knockout mice lacking the 5-HT receptor, when compared to normal mice, these animals showed less intoxication in response to a single dose of alcohol, indicating that 5-HT receptor activity is responsible for some of alcohol's intoxicating effects (David M. Lovinger, Ph.D. Serotonin's Role in Alcohol's Effects on the Brain.

2.11 Serotonin transporters and alcohol

Alcohol is a highly addictive substance. It has a significant impact on several neurological pathways and causes significant changes in the brain. The dopaminergic, serotonergic, -amino butyric acid (GABA), and glutamate pathways are among the neurological pathways known to be affected by alcohol consumption. Because of the well-established link between serotonin depletion, impulsivity, and alcohol-drinking behavior in rats and humans (Lappalainen *et al.*,1995) .

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The neurotransmitter serotonin has long been a target of interest for potential pharmacotherapy for alcoholism. Pharmacological compounds that target the serotonin system by inhibiting neuronal reuptake of serotonin, thus prolonging its actions, or by blocking specific serotonin receptor subtypes have been shown to suppress alcohol-reinforced behavior in rats, according to (Johnson BA .2008). Serotonin release in the nucleus accumbent of rats is suppressed during alcohol withdrawal, and this reduction is partially reversed by self-administration of alcohol during withdrawal (Weiss F et al .1996).

Chapter Three

Material and Method

3. Materials and Methods

3.1. Equipment's, laboratory tools and chemicals

Many equipment's, laboratory tools and chemicals were used in the present study and they are summarized in tables (3-1 and 3-2).

Table (3-1): Chemicals used during this study

NO.	Chemical	company
1	Agarose	Bio Basic/Canada
2	-D2000 DNA ladder	Biosharp/Korea
3	DNA Extraction kit	Geneaid/Korea
4	Ethanol	Biosolve company/USA
5	Ethidium Bromide	Promega/USA
6	Human serotonin ELISA Kit Cat. No E1128Hu	Bioassay technology/China
7	Loading Dye	Promega/USA
8	Master Mix	Promega/USA
9	Proteinase K	Proteinase K
10	TBE Buffer	Bio-Basic/England

Table (3-2): Instrument used during this study

No.	Tools	Supplying company
1	Autoclave	Haramaya/Japan
2	Balance	Precisa/UK
3	Centrifuge; Cooling Centrifuge	Hettich/Germany
4	Deep Freeze	GFL/Germany
5	Digital Camera	Sony/Japan
6	Distillater	GFL/Germany
7	Elisa reader and washer	Biotek/USA
8	Gel electrophoresis unite	Cleaver scientific/Japan
9	Photo documentation	Velber/France
10	Vortex mixer	Bioneer/Korea
11	Water bath	GFL/Germany

3.2 study design and subjects

This study was done on a group of prisoners in the Babylon Province, who were drug users, for period from May to July 2021. This study included 40 persons who abuse taken, 30 persons were not abuse apparently, the group was selected as a control group. The ages of persons and control group ranged between eighteen to twenty-five years.

3.2.1 Blood Samples

Five venous bloods collected from each sample of the study, samples were placed in tubes containing a gel, the blood was separated by centrifuge at 3000 rpm for 15 minutes, and the remaining serum was distributed to Eppendorf tube and then stored in a deep freeze at -20°C until

3.3 Experimental design

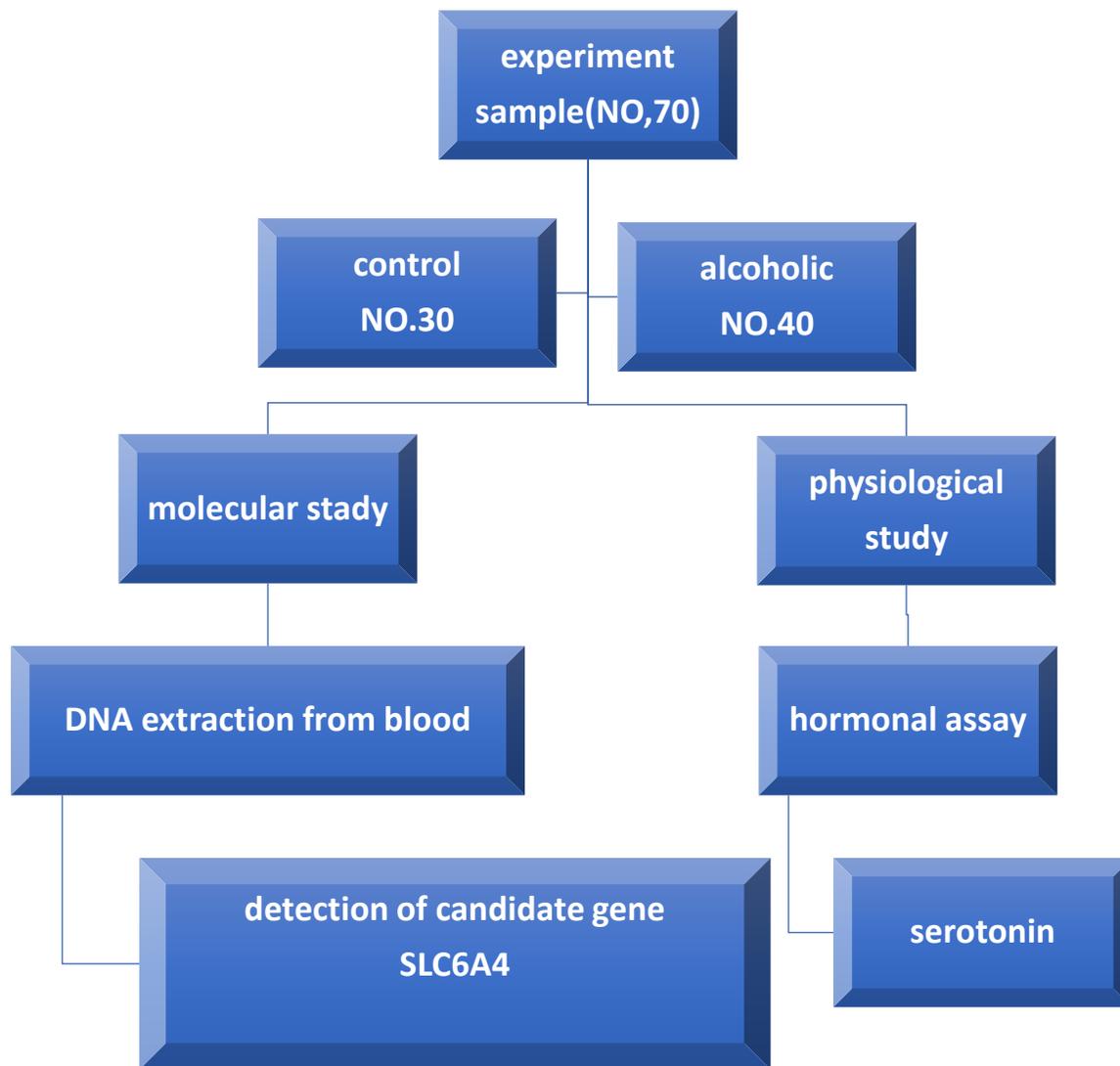


Figure (3-1) study design

3.4. Physiological Study

3.4.1 Human serotonin capacity test(human serotonin ELISA kit,no E1128Hu)

Human Serotonin was determined as a leaflet of kit:

- 1-All solutions were prepared at room temperature.
- 2-About 50 µl of standard was added to stander well, while 40 µl of sample was added to sample well.
- 3-The anti- ST- antibody added to sample well (10 µl), then 50µl streptavidin-HRP was added also to both sample and stander wells with mixing.
- 4-Incubated at 37 °C for 60 min, then plate was washed 5 times by WB.
- 5-Substrate solution (A and B) was added (50 µl), then incubated at 37 °C in dark.
- 6-Stop solution was added (50 µl).
- 7-Detected OD at 450 nm within 10 min.

3.5 Genetic study

3.5. 1. DNA Extraction

Genomic DNA from white blood cells (WBCs) for all groups and control group were extracted by using DNA extraction kit (Favorgen) table (3-4) according to the following:

A. Kit Contents

Table (3-4) Genomic DNA Kit contents

Cat. No. / preps	FABGK 100 (100 Preps)	FABGK 300 (300 Preps)
RBC Lysis Buffer	135 ml	405 ml
FATG Buffer	30 ml	75 ml
FABG Buffer	40 ml	100 ml
W1 Buffer	45 ml	130 ml
Wash Buffer	25 ml	50 ml
Elution Buffer	30 ml	75 ml
FABG Column	100 pcs	300 pcs
2 ml Collection Tube	200 pcs	600 pcs

3.5.1.B Special Protocol of DNA extraction (for frozen blood)

Step 1- Sample preparation

1. A 200µl blood was transferred up to a 1.5ml microcentrifuge tube.
2. A 40µl proteinase k (10 mg / ml) was added to the sample and briefly mixed. Then incubate for 15 minutes at 60 °C.

Material and Method

Step 2- Cell Lysis

1. A 200µl FABG Buffer was added to the sample and mixed by vortex.
2. Incubated in 70 °C water bath for 15 minutes to lyse the sample. During incubation, invert the sample every 3 minutes.
3. Elution buffer was preheated (for step 5 DNA Elution) in a 70 °C water bath.
4. (Optional step): If RNA –free genomic DNA is required, 5µl of 10 mg/ml RNase was added to the sample and mix by vortex. Then incubate for 5 minutes at the room temperature

Step 3- Binding

1. A 200µl ethanol (96-100%) was added to the sample and vortex for 10 seconds. (Pipetting if there is any precipitate).
2. A FABG Column was Placed to a 2ml collection tube. Transfer the sample mixture (including any precipitate) carefully to FABG Column. Centrifuge for 5 minutes at full speed (14000 rpm) and discard the 2ml collection tube. Place the FABG Column in a new 2ml collection tube.

Step 4 – Washing

1. FABG Column with 400µl W1 Buffer was washed. Centrifuge for 30 seconds at full speed (14000 rpm) and discard the flow- through.
2. the FABG Column was placed back in the 2ml collection tube. Wash FABG Column with 600µl Wash buffer (ethanol added). Centrifuge for 30 seconds at full speed (14000 rpm) and discard the flow-through.
3. the FABG Column was placed back into the 2ml collection tube. Centrifuge for an additional 3 min at full speed (14000 rpm) to dry the column.

Material and Method

Step 5 – Elution

1. the dry FABG Column was placed to a new 1.5ml microcentrifuge tube.
2. A 100µl of preheated Elution buffer or TE was added to the membrane center of FABG Column. Stand FABG Column for 3-5 min or until the buffer is absorbed by the membrane.
3. Centrifuge for 30 seconds at full speed (14000 rpm) to elute the DNA.

Step Final – Pure DNA

The DNA fragment was stored at -20 °C.

3.5.2 Gel electrophoresis protocol for DNA of blood.

3.5.2.1. Tris Borate EDTA Buffer preparation (1X TBE)

This solution was Prepared by adding 950 ml Distill water to 50 ml 5,0X TBE (Promiga/ Germany), with a modification in dilution by adding 25 µl TBE to 475 ml D.W. to get 0.5 X (Sambrook and Russel, 2001).

2.5.2.2 Preparation of Agarose

- 1- One hundred ml of 1X /or 0.5X TBE buffer was powered in conical flask.
- 2- Volume of 1.2 mg agarose powder (Biostatic) was added to the buffer.
- 3- The solution was heated to boiling using a heater until all gel particles were melted.
- 4- The solution was left to cool down to 55-60 °C.
- 5- One µl of the Ethidium bromide (10 mg/ml) was added to agarose solution, and mixed. The mixture was casted in a horizontal tray (Sambrook and Russel et al. 2001).

Material and Method

3.5.3.3 Preparation of Horizontal Agarose Gel

1. Fix the comb in 1 cm was fixed away from one edge of a gel tray, the agarose solution was poured into the gel tray.
2. The agarose was allowed to solidify at room temperature for 45 min.
3. The fixed comb was carefully removed, the wells of gel filled with the DNA, and tray was placed in the gel tank, which was filled with 1X TBE buffer until the buffer reached 3-5 mm over the surface of the gel (Sambrook & Russel et al. 2001).

3.6 Reconstituting and diluting primers

Bioneer created the primer in a clean room following strict ISO 9001:2000 guidelines to provide a DNase /RNase and DNA-free environment. Bioneer® primers were frequently delivered lyophilized. A lyophilized primer's units were supplied as a mass in picomoles . Reconstitute the primer in sterile 1X TE (1 mM Tris, 0.1mM EDTA, pH 8.0) or sterile, nuclease-free H₂O to make a stock of primers. The manufacturer provided the amount of TE or sterile, nuclease-free H₂O to be added to each primer to make a master stock, which would then be used to make working stock.

The following steps were followed for reconstituting and diluting the primers:

- 1-Spin down the tube before opening the cap.
- 2-Add the desired amount of water according to the oligos manufacturer to obtain a 100 pmoles/μl (Master Stock).
- 3-Vortex properly for re-suspend the primers evenly.
- 4-Transfer 10 μl of the master stock to a 0.2 ml eppendorff tube that contains 90μl of sterile, nuclease-free H₂O (Working Stock).
- 5-The master stock was stored at -20 C°.

Material and Method

6-The working stock was stored at -20 C°.

7-The working stock was thawed on ice and vortex before using in PCR and then stored at -20 C°.

Table (3-3): Primer sequences used to amplify the *SLC6A4* promoter region.

DNA Primer	Sequences	Amplicon size (kb)	References
5- SLC6A4	F:5'-ATGGGTTCCAGAGTCAATCCTTTGCGTTTTCTGTTGCCCT-3' R:5'-GAAAGGTCTGGAGTCTTGATGAGGGACTGAGCTGGACAACCAC-3'	Long allele/20-23bp Short allele/17-24 bp	(Mariana et al.,2020)

3.7 Polymerase chain reaction amplification analysis

As a first stage, PCR optimization was carried out at a temperature gradient ranging from 49 to 62.1 degrees Celsius. The PCR reaction mixture comprised of 250 ng template DNA, 400 M of each dNTP, 12.5 l buffer containing 1 U GoTaq DNA polymerase (Promega), 10 M of each primer, and 3 mM MgCl₂ in a total reaction volume of 25 l after the optimum annealing temperature (57.8 C) was determined. The GTC Series thermocycler (Clever Scientific /UK) was used to carry out the amplification processes. The following program was set in the thermocycler to amplify the target DNA fragments when the optimum annealing temperature was determined.

Chapter Four

Result and Discussion

Result and Discussion

The result of present study included estimation of serotonin level in drinker and control group, the genetic study included DNA extraction from sample and polymerase chain reaction of serotonin transport gene (SLC6A4) and static analysis. only in drinker or alcoholism

4.1: The baseline characteristics and alcohol level of study groups

The socio demographic distribution of study parameters were exhibits in table (4-1).

Variables	Dru	Ctr	Sig
Age	29.8750±1.43500	26.7667±0.80898	0.089
BMI	26.6977±0.56971	24.4970±0.68664	0.016
Duration	3.8500±0.43346	-	-
Alcohol level	74.2750±6.50453	0	-
Independent t test , p<0,05			

Table (4-1) the baseline characteristics and alcohol level of study groups .

4.1.1: The distribution of study groups according to age categories

The distribution of study groups according to age categories show that the first categories shows that less than 30 years 55% at drink was compares with controls 63.33% and for more than 30 years for drunks was 45% compares with controls in which percentage 36.66%.

Result and Discussion

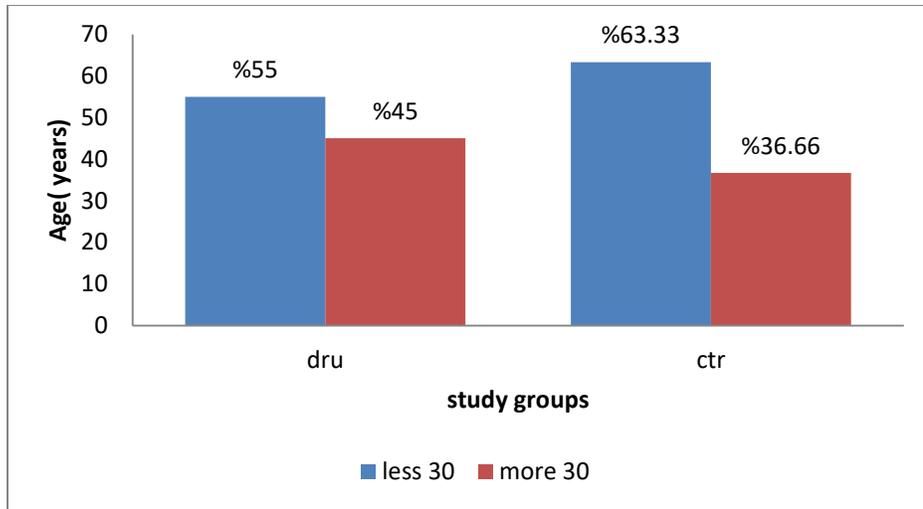


Figure (4-1) distribution of study groups according to age .

Alcohol consumption is a lifestyle behavior that has been extensively studied for potential benefits and risks to health (Thun *et al.*, 1997). It has been suggested that older persons may be at increased risk of adverse consequences of alcohol because of a diminished volume of distribution for alcohol due to a decrease of lean body mass (Vestal *et al.*, 1976). Marsha *et al.*,(2001) reported by their study that four age categories (45–49, 50–54, 55–59, and 60–64 years) were used in the evaluation of the association. The proportion of drinkers and, among drinkers, the amount of alcohol consumed.

Excessive alcohol consumption during adolescence represents a concern due to its numerous negative consequences –difficulty in emotional regulation, increased risky sexual behaviors, fighting and physical aggression, neurotoxic effects on memory, attention and learning, and changes in brain structures and their functionality (Bajac *et al.*, 2016). In Spain, 1 out of 3 students aged 14-18 years acknowledged having engaged in this type of consumption drugs (Simões *et al.*, 2018). During pre-adolescence (around age 11) differences are barely perceptible and increase with age (Goldstein *et al.* 2013; Kuntsche *et al.*, 2015).

Result and Discussion

4.1.2: The distribution of the study group by Body mass index (BMI).

Three categories of BMI were dependent in present study, less than 30 (40%) and more than 30 (17,5%) kg/m² and(42%) for over weight 25 – 29,9 kg/m² in alcoholism's in compare with control group figure (4-2).

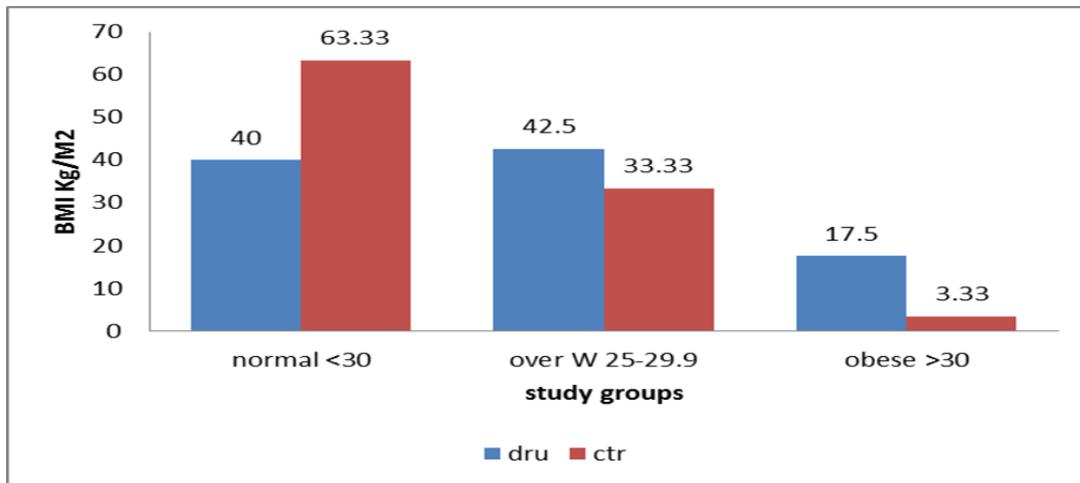


Figure (4-2) distribution of study groups according to BMI kg/m²

The amount and frequency of alcohol consumed have been found to correlate with BMI. The binge drinkers and the heavy drinkers often had a significantly higher BMI than those who participated in only light-to-moderate drinking. The National Institute on Alcohol Abuse and Alcoholism's (NIAAA, 2007). Using national surveys in the United States examined quantity, frequency, and average volume to categorize alcohol consumption based upon participant information. They found that people who consumed four or more drinks per day had significantly higher BMIs than those who consumed one drink per day (Mary *et al.*, 2012).

Alcohol is high in calories, so drinking alcohol may contribute to higher BMIs and the poor health choices of college-age students who are already overweight, particularly freshmen female students who drink four or more drinks per

Result and Discussion

day and male students who consume five or more drinks per day. Elevated triglycerides and low high-density lipoprotein cholesterol levels are the result of continued high carbohydrate intake, especially if students are already overweight when they first start to drink (Park *et al.*, 2003).

4.1.3: The distribution of study groups according to home

The distribution of study groups according to home the percentage of drinking peoples (17.5%) in rural and (82.5%) urban fig (3-4).

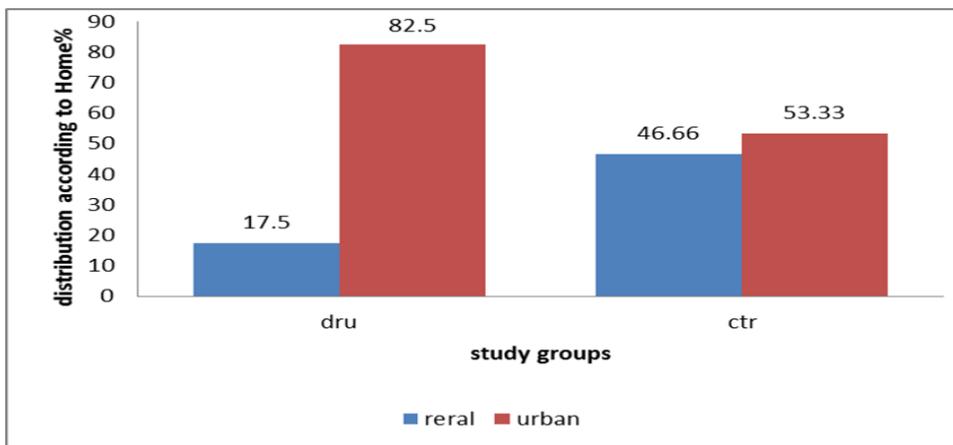


Figure (4-3) distribution of study groups according to home

A number of social and cultural factors predict increased alcohol use, including discrimination and its related stigma. The role of discrimination and stress in health-related risk behaviors, including alcohol use, is well established (Dawson *et al.* 2005; Hatzenbuehler 2009).

The stress and coping framework frequently is applied to explain the influence of discrimination and stigma on health (Pascoe and Smart Richman 2009).

For example, socioeconomic status (SES) indicators (i.e., education, income, and occupation) usually are strong predictors of health behaviors and outcomes and tend to be positively associated with health. People with higher SES tend to drink more

Result and Discussion

frequently than others (Huckle *et al.* 2010). Among drinkers, low-SES groups tend to drink larger quantities of alcohol (Huckle *et al.* 2010). Like other health issues, alcohol use can be linked to a complex array of factors ranging from individual-level (i.e., genetics) to population-level (i.e., cultural and societal factors) characteristics (Berkman *et al.* 2000).

On a population level, emerging research has documented the relationship between social determinants and health (Berkman and Kawachi 2000; Berkman *et al.* 2000) and, specifically, the social epidemiology of alcohol use (Bernstein *et al.* 2007; Galea *et al.* 2004). Social capital theory suggests that social networks and connections influence health (Berkman *et al.* 2000). Individuals who have higher levels of social support and community cohesion generally are thought to be healthier because they have better links to basic health information, better access to health services, and greater financial support with medical costs. (Berkman and Kawachi 2000).

The reason for the high rate of alcoholics in cities compared to rural areas may be due to the nature of life in the city in terms of multiple relationships and sometimes the level of economic income.

4.1.4: The distribution of drunks according to criminal behaviors

The fig (4-4) show the distribution of drunks according to criminal behaviors 60% violent,17% normal drink,12% paraphilia's, 8% drunk with possession of a drunk and 3% accident.

Result and Discussion

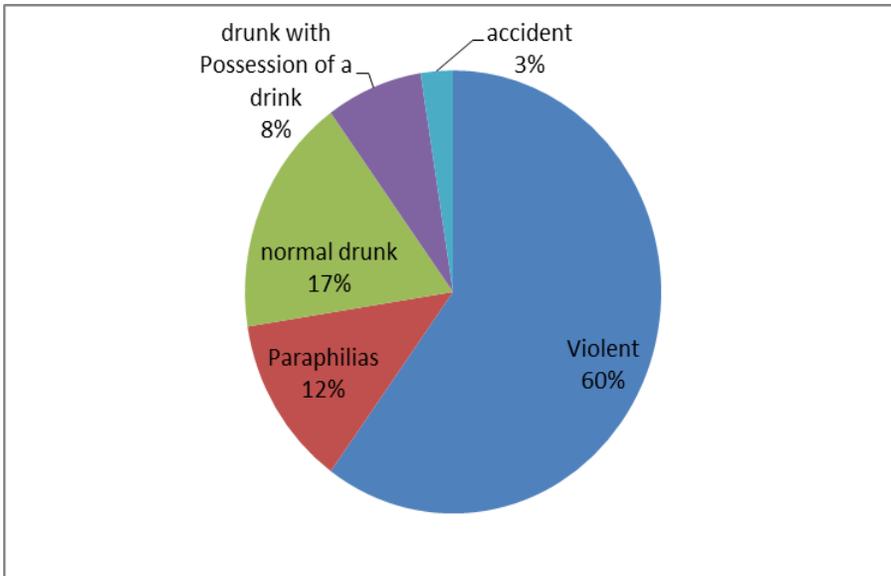


Figure (4-4) distribution of drunks according to criminal behaviors

According to DSM V, criminal behavior, aggression, impulsivity, and irresponsibility are common in individuals diagnosed with ASPD (Holzer, and Vaughn, 2017). Therefore, it is an expected situation that most of the prisoners in prisons will meet the criteria for ASPD. Many studies, which examine the distribution of ASPD according to genders, show that the frequency of this disorder and exhibiting aggressive behavior is lower in women compared to men (Hodgins,2020).

4.1.5: The distribution of drunks according to duration of abuse

Figure (4-5) show that the majority of duration percent was 65% to category more than 5 years and 30% between 5-10 years and the lesser was less than 10 year was 5% .

Result and Discussion

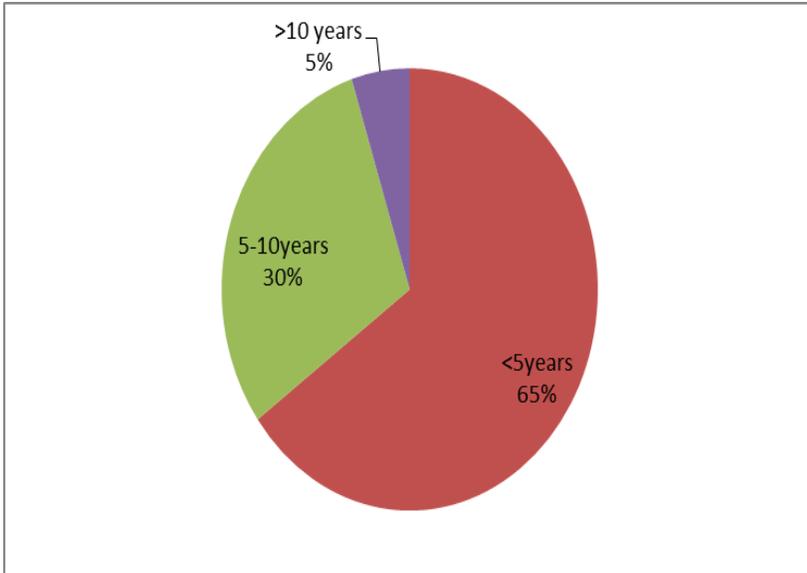


Figure (4-5) distribution of drunks according to duration of abuse

Alcohol is one the most widely used and abused drugs in the world and the number of annual alcohol-attributed deaths exceeds 3 million (WHO,2014). Drugs of abuse, including alcohol, increase dopamine release in the striatum (WHO,2018).

The World Health Organization has estimated that as of 2016, there were 380 million people with alcoholism worldwide (5.1% of the population over 15 years of age) (WHO,2018). As of 2015 in the United States, about 17 million (7%) of adults and 0.7 million (2.8%) of those age 12 to 17 years of age are affected (Cherpitel,2007; Littrell ,2014). Alcoholism is most common among males and young adults. Geographically, it is least common in Africa (1.1% of the population) and has the highest rates in Eastern Europe (11%),The International ISBN Agency(WHO,2018). Alcoholism directly resulted in 139,000 deaths in 2013, up from 112,000 deaths in 1990(Littrell ,2014) . A total of 3.3 million deaths (5.9% of all deaths) are believed to be due to alcohol(WHO,2018). Alcoholism reduces a person's life expectancy by approximately ten years (Schuckit MA (2014).

4.2 Serotonin Level

The results of statistical analysis showed significant differences between serotonin concentration between drinker and control, p. value (**0.0101**) as shown in Figure (4-6).

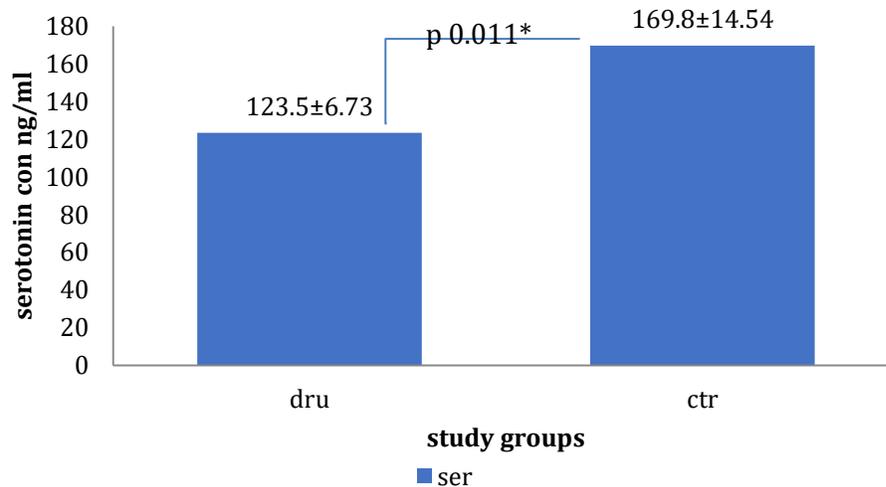


Figure (4-6) the serotonin concentrations differences in study groups (independent t test at $p < 0.05$).

Serotonin is an important brain chemical that acts as a neurotransmitter to communicate information among nerve cells. Serotonin's actions have been linked to alcohol's effects on the brain and to alcohol abuse, Since the seminal studies by Asberg and collaborators in the mid-1970s ,most studies have shown that impulsive aggression and suicidal behavior are correlated with low levels of 5-HT transmission, this association is stronger for more severe phenotypes: lethal and/or violent suicidal behavior and violent aggressive behavior ,The magnitude and duration of serotonergic activity is believed to be regulated mainly by the human serotonin transporter (5-HTT), which controls the uptake of serotonin from the synaptic junction (Åsberg *et al.*,1976; Sher and Mann,2003).

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Pharmacological studies have also provided evidence that the serotonin transporter is involved in modulating aggressive behavior, as shown by the consistent response of patients to 5-HT reuptake inhibitors. Several recent controlled trials have reported that specific serotonin reuptake inhibitors (SSRI) are effective at reducing the recurrence of suicide attempts and decreasing impulsive aggression traits in subjects with and without personality disorders these main effects were independent of an antidepressant effect (Coccaro and Kavoussi, 1997; New *et al.*,2004).

The human 5-HTT is encoded by a single gene (SLC6A4), located on 17q12.67. A biallelic functional polymorphism in the 5' regulatory promoter region (termed 5-HTTLPR), involving two alleles, corresponding to a 44-bp insertion (L-allele) or deletion (S-allele), regulates transcription of the 5-HTT gene, the S promoter is less active than the L promoter, resulting in lower levels of 5-HT uptake. The shorter allele has been found to be associated with affective disorders and with anxiety-related personality traits, such as harm avoidance and neuroticism (Bondy *et al.*,2000; Courtet *et al.*,2001).

Serotonin plays an important role in mediating alcohol's effects on the brain. Alcohol exposure alters several aspects of serotonergic signal transmission in the brain. For example, alcohol modulates the serotonin levels in the synapses and modifies the activities of specific serotonin receptor proteins. Abnormal serotonin levels within synapses may contribute to the development of alcohol abuse, because some studies have found that the levels of chemical markers representing serotonin levels in the brain are reduced in alcoholic humans and chronically alcohol-consuming animals. Moreover, SSRI's and receptor antagonists can reduce alcohol consumption in humans and animals, although these agents are only moderately effective in treating alcohol abuse. Serotonin is not the only

Result and Discussion

neurotransmitter whose actions are affected by alcohol, however, and many of alcohol's effects on the brain probably arise from changes in the interactions between serotonin and other important neurotransmitters (George *et al.*,2002).

Alcohol interferes with the function of serotonin receptors. When activated by serotonin binding, the 5-HT₃ receptor rapidly increases neuron activity by generating electrical signals, Acute alcohol exposure enhances the electrical signals generated by the 5-HT₃ receptor. This change in receptor function results from alcohol's direct action on the receptor protein or on molecules closely associated with the receptor in the cell membrane, increased 5-HT₃ receptor function probably causes excessive stimulation of neurons in brain regions receiving information from serotonergic neurons (A Kenna, 2010).

The serotonin and alcohol levels in drinks according to age categories .

The serotonin concentration of study groups according to age categories show that the first categories less than 30 years were 151.31 ± 23.07107 ng/ml and 89.50 ± 12.32253 ng/ml more than 30 years were .There was no significant difference between age , serotonin and alcohol concentration at heavy drinker table (4-2).

Variables	Ser ng/ml	Alcohol mg/cm³
Age categories		
<30	151.31 ± 23.07107	69.04 ± 8.38610
>30	89.50 ± 12.32253	80.66 ± 10.25396
Sig	0.33*	0.381
Independent t test , p<0,05		

Table (4-2) the serotonin and alcohol levels in drinks according to age categories .

Result and Discussion

Drugs of abuse, including alcohol, increase serotonin release in the striatum (Di Chiara and Imperato, 1988). Alcohol use among adolescents is heterogeneous, ranging from low, normative use to heavy, pathological use. Alcohol is the most frequently used substance, as it is generally the easiest for adolescents to access (WHO, 2018). The average age of initiation for alcohol use among US and Australian adolescents is 15 years (Richmond et al., 2017; Aiken et al., 2018). Across Europe, most adolescents begin drinking alcohol between ages 12 and 16, with 25% of adolescents in this region first consuming alcohol by age 13 (WHO, 2002–2014, 2018). The worldwide estimate of adolescents (age 15–19) who drank alcohol in the past month is 27%, ranging from 1 to 44% across countries. Higher rates of past month adolescent drinking occur in higher income countries; the highest rates are observed in the European region (44%), and the lowest rates are observed in the Eastern Mediterranean region (1.2%; 33, 37). Past month alcohol use among adolescents in other countries ranges from 38% in the Americas and Western Pacific regions, to 21% in Africa and Southeast Asia, and 14% in Japan (Morioka et al., 2013; WHO, 2018).

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The serotonin and alcohol levels in drunks according to BMI categories .

The serotonin concentration of study groups according to BMI categories show that the first categories less than 25 were 137.62 ± 23.95497 ng/ml and 112.85 ± 32.50160 ng/ml more than 30 were non significant table (4-3).

Variables	serng/ml	Alcohol mg/cm ³
BMI		
<25	137.62±23.95497	82.50±12.89477
25-29.9	114.58±22.91160	69.2941±8.24120
>30	112.85±32.50160	67.57±11.755
Sig	0.304	0.596
ANOVA one way p<0.05		

The most impressive findings have shown that the reinforcing effects of frequently abused drugs are associated with large and rapid increases in serotonin (Volkow et al.,2003). Pirola and Lieber (1972) reported on the hypermetabolic effect of alcohol more than thirty years ago, however this applies only to higher consumption rates (Pirola and Lieber ,1972, 1976; Hodgkins et al.,2004). In their study, Pirola and Lieber found that when a group of alcoholics had fifty percent of carbohydrate calories replaced with ethanol, a small but significant decrease in body weight was noted (Pirola and Lieber ,1972).

The serotonin and alcohol levels in drunks according to Home categories .

The serotonin concentration according to home the drinking peoples **133.142±33.007** ng/ml in rural and **121.45±16.389** ng/ml in urban as show in table was non-significant table (4-4).

Variables	Ser ng/ml	Alcohol mg/cm3
Home		
Rural	133.142±33.007	57.00±10.54017
Urban	121.45±16.389	77.939±7.462
Sig	0.765	0.226
Independent t test , p<0,05		

Table (4-4) the serotonin and alcohol levels in drunks according to Home categories .

A number of social and cultural factors predict increased alcohol use, including discrimination and its related stigma. The role of discrimination and stress in health-related risk behaviors, including alcohol use, is well established (Dawson et al. 2005; Hatzenbuehler 2009).

The serotonin and alcohol levels in drunks according to duration of abuse categories .

The serotonin concentration according to duration of abuse show the drinking peoples that less than 5years ware **142.88±20.42429** ng/ml and more than

Result and Discussion

10 years were 72.0000 ± 2.00000 ng/ml was significant rather than alcohol level non-significant as shown in table (4-5).

Variables	Ser ng/ml	Alcohol mg/cm³
Duration		
<5	142.88±20.42429	65.07±7.20596
5-10 years	90.0833±15.21983	86.9167±12.09461
>10	72.0000±2.00000	118.00±50.0
Sig	0.189	0.093
ANOVA one way p<0.05		

Table (4-5) the serotonin and alcohol levels in drunks according to duration of abuse categories .

The serotonin and alcohol levels in drunks according to criminal behaviors categories .

The serotonin and alcohol concentration according to criminal behaviors categories showed. non-significant as shown in table (4-6)

Criminal behavior	Ser ng/ml	Alcohol mg/cm³
Violent	93.708±16.29167	76.2917±8.83
Paraphilias	128.20±30.87459	67.0000±10.52
normal drunk	161.14±33.05942	74.8571±18.66
drunk with Possession of a drink	288.00±17.92577	48.6667±1.33
Accident	58.00±0	135.0000±0
Sig	0.002	0.240
ANOVA one way p<0.05		

Table (4-6) the serotonin and alcohol levels in drunks according to criminal behaviors categories.

4.2.6: Correlation between serotonin levels and alcohol levels.

When study the correlation between serotonin levels and alcohol levels, non-significant invers correlation fig (4-12)

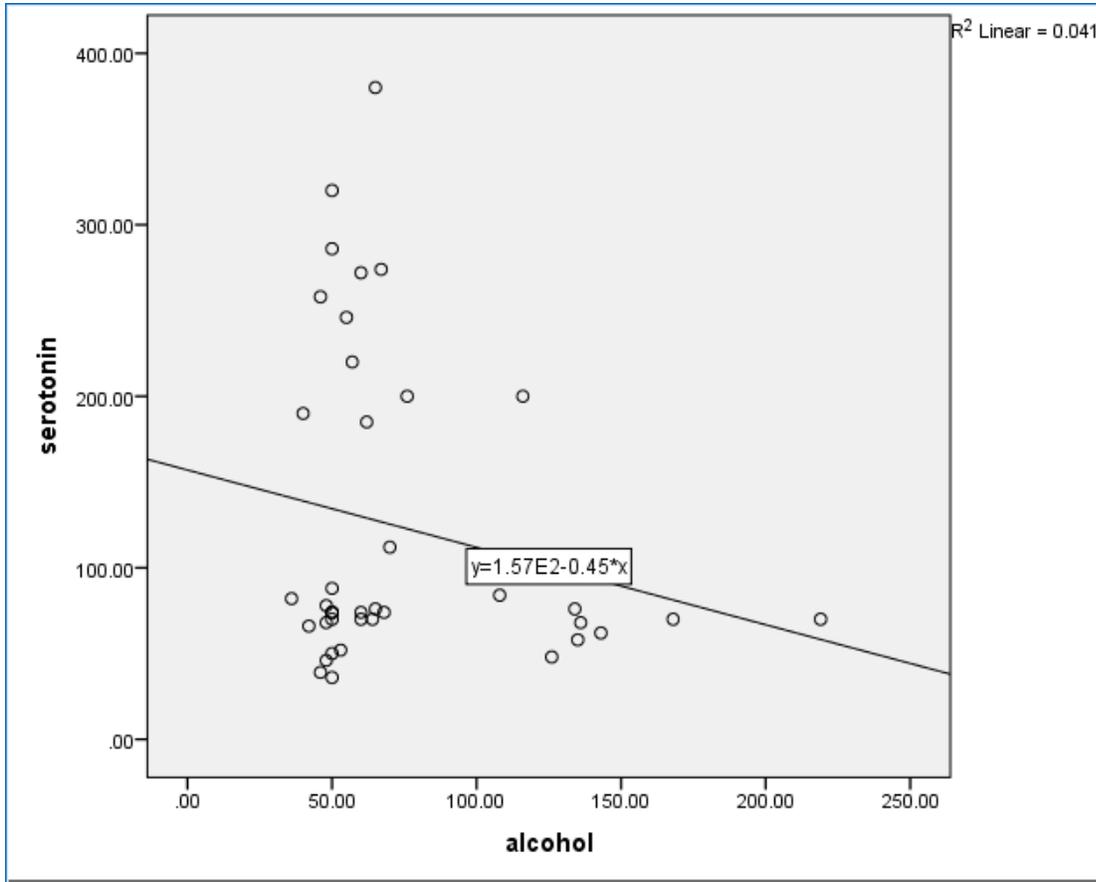


Figure (4-7) correlation between serotonin levels and alcohol levels, non-significant invers correlation (r 0.202, P 0.212).

4.3 Genetic study

4.3.1 DNA extraction

DNA was extracted from whole blood of Drinker and control, its concentration was (50-150 ng) and purity was (1.8-2.1), Figure (4-7) showed the electrophoresis pattern of DNA extract.

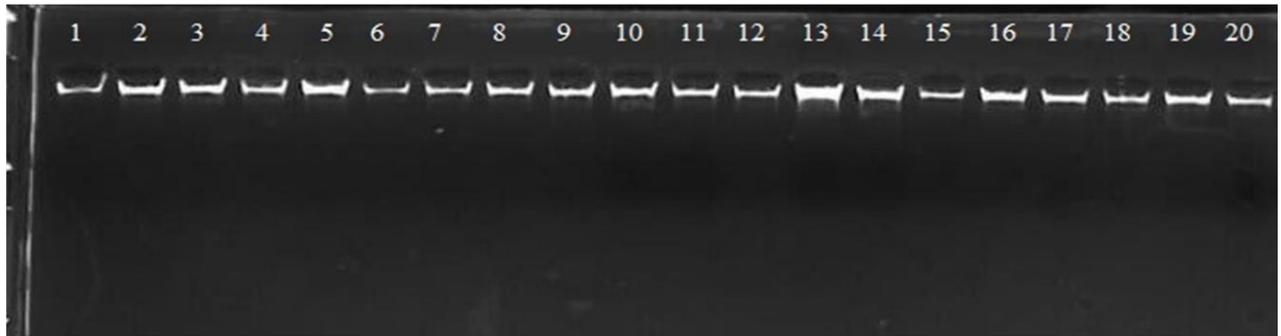


Figure (4-7): Electrophoreses pattern of DNA extracted from whole blood of drinker and control ,1% Agarose ,75 v, 20 mA for 1 hour (10µl in each well). lane 1-10 DNA of drinker, lane 11-20 DNA of control.

4.3.2 Amplification of serotonin transport gene (SLC6A4)

The PCR amplification of the SLC6A4 polymorphic region was performed using the DNA obtained from individuals constituting the case and control groups. PCR products were visualized on 1% agarose gel and separated according to their length to detect polymorphism of the SLC6A4. Depending on the size of the amplicon obtained, individuals were marked as having L (572 bp) or S (528 bp) genotype.

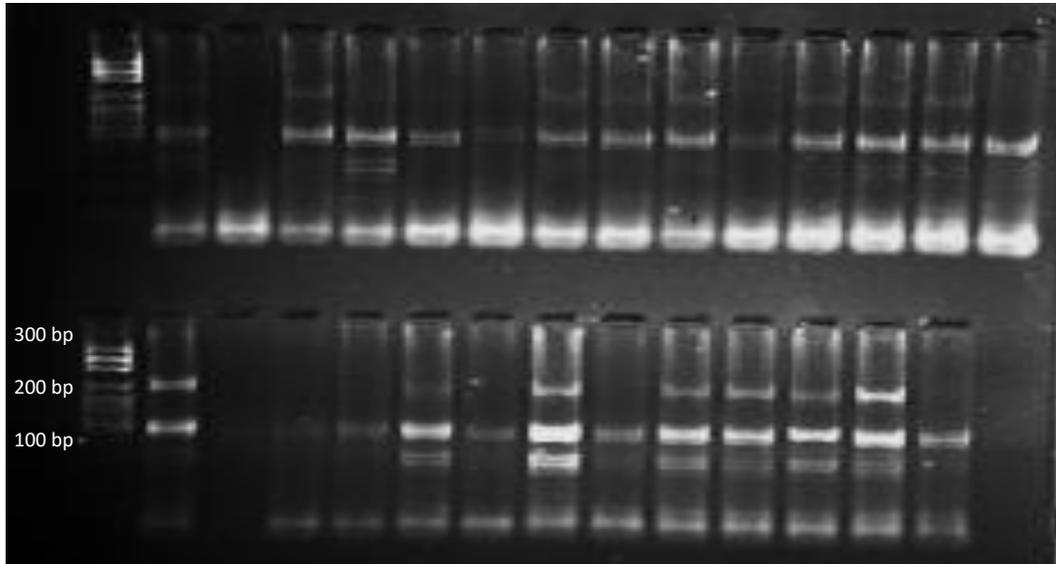


Figure (4-8) Electrophoreses pattern of PCR product serotonin transporter (SLC6A4) in study group, 1% agarose, 20 mA, 70 v. for 40 min.

The human serotonin transporter gene (SLC6A4) is considered the most researched genetic variant in psychiatry, psychology, and neurobiology, the most common polymorphism in the promoter region (5-HTTLPR) of the SLC6A4 gene leads to the generation of two different variants. These S and L variants of the 5-HTTLPR region alter the transcriptional activity of the SLC6A4 promoter at different stages (Hensler, 2010).

The result of current study show there was four genotyping of (SLC6A4) gene polymorphemic (Deletion, LL, SS, and LS), the more frequent genotyping in drinker was LL (21) while in control was (18) and genotyping SS also more frequent in drinker (18) while in control was (6) there is no significant difference in study groups.

Table (4-1) genotyping of serotonin transporter (SLC6A4) in drunks and control group

Genotyping	Dru	Ctr	Odd ratio (CI%)	Sig
LL (513 bp)	21	18	0.3889 0.1271 to 1.1896	0.097
SS (469bp)	18	6	2.8462 0.0511 to 158.6260	0.610
LS (513/469 bp)	0	0		

The result of our study agreement with study conducted by Sah *et al.*,2021 that show Both basal and stimulated in vitro transcription of the L variant of the SLC6A4 gene were found to be higher than the S allele. Consistent with this result, both basal and stimulated serotonin uptake in the lymphoblast cell lines and platelets of homozygous individuals for the L allele is two times higher than the cells carrying the S allele in one or two copies (Iofrida *et al.*,2014). Thus, there are differences in serotonin uptake in the brain between individuals depending on variations in SLC6A4 mRNA expression levels and SERT protein amounts (Murphy *et al.*,2004). Various studies show that the S allele is associated with increased psychological sensitivity to stress. This finding poses an increased risk for many mental health related conditions, including depression, anxiety, suicide, and stress-related substance abuse (Maillet and Rajah ,2014).

Several studies show that the SLC6A4 gene 5-HTTLPR polymorphisms may have a relation with personality disorders. However, the research on the effect of these polymorphisms on the ASPD is limited. A study that examined the relationship between antisocial personality disorder and SLC6A4 polymorphisms

Result and dissection

in young adults with low income reported young adults with S allele were more sensitive to antisocial personality disorder (Lyons-Ruth *et al.*,2007).

In another study, convicts with S/S or S/L genotypes in terms of 5-HTTLPR polymorphism have been found to have a higher risk of having, ASPD symptoms (Garcia *et al.*,2010). When the relationship between the short allele causing low expression and type 2 alcoholism (early-onset alcoholism accompanied by ASPD) has been studied in alcoholics, the L , A. allele thought to provide high expression was found at lower levels among type 2 alcoholics (Wu *et al.*,2008).

Table (4-2) genotyping of serotonin transporter (SLC6A4) effect in the serotonin level of drunks and control group

Genotyping	Dru	Ctr
LL (513 bp)	122.76±20.56	158.11±6.82407
SS (469bp)	126.44±22.23	207.66±13.23799
LS (513/469)	0	0
Sig	0.735	0.271
ANOVA one way p<0.05		

Our study shows elevated in serotonin level with the genotyping (SS) Mean +SD 126.44±22.23, while decrease in hormone level with the genotyping Deletion 72.00±4.00 while no hormone level with genotyping LS in both drinker and control.

In a study conducted with Chinese male individuals, the S allele carriers in those who committed excessive violence were found to be at a higher frequency than the control group. However, no statistically significant relationship was found

Result and dissection

between the 5-HTTLPR polymorphisms and the ASPD in the criminal group (Lee *et al.*,2003). On the other hand, in a study conducted by Japanese researchers, no evidence was found to support the relationship with the S/S genotype in individuals who exhibit antisocial behavior and experience severe alcoholism problems (Wang *et al.*,2016).

As can be seen from the literature, there are differences in the relation between the ASPD and 5-HTTLPR genotypes in different societies. According to the results of our study, there is no significant difference between the case and control groups in terms of the distribution of 5-HTTLPR polymorphisms. Therefore, it has not been possible to conclude that a particular 5-HTTLPR genotype is common in individuals diagnosed with ASPD with a criminal history.

Conclusion

and

Recommendation

Conclusion and Recommendation.....

Conclusion:

From present study we can concluded that :

1. The serotonin level was lower in alcoholic drinkers compared to the control.
2. The percentage of alcoholic drinkers in the city is higher than in the countryside.
3. The duration of alcohol consumption effect of serotonin concentration for alcoholism and criminal behaviors.

Recommendation

1. Measurements of other hormones that associated with serotonin or alcohol.
2. Study other genes that associated with alcohol abuse .
3. Used other technologies like RT-PCR.
4. Conducting a study that includes drug users and making a comparison between the types.

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الخلاصة

أجريت الدراسة على (40) فرد من مدمني الكحول و 30 عينة كمجموعة سيطرة ، واستمرت الدراسة ثلاث أشهر للفترة من 2020/5/23 - 2021/7/22 وأجريت هذه الدراسة في جامعة بابل - كلية العلوم - قسم علوم الحياة - مختبر الحامض النووي ، تهدف هذه الدراسة إلى تقدير مستوى السيروتونين والكشف عن تعدد الأشكال لجين نقل السيروتونين وتأثيره على السلوك الإجرامي ، وأجريت الدراسة الفسيولوجية بتقدير مستوى هرمون السيروتونين في كل من مدمني الكحول والسيطرة والمقارنة بينهم ، تم تطبيق تفاعل البلمرة المتسلسل لتضخيم جين نقل السيروتونين واكتشاف تعدد الاشكال الجيني في هذا الجين والمرتبط بتركيز السيروتونين.

أظهرت نتيجة الدراسة الحالية أن المدمنين كانوا أعلى في الفئة العمرية (أقل من 30) سنة (55%) بينما 45% في الفئة العمرية (أكثر من 30) ، وصنفت مجموعة الدراسة والمجموعة السيطرة المسجلة في الدراسة الحالية إلى ثلاث مجموعات حسب مؤشر كتلة الجسم إلى الوزن الزائد 25-29 ، الوزن الطبيعي والسمنة ، ، تبين أن غالبية المتعاطين كانوا يعانون من زيادة الوزن بين (30-40) كجم / م 2 (42.5%) بينما (40%) منهم طبيعيون و (17.7%) بدناء ، في حين أن غالبية مجموعة السيطرة كانوا من الوزن الزائد بين (30-40) كجم بنسبة (3.33%) ، (33.3%) بدناء و (63.33%) كانوا طبيعيين.

كان توزيع المرضى المقربين حسب المنطقة (82.5%) من المناطق الحضرية بينما كان (17.5%) من المناطق الريفية بينما كانت غالبية السيطرة (53%) من المناطق الحضرية و (46.6%) من المناطق الريفية، وكانت غالبية المرضى من المناطق الريفية. كانت النسبة المئوية للمدة 65% للفئة أكثر من 5 سنوات و30% بين 5-10 سنوات وأقل من 10 سنوات كانت 5%، وأظهرت نتائج التحليل الإحصائي لمستوى السيروتونين فروق معنوية بين تركيز السيروتونين بين المتعاطين والسيطرة.



جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل- كلية العلوم
قسم علوم الحياة

تأثير تعدد الأشكال الجيني لناقل السيروتونين في السلوك الإجرامي الكحولي

بحث مقدم الى كلية العلوم /جامعة بابل
وهو جزء من متطلبات نيل درجة الدبلوم العالي في علوم الادلة الجنائية
من قبل

سحر احمد مهدي كاظم

(بكالوريوس علوم حياة-2012-جامعة الكوفة)

اشراف

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