SIGNIFICANCE OF SOME COAGULATION PARAMETERS IN WOMEN WITH UNEXPLAINED RECURRENT IMPLANTATION FAILURES

ABSTRACT

Unexplained recurrent implantation failures are currently a public health problem especially in Sub Saharan Africa. It is a medical condition that has been attributed to many factors including abnormal coagulation. The presented study was conducted to determine the levels of coagulation parameters involving the Platelet count (PLT), Activated Partial Thromboplastin (APTT), Prothrombin Time (PT) and the International Normalized Ratio (INR) in women with unexplained recurrent implantation failures and healthy controls. A total of 80 subjects involving 40 cases and 40 controls aged 20-35 years were recruited for the study. Blood samples were collected from the subjects by venipuncture. The PLT was determined with the Mindray 530BC automated analyzer, Mindray, Japan, the APTT and PT were determined using Haemoscan reagent purchased from Fortress diagnostics, United Kingdom while the INR was calculated from the PT and ISI values. The data was analyzed with SPSS version 23 using Student test. Results were presented as Mean ± SD from the mean and $p<0.05$ considered as significant. The results revealed significant increase in the PLT (225 ± 41.6) in the women with unexplained recurrent implantation failures compared to PLT (189 ± 26.61) for the control. There was also a significant decrease in APTT (36.6±6.6), PT(10.98±0.7),INR(0.57±0.07) in the women with unexplained recurrent implantation failures compared to APTT(43.4±1.0),PT(16.5±1.2) and INR(1.02±0.18) respectively for the control. This finding supports the claim for abnormal coagulation profile in women with unexplained recurrent implantation failures.

Key words: Coagulation, unexplained recurrent reproductive failures, Nigeria

INTRODUCTION

Unexplained recurrent implantation failure is defined as the repeated failures of embryos to implant in a healthy woman aged less than 40 years resulting from a failure of the embryo-endometrial immune crosstalk in at least three or more assisted reproductive therapy cycles following the transfer of competent embryos (1,2).Embryo implantation is a complex process involving apposition, adhesion and invasion of a competent embryo into a receptive

endometrium within a specified period referred to as the window of implantation(3-5).It is considered the rate limiting step in assisted reproductive therapy cycles(6-9).Although advances in biomedical research have given rise to innovative strategies to improve the chances of successful implantation during assisted reproductive therapy cycles, the prevalence of unexplained recurrent implantations failures remains as high as 20%(10). In some clinics, patients may have to undergo as many as seven therapy cycles to achieve successful implantation (10). This is quite challenging especially for a low economic resource setting such as Nigeria considering the cost of payment for repeated therapy cycles. Studies reported that the Assisted Reproductive Program induces changes in the coagulation profile which can alter the embryo-endometrial crosstalk during the window of implantation resulting in failures of implantation (11,12). However, there is currently a paucity of scientific literature on the coagulation profile of Nigerian women with unexplained recurrent reproductive failures. The aim of the present study is therefore to determine the levels of the coagulation parameters involving the Platelet Count, Activated Partial Thromboplastin Time, Prothrombin Time and the International Normalized Ratios in women with unexplained recurrent implantation failures compared to healthy controls.

MATERIALS AND METHODS

Study Area

The study was conducted in Enugu State in the South East geopolitical zone of Nigeria. The state derived its name from her capital and largest city, Enugu. It has an area of 7,161km^2 with a population of 3,267,837 comprising mainly the Igbo tribe of South Eastern Nigeria. It lies between longitudes 6^o 30 'E and 6^o 55 'E and latitudes 5^o 15 'N and 7^{o}15'E. It consists of three senatorial divisions namely Enugu East, Enugu North and Enugu West (13). The ESUT Teaching Hospital is the major tertiary health facility for the State and is located at the centre of the Enugu metropolis (Parklane) for easy accessibility to Enugu residents.

Study Design

This is a cross-sectional case-controlled survey in which women with unexplained recurrent implantation failures served as the cases while age-matched healthy women with good Obstetrics and Gynecology history served as the controls. Blood samples were collected from subjects irrespective of any center in Nigeria where she had been offered an assisted reproductive therapy service.

Sample Size

The means of the differential leukocyte ratios was used to calculate a minimum sample size of 70. Group sample sizes of 35 cases and 35 controls achieved 80% power to reject the null hypothesis of zero effect size when the population effect size is 0.70(moderate to large) and the significance level(alpha) is 0.05 using a two-sided two-sample equal-variance t-test.

Subjects Recruitment

Subject selection was based on a simple random sampling procedure from a population of women with unexplained recurrent implantation failures and healthy women who gave their consent to participate in the study.

Inclusion Criteria

1. A total of 40 aged 20-35 years women who failed to achieve clinical pregnancy after a transfer of at least 3 good quality embryos in at least 3 assisted reproduction therapy cycles served as the cases.
2. A total of age-matched 40 women with good obstetrics and gynecology history served as the controls.

Exclusion Criteria

1. Women diagnosed with hematological disorders, hormonal disorders, infections, thyroid disorders, autoimmune disorders, systemic disorders such as diabetes mellitus.
2. Women with existing or previous ultrasonographic evidence of uterine malformations.
3. Women with a history of smoking, contraception, alcohol or substance abuse.
4. Rhesus negative women with rhesus positive partners.
5. Women with Body Mass Index (BMI) greater than 24.99kg/m2 and/or age greater than 40 years.

Blood Sample Collection

Blood was collected from subjects using venipuncture (14). Subjects were made comfortable in a sitting position. A tourniquet was gently applied 2-5cm just above the antecubital fossa. The antecubital fossa was cleaned using 70% alcohol in cotton wool. A hypodermic syringe and 21G needle were inserted into the lumen of the antecubital vein and seven milliliters(7ml) of blood was drawn quickly by a non-traumatic pulling of the syringe piston. About 5ml was dispensed into an EDTA bottle which was gently mixed for the determination of the platelet count while 1.8ml was dispensed into a Trisodium Citrate bottle for the estimation of the Activated Partial Thromboplastin Time, Prothrombin Time and International Normalized Ratio.

Determination of the platelet count (PLT)

The values of the platelet count were determined by performing an automated full blood count using the Mindray 530BC automated analyzer, Mindray Japan. The samples were aspirated by letting the machine sample probe into the sample bottles and the probe button was tapped.

Approximately 20ul of blood was aspirated and the values were displayed in the screen after about 30seconds as part of the full blood count results (15).

Determination of the Activated Partial Thromboplastin Time(APTT)

The APTT was estimated using a Haemoscan reagent obtained from Fortress Diagnostics, United Kingdom. 0.2ml of the Kaolin/platelet mixture (homeostat reagent) was dispensed into a small tube.0.1ml of the plasma sample was then added and content mixed and incubated for 2 minutes. With the tube being tilted at intervals, 0.1ml of 0.025M calcium chloride was added and the stopwatch was started immediately. The tube was tilted back and forth for clot formation and the time for clot to form was recorded (16).

Determination of the Prothrombin Time (PTT)

The prothrombin time was determined using a plasmascann reagent obtained from Fortress Diagnostics, United Kingdom. 0.2ml of the thromboplastin/calcium reagent (plasmascann reagent) was dispensed into a small tube and placed in a water bath at $37^{o}C$ for about 2 minutes. 0.1ml of plasma sample was added using a calibrated capillary pipette. The contents were mixed and the stopwatch started. The tube was held in a water bath and the mixture tilted back and forth until a clot was formed. The time at which a clot was formed was recorded as the PT in seconds (16).

Determination of the International Normalized Ratio (INR)

The international normalized ratio was determined using the PT and ISI values (17)

$$INR = \frac{\text{Prothrombin time of subject}}{\text{Prothrombin time for laboratory reference plasma)}} ISI$$

Where ‘ISI stands for international sensitivity index for the thromboplastin reagent

Data Analysis

Data was analyzed using SPSS version 23 (SPSS Inc. Chicago). Statistical significance was defined as $p < 0.05$. Differences in the coagulation parameters between the cases and controls were tested using t-test.

RESULTS

There was significant decrease ($p<0.05$) in the mean values of the Platelet Count (PLT), Activated Partial Thromboplastin Time(APTT), Prothrombin Time (PT) and the International Normalized Ratio(INR) in the women with unexplained recurrent implantation failures compared to the healthy control women with good obstetrics and gynecology history.

Table 1: Coagulation parameters in the study cases and controls

Parameters	Controls (n=40)	Cases (n=40)	T-test (p-value)
PLT($x10^9$/l)	189 ± 26.61	225±0.41.60	0.001*
APTT (secs)	43.4 ± 1.0	36.6±6.6	0.032*
PT (secs)	16.5 ± 1.2	10.98±0.7	0.000*
INR	1.02 ± 0.18	0.57±0.07	0.002*

Key: PLT =Platelet Count, APTT= Activated Partial Thromboplastin Time, PT= Prothrombin Time, INR = International Normalized Ratio, *significant at $p<0.05$,Data expressed as Mean ±SD.

DISCUSSION

Blood coagulation may play a crucial role in blastocyst implantation process with its alteration resulting to implantation failures in assisted reproductive therapy cycles. In the present study, we conducted a case-controlled cross-sectional observation study in women with unexplained recurrent implantation failures to explore the relationship between alteration in blood coagulation parameters and successful implantation in assisted reproductive therapy cycles and to identify the biomarkers of coagulation alteration related to assisted reproductive therapy outcome. The present findings revealed a significant decrease in the activated partial thromboplastin time, the prothrombin time and the international normalized ratios in women with unexplained recurrent implantation failures compared to healthy controls. These parameters when decreased in patients are markers for the activation of coagulation cascade both in the intrinsic and extrinsic pathways leading to a mild hypercoagulation which may be the underlying factor predisposing to microvascular circulatory failure due to formation of microclot (microthrombi) and development of nonspecific inflammatory reaction against the implanting blastocyst. Our present finding agrees with some studies which have reported that the in vitro fertilization program during assisted reproductive therapy may induce coagulopathy in subjects which may affect the process of implantation during assisted reproductive therapy cycles (11,18).However, this do not agree with findings of Gerotziafas et al(19) who reported a hypercoagulable state of cellular origin which appears at baseline investigations prior to hormonal treatment in patients and subsequent implantation failures during assisted reproductive therapy.

The present study also recorded a significant increase in the platelet counts of the women with unexplained recurrent implantation failures compared to the healthy controls. This agrees with the findings of Balandina et al (20) who reported significant increase in platelet counts of women with repeated implantation failures during in vitro fertilization therapies. Platelets play major roles in coagulation and the pathological state of hypercoagulation. Platelets when activated releases stored substances such as serotonin, thromboxane A2 and ADP which helps to initiate and promote aggregation which activates coagulation and subsequent formation of microthrombi.

CONCLUSION

Significant decrease in the activated partial thromboplastin time, prothrombin time as well as the international normalized ratio and significant increase in the platelet count among the women with unexplained recurrent implantation failures supports a role for alteration in coagulation parameters in failure of implantation during assisted reproductive therapy cycles. This suggests the use of coagulation parameters for an accurate prediction of prethrombotic state and implantation failure during assisted reproductive therapy. The implication of this is that an integration of anticoagulant therapy in the in vitro fertilization program may help reduce the risk

of implantation failures in patients with unexplained recurrent implantation failures and improve successful outcome.

Ethical Approval and Consent

Ethical clearance was obtained from the Ethical Review Committee of the Enugu State Ministry of Health. The purpose of the research was explained to the participants and only participants who gave verbal and written informed consent were recruited for the study.

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