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Program of Medical Laboratory Sciences
Department of Hematology



**Determination PT and APTT in pregnant women in Khartoum
during the period from June to October 2022.**

**A dissertation submitted to Napata College in partial
fulfillment of Bachelor in Medical Laboratory Sciences**

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Dr.Amani Ashmig.

2022

الآية

"بسم الله الرحمن الرحيم"

قال الله تعالى:

{يَخْلُقُكُمْ فِي بُطُونِ أُمَّهَاتِكُمْ خَلْقًا مِّنْ بَعْدِ خَلْقٍ فِي ظُلُمَاتٍ ثَلَاثٍ ذَلِكُمْ اللَّهُ رَبُّكُمْ لَهُ الْمُلْكُ {
إِلَّا إِلَهَ إِلَّا هُوَ فَآَنَى تُصْرَفُونَ

صدق الله العظيم.

سورة الزمر

آيه (6)

Declaration

We declare this subject matter presented in this graduation project is original and has only been submitted to Napata College to obtain the Bachelors.

Dedication

After appreciation and respect ,we de parents this great success to our parents for their strength and efforts throughout our study journey in order to present this research .we also extend our thanks to everyone who stood with us and helped us in this research that we did ,we are thank for the efforts of Dr. Ahmed Hassan ,Dr.Ramy and Dr. sally Magdy , for whom we extend our sincere thanks and gratitude to our honorable brother Mazen Othman, and we especially mention in our prayers to the late mather of our colleague Nesreen Gaffer . may god have mercy on her and protect her from the fire . we spent the best school years with them.

Acknowledgment

Praise be to God, Lord of the Worlds, and prayers and peace be upon the most honorable prophets and messengers, our master Muhammad, his family and companions, and those who followed them in goodness until the Day of Judgment, and after. I thank God Almighty for His bounty as He made it possible for us to accomplish this work by His grace, to Him be praise first and foremost.

Then I thank those good people who helped us during this period, led by my supervisor professor Dr. Amani Ashmiq, who strengthened our resolve and desire. In it, and strengthen our resolve for it, it has a reward from God and from us its appreciation and enjoyment of health and wellness, and the benefit of its knowledge.

I also thank the organizers of the whole Napata college . On top of them are His Excellency **Dr. /Solafa Saif Aldeen** , the Rector of the University, and His Excellency the Dean of the College, **Dr. Al-Zubair Bashir Taha**, and I wish them all the best for their interest in the students of the College in general and the graduates in particular.

Abstract

As a result of the typical physiological needs of pregnancy and childbirth, the hemostatic system develops a physiological hypercoagulable condition and the levels of coagulation factors I, VII, IX, X and XII rise during pregnancy. The aim of this study was to compare pregnant women to non-pregnant women in terms of coagulation changes during all three trimesters of pregnancy. The research was conducted 50 pregnant women, aged range between 20-39 year, who attended Dar ALelag Medical laboratory in Khartoum-Sudan during the period from June 2022 to January 2023, followed by PT, & PTT and compared with other 50 non-pregnant women which serve as controls. PT, & PTT showed decrease in third trimesters in pregnant women and PT also decrease in second trimester, while PTT decrease in all three trimesters in pregnant women when compared with non-pregnant women. These parameters also decrease in third trimester when compared with first trimester. In good, a condition of physiological low-grade intravascular coagulation characterizes normal pregnancy. In our research, in each trimester, there were alterations in the levels of coagulation parameters. In addition, the ranges for coagulation measures were supplied, which may help clinicians track coagulation in pregnant women more effectively.

Abbreviation

Abb:full structures

APC: Activated protein C

APTT: Activated partial thromboplastin time

HCG: Human chronic gondatropin

HMWK: High molecular weight kinogen

HPL: Human placenta lactogen

INR: International standard ratio

KCCT: koalincephalincoltting time

PT: prothombin time

PTTK: Partial thromboplastin with koalin

TEG: Thromboelstography

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Chapter one: Introduction.

1. Introduction

1.1 Hemostasis:

Hemostasis is process of formatting clot in the wells of damaged blood vessels and preventing blood loss while maintaining blood in a fluid state within the vascular system.

A collection of complex interrelated systemic mechanisms operates to maintain a balance between coagulation and anticoagulant.(Kim Eatal,2012).

The hemostasis contains two pathway primary hemostasis (platelet clotting) and secondary hemostasis (coagulation cascade).

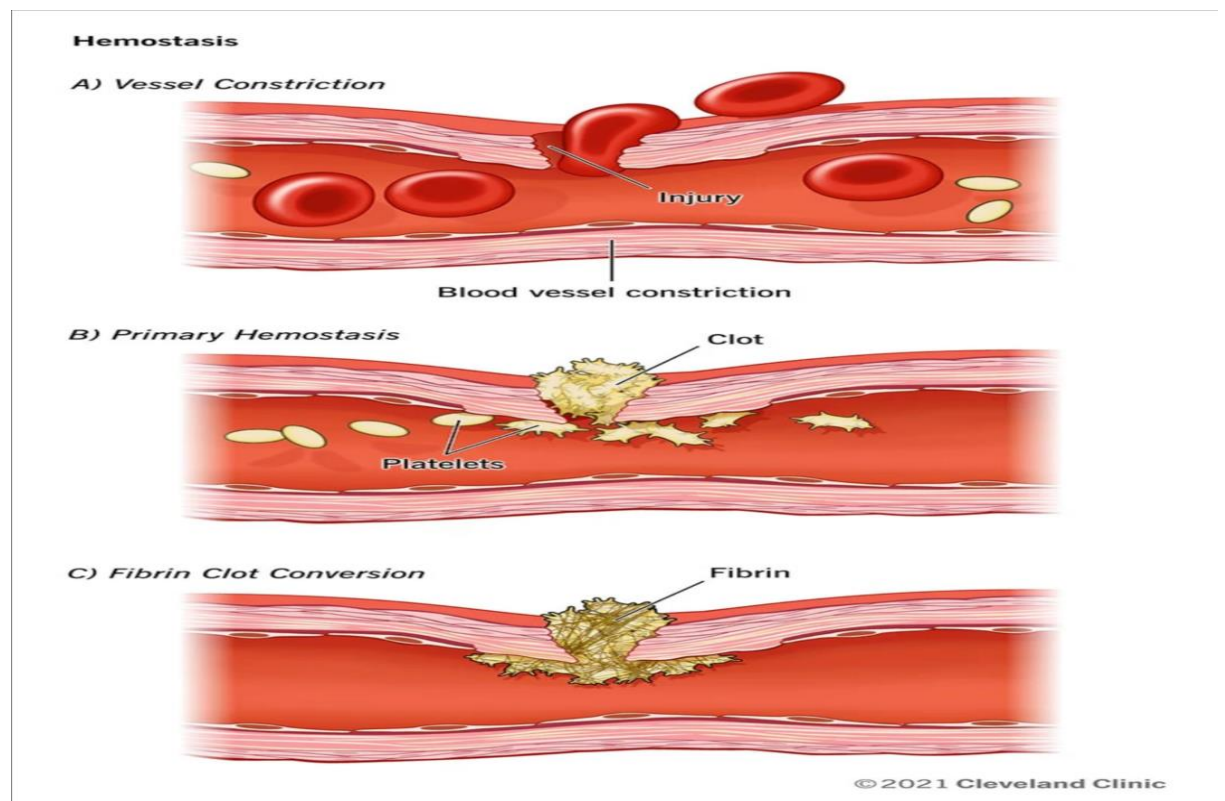


Figure 1.1: hemostatic stage

1.1.1 Primary hemostasis:

The primary hemostasis is when the body forms a temporary plug to seal an injury. To accomplish that, platelets that circulate in the blood stick to the damaged tissue and activate. That activation means they can "recruit" more platelets to form a platelets "plug" to stop blood loss from the damaged area. That clot works much like a cork or bottle stopper, keeping blood in and debris or germs out. Primary hemostasis may also involve constricting.

1.1.2 Secondary hemostasis :

Coagulation system the process by which a blood clot is formed in order to stop bleeding.

Coagulation consists of three pathways, the extrinsic common and intrinsic pathway, that interact together to form a stable blood clot. The extrinsic and intrinsic coagulation pathways both lead into the final common pathway by independently activating factor. The extrinsic pathway involves initiation by factor III (i.e, tissue factor) and its interacting with factor VII. Whereas, factor XII, XI, IX, and VIII are utilized in the intrinsic pathway. Then, the common pathway uses factor X, V, II, I, and XIII.

1.1.2.1 Extrinsic pathway:

The extrinsic pathway begins when there is injury to the endothelial tissue (i.e, skin tissue), exposing tissue factor. Factor VII is present in the blood and requires vitamin K to be activated.

Meanwhile, the intrinsic pathway begins when factor XII or the Hageman factor.

1.1.2.1.1 Prothrombin time and International Standard Ratio:

The prothrombin time (PT) test measures how long it takes for a clot to form in a blood sample. INR (International Standard Ratio) is a type of calculation based on the results of a PT test.

Prothrombin is a protein made by the liver. It is one of several substances known as coagulation (clotting) factors. When you get a cut or other injury that causes bleeding, clotting factors work together to form a blood clot. How quickly your blood clots depend on the amount of clotting factors in your blood and whether they're working properly. If your blood clots too slowly, you may bleed a lot after the injury. If the blood clots too quickly, dangerous clots may form in the arteries or veins.

The prothrombin time measures the clotting time of recalcified plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system. Although originally thought to measure prothrombin, the test also depends on factors V, VII and X and on the fibrinogen concentration of the plasma

1.1.2.2 Intrinsic pathway:

The intrinsic pathway begins when factor XII or the Hageman factor is exposed to collagen, kallikrein, and (HMWK) and is subsequently activated. Factor XI into XIa activates factor IX then, factor VIII is found in the blood and is often activated by thrombin (factor IIa).

1.1.2.2.1 Activated Partial Thromboplastin Time:

Activated partial thromboplastin time Specific variations of the APTT test are known as the (PTTK) and (KCCT), reflecting the methods used to perform the test.

1.1.2.3 Common pathway

The common pathway may result after the activation of factor Xa, Va, and calcium bind together ,forming a prothrombinase complex then activates prothrombin (factor II) into thrombin (factor IIa) .next, thrombin cleaves fibrinogen (factor I) into fibrin (factor Ia) .afterwards, thrombin cleaves the stabilizing factor (factor XIII) into XIIIa . Factor XIII binds with calcium to then create fibrin crosslinks to stabilize the clot. Thrombin has several functions, including activating platelets (cell fragments involved in clot formation) and activating factors V ,VIII, and IX

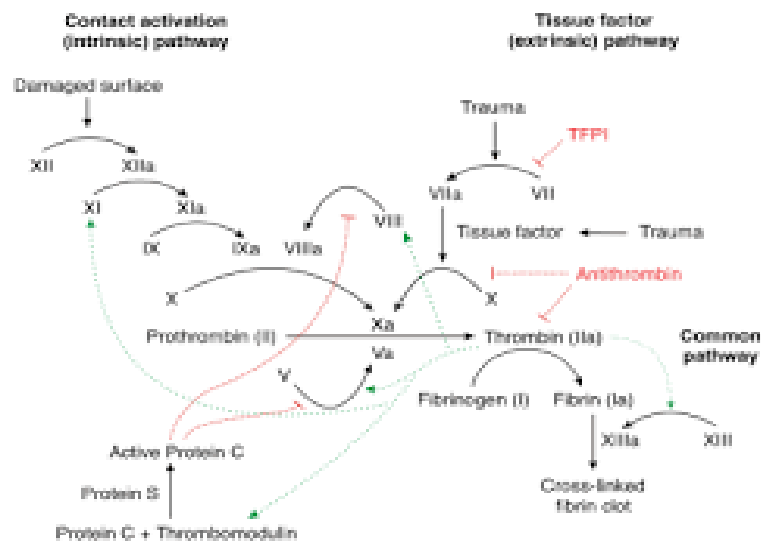


Figure 1.2: coagulation cascade

1.2Pregnancy:

Pregnancy term used to describe the period in which a fetus develop inside woman's womb. That leads to pressure in the blood system, leading to physiological changes the total blood volume increases by about 1.5 liter.

Decreases hemoglobin, increases plasma size by about 10–15 % at 6–12 weeks of gestation, and increases the mass of red cells(driven by an increase in maternal erythropoietin production) also increases to 25%, but relatively less, which does not lead to an increase in hemoglobin. This is accompanied by changes in coagulation (State of hypercoagulation) and produces an increase in factors (I-II-VII-VIII-IX-XII) and also accompanied by low levels fibrinolysis such as protein s, which acts as a activation for protein C. Luckily it's important in reducing of blood loss in the event of birth and reducing clots during and after pregnancy. Unfortunately the hypercoagulable state, thromboembolism is one of the leading causes of death associated with pregnancy. The hypercoagulability of blood during pregnancy has been confirmed with (TEG) and is thought mainly due to the increased production of factor VII and fibrinogen. Although many of the coagulation factors are increased during pregnancy, none are quite to the extent of factor VII and fibrinogen. The risk of developing a venous thromboembolism increases about 100-fold when a parturient is admitted to a hospital and also seems to be worse during the third trimester. This is thought to be due to changing hormonal levels, in particular increased estrogen as pregnancy progresses. Also the hormones can effect by pregnancy like (HCG) , (HPL) ,estrogen ,and progesterone.

1.2.1: Coagulation change:

Pregnancy is associated with significant changes in the hemostatic profile. The activity of fibrinogen, coagulation factor VII, VIII, X and XII, vWF and ristocetin increases significantly with the progression of pregnancy. Increased levels of clotting factors are due to increased protein synthesis mediated by elevated estrogen levels. In laboratory experiments, pregnant plasma has been shown to be able to increase thrombin production (*Boer et al,1989*).

Thus, pregnancy is a thrombotic condition. In pregnancy, the APTT is usually shortened, up to 4 seconds in the third trimester, largely due to the hormone-influenced increase in factor VIII. However, no noticeable changes occur in PT(*Ramsay,2010*) .

There are changes in the levels and activity of natural anticoagulants as well. In addition, the levels and activity of Protein C remain in the same rang. Total and free (ie, bioavailable) protein S levels gradually decline as pregnancy progresses. Antithrombin levels and activity are usually stable throughout pregnancy, decrease during labor and rise again shortly after delivery. Acquired active protein C (APC) resistance has been found during pregnancy, even in the absence of factor V Leiden and antiphospholipid antibodies(*Clark et al,1998*) .

This has been attributed to the high factor VIII and factor V activity and low free Protein S levels. Hence, APC sensitivity ratio does not serve as a screening test for Factor V Leiden during pregnancy.Coagulation factors remain elevated for up to 8–12 weeks post-partum and assays for them may be falsely negative during this period.

Rational:

Generally used to assess coagulation disorders in patients with abnormal bleeding due to deficiencies within the intrinsic coagulation cascade, for monitoring anticoagulant therapy with unfractionated heparin and for detecting inhibitors of blood coagulation which tend to produce prolonged PT and APTT. On the other hand, shortened PT and APTT has long been thought of as an artifact secondary to problematic blood collections, storage or processing leading to in-vitro activation. However, recent evidence suggests that shortened and APTT might actually reflect in-vivo events that might be associated with hypercoagulability. Use of different commercial reagents was also one of the controversies for a shortened PT and APTT but they have also been found to have a good agreement.

Objectives:**General objective:**

-To evaluate the influence of normal pregnancy on blood coagulation and to explore change in activity from first trimester through the third trimester.

Specific objectives:

- To estimate PT and APTT in case.
- To estimate PT and APTT in control.
- To correlate the result of test control.
- To correlate the result with possible risk factor.

Chapter Tow:
Previous
studies.

2. Previous studies

The reference intervals for PT, and APTT at first trimester were 10.90-13.85 s, 24.60-39.28 s, respectively. The reference intervals at second trimester, 9.70-12.64 s, 24.16-35.43 s, respectively. For the third-trimester, the intervals were 9.20-11.95 s, 23.90-35.51 s, the third-trimester group showed decreased PT, APTT as compared with the other groups. In this study, level changes of coagulation parameters in different trimesters were observed. And the ranges for coagulation parameters were presented, which may provide some reference for clinicians to more accurately monitor the coagulation in pregnant women. (Wengonget *al*,2021)

Compare pregnant women to non-pregnant women in terms of coagulation changes during all three trimesters of pregnancy. PT and PPT showed decrease in third trimesters in pregnant women and PT also decrease in second trimester, while PTT decrease in all three trimesters in pregnant women when compared with non-pregnant women. These parameters also decrease in third trimester when compared with first trimester. In , a condition of physiological low-grade intravascular coagulation characterizes normal pregnancy. In this research, in each trimester, there were alterations in the levels of coagulation parameters. In addition, the ranges for coagulation measures were supplied, which may help clinicians track coagulation in pregnant women more effectively. (Mazinet *al*, 2020-2021).

Study was carried out to determine the Prothrombin Time (PT), Student t-test analysis revealed a statistically significant decrease (P-value =0.002)

In the mean PT of the pregnant women (12.82 seconds with SD of ± 1.84) compared to the control group (14.29 seconds with SD of ± 1.75). The study also showed a significant gradual decrease in the mean PT as pregnancy progresses toward term as 13.85 ± 1.68 , 13.21 ± 1.51 and 11.40 ± 1.47 in the first, second and third trimesters respectively (P- value=0.001). (Moses D Lugos *et al*,2018)

The means of the APTT were significantly lower in the first, second and third trimesters compared with controls (35.59 ± 4.95 seconds, 32.22 ± 5.79 seconds and 29.60 ± 3.66 seconds, respectively, vs. 40.55 ± 5.95 seconds; $p = 0.01$). (Ibehet *al* , 2015)

Chapter Three: Materials and Methods .

3. Materials and Methods

3.1 Study design:

Case control study

3.2 Study area and duration:

This study conducted random in Khartoum Sudan hospital. During the period from July 2022 to compare the coagulation factor parameters between pregnant lady and non-pregnant lady.

3.3 study population:

The data and samples were collected from 50 pregnant lady and 50 from healthy non-pregnant lady.

3.3.1 Inclusion criteria:

Sample will be collected from healthy pregnancy lady, included all different age.

3.3.2 Exclusion criteria:

Autoimmune disease, and cancer will be excluded.

3.4 Methods

3.4.1 Equipment:

- cotton
- 70% alcohol
- Syringes
- Trisodium citrate
- APTT and PT reagent
- Coagulometer

3.4.2 Sample collection:

Venous blood was drawn after sterilization by 70% alcohol, syringe was used to puncture the antecubital fossa area of non-dominant hand.

3.4.3 Measurement of coagulation profile:

APTT and PT were measured by automated technique using automated hematology coagulometer (STAGO).

3.4.3.1 Coagulometer information:

Steel ball coagulometer Semi automated no was (37) from STAGO company.

3.4.3.2 Principle:

The ball coagulometer was used where a stainless steel ball is kept within a magnetic field. It provides the clotting time in seconds, due to the displacement of the ball by a fibrin thread. The magnetic sensor detects this change in position and the time measurement is stopped.

3.4.3.3 Procedure:

3.4.3.3.1 Prothrombin time:

- Add caveat in incubation place with the steel-ball .
- Add 50 ml of sample and waited 5 min.
- Remove the mixture to measurement place and add 100 ml of thromboplastin reagent and read the result.

3.4.3.3.2 Activated partial prothrombin time:

- Add caveat in incubation place with the steel-ball
- Add 50 ml of sample (ppp) and R₁(Kaolin mixed with phospholipids) and wait for 10 min
- Remove the mixture to measurement place and add 50 ml of Calcium chloride and read the result.

3.5 Data analysis:

The data of this research we analyzed by SPSS programmer using mean independent and T test one way nova.

Chapter four: Results.

4. Results

This is a case control study that conducted in Khartoum Sudan hospitals during period July 2022 ,to compere the coagulation parameter(PT,INR and APTT)between pregnant lady and non-pregnant lady.100samples collected from lady 50 pregnant 17 from first trimester 15 from second trimester and 18 from third trimester and 50 from non-pregnant lady.

Fig4.1: shows that the mean of PT in pregnant lady was 15.804seconds and in non-pregnant lady 15.798seconds.

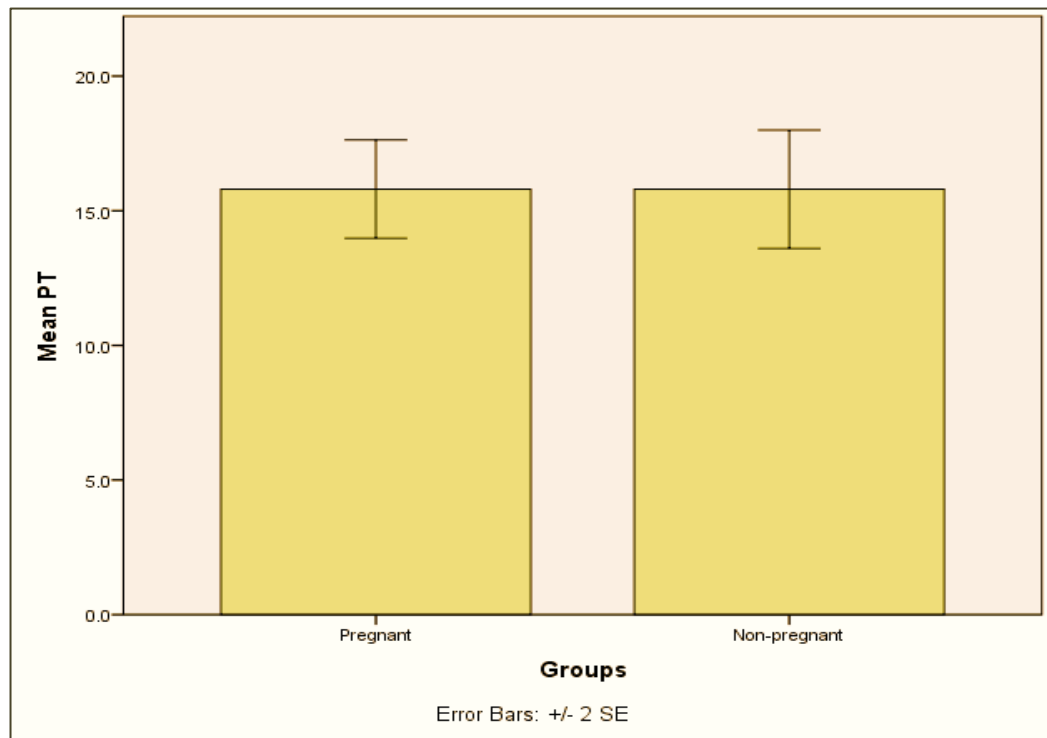


Fig4.1: Variation of PT between pregnant and non-pregnant lady.

Fig4.2: shows that the mean of INR in pregnant lady was 1.155 and in non-pregnant lady 1.13.

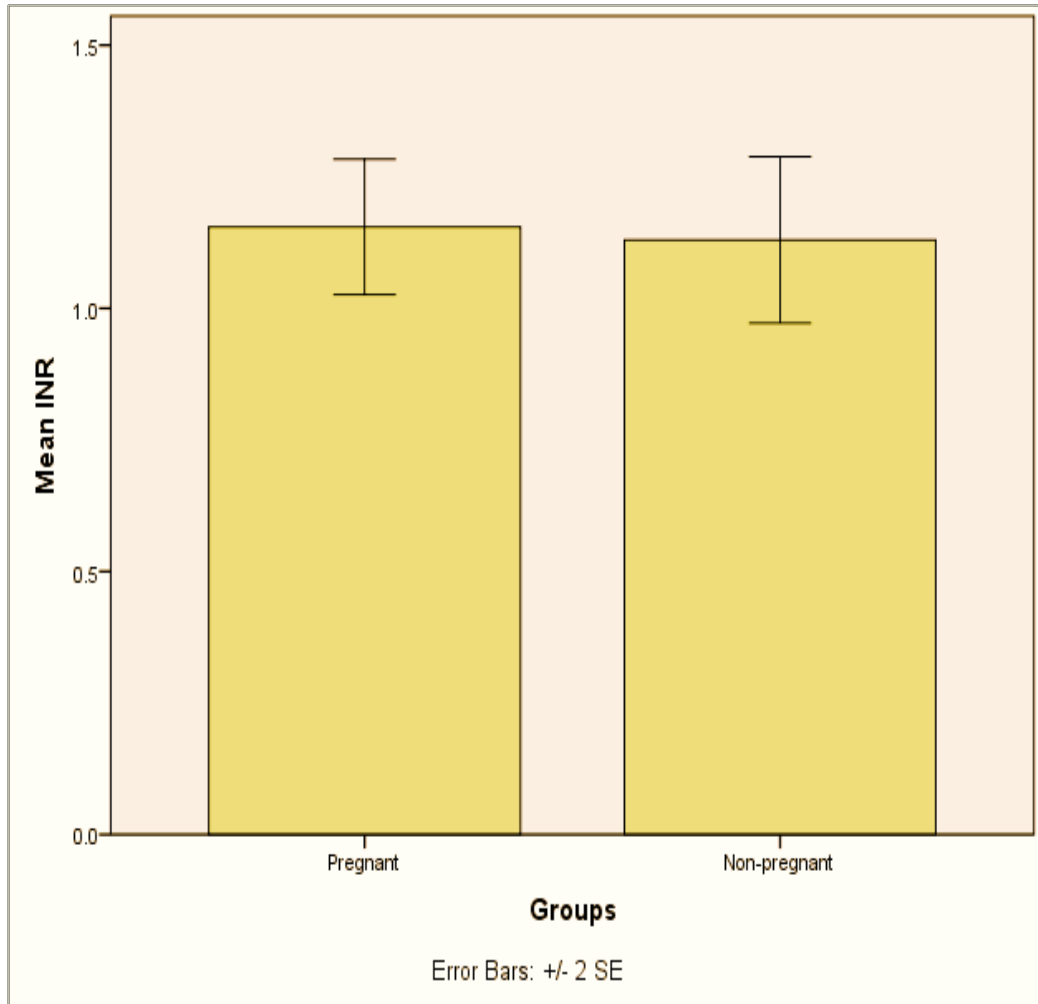


Fig4.2: Variation of INR between pregnant and non-pregnant lady.

Fig4.3: shows that the mean of APTT in pregnant lady was 28.702 seconds and in non-pregnant lady 31.69 second.

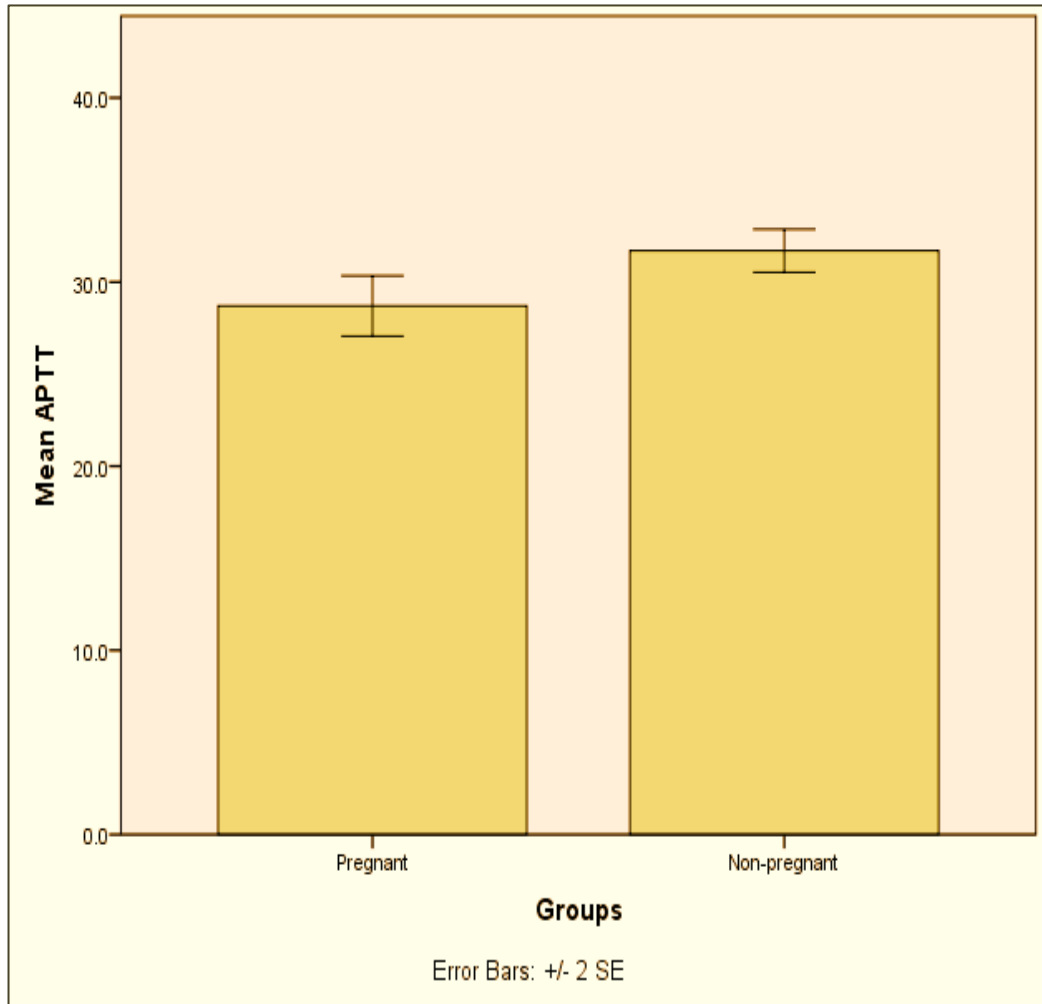


Fig4.3: Variation of APTT between pregnant and non-pregnant lady.

Fig4.4: shows that the mean of PT in pregnant lady at first trimester 21.171seconds, second trimester13.933seconds and third trimester 12.294seconds with non-pregnant lady15.798seconds.

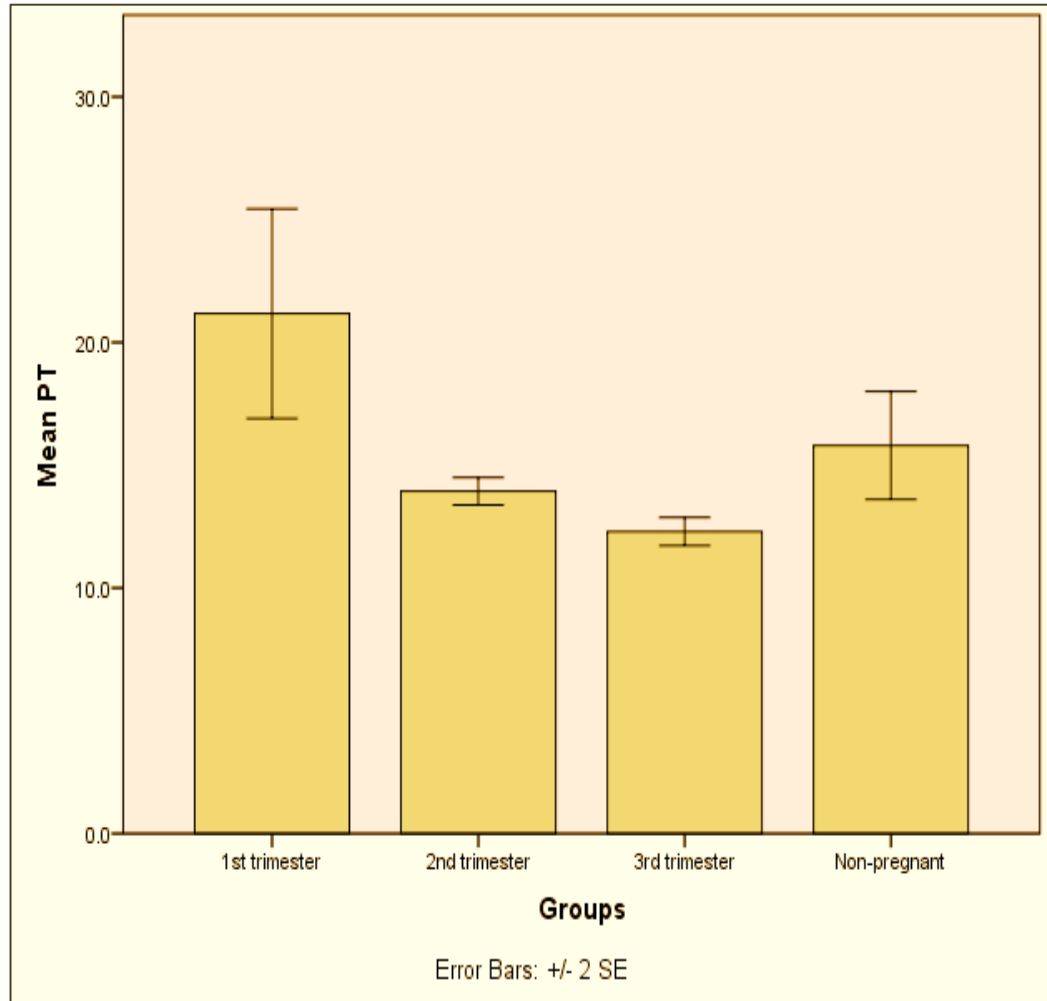


Fig4.4: Variation of PT between pregnant at different trimester and non-pregnant lady.

Fig4.5: shows that the mean of INR in pregnant lady at first trimester 1.608 ,second trimester 1.02 and third trimester 0.839 with non-pregnant lady 1.13.

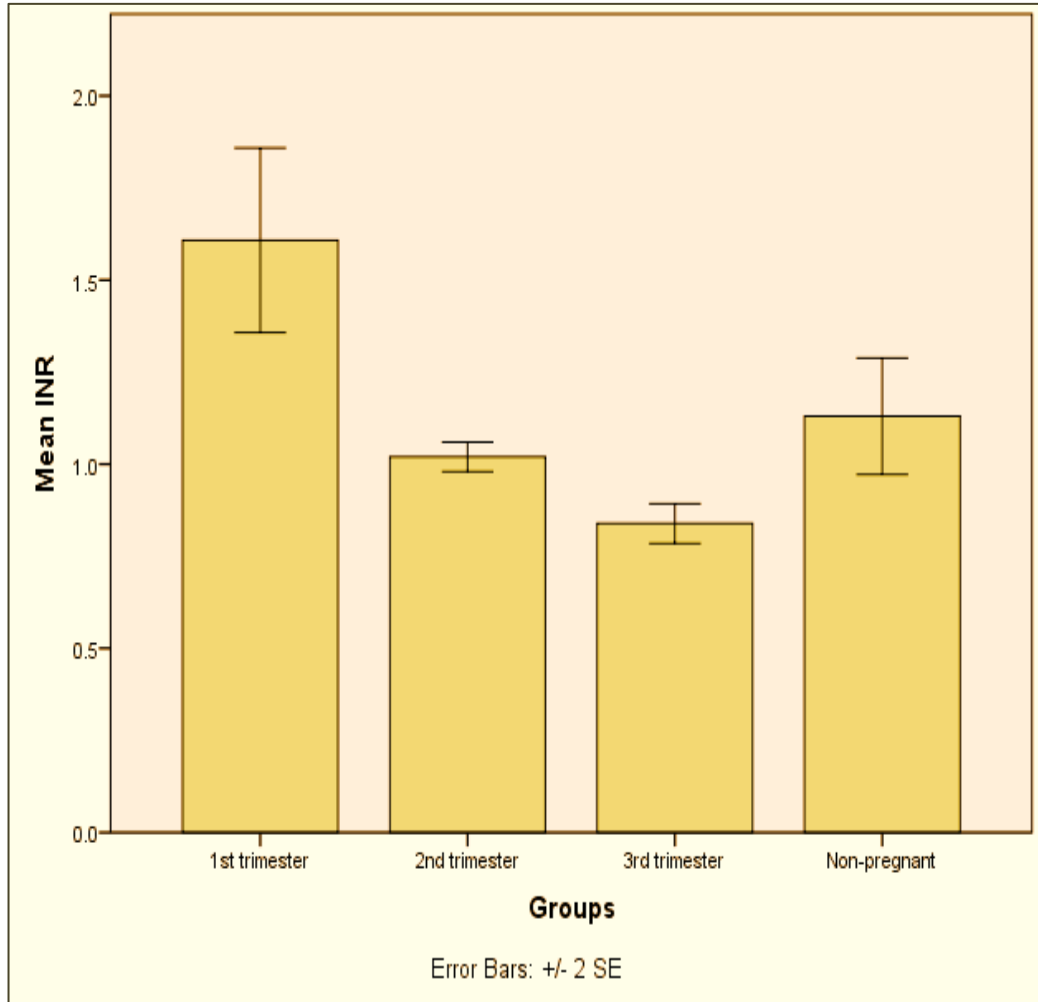


Fig4.5: Variation of INR between pregnant at different trimester and non-pregnant lady.

Fig4.6: shows that the mean of APTT in pregnant lady at first trimester 29.394 seconds ,second trimester26.707 seconds and third trimester 29.711seconds with non-pregnant lady31.69seconds.

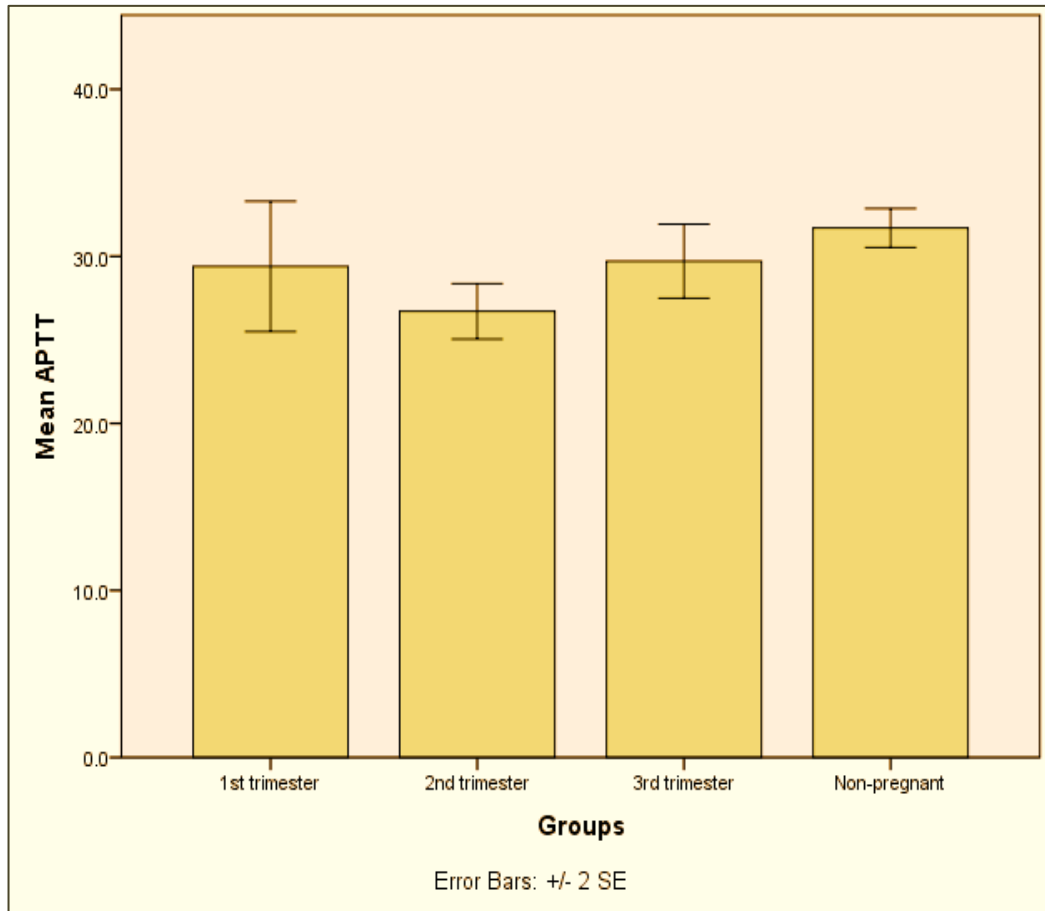


Fig4.6: Variation of APTT between pregnant at different trimester and non-pregnant lady.

Table4.1:The mean and P value of (PT,INR and APTT) in the pregnant lady at all trimester and non-pregnant lady.

One Way ANOVA-Test						
Parameters	Groups	Number	Mean	Std.Deviation	Std.Error	P value
PT	1 st trimester	17	21.171	8.7983	2.1339	0.001**
	2 nd trimester	15	13.933	1.0834	0.2797	
	3 rd trimester	18	12.294	1.2221	0.288	
	Non-pregnant	50	15.798	7.7763	1.0997	
APTT	1 st trimester	17	29.394	8.0325	1.9482	0.008**
	2 nd trimester	15	26.707	3.2132	0.8297	
	3 rd trimester	18	29.711	4.697	1.1071	
	Non-pregnant	50	31.69	4.12	0.5827	
INR	1 st trimester	17	1.608	0.5167	0.1253	0.000**
	2 nd trimester	15	1.02	0.0775	0.02	
	3 rd trimester	18	0.839	0.1145	0.027	
	Non-pregnant	50	1.13	0.5596	0.0791	

Table 4.1:we showed the mean,std.deviation for each trimester and parameter also show the p.value in (PT=0.001) significant , (INR=0.000) significant and (APTT=0.008) insignificant.

Chapter five:

Discussion

5. Discussion

This is descriptive study in Khartoum hospitals during the period from June 2022 to December 2022, to detect the variation of PT, INR and APTT between pregnancy and non-pregnant lady attending Khartoum Sudan lady. 50 sample were collected from pregnancy, 50 from non-pregnant lady.

The mean of PT in pregnancy was (15.804) and the mean of PT and in non-pregnant lady was (15.798) insignificant difference in PT ($P=0.997^*$), this show differently in comparison with other study in PT ($P=0.002$) (Moses D Lugos *at al*, 2018).

The mean APTT in pregnancy lady was (28.702) and in normal lady was (31.69) there is significant in ($P=0.004^*$), this show similarity in comparison with other study in which the p value of APTT was ($p=0.01$) (Ibehat *al*, 2015).

Also in other study we similar with APTT which it decrease in all three trimester, and difference with it at PT (Wengonget *al*, 2021).

Chapter six:
Conclusions
and
recommendati
on's.

6. Conclusion and Recommendation

6.1 Conclusions:

We chose 50 samples from non-pregnant lady as control.

The frequencies of trimester individuals were 50 pregnancy and 50 lady while the frequencies of PT and APTT.

We compared the pregnant lady results with results of 50 non-pregnant women which serve as controls. PT & APTT showed decrease in third trimesters in pregnant women and PT also decreases in second trimester, while APTT decrease in all three trimesters in pregnant women when compared with non-pregnant women. These parameters also decrease in third trimester when compared with first trimester.

6.2 Recommendations

Determination the variation of coagulation parameters between pregnant lady and non-pregnant lady in correlation to age, medication, platelet count and function and other coagulation parameter e,g(Thrombin time, Bleeding time and Clotting time ...ect).

Determination the variation of chemical parameters between pregnancy and non-pregnant lady like(Proteins and Steroid hormone... ect)

Determination the variation of PT and APTT between pregnancy and non-pregnant lady by using larger samples and other biostatistics' test.

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Rafidain University College, Iraq.(COAGULATION CHANGES IN DIFFERENT TRIMESTERS OF PREGNANCY).

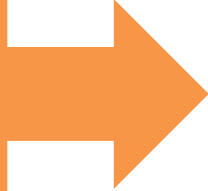
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Appendixes

Questions to collect the samples:



بسم الله الرحمن الرحيم

اسم المريضة:.....

العمر:.....

عمر الحمل:

الشهور الثلاث الاولى

الشهور الثلاث الوسطى

الشهور الثلاث الاخيرة



**Semi-automated
coagulometer**



Cuevate



Steel ball :