الخلاصة

يعتبر ادمان الكحول احد مشاكل الطب الشرعي ، وذلك بسبب زيادة حوادث السيارات والجرائم ، لذالك هدفت الدراسة الحالية الى استخدام الانترلوكين 10 الحركي الخلوي والكلوبيولين المناعي IgA كمؤشر جنائي على إدمان الكحول واستخدام المصول العدلية للكشف عن مدمني الكحول وما يرتبط به من معايير مناعية. وشملت الدراسة 25 فردا من مدمني الكحول من مناطق مختلفة من محافظة النجف الأشرف ومن بعض السجناء في سجن شرطة النجف من متعاطي الكحول للفترة من شهر حزيران الى شهر آب (2021) وشملت هذه الدراسة ايضا 20 آخرين غير مدمنيين على الكحول كمجموعة سيطرة . وقد تم اختيار المجموعة الضابطة كمدمنيين تراوحت أعمارهم بين (20- 65) سنة.

اختبرت عينات الإدرار للكشف عن متعاطي الكحول من المدمنيين ونوعها باستخدام Pannal rabid kite ثم جرى تقدير تراكيز IgA , Interleukine 10 في مصول كلا المجموعتين بواسطة تقنية الاحتزاز المناعي المرتبط بالانزيم ELISA اظهرت النتائج وجود تراكيز عالية من IL-10 الحركي الخلوي في المدمنيين مقارنة بمجموعة السيطرة اذ بلغ 363.57 بيكوغرام/مل والتحكم 229.62 بيكوغرام/مل على التوالي مع ارتفاع معنوي (0.0001) وكذلك بينت النتائج ارتفاع تركيز الكلوبيولين المناعي (IgA) في المدمنيين مقارنة بمجموعة السيطرة اذ بلغ 448.00 ملغرام/ دستليتر والتحكم 181.88 ملغرام/ دستليتر على التوالي مع وجود ارتفاع معنوي (0.0001).

ونستنتج من هذه الدراسة ان استهلاك الكحول يسبب التهابات وارتفاع مستويات Interleukine 10 الحركي الخلوي والكلوبيولين المناعي المصلي (IgA).

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل كلية العلوم

قسم علوم الحياة

العلاقة بين IL-10 و IgA المصلي مع متعاطي الكحول

بحث

مقدم الى كلية العلوم /جامعة بابل

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

من قبل

مجيب حسن كريم عيسى

بكالوريوس علوم الحياة

جامعة الكوفة /2013

اشراف

أ.م.د شيماء جاسم محيميد السلطاني

١٤٤٣هـ **٢٠٢١م**

Summary:

Alcoholism is considered a problem in forensic medicine, due to the increase in car accidents and crimes, so the current study aimed to use interleukin 10 and IgA as a forensic indicator in alcoholism and the use of forensic serology to detect alcoholics and its associated immunological parameters. The study included a group of alcohol addicts from different regions of Najaf Governorate and some prisoners in the Najaf Police Prison who abused alcohol for the period from June to August 2021, and this study included 25 of them alcoholics and 20 others who are non-alcoholic . The control group was selected as addicts whose ages ranged between (20-65) years.

Urinary samples were tested to detect alcohol abusers of addicts and their type using the Pannal rabid kite, then serum tests were conducted on to estimate the level of IgA and Interleukine-10 by using ELISA technique . The results appeared that high concentrations of IL-10 in addicts compared to the control groups which reched to 363.57 pg/ml, and (229.62 pg/ml) respectively with a significant increase 0.0001, also the levels of immunoglobulin IgA was higher in the addicts compared to the control groups which reched 448.00 mg/deciliter and 181.88 mg / deciliter respectively with a significant increase (0.0001).

The study concluded that alcohol consumption causes inflammation, and levels of Interleukine-10 and serum Immunoglobulin IgA.

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Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Babylon
College of Science
Department of Biology

Association of Serum Interleukin 10 and IgA with Abuse Substance Addicted

A Research

**Submitted to the Council of College of Science/University of Babylon
in Partial Fulfillment of the Requirements for the
Degree of Higher Diploma in Science/ Forensic Evidences
By**

Mujeep Hassan Kareem Eissa

B.Sc. Biology / University of Kufa (2013)

Supervised by

Asst.Prof.Dr.Shaimaa Jassim Mohemeed AL Sultany

2021 A.D **1443A.H**

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

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

صَدَقَ اللهُ العَلِيِ العَظِيمِ

سورة المائدة الآية ٩٠

Supervisor Certification

I certify that this research was done under my supervision at university of Babylon as a partial requirement for the Degree of Higher Diploma in Forensic Evidences.

Supervisor

Assist.Prof.

Dr. Shaimaa Jassim Mohemeed AL Sultany.

Microbiology

University of Babylon

Date: / / 2021

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

Assistant Professor

Dr. Adi Jassim Abd ALrezzaq

Head of Biology Department

College of Science

University of Babylon

/ / 2021

Dedication

To whom mighty Allah crowned with dignity , to whom that taught me to give without a return , to whom that hold his name with pride my father May the God have mercy on him and placed him into his havens .

To the source of love and affection , to the one who blessed my days by her prayers : my mother .

To whom that her heart was longing to see me holding my certificate my partner in life and affectionate heart you are the source of happiness at home my wife (Um mohammed).

I dedicate this work ...

Mujeep 2021

Acknowledgements

First of all, I would like to praise Allah the Almighty, for helping me to complete this work

It is a pleasure to express my deep appreciation to supervisor **Dr. Shaimaa Jassem Mohemeed AL Sultany** throughout the course of preparing my thesis

Thanks to the Dean of the College of Science/ University Babylon and presidency of the Department of Biology for providing all the needed facilities, which are essential for the successful completion of the present work

I would like to thank everyone who helped me collect samples and the staff of the Public Health Laboratory in Najaf.

For the pure blood and pure souls of the martyrs and the men who defended the homeland and its sanctities.

Last but not least , I would like to thank all the members of my family for their enthusiasm and encouragement.

I wouldn't have made it without you all.

Mujeep 2021

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I. INTRODUCTION

Alcohol affects many organs, including the immune system, with even moderate amounts of alcohol influencing immune responses. Although alcohol can alter the actions of all cell populations involved in the innate and adaptive immune responses, the effect in many cases is a subclinical immunosuppression that becomes clinically relevant only after a secondary insult (e.g., bacterial or viral infection or other tissue damage). 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. The proinflammatory effects of chronic alcohol play a major role in the pathogenesis of alcoholic liver disease and pancreatitis, but also affect numerous other organs and tissues. In addition to promoting proinflammatory immune responses, alcohol also impairs anti-inflammatory cytokines. Chronic alcohol exposure also interferes with the normal functioning of all aspects of the adaptive immune response, including both cell-mediated and humoral immune responses. All of these effects enhance the susceptibility of chronic alcoholics to viral and bacterial infections (Miyake and Kaisho 2014 ; Mogensen 2009 ; Newton and Dixit 2012) .

Interleukin 10 (IL-10) is a cytokine with potent anti-inflammatory features that plays a central role in limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis. Dysregulation of IL-10 is associated with enhanced immunopathology in response to infection in addition increased risk for development of many autoimmune diseases. Thus a fundamental understanding of IL-10 gene articulation is critical for our comprehension of disease progression and resolution of host inflammatory response (Mosser and Zhang, 2008).

Interleukin 10 (IL-10) is a potent anti-inflammatory cytokine that plays a crucial, and often essential, role in preventing inflammatory and autoimmune pathologies (Sabat *etal*, 2010)**.**

Deficiency or aberrant expression of IL-10 can enhance inflammatory response to microbial challenge but also lead to development of inflammatory bowel disease and a number of autoimmune diseases (Fitzpatrick *etal* 2008 and NIAA, 2019).

Conversely, some pathogens can harness the immunosuppressive capacity of IL-10 to limit host immune response, leading to persistent infection (Brooks , 2006 ; Roncarolo , 2006).

In human serum, the predominant IgA form is monomeric, i.e., comprises 2HC and 2LC, with a subclass distribution of about 90% IgA1 and 10% IgA2. In contrast, the main molecular form found at mucosal surfaces, known as secretory IgA (SIgA), is dimeric, although some higher molecular weight species, including trimers and tetramers, are also present. Here the relative proportion of the two subclasses is more closely matched; an average distribution being about 40% IgA1 and 60% IgA2, though this varies depending on the particular mucosal site sampled (Chintalacharuvu, *etal* , 1994; Fitzpatrick *etal* 2008).

1.1 Aim of Study

The aim of present study was to determine the relationship between levels of Interleukin 10 , IgA and alcoholic addicted persons.

2 .Literature of Review

2.1 Alcohol

Beverages have been a part of social life for millennia, yet societies have always found it difficult to understand or restrain their use. Alcohol's combined effects on both innate and adaptive immunity significantly weaken host defenses, predisposing chronic drinkers to a wide range of health problems including infections and systemic inflammation. Alcohol consumption does not have to be chronic to have negative health consequences. In fact, research shows that acute binge drinking also affects the immune system. There is evidence in a number of physiological systems that binge alcohol intake complicates recovery from physical trauma, alcohol impairs recovery from three types of physical trauma burn, hemorrhagic shock, and traumatic brain injury by affecting immune homeostasis The combined effect of alcohol and injury causes greater disruption to immune function than either challenge alone (WHO, 2005) .

Alcohol exposure, and particularly chronic heavy drinking, affects all components of the adaptive immune system. Studies both in humans and in animal models determined that chronic alcohol abuse reduces the number of peripheral T cells, disrupts the balance between different T-cell types, influences T-cell activation, impairs T-cell functioning, and promotes T-cell apoptosis. Chronic alcohol exposure also seems to cause loss of peripheral B cells, while

simultaneously inducing increased production of immunoglobulins (Sarkar *etal*, 2015), as illustrated in figure (2-1) .

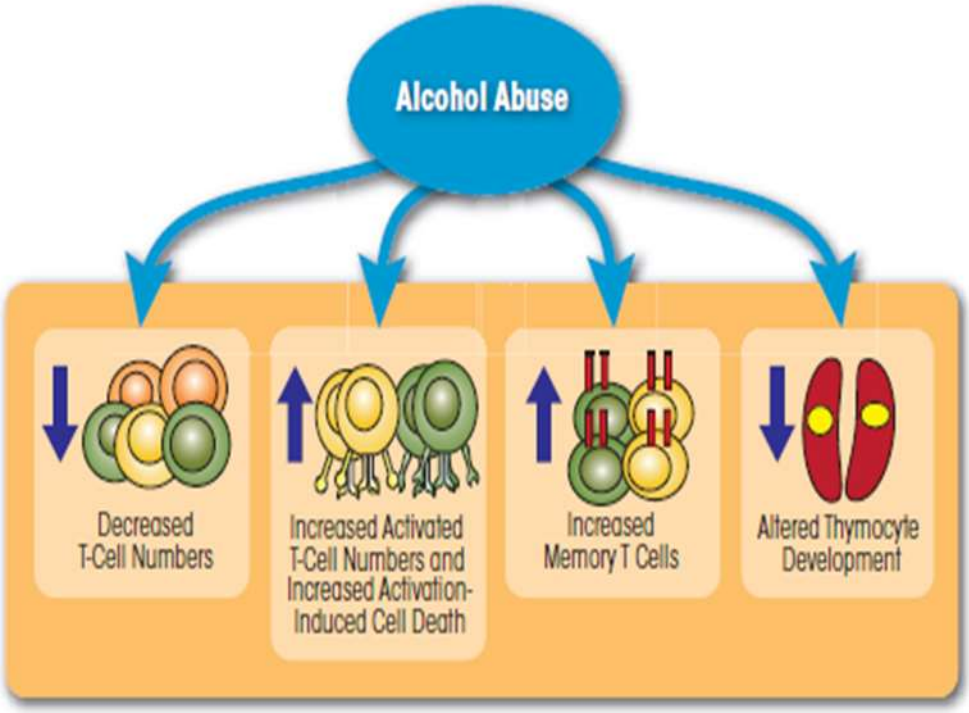


Figure (2- 1) Alcohol abuse affects both the number and function of T cells. Chronic alcohol consumption decreases the number of circulating T cells, increases the number of activated T cells, accelerates differentiation of T cells to a memory phenotype, and interferes with thymocyte development(Pasala *etal*., 2015).

In particular, the levels of antibodies against liver-specific auto antigens are increased in patients with alcoholic liver disease and may promote alcohol-related liver damage. The chronic alcohol exposure in uterus interferes with normal T-cell and B-cell development which may increase the risk of infections during both childhood and adulthood. Alcohol's impact on T cells and B cells

increases the risk of infections (e.g., pneumonia, HIV infection, hepatitis C virus infection, and tuberculosis) impairs responses to vaccinations against such infections, exacerbates cancer risk, and interferes with delayed-type hypersensitivity. In contrast to these deleterious effects of heavy alcohol exposure, moderate alcohol consumption may have beneficial effects on the adaptive immune system including improved responses to vaccination and infection. The molecular mechanisms underlying ethanol's impact on the adaptive immune system remain poorly understood (Pasala *etal*., 2015).

2.2 Alcohol's Effects on the Immune System

Alcohol can modulate the activities of all of these cell populations by affecting the frequency, survival, and function of most of these cells, thereby interfering with pivotal immune responses. However, unlike other mechanisms that cause classical immunocompromised states, such as HIV or tuberculosis infection, alcohol use typically results in a subclinical immunosuppression that becomes clinically significant only in case of a secondary insult. For example, chronic alcohol consumption increases the risk and severity of chronic infections with HIV; hepatitis C virus (HCV); or *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis, and promotes post-trauma immunosuppression (Dolganiuc *etal*., 2003).

Emerging evidence also suggests that 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 (i.e., the host microbiome). This microbiome is composed of the normal microorganisms found in and on the body (i.e., commensal microorganisms), 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 the microbiome of the gut, oral cavity, skin, and other mucosal surfaces (Chen and Schnabl 2014; Leclercq *etal*. 2014a,b).

These alcohol-induced changes could have clinical significance because the composition of the microbiome sends important pathogenic as well as homeostatic signals for the functions of host immunity. For example, chronic alcohol use is associated with changes in the gut microbiome, both increasing the microbial content in the first part of the large intestine (i.e., cecum) and changing the abundance of different types of microorganisms in the gut (Chen and Schnabl 2014; Fouts *etal*. 2012 and Yan *etal*. 2011), and this may alter the levels of LPS released by certain types of bacteria in the gut, which can contribute to inflammation in alcoholic liver disease as well as in liver cancer (i.e., hepatocellular carcinoma) (Szabo and Bala 2010 ; Gao *etal*, 2011 ; Chassaing *etal*, 2014).

2.3 alcohol abuse

Alcohol abuse encompasses a spectrum of unhealthy alcohol drinking behaviors, ranging from binge drinking to alcohol dependence, in extreme cases resulting in health problems for individuals and large scale social problems such as alcohol-related crimes, Alcohol abuse was a psychiatric diagnosis in the DSM-IV, and has been merged with alcohol dependence into alcohol use disorder in the DSM-5. Globally, excessive alcohol consumption is the seventh leading risk factor for both death and the burden of disease and injury. In short, except for tobacco, alcohol accounts for a higher burden of disease than any other drug. Alcohol use is a major cause of preventable liver disease worldwide, and alcoholic liver disease is the main alcohol-related chronic medical illness. Alcohol use disorder can effect people from all

walks of life. There are many factors that play a role in causing someone to obtain an Alcohol Use Disorder (AUD), genetics, psychiatric conditions, trauma, environments (Koob and Mason, 2016).

2.4 Addiction

Addiction, or to give its jargon term, is the compulsive use of a drug on a regular basis in order to experience its psychoactive effects or to avoid the discomfort of its absence. There are different types of dependence, ex. dependence on the opiate type, dependence on the alcohol type and so on. Many drugs result in compulsive use after repeated exposure to them. Examples include heroin and other opiates, alcohol, tranquillisers, nicotine, cocaine. Sometimes the dependence is physical, sometimes it is psychological. The latter is the most difficult to deal with (WHO, 2005).

2.5 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 and physical trauma, including poor wound healing The gastrointestinal system is typically the first point of contact for alcohol as it passes through the body and is where alcohol is absorbed into the bloodstream (Sarkar *etal*.,2015).

One of the most significant immediate effects of alcohol is that it affects the structure and integrity of the GI 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 the circulation(Engen *etal*.,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 IL-10 .also Chronic alcohol exposure interferes with the normal 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 the microbiome of the gut, oral cavity, skin, and other mucosal surfaces (Chen and Schnabl 2014)

2.6 Alcohol abuse with crime

Data suggests that engaging in prolonged drinking or binge drinking significantly increases your risk of committing violent offenses (Håkansson *etal*.2013) .

2.6.1 Robbery

Many cities across the world have seen a steady increase in robberies and property-related crime. A number of these robberies – roughly 15 percent – have been linked to alcohol use. Alcohol can intensify a robber's feelings of desperation and cause them to steal someone's money or property. While some robbers desire a better lifestyle or want to make a quick buck, others can turn into repeat offenders. The consequences of robbing someone are harsh and may entail time in jail, criminal charges on your record, fines and other legal troubles (Zorza, J, 1991).

2.6.2 Sexual assault

A sexual assault is a forced sexual act and may involve touching, kissing and intercourse. An estimated 37 percent of sexual assaults and rapes are committed by offenders who were under the influence of alcohol. For perpetrators, drinking may intensify their aggressive behavior. This can make them become more forceful when someone tries to resist them. Sexual assault can occur when there is a lack of consent, as well as when the victim is unable to give consent due to intoxication or mental state. (Zhang, 2016).

2.6.3 Aggravated assault

A common warning sign of alcohol abuse is irritability and extreme mood swings. Because of this, some individuals turn violent after an episode of heavy drinking. Poor decisions and impaired judgment, combined with aggression and hostility, can quickly become dangerous. If violent thoughts and feelings are acted on, it can lead to an aggravated assault charge. About 27 percent of aggravated assaults are committed by individuals who have used alcohol. Aggravated assault means causing serious injury, such as bodily harm to another person. Criminal charges are much stricter if a weapon is involved (Håkansson *etal*., 2013).

2.6.4 Intimate partner violence

Alcohol can play a dangerous role in intimate partner violence, leading to aggression, intimidation, forced sexual activity and other forms of controlling behavior. Intimate partner violence happens when a romantic partner causes physical, psychological or sexual harm to their significant other. An estimated two-thirds of victims suffering from violence by a current or former spouse or partner report that the perpetrator had been drinking, compared to less than one-third of stranger victimizations. Having a partner who is a heavy drinker can cause significant hardships, including financial difficulties, child care problems, infidelity, as well as other challenges (Zorza J, 1991).

2.6.5 Child abuse

Stress, money trouble, professional instability and a host of other factors can influence the amount of alcohol a person consumes. However, alcoholism not only affects an individual, it impacts family members and friends – including children. Research studies have shown a link between parents who abuse alcohol and the risk of child neglect and abuse. Roughly four in ten child abusers have admitted to being under the influence of alcohol during the time of the offense. Children who are victimized at a young age have an increased risk of developing behavioral and physical problems as they get older. (Spatz Widom and Hiller-Sturmhöfel, 2001).

2.6.6 Homicide

Alcohol is involved in more homicides across the world compared to other substances, like heroin and cocaine. In fact, about 40 percent of convicted murderers had used alcohol before or during the crime. Excessive drinking can lead to more severe forms of violence that can quickly escalate to extremely dangerous situations. The short- and long-term effects of alcohol

blur a person's mental state, contributing to an increased risk of committing violent crimes. There are strict legal punishments in place for homicide convictions and can land you in jail for many years, or even the rest of your life (Sharps *etal*, 2003).

2.7 Effect of alcohol abuse on Immunoglobulin A (IgA)

Chronic ethanol ingestion to trigger the formation of antibodies that recognize acetalde- hyde-protein condensates. assays for immunoglobulin (IgA) antibodies to acetaldehyde-derived adducts were performed on group of alcohol consumers, Anti-acetaldehyde (Ach)-adduct antibodies of each Ig isotype were found from the alcohol abusers. The main metabolite of ethanol is Ach, which accumulates in the liver and blood as a result of excessive alcohol consumption.' Ach has been shown to be capable of conjugating covalently with exposed proteins, particularly under reducing conditions, and these modified proteins, in turn, may trigger (Ig) formation that is directed against Ach-derived protein ad- ducts.27 Previously reported that the antibody responses against Ach-modified proteins in alcoholics consist exclusively of IgA. Because the IgA titers were also found to correlate with the amount of alcohol consumed, not with the degree of liver disease severity as assessed by biochemical data, it was further suggested that such titers could serve as markers of alcohol consumption. The present work was set out to clarify the association between the antibodies to Ach-derived epitopes, ALD, and the amount of alcohol consumption. Antibodies of different isotypes were measured from patients representing a wide range of clinical and morphological severity of ALD and the amount of alcohol consumption (Håkansson *etal*., 2013) .

2.8 Effect alcohol on cytokine IL-10

Cytokines are a class of multifunctional proteins that are implicated in cellular 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 (anti -inflammatory) 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 variety of tissues including plasma, lung, liver, brain (Crews *etal*., 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 lipopolysaccharide to “leak” into systemic circulation which promotes secretion of prionflammatory cytokines including tumor necrosis factor-alpha and interleukin- IL-10 through Toll-like Receptor mediated activation of transcription factors, such as nuclear factor-κB. (Leclercq *etal*.,2012) .

High dose alcohol exposure can induce neuroimmune signaling even after 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-10, and monocyte chemoattractant protein 1 (MCP-1, also known as CCL2) were elevated in the liver, serum, and brain (Qin *etal*.,2008).

Increases in serum cytokine levels subsided by 9 hours with clearance of blood 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 *etal*.,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 exposurefavors 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 *etal*.,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 number of infectious diseases (Romeo *etal*., 2007)

2.9 Studies in Iraq

Alcohol addiction contributes to a high burden on the society in terms of years that people spend with disability or in poor health because of alcohol-related illnesses or injuries .one Iraqi study found that significant association between patients knowledge with regard age with alcohol drink groups and no association with regard occupational status so low level knowledge cocering alcohol addicted. Iraq Mental Health survey (IMHS) during 2006-2007 reported a prevalence of < 1% for both alcohol and drug abuse disorders. IMHS was an effort to obtain basic descriptive data on the prevalence and correlates of mental disorders in Iraq and drug abuse at youth centers in Baghdad ، High rates of alcohol and drug abuse were recorded. A tendency for early age of onset in alcohol and drugs was observed. High household density and low educational level are important factors in alcohol and drug abuse also In Al Najaf city found that high addicted person with alcohol (Abogelal and Abd , 2019).

3. Materials and Methods

3.1 Materials

3.1.1 Chemicals and devices.

Chemicals and device utilized in the current study were illustrated in Tables (3-1) and (3-2).

Table (3-1) : Equipment and Instruments.

Items	Manufacturer
Disposable syringe 5 ml	Changzhou Tongda Medical Appliance/ Chine
Disposable cups 10ml	Shanghai Orsia Medical Technology /Chine
Gel tubes 5 ml	Shanghai Orsia Medical Technology /Chine
Gloves	Bio Basic /China
Incubator Model INB 400	Memmert /Germany
Medical cotton	Kardelen / Turkey
Refrigerator double door model SR32EMB	Cool tech /Korea
Micropipettes (0.5-5,100-1000)µl	Huawei and dragon med- Germany
Eppendorf tube 1.5ml	Shang Yu Yite Plastic /China
Alcohol 70%	Greet Med/ China
Cool box	International Haotian Technology/ China

Table (3-2) Chemicals and Reagents :

Components	Quantity
Standard Solution (1600pg/ml)	0.5ml x1
Pre-coated ELISA Plate	12* 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated human IL-10 Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

3.1.2 Kits.

Kits and its components that used in current study was illustrated in tables (3-3) (3-4).

Table (3-3) Kits used at work

Kits	Company /Country
Human Interleukin 10 ELISA Kit 96 test	Shanghai / Chine
Immunoglobulin A (IGA) ELISA Kit 96 test	Elabscience / Chine
Alcohol Screening test Kite	Ballymena / UK
Alcohol Rapid Test Panel (Urine) Kite	Hangzhou /Chine

Table (3-4): kit components of current study

Item	Specifications	Storage
Micro ELISA Plate(Dismountable)	96T: 8 wells ×12 strips 48T: 8 wells ×6 strips	-20℃, 6 months
Reference Standard	96T: 2 vials 48T: 1 vial	
Concentrated Biotinylated Detection ×Ab (100)	96T: 1 vial, 120 μL 48T: 1 vial, 60 μL	
Concentrated HRP ×Conjugate (100)	96T: 1 vial, 120 μL 48T: 1 vial, 60 μL	-20℃(Protect from light), 6 months
Reference Standard & Sample Diluent	1 vial, 20 mL	2-8°C, 6 months
Biotinylated Detection Ab Diluent	1 vial, 14 mL	
HRP Conjugate Diluent	1 vial, 14 mL	
Concentrated Wash ×Buffer (25)	1 vial, 30 mL	
Substrate Reagent	1 vial, 10 mL	2-8℃(Protect from light)
Stop Solution	1 vial, 10 mL	2-8°C
Plate Sealer	5 pieces	
Manual	1 copy	
Certificate of Analysis	1 copy	

3.2 Methods

3.2.1 Patients and Control Groups

This study was conducted on a group of alcoholics and other group who did not use alcohol (control), where samples were collected from alcoholics in different areas of Najaf governorate and prisons for the period from June to August 2021, and the study included 25 samples of alcoholics and 20 who did not use alcohol as control group, The age of participants in the control group and alcoholics ranged between (20-65) years and the levels of interleukin 10 and Immunoglobulin (IGA) where estimated by ELISA technique in a laboratory Public Health in Najaf Governorate.

3.2.2 Blood Samples

Five ml of venous blood were collected from each person .Samples were placed in tubes containing a gel . The blood was separated by centrifuge at 3000 rpm for 15 minutes ,and the separated serum was distributed to Eppendorf tube and then stored in a deep freeze at -20C^0 until used (Fitzpatrick *etal* 2008).

3.3 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. (Not applicable in Scotland) . 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 taking a decision to drive or do dangerous tasks, as if the test is not used correctly it can give erroneous results. The limitations below should be studied carefully before use.

(1) Easy to use (2) Individually packaged (3) Results in 2 minutes

3.3.1 Alcohol Screening test

The test was allowed to reach room temperature (15-30°C) prior to testing. The test should be taken after 15 minutes waiting after last alcohol consumption or after drinking a glass of wate, as the following:

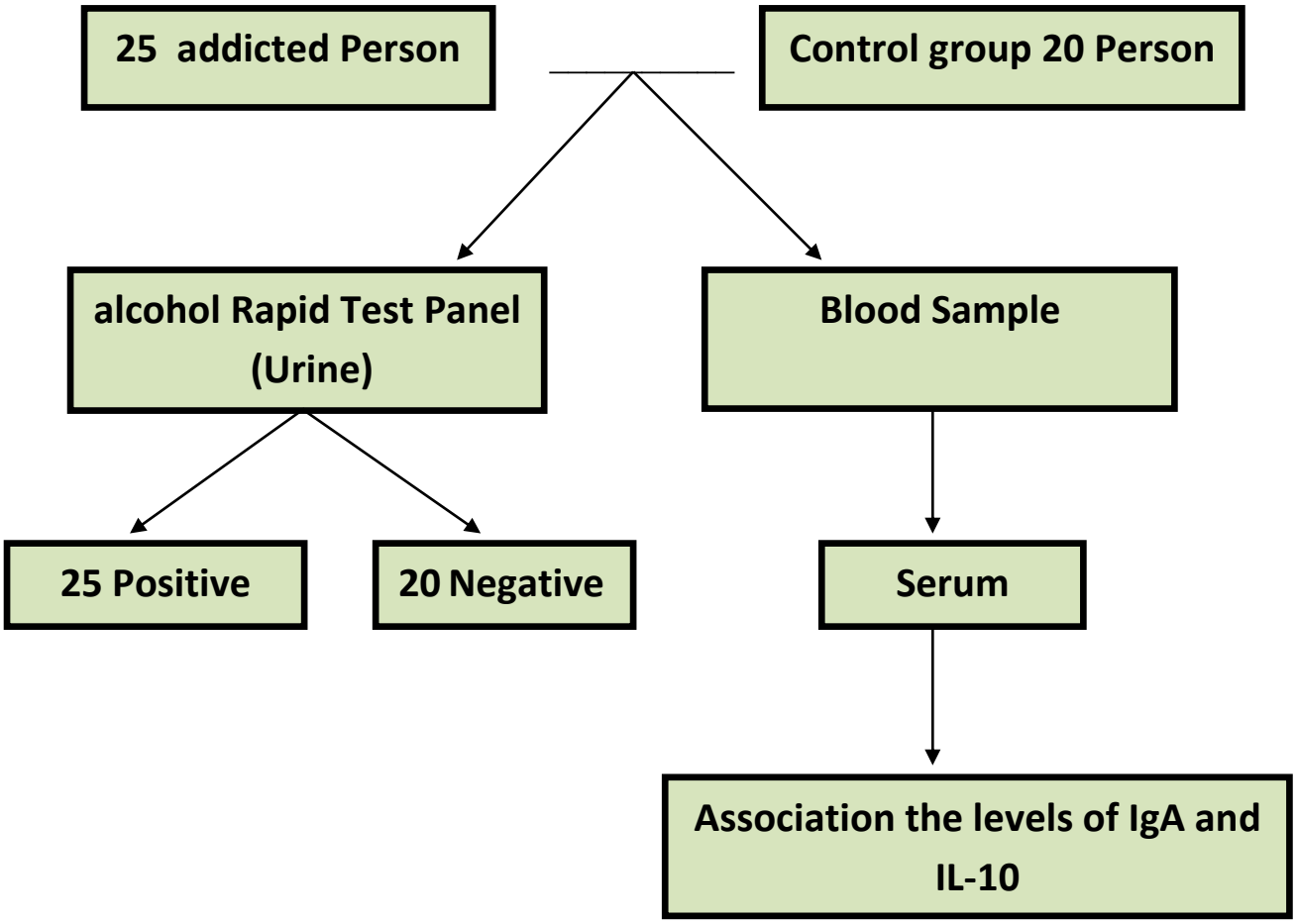
1-The pouch was brought to room temperature before opening and it was used as soon as possible.

2-The detector was removed from the sealed pouch by tearing the sealed pouch at the pre-cut mark to avoid touching the Mouthpiece. The Mouthpiece wasn't touched to avoid any contamination.

3-The middle of the detector was held using the (my) left and right index finger and thumb, then the detector was firmly squeezed to break the Inner Glass Tube containing the yellow crystals. The detector shouldn't be crushed or bent. The test was performed as soon as possible after breaking the Inner Glass Tube.

4. The middle of the detector was held horizontally, then a deep breath was taken and blown hard into the mouthpiece of the detector in one continuous breath for 12 seconds. Then the detector was shaken slightly to distribute the crystals evenly in the Test Window. Note: Failure to blow hard or to blow in one continuous breath for 12 seconds into the detector may cause erroneous results. Inhalation shouldn't be done while blowing into the detector.

3.4 study design:



3.5 Assay Procedure of IL-10

1- Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.

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

3- Add 50µl standard to standard well. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.

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

5- Remove 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 each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.

6- Add 50ul substrate solution A to each well and then add 50ul substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7- Add 50ul Stop Solution to each well, the blue color will change into yellow immediately.

8- Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minuets after adding the stop solution.

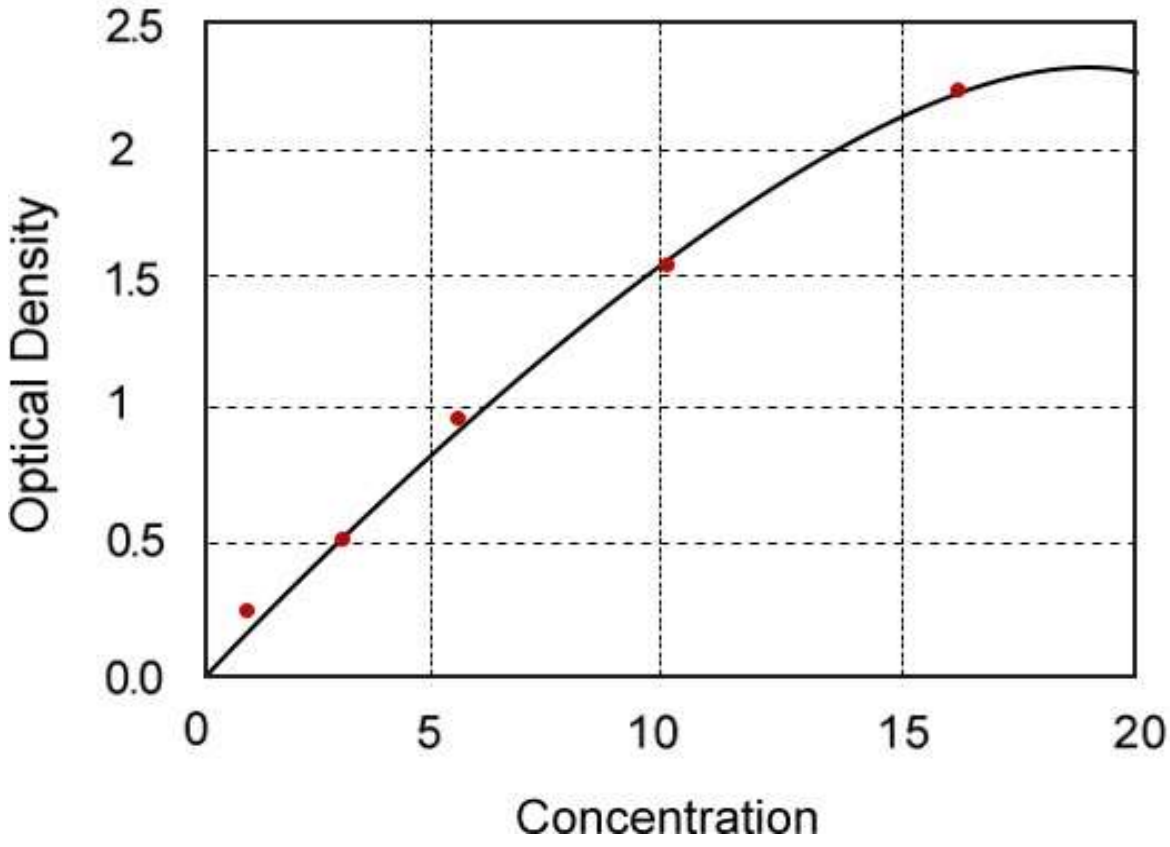


Figure (3-1) standard curve for estimate the level of IL-10

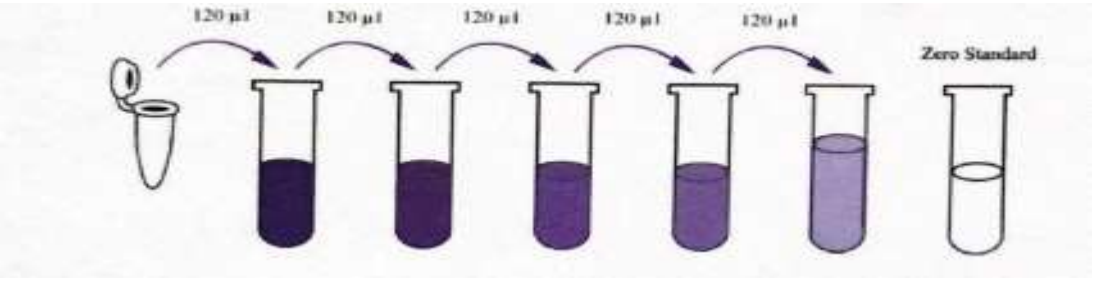
3.6 Reagent Preparation

All reagents should be brought to room temperature before use and 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, as illustrated in table (3-5) and Figure (3-2).

Dilution of standard solutions suggested are as follows:

Table (3-5)

800pg/ml	Standard No 5	120µl Original Standard+120µl Standard Diluent
400pg/ml	Standard No 4	120ul Standard No.5+ 120ul Standard Diluent
200pg/ml	Standard No 3	120µl Standard No.4 + 120µl Standard Diluent
100pg/ml	Standard No 2	120µl Standard No.3 + 120µl Standard Diluent
50pg/ml	Standard No 1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
1600pg/ml	800pg/ml	400pg/ml	200pg/ml	100pg/ml	50pg/ml

Figure (3-2) Wash Buffer Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of Ix Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals completely dissolved .

3.7 Principle assay of interleukin 10

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human IL-10 antibody. IL-10 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human IL-10 Antibody is added and binds to IL-10 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated IL-10 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in

proportion to the amount of human IL-10. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

3.8 Immunoglobulin A (IgA)

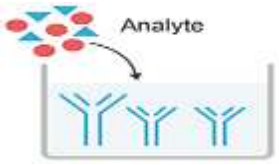
The levels of serum IgA were estimated in addicted control groups by using ELISA technique.

3.8.1 Principle Immunoglobulin A (IgA) test

The ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IgA. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IgA and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human IgA, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human IgA. You can calculate the concentration of Human IgA in the samples by comparing the OD of the samples to the standard curve.

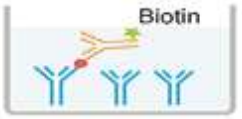
3.8.2 Assay Procedures for Immunoglobulin A (IgA):

1. Add 100µL standard or sample to the wells. Incubate for 90 min at 37°C



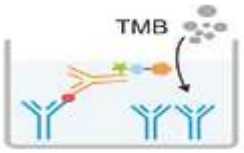
2. Discard the liquid, immediately add 100µL Biotinylated Detection Ab working solution to each well. Incubate for 60 min at 37°C

3. Aspirate and wash the plate for 3 times.

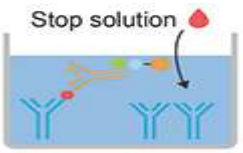


4. Add 100µL HRP conjugate working solution. Incubate for 30 min at 37°C. Aspirate and wash the plate for 5 times.

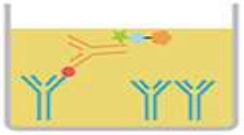
5. Add 90µL Substrate Reagent. Incubate for 15 min at 37°C.



6. Add 50µL Stop Solution



7. Read the plate at 450nm immediately. Calculation of the results.



4. Results and Discussion

4.1 Alcohol Screening

Alcoholics were detected by taking a urine sample placed in a special container, then we placed the analysis strip from the examination kit, then waited for (2-3) minutes, then read the result where it is positive after one line appears in red.

4.2 The level of serum IL-10 and IgA

IL-10 cytokine were estimated by using Enzyme Linked Immunosorbent assay (ELISA) were used for quantification of human IL-10. The result of current study were calculated by using standard curve fit equation. The Means of IL-10 concentration in serum of patient was 363.57±6.52 pg/ml while in control group it reached to was 229.62±21.35 pg/ml with high significantly (0.001), as also, the results mentioned that the levels of IgA was higher in addicted person compared with control group which reached 448.00±15.36 (ml/deiliter) and 181.88±14.39 (ml/deiliter) respectively, as shown in table (4-1) and Figure (4-1) and (4-2).

Table (4-1) The levels of serum IL-10 and IgA alcoholism persons and control group.

Groups / Parameters	Control	Alcoholic	p-value
	Mean±S.E		
Interleukin-10	229.62±21.35	363.57±6.52	**0.0001****
IgA	181.88±14.39	448.00±15.36	**0.0001****

(*)mean significant difference in comparison with control at the 0.0001 Level

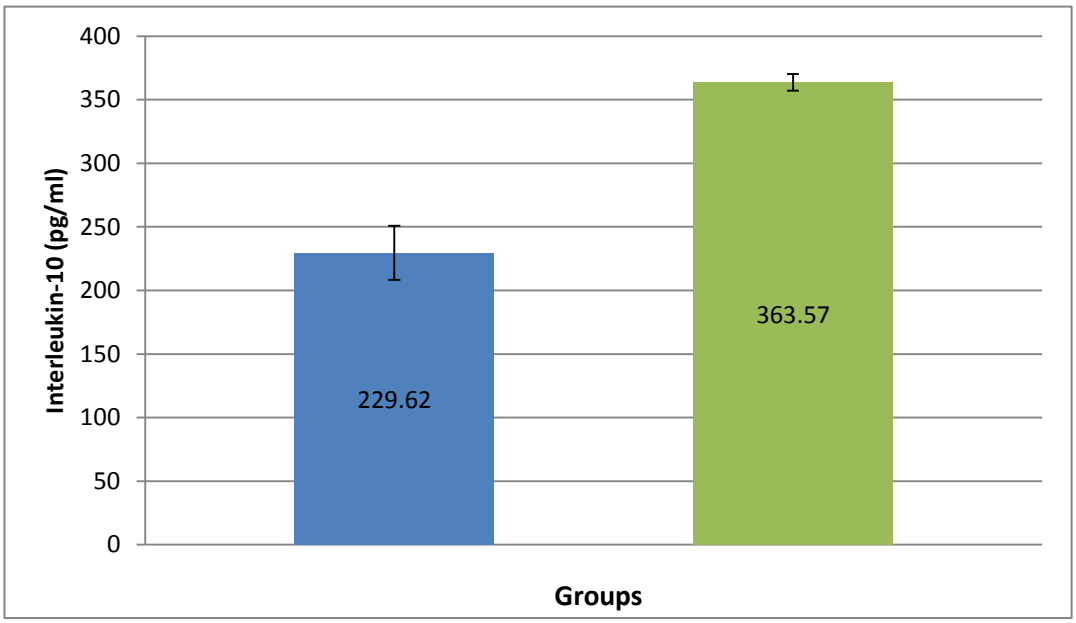


Figure (4-1): Correlation between Interleukin-10 For alcoholics and normal people

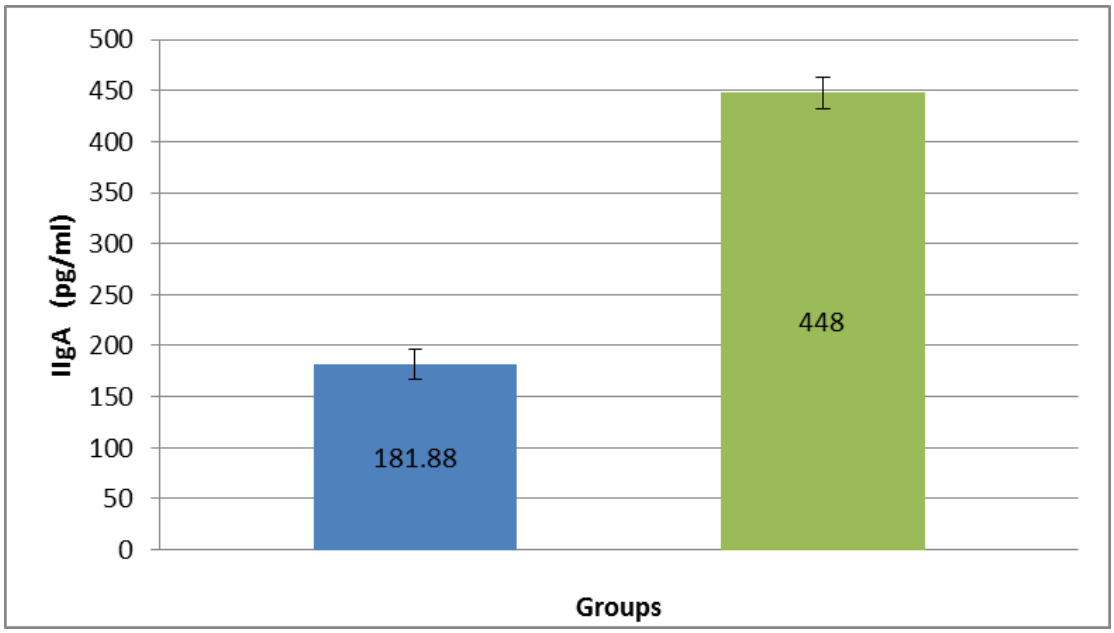


Figure (4-2): Correlation between Immunoglobulin A (IgA) For alcoholics and normal people

The results of current study was differ from other study that found alcohol was decreased in experimental study(Braun *etal* .,1995) . also another study found alcohol inhibited IL-10–induced CC chemokine (CCL3 and CCL4) expression by NK cells. Functional tests demonstrated that this reduced expression of CC chemokines 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. Alcohol inhibited IL-10– induced NF-κB p65 protein expression and calcium mobilization by NK cells (Guo *etal*. 2005).

Like all proteins and cytokines, IL-10 is recognized by its specific receptor. The IL-10 receptor is expressed by most hematopoietic cells and is part of the type-II cytokine receptor family. The receptor is composed of two types of chain called IL-10R1 and IL-10R2, which span the membrane of the cell . IL-10R1 is important in the binding of IL-10 to the receptor. IL-10R2 is an ‘accessory’ subunit meaning it is not critical in the binding of IL-10, but is important in activating signaling pathways downstream of the receptor. Ultimately, both IL-10R1 and IL-10R2 are required for a functional IL-10 receptor (Moore *etal*., 2001)

4.3 The Correlation between IL-10 and IgA in alcoholics and control groups

The Correlation between IL-10 and IgA was positive and p value was significant at (p-value 0.042) as show in figure (4-3).

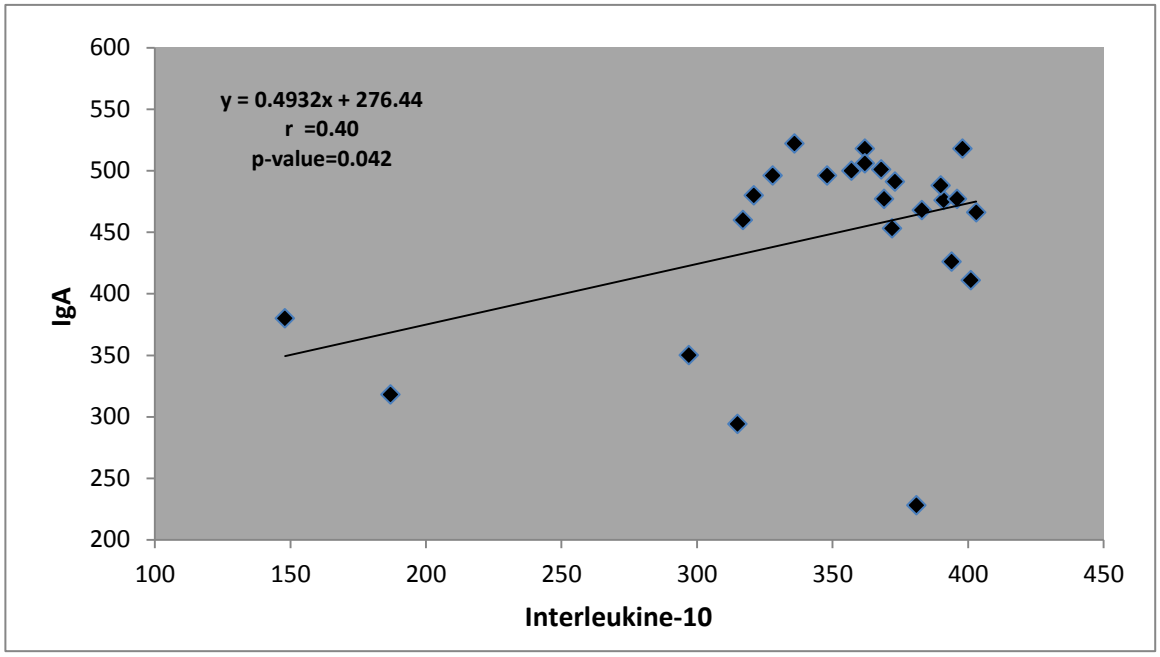


Figure (4-3): Correlation between Interleukin-10 and IgA

4.4 The effect of age stratum on levels of IgA and IL – 10 in alcoholic and control groups .

The alcoholics and control groups can be divided in to two groups (20-40) years and (41-65) years . The levels of IgA and IL-10 showed that no significant differences between age categories of addicted alcohol, as well as between addicted alcohol and control group as shown in table (4-2).

Table (4-2) effect of age in control for IL10, Immunoglobulin A (IgA)

Parameters \ Age (year)	20-40	41-65	p-value
	Mean±S.E		
Interleukin-10	440.00±21.84	458.18±21.90	**0.568**
IgA	367.00±8.01	323.55±25.17	**0.045***

(*)mean significant difference in comparison with control at the 0.045 level

Conclusions and Recommendations

Conclusions :

The abuse alcohol was effect on some immunological parameters .

Recommendations :

1-With the spread of analytical devices in schools, factories, laboratories, communities and analysis randomized members of the community can remove the alcohol for easy sampling through urine and continued presence of alcohol from days to weeks after abuse.

2-The police should be authorized to conduct alcohol analysis for those suspected, in particular traffic men samples are taken randomly on snares in highways especially for transport vehicles and taxi, since most of the serious accidents of these vehicles as a result of the drivers' use of various alcohol types, the analysis has a urine sample it is not a blood sample and anyone can easily take the sample .

Chapter One

INTRODUCTION

Chapter Two

Literatures Reviews

Chapter Three

Materials and Methods

Chapter Four
Results
and
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Conclusions and Recommendations

References

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