بسم الله الرحمن الرحيم

كلية نبتة
NAPATA COLLEGE

Napata College

Medical Laboratory program

Department of Hematology

ASSESSMENT OF COAGULATION PROFILE AMONG METHAMPHETAMINE SUDANESE ADULTS ABUSERS IN KHARTOUM STATE

A graduation project submitted to Napata College in partial of fulfillment of BSc. Degree (Honor) in hematology

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الأية

بسم الله الرحمن الرحيم

(يرفع الله الذين امنوا منكم والذين أوتوا العلم درجات والله بما تعملون خبير)

المجادله 11

Dedication

We dedicated this thesis to Allah the Almighty, thank you for the guidance, strength, power of mind, protection and skills and for giving us healthy life.

This study is wholeheartedly dedicated to our beloved parents, who have been our source of inspiration and gave us strength when we thought of giving up, who continually provide their moral, spiritual, emotional, and financial support.

To our brothers, sisters, relatives, mentor, friends, and classmates who shared their words of advice and encouragement to finish this study.

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Abstract:

Background: Methamphetamine is strong addictive drug that has serious physiological and psychological effects on human; we performed this study to assess the coagulation profile among Methamphetamine Sudanese adult abusers in Khartoum state.

Method: An analytical case control study aimed on testing coagulation parameters on methamphetamine abuser as a case group and nonusers as a control group, the recorded results were analyzed using SPSS program.

Results: A 50 samples of case group and 50 samples of control group were tested against each other for coagulation tests. The analyzed results revealed that males were abusing methamphetamine were much more than females. The analysis also shows that the addicts who have been abusing meth for a long time have a higher PT, APTT and INR than the patients who using it recently. The platelets of methamphetamine abusers were a bit lower than the people with no history of abusing.

Conclusion: The findings suggest that the study populations are at higher risk of coagulopathies. Planning of drug awareness programs and developing of the rehabs centers would help to reduce the effect of Methamphetamine drug.

الخلاصة:

الخلفية: الميثامفيتامين عقار قوي يسبب الإدمان وله آثار فسيولوجية ونفسية خطيرة على الإنسان. أجرينا هذه الدراسة لتقييم ملف التخثر بين متعاطي الميتامفيتامين السودانيين البالغين في ولاية الخرطوم.

الطريقة: دراسة حالة تحليلية شواهد تهدف إلى اختبار معاملات التخثر على متعاطي الميتامفيتامين كمجموعة حالة وغير المستخدمين كمجموعة ضابطة ، تم تحليل النتائج المسجلة باستخدام برنامج.SPSS

النتائج: تم اختبار 50 عينة من مجموعة الحالات و 50 عينة من مجموعة التحكم مقابل بعضها البعض لاختبارات التخثر. كشفت النتائج التي تم تحليلها أن الذكور كانوا يتعاطون الميتامفيتامين أكثر بكثير من الإناث. يُظهر التحليل أيضًا أن المدمنين الذين يتعاطون الميثامفيتامين لفترة طويلة لديهم مستوى أعلى من PT و APTT و INR من المرضى الذين يتعاطونه مؤخرًا. كانت الصفائح الدموية لمتعاطي الميتامفيتامين أقل قليلاً من الأشخاص الذين ليس لديهم تاريخ من سوء المعاملة.

الخاتمة: تشير النتائج إلى أن السكان الذين شملتهم الدراسة معرضون بشكل أكبر للإصابة باعتلال التخثر. من شأن تخطيط برامج التوعية بالعقاقير وتطوير مراكز إعادة التأهيل أن يساعد في تقليل تأثير عقار الميثامفيتامين.

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List of abbreviations:

Abbreviation	Full name
MA	Methamphetamine
HIV	Human Immunodeficiency Virus
CM	Crystal Meth
VWF	Von Willebrand Factor
PT	Prothrombin Time
APTT	Activated Partial Thromboplastin Time
INR	International Normalized Ratio
TTP	Thrombotic Thrombocytopenic Purpura
MDMA	Methylene Dioxin Methamphetamine
DIC	Disseminated Intravascular Coagulation
TF	Tissue Factor
QC	Quality Control
EDTA	Ethylene Diamine Tetra Acetic Acid

Chapter One

Introduction and literature review

1.1 Introduction:

Methamphetamines, sometimes known as "meth," "crystal meth," and "ice" on the street, are potent and addictive stimulants that can be smoked, snorted, eaten, injected, or taken orally. . It is also among the most often used illicit substances, Methamphetamine is accessible in a variety of dosage forms, including ice powder, and tablets, with varying pharmacokinetic features.[13]. . Due to the psychotropic qualities, inflated self-confidence, and sensation of satisfaction.[1]. Using legal and easily accessible components, such as ephedrine, pseudoephedrine, red phosphorous, iodine, ammonia, paint thinner, lye, camping gasoline, drain cleaner, and lithium, MA is manufactured, or "cooked," fast, reasonably simply, and inexpensively. Recipes can be found online, and local hardware and drug stores have these ingredients and culinary supplies. Many of the chemicals used in the manufacture of MA are explosive, and the waste that is produced is poisonous and caustic.[2]. Meth is an extremely addictive substance, and long-term abuse can cause psychiatric issues. Chronic meth users experience memory and executive functioning problems, as well as greater rates of anxiety, depression, and most significantly psychosis.[3].

MA is a stimulant, and as such, it has similar physiological and psychological effects to cocaine. Dopamine, norepinephrine, and serotonin are stimulated to release and their reuptake is blocked by MA. This overabundance of neurotransmitters causes euphoric feelings, diminished inhibitions, invincibility feelings, increased alertness, enhanced sexual experiences that lead to inappropriate sexual behavior, and hyperactivity brought on by increased energy for extended periods of time. Increased heart and respiration rates, heat, chest pain, hypertension, decreased appetite, anorexia, irritability, confusion, tremors, convulsions, anxiety, aggressiveness, and psychotic symptoms like hallucinations and paranoia are some of the harmful short-term consequences. Then comes physical and mental weariness, headaches, irritation, impaired focus, hunger, low energy, and a need for more MA. Cognitive impairments and changes in the brain that result in symptoms similar to those of Parkinson's disease can occur. Long-term use of MA use is associated with neurotoxicity and neurodegeneration .[2]. Teenagers who have a history of

MA abusing are associated with increased rates of suicidal ideation and suicide attempts that are disproportionate to population-based surveys during the same period.[4].

Methamphetamine (MA) not only affects the nervous system but also has cardiac toxicity and immunosuppressive features. The cardiovascular symptoms of acute MA use include tachycardia, atrioventricular arrhythmias, myocardial ischemia and hypertension, resulting in cardiac lesions. Chronic abuse of MA causes cardiomyopathy including cellular infiltration, myocardial hypertrophy, myocardium rupture and fibrosis. The second main problem with MA use is its potential to disrupt the immune system function leading to suppression of mitogen-stimulated lymphocyte, a reduction in circulating lymphocyte numbers and alternation T-lymphocyte cytokine secretion as well as B cell proinflammatory cytokine secretion. Concomitant MA use and Human Immunodeficiency Virus (HIV) infection not only increases the incidence of immunosuppression, but also increases the heart disease occurrence with a coincidentally complication of AIDS or AIDS medications.[5]. Long term CM use can lead to severe damage inside the oral cavity. When CM is used over a long period of time and in the absence of treatment, clinical symptoms in terms of Meth mouth syndrome cannot be avoided.[6].

After the integrity of the vascular system has been restored, hemostasis permits an organism to seal off broken blood vessels, maintain blood fluidity, and dissolve blood clots. From zebrafish to humans, the hemostatic system is a highly conserved process in which blood clotting, also known as coagulation, plays a significant role. Greek philosopher Plato first explained how blood creates fibers after leaving the body's heat two thousand years ago. Additionally, he was the first to use the term "fibrin," which is currently used to describe a crucial blood clotting protein that makes up those fiber formations. It's interesting to note that Plato's theory on blood clotting, which other ancient thinkers like Aristotle and Galen shared, remained the dominant idea until the end of the 18th century. On the biological mechanism of coagulation, significant advancements were made throughout the 19th century. The importance of platelets in hemostasis was first understood around 1865. It was suggested that a fictitious protein known as "thrombin" may cause fibrin to develop.

The majority of the crucial coagulation factors were identified in the 20th century. The earliest coagulation model was created in 1905 by Morawitz, who proposed that tissue factor—now

known as Thromboplastin was generated by injured blood arteries and converted Prothrombin into thrombin in the presence of calcium. The subsequent conversion of fibrinogen into fibrin by thrombin led to the development of a blood clot. This four-clotting factor model, however, was unable to completely explain the intricate process of coagulation. Many of the remaining factors, including the von Willebrand factor (VWF) and factors V, VII, VIII, IX, and XI, had been defined by the 1950s (FV, FVII, FVIII, FIX, FXI). Some of these variables, such as FVIII insufficiency in hemophilia A and FIX deficiency in hemophilia B, have been related to bleeding disorders. Two separate teams developed a waterfall- or cascade-like coagulation model in the 1960s. This model was therefore appropriately called the "coagulation cascade model." Each clotting factor is made up of a proenzyme that, when activated by an upstream clotting factor, becomes an active enzyme. It was also proposed that FX activation is the result of the convergence of two distinct cascades. These are referred to as the intrinsic pathway since all of the necessary components are found in the blood and the extrinsic pathway because an external agent is required (TF from the extravascular tissue). Once blood contacts hydrophilic surfaces in vitro, the intrinsic pathway is initiated. Prekallikrein is converted into kallikrein by auto-activated FXII, which then initiates the activation of Prothrombin, FXI, FIX, and FIX. Starting with TF and activated FVII, the extrinsic pathway directly triggers the successive activation of FX and prothrombin.

Coagulation profiles are screening tests that look for abnormal blood clotting. They include (Activated Partial Thromboplastin Time, Prothrombin Time, Thrombin Time, Fibrinogen, D-dimer). Depending on the coagulation cascade pathways, the test results when analyzed collectively are used to identify coagulation factor deficiencies and defects, the presence of coagulation factor inhibitors, the efficacy of anticoagulant medications, hereditary conditions, severe infections, and liver diseases. Prior to surgery, the bleeding profile may also be used to check appropriate blood coagulation.[7].

1.2 Literature review:

A preliminary study was conducted in East-Malaysia associated with 3–4 methylene-dioxin-methamphetamine (MDMA) abuse, it showed that methamphetamine cause coagulopathy and being one of the causative agents in triggering massive detrimental responses like disseminated intravascular coagulation (DIC) (Navkiran Singh Gill, 2017).[9].Other study regarding methamphetamine users was conducted in Indiana in United states, the outcome was: high values for INR and d-dimer, undetectably low fibrinogen, decrease in hemoglobin, Thromboelastography results were unobtainable due to lack of clot formation with improve (Coghlan, 2022).[10]. In a case report Associated with Methamphetamine Intoxication, the investigation results were: e Thrombotic Thrombocytopenic Purpura (TTP) with the rise of D-Dimer, prolonged Activated Partial Thromboplastin Time (Navid Khosravi1, 2021) .[11].The methamphetamine cause thrombosis in small or medium-sized vessels, such as coronary arteries and mesenteric vessels (Alla VM, 2011) .[12].

1.3 Justification:

Abusing of methamphetamine can produce a lot of disorders like inducing of myocardial infraction and liver and hematological disorders, and one of the most serious hematological disorders is thrombus formation, so we conducted this study to predict thrombus formation and to avoid the subsequent complications by assessment of coagulation profile. Also there are no enough previous studies are performed in Sudan regarding this topic.

1.4 General objectives:

Assessment of coagulation profile among Methamphetamine Sudanese adult abusers.

1.5 Specific objectives:

- To estimate the Prothrombin Time (PT)
- To estimate the Activated Partial Thromboplastin Time (APTT)
- To calculate the International Normalized Ratio (INR)
- To count the platelets
- To compare these results of coagulation between the case group and control group
- To correlate between the duration of abusing and the results of coagulation
- To correlate between the coagulation results and doses per day

Chapter Two

Materials and Methods

2.1 Study Design:

This is analytical case control study

2.2 Study Area and Population:

This study was done in Khartoum state during the period from June 2022 to October 2022 in (Abd Alaal Al Edreesy hospital).The targeting group in this study is Sudanese methamphetamine abusers.

2.3 Sample size:

The sample size formula is determined in two steps. First, we calculate the sample size for the infinite population and second we adjust the sample size to the required population. The sample size formula can be given as:

$$\text{Unlimited population: } n = \frac{z^2 \times \hat{p}(1-\hat{p})}{\varepsilon^2}$$

$$\text{Finite population: } n' = \frac{n}{1+\frac{z^2 \times \hat{p}(1-\hat{p})}{\varepsilon^2 N}}$$

Where=

z is the z score
ε is the margin of error
N is the population size
p̂ is the population proportion

Note: Z score is determined based on the confidence level.

Confidence level = 95%

Margin of error = 13.5%

Population proportion = 50%

Population size = 1000

Using previous formula the samples are 100 samples, 50 samples collected from adult known methamphetamine abusers as a case group and 50 samples from Sudanese adult with no history of abusing as a control group. This was randomly selected from the study area.

2.4 Sampling technique:

Random sample technique.

2.5 Inclusion and exclusion criteria:

2.5.1 Inclusion: In this study we are targeting methamphetamine Sudanese abusers in the ages between (18-50) years old.

2.5.2 Exclusion: We will exclude the following cases:

- Patients with underlying diseases that can cause clotting prolongation.
- Patients under anticoagulant treatment.

2.6 Ethical considerations:

A permission from all the volunteers were taken using a consent form before starting collection, the patients were informed with all the information and will be highly secured, also will not be used for any other purposes scientific inquiry.

2.7 Sample and data collection:

2.7.1 Data collection:

The clinical data and history of participants were obtained using questionnaire.

2.7.2 Specimen collection:

Venous blood sample was collected from those who fit the inclusion criteria using sterile dry plastic syringes and tourniquet to facilitate the blood flow in the syringe. The site of puncture was cleaned using 70% Alcohol and the amount of blood sample was 5ml, which collected on tri sodium citrate container from each volunteer. A platelet poor plasma was prepared by centrifuging the sample for 15 minutes at 4000 rpm to obtain Sample the plasma for coagulation tests. A 2.5 ml of blood was collected on EDTA container and process the sample for platelets count.

2.8 Analytic method:

2.8.1 PT and APTT Method:

The samples were analyzed for PT and APTT using fully automated STA Compact Max analyzer. The STA Compact Max is constructed on the most durable platform available. The Compact Max is a powerful, high-efficiency analyzer with higher throughput that has a wide range of test options, making it the ideal system option for mid-sized laboratories. Due to the system's innovative sample management technique, STAT samples can be processed quickly and with a moderate throughput without affecting the instrument's time to result. The Compact Max is equipped with 96 sample places and 45 reagent positions, making it easy to manage the workload with little assistance from the operator. Full auto-verification, repeat/reflex testing, a complete QC package, accreditation tools, automated maintenance logs, and other features are all provided by Integrated STA Coag Expert Software. TAT monitoring and maintains 5 years of patient archives onboard. The Compact Max is available with an optional cap-piercing system to reduce bio-hazard exposure risks.

2.8.2 Platelets count method:

After thoroughly mixing each sample, thin air-dried blood smears were prepared, stained by manually with a RAL Diff-Quick stain, and studied using an X100 oil-immersion lens for light microscopy.

Platelets then calculated carefully on the one layer area in ten fields ,and the total platelets count was calculated using this formula:

Total platelets count = sum of Plts x 20000/10

2.9 Statistical analysis:

Data were analyzed with SPSS program (Statistical Package for Social Science) Version (2022).

Chapter Three

Results

3. Results:

Table 1: This table shows the means of ages per years, duration per months and dose per weeks:

Descriptive Statistics						
Groups	Variables	Number	Minimum	Maximum	Mean	Std. Deviation
Non-users	Age (Years)	50	21	48	27	6
Methamphetamine user	Age (Years)	50	20	50	28	6
	Duration (months)	50	1	23	10	6
	Dose per week	50	1	6	2	1

Table 2: Comparison between genders in control and case groups

Variables		Groups		
		Methamphetamine user	Non-users	Total
Gender	Male	46	39	85
		54.10%	45.90%	100.00%
	Female	4	11	15
		26.70%	73.30%	100.00%
Total		50	50	100
		50.00%	50.00%	100.00%
Pearson Chi-Square P value=0.049**				

Table 3: This table correlates between the (duration of abusing, dose per weeks) and coagulation parameters:

Correlations					
Variables		PT (Second)	INR	PLT(*10³\mm³)	APTT (Second)
Duration (months)	Pearson Correlation	0.439**	0.423**	-0.04	-0.290*
	Sig. (2-tailed)	0.001	0.002	0.781	0.041
	Number	50	50	50	50
	Strength and direction	Weak positive	Weak positive	Weak negative	Weak negative
Dose per week	Pearson Correlation	0.206	0.178	0.056	0.065
	Sig. (2-tailed)	0.151	0.217	0.701	0.653
	Number	50	50	50	50
	Strength and direction	Weak positive	Weak positive	Weak positive	Weak positive
** Correlation is significant at the 0.01 level (2-tailed).					
* Correlation is significant at the 0.05 level (2-tailed).					

Table 4: This table sums up the result of each parameter among both users and nonusers:

Independent-Test						
Parameters	Groups	Number	Mean	Std. Deviation	Std. Error Mean	P value
PT (Second)	Methamphetamine user	50	14.314	1.3035	0.1843	0.00000001**
	Non-users	50	12.752	1.008	0.1425	
INR	Methamphetamine user	50	0.9482	0.08395	0.01187	0.003**
	Non-users	50	0.9046	0.05905	0.00835	
PLT(*10³\mm³)	Methamphetamine user	50	227.5	74.001	10.465	0.000003**
	Non-users	50	296.08	63.215	8.94	
APTT (Second)	Methamphetamine user	50	35.524	3.3827	0.4784	0.00000001**
	Non-users	50	26.898	4.1472	0.5865	

Figure 3.1: INR among methamphetamine users rather than nonusers:

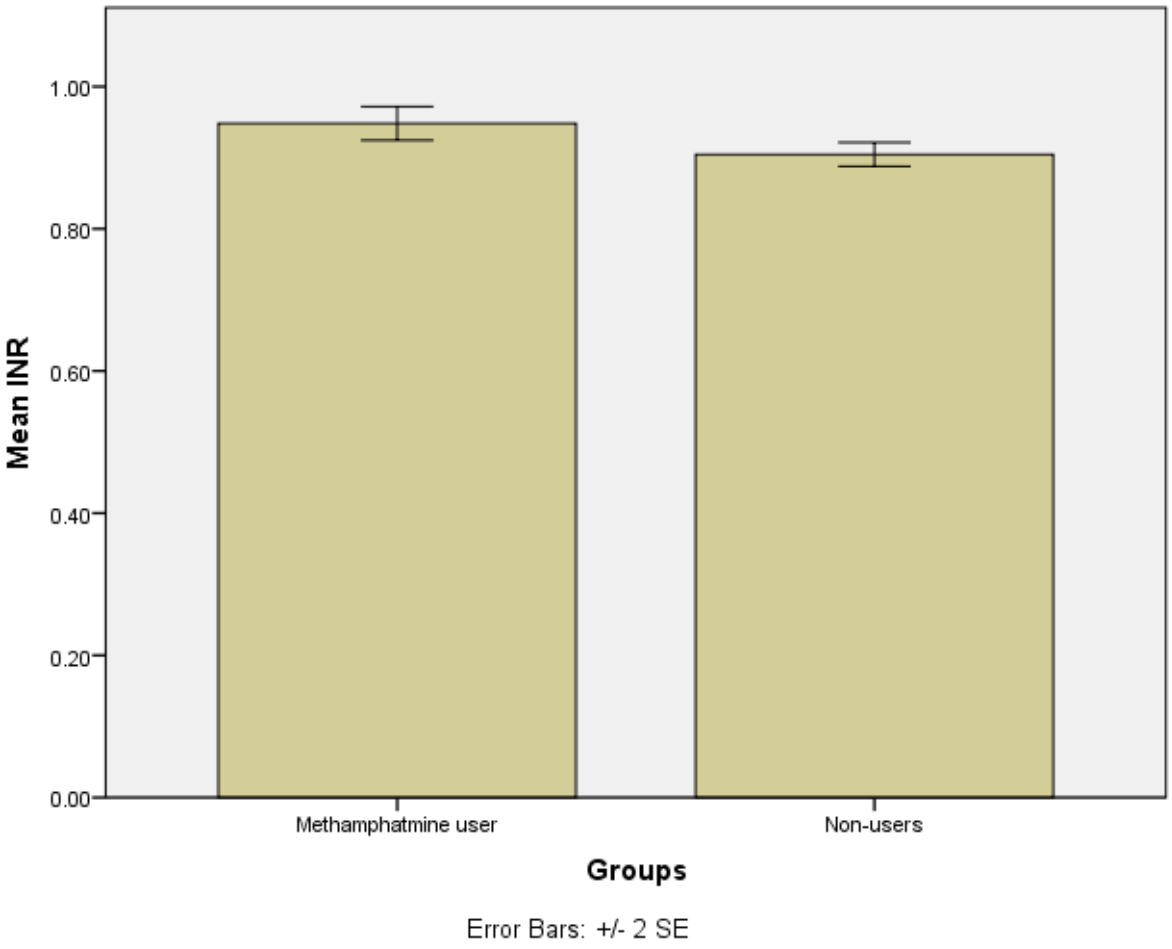


Figure 3.2: The mean of platelets count on both case and control group:

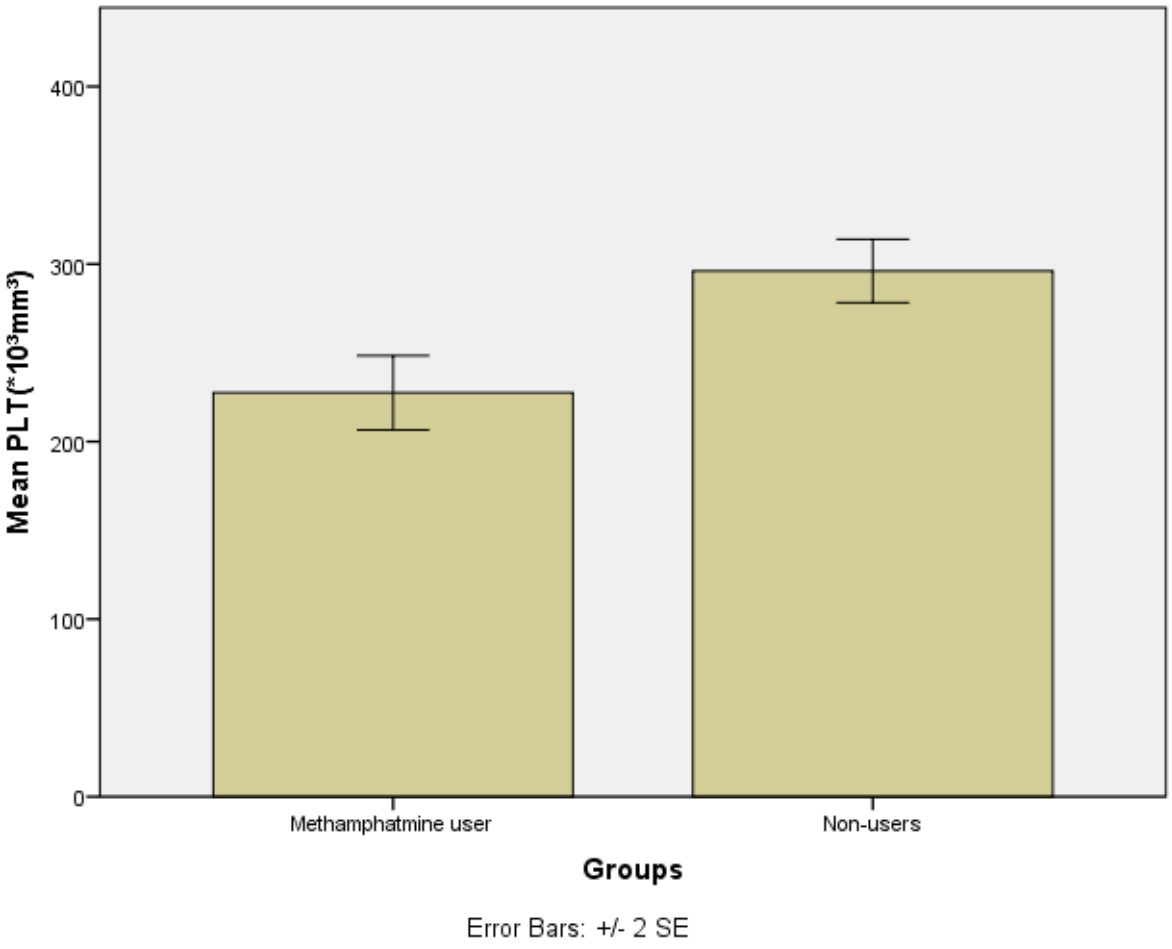
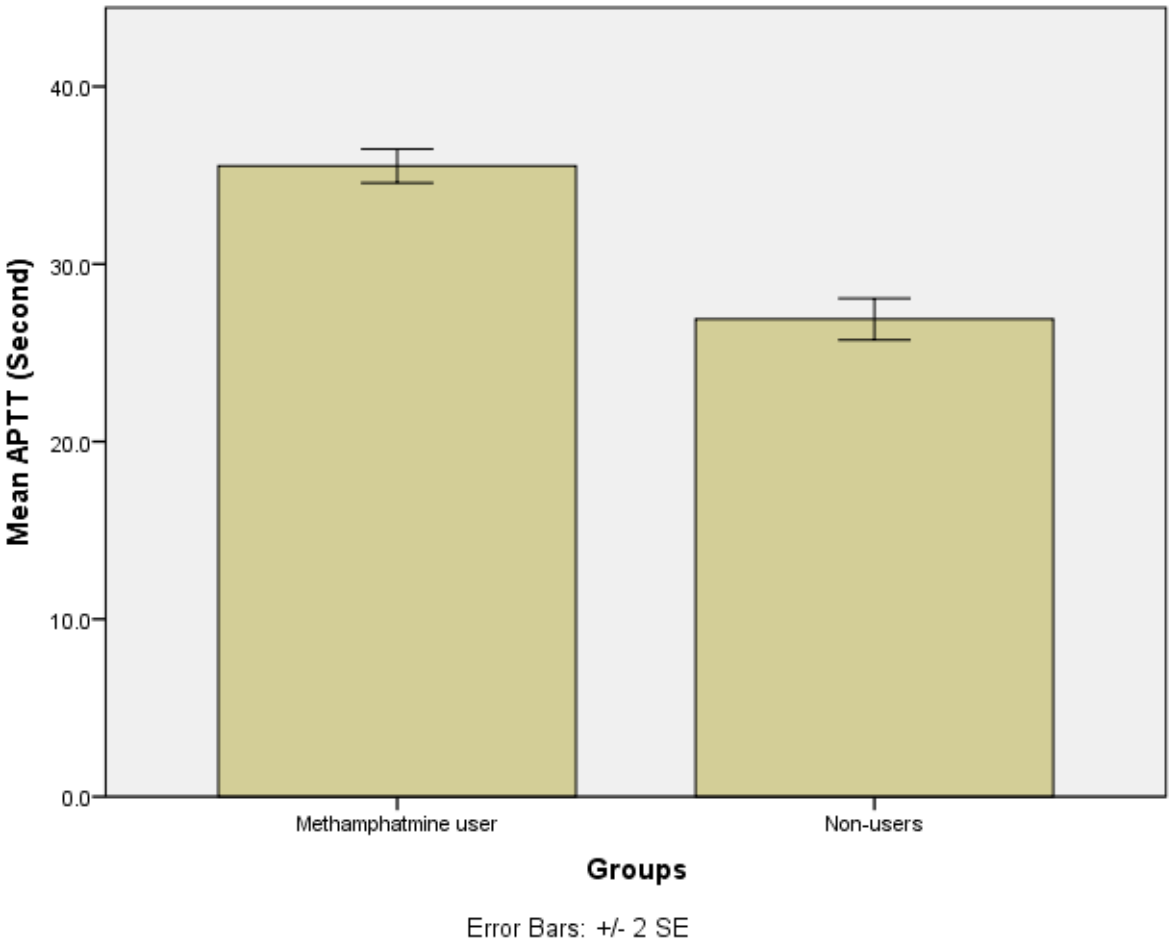


Figure 3.3: The mean of APTT results among case and control groups:



Chapter four

Discussion and conclusion

4.1 Discussion:

- This is a study of a 100 sample, 50 samples were collected from known methamphetamine abusers according to inclusion criteria and 50 samples collected from healthy individuals as a control group, both groups were tested for coagulation profile.
- The mean of ages of the control group was 27 years while the mean of ages of the case group was 28 years.
- Most of the methamphetamine users included in this study were abusing meth for minimally 1 month and maximally 23 months, by mean of 10 months.
- The number of doses taken per week among the users group varied from 1 to 6 by mean of 2 doses per week.
- Most of the individuals included in this study were males (46).
- There was a weak positive correlation between the INR value and the dose taken per weak.
- The INR data analysis showed a weak positive correlation with the duration of abusing per months.
- The results of platelets count showed a weak negative correlation with the abusing duration per months.
- A weak positive correlation present between the platelet count results and the number of doses per week
- There was a weak positive correlation between the PT results and the doses taken per week.
- There was a weak positive correlation between the PT results and the duration per months.
- The APTT results showed a weak negative correlation with the duration per months.
- A weak positive correlation also present between the APTT results and the number of dose per weak.
- The PT results obtained from the case group were higher than the control group results.

- The mean of the APTT results of the case group was (35.5) which is higher than the mean of the APTT results obtained from the control group (26.8).
- There were a significant difference in the platelets count between the case group and the control group.
- Our study was similar to another study that was conducted in East-Malaysia by Navkiran Singh Gill, 2017 associated with 3–4 methylene-dioxin-methamphetamine (MDMA) abuse, it showed that methamphetamine cause coagulopathy and being one of the causative agents in triggering massive detrimental responses like disseminated intravascular coagulation (DIC).
- Our results also matching another study results, the study was conducted in in Indiana United States by Coghlan in 2022 it revealed a significant increase in the INR values among methamphetamine users.

4.2 Conclusion:

This is a case control study conducted in Khartoum state, Sudan in the period from September 2022 to October 2022; we studied the coagulation parameters on Sudanese adults methamphetamine abusers. Our data analysis results showed a higher PT, APTT and INR on the case group and lower platelets count than the healthy nonusers control group.

4.3 Recommendations:

- We recommend assessing other coagulation parameters such as D-dimer, TT, and fibrinogen level to confirm coagulopathies.
- We also recommend trying larger sample size to confirm the significance.
- We advise using automated techniques for platelets count for much accurate results.

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