Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Babylon

College Science

Department of Biology

Association between IL-2,GPT,GOT,Criatinin and blood urea in alcoholic addicted as forensic markers

A Research

Submitted to the College of Science/University of Babylonin
Partial of the Requirements for the
Degree of Higher Diploma in Science/ Forensic Evidence
By

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(2013)

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2021 AD **1443AH**

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

يَا أَيُّهَا الَّذِينَ آمَنُوا إِنَّمَا الْخَمْرُ وَالْمَيْسِرُ وَالْأَنْصَابُ وَالْأَزْلَامُ
رِجْسٌ مِنْ عَمَلِ الشَّيْطَانِ فَاجْتَنِبُوهُ لَعَلَّكُمْ تُفْلِحُونَ

صَدَقَ اللهُ العَلِيِ العَظِـيمِ

سورة المائدة الآية 90

Certification

I certify that this research was done under my supervision at university ofBabylon as a partial requirement for the Degree of

Higher Diploma in Forensic Evidences.

Dr. Frial Gemeel Abd.

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University of BabylonDate: / /2021

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

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Committee Certification

We, the examining committee, certify that we have read the thesis entitled (**Association between IL-2,GOT,GPT,Creatinin and blood urea in alcohol addicted as forensic marker**) and examined the student (Walaa Abedul Ameer Yaseen Abdullah) in its contents and that in our opinion it is accepted as a thesis for a degree of high diploma in forensic evidences with Excellent Degree.

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Date: / /2021

Dedication

To whom Allah sent as mercy to the

worlds……prophetMohammed and his good family

and the goodness

To my dear father.
You are the father and the teacher generous in guiding usthe right direction in the paths of life taught us the love of God and obedience him and the love of science

To my dear mother.
who was our chest, affectionate and safe, at the time ofthe crisis, we ask God to prolong her life and grant her health

To my dear wife.

(Halaa) my partner in life and affectionate heart you

are the source of happiness at home .

Walaa Abed Al-ameer Yaseen

Acknowledgements

After the help of "Allah" for me in completing this study first of all I would like to thank and gratefulto his almighty of all creatures for entire me blessing during the pursuit of my research.

I thanks to the deanship of Science College, Babylon University for giving me the opportunity to get the requirements of this study .

My deepest thanks to my supervisor Dr. Frial Gemeel Abd for her constant advice,support, knowledge and experience throughout the duration of this work. She was the best help along the research with constant encouragement.

I would like to thank all those who helped me get samples and thank the members of the Public Health Laboratory in Najaf ALashraf.

To pure blood and pure souls to the martyrs and men who defended the homeland and the sanctities of the homeland.

Abstract

Alcohol addiction is one problem in forensic ,because it increased accidental cars and crimes the present study was aimed to detect the effect of alcohol on IL2 , liver and kidney function . The experiment were conducted to study was don for 40 individuals included a group of prisoners in the Najaf police prison (Najaf governorate) who were alcohol users for the period from Jun to August 2021 .This study included 40 user , 20 participants of them not alcohol addicted apparently , the group was selected as a control group .The age of participants in the control group ranged between (22-65) years .

Alcohol screening by the Breath Alcohol Detector ,then the blood was collected from patients and control groups , the separated serum was distributed to Eppendrof tube and then stored in a deep freeze used to determine IL-2 concentrations (estimation by ELISA)
,and liver enzymes (GOT,GPT) was determined .The results appeared high concentrations of IL-2 in user(708.875pg/ml±SD) compared control groups while non alcoholic user was (308.250pg/ml±SD) with high significantly (0.001),while liver enzymes found GOT was significantly higher(36.275±11793MS±SD)when compared to control ($p<0.000$) and GPT was significantly higher(44.625±16.589M±SD) when compared to control ($p<0.023$), The results showed was no significantly for serum criatinin when compared to control ($p> 0.0456$) and b.urea was significantly higher (34.400±5.400 M±SD) when compared to control ($p<0.025$)

It concluded from current study the consumption of alcohol induced inflammatory cytokineand increased inflammation on liver and kidney.

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Lit of acronyms

ADH	Alcohol dehydrogenease
ALD	Acute liver disese
AUD	Alcohol use disorder
B.UREA	Blood urea
CKD	Chronic kidney disese
Cr	Criatinin
DAMP	Damage-associated molocular pattern
ELIZA	Enzyme linked immune sorbent
GOT	Glutamic oxaloacetic- transamenase
GPT	Glutamic pyruvic-transaminase
KCs	Kuffper cells
PAMP	Pathogen-associated molocular pattern
ROS	Reactive oxygen species
S.Criatinin	Serum criatinin

Chapter One

Introduction

Introduction

Alcohol use disorder (AUD) confers a prodigious burden of disease, disability, Alcoholism is a chronic, relapsing brain disorder that is characterized by compulsion to seek alcohol, loss of control in limiting alcohol intake, and experiencing a negative emotional state during withdrawal (Koob, 2014).

With 3.3 million attributable deaths each year globally, alcohol is responsible for approximately 10 times the mortality rate for all illicit drugs combined, as well as 5.1% of the total global burden of disease (Kelly *et al*.;2020) .

Addiction to drugs or alcohol can be defined as a chronic, relapsing disorder that has been characterized by (*i*) a compulsion to seek and take drugs, (*ii*) the loss of control over drug intake, and (*iii*) the emergence of a negative emotional state (e.g., dysphoria, anxiety, and irritability) that defines a motivational withdrawal syndrome when access to the drug is prevented .Addiction has been conceptualized as a three-stagecycle—binge/intoxication,withdrawal/negativeaffe,and preoccupation/anticipation—that worsens over time and involves allostatic changes in the brain reward and stress systems that lead to compulsive alcohol taking and seeking. Two primary sources of reinforcement, positive and negative reinforcement, have been hypothesized to play a role in this allostatic process . Positive reinforcement is defined as the process by which the presentation of a stimulus increases the probability of a response. Negative reinforcement

is defined as the process by which the removal of an aversive stimulus increases the probability of a response (Koob, 2013,Cui *et al*.,2015).

Excessive alcohol consumption is a global healthcare problem. The liver sustains the greatest degree of tissue injury by heavy drinking because it is the primary site of ethanol metabolism. Chronic and excessive alcohol consumption produces a wide spectrum of hepatic lesions, the most characteristic of which are steatosis, hepatitis, and fibrosis/cirrhosis(Osna *et al*.,2017).

Other studies have shown that about 20–36% of patients with chronic kidney disease consume alcohol either occasionally or daily, and 10% of patients even drink heavily .Some studies reported that alcohol consumption was associated with the development or progression of chronic Kidney disease CKD (Lee *et al*.,2021).

Alcohol is a known modulator of the immune system affecting innate aswell as adaptive arms of the host immune response. Excessive and chronicheavy drinking, as typified in alcohol use disorder(Neupane *et al*.,2016).

The aim of study

This study aimed to detriment interleukin -2 ,liver and some of kidney function .to achieves this following steps:

1. Detect alcohol addicted groups by breathing test ,urine rabid test and blood collected from alcoholism user and control (non-alcoholic drinking).
2. Estimation the IL-2 concetration in serum
3. Estimate some Liver and kidney function serum Got(glutamic oxaloacetic transaminase, Gpt (glutamic pyruvic transaminase) and blood urea, s-cratinin.

Chapter Two

Literature Review

2-1 :Alcohol

Alcohol consumption has been a part of socio-cultural practices worldwide. According to the World Health Organization report in 2016, about 43% of the world's population over 15 years old reported drinking in the past 12 months(World Health Orgaization, 2018)) According to the Korean National Health and Nutrition Examination Survey (2013), the drinking rate of men and women was 75.3% and 45.7%, respectively. Alcohol consumption has various effects on health. Although its obvious adverse health effects include liver cirrhosis, cancers, seizure, pancreatitis, poisoning, etc., previous studies have reported that light to moderate alcohol consumption has some beneficial effects such as reduction in the risk of cardiovascular disease and type 2 diabetes(Baliunas *et al*.,2009 ,Lee *et al*.,2021)

2-2:Effect of alcohol on liver

Beverage alcohol is chiefly metabolized in the main parenchymal cells of the liver , hepatocytes, that make up about 70 percent of the liver mass. ADH is the most catalytically efficient ethanol-metabolizing enzyme. It reaches its half-maximal velocity when circulating ethanol levels are about 5 to 10 milligrams per deciliter, well below levels that cause intoxication. ADH-catalyzed ethanol oxidation uses nicotinamide adenine dinucleotide (NAD^+) as a cofactor, generating reduced NAD^+ (NADH) and acet aldehyde. The latter compound is highly reactive and toxic(Brooks and Zakhari 2014).

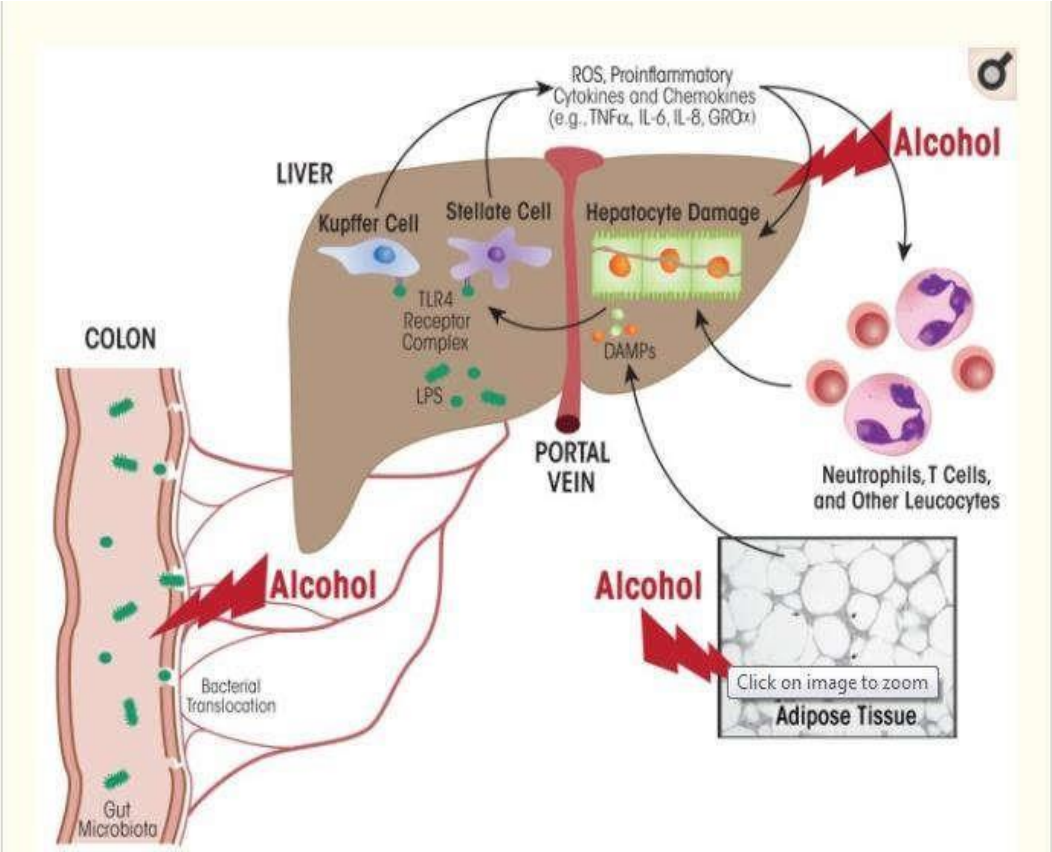
Alcoholic hepatitis occurs in about 30 to 40 percent of individuals reporting chronic alcohol abuse. It represents the most serious form of acute liver disease (ALD) and is associated with high short-term mortality. Central to the progression of alcoholic hepatitis are resident and infiltrating immune cells called macrophages, which have important roles in inducing liver inflammation. The resident macrophages in the liver, represent up to 15 percent of liver cells and 50 percent of all macrophages in the body. They reside in the liver sinusoids and provide the first line of defense, serving as potent innate immune cells. In contrast, infiltrating macrophages are recruited as immature cells from the bone marrow, and their differentiation into macrophages in the liver only occurs during inflammation(Lefkowitch, 2005).

The ability of macrophages to regulate inflammation depends on their polarization. their ability to develop into one of two different functional states, namely M1 (i.e., proinflammatory) or M2 (i.e., anti- inflammatory) macrophages. The polarization to either phenotype depends on the microenvironment, including circulating growth factors, cytokines, and pathogen-associated molecular pattern (PAMP) as well as damage-associated molecular pattern (DAMP) molecules. Because the liver is exposed to countless antigens, pathogens, and toxic substances that come from the intestine via the portal circulation, it must be protected from developing an immune response to such exposure. As a result, Kuffer cells (KCs) usually have tolerogenic properties proinflammatory M1 phenotype. Usually, ALD progression from liver steatosis to

inflammationrequires a second insult in addition to the alcohol exposure, such as another toxic insult, nutritional factor, or viral infection (Tsukamoto etal.2009).

These effects are related to the stage and severity of the alcoholic hepatitis; in severe cases, KCs differentiate to the proinflammatory M1 phenotype, whereas in mild forms, KCS switch to the anti-inflammatory M2 phenotype. As inducers of inflammation, KCs release multiple prion flammatory cytokines, including TNFa, interleukins, and chemokines that attract inflammatory cells from circulation. KCs also are an abundant source of reactive oxidation species (ROS) that exacerbate oxidative stress in the liver.

The activation of KCS to produce proinflammatory cytokines and promote free-radical formation via induction of the reduced nicotinamide adeninedinucleotide phosphate (NADPH) oxidase and CYP2E1. The resulting reactive oxygen and nitrogen species promote the release of proin flammatory cytokines, which in turn increase inflamma some. Inflamma somes are innate immune system sensors that regulate the activation of caspase-1 and induce inflammation in response to microbial/ viral pathogens, molecules derived from host proteins, and toxic insults (e.g., alcohol exposure) as shown in figure 1.



Figure(2-1) gut–liver axis. A major factor in the initiation of the inflammatory response by resident macrophages of the liver (i.e., Kupffer cells) is endotoxin or lipopolysaccharide (LPS), a cell- wall component of Gram-negative bacteria that translocates from the gut lumen into the portal circulation to reach the liver. Enhanced circulating endotoxin levels in alcoholic hepatitis are caused by alcohol-induced qualitative and quantitative changes in the bacteria that inhabit the gut (i.e., gut microbiota) and increased gut leakiness. In the liver, LPS activates Kupffer cells and hepatic stellate cells by interacting with toll-like receptor 4 (TLR4). These cells produce reactive oxygen species (ROS) as well as proinflammatory cytokines and chemokines that together with alcohol contribute to hepatocyte damage. Other factors contributing to hepatocyte damage include alcohol-induced activation of various immune cells (i.e., neutrophils, T cells, and other leukocytes) as well as alcohol's effects on the fat (i.e., adipose) tissue, which results in the production of damage-associated molecular pattern (DAMP) molecules (Osna *et al.*,2017)

:

2-3 Alcohol and Kidney

Kidney function, assessed by measurement of the glomerular filtration rate declines by about 8 mL/min/1.73 m^2 per decade after age 40 years. The decline in kidney function may be accelerated due to various factors such as hypertension, diabetes, primary renal disorders, and some medications causing kidney injury. It is a noteworthy problem that patients with impaired kidney function also consume alcohol. Previous studies have shown that about 20–36% of patients with chronic kidney disease (CKD) consume alcohol either occasionally or daily, and 10% of patients even drink heavily(Bundy *et al*.,2018).

Notwithstanding, the association between alcohol consumption and kidney function has received relatively less attention and studies have been inconclusive. Some studies reported that alcohol consumption was associated with the development or progression of CKD. In other studies, however, alcohol consumption was not associated with kidney function; rather, it was inversely associated with the risk of CKD. There are several potential mechanisms to explain the inverse association between alcohol consumption and decline in kidney function (White *et al*.,2009).

First of all, polyphenolic compounds in alcoholic bevek,rages exhibit antioxidant and anti-inflammatory properties, both of which may have renal protective effects. Among patients with type 2 diabetes, resveratrol, a polyphenolic compound, was found to reduce the levels of serum criatinin, urea nitrogen, and total cholesterol, suggesting improved kidney function and lipid profile .

Moreover, even polyphenol-free alcoholic beverages have been found to exert anti-inflammatory or antioxidant effects, even though they were

less effective than those with high amounts of polyphenol. Second, alcohol consumption is associated with an increase in insulin sensitivity. Given that insulin resistance and concomitant hyper insulinemia are associated with renal dysfunction in the general population the improvement of insulin sensitivity due to alcohol consumption may have a beneficial effect on kidney function. In conclusion, more alcohol consumption was associated with lesser decline in kidney function over 12 years among the general population in Korea, especially men. Although we found a favorable effect of alcohol consumption on kidney function, our results should not be used to encourage or justify excessive alcohol consumption for kidney health because of many other known health or social problems associated with drinking, especially excessive drinking.(estruch,*et al* 2011)

However, there is a lack of grounds that alcohol consumption should be discouraged just for kidney health. Considering both the beneficial and detrimental effects of alcohol consumption, additional studies are needed to determine the appropriate amount of alcohol consumption. Moreover, the effects of alcohol consumption among patients with moderate to severe renal impairment need to be studied further(Lee *et al*., 2021).

2-3 :Immunity against alcohol

There are long observed an association between excessive alcohol consumption and adverse immune-related health effects such as susceptibility to pneumonia., this association has been expanded to a greater likelihood of acute respiratory stress syndromes ,sepsis, alcoholic liver disease, and certain cancers; a higher incidence of postoperative complications; and slower and less complete recovery from infection andphysical trauma, including poor wound healing The gastrointestinal system is typically the first point of contact for alcohol as it passes throughthe body and is where alcohol is absorbed into the bloodstream(Sarkar *et al.*,2015). One of the most significant immediate effects of alcohol is that it affects the structure and integrity of the gastric intestinal tract. alcohol alters the numbers and relative abundances of microbes in the gut microbiome, an extensive community of microorganisms in the intestine that aid in normal gut function. These organisms affect the maturation and function of the immune system. Alcohol disrupts communication between these organisms and the intestinal immune system. Alcohol consumption also damages epithelial cells, T cells, and neutrophils in the GI system, disrupting gut barrier function and facilitating leakage of microbes into thecirculation(Engen *et al.*,2015).

Alcohol's specific effects on the innate immune system depend on the pattern of alcohol exposure, with acute alcohol inhibiting and chronic alcohol accelerating inflammatory responses. In addition to promoting proinflammatory immune responses, alcohol also impairs anti-inflammatory cytokines. Chronic alcohol exposure also interferes with thenormal functioning of all aspects of the adaptive immune response, including both cell-mediated and humoral responses.

All of these effects enhance the susceptibility of chronic alcoholics to viral and bacterial infections and to sterile inflammation. alcohol may affect immune functions by altering the balance and interactions between the host immune system and the entirety of microorganisms found in the host. This microbiome is composed of the normal microorganisms found in and on the body which are needed for the body's normal functioning, and disease-causing pathogens. Increasing evidence suggests that alcohol may modulate the composition of pathogenic and commensal organisms in themicrobiome of the gut, oral cavity, skin, and other mucosal surfaces (Chenand Schnabl 2014) .

2-4:Effect alcohol on cytokine

Cytokines are a class of multifunctional proteins that are implicated incellular communication and activation. Cytokines are critical to the development and functioning of both innate and adaptive immune response, and not just limited to the immune system, but also involved in developmental processes. The cytokines could be of type Th1 (proinflammatory) or Th2 (antiinflammatory) depending upon their role in the immune system. Cytokines impact tissues in a complex manner that regulates inflammation, cell death, cell proliferation, cell migration, and healing mechanisms. Alcohol is known to alter cytokine levels in a varietyof tissues including plasma, lung, liver, brain (Crews *et al*., 2006).

Alcohol is a known modulator of the immune system affecting innate as well as adaptive arms of the host immune response.

Excessive and chronic heavy drinking, as typified in alcohol use disorder ,it induce systemic and central nerves system inflammation .One widely proposed mechanism for innate immune response in chronic heavy alcohol consumption involves alcohol-induced changes in the composition of gut microbiome and compromised gut wall integrity , allowing bacterial products such as lipopolysaccharid) to "leak" into systemic circulation which promotes secretion of proinflammatory cytokines including tumor necrosis factor- alpha and interleukin- (IL-) 1β through Tolllike receptor mediated activation of transcription factors, such as nuclear factor-hB. (Leclercq *et al*.,2012) .

High dose alcohol exposure can induce neuroimmune signaling evenafter a single alcohol binge and immune stimuli such as LPS may not be necessary for inducing these changes. A study demonstrated increased hippocampal IL-10 content in adult rats one hour after a single intoxicating intragastric dose of ethanol (5 g/kg). In mice pretreated with ethanol, Qin and colleagues found that LPS-induced production of TNF-α, IL-1β, and monocyte chemoattractant protein 1 (MCP-1, also known as

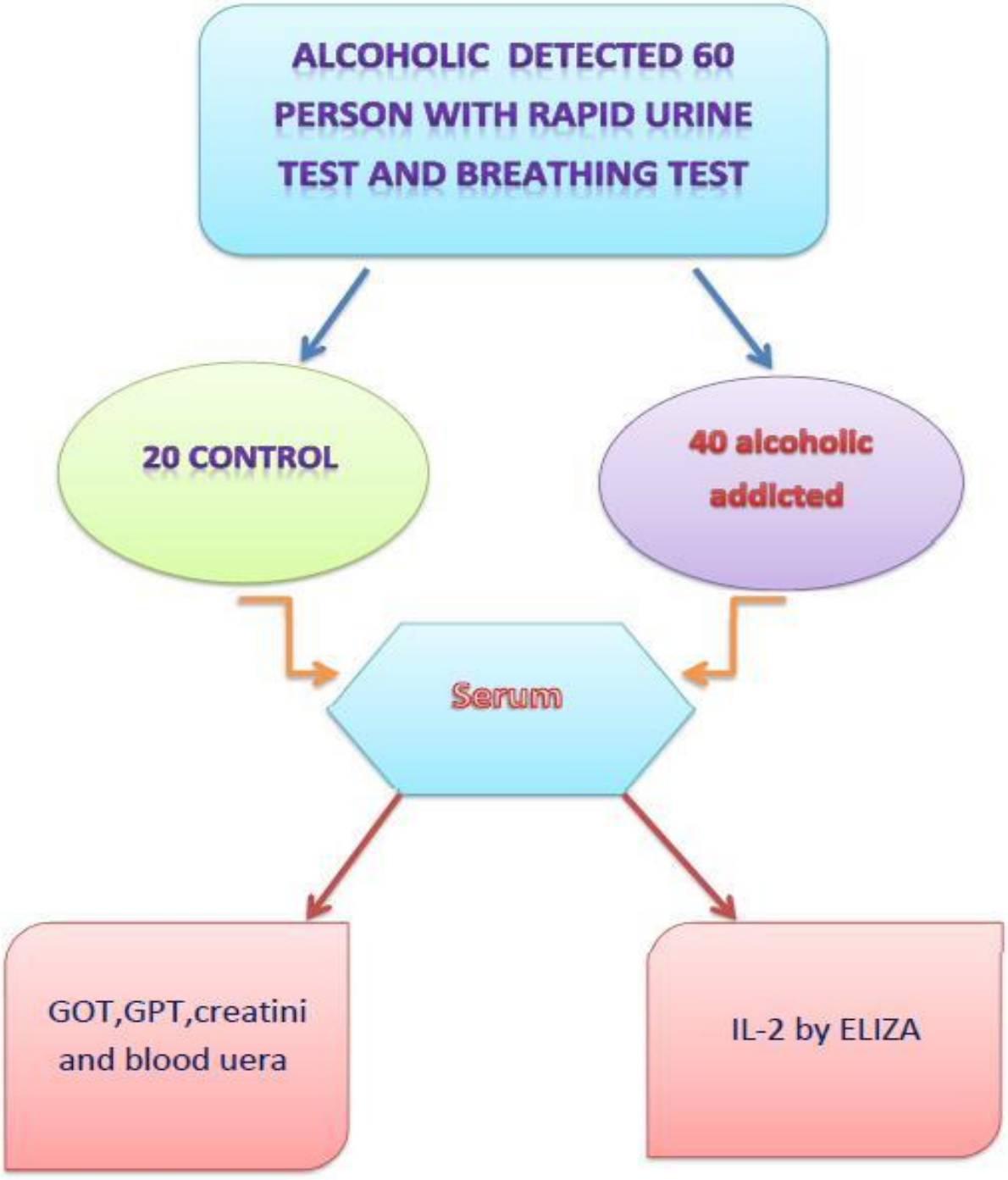
CCL2) were elevated in the liver, serum, and brain (Qin *et al*.,2008). Increases in serum cytokine levels subsided by 9 hours with clearance ofblood alcohol content. Importantly, the same group discovered that a single immune stimulus was sufficient to activate brain microglia to produce chronically elevated inflammatory factors in rodent models (Qin*et al*.,2007).

These lines of evidence suggest that occasional ethanol intoxication can have far-reaching consequences through neuroimmune modulation. However, the nature of those consequences is unclear because of the paucity of experimental alcohol studies in humans. Generally, acute alcohol exposure favors anti-inflammatory response and chronic alcohol consumption favors proinflammatory cytokine release. For instance, healthy men and women 20 minutes after binge alcohol consumption were found to have elevated blood leukocytes, monocytes, natural killer cells, and LPS-induced TNF-α production which switched towards anti- inflammatory direction after 2 hours. The dynamic of the immune response, thus, seems to be more complex and depends on the dose as well as time duration since alcohol intake. (Neupane *et al*.,2016).

Low levels of ethanol are commonly consumed as part of normal daily behavior. It is commonly accepted that moderate amounts of polyphenol rich beverages such as beer or wine may have beneficial health effects including on the immune system. On the other hand, high doses of alcohol consumption can directly suppress a wide range of immune responses, and alcohol abuse is associated with an increased incidence of a numberof infectious diseases (Romeo *et al.*, 2007).

Chapter Three

Materials & Methods


ALCOHOLIC DETECTED 60 PERSON WITH RAPID URINE TEST AND BREATHING TEST
20 CONTROL
40 alcoholic addicted
Serum
GOT,GPT,creatini and blood uera
IL-2 by ELIZA

3.Materials and Methods.

3.1 Materials

3.1.1 Chemicals and devices.

Chemicals and devices utilized in the current study are illustrated in Table (3-1).

Table (3-1) chemicals and devices of study

Items	Manufacturer
Alcohol 70%	greet Med/ China
Cool box	international Haotian Technology/ China
Centerfuge	ALS DM04125
Disposable syringe 5 ml	changzhou Tongda Medical Appliance/ China
Eppendorf tube	shang Yu Yite Plastic /China
Gel tubes	shanghai Orsia Medical Technology /Chine
Gloves	bio Basic /China
Graduate cylinder	zhejiang, China
Medical cotton	kardelen / Turkey
Micropipettes different size	huawei and dragon med- Germany
Mindray biochemistry analyzer Bs-120	mindray limted combany / Korea
Refrigerator SR32EMB	cool tech /Korea

3.1.1 Kits.

The kits were used in this study are listed in table(3-2) .

table (3-2) study kits.

Kits	**Company /Country**
Alcohol screening test kite	Ballymena/UK
Interlokin 2 eliza kit 96 test	Bioassay technology laboratory Korea
Midray biochemistry kits	Korea
Rapid test panel(Urine) kit	Hangzhou /China

3.2 Methods

Ethical considerations

The approvals were obtained from all the participants (patients and healthy) and also agreed to study scientifically and morally by the prisoners in the Najaf police prison (Najaf governorate).

3.2.1 Patients and Control Groups

This study was done on a group of prisonerse Najaf police prison(Najaf governorate) who were alcohol users for the period from Junto August 2021. This study included 40 patients 20 participants of them not alcohol apparently the group was selected as a the age of participant in control and alcoholsim group range between (22-65) years.

Laboratory tests were conducted for detecting the effect of alcohol on immunity in Public Health Laboratory at AL Najaf city.

3.2.2 Blood Samples

Five ml of ventus blood were collected from each person Samples were placed in tubes containing a gel. The blood was

separated by centrifuge at 4500 rpm for 5 minutes and the separated serum was distributed to Eppendorf tube and then stored in a deep freeze at -30c until used

3.3 Urine Samples

Five to ten milliliters urine were collected from each person.

samples a placed in disposable cups and distributed in rapid test.

3.4 Alcohol Screening

The Breath Alcohol Detector is for rapid detection of the presence of alcohol in the exhaled breath and provides. relative Blood Alcohol Concentration (BAC) at 0.05%, 0.03% under the

UK legal limit for driving. If correctly used it provides a reliable method and clear warning to help in the prevention of drunk driving.
It is not intended to be used as the only source of information when takinga decision to drive or do dangerous tasks, as if the test is not used correctlyit can give erroneous results. The limitations below should be studied carefully before use.

- Easy to use
- Individually packaged
- Results in 2 minutes
- Rabid test

Gloutamin-oxaloacetic transaminase and Gloutamic-pyuvric transaminase measurement Method

- Serum was taken (after blood centrifuged with 4500 rpm).
- The serum was put in clean can tube to transfer it to instrument. Be sure of found clean and enough number of cuviat.
- Selected the position in auto sampler pleat .
- Put the sample .
- Selected the position in auto sampler pleat .
- Put the sample .
- Programed the instrument dependently of position of sample andselect the type of test(GOT,GPT) and press run .

After 15 mints the result would be get .(According to the company direction)

Principle of Gloutamin-oxaloacetic transaminase (GOT) Test

(MDH-Malate dehydrogenase. EC1.1.1.37) In the assay reaction, the AST catalyzes the reversible transamination of L-aspartate and a-oxoglutarateto oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase with NADH being oxidized to NAD. The rate of the photometrically determined NADH decrease is directly proportional to the rate of formation of oxaloacetateand thus the AST activity.(According to the company direction).

Principle of Gloutamin-pyurvic transaminase (GPT) Test

Alanine aminotransferase catalyzes the reversible transamination of L- alanine and a-oxoglutarate to pyruvate and L-glutamate. The pyruvate isthen reduced to lactate in the presence of lactate dehydrogenase (LDH).

with the concurrent oxidation of reduced ß-nicotinamide adenine dinucleotide (NADH) to B-nicotinamide adenine dinucleotide (NAD). Thischange in absorbance is directly proportional to the activity of ALT in the sample.(According to the company direction),

Blood urea ,Serum creatinine Method

- Taken 200µg/dl serum after centrifuge patient blood sample.
- The serum was put in clean can tube to transfer it to instrument.
- Selected the position in auto sampler pleat .
- Put the sample .
- Programed the instrument dependently of position of sample and select the type of test(B.uera, S.creatinine) and press run .

After 15 mints the result would l be get .

Principle of createnin Test

$2H_2O2$ + 4-aminoantipyrine + ESPMT : Quinonimine + $4H_2O$

The absorbency increase at 546 nm of the product Quinonimine is directlyproportional to the concentration of creatinine.

Principle of urea test

UREA + $2H_2O$: $2NH_4$ + CO_3

a-Oxoglutarate +NH4 + NADH : L-Glutamate + NAD+ + H_2O

Urea is hydrolyzed by urease, and one of the products, ammonia, helps to turn NADH to NAD* with the catalysis of GLDH. The absorbency decreaseis directly proportional to the concentration of urea.

Interleukin-2concentrations

Assay Procedure

1. All reagent were preperd solutions and samples as instructed. Bring all reagents temperature before use. The assay is performed. room temperature.

2. The number of strips were determined required for the assay. Insert the stripsin the frames for use. The unused strips should be stored at 2-8°C.

3.The stander was added 50µl to standard well.

4. The sample 40µl added to sample wells and then add 10µl anti-IL-2 antibodyto sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Covered the plate with a sealer. Incubate 60 minutes at 37°C.

5. Removed the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for eachwashed. For automated washed, aspirated all wells and washed 5 times with washed buffer, overfilling wells with wash buffer. Blot the plate onto papertowels or other absorbent material.

6.50µl from substrate solution A was added to each well and then added 50µl substrate solution B to each well. Incubated plate covered with a new sealer for 10 minutes at 37°C in the dark.

7. 50µl from Stop Solution was added to each well, the blue color will change into yellow immediately.

8. Determined the optical density (OD value) of each well immediately used a microplate reader seted to 450 nm within 10 minuets after added the stop solution.

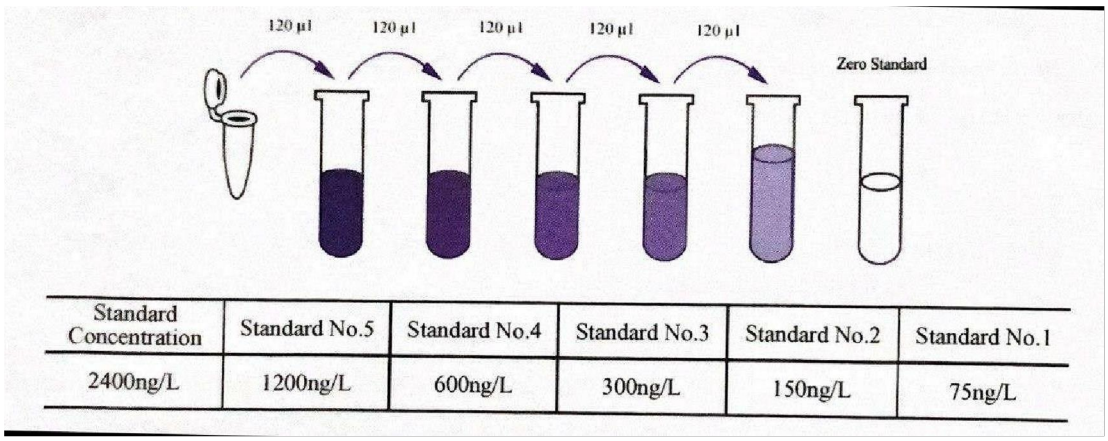
Reagent Preparation

■ All reagents have been reduced to room temperature before use.

■Standard Reconstitute the 120µl of the standard (2400ng/L.) with 120µl of standard diluent to generate a 1200ng/L standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (1200ng/L) 1:2 with standard diluent to produce 600ng/L, 300ng/L, 150ng/L and 75ng/L solutions. Standard diluent serves as the zero standard(0 ng/L). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions according the table 3-3 and figar 3-1.

Table3-3 Standerd Solution suggested by combany

1200µg/dl	Standard No 5	120µl Original Standard + 120µl Standard Diluent
600 µg/dl	Standard No 4	120ul Standard No.5+ 120ul Standard Diluent
300µg/dl	Standard No 3	120µl Standard No.4 + 120µl Standard Diluent
150 µg/dl	Standard No 2	120µl Standard No.3 + 120µl Standard Diluent
75µg/dl	Standard No 1	120µl Standard No.2 + 120µl Standard Diluent



Figar 3-1 standared Preparinng for IL-2

▪Wash Buffer Dilute 20ml of Wash Buffer Concentrate 25x into deionizedor distilled water to yield 500 ml of Ix Wash Buffer. If crystals have formedin the concentrate, mix gently until the crystals completely dissolved.

Consitration of IL2	M±SD	P value(sig)
patient	708.875 ± 632.072	0.001**
Control	308.250± 115.503	

Chapter Four
Results
and
Discussion

4-1 :- Interleukin -2 cytokine detection

Interlukin-2 cytokine were estimated by using Enzyme Linked Immunosorbent assay (ELISA)

The result of this test were calculated by using standard curve fit equation.Mean of IL-2 concentration in serum of patient was (708.875pg/ml M±SD) while control was (308.250pg/ml M±SD)with high significantly 0.001. shown in Table (4-1).

Table (4-1)Concentration of IL-2 between alcoholism persons and control in serum

Consitration of IL2	M±SD	P value(sig)
patient	708.875 ± 632.072	0.001**
Control	308.250± 115.503	

M= mean
SD= stander difftion
**** = Siginificant**

The results were differ from other study found alcohol decreased in experimental study(Braun *et al* .,1995) . Also another study found Alcohol inhibited IL-2–induced CC chemokine (CCL3 and CCL4) expression by NK cells. Functional tests demonstrated that this reduced expression of CCchemokines was associated with diminished anti-HIV ability of NK cells. Alcohol also reduced the ability of NK cells to response to CCL3-mediated chemotaxis this in cell line (Guo *et al.* 2005). Alcohol inhibited IL-2– induced NF-*κ*B p65 protein expression and calcium mobilization by NK cell.

IL2 is the lymphocytotrophic 15.5 kDa glycoprotein in IL-2 is located in the plasma membrane and expressed in normal tissues,the endothelial cells, and the intestinal epithelium; this unique interleukindemonstrates an active role in the growth sprouting and differentiation of the T and B cellular groups and non-lymphoid cells and promotes the cytolytic properties of the natural cells (NK cells). Biochemically, the IL-2 receptor complex (IL-2R) encompasses three subunits (α, β, γ) deciphered by different and unrelated genes. It is synthesized by CD4+ T helper (Th) lymphocytes (Nelson *et al ,1998*)

4-2 Liver function test GOT and GPT

The results showed GOT (36.275pg/ml M±SD) was significantly higher when compared to control (p<0.000) and GPT was significantly higher (44.625pg/ml M±SD) when comparedto control (p<0.023) as in table (4-2)

Table (4-2) GPT and GOT persons

Parameters	Patient M±SD	Control M±SD	P value (sig)
GPT	44.625±16.589	32.687±7.964	0.000***
GOT	36.275±11.793	33.812±9.152	0.023*

Long term alcohol consumption can cause alcoholic liver disease it leads to developed alcoholic hepatitis and cirrhosis, most of the liver damage caused by alcohol is attributed to alcohol metabolism and by products of that metabolism, Liver injury may be caused by direct toxicity of alcohol by products and also by inflammation that is induced secondarily by these same compounds, one half of heavy drinkers develop alcoholic hepatitis

or cirrhosis ,the study of .Abdullah in 2012 found high concentrations ofliver enzymes in alcoholism patients .

4-2-1 :-Correlation between IL2 and liver function test

Correlation between IL-2 and GPT was positive correlation and p value was significant at p<0.05 Show in table(4-3) and figure (4-1) and.

Table (4-3) Correlation between IL2 and GPT

Parameters	M±SD	P_value	Correlation (r)
IL2	708.874±632.072	0.000***	0.587**
GPT	44.625±16.589		

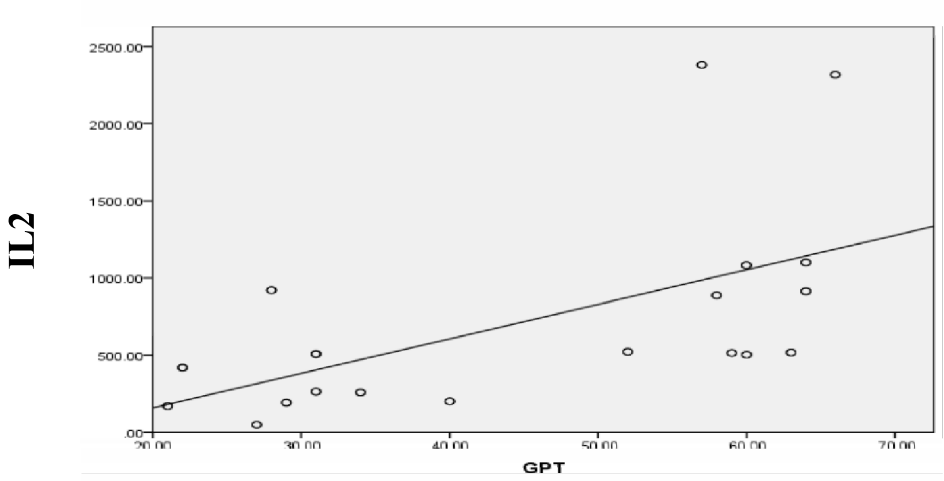


Figure (4-1) correlation between IL2 and GPT

while the correlation between IL-2 and GOT was positive correlation and p value was significant at p<0.05 Show in table (4-4) and figure (4-2) .

Table (4-4) Correlation between IL2 and GOT

Parameters	M±SD	P_value	Correlation (r)
IL2	708.875±632.672	0.000***	0.645**
GOT	36.275±11.793		

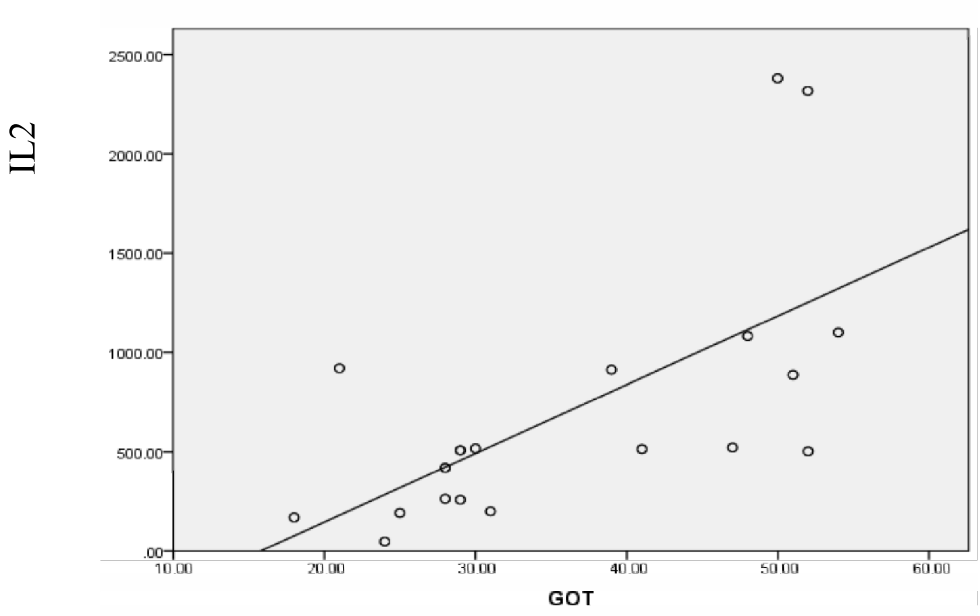


Figure (4-2) correlation between IL2 and

GOT4-3:- Kidney function test S.ceratnin and b.urea

The study includes 40 participated patient aged 20-65 years

Results higher significantly showed of s.cratinin (0.850pg/ml M±SD) when compared to control (p>0).

(0.456) and b.urea was significantly higher (34.400pg/ml M±SD) when compared to control (p<0.025) show in table (4-5) Both acute and chronic alcohol consumption can compromise kidney function, particularly in conjunction with established liver disease. Epestein expressed in 1997 that investigators have observed alcohol-related changes in the structure and function of thekidneys and impairment in their ability to regulate the volume and composition of fluid and electrolytes in the body. Chronic alcoholicpatients may experience low blood concentrations of key electrolytes as

well as potentially severe alterations in the body's acid-base balance. In addition, alcohol can disrupt the hormonal control mechanisms that govern kidney function. By promoting liver disease, chronic drinking has further detrimental effects on the kidneys, including impaired sodium and fluid handling and even acute kidney failure (Epestein ,1997).

Table (4-5) Comparison of serum cratinin and blood urea in addected and control

Parameters	Patient	Control	P value (sig)
	M±SD	M±SD	
Serum ceratnin	0.850 ±0.173	0.768±0.162	0.456
blood urea	34.400±5.400	23.625±7.649	0.025*

4-3-1:-Correlation between IL2 and serum cratinin test

Correlation between IL-2 and serum ceratnin was positive correlation andp value was significant at $p<0.05$ as a table (4-6) and figure (4-3) Burton and Pallett in (2021) explained the relation between il-2 and liver found the individuals presenting with decompensated cirrhosis have increased serum concentrations of soluble-CD25 (a surrogate marker associated with IL-2-induced immune activation), rendering $CD4^+$ T cellsin these patients more sensitive to IL-2 signalling. In further support of IL-2 imprinting, cT_{FH} in decompensated individuals express less TCF1 – a transcription factor upstream of Bcl-6 that is inhibited by IL-2

Table (4-6) correlation between IL2 and serum.ceratnin

Parameters	M±SD	P_value	Correlation (r)
IL2	708.875± 632.072	0.000***	0.623**
s.ceratnin	0.850±0.173		

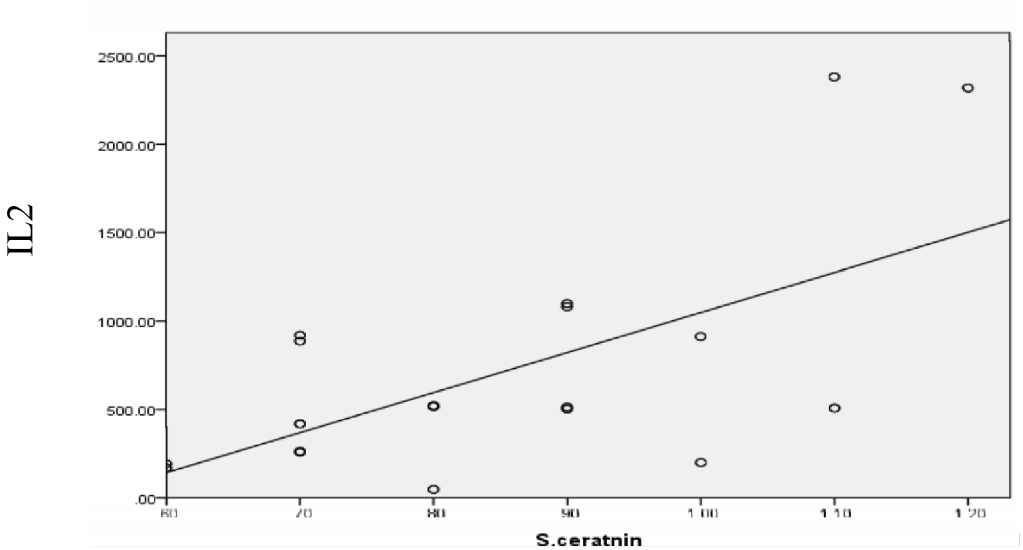


Figure (4-2) Correlation between IL-2 and serum ceratnin

So the correlation between IL-2 and blood urea was positive correlation and p value was significant at $p<0.05$ as table (4-7) and figure (4-4).

Table(4-7) Correlation between IL2 and blood urea

Parameters	M±SD	P_value	Correlation (r)
IL2	708.875± 623.072	0.000***	0.536**
blood urea	34.400±5.400		

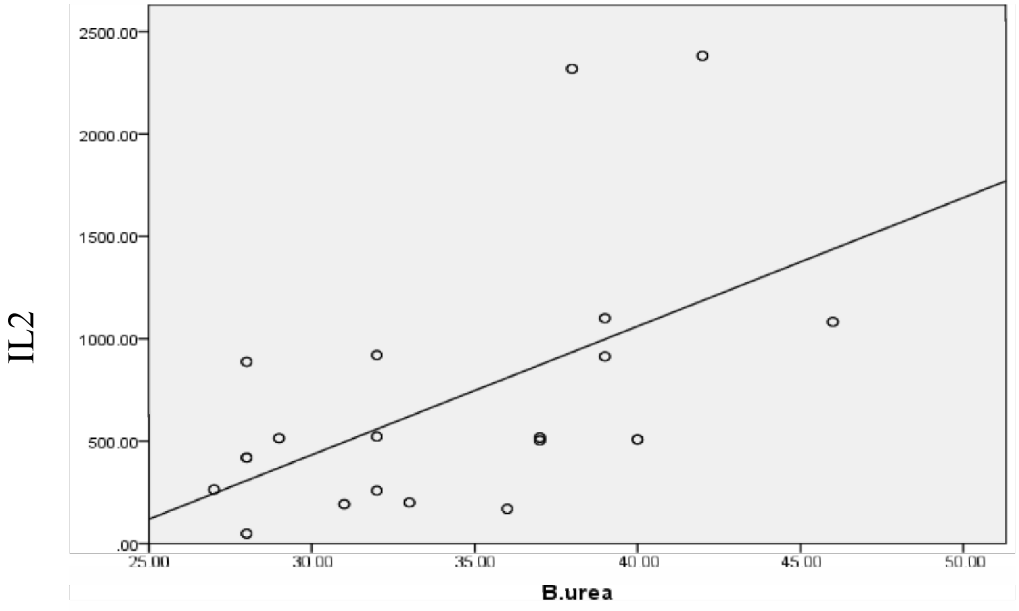


Figure (4-4) Correlation between IL2 and blood urea

When divided the patients to three age groups table(4-8) the results were appeared concentration of IL-2 , S.ceratnin and B. urea were no significantly different among groups while there were significantly different in GPT and GOT . While in control appears the result the S.ceratnin, B.urea,GOT and GPT there were significantly but the IL2 show that no significantly between age group shoe in table (4-9).

Table (4-8) Effect of age in patients

Age / Parameters	M±SD			P value (sig)
	20-30 years	31-40 years	41-65 years	
IL 2	158.33 ± 94.28	812.38±568.21	790.80±814.33	0.065
s.ceratnin	0.700±0.09	0.88±0.14	0.88±0.24	0.069
b.urea	32.00± 3.58	34.83±5.99	39.30±16.04	0.280
GPT	23.67±4.93	49.96±14.17	42.200±19.48	0.002**
GOT	23.67± 4.93	40.88±10.98	32.800±10.27	0.002**

Table (4-9) effect of age in control for IL2, GOT,GPT, B.urea and S.ceratnin

Age / Parameters	M±SD			P value(sig)
	20-30 years	31-40 years	41-65 years	
IL 2	246.50 ± 52.64	262.67± 45.61	350.89±136.84	0.256
S.ceratnin	0.60±0.00	0.73±0.06	0.86±0.16	0.016*
B.urea	23.50±1.73	32.67±4.62	36.67±6.58	0.006**
GPT	23.50±3.00	28.33±0.58	38.22±5.63	0.000***
GOT	20.25± 1.50	34.00±1.73	39.78±4.84	0.000***

Conclusions

1-Alcohol consumption induced inflammatory cytokine interleukin 2.

2- Alcohol effect on liver by increasing concentrations of its enzymes (GOT , GPT)
.

3- Alcohol increased the kidney enzymes (serum cratinin , blood urea).

4- there were positive correlation between interleukin and liver (GOT , GPT) and kidney enzymes (serum cratinin , blood urea).

Recommendations

Study the polymorphism of interleukin 2 in alcoholism patient

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الخلاصة

يعد الادمان على الكحول احد المشاكل في الطب الشرعي ، وذلك بسبب زيادة في حوادث السيارات والجرائم ، لذلك هدفت الدراسة الحالية إلى دراسة تأثير متعاطي الكحول على الحركيات الخلوية والكبد والكلى. اشتملت الدراسة على مجموعة من الموقوفين لدى شرطة النجف وتجمعات للمتعاطين وحلات في طوارئ مستشفى الحكيم في النجف الاشرف من متعاطي الكحول للفترة من حزيران إلى اب 2021 ، وشملت هذه الدراسة 40 مريضاً منهم 20 مشاركاً ليسوا مدمنين على الكحول على ما يبدو ، وقد تم اختيار المجموعة كمدمنين. المجموعة السيطرة تراوحت أعمار المشاركين في المجموعة السيطرة بين (22-65) سنة.

فحص الكحول بواسطة كاشف الكحول في التنفس وكذلك عن طريق الفحص السريع للإدرار , ثم تم جمع الدم من المرضى ومجموعات السيطرة ,وتم توزيع المصل المفصول على انبوب ابندروف ثم تخزينه في تجميد عميق يستخدم لتحديد تركيزات الحركي الخلوي 2 ويكون التقدير بواسطة جهاز الاليزا , وانزيمات الكبد تم تحديد (الالانين ,الاسبارتات) وضهرت النتائج بتركيزات عالية من الحركي الخلوي 2 في مرضى مقارنة بمجموعات السيطرة (708.875) بيكو غرام/ مل بينما كانت مجموعة السيطرة (308.250) بيكو غرام/مل مع ارتفاع معنوي (0.001) بينما وجدت انزيمات الكبد(Got) اعلى بكثير عند المقارنة بالسيطرة ($p<0.000$) وكان (Gpt) اعلى بشكل ملحوظ عند مقارنة مجموعة السيطرة ($p<0.023$) وأظهرت النتائج عدم وجود معنوية عند فحص الكرياتنين عند مقارنتها بمجموعة السيطرة ($p>0.0456$) وكذلك نسبة اليوريا في الدم تعطي علامة معنوية عالية في حال مقارنتها مع السيطرة ($p< 0.025$)

يستنتج من الدراسة الحالية ان استهلاك الكحول بصورة مستمرة يسبب زيادة الحركيات الخلوية IL2 ويزيد التهاب الكبد والكلى.

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

الأرتباط بين IL2 , GPT,GOT ,الكرياتنين و يوريا الدم في مدمني الكحول كأدلة جنائية

بحث

مقدم الى كلية العلوم / جامعة بابل

وهو جزء من متطلبات نيل درجة الدبلوم العالي في العلوم / الادلة الجنائية

من قبل

ولاء عبد الامير ياسين عبد الله

بكالوريوس/علوم كيمياء

(2013)

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1443 هـ 2021م