

## Evaluating some haemostatic profiles and platelet aggregation in gestational diabetes mellitus subjects

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### Abstract

**Background:** Hypercoagulability has been linked to an increase in risk factors, making diabetes a major cause of morbidity and mortality in low-income countries like Nigeria. This research examined some haemostatic parameters in women with gestational diabetes mellitus (GDM).

**Methodology:** A total of 100 subjects consisting of 40 GDM, 30 non-gestational diabetes pregnant women (NGPW) attending the ante-natal clinic of the hospital and 30 women with neither diabetes nor pregnancy (NDNP) were enrolled as controls in this study. Ten milliliters of blood was collected and dispensed into an appropriate anticoagulant bottle. Prothrombin time (PT), Activated partial Thromboplastin Time (APTT), complete blood count (CBC) and fasting blood sugar (FBS) was estimated using standard techniques.

**Results:** There was a significantly higher in the mean values of FBS, PT, APTT and WBC among GDM and NGPW when compared with NPNP ( $p < 0.05$ ), while PCV was lower. FBS has positive correlation with PT and APTT while it shows negative correlation with platelet. APTT had a little edge over PT with higher area under the ROC curve of 0.997 than PT among GDM.

**Conclusion:** In this study, women with GDM have considerably longer PT and APTT than NGPW and NDNP. Therefore, there may be possibility of haemorrhagic complications in gestational diabetes.

**Keywords:** APTT, Gestational diabetes, Coagulation, Atherosclerosis, Hyperglycaemia

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Received: September 22, 2022

Accepted: April 22, 2023

Published: June 30, 2023

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<http://dx.doi.org/10.4314/rejhs.v11i2.9>

## Évaluation de certains profils hémostatiques et agrégation plaquettaire chez des victimes diabétiques gestationnels

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### Résumé

**Contexte général de l'étude:** L'hypercoagulabilité a été associée à une augmentation des facteurs de risque, faisant du diabète une cause majeure de morbidité et de mortalité dans les pays à faible revenu comme le Nigéria. Cette recherche a examiné certains paramètres hémostatiques chez les femmes atteintes de diabète sucré gestationnel (DSG).

**Méthode de l'étude :** Un total de 100 victimes comprenant 40 DSG, 30 femmes enceintes diabétiques non gestationnelles (FEDN) fréquentant la clinique prénatale de l'hôpital et 30 femmes sans diabète ni grossesse (FSDG) ont été recrutés comme témoins dans cette étude. Dix millilitres de sang ont été prélevés et distribués dans une bouteille d'anticoagulant appropriée. Le temps de prothrombine (TP), le temps de thromboplastine partielle activée (TTPA), la formule sanguine complète (FSC) et la glycémie à jeun (LGJ) ont été estimés à l'aide de techniques standard.

**Résultat de l'étude :** Il y avait une augmentation significative des valeurs moyennes de LGJ, TP, TTPA et WBC chez les DSG et FEDN par rapport au NPNP ( $p < 0,05$ ), tandis que le PCV était inférieur. Le LGJ a une corrélation positive avec le TP et le TTPA alors qu'il montre une corrélation négative avec les plaquettes. TTPA avait un petit avantage sur PT avec une aire sous la courbe ROC de 0,997 plus élevée que PT parmi les DSG.

**Conclusion :** Dans cette étude, les femmes atteintes de DSG ont un TP et un TTPA considérablement plus longs que le FEDN et le FSDG. Par conséquent, il peut y avoir une possibilité de complications hémorragiques dans le diabète gestationnel.

**Mots-clés :** TTPA, diabète gestationnel, coagulation, athérosclérose, hyperglycémie

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## INTRODUCTION

Globally, diabetes affects more than 370 million people, resulting in approximately five million deaths, and more than 471 million USD are spent yearly on treatment (1,2). Nearly 3.96 million people aged 20-79 die every year because of diabetes. Death rates from diabetes are between 6 and 15 % in Africa and North America, respectively, suggesting that regional differences exist (3,4). According to Fadairo et al. (5), 4.3% of the population in Africa has diabetes, that is about 15 million people. As a form of diabetes, gestational diabetes is characterized by intolerance to carbohydrates of varying severity that develops or manifests for the first time during pregnancy. This is associated with insulin deficiency in the context of insulin resistance (6,7). Obesity, a history of gestational diabetes, a family history of type 2 diabetes, and polycystic ovarian syndrome are all risk factors. A healthy weight and prenatal exercise are preventative measures. Insulin injections, a diabetic diet, and regular physical activity are all effective ways to control gestational diabetes (8). Diet and exercise allow a significant number of women to control their blood sugar levels. Pregnancies are affected by gestational diabetes at a rate of 3-9%, depending on the community. It typically occurs in the third, fourth, and sometimes fifth months of pregnancy. It affects 1% of individuals younger than 20 years of age and 13% of those older than 44 years of age (6,9,10).

It has been noted that patients with gestational diabetes mellitus have a higher risk of coagulation abnormalities and thromboembolic events (11). Many signaling pathways are disrupted, and metabolic alterations such as insulin resistance, hyperglycemia, and dyslipidaemia promote platelet adhesion, activation, and aggregation, which is platelets' primary function (12). In women with gestational diabetes, increased platelet activation and release of prothrombotic and proinflammatory agents has been linked to systemic inflammation, oxidative stress, impaired calcium metabolism, decreased nitric oxide bioavailability, and increased phosphosylation and glycosylation of cellular proteins (12,13,14). Increased mean platelet volume (MPV) indicates more active platelets due to elevated levels of prothrombotic substances like thromboxane A<sub>2</sub>, thromboxane B<sub>2</sub>, platelet factor 4, serotonin, and platelet derived growth factor (15).

Mortality in people with diabetes is primarily due to atherothrombotic complications. From the beginning of pregnancy, pregnant

women have higher rates of coagulation than non-pregnant women, and this trend may be much more pronounced in women with GDM (16). Consistent with this, increased coagulation indices are a hallmark of late pregnancy alongside hormonal shifts and the risk of hepatic embolism. However, diabetes makes hypercoagulation worse, which might be a problem during pregnancy (17). The evaluation of coagulation issue management in gestational diabetes and its integration into optimal care would be improved by this study.

## MATERIALS AND METHODS

### Study Design

This is a descriptive, comparative study. The study was conducted in 2021 between January and August. The study was carried out at the Federal Medical Centre (FMC), Owo, which is a tertiary medical facility in Nigeria. A total of 100 subjects consisting of forty (40) gestational diabetic subjects (GDM), thirty (30) age non-gestational diabetes pregnant women (NGPW) attending the ante-natal clinic of the hospital and thirty (30) women with neither diabetes nor pregnancy (NDNP) were enrolled as controls in this study.

**Ethical Considerations:** The research approaches employed in the clinic were properly explained to the individuals taking part in this study before they were requested to sign a written consent. The Health Research Ethics Committee, Federal Medical Centre, Owo granted ethical approval for this study with reference number FMC/OW/380/VOL.CXVII/133.

### Inclusion and Exclusion Criteria

**Inclusion Criteria:** The study enrolled women aged 18 to 50 who had gestational diabetes. The control group included apparently healthy pregnant women who did not have gestational diabetes, as well as women between the ages of 18 and 50 who did not have diabetes or were not pregnant.

**Exclusion Criteria:** Participants with known comorbidities, such as hypertension, HIV, hepatitis, cancer, oral anticoagulant treatment, bleeding tendencies, and so on, as well as breastfeeding mothers, were excluded from the study. The controls were subjected to the same exclusion criteria as the subjects.

### Collection and Storage of Samples

A tourniquet was applied to each participant's arm above the elbow while blood

was drawn. Following a 12-hour fast, ten milliliters (10ml) of venous blood was obtained from each individual using aseptic technique. Four milliliters of venous blood were dispensed into a 5 milliliter sterile vacutainer bottle containing 0.5 milliliters of 3.2% tri- sodium citrate solution in a blood- citrate, 9:1 (v/v) ratio as an anticoagulant and gently mixed by inverting the container several times for the determination of PT and APTT. After centrifuging the blood for 10 minutes at 2000g/m, the platelet poor plasma was collected for coagulation investigation. Tests were carried out within three hours of collecting the samples. Similarly, 3ml of venous blood was dispensed into a fluoride oxalate container for glucose estimation and 3ml of blood into an ethylene diamine tetraacetic acid (EDTA) bottle for complete blood count.

### Analytical Methods

The glucose oxidase method was used to determine plasma fasting blood glucose using reagents supplied by Randox Laboratories Ltd. (UK). The PT and APTT were determined using the standard procedures of Dacie et al. (18). A haematological analyzer was used to determine the complete blood count (CBC). Weight and height were measured for all participants, and body mass index (BMI) was determined as described by Atere et al (19).

### Statistical analysis of data

A statistical package for social sciences version 23.0 (SPSS Inc, Chicago, IL) was used for the analysis of data appropriately. A one-way analysis of variance (ANOVA) was used to compare the groups. Correlation and the ROC curve were also applied to examine the relationship between variables. For all quantitative values, data were presented using mean  $\pm$  standard deviation (mean  $\pm$  SD). The 95% confidence interval was used as the level of significance, and a P value less than 0.05 was considered significant.

### RESULTS

A total number of 100 subjects comprising 40 gestational diabetic subjects (GDM) with mean age (27.70 $\pm$ 3.98) years, 30 non-gestational diabetes pregnant women (NGPW) with mean age (27.93 $\pm$ 4.76) years and 30 women with neither diabetes nor pregnancy (NDNP) with mean age (27.40 $\pm$ 4.38) years were studied. Table 1 shows comparison of anthropometric indices in GDM, NGPW and NDNP. Pregnant women had significantly higher

systolic blood pressure (SBP) than women who were not pregnant or had diabetes.

In table 2, biochemical parameters in GDM, NGPW and NDNP were compared using independent One Way Analysis of Variance (ANOVA). There was a significantly higher in the mean values of FBS, PT, APTT and WBC among GDM and NGPW when compared with NDNP ( $p < 0.05$ ), while PCV was lower. Posthoc statistical analysis showed a significant higher in mean values of FBS, PT, APTT and PCV, but lower WBC mean value when GDM group was compared with NGPW group ( $p < 0.05$ ).

Figures 1-3 show correlation of plasma levels of FBS in gestational diabetic subjects with haematological parameters. FBS has positive correlation with PT and APTT (Figure 2 and 3) even though it not statistically significant and considerably positive association with PCV (Figure 1) while it shows negative correlation with platelet. The diagnostic performance of PT, APTT and platelet were determined as diagnostic tool in Gestational Diabetic subjects' coagulation study. APTT had a little edge over PT with higher area under the ROC curve (AUROC) of 0.997 than PT with area 0.986 (Figure 4).

### DISCUSSION

Diabetes has been linked to several disease burdens in developing countries such as Nigeria, making it one of the leading causes of morbidity and mortality, with a reported increase in risk factors leading to a variety of health issues such as kidney failure, cardiovascular disease, and blindness (10,20). Gestational diabetes mellitus (GDM) is a pregnancy condition characterized by spontaneous hyperglycemia during pregnancy (12,21).

In this study, a total number of 100 subjects comprising 40 GDM with mean age (27.70 $\pm$ 3.98) years, 30 NGPW with mean age (27.93 $\pm$ 4.76) years and 30 NDNP with mean age (27.40 $\pm$ 4.38) years were studied. The difference in the mean age of the study respondents was not statistically significant when compared across the groups in this study. This result contradicts with earlier research work by Liu et al. (22), which demonstrated that the GDM-affected women were much older than NGPW. Pregnant women had significantly greater systolic blood pressure (SBP) than women who were not pregnant or who had diabetes. Contrary to the findings of Bakker et al. (23), that indicated no discernible distinction between pregnant women and women who were not pregnant or diabetic.

This study also shows a significantly

higher in the mean values of FBS, PT, APTT and WBC among GDM and NGPW when compared with NDNP ( $p < 0.05$ ), while PCV was lower in both GDM and NGPW groups. Post hoc statistical analysis showed a significant higher in mean values of FBS, PT, APTT and PCV, but lower WBC and platelets mean value when GDM group when compared with NGPW group ( $p < 0.05$ ). This is not in line with a study conducted at Federal Medical Center, Owerri, Nigeria by Okorie *et al.* (24) who showed that PCV in gestational diabetic women ( $33.84 \pm 3.26\%$ ) was significantly ( $p < 0.05$ ) higher when compared to controls ( $32.06 + 2.15\%$ ) but in agreement with platelet count showing a significantly lower Platelet count ( $p < 0.05$ ) in gestational diabetic women when compared to the control subjects.

The APTT is a performance measure that assesses both the intrinsic and common coagulation pathways for efficacy. Apart from detecting irregularities in blood clotting, it can also be used to track the therapeutic effects in patients on heparin therapy who are at risk of thrombosis. It is utilized in conjunction with PT, which is a test that examines the extrinsic pathway. Patients who have their PT and APTT shortened are at a higher risk of thrombosis (25,26). Thrombophilia is caused by coagulation abnormalities that occur as a result of diabetes mellitus, according to numerous studies. Hyperglycemia, which is also a distinguishing hallmark of diabetes, appears to be the cause of the coagulation abnormalities seen in diabetic individuals (27,28).

The Prothrombin time of GDM was found to be substantially longer than that of NGPW and NDNP in this study. The diabetic participants' APTT was likewise substantially longer than the controls', despite the fact that the readings were within normal limits. This finding is consistent with the outcome of previous studies by Alao *et al.* (28) and Fadairo *et al.* (5), which discovered a notable difference in the mean value of the PT between diabetic and control patients. According to McFarlane (29), these changes could be the result of circulatory disturbances in diabetic patients, which include changes in platelet count and activity, coagulopathy, fibrinolytic aberration, haemorrhagic factors, and abnormalities in endothelial metabolism.

Additionally, in this investigation, FBS had positive correlation with PT and APTT, though not statistically significant and considerably positive association with PCV ( $p < 0.05$ ) while it showed negative correlation

with platelet. The correlation between FBS and PCV is in agreement with the study by Akinloye *et al.* (30) who also showed a positive correlation between fasting plasma glucose (FPG) and PCV, while it is in disagreement with Nnenna *et al.* (31) who observed significant correlation between PT and APTT. This finding adds to the evidence that hyperglycemia plays a role in the development of prothrombotic alterations, as shown in clamp experiments (32). In addition to the faster development of atherosclerosis in diabetic individuals, a higher risk of thrombotic events was identified, which was attributed to a combination of elevated procoagulant activity and decreased fibrinolytic ability (32,33). Deficiencies in specific haematological parameters caused by pathological abnormalities may have an impact on other blood parameters. Uko *et al.* (34) showed that Type 1 Diabetes impacts the number of platelets, total white blood cells, and packed cell volume. The activated partial thromboplastin time is one of the haemostatic measures in diabetic patients, according to Zhao *et al.* (25).

The diagnostic performance of PT, APTT and platelet were determined as diagnostic tool in Gestational Diabetic subjects' coagulation study. APTT had a little edge over PT with higher area under the ROC curve (AUROC) of 0.997 than PT with area 0.986. This reiterated earlier statement made that 'the APTT is a performance measure that assesses both the intrinsic and common coagulation pathways for efficacy'. However, women with GDM alone or when diagnosed with diabetes are at increased risk of cardiovascular disease (CVD) in future (33,35).

## CONCLUSION AND RECOMMENDATION

The results of this study revealed that women with GDM had considerably longer PT and APTT when compared to non-gestational diabetes pregnant women (NGPW) and women who neither diabetic nor pregnant (NDNP). In addition, platelet levels in GDM patients were considerably lower than in NGPW and NDNP patients. Changes in these parameters may predispose pregnant women with gestational diabetes to bleeding disorders.

As a result, in patients with gestational diabetes, clinicians should not rule out the risk of bleeding and associated complications. Therefore, coagulation testing should be a key component of the screening procedure for pregnant women with gestational diabetes.

**Conflict of interest:** The authors have confirmed

that they have no competing interests.

**Funding:** Self-sponsored

**Acknowledgments:** The authors would like to thank the whole medical personnel of the Antenatal Clinic at the Federal Medical Centre, Owo, as well as all of the participants, for their assistance throughout this study.

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**Table 1: Comparison of anthropometric Indices in gestational diabetic subjects (GDM), pregnancy without gestational diabetes (NGPW) and women without diabetes or pregnancy (NDNP)**

	GDM (n=40)	NGPW (n=30)	NDNP (n=30)	P-Value
Age (Years)	27.70±3.98	27.93±4.76	27.40±4.63	0.896
SBP (mmHg)	117.63±5.99 <sup>c</sup>	123.17±7.82 <sup>a,b</sup>	115.50±5.47 <sup>c</sup>	0.000*
DBP (mmHg)	75.00±7.25	77.67±6.53	76.50±6.45	0.265
BMI (Kg/m <sup>2</sup> )	22.73±1.54	22.59±1.76	22.59±1.76	0.900

\* significant at p=0.05

a = significantly different from NDNP, b = significantly different from GDM group, c = significantly different from NGPW group

Key: n=sample size, SBP= Systolic blood pressure, DBP= Diastolic blood pressure, BMI= Body mass Index

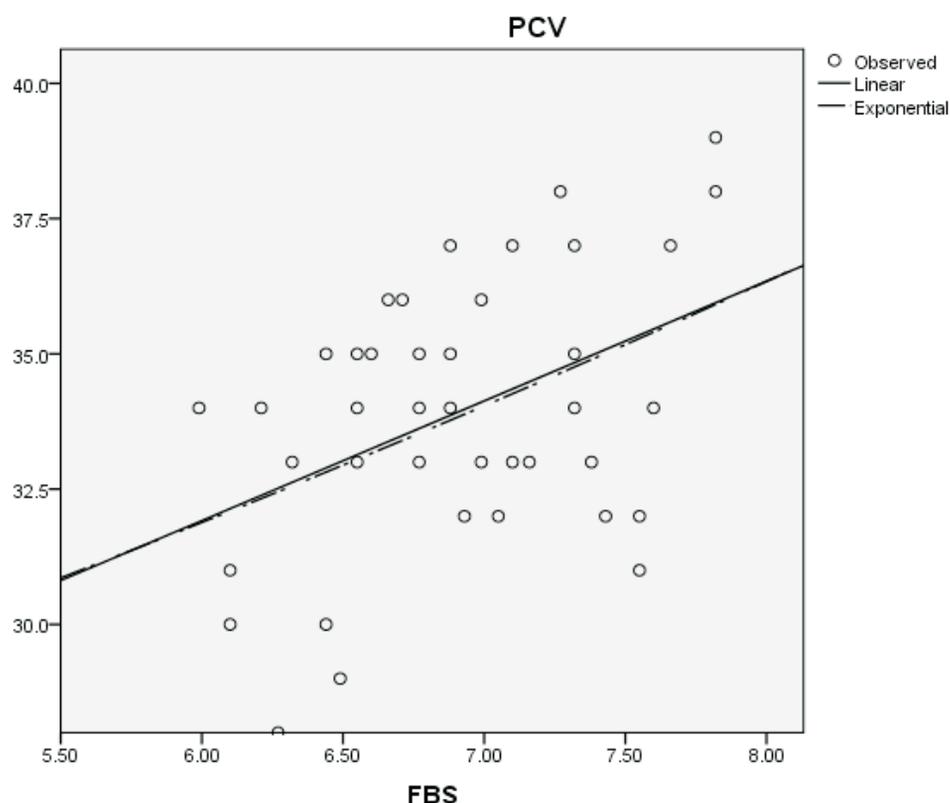
**Table 2: Biochemical parameters in in gestational diabetic subjects (GDM), pregnancy without gestational diabetes (NGPW) and women without diabetes or pregnancy (NDNP)**

	GDM (n=40)	NGPW (n=30)	NDNP (n=30)	P-Value
FBS (mmol/L)	6.91±0.49 <sup>a,c</sup>	4.50±0.43 <sup>b</sup>	4.40±0.26 <sup>b</sup>	0.000*
PT (Secs)	14.32±0.45 <sup>a,c</sup>	13.26±0.36 <sup>a,b</sup>	12.62±0.37 <sup>b,c</sup>	0.000*
APTT (Secs)	43.14±1.03 <sup>a,c</sup>	39.64±0.63 <sup>a,b</sup>	36.76±0.53 <sup>b,c</sup>	0.000*
PCV (%)	33.93±2.51 <sup>a,c</sup>	31.93±2.63 <sup>a,b</sup>	35.97±3.17 <sup>b,c</sup>	0.000*
WBC (xU/L)	8.11±1.07 <sup>a</sup>	8.67±1.58 <sup>a</sup>	5.50±1.45 <sup>b,c</sup>	0.000*
Platelet (xU/L)	177.85±15.29 <sup>a,c</sup>	196.30±15.28 <sup>b</sup>	203.00±23.28 <sup>b</sup>	0.000*

\* significant at p=0.05

a = significantly different from NGPW, b = significantly different from GDM group, c = significantly different from NDNP group

Key: n=sample size, FBS= Fasting blood sugar, PT= Prothrombin time, APTT= Activated Partial Thromboplastin Time, WBC= White blood cells



**Figure 1: Correlation between FBS and Packed Cell Volume (PCV) in Gestational Diabetic Subjects**

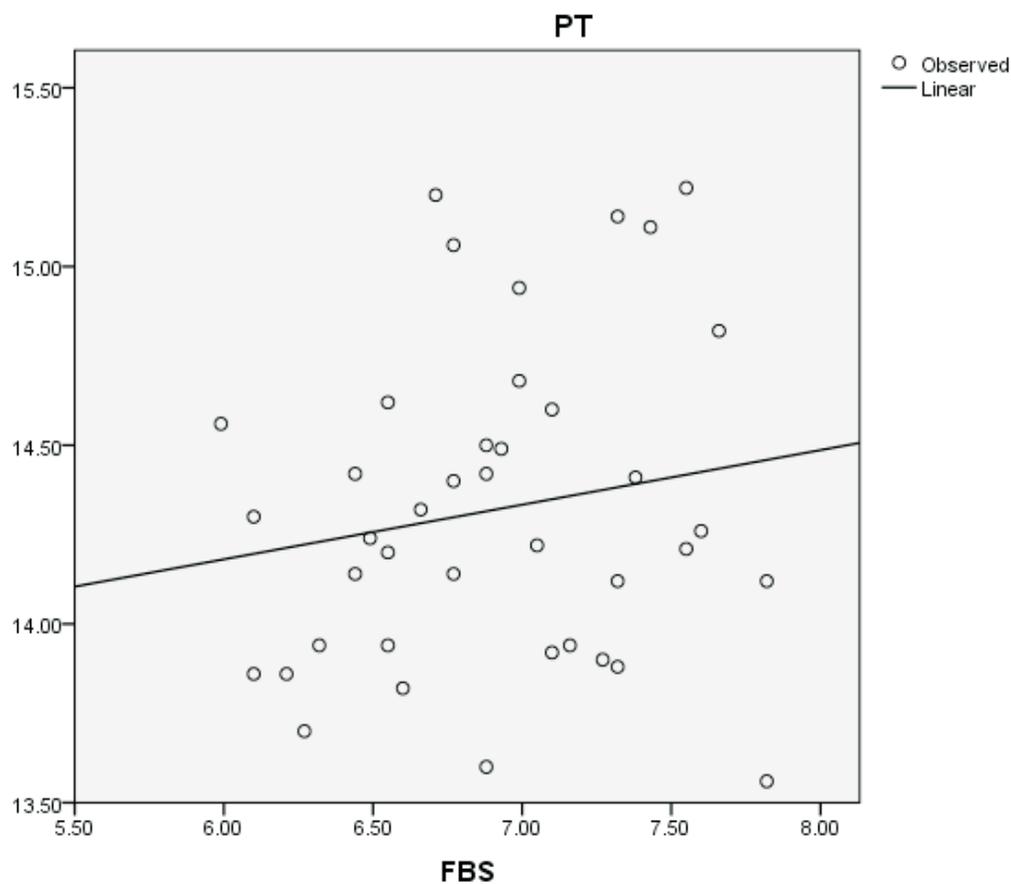


Figure 2: Correlation between FBS and PT in Gestational Diabetic Subjects

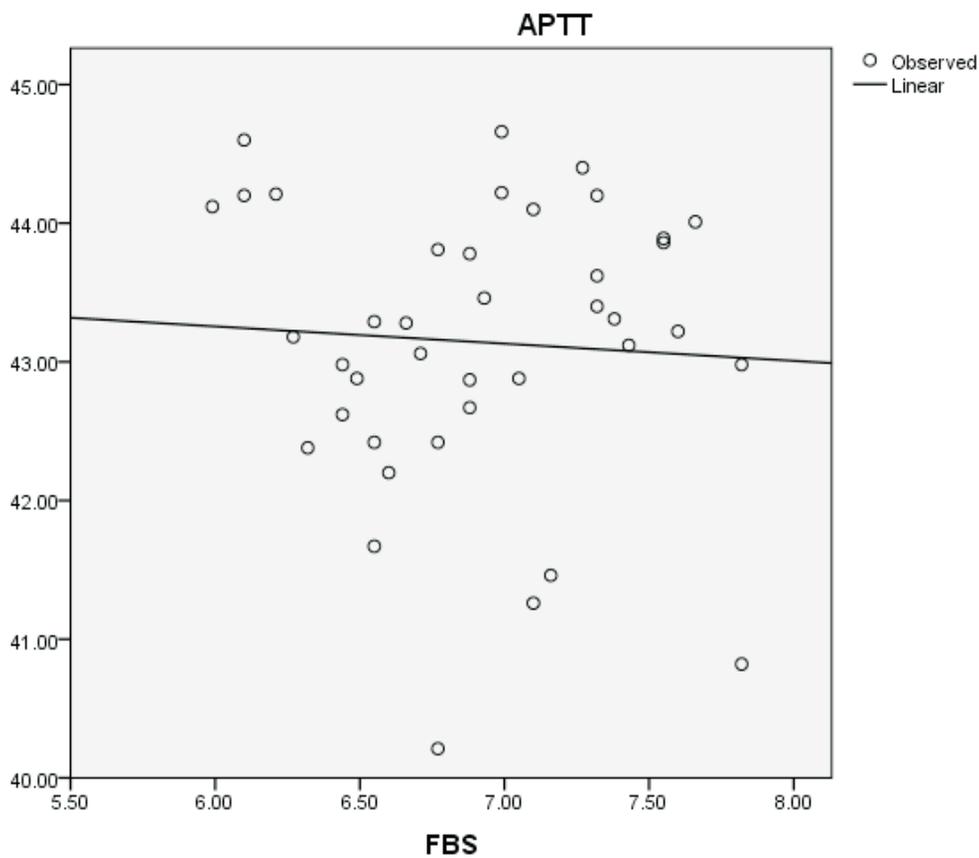
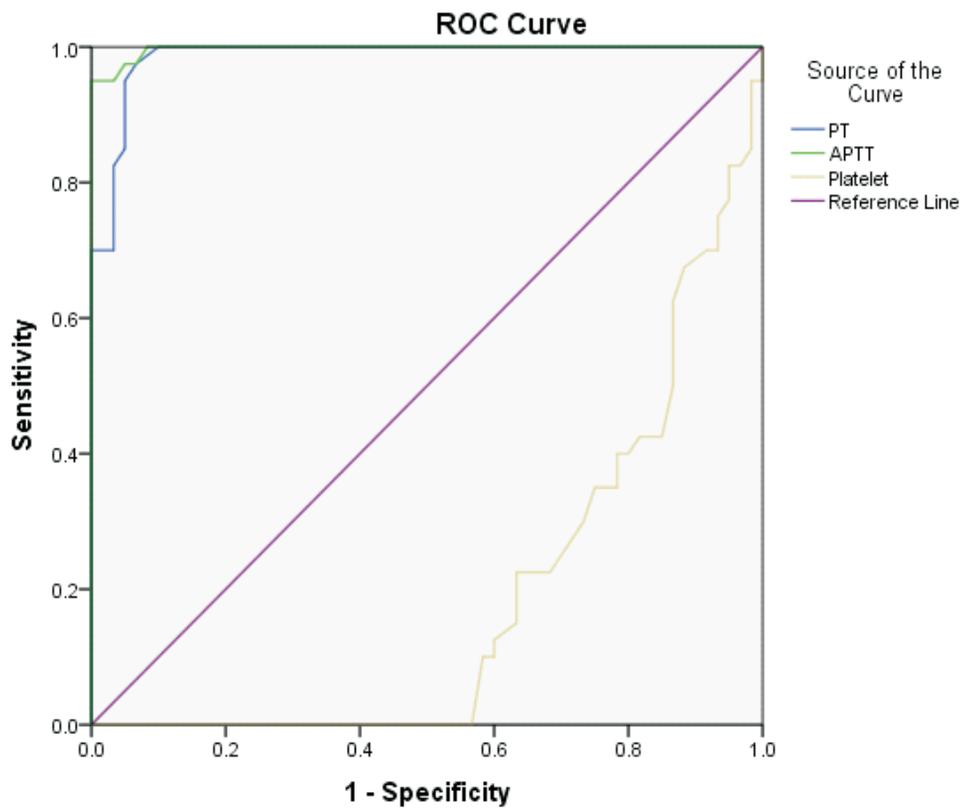


Figure 3: Correlation between FBS and APTT in Gestational Diabetic Subjects



Diagonal segments are produced by ties.

Figure 4: The ROC Curve of blood levels of PT, APTT and Platelet as diagnostic tool in Gestational Diabetic subjects coagulation study