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D-dimer Concentrations in Women with Uncomplicated Miscarriage and Intrauterine Fetal Death: A Prospective Study

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Abstract

The use of D-dimer in identifying women at increased risk of thrombosis/ hemorrhage in pregnancy is understudied. This study aimed to determine the prevalence of coagulation disorders in women with uncomplicated miscarriages and intrauterine fetal death (IUFD) by using the D-dimer concentration and to examine its association with gestational age using curve estimation. Fifty women were diagnosed with fetal death during the study period. Of these, 22cases (44%) were IUFD > 22 weeks, and 28 cases (56%) miscarriage had no pregnancy-associated complications. Both groups were similar in age, prior miscarriage, parity, hemoglobin, red blood cell indices and duration of fetal death p>0.05. No significant differences were observed in PT, aPTT, INR, platelet counts, or fibrinogen p >0.05. The IUFD group had significantly elevated concentrations of FDP and D-dimer (p=0.037), compared to miscarriage group. Also, the study showed 9.1% of IUFD group and 3.6% of miscarriage group at risk of devolving thrombosis. Also, 18.4% of women with IUFD had low platelet counts (counts < $150x10^9$) versus none in the miscarriage group, p=0.019. There was no association between gestational age and D-dimer in both miscarriage and IUFD cases. Based on the result, 9% of women with IUFD and 3.6% of women with uncomplicated miscarriage were at increased risk of thrombosis .

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Keywords: intrauterine fetal death; miscarriage; coagulopathy; clotting tests.

1. Introduction

Fetal death is defined by the National Center for Health Statistics as "death prior to the complete expulsion or extraction from its mother of a product of human conception, irrespective of the duration of pregnancy and which is not an induced termination of pregnancy"[1].

The problem of nonviable pregnancy is when and how to deliver. There is a general agreement that treatment of fetal death should include active (surgical and medical) and expectant management. Despite many studies performed to determine safety and effectiveness of expectant management versus surgical treatment, there is no established protocol for specific management and each case should be managed individually. It is important to offer both the options of termination and expectant management to women once fetal death has been diagnosed [2, 3]. It was found that expectant management is associated with increased rate of infection and maternal coagulopathy [3]. However, surgical option is associated with many complications including infection, uterine perforation and bowel damage [4, 5].

Disseminated intravascular coagulation (DIC) complicated 25% of patients who retain a dead fetus for more than 4 weeks. The risk of DIC in expectant management can be reduced by performing full blood counts and coagulation tests on a weekly basis [6,7].

The authors in [8] found that the total fibrinogen concentrations increase progressively with advancing gestational age. In consumptive coagulopathy in fetal death, tissue factor is the primary activating moiety for the extrinsic pathway of coagulation at different gestational age [8]. The D-dimer cuff-off point of 500 ng/mL has been validated in numerous studies as positive results to initiate treatment [9, 10]. On the other hand, a D-dimer concentration of < 500 µg/L is a robust means of excluding embolism when measured using a high-sensitivity assay system [10]. This study was conducted to determine the D-dimer levels in women with miscarriages and IUFDs and identify the percentage of women that are at risk of developing thrombosis using a cut-off level of D-dimer greater than 500 ng/mL and its association with gestational age using curve estimation.

2. Materials and Methods

From August through December 2014, 50 cases of fetal death were diagnosed in a prospective study. The study was approved by the Department of Obstetrics & Gynecology, Gezira College of Medicine as well as the associated Institutional Research Ethical Committee. The study was conducted according to the ethical standards laid down in the Helsinki Declaration of 1975 and revised in 1983. All women gave their informed consent prior to their inclusion after they were informed about objectives of the study. Fetal death was diagnosed based on clinical examination and confirmed by ultrasound examination demonstrating the absence of cardiac pulsation.

2.1 Study population

Cases (n=50) of fetal death were included in the study. Patients were divided according to survival potential into 28 cases of miscarriage group ≤ 22 weeks' gestations and 22 cases > 22 weeks gestations .

Inclusion Criteria: Cases were excluded if any of the following abnormalities were detected: 1) history of bleeding; 2) chronic disease; 3) history of chronic liver disease;4) patients on anticoagulant therapy;5) placental abruption;6) placenta previa; or 7) hypertensive disorder of pregnancy 8) evident of sepsis.

Data recorded included demographic characteristics, maternal age, parity, gestational age, and estimation of duration of fetal death. Investigations performed for all patients included Prothrombin Time (PT), activated partial thromboplastin time (APTT), platelet count, fibrinogen level, and fibrinogen degradation products (FDPs), and D-dimer. Other basic blood parameters were Hb%, MCH, MCV, MCHC, and hemoglobin.

2.2 Blood sampling

For fibrinogen, FDPs, PT, and APTT tests, blood was collected by a clean venipuncture in plastic tubes containing 0.109 M sodium citrate in a ratio of 9 parts of blood and 1 part anticoagulant. The venous sample was allowed to enter the syringe without pressure.

2.2.1 Sample preparation:

Blood samples were centrifuged at 4000 rpm for 15 minutes to collect platelet poor plasma (PPP). For platelet count, the sample was collected in clean EDTA venipuncture tubes.

2.3 Prothrombin time (PT)

PT was determined by the one-stage procedure in which 0.2 mL of thromboplastin-calcium reagent (Diagnostic Reagents Ltd, Thame, UK). This was then placed in a clotting tube in a water bath for 2 or 3 minutes at 37°C. About 0.1 mL of plasma was added; the tube was tilted gently for a 2- or 3-seconds and a stopwatch was used to calculate time from addition of plasma and formation of a clot.

Activated partial thromboplastin time (aPTT).

Approximately 0.1 mL of plasma was added to 0.2 mL of Kaolin platelet substitute (Diagnostic Reagents Ltd), and the mixture was placed in a clotting tube. The mixture was then gently tilted for exactly 2 minutes. Then 0.1 mL of calcium chloride was added. The tube was tilted for 2- to 5-seconds, and the clotting time was calculated by a stopwatch and recorded. The test was performed twice for both the control and the subject samples.

2.4 Platelet counts

We used a Sysmex blood counter (SysmexKx 21N) (Sysmexcorporation; Mundelein, Illinois, USA). Whole blood diluted with 1% ammonium oxalate at a ratio of 1:19. Platelets were counted in the small central square,

and the calculation followed the simple principle: Dilution factor × cells counted × chamber depth/area of the chamber counted .

Packed cell volumePCV was estimated using the microhematocrit centrifuge (Andreas Hettich GmbH and Co, KG, Tuttlingen, Germany). A heparinized capillary tube was filled with blood, and the empty portion was sealed. The results were expressed as percentages.

2.5 Quantitative D-dimer concentration (D-dimer)

The D-dimer concentration was determined by using the ELISA (Reference number 259906, Lot number RH92A00; TechnoicloneGmDH, Vienna, Austria) with the Technozym D-dimer ELISA kit. Then, 100 μL of the controls and the diluted samples were placed in the test wells and covered with film. It was then incubated at 37°C for 60 minutes. Then, 200 μL of pH 7.3 buffer was used to wash the test wells. Anti-dimer was pipetted into the wells and covered with film and incubated for 60 minutes. The mixture was washed three times using 200 μL of washing buffer. Then 100 μL of chromogen tetraethyl benzidine was added to the test wells, covered with a film, and incubated for 10 minutes. After 10 minutes, a solution of 100 μL was added and shaken for 10 minutes; a golden yellow color appeared. We used a plate reader (Stat Fax®; Awareness Technology, Inc, Palm City, FL) at 450 mm within 10 minutes to measure the absorbance. The D-dimer concentration of the samples and control (high and low) values were obtained from the standard calibration curve by plotting the absorbance against the concentration of the reference standard.

3. Statistical analysis

The Statistical Package for Social Sciences (SPSS 15 for Windows) was used for data analysis and statistics. A chi-square test was used to compare the association between categorical variables, and the Student t-test was used to compare the means of continuous data. The normality of the distribution was examined using the Kolmogorov–Smirnov test. Correlation analysis was used to determine the relationship between the variables. A p-value of <0.05 was considered statistically significant.

4. Results

As expected, women who miscarried had a lower gestational age (15.811±4.0629 vs. 29.127±4.5786 weeks, $p<0.001$) than those with intrauterine fetal death (IUFD). Both groups were similar in other characteristics, maternal age (31.32±6.348 vs. 30.23±7.393years ,p=0.584), parity(3.04±2.301 vs. 3.05±2.627 deliveries=0.989), estimated duration of fetal death(13±1.6 vs. 12±2.8,p=0.116 days),Hb% concentrations (12.171±1.1356 vs. 11.873±1.9372gm,p=0.526), RBS (4.271±.3650 vs. 4.182±.4866,p=0.476)and RBC indices $p>0.05$ (Table 1).

As shown in Table 2, both groups had similar values of PT, aPTT, INR, fibrinogen $p>0.05$. The IUFD group had significant differences in the concentration of FDP and D-dimer (1.46±.744± vs. 2.00±.756, p=0.016) and (0.9761±1.37698 vs. 1.9255±1.75736, p=0.037) versus the miscarriage group, respectively.

Further analysis for extreme values showed that 3.6% of women with miscarriage had D-dimer levels exceeding 500 versus 9.1% in the IUFD group, p= 0.415. Also, 18.4% of women with IUFD had thrombocytopenia versus none in the miscarriage group, p=0.019.Data are presented as mean±SD and number (percentage), a p value of <0.05 is considered significant.

Table 1: Baseline characteristics and some basic laboratory tests between women with Miscarriage and those with intrauterine fetal death

Variable	Miscarriage group (n=28)	IUFD group (n=22)	P value
Age in years	31.32±6.348	30.23±7.393	0.584
abortions	1.50±.638	1.41±.734	0.648
Parity	3.04±2.301	3.05±2.627	0.989
gestation age/weeks	15.811±4.0629	29.127±4.5786	0.000
Duration of fetal death	13±1.6	12±2.8	0.116
hemoglobin	12.171±1.1356	11.873±1.9372	0.526
MCV	36.582±3.4022	35.755±5.1229	0.518
RBS	4.271±.3650	4.182±.4866	0.476
MCV	86.407±4.4903	85.064±2.7159	0.502
MCH	28.625±1.2560	28.232±1.6234	0.690
MCHC	33.186±1.2560	32.886±1.6234	0.480

Abbreviations: prothrombin time, PT; activated partial thromboplastin time, aPTT; packed cell volume, PCV, Hb%, hemoglobin.

Table 2: comparison of test values between women with miscarriage and those with intrauterine fetal death

Variable	Miscarriage group (n=28)	IUFD group (n=22)	P value
PT	14.6±1.4	14.382±1.4	0.461
aPTT	33.2±3.5	34.909±2.6	0.054
INR	1.1±.14	1.042±.15	0.458
Fibrinogen mg/dl	486±127.5	450±143.6	0.431
FDP	1.5±.7	2.00±.756	0.007
D-dimer ug/ml	0.981±1.4	1.9±1.8	0.020
D-dimer >500 ug/ml	1(3.6)	2(9.1)	0.415
Platelets(<150x10^9)	(0)	4(18.4)	0.019

Abbreviations: prothrombin time, PT; activated partial thromboplastin time, aPTT; packed cell volume, PCV,

fibrin degradation product, FDP.

Figure 1, presents the association analysis by curve estimation and illustrated that there were on association between the D-dimer levels and gestational age in miscarriage group, R2 = 0.026, P = 0.485.

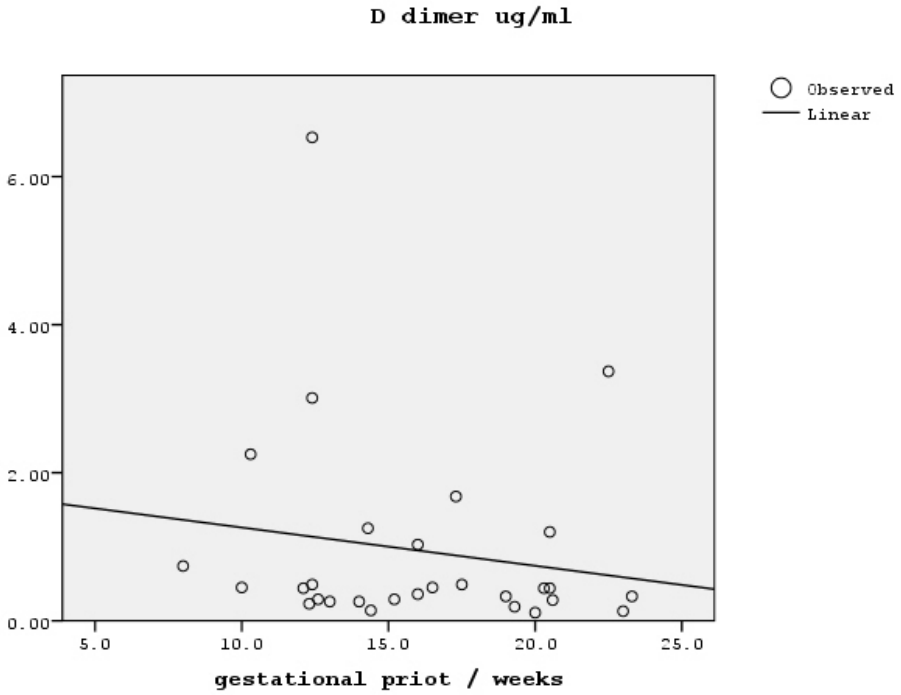

Figure1: Regression Curve estimation of the association of the D-dimer values and gestational ages in women with miscarriage.

Figure 2 presents the association analysis by curve estimation and illustrated that there was no association between the D-dimer levels and gestational age in IUFDgroup, R2 = 0.026, P= 0.485.

5. Discussion

This study shows that the concentrations of the D-dimer are significantly elevated in IUFD group. Moreover, 9.1% of women with IUFD and 3.6% of miscarriage group had an elevated D-dimer above the cut-off point (500 ug/ml) with a possibility of risk of thrombosis. Also, in the IUFD group, 18.4% had thrombocytopenia (150x109). Further, the D-dimer correlated positively and significantly with gestational age in a linear fashion.

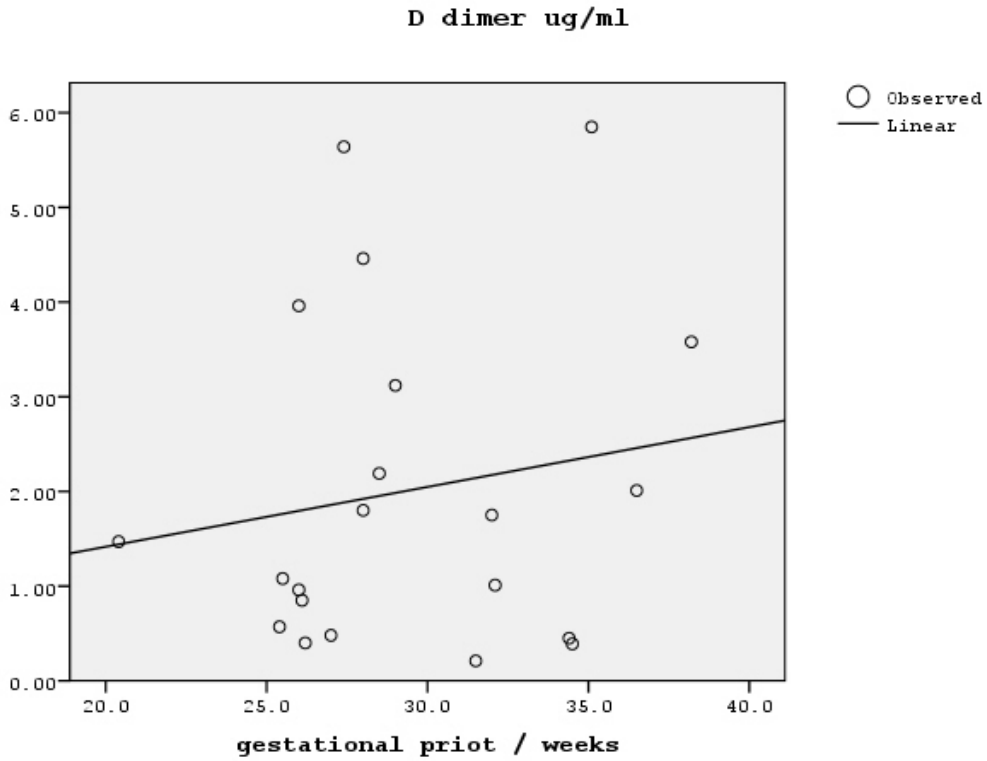

Figure2: Regression Curve estimation of the association of the D-dimer values gestational ages in women with IUFD

The coagulation tests including D-dimer are unreliable in diagnosing disseminated intravascular coagulation especially in normal pregnancy as their values are often increased [9]. Nevertheless, the D-dimer cuff-off point of 500 ng/mL has been validated in numerous studies as positive results to initiate treatment [10]. During pregnancy, a D-dimer value >500 ng/mL is associated with a three-fold risk of maternal mortality [11]. In contrast, the Royal College Obstetricians and Gynecologists in their guidelines reported that a low level of D-dimer in pregnancy is likely to suggest the absence of thrombosis [12].

The D-dimer is more specific for fibrinolysis activity than FDPs, as its formation requires the action of thrombin (to activate factor XIII) to produce cross-linked fibrin and the cleavage of this fibrin by plasmin [13]. In this study, abnormally elevated D-dimer is seen more frequently in IUFD (9.1%) group than miscarriage group (3.6%). Although both groups has similarly estimated a duration of fetal death (0.116), the relatively high frequency of elevated D-dimer in IUFD group is difficult to interpret from the current study. However, additional factors might be acquired as pregnancy advances.

The 9.1% risk of coagulopathy for uncomplicated IUFD in this study is almost comparable to the reported 10.4% and 11% rates for complicated IUFD [14 - 16]. The later studies had estimated frequencies of coagulopathy during labor and within 24 hours following fetal death and utilized PT, aPTT and fibrinogen levels for assessing coagulopathy. Also, we reported 3.6% risk of coagulopathy among women with miscarriages.

There are no previous studies evaluating the prevalence of coagulopathy in women who miscarriage. Indeed, the prevalence of DIC in complicated miscarriage and its severity correlated with the degree of sepsis [17].

The results of D-dimer should be interpreted cautiously depending on the test is used to perform the assay [18]. Studies have shown that the D-dimer can be elevated in pregnancy, advanced age, trauma, post-operative periods, inflammatory states, and increased gestational age [19].

In the present study, the average maternal age was 31 versus 30 years for miscarriage and IUFD groups respectively fall within the reproductive age. Therefore, the rise in the D-dimer in the present study cannot be explained by age as previously mentioned [19, 20]. Regression curve estimation showed that there was no association between the D-dimer level increases with gestational age ($p>0.05$). Jeremiah et al. [17] reported that the D-dimer values increased progressively with advancing gestational in normal pregnant population while Eichinger [21] failed to demonstrate this association in normal pregnancy.

Women with IUFD had significant laboratory evidence of impaired coagulation prior to delivery versus the miscarriage group. That is, there was a significant difference in platelet count, and D-dimer levels. Hence, coagulopathy may be a significant clinical problem in women with IUFD and therefore, early pregnancy termination to reduce the risk of thrombosis. The risk of thrombosis in miscarriage is low unless it is complicated with sepsis [22].Fibrinogen level, PT, and aPTT did not differ significantly between miscarriage and IUFD groups; instead all fall within normal pregnancy ranges. In one study, authors found that fibrinogen is the least sensitive test for diagnosing coagulopathy, and it is the last finding in DIC [23, 24].

The limitation of this study are its relatively small sample size due to financial constraints and the gathering of data from a single center rather than multiple centers data, which is more informative and representative of the general population.

6.Conclusion

We conclude based on the findings of the study that coagulopathy occurs in 9% of women with uncomplicated IUFD and 3.6% of women with uncomplicated miscarriage. Increased prevalence of coagulopathy in women with IUFD, though not significant prone them to increased risk of bleeding compared to miscarriage group. Therefore, early pregnancy termination may reduce such risk. Further studies are warranted to investigate these findings.We recommended that D-dimer can be used as screening tests for women at risks of thrombo-hemolytic disorders.

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Conflict of Interest: None to declare

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