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Evaluation of platelet count, d-dimer and fibrinogen levels in women on injectable contraceptives

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ABSTRACT

Oral contraceptive use is a widely-accepted risk factors for venous thromboembolism. The role of injectable contraceptives as a risk factor for venous thromboembolism is controversial. The aim of the study was to determine the platelet count, d-dimer and fibrinogen levels in women on injectable contraceptives in Port Harcourt, Nigeria. This is a hospital based cross sectional comparative study, in which d-dimer, fibrinogen levels and platelet count were evaluated in serum, citrated plasma and EDTA samples of 80 women on injectable contraceptives and 80 controls who were not on hormonal contraceptives. D-dimer was analyzed using ELISA based methods, fibrinogen was analyzed using modified Clauss method while platelet count was assessed using a full blood count auto analyzer. The mean d-dimer levels between the cases and controls did not differ significantly (p-value 0.143). The frequency of occurrence of an elevated d-dimer level was also not significant (p-value 1.000). The mean fibrinogen levels in cases and controls were not statistically significant. (p-value 0.145). None of the cases nor controls had a high level of fibrinogen. Although the mean platelet count was normal in both populations, the cases had a significantly lower platelet count (214.16 ± 65.42 X 10^9/L) compared to the controls (244.18 ± 77.34 X 10^9/L), p = 0.03; however the frequency of having a high platelet count was not significant. The study found no significant difference between D-dimer and fibrinogen levels between women on injectable contraceptives and non-users. Although it found a statistically significant lower platelet count on injectable contraceptive users. However, no clinical significance could be adduced as the mean platelet count for cases and controls were within normal limits.

Keywords: Oral contraceptive, platelet count, d-dimer and fibrinogen.

INTRODUCTION

Contraception is an intentional prevention of conception through the use of various devices, sexual practices, chemicals, drugs, or surgical procedures [1]. Several modern contraceptive methods exist, and their usage has been on the increase in many parts of the world, especially in Asia (66%), Latin America and the Caribbean (73%) but has remained low in sub-Sahara Africa (22%). The current prevalence rate for contraceptive use in Nigeria is

approximately 11%–13%, this figure highlights the setbacks to the use of birth control which includes unavailability, religious beliefs, poor health care services, disapproval from spouse, and ignorance about the effects of birth control [2]. Among the hormonal contraceptives, injectables are becoming more popular than oral contraceptive pills, especially in Rivers State in the southern part of Nigeria. These findings are from records obtained from the family planning units of University of Port Harcourt Teaching Hospital (UPTH) and Braithwaite Memorial Specialist Hospital (BMSH) both in Port Harcourt. Injectable contraceptives are also the most effective and frequent form of contraception used by African women, basically because of its effectiveness and convenience. This study therefore seeks to evaluate the platelet count, D – dimer and fibrinogen levels in women on injectable contraceptives in Port Harcourt and determine their risk of thromboembolic events.

Before the 20th century, different forms of contraception were practiced. Some were so precarious that it resulted to infertility or death. In the vagina, honey acts as a spermicide and lint soaked in juice of acasia was used to block sperm [3]. Breast-feeding of up to three years which is still practiced today was used by our ancestors to prevent conception. It is believed that continuous breast feeding of a child prevents ovulation [4]. Coitus interruptus was mentioned in the bible; in the book of Genesis, Onan refused to ejaculate inside his deceased brother's wife to avoid fathering a child for him. Silphium (a plant with contraceptive and abortificient properties) was used in ancient Greece as a form of contraceptive [5]. The oldest condom made from animal gut was found in the foundations in Dudley castle in England, this dated back to 1640. It was only in the 20th century however that condoms became publicly available [6]. The first intrauterine contraceptive device was developed and marketed by Richard Richter in 1909 and Ernst Gräfenberg in 1920s. Although oral contraceptive pills were made available to the public in 1960s, it was first developed in the 1950s following studies done by Gregory Pincus and John Rock with the help of Planned Parenthood Federation of America [7]. Contraceptive options presently available are; hormonal contraceptives (oral contraceptives, skin patch, vaginal ring, implants, injectables), intrauterine devices (IUDs), - which contain either a hormone or copper, barrier devices with or without spermicides (diaphragm, cervical cap, sponge, condom), fertility awareness methods (temperature, cervical mucus, calendar,), female sterilization (tubal ligation) or male sterilization (vasectomy) [8].

OBJECTIVES

To compare the platelet count, d-dimer and fibrinogen levelsin women on injectable contraceptives with those that are not on contraceptives.

RATIONALE FOR STUDY

Based on departmental records, injectable contraceptive use is on the increase at the University of Port Harcourt Teaching Hospital and Braithwaite Memorial Hospital. There are some reports of an increased risk of thrombosisin women on injectable contraceptives. There is limited data on the thrombotic risk of Nigerian women on injectable contraceptives. This study seeks to compare the platelet count, d-dimer and fibrinogen levels in women on injectable contraceptives with those that are not on contraceptives in Port Harcourt attending University of Port Harcourt Teaching Hospital and Braithwaite Memorial Hospital family planning unit, thereby providing a baseline data for monitoring women on injectablecontraceptives

MATERIALS AND METHODS

Study Location

This study was conducted at the University of Port Harcourt Teaching Hospital (UPTH), a Federal Government tertiary institution and Braithwaite Memorial Specialist Hospital (BMSH), a state owned tertiary institution. Both tertiary health institutions are situated in Port Harcourt, Rivers State and have several specialties including Haematology, Obstetrics & Gynaecology and Paediatrics. These hospitals also serve as the main referral centres for Rivers State and its environs.

STUDY DESIGN

This is a cross sectional comparative study.

STUDY POPULATION

Women on injectable contraceptives attending the family planning clinic in two tertiary health care centres – University of Port Harcourt Teaching Hospital and Braithwaite Memorial Hospital in Port Harcourt metropolis comprised the subjects. While thecontrols comprised of apparently healthy women between the ages of 18 and 45 who were not on any form of contraceptives and who gave their informed consent to participate.

Inclusion Criteria for the Cases

1. Attendees of the family planning clinic on injectable contraceptives.
2. Non–pregnant women of reproductive age (18-45) who have been on injectable contraceptives

Exclusion Criteria for the Cases

1. Women with chronic illness (except hypertension and diabetes mellitus), malnutrition or on anticoagulant therapy.
2. Past and present history of thrombosis

The controls were apparently healthy women (except those with a history of

diabetes mellitus or hypertension) between the ages of 18-45 who gave consent to participate in the study and had never been on hormonal contraceptives.

ETHICAL CONSIDERATION

Ethical approval was obtained from University of Port Harcourt Teaching Hospital. The study objectives and procedure for sample collection and follow up was explained to each participant before signing the consent form. Confidentiality and animosity were maintained in the course of the research.

SAMPLE SIZE ESTIMATION

Consecutive sampling was used

The sample size was calculated from the formula $n = Z^2pq/d^2$. Where n = the required sample size (when population is >10,000). Z= the standard normal deviation, usually set at 1.96 which corresponds to 95% confidence interval. P= the proportion of the target population estimated to have a particular characteristics. In this research, data from United Kingdom revealed that 3% of women are on injectables, there are varying or controversial literature concerning the number of women on injectable contraceptives in Nigeria. Some studies gave 7.9% while others gave 3.8%. However due to absence of local prevalence in Port Harcourt, 5% prevalence was used to calculate the sample size. Q = 1.0 – p = 1.0 – 0.05 = 0.95 D = the degree of accuracy which is usually set at 0.05. With this formula n = 72 Applying this formula, a minimum sample size of 72 was determined. However given 10% non – response rate, a minimum sample size of appropriately 80 was recruited into the study.The control sample was taken from women within the ages of 18-45 years that are not on hormonal contraceptives.

SAMPLE COLLECTION AND PREPARATION

After filling the questionnaire, venous blood was collected by venipuncture following standard sterile procedure into 2 vacuum tubes. Blood measuring 5ml was put into an EDTA bottle and 4.5ml into a bottle containing 0.5ml of 0.129M sodium citrate to ensure adequate blood-anticoagulant ratios. Samples were properly mixed after sample collection. Samples were also collected for C–reactive protein (CRP) and erythrocyte sedimentation rate (ESR) levels assessment, as controls for d-dimer and fibrinogen testing to rule out acute phase reaction. Those with high CRP or ESR values were excluded. The sample in the EDTA bottle was used to evaluate the platelet count of the participants. Samples in the sodium citrate bottle which was for fibrinogen assay and d-dimer testing were centrifuged at 3500 rpm for seven minutes and stored at room temperature. If testing was not done

within four hours of collection, the plasma was separated immediately and stored at -20°C for two weeks.

Materials

1) Auto haematology analyzer (model: BC0800)
2) Glass test tubes
3) Stop clock
4) Water bath
5) Precision pipettes (capable of delivering between 0-200ul)
6) Racks
7) Microplate reader capable of measuring absorbance at 450nm
8) Precision pipettes to deliver 2ul to 1ml volumes
9) Adjustable 1-25ml pipettes for reagent preparation
10) 100ml and 1litre graduated cylinders
11) Absorbent paper
12) Distilled water or deionized water
13) Log-log graph paper or computer and software for ELISA data analysis
14) Test tubes to prepare standard or sample dilutions

Reagent

1) Calibration/standard plasma
2) Control plasma
3) Bovine Thrombin solution
4) Imidazole Buffer
5) Distilled water
6) D-dimer Microplate of 96 wells coated with antihuman d-dimer
9) Wash Buffer Concentrate
3) Standard Protein of Human d-dimer
4) Detection Antibody d-dimer of biotinylated anti-human d-dimer
5) HRP-Streptavidin Concentrate
6) TMB One-step Substrate Reagent
7) Stop solution (Sulfuric acid)
8) Assay Diluent buffer

METHODOLOGY

D-Dimer and Fibrinogen testing was carried out in duplicate for all the samples. Full blood count was carried out on the sample in an EDTA bottle using an autoanalyzer.The normal reference range of platelet count is 90-350 x 10^9/L. Fibrinogen Assay. Fibrinogen Assay was done using a modified Clauss Fibrinogen Reagent Kit. A modified Clauss Fibrinogen Reagent Kit has a high thrombin concentration that makes the test virtually insensitive to heparin (10 I.U./mL) and enhance clot detection.

Fibrinogen assay procedure

1) Standard plasma was diluted with imidazole buffer solution. This gave a range of fibrinogen concentration (i.e. 1in 5, 1in 10, 1 in 20 and 1in 40).
2) 200ul of the solution was warmed in a water bath, at 37^0c.
3) 100ul of thrombin solution was added and the clotting time measured.

4) A plot of the clotting time against fibrinogen concentration in seconds and g/l respectively was made on a log/log graph paper.
5) 1in 10 dilution of each test samples was made with imidazole buffer solution.
6) 200ul of this test sample dilutions was clotted with 100ul of thrombin solution and clotting times was noted.
7) The fibrinogen concentrations of each test sample was read off the graph.

Normal range approximately 1.5 -4.0g/l

D – Dimer Assay

D-dimer testing was measured with IMUCLONE® D-dimer ELISAmethod following the manufacturers instruction

1) 100ul of sample was added to each well
2) The microplate was incubated at room temperature for 2.5 hours
3) The microplate was washed 4 times with wash solution, decanted and blotted with absorbent paper.
4) 100ul prepared biotin antibody was added to each well.
5) The microplate was then incubated at room temperature for 1 hour.
6) The microplate was once again washed 4 times with wash solution, decanted and blotted with absorbent paper.
7) 100ul prepared Streptavidin solution was then added.
8) Thereafter the microplate was incubated at room temperature for 45 minutes.
9) Subsequently it washed 4 times with wash solution, decanted and blotted with absorbent paper.
10) 100ul TMB One-Step Substrate Reagent was added to each well.
11) The microplate was then incubated at room temperature in the dark for 30 minutes.
12) 50ul of Stop solution was added to each well.
13) The Absorbance was read at 450nm immediately.

Plasma normal level < 200ng/ml

PRECAUTIONS

1) Contact was avoided with the skin and mouth, even when the reagent did not contain reactive components.
2) Donor blood and materials used for the preparations were handled as potentially infectious agents, although the blood had been found to be sero-negative to HIV, HCV and HbsAg.

DATA ANALYSIS

Data entry and analysis were done using the Statistical Package for Social Sciences (SPSS) software version 22.0. The quantitative variables such as d-dimer, fibrinogen and platelet valueswere summarized as means ± standard deviation while frequencies and proportions were used to

summarize categorical variables. Differences in means between groups (women on injectable and controls) were compared using the student t-test.Chi-square and Fishers exact test were used as appropriate to compare differences in proportions.Probability values less than 0.05 ($p < 0.05$) were considered as significant.

RESULTS

A total of 160 women were recruited for this study, comprising of 80 women on injectable contraceptives (cases) and 80 women not on any form of contraceptives (controls). Both groups were within the 18-45 years age bracket. The study was conducted over a period of 24 months (July 2015 to June 2017).

Comparing the mean d-dimer, fibrinogen levels and platelet count in the study group

The mean d-dimer levels in the cases and controls were 74.37±49.37 g/L and 86.44±54.24 g/L respectively.There is no significant difference in both means (P = 0.143).Also, the mean fibrinogen levels in cases and control were 2.77±0.76g/L and 2.52±0.92g/L respectively. Similarly the means were not significantly different from each other (p = 0.08). None of the cases nor controls had a high fibrinogen value, however 3.75% of the cases had a low fibrinogen value while 7.50% of the control group had low fibrinogen values. On the other hand, the mean values for platelet count was significantly higher in the controls than the cases. Their respective values are 244.18±77.34 X 10^9/L and 214.16±65.42 X 10^9/L, with a p-value of 0.039 as shown in table IV.There were 3 (3.75%) of the cases and 2 (2.5%) of the controls with a low platelet count.

Table I: Socio-demographic Characteristics of Respondents

Age in years	Cases n (%)	Control n (%)	Total n (%)
≤20 years	0 (0.00)	19 (23.80)	19 (11.90)
21 – 30 years	19 (23.80)	48 (60.00)	67 (41.90)
31 – 40 years	39 (48.80)	7 (8.80)	46 28.80)
>40 years	22 (27.50)	6 (7.50)	28 (17.50)
	*Chi square = 62.956; p-value =0.0001**		
Marital status			
Single	1 (1.20)	68 (85.00)	69 (43.10)
Married	79 (98.80)	12 (15.00)	91 (56.90)
	*Chi square = 114.388; p-value = 0.0001**		
Educational level			
Primary	9 (11.30)	0 (0.00)	9 (5.70)
Secondary	25 (31.20)	0 (0.00)	25 (15.70)
Tertiary	46 (57.50)	80 (100.00)	126 (78.80)
	*Fisher's exact test = 50.030;p-value = 0.0001**		
Employment status			

Unemployed	12 (15.00)	64 (80.00)	76 (47.50)
Employed	68 (85.00)	16 (20.00)	42 (26.20)
*Chi square = 79.960; p-value = 0.0001**			

*Statistically significant

Table II: Comparison of Mean D-Dimer, Fibrinogen and Platelet Count among Cases and Controls

Variables	Cases Mean ± S.D	Control Mean ± S.D	t-test	p-value
D-Dimer	74.37±49.37	86.44±54.24	-1.472	0.143
Fibrinogen	2.77±0.76	2.52±0.92	1.467	0.145
Platelet count	214.16±65.42	244.18±77.34	-2.091	0.039*

S.D- Standard deviation *Statistically significant

DISCUSSION

Injectable contraceptives are generally believed not to be associated with any thrombotic risk, but there are conflicting reports on thrombotic risk in women on injectable contraceptives [1]. Some researchers have reported an increased risk of venous thromboembolism. However most of the available studies were conducted in non-African populations. There is paucity of studies on this in our environment [2]. In this study of apparently healthy women, between the ages of 18 and 45 years, the mean ages of the cases and controls were significantly different with the cases being older than the controls, although they were both within the age range specified for the study. More married women were on injectable contraceptives than those in the control group, only one single woman was on injectable contraceptives. This study observed that the mean d-dimer levels in both cases and controls although low, were within the normal reference range. The difference in mean were also not significantly different in both cases and controls.This is in keeping with three other studies conducted by Goldstein *et al* (2007) [9], Gohar *et al* (2000) [10] and Vaneska *et al* (2017) [11]. In these individual prospective studies of women followed up over time, they observed a reduction in d-dimer levels. This observation may suggest a protective effect of injectable contraceptives, with a decrease in thrombogenic risk. Unlike the index study which is cross-sectional, we could not determine if d-dimer levels will further reduce over time in these women on injectable contraceptives as observed by authors stated earlier. Gohar *et al* (2000) [10] and Syed *et al* (2002) [12] observed that the fibrinogen levels in both cases and controls were within the normal range and they were not significantly different. This is similar to the finding in this study

where fibrinogen levels were observed to be within the normal reference range in both the cases and controls. This was also found not to be statistically significant. Mean fibrinogen levels were of normal reference values in cases and controls and this was not significantly different. This observations reflect a normal in fibrinolytic potential of women on injectable contraceptives. Although the mean platelets count of both groups were within the normal reference range, the platelet count of the controls was significantly higher than that of the cases. The Joseph et al had a different observation. In a related study involving 180 women on oral and injectable contraceptives and 100 women that were non-users, he recorded no significant difference in the mean platelet count of both cases and controls. Reports have shown that long term use of hormonal contraceptives (OCP) can lead to reduced platelet counts, however there are no published reports on the effect of injectable contraceptives on platelet count.

CONCLUSION

The study found no significant difference between D-dimer and fibrinogen levels between women on injectable contraceptives and non-users. Although it found a statistically significant lower platelet count on injectable contraceptive users. However, no clinical significance could be adduced as the mean platelet count for cases and controls were within normal limits.

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